A thesis entitled

"STUDIES ON THE SYNTHESIS AND REACTIVITY OF SOME POTENTIALLY CYTOTOXIC COMPOUNDS"

submitted by

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in part fulfilment of the requirements for the
Degree of Doctor of Philosophy
of the University of London
in the Faculty of Science

Imperial College,
London, S.W.7.

June, 1965.

ABSTRACT

The new investigations described in this thesis have been mainly concerned with the synthesis of a range of α-halo-ketones and their conversion into enol esters. The latter, being vinyl halides, were expected to be much less reactive than the parent α-halo-ketones, but capable of being reconverted into them by enzymic fission *in vivo*.

A method has been developed for the synthesis of α-acetoxy-α'-halostilbenes in moderately good yields, and these compounds represent the first reported examples of enol esters derived from acyclic α-halo-ketones. Their reactivities and those of the α-halo-ketones towards pyridine in aqueous ethanol have been measured, the results confirming that enol acetylation considerably reduces the reactivity. Evidence is presented for the structure of the products formed in the reaction of the α-halo-ketones with pyridine.

Some enol phosphates derived from 2-haloacetophenones and from bromopyruvic acid have been synthesised, and some exploratory studies made on methods by which they might be converted into the free phosphoric acid derivatives.

Certain other halides of potential biological interest, including α-halo-orthoesters and some amide substituted halides of the lachrymator type have been synthesised.

ACKNOWLEDGEMENTS

I would like to express my gratitude to Professor L.N.Owen for his constant guidance and encouragement throughout the course of this work, and to my colleagues in the Armstrong Research Laboratory for many helpful discussions.

I am also indebted to Imperial College for a research assistantship and to the University of London for the award of the Arthur Jubber Postgraduate Studentship and a subsequent studentship.

I also wish to thank the staff of the Micro-analytical Laboratories for analyses, Mrs.A.I.Boston and Mr.E.A.King for spectrographic data, Dr.J.A.Elvidge for his assistance in interpretation of N.M.R. spectra and Mrs.T.K.Perkins for typing the manuscript.

Finally I thank my Wife for her continual help and encouragement, and her parents, Mr. and Mrs.E.A. Sharpley, whose many kindnesses and sacrifices I shall never be able to repay.

Armstrong Laboratory,
Imperial College.

D.J.Cooper,
June, 1965.

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Chemotherapy of Cancer.

As far as is known at present, the chemical constituents of neoplastic tissue are qualitatively identical to those of the parent, normal tissue. Many quantitative chemical differences between cancerous and normal cells have been noted; e.g. enzyme distributions and activities, rate of synthesis of proteins, rate of synthesis of nucleic acids, pH etc. It seems likely that the major qualitative differences will be found in the cell genotypes, but as yet methods of chromosome analysis are so crude as to be of little use. Busch et al.[1] have recently advanced some evidence for the presence of a specific nucleoprotein in cells of the Walker 256 rat carcinoma, but conclusive proof is still required.

The aim of cancer chemotherapy is to destroy, selectively, the neoplasm, with the minimum possible damage to the host. Clearly this problem is complicated by the similarity of host and parasite tissues. Chemotherapeutic agents must be designed to take account of the quantitative chemical differences between host and parasite and, as Danielli[2,3] has pointed out, the more of these variables that a drug depends upon for its activity, the greater will be its selectivity. Thus workers in the field of cancer chemotherapy have concentrated upon the

elaboration of small molecules, known to be cytotoxic to some degree, with the object of increasing the selectivity, whilst leaving the active centre intact (or reproducible *in vivo*.) To date, cytotoxic agents have tended to fall into three groups : antimetabolites, hormones and the biological alkylating agents.

The antimetabolites have been confined largely to purine, pyrimidine and folic acid antagonists. In almost every case the antimetabolite is a derivative of the parent metabolite. Antimetabolites have been among the more successful cytotoxic agents. Purine, pyrimidine, and folic acid antagonists have been reviewed by Timmis[5].

Hormone treatment has been confined to highly specialised forms of cancer and the compounds used have been steroids with an oxygen function at C_{11}, to which the cytotoxicity is thought to be due.

This thesis will be concerned largely with alkylating agents, and for this reason the antimetabolites and hormones will not be further discussed.

Biological Alkylating Agents.

Compounds which can function as alkylating agents under physiological conditions of temperature and pH and in aqueous media, are termed biological alkylating agents, and have recently been comprehensively reviewed by

Ross[4]. There are many types e.g. benzyl halides, sulphonic acid esters, phosphoric acid esters, chloromethyl ethers, 2-chloroethyl sulphides, epoxides, ethylene-imines, diazo-alkanes, activated ethylenic compounds, α-halogeno-ketones, α-halogeno-esters and nitrogen mustards. The nitrogen mustards are the most widely studied of these agents and many of the principles of cytoxic drug design can be exemplified by reference to them. Thus, although this thesis is concerned mainly with α-halo-ketones and α-halo-esters, on which little work in the cancer field has been done, some discussion of the chemical and biological properties of the nitrogen mustards, as typical alkylating agents, is an essential introduction.

Nitrogen Mustards.

Prelog et. al.[6] and Ward[7] observed that methyl di-2-chloroethyl amine (HN2) and tri-2-chloroethyl amine (HN3), respectively, were analogous to sulphur mustard gas (I) in possessing vesicant properties. In addition

$CH_3.N(CH_2.CH_2Cl)_2$ (HN2) $N(CH_2.CH_2Cl)_3$ (HN3) $S(CH_2.CH_2Cl)_2$ (I)

the mustards exerted a leucopenic effect, i.e. they

produced a drop in the white blood cell count. This latter effect led to trials of a number of nitrogen mustards for the treatment of leukaemia. Later it became evident that nitrogen mustards were active against rapidly dividing cells in general and their use against cancer was extended. Comprehensive investigation of the chemical and biological effects of these compounds have since been carried out, but their initial promise as curative agents has not been fulfilled. The bulk of the work on nitrogen mustards has centred about the difunctional compounds (II), i.e. derivatives of HN2, in which many diverse

$$R.N(CH_2.CH_2Cl)_2$$

(II)

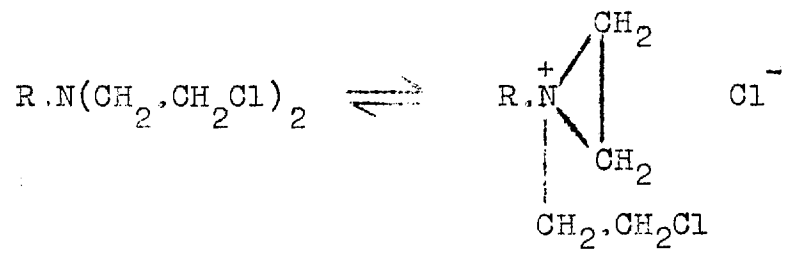
groups R have been tried.

The Reactions of the Nitrogen Mustards.

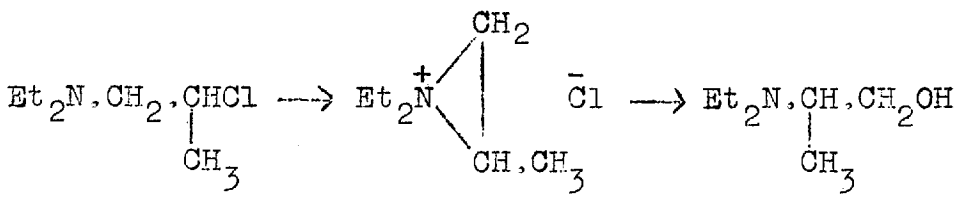
Hydrolysis

Aliphatic nitrogen mustards (II, R = alkyl) ionise rapidly in aqueous media, by a unimolecular process, giving rise to one equivalent of halide ion. In the absence of charged nucleophiles, one equivalent of hydrogen ion is then slowly liberated. The initial step is formu-

lated as formation, by the basic nitrogen atom, of a cyclic ethylene imonium ion:



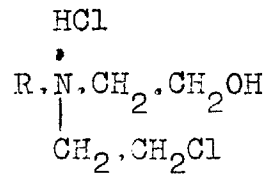
e.g. in the case of HN2 at 37°C this process is complete in about 2 minutes. The cyclic ethylene imonium ion can then undergo bimolecular attack at a rate dependent on the nature and concentration of the nucleophiles present in the system. Evidence for the existence of the cyclic ion has come from two quarters: Golumbic et. al.[8] and Fruton and Bergmann[9] were able to isolate derivatives such as picryl sulphonate salts from aqueous solutions, whilst Ross[10] showed that (III) gave the rearranged product (IV) on hydrolysis and this can only be explained by the formation of a cyclic



(III) (IV)

ion and subsequent S_N2 ring opening. In general, nucleophilic attack on the cyclic ethylene imonium ion is bimolecular in the simple aliphatic nitrogen mustards.

After one equivalent of hydrogen halide has been liberated hydrolysis ceases due to formation of the hydrochloride (V). In buffered solutions (V) can be hydrolysed



(V)

to completion.

In the case of the aromatic nitrogen mustards (II, R= aryl) the basicity of the nitrogen atom is very much less than that of the aliphatic compounds; consequently formation of a cyclic imonium ion is not possible. Thus the aromatic nitrogen mustards tend to hydrolyse by an S_N1 mechanism, the rate determining step being ionisation of the halide to give a carbonium ion. This mechanism is characterised by simultaneous liberation of halide and hydrogen ion, a retardation of the reaction on adding halide ion, an acceleration on adding other nucleophiles and by placing electron repelling substituents on the aromatic

ring. The aromatic nitrogen mustards are therefore more efficient as biological alkylating agents than their aliphatic analogues, since the rate of alkylation is not dependent on the concentration of nucleophilic centres in the system. They also offer greater possibilities for modification of reactivity by various types of substitution.

Variation of hydrolysis rates.

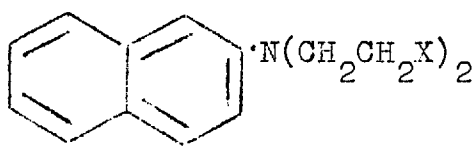
Ross [12] has shown that the extent of hydrolysis, under specified conditions (50% aqueous acetone, containing 20 m.mole of nitrogen mustard per litre, boiled for 30 min. the liberated hydrogen halide being determined volumetrically), can be correlated with the cytotoxic effects of the mustard. He found[13] that, in general, the "hydrolysis rate" under these conditions must be 20% or more for the compound to show inhibitory effects towards the Walker 256 carcinoma.

The rate of hydrolysis of a nitrogen mustard, under given physical condition, will depend on two parameters (i) the nature of the group R and (ii) the nature of the β-halogens.

(i) If the group R is electron releasing then, in general, the rate of hydrolysis will be increased in the case of the aromatic nitrogen mustards (S_N1 rate-determining step) and decreased for the aliphatic nitrogen mustards

(S_N2 ring opening is rate-determining step). The converse will be true if R is electron withdrawing.

(ii) On substituting bromine for chlorine in nitrogen mustards there is a marked increase in the rate of hydrolysis. The corresponding iodo-compounds, however, have a lower rate of hydrolysis than the bromo-compounds e.g.



X	% hydrolysis
Cl	15
Br	80
I	64

It is likely that a mixed mechanism of hydrolysis operates in the case of the iodides and this is supported by the reactive sequence Cl < Br < I in the case of the 3-halogeno-propylamines, where the mechanism is bimolecular. That the iodo-compounds have been found to be of little use as cytotoxic agents emphasizes the importance of the S_N1 mechanism in this group of compounds.

Sites of alkylation *in vitro*.

At physiological pH (about 7.5 in most biological systems) the number of groups which can be alkylated by biological alkylating agents is severely limited. Only those groups existing in a reactive nucleophilic form

at pH 7.5 will be readily alkylated. For proteins, Ross[14] gives the following table of reactive fractions (f) of the main functional groups at pH 7.5 :

Table I

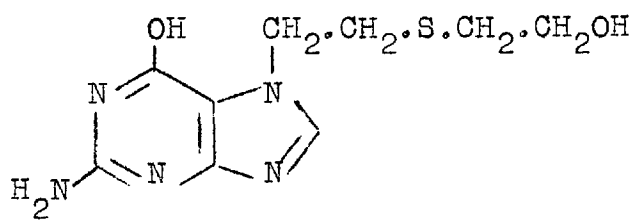
Group	pKa	f at pH 7.5
α-Carboxyl	3.0 - 3.2	0.9999
Carboxyl (aspartyl)	3.0 - 4.7	0.9999 - 0.999
Carboxyl (glutamyl)	4.4	0.999
Phenolic hydroxyl (tyrosine)	10.4	0.001
Sulphhydryl (terminal cysteinyl)	7.9- 8.5	0.01 - 0.06
Sulphhydryl (non-terminal cysteinyl)	10.8	0.0005
Imidazolium (histidine)	5.6 - 7.0	0.99 - 0.76
α-Ammonium	7.6 - 8.4	0.44 - 0.11
ε-Ammonium (lysine)	9.4 - 10.6	0.01 - 0.001
Guanidinium (arginine)	11.6 - 12.6	0.0001 - 0.00001

It can be seen that whilst carboxyl groups can be readily alkylated under these conditions, amino groups cannot. Sulphhydryl and phenolic hydroxyl groups are even less readily alkylated. Similar arguments can be extended to other biologically important molecules. Table II[14a] gives the reactive fraction (f) of functional groups in nucleic acid constituents.

Table II

Group	pKa	f at pH 7.5
Primary phosphoryl	2.0	0.9999
Secondary Phosphoryl	6.0	0.96
Aromatic Hydroxyl (uracil, thymine)	10.2	0.002
Aromatic hydroxyl (guanine)	10.1	0.0025
Aromatic amino (guanine)	2.3	0.9999
Aromatic amino (adenine, cytosine)	3.7/4.2	0.999
Sugar hydroxyl	13	10^{-5}

The ring nitrogen atoms of guanine, adenine and cytosine are seen to be highly reactive to alkylating agents. Lawley and Brookes[15] isolated (VI) and (VII) from acid hydrolysates of nucleic acid pretreated with sulphur mustard gas, indicating the N_7 position as the most reactive site of guanine.



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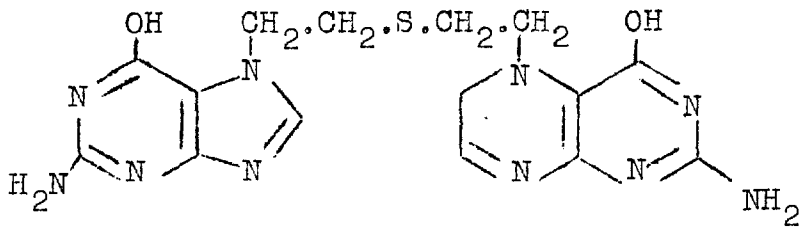


(VII)

Reaction of alkylating agents in vivo

Studies on the in vivo reactions of alkylating agents have been carried out using two approaches:

(i) Agents labelled with a radioactive tracer atom have been administered to biological systems to seek to determine any localisation of the reaction site. In general this has not proved particularly informative, mainly owing to the difficulties encountered because of redistribution of the tracer by secondary processes other than alkylation.

(ii) Chemical analysis has been employed to determine the exact sites of alkylation. Thus Lawley and Brooks[15] administered sulphur mustard gas to mice bearing an ascites tumour. Analysis of the RNA, DNA and protein components of the tumour showed the nucleic acids to be the main components attacked. Hydrolysis of the RNA and DNA

fractions and subsequent examination by autoradiography and paper chromatography gave guanine derivatives as the only identifiable alkylated moities.

It seems likely that alkylation of nucleic acids is the most important action of the nitrogen and sulphur mustards. The resulting alkylated nucleic acids are considerably less stable than the parent molecules, thus affording one possible mechanism for the biological action of the mustards. The fact that the monofunctional nitrogen mustards (VIII) do not inhibit tumour metabolism

$$R_2N.CH_2.CH_2X \qquad \text{(VIII)}$$

has led Timmis[16] to suggest that alkylation of nucleic acid by one arm of the alkylating agent may be followed by irreversible alkylation, by the remaining alkylating group, of enzymes essential to nucleic acid synthesis. An alternative possibility may be simple cross-linking of DNA strands and consequent inhibition of the replication process.

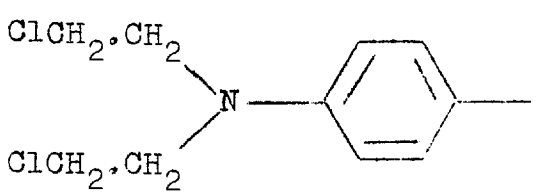
Approaches to specific cytotoxicity.

Whilst there are no qualitative differences between neoplastic and normal cells there are many quantitative differences, e.g. enzyme activities, protein

synthesis, nucleic acid synthesis, adaptation and resistance to drugs, pH etc. In addition such variables as permeability properties and active transport may be incorporated into drug design. Nitrogen mustards have been prepared which make use of one or more of these variables with a corresponding increase in cytotoxicity. Some of these are described below.

Enzymic activation.

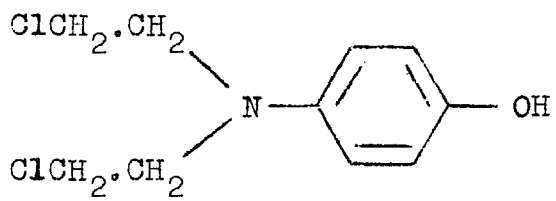
Generation of cytotoxic compounds from non-toxic compounds by enzymic action *in vivo* is referred to as enzymic activation. In view of the many differences in enzyme activities in normal and cancerous cells (for an excellent review see Bergel[17]) this represents an attractive method of obtaining selective cytotoxicity. Consider the nitrogen mustard (IX). Substitution in the p-position by an amino group gives (X) in which the



(IX), X = H
(X), X = NH_2
(XI), X = $NHCOCH_3$

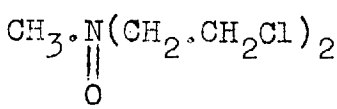
alkylating power is greatly enhanced by the electron releasing substituent. There is a corresponding increase in overall toxicity to both host and tumour. If the

p-amino group is acetylated to give (XI), the alkylating power falls, due to the electron withdrawing effect of the N-acetyl group. Biological systems contain enzymes capable of deacetylating (XI) to give (X) *in vivo* and it transpires that these enzymes have greater activity in some tumour cells than in normal host cells. As a result (XI) tends to have greater cytotoxicity, but less toxicity toward the host, than either (IX) or (X). Similarly, Hebborn and Danielli[3] have shown that the Walker 256 carcinoma contains enzymes of high activity, capable of hydrolysing the acetate and benzoate esters of the nitrogen mustard (XII).



(XII)

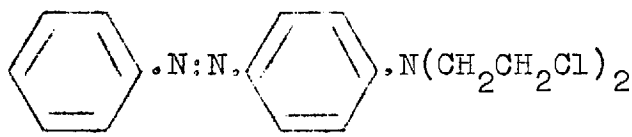
In the aliphatic series the nitrogen mustard (XIII), "Nitromin"[18], an oxidation product of HN2, has



(XIII)

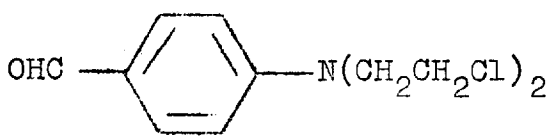
been reported to have a lower host toxicity than HN2 and to cause complete regressions of the Yoshida sarcoma.

Presumably (XIII) is reduced *in vivo* to HN2 by a reductase having high activity levels in the sarcoma. Activation by reductive enzymes has also been observed for azobenzene derivatives, such as (XIV) which is reduced *in vivo* to the nitrogen mustard (X).



(XIV)

Enzymic activation by oxydation is thought to occur in the case of (XV) which, despite a low hydrolysis



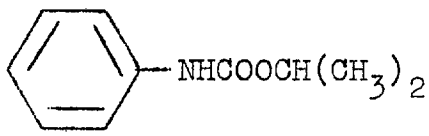
(XV)

rate[19], shows moderate anti-tumour activity. Oxidation to the carboxylic acid occurs, *in vivo*, and this compound, at physiological pH, exists in the reactive anionic form.

Adaptation and resistance.

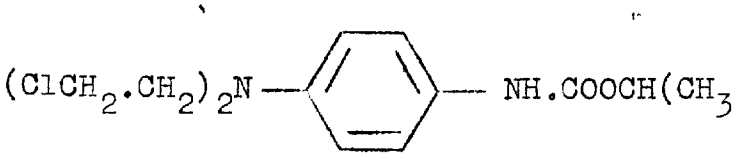
Tumour cells, and to a lesser extent the host cells, are able to develop resistance to cytotoxic agents. In the tumour such resistance could arise by (a) the

evolution of detoxifying enzymes, (b) deletion of activating enzymes or (c) development of an improved active transport mechanism resulting in rapid elimination of the drug. Thus the Walker 256 carcinoma readily develops resistance to urethane. Danielli[20] has shown that in the case of the urethane (XVI) the tumour develops an enzyme capable of



(XVI)

liberating aniline from the compound. This formation of an adaptive enzyme can be utilised to produce effective cytotoxic agents; e.g. Danielli[20] administered the urethane (XVI) to a rat carrying a Walker tumour. When full resistance to the compound had been induced, the urethane (XVII) was administered, whereupon the detoxifying enzyme for (XVI) became an activating enzyme for (XVII) and 80% complete regression of the tumour was noted, compared to only 10% in the case of (XVI) alone and 0% for



(XVII)

(XVII) alone. This process of potentiation has been used extensively with other nitrogen mustards and may well prove to be of use with other cytotoxic compounds.

For further information on this and other topics discussed above, the reader is referred to the book by Ross[4].

The Reaction of α-Halo-ketones with Nucleophiles.

α-Halo-ketones (and α-halo-esters, which they closely resemble) contain the grouping (XVIII). The chemical reactivity of the halogen atom in these compounds

— CO.CHX —	— CH_2.CHX —
(XVIII)	(XIX)

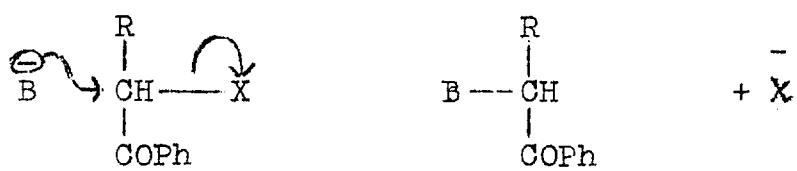
is very much greater than that of the halogen atom of the corresponding alkyl halides (XIX). This, at first sight, is somewhat surprising since polarisation of the carbonyl group renders the halogen atom more positive and it might thus be expected to be less readily displaced than the halogen of the corresponding alkyl halide. Reaction of α-halo-ketones with nucleophilic reagents can lead to a variety of products other than the simple substitution product, e.g. epoxides, saturated and unsaturated ketones

and rearranged acids and acid derivatives have been obtained. This complexity of products arises from the existence of a number of possible sites of attack for the nucleophilic reagent, the favoured site depending on the nature of the ketone, the nucleophilic reagent and the reaction conditions. In this thesis only ketones of type (XX) and (XXI) will be considered and in these particular cases the

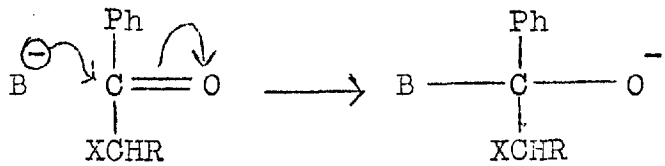
$$\begin{array}{l} Ph.CO.CHR \\ \qquad\quad | \\ \qquad\quad X \end{array} \qquad \begin{array}{l} (XX)\ R = H \\ (XXI)\ R = Aryl \end{array}$$

number of possible products is somewhat reduced by the absence of α' and β hydrogen atoms. There are four possible sites of attack for nucleophilic reagents: (a) the α-carbon; (b) the carbonyl group; (c) the α-hydrogen atom; (d) the α-halogen atom.

(a) Attack on the α-carbon atom leads to "normal " S_N2 substitution:

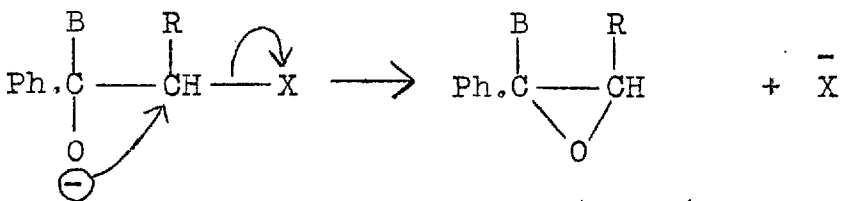


(b) Attack on the carbonyl group leads to the oxy-anion (XXII):



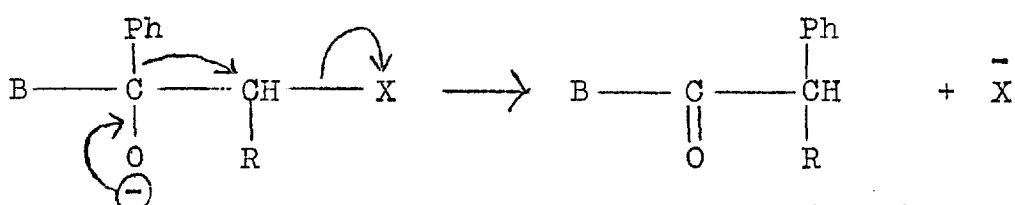
(XXII)

which can then eliminate halide ion in three ways; (i) to give an epoxide (XXIII):



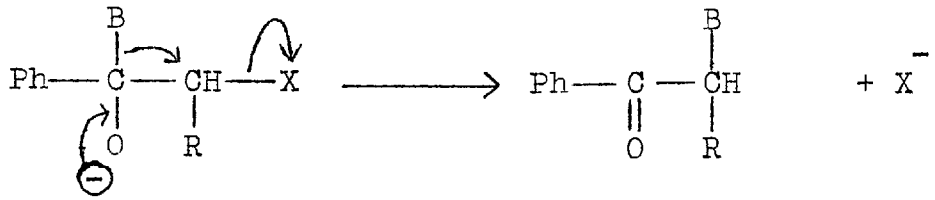
(XXIII)

(ii) to give an acid or acid derivative (XXIV):



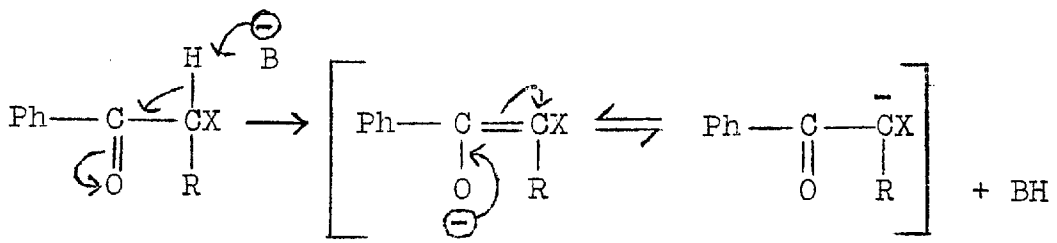
(XXIV)

(iii) to give a ketone identical with the "normal" substitution product:



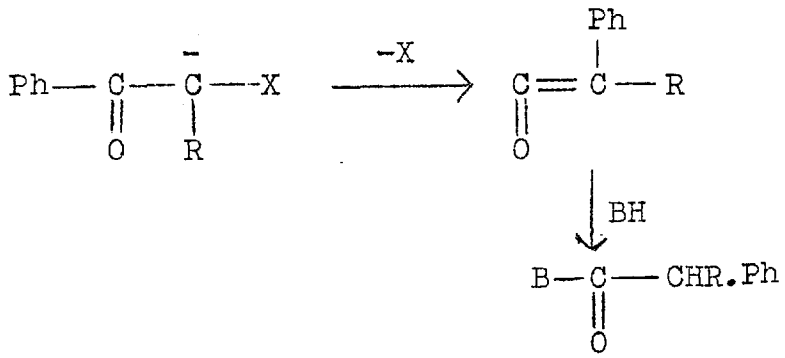
(XXV)

(e) Attack on the α-hydrogen atom produces the anion (XXVI) which can undergo rearrangement to produce



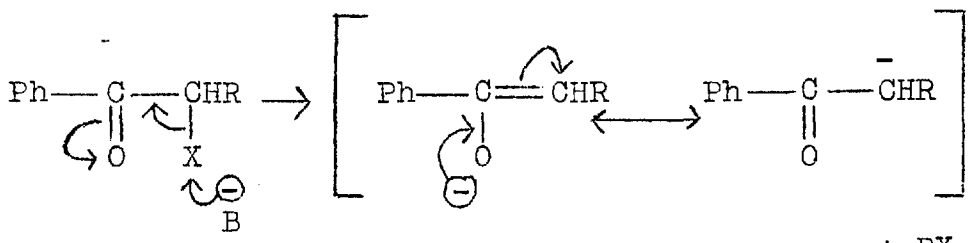
(XXVI)

an acid or acid derivative (XXVII):

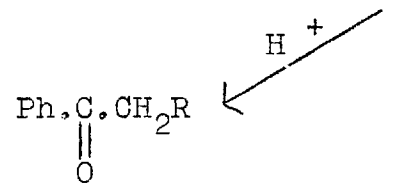


(XXVII)

(d) Attack on the α-halogen results in formation of a saturated ketone (XXVIII) via the anion (XXIX):



(XXIX)



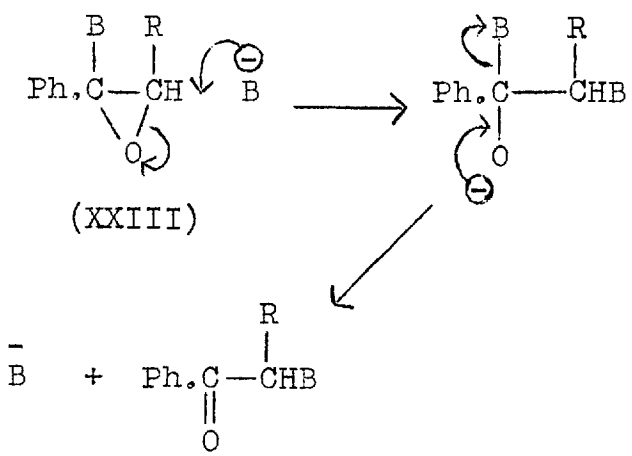
(XXVIII)

The rate determining stage of all of these reactions has been found to be bimolecular with, as yet, no exceptions; i.e. rate $\propto$ [nucleophile] . [α-halo-ketone] .

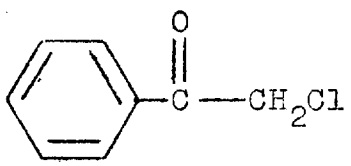
From the point of view of their use as alkylating

agents, only α-halo-ketones which undergo predominantly substitution with nucleophiles will be of importance. This condition is fulfilled by the 2-haloacetophenones discussed above.

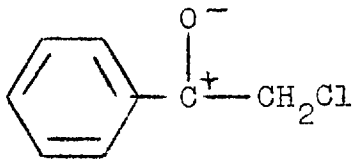
The exact mechanism of the substitution reaction is still not clear. It could occur by direct replacement of the halogen as in (a) or by rearrangement of the oxy-anion (XXII) as in (b) (iii). Alternatively, isomerisation of the epoxide (XXIII) may occur:



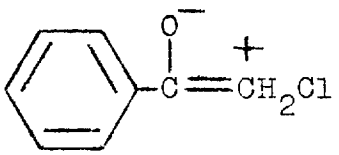
Baker[21] considered that the valence bond representations of phenacyl chloride, (XXX), (XXXI), (XXXII) and (XXXIII), presented a prima facie case for initial attack by the nucleophile on the carbonyl group; this would lead to a mechanism going via the oxy-anion (XXII).



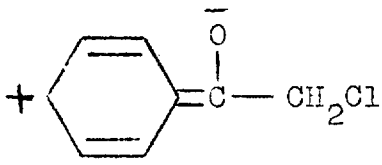
(XXX)



(XXXI)



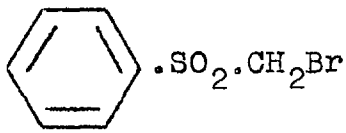
(XXXII)



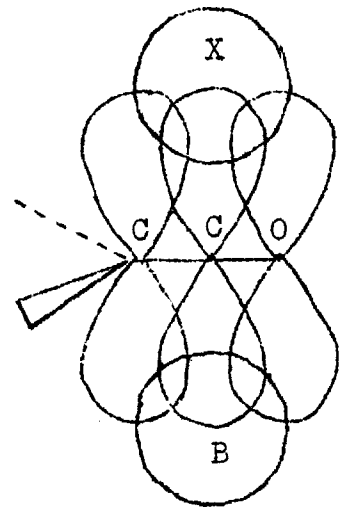
(XXXIII)

He had earlier shown[22] that the reaction of substituted 2-haloacetophenones with pyridine in dry acetone, is facilitated by introduction of electron-attracting groups into the ring and retarded by electron-donating groups as is required by this mechanism. In addition Thomson and Stevens[23] found that phenyl bromomethyl sulphone (XXXIV) did not react with either benzyl dimethylamine or piperidine despite the powerful negative inductive effect of the SO_2 group and this again lends support to the idea of initial attack at the carbonyl-group of the 2-halo-acetophenones.

Streitwieser[24] has suggested that direct S_N2 replacement of the halogen of α-halo-ketones may go through a transition state (XXXV) stabilised by π-bond overlap, with a consequent enhancement of the reaction rate.



(XXXIV)

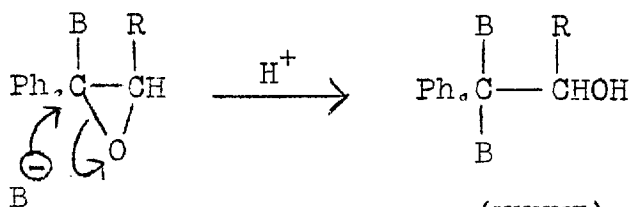


(XXXV)

The observation by Baker[25] that 2-bromacetophenone reacts about sixty times as rapidly with pyridine as does 2-chloroacetophenone supports initial attack on the α-carbon atom, since the rates of addition of pyridine to the carbonyl groups of these ketones would not be expected to differ by such a large amount. Pearson et. al.[26] measured the rate constants for the reactions of thiourea and pyridine with a series of α-bromo-ketones in methanol. They found that the bromo-ketones were more reactive towards thiourea than towards pyridine. Thiourea is the stronger nucleophile but the weaker base; rates of addition of reagents to a carbonyl-group are usually a function of their basicity and not nucleophilicity, whilst for S_N2 displacement of a halogen from an alkyl halide the reverse is true. Thus the results favour initial attack of the nucleophile at the α-carbon atom.

The possibility of addition of an amino-group of thiourea to the carbonyl group was excluded by observing that the rate of reaction was not affected by the addition of acid; addition of an amino-group to a carbonyl-group is invariably acid catalysed.

Isomerisation of the epoxide (XXIII) seems unlikely as an important mechanism for formation of the simple substitution product, since the second mole of nucleophile would be expected to attack the carbon atom of lowest electron density and in most cases this would give rise to the product (XXXVI):



(XXXVI)

In some experiments[27,28,29] hydroxyketals corresponding to (XXXVI) have been isolated. This mechanism is further opposed by the work of Pearson et.al.[26] In a competition reaction, between aniline and pyridine, for 2-bromoacetophenone, they observed that the ratio of the rate constants was equal to the ratio of the products. If the mechanism of substitution involves an epoxide intermediate then the

closing and opening of the epoxide ring would have to occur with equal relative rates to obtain these results; this is extremely improbable.

Kinetic Work on the 2-Haloacetophenones.

The first detailed investigation of the reactivity of the 2-haloacetophenones was made by Matheson and Humphries[30]. They measured the rates of reaction of 2-chloro-, 2-bromo- and 2-iodo-acetophenone with aniline, in alcohol, at 40°. The aminolysis:

$$Ph.CO.CH_2X + PhNH_2 = Ph.CO.CH_2NHPh + HX$$

was followed by Volhard estimation of the liberated halide ion. It was found that, if equimolar quantities of ketone and aniline were used, the reaction did not go to completion due to salt formation between unchanged aniline and liberated hydrogen halide. If a large excess of aniline was employed the reaction became pseudounimolecular; the reactivities of the 2-haloacetophenones were compared in terms of the pseudounimolecular rate constant, k, and are set out in table III.

Table III

Reaction of 2-haloacetophenones (0.2 Mole) with aniline (5 Moles) in alcohol at 40°

Compound	$10k\ min.^{-1}$
2-chloroacetophenone	0.67
2-bromoacetophenone	1.66
2-iodoacetophenone	1.90

The order of reactivity I > Br > Cl is as expected for an S_N2 type reaction.[31]

Baker [22,25] extended this work, using similar experimental procedures, to cover the reactions of a variety of substituted 2-haloacetophenones with pyridine and aniline in 90% ethanol and with pyridine in anhydrous acetone. His results are summarised in Tables IV, V and VI.

Table IV

Values of $10^3k(min.^{-1})$ for the interaction of $R.C_6H_4.CO.CHR'X$ with aniline (0,25 Mole) in M/40 solution in 90% alcohol at 30.5°.

R	R' = H			R' = Me	R' = NO_2
	X = I	X = Br	X = Cl	X = Br	X = Br
p-MeO	10	-	-	-	-
p-Me	11	11.4	-	-	-
H	14.8	14.5	0.15	0.7	-
m-NO_2	47	44	-	2.6	-
p-NO_2	41.4	35	-	-	0.025

Table V

Values of 10^3k ($min.^{-1}$) for the interaction of $R.C_6H_4.CO.CH_2X$ with pyridine (0.25 Mole) in M/40 solution in 90% alcohol at 30.5°

R	X = I	X = Br	X = Cl
H	5.0	6.5	0.11
m-NO_2	8.2	11.3	0.15

Table VI

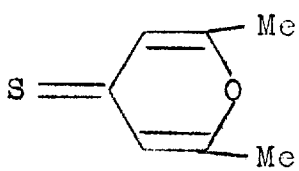
Values of 10^3k, (l. $g.mole^{-1}$ $sec.^{-1}$) for the interaction of $R.C_6H_4.CO.CH_2Br$ and pyridine in 0.025 M solution in dry acetone.

R	10^3k	
	20°	40°
H	0.79	2.83
p-Me	0.74	2.62
o-Me	0.55	-
2,4-Me_2	0.50	1.82
2,4,6-Me_3	Not measurable	
p-t-Bu	0.57	2.00
p-NO_2	ca 1.9	-
o-NO_2	ca 0.24	-

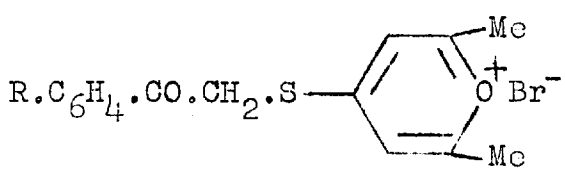
The results in tables IV and V are in general agreement with an S_N2 mechanism analogous to that for alkyl halides. In Table VI it can be seen that although

substitution by a nitro-group in the p-position in 2-bromoacetophenone increases the reaction velocity about 2.5 times, similar substitution in the o-position reduces the velocity to about one-third of that of the unsubstituted parent. Baker attributes this result to a direct effect of the negative end of the nitro-group dipole on the $-CO.CH_2Br$ side chain.

Ozog et. al.[32] examined the reaction of 2,6-dimethyl-4-thiopyrone (XXXVII) with substituted 2-bromoacetophenones to give the pyrylium salts (XXXVIII).



(XXXVII)



(XXXVIII)

In benzene or acetone solution the reaction was found to be first order with respect to each of the reacting species. The effect upon the rate of reaction, in benzene solution, of m and p-substituents was determined and it was shown that the results (Table VII) can be described by the Hammett equation. The reaction was followed by weighing the precipitated pyrylium salt.

Table VII

Reaction of $R.C_6H_4.CO.CH_2Br$ and 2,6-dimethyl-4-thiopyrone in 0.2 M solution in benzene.

R	10^4k l.mole^{-1} sec.$^{-1}$	
	25.4°	14.8°
p-MeO	13.5	6.78
p-CH_3	16.3	7.80
H	18.7	9.05
p-Br	30.8	15.2
p-Cl	31.6	15.8
m-Br	34.0	16.8
m-NO_2	93.5	46.5
p-NO_2	107	54.5

Again the results are in general agreement with an S_N2 mechanism for the reaction.

Some Biological Properties of α-halo-ketones and α-Halo-esters

Many of the α-halo-ketones and α-halo-esters belong to the class of compounds known as the lachrymators. Their lachrymatory powers are due to the presence of an activated halogen atom, which, as discussed above, is rendered positive by the adjacent carbonyl group. Ford-Moore[33] has shown that there is a quantitative correlation

between the positiveness of the halogen and the lachrymatory power. The commoner lachrymators have been reviewed by Jackson and Jackson[34].

In biological systems the lachrymators behave as enzyme inhibitors. Mackworth[35,36] investigated this property and found that many enzymes were not inhibited. Those that were, were all found to be enzymes depending on thiol-groupings for their activity. Furthermore, these enzymes were poisoned by any lachrymator regardless of its chemical structure, although the lachrymators deriving their powers from an activated halogen atom were by far the most efficient poisons. This evidence suggests that the enzyme inhibition is brought about by irreversible reaction of the lachrymator with the free thiol-groups of the enzyme. Further experimental work has confirmed this view.

Dixon[37] has shown that the thiol-groups of cysteine and of proteins are rapidly and irreversibly alkylated by 2-chloro- and 2-bromo-acetophenone, e.g.

$$CySH + BrCH_2.CO.Ph = Cy\text{-}S\text{-}CH_2.CO.Ph + HBr.$$

It was shown that[38] all of the thiol-groups present in denatured muscle protein were alkylated on exposure to a M/1000 solution of 2-bromoacetophenone for 5 minutes at

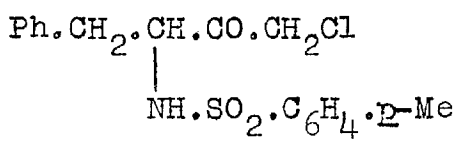
room temperature. The possibility that the reaction of thiol-groups with the protein involved oxidation of the thiol-groups to disulphides was discounted by showing that reduction of the alkylated protein with glutathione and cyanide did not reproduce the free thiol-groups.

Some thiolic enzymes can be reversibly oxidised to inactive disulphide forms. Van Heynigen[39] was able to show that such enzymes, in their thiolic form, are inactivated by ethyl iodoacetate, a potent lachrymator. In the disulphide form the enzymes were not inactivated and removal of the ethyl iodacetate (by addition of excess cysteine) and subsequent reduction, regenerated the active thiolic enzyme. Similar results were obtained by Mackworth[35] working with the enzyme papain.

Hirade et. al.[40] investigated the toxicity of a variety of lachrymators. They found that a correlation existed between the "thiol reactivity" of the lachrymators and their toxicity; no such parallelism could be found between the "amino-reactivity" and toxicity.

Whilst the most outstanding reactivity of lachrymators is towards thiol-groups, some cases have been reported of enzyme poisoning by reaction at alternative sites. Chen-Lu Chu et. al.,[41] showed that 2-bromo-4'-nitroacetophenone would inhibit papain, pancreatic ribonuclease,

α-chymotrypsin and insulin. Their results suggest that, at least in the cases of insulin and ribonuclease, inactivation is brought about by alkylation of an imidazole group of a histidine residue. Yu-Kun Sun and Chan-hu Tsou[42] found that, at pH 5, ethyl bromoacetate or ethyl iodoacetate, in the presence of excess cysteine, were strong inhibitors of papain. Furthermore, after complete inhibition of papain by ethyl bromacetate, the thiol content of the enzyme was unchanged. Hydrolysis of the enzyme and paper chromatography of the hydrolysate indicated that S-carboxymethylcysteine was absent. The authors suggest that histidine and not cysteine is the site of attack of the lachrymator. Schoellmann and Shaw[43] synthesised N-tosylphenylalanylmethyl chloride (XXXIX) and showed that it inhibited chymotrypsin at pH 7.2. The point of attack



(XXXIX)

of the alkylating agent was shown to be a histidine residue and the authors suggest the imidazole ring as the probable site of alkylation.

In conclusion it appears that enzymes relying for their activity on the presence of thiol-groups are almost always inactivated by lachrymators; considerable evidence is available to suggest that this is due to alkylation of the thiol-groups. In addition it would seem that alkylation of histidine residues, even in certain thiolic enzymes, may play an important part in the inactivation of enzymes by lachrymators.

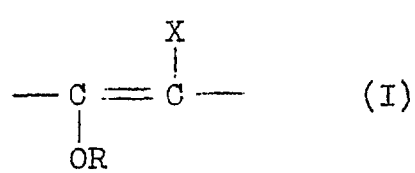
The Possible Use of α-Halo-ketones and α-Halo-esters as Cytotoxic Agents.

The behaviour of α-halo-ketones and α-halo-esters as biological alkylating agents has been discussed above with particular regard to their action as enzyme inhibitors. Because of the wide spectrum of enzymes inactivated by these compounds they will have, at suitable dosages, the ability to inhibit cell metabolism completely and thus bring about death of the cell. If this action could be directed selectively against neoplastic tissue then a useful cytotoxic drug would have been obtained. The mechanism by which these compounds alkylate is, as detailed above, exclusively of the S_N2 type; the rate of alkylation is therefore dependent on the concentration of nucleophilic centres and thus, with the reactive halides desirable, much of the drug will react prior to arriving at the tumour site. In addition, although the nitrogen mustards derive some selectivity from the considerable differences in the rate of synthesis of DNA and RNA between normal and neoplastic cells, differences in enzyme activities are not so pronounced and similar selectivity would not be expected with lachrymators, which would probably tend to be as toxic towards the host as to the tumour. Much of the work described below has been carried out with

a view to synthesising derivatives of α-halo-ketones and α-halo- esters that are inactive as alkylating agents but are amenable to conversion, *in vivo*, to the parent alkylating agent.

Derivatives of α-halo-ketones.

It has been shown (see p.18 *et seq.*) that compounds having the grouping —CO.CH_2X— are often powerful alkylating agents. Furthermore, regardless of the precise point of attack of the substituting nucleophile, there is little doubt that the carbonyl-group plays an important activating role - either by providing a labile site of attack for the nucleophile or by stabilising the transition state via π-bond overlap. Thus it might reasonably be expected that destruction of the carbonyl function would deactivate the α-carbon atom towards nucleophilic substitution. For the α-halo-ketones this can be achieved by conversion to a derivative of the unstable enolic form (I, R = H), in which, not only is the keto-group lost, but also the α-carbon becomes ethylenic and hence even less reactive towards nucleophilic substitution.



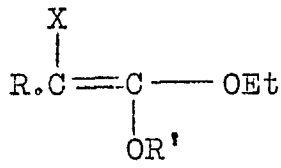
(I)

The form which this derivative shall take is governed by two considerations; firstly it must be designed such that the R—— O bond is of a type readily cleaved by an enzyme having a high activity in tumour tissue (or by an enzyme which can be induced in the tumour by prior administration of a non-toxic compound) and secondly it must be chemically stable and amenable to synthesis. The latter condition is met by three types of derivative; enol ethers (I, R = alkyl, aryl), enol esters (I, R = CO.Alkyl or CO.Aryl) and enol phosphates (I, R = phosphate, phosphate salt or phosphate ester). Enol ethers are unlikely to be useful because of the relative stability of ether linkages in biological systems. Enol esters and enol phosphates are both of interest because of the variety of esterases and phosphatases, present in biological systems, capable of cleaving the enol ester linkage. At least in the case of enol phosphates some selectivity of action towards tumour cells might be expected because the high rates of metabolism in neoplastic tissue require high activity for enzymes of the phosphatase group.

Derivatives of α-halo-esters

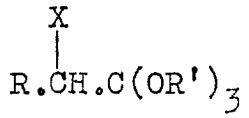
The α-halo-esters owe their alkylating powers to the ester carbonyl-group and the arguments advanced above for deactivation of α-halo-ketones will apply

equally to α-halo-esters. Whilst enolic derivatives of α-halo-esters (II) would be expected to be unreactive



(II)

towards nucleophiles, they do not appear to be amenable to synthesis and are thus of no further interest. However, the carbonyl-group of α-halo-esters can be effectively destroyed by conversion to α-halo-orthoesters (III). The α-carbon is not ethylenic in this case and consequently may

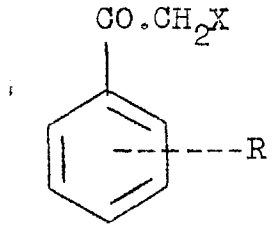


(III)

be expected to be moderately reactive towards nucleophilic reagents. Some of these compounds are known and are easily synthesised (see p.80). The orthoester grouping should undergo ready hydrolysis *in vivo*.

The 2-Haloacetophenones.

The 2-haloacetophenones (IV) were chosen for study for several reasons; they have been widely

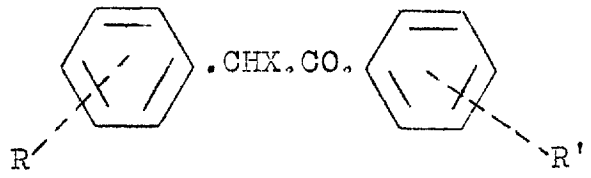


(IV)

investigated both chemically and biologically (see p.27 et seq.) and most of them are potent lachrymators. Variation of the nature and position of the group R in (IV) affords a series of compounds of widely differing alkylating powers and, furthermore, many of these compounds are known and a considerable number are available commercially. But for one special case (discussed in a separate section on p.84) the problem of synthesis in this series did not arise, those required being commercially available. The major work with these compounds centred about attempts to prepare the enol acetate derivatives. In addition, preliminary studies were begun on the synthesis of the enol phosphate derivatives.

The 2-Aryl-2-haloacetophenones.

The 2-aryl-2-haloacetophenones (V) have not been

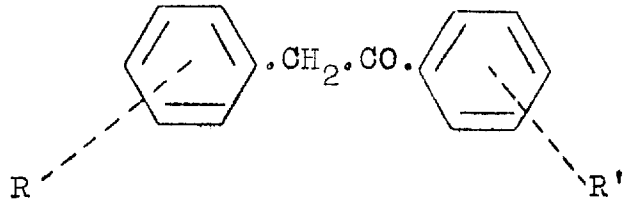


(V)

subjected, hitherto, to the same intensive investigations as the 2-haloacetophenones. No quantitative measurements of their reactivity towards nucleophiles have been made and no biological properties have been recorded. They would seem to offer the same advantages as the 2-halo-acetophenones from the point of view of derivatives of differing reactivity; in this case both rings of (V) are available for substitution. It was decided to limit initial investigation to the compounds (V) in which $R' = H$; $R = H, Cl$ or NO_2; $X = Cl$ or Br.

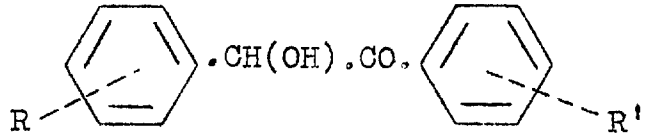
Methods for the preparation of 2-aryl-2-haloacetophenones.

There are only two important general methods available for the synthesis of the 2-aryl-2-halo-acetophenones; (i) by direct halogenation of the 2-phenyl-acetophenone (VI) and (ii) by replacement of the hydroxyl



(VI)

group of the benzoin (VII) with halogen.



(VII)

(i) Jenkins [44] prepared a number of 2-aryl-2-bromo-acetophenones by bromination of the 2-arylacetophenone, under photochemical conditions, in carbon tetrachloride solution. The yields were of the order of 80%. Recently McDonald and Schwab[45] prepared 2-chloro-2-(p-methylphenyl)-acetophenone from 2-(p-methyl-phenyl)acetophenone by chlorination, using sulphuryl chloride, in carbon tetrachloride solution.

(ii) Replacement of the hydroxyl group of benzoin (VII, R = R' = H) by chlorine has been effected by Ward[46] using thionyl chloride in pyridine solution. 2-chloro-2-phenyl- and 2-chloro-2-(p-methylphenyl)-acetophenone are the only α-chloro-ketones in this series reported to date.

The synthesis of both the α-bromo- and α-chloro-ketones of this class thus depends upon the availability of the appropriate benzoin or 2-arylacetophenone. Although many methods for the synthesis of these compounds have been described, few are of preparative importance. Some of the more useful methods are summarised below.

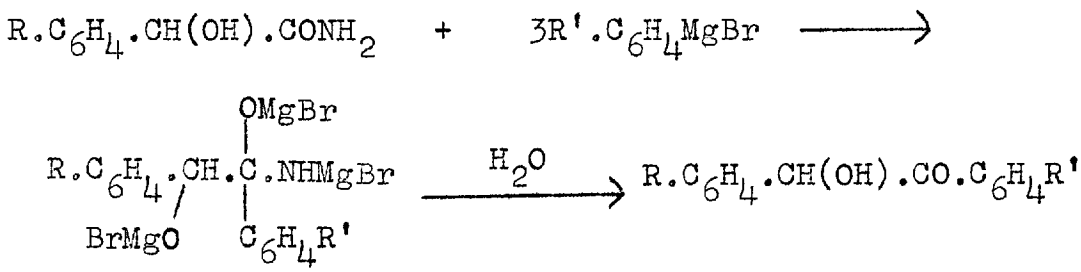
Methods for the preparation of benzoins.

Benzoins may be prepared by the classical, cyanide catalysed condensation of aromatic aldehydes as described by Lapworth[47,48] . The method is not universally applicable and is virtually useless as a synthetic method for preparing unsymmetrical benzoins. For this group of compounds, three useful general preparative methods are available. Fuson et.al.[49,50,51] have shown that benzoins can be prepared in 35-90% yield by the condensation of phenyl glyoxals with aromatic compounds in the presence of aluminium chloride:

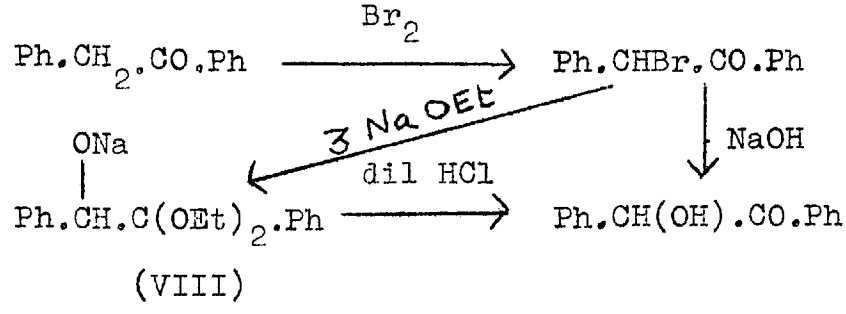
$$Ar.CO.CHO + Ar'H \xrightarrow{AlCl_3} Ar.CO.CH(OH).Ar'$$

The benzoins so prepared have the carbonyl group adjacent to the aromatic residue of the glyoxal. The necessary glyoxals can readily be prepared by selenium dioxide oxidation of the corresponding acetophenone, according to the method of Riley and Grey[52] . An alternative method

for preparing unsymmetrical benzoins utilizes the reaction between a mandelamide and a substituted phenyl magnesium bromide; e.g.



Yields are poor (20-50%) and the method is time consuming and expensive. However, it is of very general application and has been widely employed[44,53-63] The third method, and, for the purposes of the present work, the best, assumes the availability of the appropriate 2-arylacetophenone. The ketone is brominated[44] and hydrolysed[44,64-67] either directly, by the action of sodium hydroxide, or indirectly using sodium ethoxide and then dilute acid; e.g.

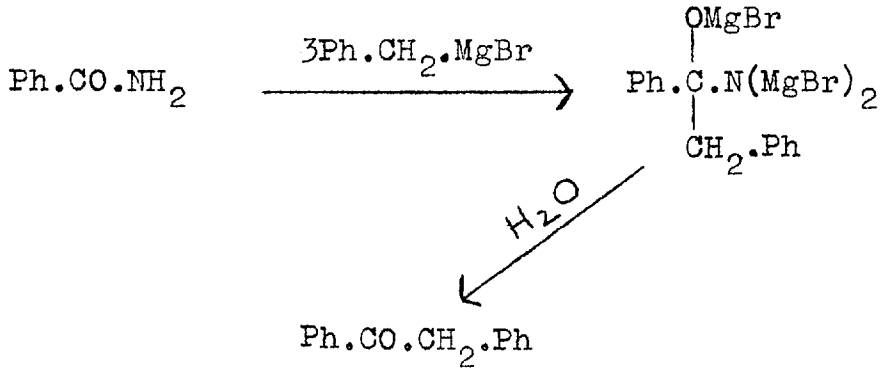


(VIII)

The method of Jenkins[44] proceeds via the ketal (VIII) giving an overall yield of 80-90%.

Methods for the preparation of 2-arylacetophenones

A number of methods are available for the synthesis of these compounds. Only two of these methods are of general preparative importance. Jenkins[68,69] obtained good yields of 2-arylacetophenones by reaction of substituted benzamides with substituted phenyl magnesium bromides; e.g.



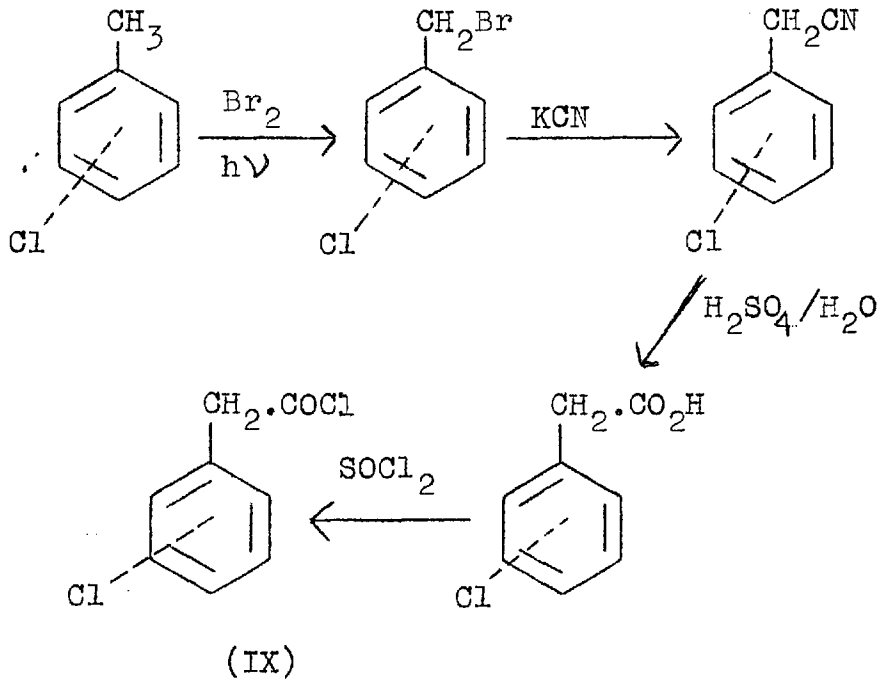
The method is time consuming and expensive, but has the advantage that it can be used to produce 2-arylacetophenones substituted in either ring. An alternative method has been perfected by Fischer et.al.[70]. They condensed substituted phenylacetyl chlorides with benzene in the presence of aluminium chloride; e.g.

$$R.C_6H_4.CH_2.COCl + PhH \xrightarrow{AlCl_3} R.C_6H_4.CH_2.CO.Ph$$

The method has obvious limitations in scope but, where applicable, good yields are obtained. For the purpose of the present investigations, described below, this was the chosen method.

The synthesis of some 2-aryl-2-haloacetophenones

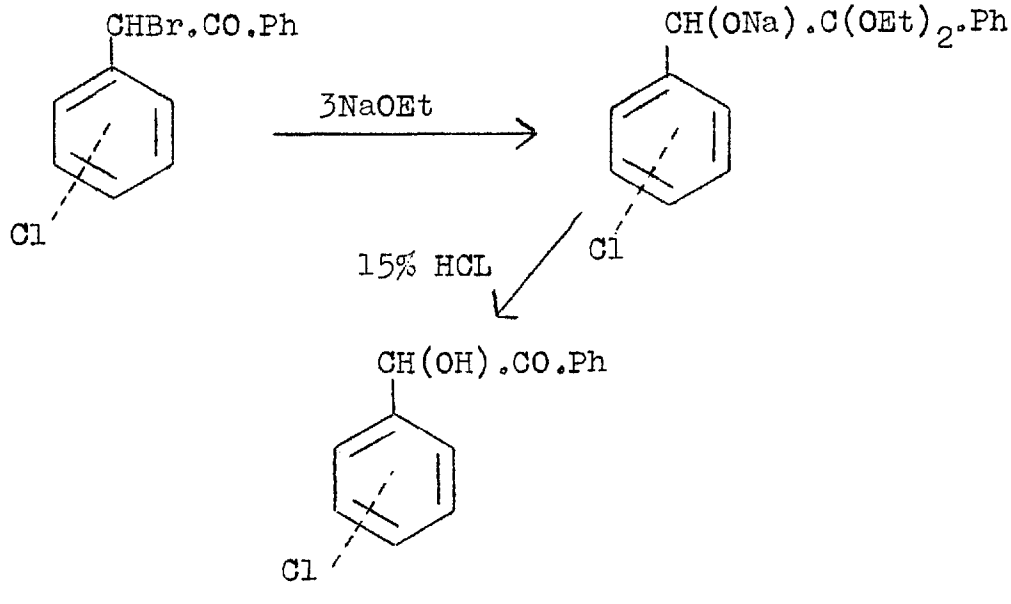
The o-, m-, and p-chlorophenylacetyl chlorides (IX) were obtained from the substituted toluenes according to the following reaction sequence:



(IX)

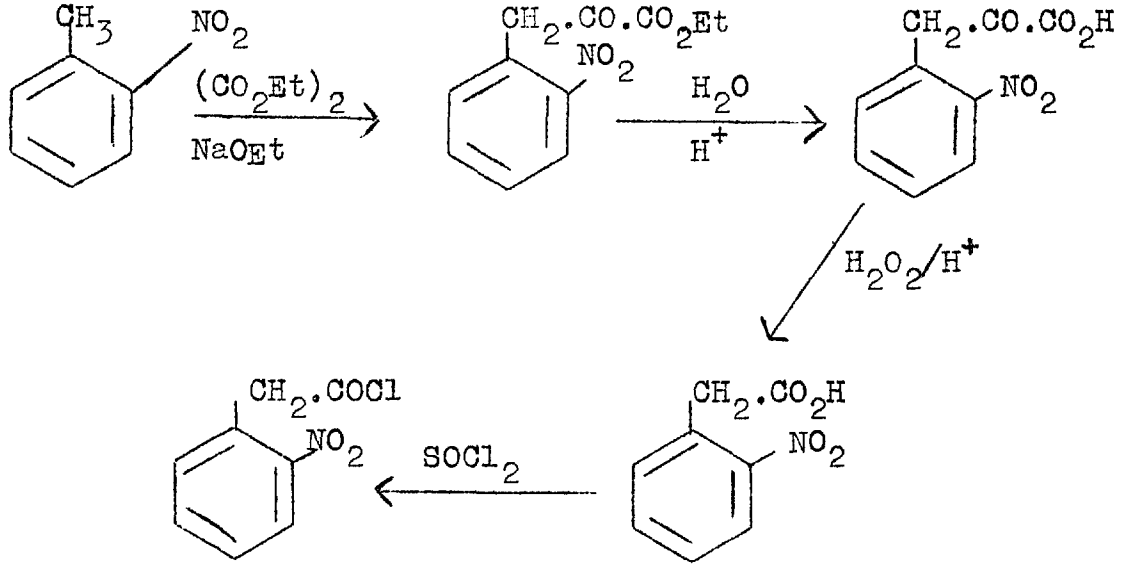
Friedel-Crafts' condensation of the acid chlorides with benzene afforded 2-(o-chlorophenyl)- , 2-(m-chlorophenyl)- and 2-(pchlorophenyl)- acetophenones in 65,70 and 63% yield respectively. The ketones were brominated

in carbon tetrachloride solution, illuminated by a 500w tungsten lamp, to give 2-bromo-2-(o-chlorophenyl)-, 2-bromo-2-(m-chlorophenyl)- and 2-bromo-2-(p-chlorophenyl)-acetophenones respectively, all in 80% yield. 2-Bromo-2-(o-chlorophenyl) acetophenone was obtained crystalline when kept standing for several months at 0°, The m-isomer could not be crystallised (and was purified by distillation under reduced pressure), but the p-isomer crystallised readily. All of the bromo-ketones were converted to the corresponding benzoins, in excellent yield, via the benzoin diethyl ketal (monosodio-derivative) and subsequent hydrolysis;



Treatment of the benzoins with thionyl chloride in dry pyridine gave moderately good yields of the corresponding chlorides. 2-Chloro-2-(o-chlorophenyl)- and 2-chloro-2-(p-chlorophenyl)-acetophenones were obtained crystalline with some difficulty; the m-isomer could not be crystallised, but distillation under reduced pressure afforded a pure specimen.

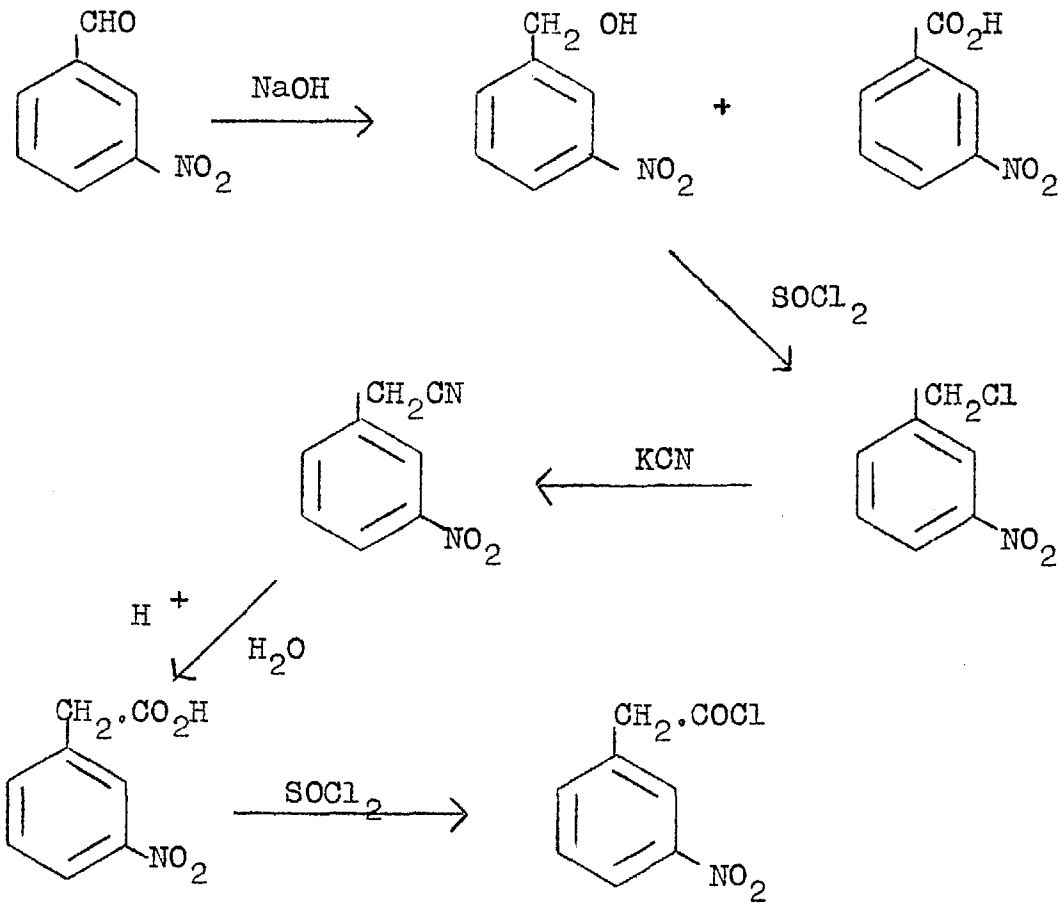
o-Nitrophenylacetyl chloride was obtained from o-nitrotoluene according to the scheme:



Friedel-Crafts' condensation of the crude acid chloride with benzene gave an oily product, but a moderate yield of very pure 2-(o-nitrophenyl)acetophenone was obtained by chromatography on alumina. This method of purification is

very successful when applied generally to the products of Friedel-Crafts' reactions of this type; a very high column loading can be used with little loss of purity of the product. Bromination of the ketone in the usual way gave an excellent yield of 2-bromo-2-(o-nitrophenyl)-acetophenone. This material separates from methanol as colourless needles, but when filtered off and kept for a few hours it becomes dark brown, and despite repeated attempts, a correct bromine analysis could not be obtained; the percentage bromine showed a tendency to increase as the specimen was kept, an effect which cannot be readily accounted for.

m-Nitrophenylacetyl chloride was obtained from m-nitrobenzaldehyde according to the scheme:

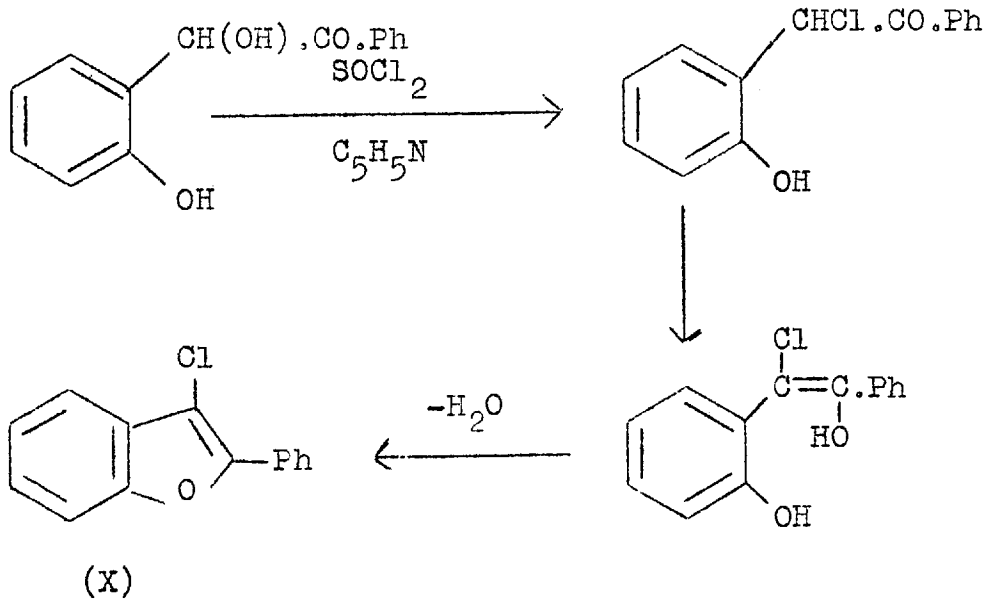


Friedel-Craft's condensation of the crude acid chloride with benzene, followed by purification of the product by chromatography on alumina, gave pure 2-(m-nitrophenyl)acetophenone. Bromination of this product in the usual way gave 2-bromo-2-(m-nitrophenyl)acetophenone, in excellent yield, as a pale-yellow, stable solid that analysed well.

2-(p-Nitrophenyl)acetophenone was obtained by

Friedel - Crafts condensation of p-nitrophenylacetyl chloride and benzene. Purification was achieved by crystallisation from ethanol. Bromination of the ketone gave 2-bromo-2-(p-nitrophenyl)acetophenone, in good yield, as a pale-yellow, stable solid; a good analysis was obtained. Treatment of the bromide with sodium ethoxide and then dilute hydrochloric acid gave only a small amount of brown tar; no benzoin could be isolated. Attempts to chlorinate 2-(p-nitrophenyl)acetophenone directly were unsuccessful even under photochemical conditions. No further attempts were made to obtain the chloro-compounds in the nitro-substituted series since it seemed, from results with other compounds, that they would have little biological interest and limited chemical interest.

2'-Hydroxybenzoin was prepared, using the method of Asahina and Teresaka[57], from o-hydroxybenzaldehyde cyanohydrin and phenyl magnesium bromide; the yield was very poor. Attempted conversion of the benzoin to 2-chloro-2-(o-hydroxyphenyl)acetophenone, using thionyl chloride in dry pyridine, gave a product which analysed quite well for $C_{14}H_9ClO$ and not $C_{14}H_{11}ClO_2$. A possible structure for this compound is 2-chloro-1-phenylbenzofuran (X), arising from dehydration of the enolised chloro-ketone:



(X)

The infra-red absorption at 1705 cm.$^{-1}$ has not, however been reported as characteristic of the benzofuran system.[71]

4'-Hydroxybenzoin was prepared, in low yield, by reaction of p-hydroxybenzaldehyde cyanohydrin with phenyl magnesium bromide. Treatment of the product with thionyl chloride in dry pyridine failed to give any isolable chloro-ketone. This may well be due to intermolecular alkylation of the phenolic grouping.

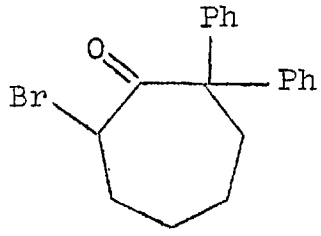
o-Methylphenylacetyl chloride was obtained from o-xylene using a synthetic scheme similar to that outlined above for the chlorophenylacetyl chlorides. Friedel - Crafts' condensation of the chloride with benzene and purification by chromatography on alumina gave 2-(o-methylphenyl)acetophenone. Bromination of this compound in the

usual way gave a brown oil which could not be crystallised and decomposed on distillation. Nuclear magnetic resonance studies on this compound showed the presence of a methyl group (τ,7.5) and a low-field lone proton (τ,3.4) thus proving the material to be essentially 2-bromo-2-(o-methylphenyl)acetophenone; bromination had, as expected, occurred exclusively alpha to the carbonyl group and not in the methyl side chain. Attempted hydrolysis of the bromo-ketone yielded a yellow oil that rapidly decomposed at room temperature.

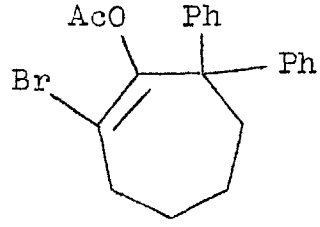
Enol-acetylation of α-Halo-ketones

While the preparation of enol esters from ketones containing an enolisable α-hydrogen atom has long been known, only two reports of enol esters derived from α-haloketones have appeared in the literature.

Lyle and Covey[72] treated 2,2-diphenyl-7-bromo-cycloheptanone (XI) with phenyl lithium in boiling benzene; addition of acetyl chloride then gave the enol acetate (XII) in 9% yield.

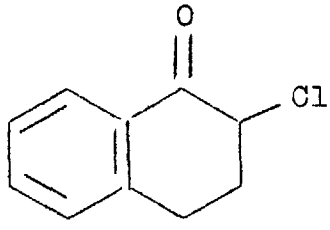


(XI)

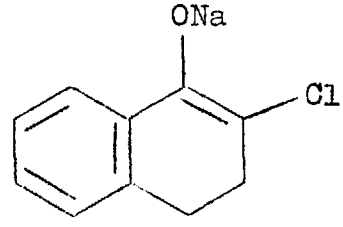


(XII)

Rutherford and Stevens[73] reported a similar method of more general applicability. 2-Chloro-α-tetralone (XIII) was treated with sodium methoxide, in ether, at -80°

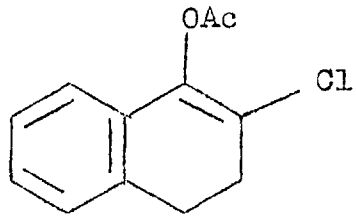


(XIII)



(XIV)

giving the sodium enolate (XIV). Treatment of the equilibrium mixture with acetyl chloride then gave the enol acetate (XV) in 65% yield. Similarly were prepared the enol

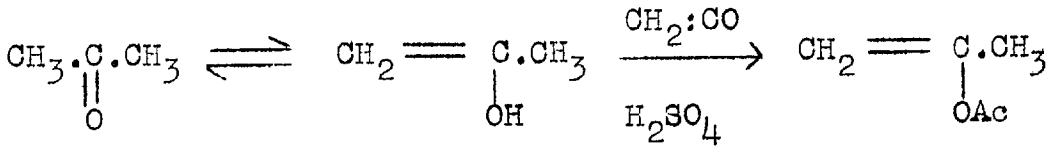


(XV)

acetates of 2-bromo-α-tetralone and 2-bromo-1-indanone and the enol caproate of 2-chlorocyclohexanone. In these cases the yields were poorer (23-47%).

Whilst reports of the enol esters of α-halo-ketones have been so sparse, a variety of methods have been developed for the preparation of the enol esters of other types of ketone. Some of the methods are not applicaple to α-halo-ketones, e.g. the widely used enol acetylation of ketones with potassium acetate and acetic anhydride[74] leads, as expected, to loss of the halogen atom when applied to α-halo-ketones[75]. Other methods. however, would appear to be extendable to α-halo-ketones, at least in principle.

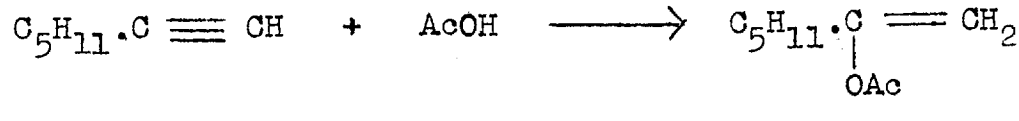
Keten, in the presence of a strong acid catalyst, will acetylate many ketones[76-79] Thus acetone reacts readily with keten in the presence of sulphuric acid, giving isopropenyl acetate (XVI). Hagemeyer and Hull[80]



(XVI)

have shown that isopropenyl acetate itself is a good enol acetylating agent in the presence of acid catalysts, presumably due to the formation of keten *in situ*. The acetone formed in the reaction can, in the case of higher boiling carbonyl compounds, be distilled off and good yields of enol acetates are obtained with many simple aldehydes and ketones. It seems reasonable to suppose that this method should be applicable to α-halo-ketones.

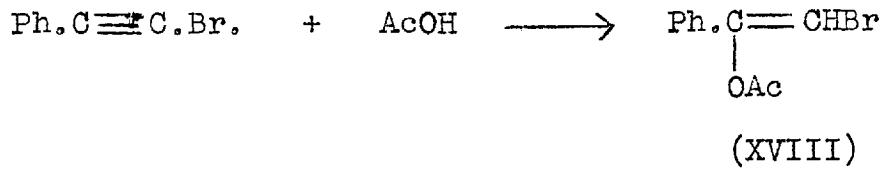
Hennion *et al.*[81] have shown that acetic acid will add across the triple bond of amyl acetylene, in the presence of a mercuric oxide - boron trifluoride catalyst, to give 1-amylvinyl acetate (XVII):



(XVII)

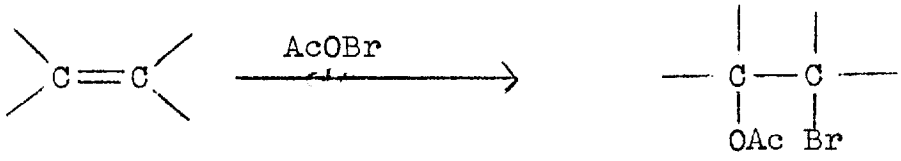
Extension of this method to bromophenylacetylene might be expected to give α-acetoxy-α'-bromostyrene (the enol acetate

of 2-bromoacetophenone (XVIII)):

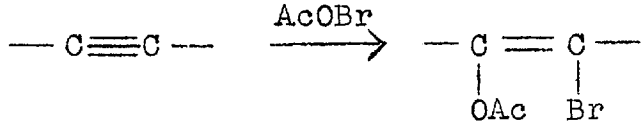


(XVIII)

although addition to the triple bond in the reverse direction is a possibility. A variation of this method has been suggested by the work of Levine and Wall.[82] These workers showed that acetyl hypobromite will add to ethylenic bonds to give bromohydrin acetates:



It is thus possible that similar behaviour with acetylenic linkages could lead to α-acetoxyvinyl halides:



The synthesis of some enol acetates derived from 2-aryl-2-haloacetophenones.

Treatment of 2-bromo-2-phenylacetophenone with a suspension of sodium methoxide in ether at -50° gave a yellow solution of the sodium enolate which, on addition of acetyl chloride, afforded the enol acetate in 22 % yield. The first stage of this reaction involves the formation of an equilibrium mixture:

$$Ph.CHBr.CO.Ph + NaOMe \rightleftharpoons Ph.C(Br):C(ONa).Ph + MeOH$$

The yield of enol acetate will thus depend upon the relative amounts of ketone and enolate present at equilibrium. To overcome this a modified method was devized. In addition to sodium methoxide (itself conveniently prepared *in situ* from one equivalent each of methanol and sodium hydride) one extra equivalent of sodium hydride was added to the reaction mixture; the methanol formed in the reaction was thus removed immediately and sodium methoxide regenerated.

$$MeOH + NaH \longrightarrow MeONa + H_2$$

Furthermore, the hydrogen evolved gives some idea of how the reaction is proceeding. Using this modified procedure with 2-bromo-2-phenylacetophenone the yield was raised to 56%. The remaining 2-aryl-2-haloacetophenones described above were, with one exception, successfully enol acetylated by this method, yields varying from 28 - 70%. The exception, 2-bromo-2-(o-nitrophenyl)acetophenone is discussed below. All of the α-acetoxy-α'-halostilbenes so prepared were crystalline solids. In chloroform solution they showed characteristic absorbtions in the infra-red spectrum at about 1200(C — O stretch), 1630 (C═C stretch) and 1760(C═O stretch) cm^{-1}; a typical spectrum - that of α-acetoxy- α'-chlorostilbene - is shown in Figure 1. The ultra-violet spectra in ethanol showed characteristic absorbtions in the 210, 230 and 280 mμ regions; a typical spectrum - that of α-acetoxy- α'-chlorostilbene - is shown in Figure 2. The more important infra-red and ultra-violet spectral properties are summarised in Tables I and II below.

Figure 1

Infra-red Spectrum of α-Acetoxy-α'-chlorostilbene

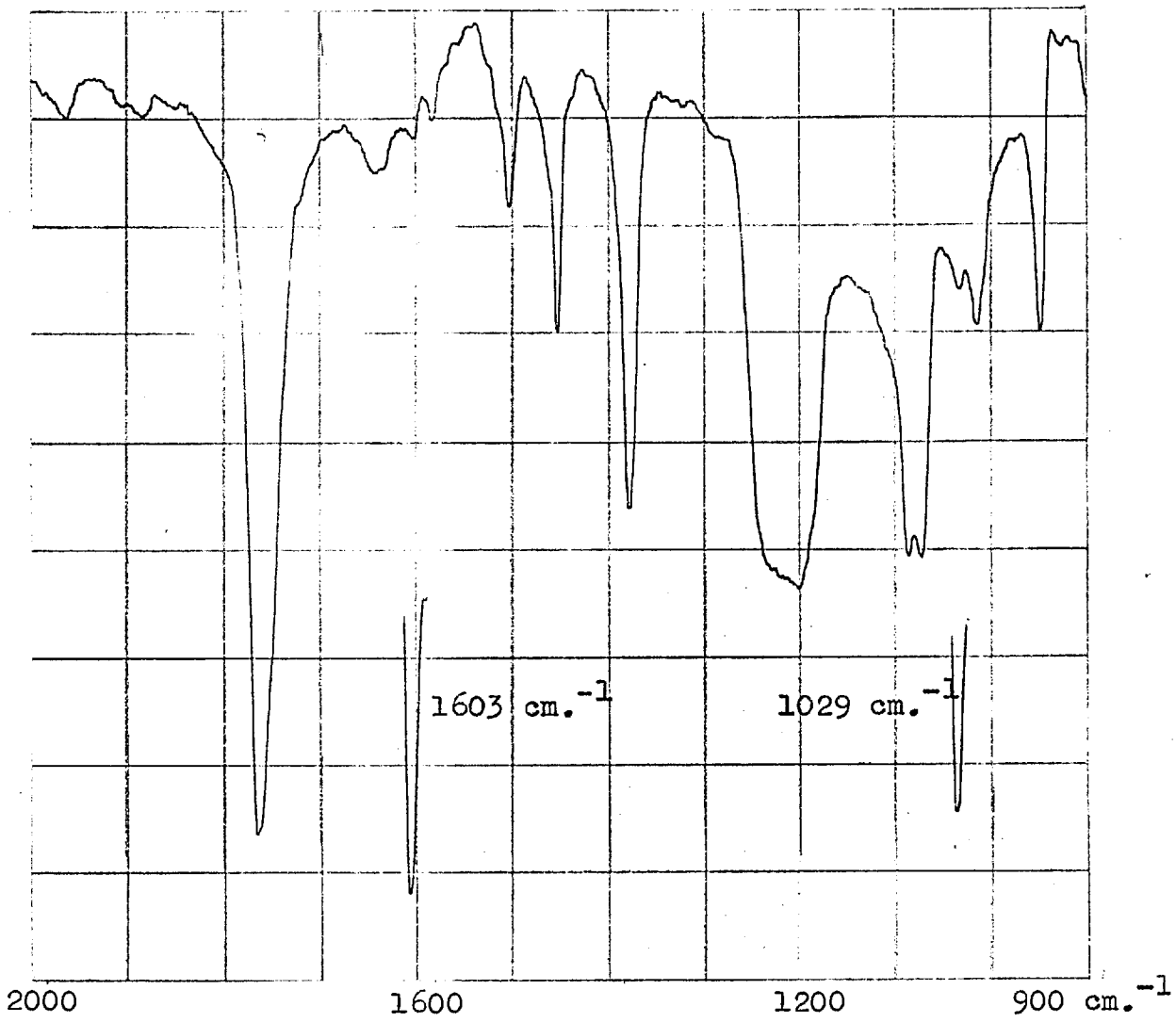


Figure 2

Ultra-violet Spectrum of α-Acetoxy-α'-chlorostilbene

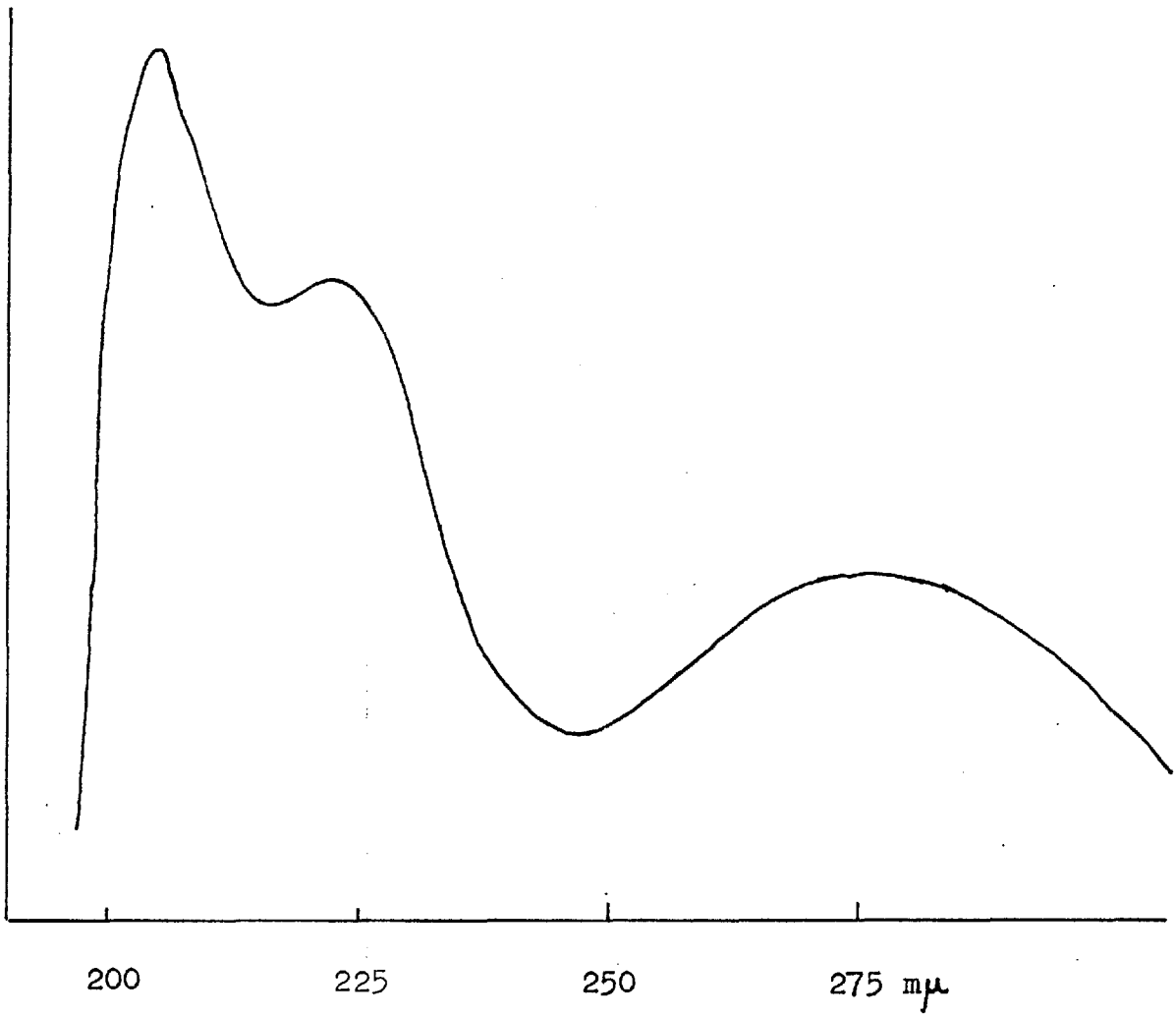


Table I

Characteristic infra-red absorbtions, in chloroform solution, of compounds of the type $R.C_6H_4C(X):C(OAc).Ph$.

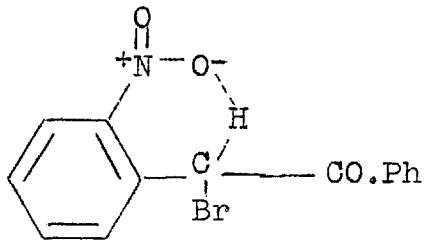
R	X	C—O cm.$^{-1}$	C=C cm.$^{-1}$	C=O cm.$^{-1}$
H	Br	1190(s)	1630(w)	1760(s)
"	Cl	1190 "	1620 "	1760 "
o-Cl	Br	1190 "	1645 "	1765 "
"	Cl	1190 "	1640 "	1765 "
m-Cl	Br	1190 "	1635 "	1765 "
p-Cl	Br	1190 "	1630 "	1760 "
"	Cl	1200 "	1640 "	1770 "
m-NO_2	Br	1190 "	1635 "	1765 "
p-NO_2	Br	1180 "	1630 "	1765 "
o-Me	Br	1200 "	1640 "	1760 "

Table II

Characteristic ultra-violet absorbtions, in ethanol solution, of compounds of the type $R.C_6H_4.C(X):C(OAc).Ph$.

R	X	$\lambda_1(\varepsilon)m\mu$	$\lambda_2(\varepsilon)m\mu$	$\lambda_3(\varepsilon)m\mu$
H	Br	205(21,300)	226(17,900)	283(7170)
"	Cl	205(24,300)	223(18,300)	276(9850)
o-Cl	Br	208(25,400)	233(15,300)	-
"	Cl	207(25,300)	-	263(10,350)
m-Cl	Br	209(28,500)	228(20,100)	285(7520)
p-Cl	Br	206(21,000)	232(19,800)	290(8060)
"	Cl	204(19,500)	229(18,000)	280(9700)
m-NO_2	Br	207(25,100)	219(26,300)	265(12,550)
o-Me	Br	214(13,400)	230(13,300)	263(8350)

2-Bromo-2-(o-nitrophenyl)acetophenone did not show any sign of enolisation when treated with sodium methoxide and sodium hydride in ether. It seems probable that hydrogen bonding between the relatively positive α-hydrogen and the o-nitro-group as in (XIX) renders the α-hydrogen unreactive towards methoxide ions from which

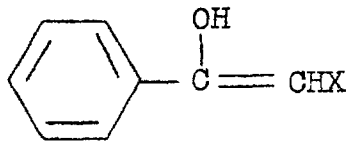


(XIX)

it will be effectively shielded. It is noteworthy that both 2-bromo-2-(o-chlorophenyl)- and 2-bromo-2-(o-methylphenyl)-acetophenones, in which the α-hydrogen atoms cannot participate in this type of hydrogen bonding, underwent enol acetylation in good yield.

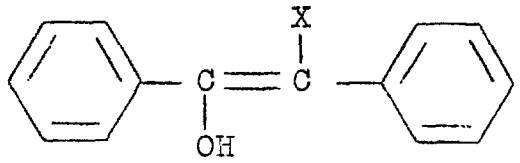
Exploration of some possible routes to the synthesis of enol acetates derived from 2-haloacetophenones.

In contrast to the results obtained with 2-aryl-2-haloacetophenones, the treatment of a variety of 2-haloacetophenones with sodium methoxide and sodium hydride in ether at low temperatures failed to give the expected sodium enolate. It would seem that these halo-ketones remain in the keto-form under these conditions, the enol form (XX) apparently being insufficiently stabilised to be formed.



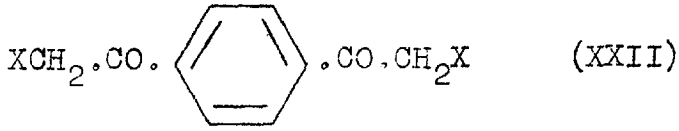
(XX)

In the case of the 2-aryl-2-haloacetophenones, formation of the sodium enolate was rapid. This is probably due to stabilisation of the enol (XXI) resulting from increased conjugation:

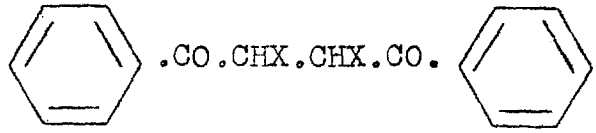


(XXI)

Some support for this point of view comes from the observation that the p-bis(haloacetyl) benzenes (XXII) and the 1,2-dibenzoyl-1,2-dihaloethanes (XXIII) both appeared to give sodium enolates at low temperatures, although

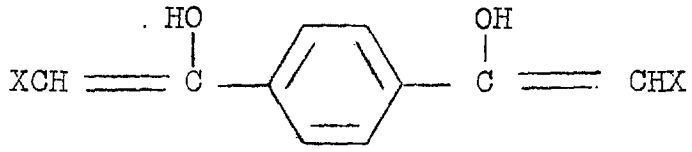


(XXII)



(XXIII)

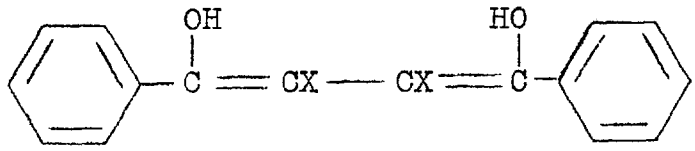
no pure enol acetates could be isolated by subsequent treatment with acetyl chloride. In the case of (XXII) the mesomeric effects of the two carbonyl groups are mutually opposed, but on enolisation (XXIV) a conjugated system is produced in which no such opposed effects are present:



(XXIV)

The diketone (XXIII) on enolisation produces a compound (XXV)

with extended conjugation:



(XXV)

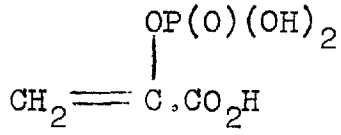
Enol acetylation of a variety of 2-haloacetophenones could not be accomplished using isopropenyl acetate in the presence of p-toluenesulphonic acid. The conditions used were such that any acetone formed as a result of enol acetylation would distil off; in no case did this occur to any detectable degree. This result can again be explained if the α-halo-ketone remains entirely in the keto form.

Addition of acetic acid to bromophenyl acetylene in the presence of boron trifluoride and mercuric oxide gave an unstable product which decomposed in air. It is possible that the enol acetate was formed but underwent polymerisation, probably catalysed by the boron trifluoride.

Addition of acetyl hypobromite to phenyl acetylene proceeded smoothly, but gave a product containing about 20% more bromine than the required compound, and the method was not pursued.

Enol Phosphates of α-Haloketones.

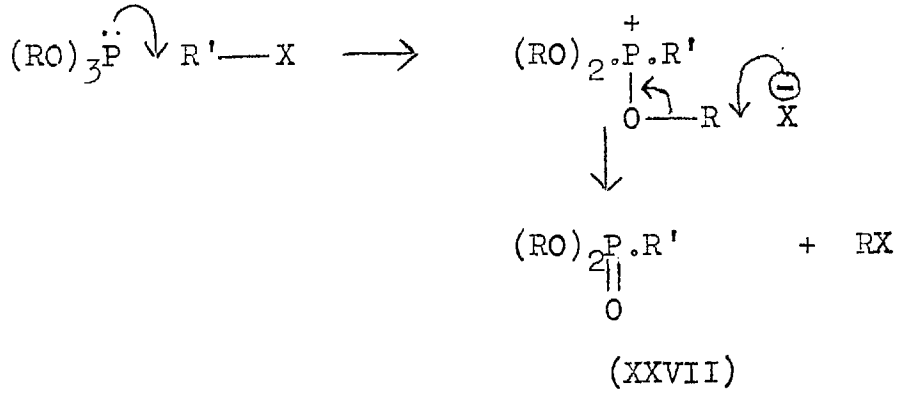
The first recognised enol phosphate, phosphoenol pyruvic acid (XXVI), was isolated[83], from natural sources, in 1934. Over the next 15 - 20 years it became apparent



(XXVI)

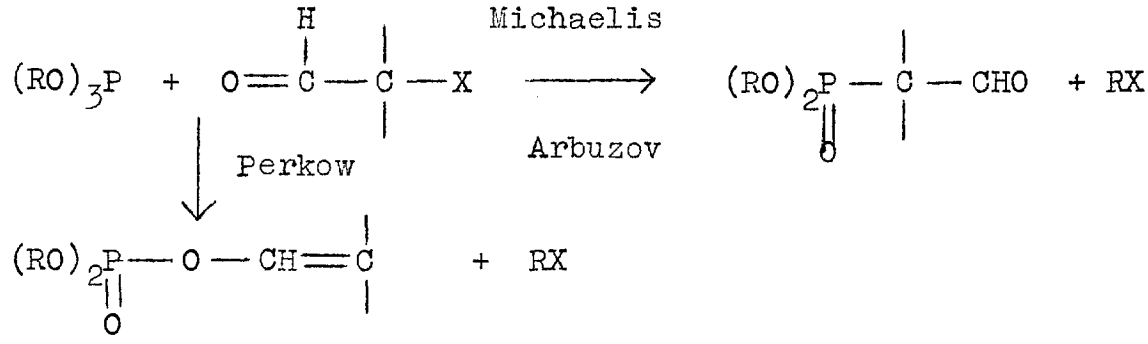
that compounds of the enol phosphate type were of some biological interest, especially in the insecticide field, and efficient methods of synthesis were sought. Only one practical general method has however been evolved.

In the early part of the century Michaelis *et al.*[84,85] and Arbuzov[86-88] reported the reaction of alkyl halides and trialkyl phosphites to give dialkyl alkyl-phosphonates (XXVII); the mechanism is now known[89] to be:



(XXVII)

Numerous workers[89] extended this reaction to α-halo-ketones, α-halo-esters and α-halo-aldehydes and the products were assumed to be phosphonates of similar type to (XXVII), but Perkow[90] showed that, in the case of α-halo-aldehydes, the reaction did not follow the Michaelis-Arbuzov scheme; instead an alternative pathway was followed giving dialkyl vinyl phosphates (XXVIII), isomeric with the phosphonates:



(XXVIII)

the precise mechanism of the Perkow reaction is uncertain.[89] This scheme was later shown to be followed by α-halo-ketones and α-halo-esters. In almost all cases both phosphonate and enol phosphate can be isolated, the yields depending on the nature of the α-halo-carbonyl compound and the physical conditions employed. A comprehensive review of these reactions has been given by Lichtenthaler.[89]

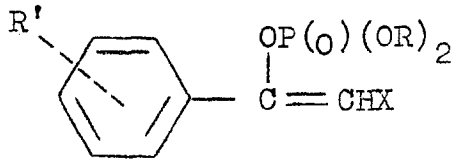
If α,α-dihaloketones are used in the Perkow reaction the product is the enol phosphate of an α-haloketone (XXIX):

$$(RO)_3P + R'.CO.CX_2.R'' \longrightarrow R'-\underset{\text{(XXIX)}}{\overset{\overset{O-P(O)(OR)_2}{|}}{C}}=CX.R'' + RX$$

appreciable amounts of phosphonate are not formed , high yields of enol phosphates being obtained. The reaction in this case is usually exothermic and often proceeds readily at 0 - 20°. The order of reactivity for a series of halo-carbonyl compounds is I > Br > Cl. The "trialkyl" phosphites used must be such that all three valences of the phosphorus atom must be occupied by groups other than H or OH; at least one of these groups must be an alkyl ester group whose alkyl residue is capable of leaving as a carbonium

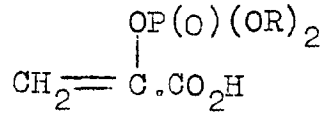
ion. Trialkyl phosphites having different alkyl moities usually eliminate the smallest group as alkyl halide.

From a biological standpoint the enol phosphates (XXX), derived from 2-haloacetophenones, are of particular



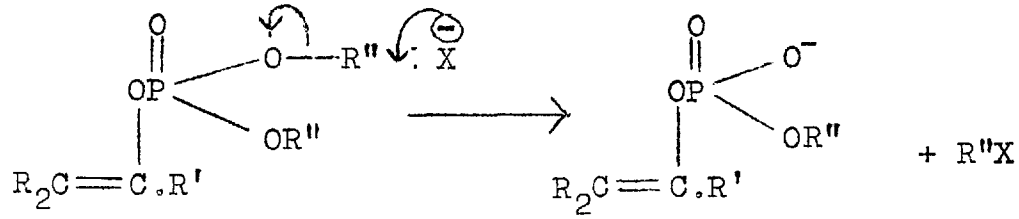
(XXX)

interest. Two such compounds are known; dimethyl 2-chloro-1-phenylvinyl phosphate (XXX, R' = H, R = Me, X = Cl)[91] and diethyl 2-chloro-1-phenylvinyl phosphate (XXX, R' = H, R = Et, X = Cl)[92,93] have both been prepared, in good yield, from 2,2-dichloroacetophenone. From the point of view of solubility and ease of transport *in vivo* it would be preferable to obtain these enol phosphates in the free acid or salt form (e.g. XXX, R = H or Na). Cramer and Voges[94] have shown that the benzyl ester groups of the dibenzyl ester of phosphoenol pyruvate (XXXI , R = CH_2Ph) can be selectively removed, by hydrogenation



(XXXI)

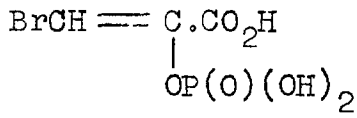
in 50% aqueous methanol using a palladised charcoal catalyst, to give the free acid (XXXI, R = H). An increase in the methanol content of the solvent resulted in increasingly facile reduction of the ethylenic double bond; the method has not been applied to other benzyl esters of enol phosphates. An alternative means of removing one alkyl group from a dialkyl vinyl phosphate has been investigated by a number of workers;[94-96] They have shown that such a compound is readily monodealkylated when heated, in a suitable solvent, with alkalihalides giving the alkali metal salt of an alkyl vinyl hydrogen phosphate; e.g.



Harvey et al.[97] have shown that monodealkylation of phosphate esters can also be effected using sodium ethyl mercaptide, although they did not investigate the particular case of dialkyl vinyl phosphates.

The achievement, by Cramer and Voges,[94] of an efficient synthesis of phospho enol pyruvic acid (XXVI) is significant because the acid is an important compound biologically, representing the last phosphorylated

three-carbon atom compound in both glycolysis and fermentation. It is therefore possible that suitably substituted phospho enol pyruvic acids may behave as anti-metabolites capable of inhibiting processes that are particularly active in neoplastic cells. In particular phospho enol bromopyruvic acid (XXXII) would be of interest as a further example of an enolic derivative of an α-halo-ketone.

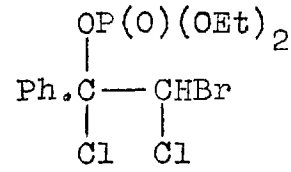


(XXXII)

Preliminary studies on the synthesis of some enol phosphates

Treatment of 2,2-dibromoacetophenone with triethyl phosphite in anhydrous tetrahydrofuran gave diethyl 2-bromo-1-phenyl vinyl phosphate (XXX, R' = H, R = Et, X = Br) isolated by fractionation under reduced pressure. This compound showed the expected[89] absorbtions at 1280 (s) (P=O) and 1630 (m) (C=C) $cm.^{-1}$ in the infra-red spectrum. An additional peak at 3490 (w) $cm.^{-1}$ may have been due to a trace of water absorbed by the compound, a difficulty experienced with alkyl phosphates by other workers.[98] On heating in absolute alcohol containing p-toluenesulphonic acid, the enol phosphate failed to undergo the expected transesterification into the triethyl

ester; this "characteristic" reaction of enol phosphates has been used extensively [89,91,99] as a proof of structure and as a means of distinguishing enol phosphates from the isomeric phosphonates. The structure was however confirmed as diethyl 2-bromo-1-phenylvinyl phosphate by conversion, using chlorine in carbon tetrachloride, into diethyl 2-bromo-1, 2-dichloro-1-phenylethyl phosphate (XXXIII). The nuclear magnetic resonance spectrum of this compound



(XXXIII)

showed τ values of 8.70 (6 protons, 2 methyl groups), 5.87(4 protons, 2 - CH_2 - groups), 3.32 (singlet, 1 proton), 2.55 (3 protons, aromatic) and 2.30 (2 protons, aromatic) in accord with its formulation as (XXXIII). It is noteworthy that the infra-red spectrum of this compound also showed a peak at 3470 (w) $cm.^{-1}$ of unknown origin.

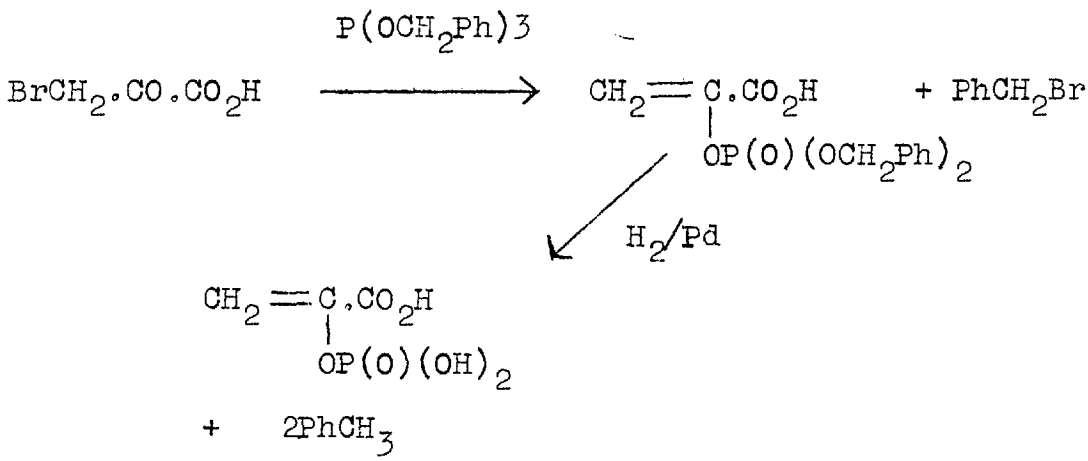
Treatment of diethyl 2-bromo-1-phenylvinyl phosphite with sodium iodide in dry acetone failed to give the monodealkylated product; even after prolonged treatment the starting material was largely recovered.

The action of tribenzyl phosphite on

2,2-dibromoacetophenone, in ether, gave dibenzyl 2-bromo-1-phenylvinyl phosphate, isolated by chromatography on silica gel - a method that gives excellent results with compounds of this type. The ester showed the expected absorbtions at 1280 (s) (P=O) and 1625 (m) (C=C) $cm.^{-1}$ in the infra-red spectrum. In addition an absorbtion at 3450 (w) $cm.^{-1}$ was observed. Treatment of the compound with sodium iodide, in dry acetone, at room temperature, resulted in a good recovery of starting material. Under more vigorous conditions (one hour reflux and 23 hours at room temperature) a poorer recovery of starting material was noted, the remaining material having undergone extensive decomposition. Hydrogenolysis of the dibenzyl ester, in methanol solution (the compound was insoluble in aqueous methanol) over 5% palladium-charcoal, appeared to go to completion (i.e. for both benzyl groups) in 25 minutes at room temperature and atmospheric pressure. The product was a water-soluble, acidic oil that rapidly decomposed giving a lachrymatory, tarry mass. The freshly prepared acid readily formed an S-benzylthiuronium salt which, after repeated crystallisation from ethanol, analysed correctly for the derivative of benzyl hydrogen 2-bromo-1-phenylvinyl phosphate. The infra-red spectrum in nujol mull, showed absorbtions at 1620 (w) and 1670 (s) $cm.^{-1}$ indicating

that the ethylenic double bond was intact. The yield of this derivative was very low and it seems likely that other reduction products are formed, thus accounting for the high uptake of hydrogen. The apparent cessation of the hydrogenolysis was later shown to be due to poisoning of the catalyst; on addition of fresh catalyst the reduction proceeds further. The rapid decomposition of the acidic hydrogenolysis product is probably due to acid catalysed hydrolysis (or alcoholysis - it is not possible to free the crude product entirely from methanol) giving rise to some phenacyl bromide with its characteristic lachrymatory properties. Further investigation of this hydrogenolysis is obviously desirable; it would seem from these preliminary results that efficient selective removal of the benzyl groups by hydrogenolysis should be possible if the right solvent-catalyst system can be found. That the conditions are probably critical is evident from the work of Cramer and Voges[94] mentioned above (p.71).

The synthesis of phospho enol bromopyruvic acid was approached along the same lines as the synthesis, by Cramer and Voges[94], of phospho enol pyruvic acid. These workers treated bromopyruvic acid with tribenzyl phosphite and then selectively debenzylated the product.

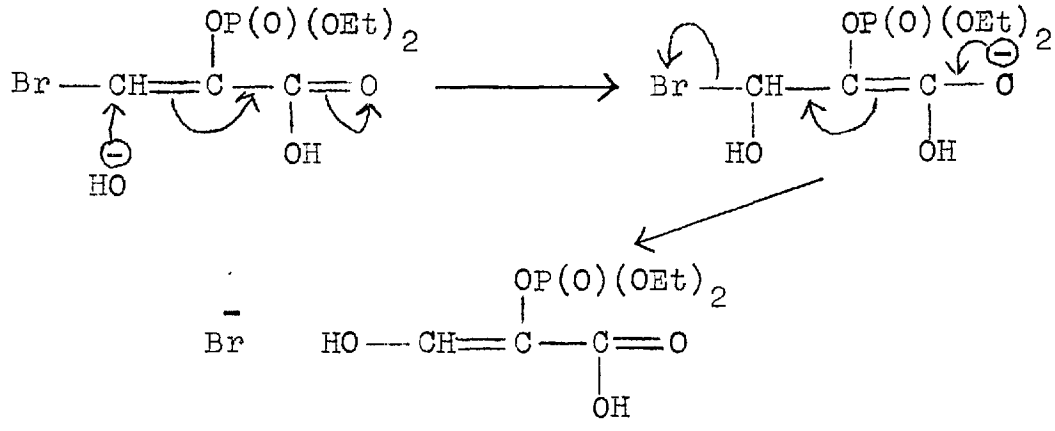


Thus phospho enol bromopyruvic acid should be obtainable from dibromopyruvic acid by a parallel series of reactions.

The preparation of dibromopyruvic acid has been described by Ponzio and Paolini.[100] Using this method (bromination in water at 100°) the product, a hydrate, had to be sublimed *in vacuo* to obtain the anhydrous compound. This laborious procedure was obviated in the present work by carrying out the bromination in anhydrous chloroform. The yields given by the two methods are similar.

In view of the fact that tribenzyl phosphite cannot be effectively purified and is normally used in the crude state, the reaction of dibromopyruvic acid with triethyl phosphite was tried initially. The reaction appeared to proceed smoothly at 0 - 10°. Working up, by extraction of the acidic product with sodium bicarbonate solution

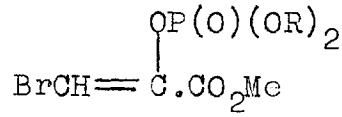
and subsequent recovery by acidification and ether extraction, gave a colourless oil. The infra red spectrum, 1262 (s)(P=O), 1630(m)(C=C), and 1722(s)(C=O) cm.$^{-1}$, indicated that the expected enol phosphate had been formed, but analysis showed that rather less than half of the calculated amount of bromine was present, whilst the phosphorus figure was considerably too high. As an excess of triethyl phosphite was used it seems unlikely that the reaction was incomplete, and the explanation probably lies in hydrolytic decomposition during the washing process. The enol linkage may be sensitive even to bicarbonate, giving rise to bromopyruvic acid which itself undergoes hydrolysis, but a more attractive hypothesis is that the bromine atom of diethyl phospho enol bromopyruvic acid may be alkali-sensitive (cf. the Michael Reaction of α,β unsaturated carbonyl compounds):



The reaction of dibrompyruvic acid with tribenzyl phosphite gave an essentially similar result.

These difficulties in work-up cannot be easily overcome if the free carboxyl group is present; because of the strength of these acids, chromatography in non-aqueous solvents was impracticable. In view of this, attention was turned to the use of an ester.

Methyl dibromopyruvate was prepared by the reaction of dibromopyruvic acid with diazomethane. The product was purified by distillation <u>in vacuo</u>. The infra-red spectrum of this compound showed a peak at 3540 (w) $cm.^{-1}$ probably due to the presence of the enol form of the keto-ester. Treatment of this ester in ether with triethyl phosphite between 0 - 10° gave a good yield of diethyl 2-bromo-1-methoxycarbonylvinyl phosphate, (XXXIV, R = Et) isolated by distillation under reduced pressure.



(XXXIV)

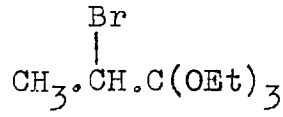
The infra-red spectrum of this compound showed absorbtions in the expected regions 1280 (s)(P=O), 1625 (m)(C=C) and 1740 (s)(C=O) $cm.^{-1}$ In addition there was a weak

band at about 3470 $cm.^{-1}$ similar to that observed with other enol phosphates (see p. 73).

Similar treatment of methyl dibromopyruvate with tribenzyl phosphite gave a product which, when subjected to chromatography on silica gel, gave material having the infra-red spectral properties expected for dibenzyl 2-bromo-1-methoxycarbonylvinyl phosphate (XXXIV, R = CH_2Ph), that is absorbtion at 1288 (s)(P=O), 1620 (m)(C=C) and 1730 (s)(C=O) $cm.^{-1}$ Analysis showed the bromine content of the compound to be considerably too low, and again it is likely that bromine was lost by hydrolysis, this time on the column of undried silica gel. It is therefore clear that, although dialkyl enol phosphates of α-halo-ketones (including simple bromopyruvic esters) can be synthesised, there are still difficulties to be overcome in the preparation of the free acids themselves.

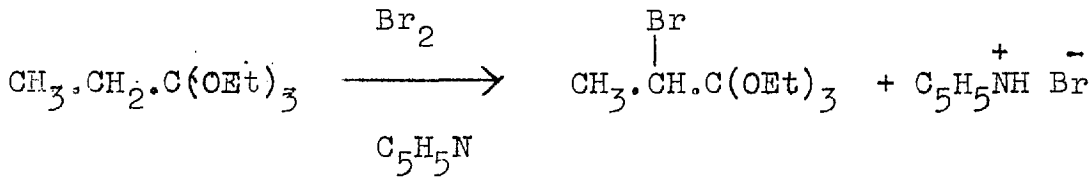
Synthesis of Some α-Halo-orthoesters.

It has been pointed out (p.40) that the α-halo-orthoesters may be moderately reactive towards nucleophilic reagents. Before embarking on a major programme of synthesis and biological tests it is therefore important to establish the toxicities of representative α-halo-orthoesters relative to the corresponding α-halo-esters.



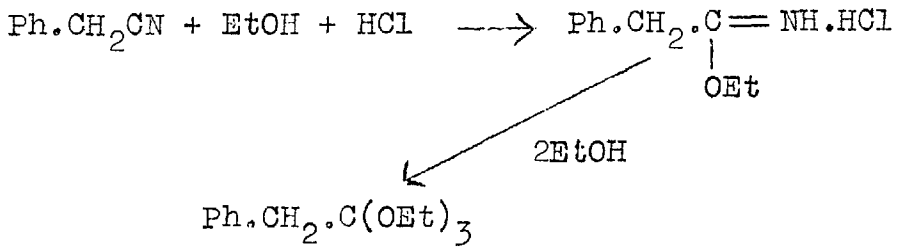
(XXXV)

Ethyl α-bromo-orthoacetate (XXXV), a known compound, was prepared from commercially available ethyl orthoacetate by bromination in pyridine - carbon tetrachloride solution, using the method of Beyerstedt and McElvain[102];

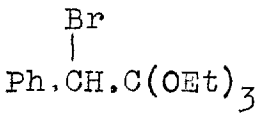


It is important in this method, which would appear to be a general one for α-halo-orthoesters, to remove hydrogen halide as soon as it is formed; this is commonly done by carrying out the reaction in the presence of excess pyridine.

Ethyl phenylorthoacetate was prepared from benzyl cyanide using the method of McElvain and Stevens[103] (which is an adaptation of the general method of Sah[104]);



The α-bromoorthoester (XXXVI) was then synthesised by a

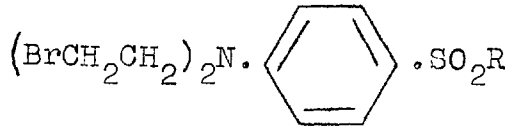


(XXXVI)

method analogous to that used for ethyl α-bromoortho-acetate (see above).

Some Amide - Substituted Alkylating Agents

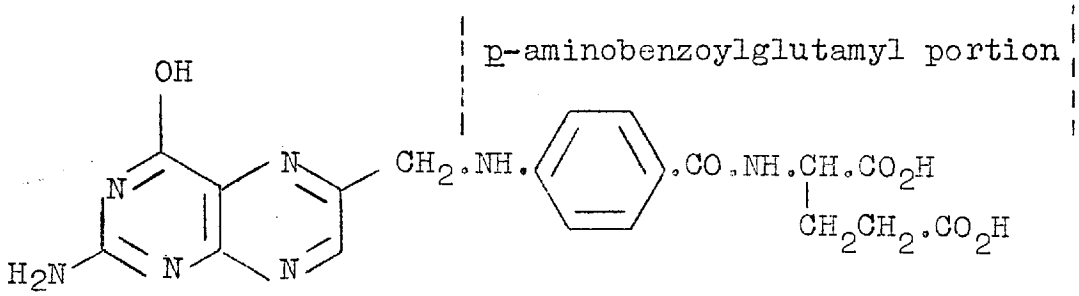
The nitrogen mustards (XXXVII, R = NH_2 and R = $NHNH_2$)[105] have been found to be highly active against



(XXXVII)

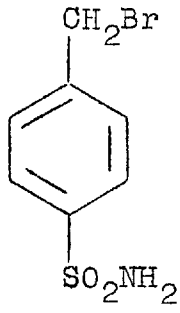
the Walker 256 rat carcinoma, often causing complete regression of the tumour. This desirable effect is however followed, after some days, by death of the animal, apparently from folic acid deficiency. That this condition is not truly present has been

shown by Hawkins and Danielli.[106] Hawkins, Owen and Danielli[107] have discussed this effect and suggest that these two compounds are of the correct molecular dimensions for adsorption on to folic acid receptors normally available to the p-aminobenzoylglutamyl portion of folic acid (XXXVIII). The receptors are then alkylated by the

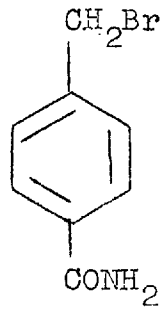


(XXXVIII)

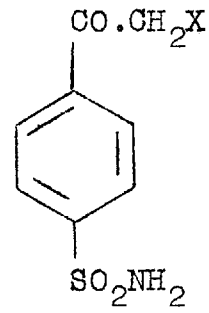
mustard and the metabolic pathway is blocked. In order to test this hypothesis alkylating agents of a similar nature were required for biological tests. The compounds selected were p-(bromomethyl)benzenesulphonamide (XXXIX), p-(bromomethyl)benzamide (XL) and 2-halo-4'-sulphonamidoacetophenone (XLI):



(XXXIX)

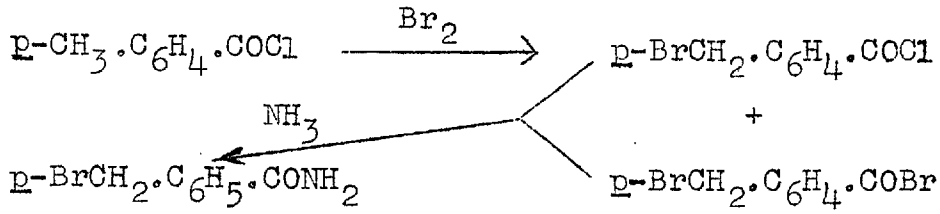


(XL)

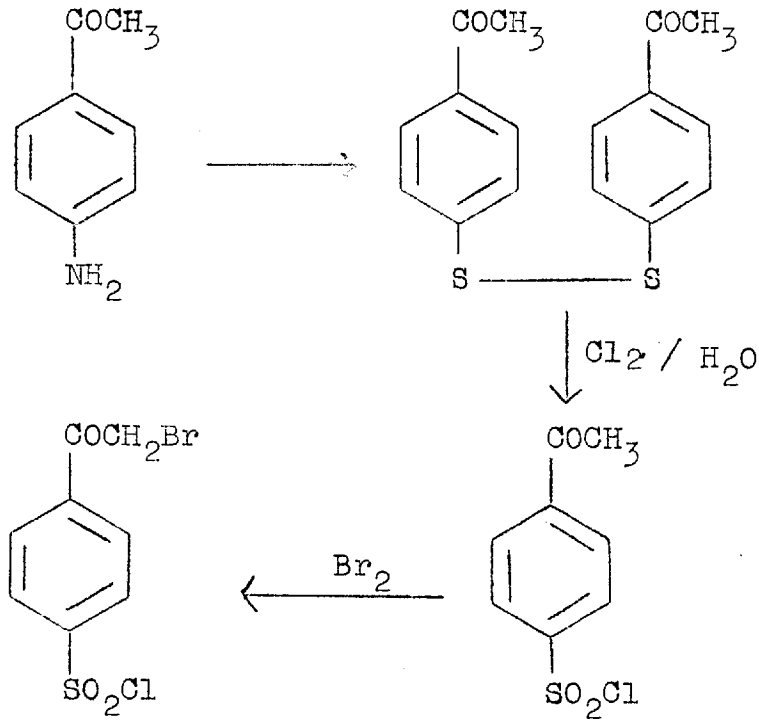


(XLI)

p-(Bromomethyl)benzenesulphonamide, a known compound, was prepared, in good yield, by standard literature methods. p-(Bromomethyl)benzamide was obtained from toluoyl chloride by bromination and treatment of the product with ammonia;



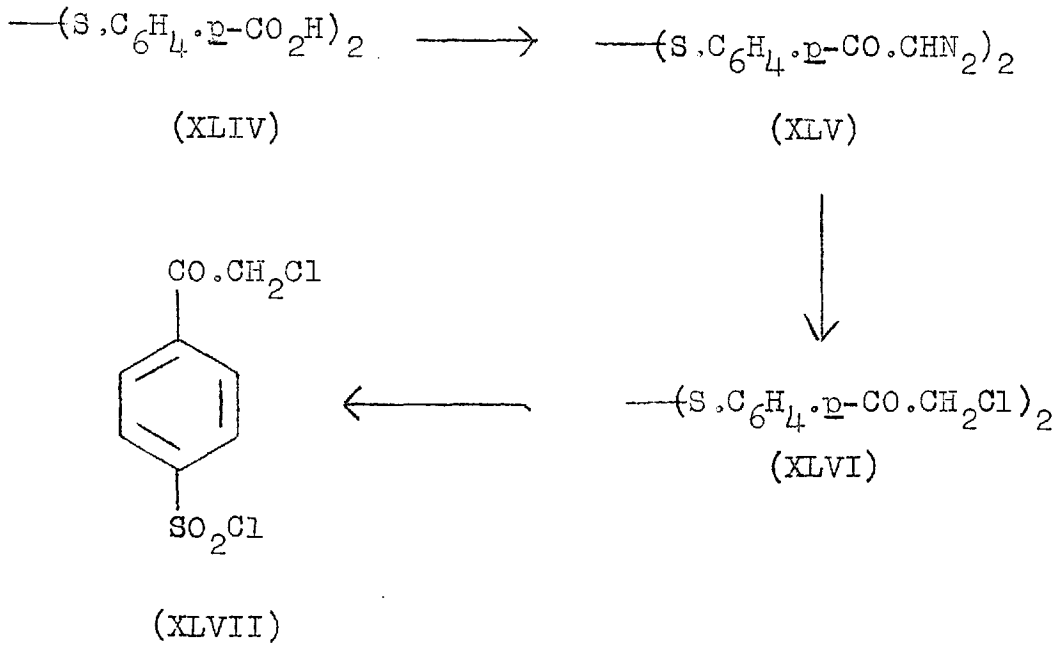
4'-(Chlorosulphonyl)acetophenone (XLII) was prepared from 4'-aminoacetophenone following the reaction scheme:



(XLIII) (XLII)

Bromination in carbon tetrachloride solution, under photochemical conditions (following induction with benzoyl peroxide), gave a good yield of 2-bromo-4'-(chlorosulphonyl)-acetophenone (XLIII). The sulphonyl chloride could not be converted to the amide, giving only gums that could not be crystallised. This is presumably because the lability of the bromo-ketone (which is activated by the powerfully electronegative chlorosulphonyl group) is of the same order, or possibly greater than, the reactivity of the sulphonyl chloride. Accordingly the synthesis of the

less active chloro-ketone was undertaken. p-Aminobenzoic acid was converted to diphenyldisulphide-4,4'-dicarboxylic acid (XLIV). The acid was treated with thionyl chloride and the resulting acid chloride (in the crude state) was added to an excess of diazomethane to give 4,4'-di(diazoacetyl)diphenyldisulphide (XLV). Reaction of this compound with hydrochloric acid afforded 4,4'-di(chloroacetyl)-diphenyldisulphide (XLVI), which on oxidation with chlorine water gave the required 2-chloro-4'-(chlorosulphonyl)-acetophenone (XLVII). The infra-red spectrum of this

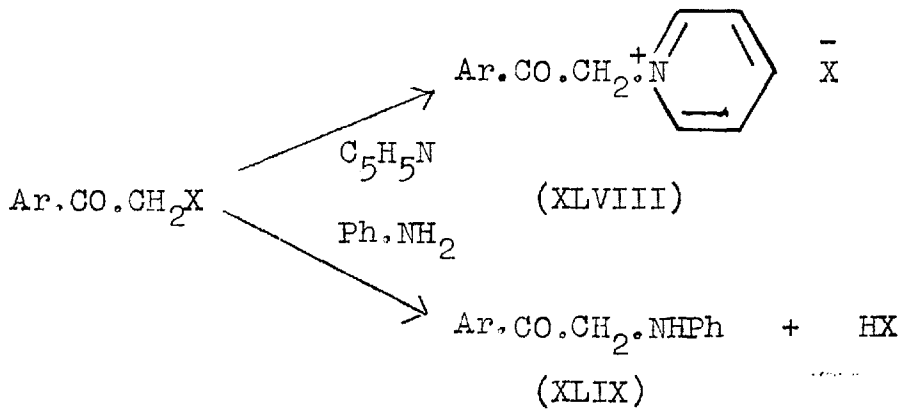


compound was very similar to that of the bromo-ketone (XLIII). In the final oxidation step a new technique was used; the

disulphide was dissolved in chloroform and shaken with an aqueous solution of chlorine suspended in carbon tetrachloride. This minimised the danger of hydrolysis of the chloro-ketone and an excellent yield was obtained. The 2-chloro-4'-(chlorosulphonyl)acetophenone reacted with ammonia in aqueous acetone to give 2-chloro-4'-sulphonamidoacetophenone (XLI, X = Cl). The infra-red spectrum showed absorbtions at 1170(s)(S=O), 1360(s)(S=O), 3380(w) and 3480(w)(NH_2) $cm.^{-1}$ consistent[108] with the assigned structure.

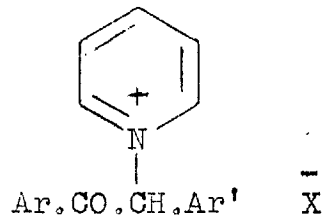
The Interaction of 2-Aryl-2-haloacetophenones with Pyridine and with Aniline

The reactions of 2-haloacetophenones with pyridine[25] and aniline[30] give rise to pyridinium salts (XLVIII) and anilides (XLIX) respectively:

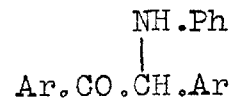


It would therefore be expected that, under similar conditions, 2-aryl-2-haloacetophenones would give the analogous derivatives (L) and (LI). It has been shown[109] that the 2-halo-2-phenylacetophenones react with aniline to give, as expected, the anilide (LI), but the reaction with pyridine has not been reported.

Treatment of 2-bromo-2-phenylacetophenone with pyridine in aqueous ethanol at 50° gave, as a main product, a colourless syrup that could not be crystallised. The material was a salt and gave a positive test for ionic

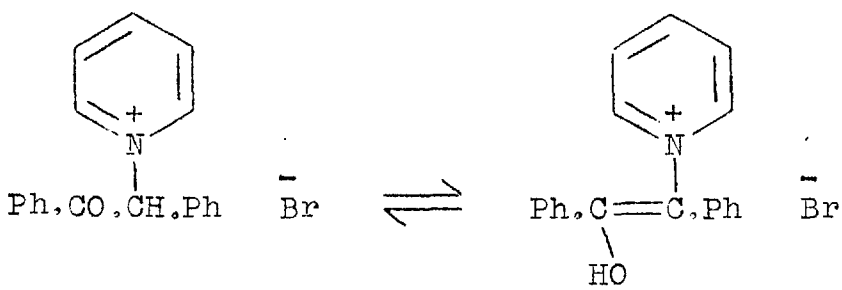


(L)



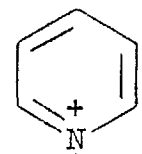
(LI)

halogen. The infra-red spectrum showed peaks at 1630(s), 1685 (s), 2450(m) and 3400(m) cm.$^{-1}$ This would suggest the presence of an ethylenic bond, a carbonyl group and a hydroxyl function; the band at 2450 cm.$^{-1}$ is mysterious and the only possible source of this absorbtion would seem to be the $\gtrless\overset{+}{N}-H$ stretching mode.[108] Excluding this last possibility (for reasons which will be discussed more fully below) the spectrum can be accounted for in terms of a keto-enol tautomeric system;



The existence of such equilibria in solutions of pyridinium salts derived from α-halo-ketones has been recorded

previously[110]. When a saturated solution of picric acid in methanol was added to the syrup obtained above, a crystalline derivative, which analysed correctly for desyl pyridinium picrate (LII), was obtained.



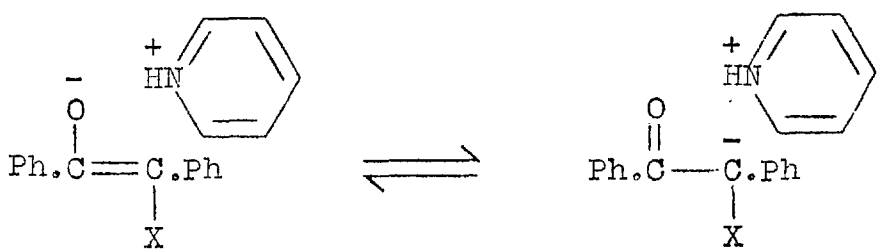
Ph.CO.CH.Ph Pic.⁻ (LII)

Reaction of 2-bromo-2-phenylacetophenone with pyridine in anhydrous benzene at 50°, afforded a colourless, crystalline solid which analysed correctly for desyl pyridinium bromide having a molecule of benzene of crystallisation. The infra-red spectrum of this compound was identical to that of the compound obtained from the similar reaction in aqueous ethanol. On treatment with methanolic picric acid solution, desyl pyridinium picrate was obtained. The bromide gave an orange-red colour with aqueous sodium hydroxide, a colour reaction characteristic of α-pyridinium, α-hydrogen ketones [111].

2-Chloro-2-phenylacetophenone on treatment with pyridine in aqueous ethanol at 100° gave, as main product, a syrup that would not crystallise. The properties of this

compound were very similar to those of the bromo-analogue previously described. The peak at 2450 cm.$^{-1}$ in the infra-red spectrum was again observed; its assignment remains a mystery. A crystalline sample of desyl pyridinium chloride was obtained, by carrying out the reaction in anhydrous benzene, as an extremely hygroscopic solid of diffuse melting point; it could not be purified. The infra-red spectrum of this material was identical to that of the syrup obtained in aqueous ethanol. Both syrup and crystalline material afforded desyl pyridinium picrate on treatment with methanolic picric acid solution.

The peak in the infra-red spectrum of both the bromide and chloride salts, at about 2450 cm.$^{-1}$, plus the apparent existence of a keto-enol system did suggest the structure (LIII) as a possibility, particularly as it was



(LIII)

known that the 2-halo-2-phenylacetophenones are enolised by base. That this was not the structure was shown by three independent physical methods;

(a) Comparison of the infra-red spectra of 2-chloro-2-phenylacetophenone and 2-bromo-2-phenylacetophenone in the 500 - 800 $cm.^{-1}$ region (see figure 3) showed that the most likely assignment for the C—Cl stretch of the chloro-compound was 730 $cm.^{-1}$ [108]. The infra-red spectrum of desyl pyridinium chloride (fig.3) in this region showed no peak between 704 and 750 $cm.^{-1}$ Doublets in the 750 - 800 $cm.^{-1}$ region were common to all three compounds. These results indicate that no covalently bound chlorine is present in the compound to which the structure desyl pyridinium chloride has been assigned.

(b) Examination of the mass spectrum of desyl pyridinium bromide (determined by Dr.E.S. Waight on an M.S.9 instrument) showed that it contained no peaks attributable to bromine-containing fragments. This would be expected for structure (L) but not for (LIII).

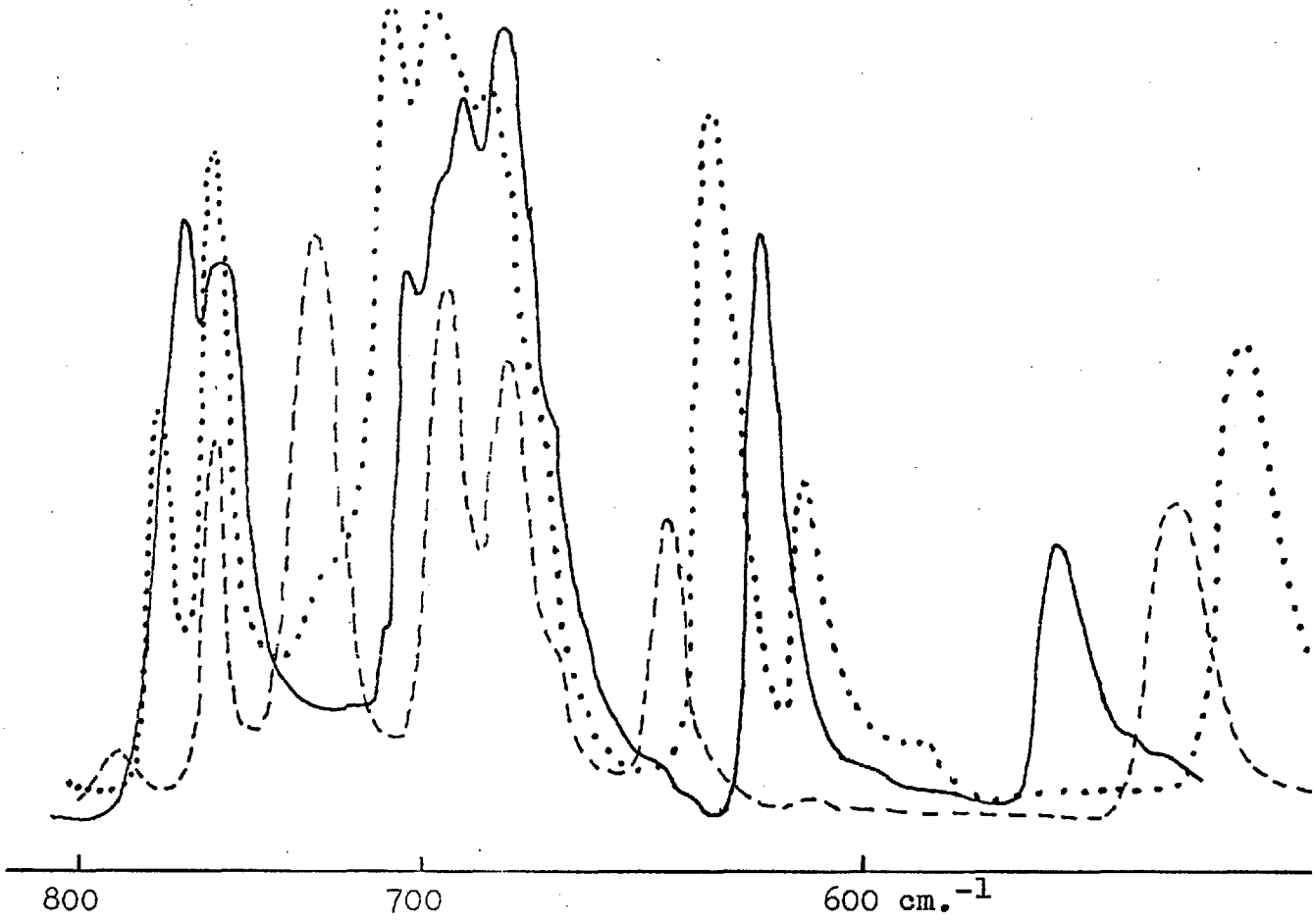
(c) The nuclear magnetic resonance spectrum of the chloride (that of the bromide was essentially the same) was consistent with its formulation as desyl pyridinium chloride. A singlet at τ 0.25, due to one proton,

Figure 3

--------- 2-Chloro-2-phenylacetophenone

·-·-·-·-· 2-Bromo-2-phenylacetophenone

———————— Desyl pyridinium chloride



can be assigned to the hydrogen alpha to the keto-group. Partially resolved peaks at 0.48, 1.44 and 2.30 arise from the α, γ and β-hydrogen atoms of the pyridine ring respectively. The hydrogen atom alpha to the keto-group appears to absorb at very low field by comparison to the absorbtions observed for similar compounds in the steroid field.[110] This effect is probably due to increased deshielding arising from the presence of the two benzene rings. It is noteworthy that resonances due to the enol form of the pyridinium salts were not apparent; this is somewhat surprising since the infra-red spectrum under similar conditions, suggests a reasonably high proportion of enol.

In addition to the physical evidence described above it was noted that small amounts of benzoic acid and ethyl benzoate were isolable from the reactions of the 2-halo-2-phenylacetophenones with pyridine in aqueous ethanol. This sort of behaviour has been observed previously for pyridinium salts derived from other types of α-haloketones.

Kinetic results.

The interaction of the 2-aryl-2-haloacetophenones with pyridine and with aniline, in aqueous ethanol, was investigated quantitatively using the method developed by

Baker[25] for the reaction of pyridine with the 2-halo-acetophenones (see p. 28) . The haloketone(0.00125 mole) was then allowed to react with pyridine (0.0125 mole) in 90% aqueous ethanol (50 ml.). The reaction was followed by Volhard estimation of the liberated halide ion and the pseudounimolecular rate constant was then calculated. The method is not highly accurate for several reasons. In the case of the bromo-ketones the reaction mixture was kept at 50°, and 5 ml. aliquots were removed at intervals for halide ion estimation; the use of a hot solution in a pipette introduces obvious errors. Titrations were kept between 2.5 and 5 ml. by addition of a suitable excess of silver nitrate. The titration is subject to an error of about ∓0.02 ml. thus introducing errors of about 1.5% at the lower end of the scale. In general therefore rate constants vary about ∓ 5% from the mean value. Unless otherwise stated the "infinity" values were obtained experimentally after a period equivalent to about ten times the reaction half-life.

The 2-bromo-2-phenylacetophenones reacted smoothly with pyridine under the above conditions. The results are set out in Table I.

Table I

Mean rate constants, at 50°, for reaction of compounds of the type $R.C_6H_4.CHBr.CO.Ph$ with pyridine.

R	$10^4 k$ ($min.^{-1}$)
H	212
o-Cl	60
m-Cl	88
p-Cl	158
o-NO_2	17
m-NO_2	98
p-NO_2	117

It is apparent from these results that these compounds are not as efficient as alkylating agents as the 2-halo-acetophenones. The results, however, are of considerable mechanistic interest. The various possible mechanisms of nucleophilic substitution have been dicussed in an earlier section (see p.18 et seq.) where it was seen that two possible sites of attack - the carbonyl group or the α-carbon - lead to different mechanisms of substitution. Rate of attack on the carbonyl group would not be expected to be particularly sensitive to relatively small changes in electron density on the α-carbon. Such a small change is brought about in going from m-NO_2 to p-NO_2 or m-Cl to

p-Cl in the above compounds (the o-substituted compounds are omitted from this particular discussion because of the additional steric factors which may be of importance in these cases). The change in rate is, however, quite considerable, a result more compatible with nucleophilic attack on the alpha-carbon as the rate-determining step. In addition, Baker[25] has shown that electron-withdrawing substituents in the m- and p-positions of 2-bromoacetophenone enhance the rate over 2-bromoacetophenone itself. He explained this as being due to the increased positiveness of the carbonyl carbon atom and hence its increased susceptibility to nucleophilic attack (o-substituted compounds were excluded because of possible steric effects). If this assumption is correct then the 2-bromo-2-phenylacetophenones substituted on either ring with electron-withdrawing substituents should show an enhanced rate over the unsubstituted compound. As can be seen from Table I, this is not the case. On the other hand withdrawal of electrons from the alpha-carbon atom of the 2-bromo-2-arylacetophenones would also be expected to increase the rate of S_N2 substitution because of the increased positive nature of the carbon atom. Since it does not, it must be assumed that the polarity of the C——Br bond is the rate-determining factor; thus electron-withdrawal diminishes

the facility with which the bromine atom can depart and hence lowers the rate. Whilst a similar argument could be used for substitution at the carbonyl group, the results obtained with the 2-haloacetophenones would seem to suggest the converse. In conclusion it is apparent that the results obtained with the 2-bromo-2-arylacetophenones do not prove that nucleophilic attack on these compounds occurs at the alpha-carbon atom. They do, however, add further doubt to the idea of initial attack at the carbonyl group.

The reaction of the 2-aryl-2-chloroacetophenones with pyridine was investigated by a similar method to that of the bromo-compounds except that the reaction was performed in sealed tubes at 100°. The mean rate constants are summarised in Table II.

Table II

Mean rate constants, at 100°, for reactions of compounds of the type $R.C_6H_4.CHCl.CO.Ph$ with pyridine.

R	10^4k (min.$^{-1}$)
H	67
o-Cl	42
m-Cl	61
p-Cl	114

All four chlorides were analytically pure. In addition all except the m-isomer are low-melting solids having sharp melting points; the m-compound is an oil. Despite the apparent purity of these compounds only the unsubstituted and the p-chloro-compounds gave (within experimental error) the calculated amount of chloride ion at "infinity", the o-chloro- and m-chloro-derivatives gave only 93.5 and 84% of the available chlorine as chloride ion, respectively. There appears to be no obvious explanation for this behaviour. The quoted rate constants were calculated from the experimental "infinity" value, and with this reservation the values for the rate constants are again mechanistically interesting. Assuming that initial attack of the nucleophile is at the alpha-carbon atom it would seem that the polarity of the C——Cl bond is the important factor for the o-chloro- and m-chloro-compounds, as in the case of the bromo-compounds. Surprisingly this does not seem to be the case for the p-chloro-compound in which the reduced inductive effect (with respect to the o- and m-compounds) is apparently insufficient to suppress the departure of the chlorine atom, but sufficient to enhance the positive nature of the alpha-carbon and hence the rate of reaction. This does not seem reasonable in view of the diminished reactivity of 2-bromo-2(p-chloro-

phenyl) acetophenone with respect to 2-bromo-2-phenylaceto-phenone.

Reaction of the 2-aryl-2-bromoacetophenones (0.00125 mole) with aniline (0.0125 mole) was carried out using the same method as for pyridine. The pseudouni-molecular rate constant decreased steadily as each reaction proceeded (see p.27), but it was still possible to establish an order of reactivity; for the compounds of the type $R.C_6H_4.CHBr.CO.Ph$ the order was p-Cl > m-Cl > p-NO_2 > H > o-Cl. In this case (excluding the o-chloro-compound because of possible steric effects) all electron-withdrawing substituents enhanced the rate with respect to the unsubstituted parent compound. The greatest enhancement of rate was produced by the group having the smallest -I effect; the decrease in reactivity then followed the increase in -I effect. Again this suggests that whilst weak withdrawal of electrons from the alpha-carbon enhances the rate of substitution by enhancing its positive nature, stronger -I groups suppress the polarity of the C——Br bond and reduce the leaving powers of the bromine atom as bromide ion.

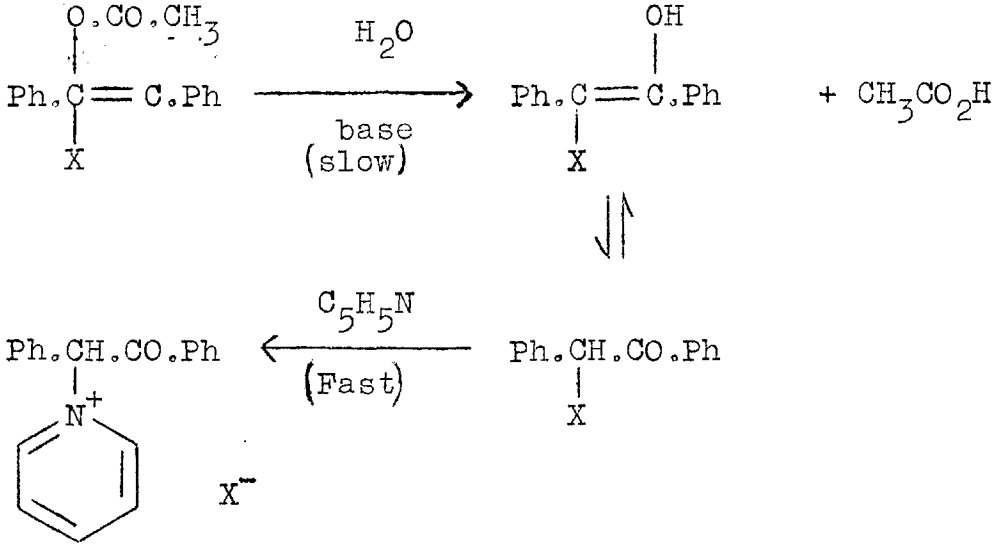
Reaction of α-Acetoxy-α'-halostilbenes with Pyridine.

The method used for the 2-aryl-2-chloro-acetophenones was used for both α-acetoxy-α'-chlorostilbene and its bromo-analogue. The "infinity" values were calculated because of the extreme slowness of the reaction. The pseudounimolecular rate constants, at 100°, were:

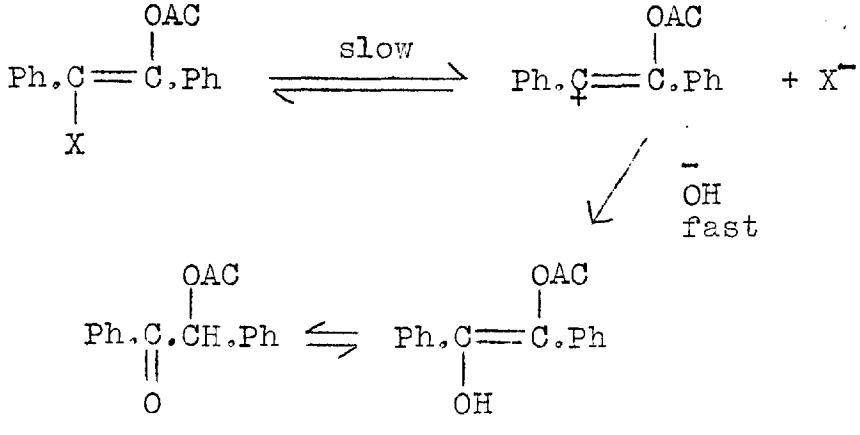
α-acetoxy-α'-chlorostilbene 1.0×10^{-4} min.$^{-1}$
α-acetoxy-α'-bromostilbene 1.9×10^{-4} min.$^{-1}$

The expected de-activation of the halo-ketones by enol acetylation is therefore established.

The relatively small difference in rates between the above two enol acetates suggests that the rate-determining step is removal of the acetyl group and generation of the parent ketone, followed by relatively fast formation of the quaternary salt;



This would seem to be more likely than, for example, a unimolecular hydrolysis of the stilbene halide:



Although unimolecular hydrolysis of some α-bromostyrenes has recently been demonstrated by Grob and Cseh,[112] a greater difference than that found above would be expected when X varies from chlorine to bromine.

BIOLOGICAL DISCUSSION

The 2-aryl-2-haloacetophenones and their enol acetates did not prove to be very effective as cytotoxic agents; however several interesting points arise from the biological results. The toxicity tests on rats showed that, in all the cases tried, the enol acetates were less toxic than the parent α-halo-ketones; unfortunately the cytotoxicity was also diminished - possibly an indication that the enol ester linkage in these compounds is relatively stable *in vivo*. 2-Bromo-2-(p-chlorophenyl)acetophenone showed a surprisingly high anti-tumour effect. It was noted that rats treated with the enol acetate (which showed little cytotoxicity) of this compound suffered delayed deaths, which again indicates that hydrolysis of the enol acetate to the parent α-halo-ketone is a slow process *in vivo*.

1,2-Dichloro-1,2-dibenzoylethane proved to be more toxic and more cytotoxic than the bromo-analogue. An examination of the extent of reaction of these two compounds in anhydrous pyridine, at 50°, for 45 min. showed the bromo compound to be the most reactive. It was noted, however, that the bromo-compound is insoluble in many more solvents than the chloro-compound. The biological inactivity of the bromo-compound may, therefore, be a solubility effect.

p-(Bromomethyl)benzenesulphonamide showed good anti-tumour effects at dosages near the LD_{50} value. 2-Chloro-4'-sulphonamidoacetophenone was equally cytotoxic at dose rates considerably less than the LD_{50} value - an encouraging result. Tests are continuing on this compound.

Provisional results with dialkyl enol phosphates are not too encouraging and it now seems desirable to test the free acid salts which may show greater biological activity.

Ethyl α-bromoorthoacetate and ethyl α-bromophenylorthoacetate were both less toxic than the corresponding normal esters - an encouraging result, in accord with theory.

EXPERIMENTAL

Numerals, in parentheses, that follow the headings throughout this section refer to the page numbers of the original notebooks.

Infra-red spectra were run on a Unicam S.P.200 spectrometer and ultra-violet spectra on a Unicam S.P.800 instrument. Infra -red spectra in the 500-800cm.$^{-1}$ region were run by Mr. E.A. King on a Grub Parsons double beam grating instrument. N.M.R. spectra were measured by Mrs.A.I. Boston using a Varian A.60 spectrometer.

Melting points were determined on a Kofler block and are uncorrected.

Substituted Acetophenones and their Enol Acetates

2-Bromo-2-phenylacetophenone (21)

This compound was prepared from deoxybenzoin, by bromination in carbon tetrachloride solution, using the method of Jenkins[44]. It had m.p. 55°; Knoevenagel[113] gives m.p. 54-55°.

α-Acetoxy-α'-bromostilbene (21, 29, 85).

(a) A well-stirred suspension of anhydrous sodium methoxide (7.04 g.), in dry ether (400 ml.), was cooled to about -50° and 2-bromo-2-phenylacetophenone (18 g.) was added in one lot. After 20 min. the solution had become deep yellow and the enolate salt began to precipitate out. After a further 10 min. acetyl chloride (20 g.) was added and the reaction mixture (now colourless) was allowed to warm to room temperature. Filtration and subsequent removal of the solvent under reduced pressure afforded a yellow, crystalline solid, which was recrystallised from methanol to give lustrous, colourless plates (4.5 g., 22%) of α-acetoxy-α'-bromostilbene, m.p. 108-109° (Found: C, 60.81; H, 4.32; Br, 25.58. $C_{16}H_{13}BrO_2$ requires C, 60.62; H, 4.14; Br, 25.22%), ν max. in chloroform 1190, 1630 (C=C), 1760 cm.$^{-1}$ (C=O); λ max. in ethanol 205 (ε = 21,300), 226 (ε = 17,900), 283 mμ (ε = 7170).

(b) Sodium hydride (0.96 g., 0.04 mole.) was suspended in dry ether (20 ml.) and treated, with stirring, with dry methanol (0.64 g., 0.02 mole.). The well-stirred suspension was cooled to about -20° and treated with a solution of 2-bromo-2-phenylacetophenone (5.5 g., 0.02 mole.) in dry ether (10 ml.); hydrogen was evolved and the solution became deep yellow in colour. When the evolution of hydrogen had ceased (after about 30 min.), acetyl chloride (3 ml.) was added, whereupon the colour of the solution was discharged. Filtration and subsequent removal of the solvent under reduced pressure, gave the crude enol acetate. Crystallisation from methanol gave pure α-acetoxy-α'-bromostilbene (3.5 g., 56%) m.p. 108-109°, identical in all respects to the material prepared by method (a).

2-Chloro-2-phenylacetophenone (17)

This compound was prepared from benzoin, by reaction with thionyl chloride in pyridine, using the method of Ward.[46] It had m.p. 68-69°; Ward gives m.p. 66-67°.

α-Acetoxy-α'-chlorostilbene (17, 41)

(a) Anhydrous sodium methoxide (5.4 g.) was

suspended in dry ether (400 ml.) and cooled to about -50°. The well-stirred suspension was treated with 2-chloro-2-phenylacetophenone (20 g.) whereupon a deep red solution of the enolate was obtained. After 10 min. acetyl chloride (20 g.) was added; the solution immediately became decolourised. Filtration and removal of the solvent under reduced pressure gave a gummy, yellow solid which recrystallised from ethanol to give colourless plates (9.0 g., 38%) of α-acetoxy-α'-chlorostilbene, m.p. 94° (Found: C, 70.55; H, 4.88; Cl, 13.10 $C_{16}H_{13}ClO_2$ requires C, 70.60; H, 4.82; Cl, 13.03%), ν max. in chloroform 1190, 1620 (C=C), 1760 cm.$^{-1}$ (C=O); λ max. in ethanol 205 (ε = 24,300), 223 (ε = 18,300), 276 mμ (ε = 9850).

Sodium hydride (0.48 g.) was suspended in dry ether (20 ml.) and dry methanol (0.32 g.) was added. The well-stirred suspension was cooled to about -20° and a solution of 2-chloro-2-phenylacetophenone (2.3 g.) in dry ether (10 ml.) was added. When the evolution of hydrogen was complete (after about 10 min.) acetyl chloride (2.5 g.) was added. Filtration and removal of the solvent under reduced pressure gave the crude enol acetate. Recrystallisation from ethanol gave colourless plates (1.85 g., 70%) of α-acetoxy-α'-chlorostilbene m.p. 94°, identical in all respects to the material prepared by method (a).

2-(o-Chlorophenyl)acetophenone (153, 159, 161, 175)

o-Chlorotoluene was brominated, under photochemical conditions,[68] to give o-chlorobenzyl bromide (b.p. 101-103°/10 mm.) in 77% yield. The bromide was converted to o-chlorobenzyl cyanide by the action of sodium cyanide in aqueous ethanol[114]; the pure nitrile (b.p. 115-120°/20mm.) was isolated by ether extraction, and subsequent distillation, in 86% yield (Misra and Shukla[114] obtained 61% yield by direct distillation of the reaction mixture). The cyanide was hydrolysed[114] to give o-chlorophenylacetic acid, m.p. 96-97° in 86% yield (Misra and Shukla[114] give m.p. 93-94°, yield 89%). 2-(o-Chlorophenyl)acetophenone was then prepared, using the general procedure of Fischer et al.[70], by Friedel-Craft's condensation of the crude acid chloride with benzene. The yield of pure ketone was 65%; it had m.p. 69-70°. Jenkins[69] gives m.p. 70.5° (corrected).

2-Bromo-2-(o-chlorophenyl)acetophenone (177)

The general procedure of Jenkins[44] was used. A solution of 2-(o-chlorophenyl)acetophenone (2.31 g.) in carbon tetrachloride (30 ml.), illuminated and warmed by a 300W tungsten lamp, was treated dropwise, with stirring, with

a solution of bromine (1.6 g.) in carbon tetrachloride (20 ml.) over a period of 20 min. Filtration through an activated charcoal pad and subsequent removal of the solvent under reduced pressure gave a yellow oil which would not crystallise. Distillation under reduced pressure gave 2-bromo-2-(o-chlorophenyl)acetophenone (2.5 g., 80%), b.p. 143°/1.4 x 10^{-2} mm., η_D^{18} 1.6267. Found: C, 54.61; H, 3.23. $C_{14}H_{10}BrClO$ requires C, 54.30; H, 3.26%). On prolonged standing at 0° a specimen of the product crystallised. Recrystallisation from ethanol gave colourless furry needles m.p. 29-30°.

2'-Chlorobenzoin. (179)

2-(o-Chlorophenyl)acetophenone (2.31 g.) was brominated to give 2-bromo-2-(o-chlorophenyl)acetophenone (see above). The crude bromide was hydrolysed by the general method of Jenkins[44], giving the crude benzoin. Crystallisation of this material from chloroform - petroleum (b.p. 40-60°) gave colourless plates (2.3 g., 93%) of 2'-chlorobenzoin, m.p. 83°. (Found C, 68.20; H, 4.38; Cl, 14.49. $C_{14}H_{11}ClO_2$ requires C, 68.15; H, 4.50; Cl, 14.38%).

2-Chloro-2-(o-chlorophenyl)acetophenone (219)

The method used was essentially similar to that of Ward[46] for 2-chloro-2-phenylacetophenone. A solution of 2'-chlorobenzoin (1.27 g.) in pyridine (0.5 g.) was cooled to 0° and treated dropwise, with stirring, with thionyl chloride (0.75 g.). The reaction mixture was allowed to stand, at room temperature, for 1 hr. Water (10 ml.) was then added and the resulting sticky yellow solid was recrystallised from ethanol giving colourless, chunky crystals (0.85 g., 64%) of 2-chloro-2-(o-chlorophenyl)-acetophenone, m.p. 44-45°. (Found: C, 63.66; H, 3.87. $C_{14}H_{10}Cl_2O$ requires C, 63.47; H, 3.81%).

α'-Acetoxy-α-bromo-2-chlorostilbene (221)

2-Bromo-2-(o-chlorophenyl)acetophenone (3.1 g.)was converted to its enol acetate by the general method (b) outlined above for α-acetoxy-α'-bromostilbene. Crystallisation of the product from methanol gave colourless prisms (1.9 g., 54%) of α'-acetoxy-α-bromo-2-chlorostilbene, m.p. 90°, (Found: C, 54.67; H, 3.50. $C_{16}H_{12}BrClO_2$ requires C, 54.65; H, 3.45%), ν max. in chloroform 1190, 1645, 1765 $cm.^{-1}$; λ max. in ethanol 208 (ε = 25,400), 233 mμ (ε = 15,300).

α'-Acetoxy-α,2-dichlorostilbene. (225)

2-Chloro-2-(o-chlorophenyl)acetophenone(4.3 g.) was converted to its enol acetate by the general method (b) given above for α-acetoxy-α'-chlorostilbene. Recrystallisation of the product from light petroleum (b.p. 40-60°), or ethanol, gave colourless, hexagonal plates (2.7 g., 54%) m.p. 65-70°. Further crystallisation from ethanol gave pure α'-acetoxy-α,2-dichlorostilbene, m.p. 71.5°. (Found: C, 62.56; H, 3.99. $C_{16}H_{12}Cl_2O_2$ requires C, 62.55; H, 3.95%), ν max. in chloroform 1190, 1640, 1765 cm.$^{-1}$; λ max. in ethanol 207 (ε = 25,300), 263 mμ (ε = 10,350).

2-(m-Chlorophenyl)acetophenone (241)

m-Chlorotoluene was brominated, under photochemical conditions[68], to give m-chlorobenzyl bromide (b.p. 110-115°/20 mm.) in 73% yield. The bromide was converted to o-chlorobenzyl cyanide by the action of aqueous, ethanolic sodium cyanide[114]; the pure nitrile (b.p. 125-130°/10 mm.) was isolated by ether extraction and subsequent distillation, in 90% yield (Misra and Shukla[114] obtained 83% yield by direct distillation of the reaction mixture). The cyanide was hydrolysed[114] to give m-chlorophenylacetic

acid, m.p. 78-79°, in 69% yield (Misra and Shukla[114] give m.p. 76°, yield 91%). 2-(m-Chlorophenyl)acetophenone was then prepared, by the method of Fischer et al.,[70] by Friedel-Craft's condensation of the crude acid chloride with benzene. The yield of pure ketone was 70% (purification was effected by distillation, b.p. 140°/ 2 x 10^{-2}mm. This material then solidified, m.p. 42-43°. Fischer et al.[70] give m.p. 43°).

2-Bromo-2-(m-chlorophenyl)acetophenone (249).

2-(m-Chlorophenyl)acetophenone (2.31 g.) in carbon tetrachloride (30 ml.) was treated with bromine (1.6 g.) in carbon tetrachloride (20 ml.) as described above for 2-bromo-2-(o-chlorophenyl)acetophenone. Filtration through activated charcoal followed by evaporation of the solvent gave a brown oil which would not crystallise. Distillation under reduced pressure gave 2-bromo-2-(m-chlorophenyl)acetophenone, (2.5 g., 80%), b.p. 154°/ 1.4 x 10^{-4} mm., η_D^{21} 1.6337. (Found: C, 53.80, H, 3.46%. $C_{14}H_{10}BrClO$ requires C, 54.30; H, 3.26%). A better analysis could not be obtained, presumably because of decomposition on distillation. The distilled material could not be crystallised.

3'-Chlorobenzoin (271)

2-(m-Chlorophenyl)acetophenone (2.31 g.) was brominated to give 2-bromo-2-(m-chlorophenyl)acetophenone. The crude bromide was hydrolysed, using the general procedure of Jenkins[44], to give the benzoin. Recrystallisation from light petroleum (b.p. 60-80°) gave colourless needles (2.3 g., 92%) of 3'-chlorobenzoin, m.p. 95°. (Found: C, 68.17; H, 4.58. $C_{14}H_{11}ClO_2$ requires C, 68.15; H, 4.50%).

2-Chloro-2-(m-chlorophenyl)acetophenone (277).

3'-Chlorobenzoin (1.27 g.) was treated with pyridine (0.50 g.) and thionyl chloride (0.75 g.), as described above for 2-chloro-2-(o-chlorophenyl)acetophenone. The product was isolated by ether extraction; distillation under reduced pressure gave 2-chloro-2-(m-chlorophenyl)-acetophenone (0.96 g., 70%), b.p. 142°/10^{-3} mm. n_D^{22} 1.6108. (Found: C, 63.08; H, 3.73; Cl, 26.51. $C_{14}H_{10}Cl_2O$ requires C, 63.47; H, 3.81; Cl, 26.75%). The compound could not be crystallised

α'-Acetoxy-α-bromo-3-chlorostilbene (251)

2-Bromo-2-(m-chlorophenyl)acetophenone (3.72 g.) was converted to its enol acetate by the general method (b)

given above for α-acetoxy-α'-bromostilbene. Recrystallisation of the product from light petroleum (b.p. 60-80°) gave colourless prisms (1.3 g., 31%) of α'-acetoxy-α-bromo-3-chlorostilbene, m.p. 84°. (Found: C, 54.81; H, 3.40. $C_{16}H_{12}BrClO_2$ requires C, 54.65; H, 3.45%), ν max. in chloroform 1190, 1635, 1765 cm.$^{-1}$; λ max. in ethanol 209 (ε = 28,500), 228 (ε = 20,100), 285 mμ (ε = 7520).

2-(p-Chlorophenyl)acetophenone (151, 155, 169)

p-Chlorotoluene was brominated, under photochemical conditions,[68] to give p-chlorobenzyl bromide, m.p. 50°. The bromide was converted to p-chlorobenzyl cyanide by the action of aqueous, ethanolic sodium cyanide;[114] the pure nitrile (b.p. 100°/0.5 mm., m.p. 30°) was isolated by ether extraction and subsequent distillation, in 86% yield (Misra and Shukla[114] obtained 71% by direct distillation of the reaction mixture). The cyanide was hydrolysed to give p-chlorophenylacetic acid m.p. 106-107°, in 94% yield (Misra and Shukla[114] give m.p. 104-105°, yield 89%). 2-(p-Chlorophenyl)acetophenone was then prepared, by the method of Fischer et al.[70], by Friedel-Crafts' condensation of the crude acid chloride with benzene. The yield of pure ketone was 63%, m.p. 136-137° (Fischer et al.[70] give m.p. 136.5°).

2-Bromo-2-(p-chlorophenyl)acetophenone (171)

2-(p-Chlorophenyl)acetophenone was brominated, in carbon tetrachloride solution, using the method of Jenkins.[44] The product had m.p. 63°, after recrystallisation from light petroleum (b.p. 60-80°). Jenkins[44] gives m.p. 62-62.5°.

4'-Chlorobenzoin (227)

2-(p-Chlorophenyl)acetophenone was brominated (see above) and the crude bromide was hydrolysed to the benzoin, using the procedure described by Jenkins[44]; the product, after crystallisation from alcohol, had m.p. 115°. Jenkins gives m.p. 116-117° (corrected).

2-Chloro-2-(p-chlorophenyl)acetophenone (239)

4'-Chlorobenzoin (2.47 g.) was treated with pyridine (1.0 g.) and thionyl chloride (1.5 g.) as described above for 2-chloro-2-(o-chlorophenyl)acetophenone. The product was recrystallised from ethanol giving colourless needles (1.98 g., 75%) of 2-chloro-2-(p-chlorophenyl)-acetophenone, m.p. 40-41°. (Found: C, 63.30; H, 3.90; Cl, 27.01. $C_{14}H_{10}Cl_2O$ requires C 63.47; H, 3.81; Cl, 26.75%).

α'-Acetoxy-α-bromo-4-chlorostilbene (223)

2-Bromo-2-(p-chlorophenyl)acetophenone (3.1 g.) was converted to its enol acetate by the method (b) given above for α-acetoxy-α'-bromostilbene. Recrystallisation of the product from light petroleum (b.p. 80-100°) gave colourless rhombs (1.4 g., 40%) of α'-acetoxy-α-bromo-4-chlorostilbene, m.p. 75°. (Found C, 54.66; H, 3.34. $C_{16}H_{12}BrClO_2$ requires C, 54.65; H, 3.45%), ν max. in chloroform 1190, 1630, 1760 cm.$^{-1}$, λ max. in ethanol 206 (ε = 21,000), 232 (ε = 19,800), 290 mμ (ε = 8,060).

α'-Acetoxy-α,4-dichlorostilbene (707)

2-Chloro-2-(p-chlorophenyl)acetophenone (5.30 g.) was converted to its enol acetate by the method (b) given above for α-acetoxy-α'-chlorostilbene. Recrystallisation from light petroleum (b.p. 60-80°) gave glittering, colourless, hexagonal plates (2.5 g., 40.7%) of α'-acetoxy-α,4-dichlorostilbene, m.p. 80-81°. (Found: C, 62.55; H, 4.02; Cl, 23.01. $C_{16}H_{12}Cl_2O_2$ requires C, 62.55; H, 3.95; Cl, 23.09%), ν max. in chloroform 1200, 1600, 1640, 1770 cm.$^{-1}$; λ max. in ethanol 204 (ε = 19,500), 229 (ε = 18,000), 280 mμ (ε = 9,700).

2-(o-Nitrophenyl)acetophenone (299)

o-Nitrotoluene was condensed with ethyl oxalate to give ethyl o-nitrophenylpyruvate. The crude ester, on hydrolysis and treatment with acidified hydrogen peroxide, gave o-nitrophenylacetic acid, m.p. 141°, in 48% yield. (The method is that of Wright and Collins[115] who give m.p. 140-141°, yield 50%). 2-(o-Nitrophenyl)acetophenone was then prepared, using the general method of Fischer et al.,[70] by Friedel-Crafts' condensation of the crude acid chloride with benzene. The ketone was purified by chromatography on alumina (Spence grade 'H') using benzene for development and elution. Crystallisation of the product from light petroleum (b.p. 40-60°) - benzene gave pure 2-(o-nitrophenyl)-acetophenone, m.p. 75°, in 31% yield. List[116] gives m.p. 73-74°.

2-Bromo-2-(o-nitrophenyl)acetophenone. (303)

2-(o-Nitrophenyl)acetophenone (2.41 g.) was brominated, in carbon tetrachloride solution, using the method of Jenkins[44]. The reaction mixture was filtered through a charcoal pad and evaporated down to a bulk of 10 ml. whereupon, on cooling, colourless needles (2.8 g., 88%) of 2-bromo-2-(o-nitrophenyl)acetophenone, m.p. 114-115°,

separated from solution. Further recrystallisation from methanol gave the pure material, m.p. 115-116° (decomp). (Found C, 52.42; H, 3.12; Br, 25.70, 26.32. $C_{14}H_{10}BrNO_3$ requires C, 52.48; H, 3.15; Br, 24.98%). The compound decomposes, on standing, to a dark brown solid.

Attempted preparation of α'-acetoxy-α-bromo-2-nitrostilbene

(305)

2-Bromo-2-(o-nitrophenyl)acetophenone (1.07 g.) was treated with sodium methoxide and sodium hydride in dry ether as described above for α-acetoxy-α'-bromostilbene. No hydrogen was evolved and there was no appreciable colour change in the solution. Addition of acetyl chloride and working up in the usual way gave only starting material (98%) The experiment was repeated at various temperatures in the range -80° to 0° with the same result.

2-(m-Nitrophenyl)acetophenone (307, 315, 325)

Using the procedure of Sprung,[117] m-nitrobenzaldehyde was converted (Cannizaro reaction) to m-nitrobenzyl alcohol. The crude alcohol on reaction with thionyl chloride, gave m-nitrobenzyl chloride, m.p. 44°, in 83% overall yield. Sprung gives m.p. 45.5-46.5°.

Treatment of the chloride

with aqueous - ethanolic sodium cyanide[114] gave m-nitrobenzyl cyanide. The crude nitrile was hydrolysed[118] to give m-nitrophenylacetic acid, m.p. 118-119°, in 41% yield (after four recrystallisations from water). Salkowski[119] gives m.p. 120°. 2-(m-Nitrophenyl)acetophenone was then prepared, using the general method of Fischer et al.[70], by Friedel-Crafts' condensation of the crude acid chloride with benzene. The ketone was purified by chromatography on alumina (Spence grade 'H'), using benzene for development and elution, followed by recrystallisation from methanol. The pure compound had m.p. 82° (yield 64%). Fischer et al.[70] give m.p. 82°.

2-Bromo-2-(m-nitrophenyl)acetophenone (331)

2-(m-Nitrophenyl)acetophenone (2.41 g.) was brominated in carbon tetrachloride solution, using the method of Jenkins[44]. The product was recrystallised from methanol giving creamy granules (2.9 g., 88%) of 2-bromo-2-(m-nitrophenyl)acetophenone, m.p. 87-88°.

(Found: C, 52.58; H, 3.27; Br, 24.78. $C_{14}H_{10}BrNO_3$ requires C, 52.48; H, 3.15; Br, 24.98%).

α'-Acetoxy-α-bromo-3-nitrostilbene (333)

2-Bromo-2-(m-nitrophenyl)acetophenone (6.40 g.) was converted to its enol acetate using method (b) described above for α-acetoxy-α'-bromostilbene. The crude enol acetate was chromatographed on alumina (Spence grade 'H') using light petroleum (b.p. 40-60°) as developer and chloroform for elution. The product was recrystallised from methanol giving pale yellow rhombs (3.5 g., 48%) of α'-acetoxy-α-bromo-3-nitrostilbene, m.p. 109-110°. (Found: C, 53.19; H, 3.26; O, 17.82. $C_{16}H_{12}BrNO_3$ requires C, 53.05; H, 3.35; O, 17.67%), ν max. in chloroform 1190, 1635, 1765 cm.$^{-1}$: λ max. in ethanol 207 (ε = 25,100), 219 (ε = 26,300), 265 mμ (ε = 12,550).

2-(p-Nitrophenyl)acetophenone (127)

Benzyl cyanide was nitrated[120] to give p-nitrobenzyl cyanide, m.p. 115°. The nitrile was hydrolysed[118] to p-nitrophenylacetic acid, m.p. 151° and the acid was then converted to 2-(p-nitrophenyl)acetophenone, by Friedel-Crafts' condensation of the crude acid chloride with benzene, using the method of Fischer et al.[70]. The pure ketone had m.p. 145°. Fischer et al.[70] give m.p. 144°.

2-Bromo-2-(p-nitrophenyl)acetophenone (137)

2-(p-Nitrophenyl)acetophenone (12 g.) in chloroform (100 ml.) was treated with bromine (8 g.) in chloroform (25 ml.) as described above for 2-bromo-2-(o-chlorophenyl)acetophenone. Filtration through activated charcoal followed by evaporation of the solvent gave a green oil which crystallised on trituration with light petroleum (b.p. 40-60°). Crystallisation from chloroform - light petroleum (b.p. 40-60°) gave pale yellow granules (8.2 g., 51%) of 2-bromo-2-(p-nitrophenyl)acetophenone, m.p. 100°. (Found: C, 52.35; H, 2.84; N, 4.45. $C_{14}H_{10}BrNO_3$ requires C, 52.48; H, 3.15; N, 4.38%).

α'-Acetoxy-α-bromo-4-nitrostilbene (141, 143)

2-Bromo-2-(p-nitrophenyl)acetophenone (6.40 g.) was converted to its enol acetate using the method (b) described above for α-acetoxy-α'-bromostilbene; anhydrous tetrahydrofuran was used as solvent in place of ether. Filtration of the reaction mixture and evaporation of the solvent gave a yellow oil which would not crystallise. The oil was taken up in ether (15 ml.) and light petroleum (b.p. 40-60°) (25 ml.) was added dropwise, with stirring, whereupon a creamy solid separated out. Recrystallisation from methanol gave pale yellow granules (2.0 g., 27.7%)

of α'-acetoxy-α- bromo-4-nitrostilbene, m.p. 114°. (Found: C, 53.16; H, 3.26; N, 3.97. $C_{16}H_{12}BrNO_4$ requires C, 53.05; H, 3.35; N, 3.87%), ν max. in chloroform 1180, 1600, 1630, 1765 cm.$^{-1}$

2'-Hydroxybenzoin (77)

This compound was prepared, in 8% yield, by condensation of o-hydroxybenzaldehyde cyanohydrin with phenyl magnesium bromide using the method of Asahina and Teresaka.[57] It had m.p. 148-153°; they quote m.p.148°.

Reaction of 2'-hydroxybenzoin with thionyl chloride (83)

2'-Hydroxybenzoin (2.1 g.) was dissolved in pyridine (1.0 g.) and treated, at room temperature, with thionyl chloride (1.5 g.). The reaction mixture was allowed to stand, at room temperature, for 1 hour. Addition of water (10 ml.) and filtration gave a colourless solid (1.32 g.). Recrystallisation from methanol gave colourless needles, m.p. 45°, which did not appear to be the expected chloro-ketone. (Found: C, 74.08; H, 4.11; Cl, 15.70. Calc. for $C_{14}H_{11}ClO_2$ C, 68.12; H, 4.50; Cl, 14.38. Calc. for $C_{14}H_9ClO$ C, 73.52; H, 3.98; Cl, 15.52%), ν max. in chloroform 1705 cm.$^{-1}$.

4'-Hydroxybenzoin (81)

Using the method of Asahina and Teresaka[57] for similar compounds, p-hydroxybenzaldehyde cyanohydrin (from 40 g. of aldehyde) was reacted with phenyl magnesium bromide giving, after hydrolysis, a colourless solid (6.0 g.) m.p. 165-170°. Recrystallisation from water-dioxan (2:1) gave glittering, colourless rhombs of 4'-Hydroxybenzoin, m.p. 187-189°. (Found C, 73.55; H, 5.20. $C_{14}H_{12}O_3$ requires C, 73.65; H, 5.31%).

Reaction of 4'-hydroxybenzoin with thionyl chloride (93)

4'-Hydroxybenzoin (1.3 g.) was dissolved in pyridine (0.62 g.) and treated dropwise, at room temperature, with thionyl chloride (0.95 g.). The reaction mixture was allowed to stand, at room temperature, for one hour. Water (10 ml.) was then added, causing a tarry mass to precipitate. This material could not be crystallised. When chromatographed on silica gel the material was lost; it was not eluted with cold methanol and more vigorous treatment of the extruded silica gel with hot methanol resulted in extensive decomposition of the organic material.

2-(o-Methylphenyl)acetophenone (291, 309)

o-Xylene was brominated, using the method of Atkinson and Thorpe[121], to give o-xylyl bromide; the reaction proceeded more easily when the reaction vessel was illuminated with a 500W tungsten lamp. Treatment of the bromide with aqueous, ethanolic sodium cyanide[114] gave o-xylyl cyanide which was then hydrolysed[114] to o-methylphenylacetic acid, m.p. 87-88° (Radziszewski and Wispek[122] give m.p. 88-89°). The acid (15 g.) was converted to the acid chloride, by treatment with thionyl chloride, and condensed with benzene using the procedure of Fischer et al.[70]. The product, a red oil, was dissolved in benzene and filtered through a column (20 x 2 cm.) of alumina. Removal of the solvent and recrystallisation of the product from methanol gave colourless plates (5.7 g., 27%) of 2-(o-methylphenyl)acetophenone, m.p. 68°. (Found: C, 85.57; H, 6.74. $C_{15}H_{14}O$ requires C, 85.68; H, 6.72%).

2-Bromo-2-(o-methylphenyl)acetophenone (311)

2-(o-Methylphenyl)acetophenone (4.7 g.) in carbon tetrachloride (45 ml.) was treated with a solution of bromine (3.6 g.) in the same solvent (45 ml.) as described above for 2-bromo-2-(o-chlorophenyl)acetophenone. Filtration of the reaction mixture through an activated

charcoal pad and subsequent evaporation of the solvent gave 2-bromo-2-(o-methylphenyl)acetophenone (5.0 g., 79%) as a brown oil which would not crystallise. The compound decomposed on standing; it distilled, b.p. $150^{\circ}/10^{-4}$ mm., with extensive decomposition. The N.M.R. spectrum of the crude bromide in deuterochloroform showed τ, 7.5 (singlet, 3 protons), 3.4(singlet 1 proton).

2'-Methylbenzoin (321)

2-Bromo-2-(o-methylphenyl)acetophenone (0.58 g.) was hydrolysed using the method of Jenkins[44]. The product, isolated by ether extraction, was a yellow oil, which would not crystallise. Within 30 minutes.this material had decomposed to a purple tarry mass.

α'-Acetoxy-α-bromo-2-methylstilbene (313)

Crude 2-bromo-2-(o-methylphenyl)acetophenone (2.89 g.) was converted to its enol acetate using method (b) described above for α-acetoxy-α'-bromostilbene. Recrystallisation of the product from methanol gave colourless chunks (1.65 g., 50%) of α'-acetoxy-α-bromo-2-methylstilbene, m.p. 88°. (Found: C, 61.78; H, 4.88. $C_{17}H_{15}BrO_2$ requires C, 61.65; H, 4.57%), ν max. in chloroform, 1200, 1600, 1640, 1760 $cm.^{-1}$; λ max. in ethanol 214

(ε = 13,400), 230 (ε = 13,300), 263 mμ (ε = 8,350).

Attempted synthesis of α-acetoxy-β-halostyrenes (3,9, 13)

(a) To a mixture of acetic acid (20 g.) and acetic anhydride (2 g.) was added the boron trifluoride - mercuric oxide catalyst described by Hennion et al.[123] Keeping the temperature at 0°, bromophenylacetylene (60 g.) was added dropwise, over a period of one hour, with vigorous mechanical stirring. The reaction mixture was then allowed to warm to room temperature and stirring was continued for 12 hr. Ether (150 ml.) was then added and, after filtration, the resulting solution was washed repeatedly with 10% sodium carbonate solution to remove the excess acetic acid. The solution was then washed with water, dried over anhydrous sodium sulphate and distilled under reduced pressure giving bromophenylacetylene (50 g. , 83%), b.p. 32-38°/10^{-4} mm. and a brown oil, b.p. 84-94°/10^{-4}mm. which decomposed violently on admitting air to the apparatus. A small amount of a white, crystalline solid remaining in the distilling flask was recrystallised from methanol giving colourless needles (0.5 g.) of a mercury complex, m.p. 210°, (Found: C, 34.54; H, 2.39; O, 13.64%). The compound was not further characterised.

(b) A solution of acetyl hypobromite in dry

carbon tetrachloride (700 ml.) was prepared from silver acetate (16 g.) and bromine (4 ml.) using the method of Levine and Wall.[82] This solution was cooled to 0°, irradiated with a 500W tungsten lamp and treated dropwise, with stirring, with phenyl acetylene (10 g.). After 10 min. the yellow colour of the acetyl hypobromite had been discharged. After washing with water, drying over anhydrous magnesium sulphate and filtering, the solvent was evaporated off and the resulting oil was distilled under reduced pressure. The distillate was collected as five fractions of which the first three (b.p. 46-76°/10^{-3} mm.) consisted mainly of phenylacetylene. Fraction four (b.p. 77-86°/10^{-3} mm.) was redistilled giving a yellow oil (b.p. 86°/5.4 x 10^{-4} mm.). (Found: C, 39.86; H, 3.51; Br, 52.7. α-Acetoxy-β-bromostyrene, $C_{10}H_9O_2Br$ requires C, 49.82; H, 3.77; Br, 33.15%). Fraction five (b.p. 87-96°/10^{-3} mm.) was redistilled giving a yellow oil (b.p. 86°/5.4 x 10^{-4} mm.). (Found: C, 40.17; H, 2.44; Br, 54.26%). Neither fraction was further characterised.

(c) Phenacyl bromide was treated with sodium methoxide and sodium hydride in dry ether as described above in method (b) for α-acetoxy-α'-bromostilbene. No hydrogen was evolved and there was no colour change in the solution. Addition of acetyl chloride and working up in

the usual way, resulted in quantitative recovery of the ketone. The experiment was repeated at various temperatures in the range -80 to -20° with similar results.

(d) 2-Bromoacetophenone (10 g.), isopropenyl acetate (40 g.) and p-toluenesulphonic acid were refluxed together for 24 hr. The excess isopropenyl acetate was then distilled off, over a period of 8 hr., under reduced pressure. The residual oil was distilled under reduced pressure giving 2-bromoacetophenone (9.0 g.), m.p.50°, mixed m.p. 50-51°. The tarry residue in the distilling flask was insoluble in ether and chloroform.

(e) Methods (c) and (d) described above for α-acetoxy-β-bromostyreme were tried with 2-chloroacetophenone with essentially similar results.

(f) Methods (c) and (d) described above for α-acetoxy-β-bromostyrene were tried with 2-bromo-4'-nitro-, 2,4'-dibromo- and 2-bromo-4'-phenylacetophenones respectively. In no case was any evidence of enol acetylation obtained and in all cases the recovery of ketone (by crystallisation) exceeded 90%.

(g) 2-Bromo-2-methylacetophenone was treated as described above in methods (c) and (d) for α-acetoxy-β-bromostyrene. There was no evidence of enol acetylation and the recovery of starting material exceeded 90% in both cases.

Some Difunctional α-Halo-ketones and Attempted Syntheses of their Enol Acetates.

p-bisBromoacetylbenzene (19).

p-Toluic acid was converted to its acid chloride which, on treatment with excess diazomethane in dry ether, gave the di-diazoketone. Reaction of the material with an excess of hydrobromic acid gave p-bisbromoacetylbenzene, m.p. 176°. The method is that of Ross[124]; he gives m.p. 176-177°.

p-bisChloroacetylbenzene (19)

The di-diazoketone obtained (see above) from p-toluoyl chloride was treated with excess hydrochloric acid giving p-bischloroacetylbenzene, m.p. 185°. Ross[124] gives m.p. 186°.

Attempted synthesis of p-bis-(α-acetoxy-β-halo-ethenyl) benzene (39)

(a) p-bisChloroacetylbenzene was treated with sodium methoxide and sodium hydride as described above in method (b) for α-acetoxy-α'-chlorostilbene but using tetrahydrofuran as solvent (p-bischloroacetylbenzene is insoluble in ether). There was a vigorous evolution of

hydrogen and the solution became deep yellow. After 30 min. acetyl chloride was added, whereupon the solution lost much of its colour. Filtration and evaporation of the solvent gave a yellow oil (νmax. in chloroform 1190, 1680, 1760 cm.$^{-1}$) which would not crystallise. Chromatography on alumina resulted in decomposition on the column; use of silica gel as adsorbent failed to give any separation of the mixture although a wide variety of solvent systems was tried.

(b) p-bisBromoacetylbenzene was treated with sodium methoxide and sodium hydride in tetrahydrofuran as described above for the chloro-compound. Hydrogen was evolved and a yellow solution formed. Addition of acetyl chloride and working up as above gave a yellow oil which would not crystallise and could not be purified by chromatography.

1.2-Dibenzoyl-1,2-dibromoethane (107)

This compound was prepared, in 76% yield, by treatment of trans-dibenzoylethylene with bromine in acetic acid solution; it had m.p. 181°. The method used was that of Campaigne and Foye[125]; They give m.p. 178-179°.

1.2-Dibenzoyl-1,2-dichloroethane (117)

trans-Dibenzoylethylene (9.44 g.) in carbon

tetrachloride (200 ml.) was treated dropwise, with stirring, with a solution of chlorine (2.84 g.) in the same solvent (55 ml.). When the addition was complete the reaction mixture was stirred, at room temperature, for 10 min. Removal of the solvent by distillation and subsequent recrystallisation from chloroform - light petroleum (b.p. 40-60°) gave colourless chunky crystals (11.8 g., 70%) of 1,2-dibenzoyl-1,2-dichloroethane, m.p. 85° (Found: C, 62.21; H, 3.64. $C_{16}H_{12}Cl_2O_2$ requires C, 62.52; H, 3.94%).

Attempted synthesis of 1,4-diacetoxy-2,3-dihalo-1,4-diphenyl-butadiene

(a) 1,2-Dibenzoyl-1,2-dibromoethane was treated with sodium methoxide and sodium hydride as described above in method (b) for α-acetoxy-α'-bromostilbene; tetrahydrofuran was, however, used as solvent in place of ether. There was a vigorous evolution of hydrogen and the solution became deep yellow. After 30 min. acetyl chloride was added, whereupon the solution was decolourised. Working up in the usual way gave a yellow oil (ν max. in chloroform 1185 (w), 1680 (s), 1760 $cm.^{-1}$ (w)) which could not be crystallised. This material decomposed on an alumina column and no separation could be achieved by chromatography

on silica gel in a variety of solvent systems.

(b) 1,2-Dibenzoyl-1,2-dichloroethane was treated with sodium methoxide and sodium hydride in tetrahydrofuran as described above for the bromo-analogue. Working up in the usual way gave a yellow oil (ν max. in chloroform, 1190 (w), 1680 (s), 1755 (w) $cm.^{-1}$) which could not be crystallised. As in the case of the bromo-compound (above) purification could not be effected by chromatography.

Reaction of 1,2-dibromo-1,2-dibenzoylethane and 1,2-dichloro-1,2-dibenzoylethane with pyridine at 50° (555, 557)

The ketone (0.0005 mole.) was dissolved in "Analar" pyridine (5 ml.) and heated in a thermostat at 50° for 45 min. The deep-red solution was then poured into dilute nitric acid (2N, 50 ml.) and extracted with ether to remove uncharged halo-ketone. The halide ion present in the aqueous phase was then estimated, gravimetrically, in the usual way. The extent of reaction was then calculated by comparing the observed amount of halide ion liberated to the theoretically possible amount. The results are as set out below:

Compound	% Reaction
1,2-dibromo-1,2-dibenzoylethane	94
1,2-dichloro-1,2-dibenzoylethane	68

Preliminary Studies on the Synthesis of the Enol Phosphates of some α-Halo-ketones.

Tribenzyl phosphite (341)

This compound was prepared from phosphorus trichloride and benzyl alcohol using the method of Cramer and Voges.[94] Analysis[126] showed a purity of 87% for the crude compound. No further purification was attempted.

2,2-Dibromoacetophenone (345)

This compound was prepared by the bromination of phenacyl bromide using the method of Evans and Brooks[127]. It had m.p. 37-39°; they quote m.p. 35-36°.

Diethyl 2-bromo-1-phenylvinyl phosphate (363, 381, 645, 661, 681)

2,2-Dibromoacetophenone (13.7 g.) in anhydrous tetrahydrofuran (20 ml.) was added dropwise, with stirring, to triethyl phosphite (12.5 g.); the reaction mixture was kept at a temperature below 30° by cooling in an ice bath. On completion of the addition the reaction mixture was distilled, under reduced pressure, through a 5 cm. Fenske column packed with glass helices. Redistillation of the product gave diethyl 2-bromo-1-phenylvinyl phosphate

(10.3 g., 64%) as a colourless oil b.p. 153-154°/10^{-4} mm., η_D^{23} 1.5334 (Found: C, 42.68; H, 4.94; Br, 24.08; P, 8.87. $C_{12}H_{16}BrO_4P$ requires C, 43.00; H, 4.82; Br, 23.85; P, 9.24%), ν max. in chloroform 1280, 1630, 3490 $cm.^{-1}$; in carbon tetrachloride the band at 3490 $cm.^{-1}$ was much weaker, relative to the other bands, than in chloroform.

Stability of diethyl 2-bromo-1-phenylvinylphosphate towards ethanolysis (677)

Diethyl 2-bromo-1-phenylvinyl phosphate (3 g.) was dissolved in absolute ethanol (25 ml.) containing p-toluenesulphonic acid (1.25 g.) and boiled under reflux for 18 hrs. The resulting solution was diluted with chloroform (50 ml.) and washed with 10% sodium bicarbonate solution until free of acid. After drying over anhydrous sodium sulphate and subsequent filtration, the solvent was removed to give a yellow oil, which had an infra-red spectrum identical to that of the starting material. Recovery 98%.

Diethyl 2-bromo-1,2-dichloro-1-phenylethyl phosphate (679)

Diethyl 2-bromo-1-phenylvinyl phosphate (3 g.) in carbon tetrachloride (10 ml.) was treated with an excess of chlorine in the same solvent. Removal of the solvent and distillation under reduced pressure gave diethyl

2-bromo-1,2-dichloro-1-phenylethyl phosphate (3.5 g., 96%), b.p. 153-158°/5.4 x 10^{-4} mm., $\eta_D^{18.5}$ 1.5300. Two further distillations under reduced pressure gave the pure compound b.p. 163°/5.4 x 10^{-4} mm., $\eta_D^{18.5}$ 1.5308. (Found: P, 7.43; total halogen, 37.50. $C_{12}H_{16}BrCl_2O_4$ requires P, 7.63; total halogen, 37.13%), ν max. in chloroform 1285, 3470 $cm.^{-1}$; τ in carbon tetrachloride 8.70 (6 protons), 5.87(4 protons), 3.32 (1 proton), 2.55 (3 protons, aromatic), 2.30 (2 protons, aromatic); on dilution the N.M.R. spectrum was unchanged.

Stability of diethyl 2-bromo-1-phenylvinyl phosphate towards sodium iodide (369, 683)

Diethyl 2-bromo-1-phenylvinyl phosphate (1.16 g.) in anhydrous acetone (3 ml.) was treated with a solution of anhydrous sodium iodide (0.5 g.) in the same solvent (5 ml.). The solution was refluxed for 30 min. and then allowed to stand, at room temperature for 18 hr. Removal of the solvent and extraction with benzene gave a white solid residue (0.47 g.) which proved to be sodium iodide. The benzene extract yielded diethyl 2-bromo-1-phenylvinyl phosphate (1.0 g.) on evaporation.

The experiment was repeated under more vigorous conditions, the reaction mixture being refluxed for 18 hr.; the result was essentially the same.

Dibenzyl 2-bromo-1-phenylvinyl phosphate (353, 693)

2,2-Dibromoacetophenone (4.17 g.) in ether (10 ml.) was added dropwise, with stirring, to tribenzyl phosphite (7.29 g. of the crude material) the temperature being kept below 30°. The reaction mixture was stirred for 1 hr. at room temperature and then chromatographed (25 x 2.5 cm. column) on silica gel (B.D.H.). Development was effected with chloroform - light petroleum (b.p. 40-60°) (1:4, 250 ml.) and chloroform (250 ml.) and the enol phosphate was then eluted with ethyl acetate. Removal of the solvent gave dibenzyl 2-bromo-1-phenylvinyl phosphate (5.97 g., 87%) η_D^{24} 1.5833. (Found: C, 56.41; H, 4.40; P, 6.20. $C_{22}H_{20}BrO_4P$ requires C, 57.51; H, 4.86; P, 6.74%), ν max. in chloroform solution 1030, 1280, 1625, 3450 $cm.^{-1}$ The compound decomposed on distillation under reduced pressure.

Stability of dibenzyl 2-bromo-1-phenylvinyl phosphate towards sodium iodide. (371)

Dibenzyl 2-bromo-1-phenylvinyl phosphate (0.74 g.) was dissolved in anhyrous acetone (2 ml.) and treated with a solution of anydrous sodium iodide (0.24 g.) in the same solvent (2 ml.) and the mixture was allowed to stand at room temperature for 24 hr. A small amount (0.036 g.)

of a yellow solid was filtered off and the acetone evaporated to give a red oil (0.85 g.) which would not crystallise. Chromatography on silica gel (B.D.H.), using the system described for dibenzyl 2-bromo-1-phenylvinyl phosphate (see above) gave only this compound (0.5 g.), identified by its infra-red spectrum. In a second run, the reaction mixture was boiled under reflux for 1 hr. and then allowed to stand at room temperature for 23 hr. In this case the recovery of starting material was less (0.3 g.) but decomposition was extensive and no pure product could be isolated other than starting material.

Benzyl hydrogen 2-bromo-1-phenylvinyl phosphate (373, 695)

Dibenzyl 2-bromo-1-phenylvinyl phosphate (1.0 g.) was dissolved in methanol (20 ml.) and the solution was refluxed with decolourising charcoal for 5 min. After filtration, the solution was hydrogenated, at atmospheric pressure, using 5% palladised charcoal as catalyst. Within 25 min. the uptake of hydrogen had almost ceased and the hydrogenation was stopped (uptake of hydrogen 95 ml. at N.T.P.; calculated uptake 97.6 ml.) Filtration, and removal of the solvent under reduced pressure, gave a colourless, viscous oil which would not crystallise and decomposed on standing overnight giving a brown,

lachrymatory mass. The hydrogenation product dissolved in water to give a strongly acid solution; neutralisation of this solution with sodium carbonate, followed by addition of aqueous S-benzyl thiuronium chloride, gave a colourless crystalline derivative. Repeated recrystallisation from ethanol gave colourless plates, m.p. 179-182°, which analysed correctly for the S-benzyl thiouronium salt of benzyl hydrogen 2-bromo-1-phenylvinyl phosphate. (Found: C 51.39; H, 4.72; Br, 14.12; P, 5.82. $C_{23}H_{24}BrO_4PN_2S$ requires C, 51.59; H, 4.53; Br, 14.93; P, 5.78%), ν max. in nujol 1620, 1670 $cm.^{-1}$

Dibromopyruvic acid (357, 367, 385, 647, 659).

Redistilled pyruvic acid was brominated, in aqueous solution, using the method of Ponzio and De Paolini.[100] The product, a hydrate, was purified and dehydrated by sublimation in vacuo and repeated recrystallisation from chloroform. The pure acid had m.p. 82-86° (Found Br, 65.3. Calc. for $C_3H_2Br_2O_3$: Br, 65.0%) and the yield was 57%. The following method proved less tedious and gave the same yield. Pyruvic acid (7.1 g.) in anhydrous chloroform (100 ml.) was treated with bromine (28 g.) in the same solvent (50 ml.). After boiling under reflux for 30 hr. the solvent was evaporated to give crude

dibromopyruvic acid (20.9 g.). Repeated crystallisation from anhydrous benzene gave the pure compound (10 g., 45%), m.p. 82-86°. Subsequent runs gave yields from 50-70%.

Methyl dibromopyruvate (651, 665)

Dibromopyruvic acid (2.46 g.) in ether (100 ml.) was treated dropwise, with stirring, with a solution of diazomethane (0.42 g.) in ether (25 ml.). The resulting solution was washed with 10% sodium carbonate solution, dried over anhydrous sodium sulphate, filtered and the solvent evaporated to leave a pale yellow oil. Distillation under reduced pressure gave methyl dibromopyruvate (1.60 g., 62%), b.p. 74.75° / 6 x 10^{-3} mm., η_D^{20} 1.5221. (Found: C, 18.78; H, 1.91; Br, 62.37. $C_4H_4Br_2O_3$ requires C, 18.56; H, 1.55; Br, 61.47), ν max. in chloroform 1740, 1765, 3540 $cm.^{-1}$

Benzyl dibromopyruvate (649)

Dibromopyruvic acid (2.46 g.) in ether (30 ml.) was treated with a solution of phenyldiazomethane (1.18 g.) (prepared by the method of Overberger and Anselme[126]) in ether (50 ml.). The reaction mixture was washed with 10% sodium bicarbonate solution and then water, dried over anhydrous sodium sulphate, filtered and the solvent

evaporated off to leave a colourless crystalline solid. Recrystallisation from benzene gave colourless needles (2.42 g., 72%) of benzyl dibromopyruvate monohydrate, m.p. 103° (softens at 85°). (Found: C, 34.10; H, 2.97; Br, 45.21; O, 18.10. $C_{10}H_{10}Br_2O_4$ requires C, 33.94; H, 2.85; Br, 45.15; O, 18.08%), ν max. in chloroform 1745, 3250, 3550 cm.$^{-1}$. The ester did not appear to lose water on heating at 60° in vacuo.

Reaction of dibromopyruvic acid with triethyl phosphite (393)

Triethyl phosphite (2.49 g.) was stirred and treated, dropwise, with dibromopyruvic acid (2.46 g.) in ether (5 ml.), the temperature being kept between 0° and 10°. After completion of the addition the reaction mixture was allowed to stand overnight at room temperature. The solvent and low-boiling materials were removed by distillation ($<60°$) under reduced pressure leaving a colourless oil. This material was poured into sodium bicarbonate solution (25 ml. 5%) and extracted with ether (3 x 10 ml.). The aqueous phase was then covered with ether (20 ml.) and acidified, at 0°, with a solution of concentrated hydrochloric acid (3 ml.) in water (10 ml.) The aqueous phase was extracted repeatedly with ether and the combined

extracts were dried over anhydrous magnesium sulphate, filtered and evaporated down to give a colourless oil (1.4 g.) ν max. in chloroform 1030, 1162, 1630, 1722 cm.$^{-1}$ (Found: Br, 11.86; P, 13.39. Calc. for $C_7H_{12}BrO_6P$: Br, 26.37; P, 10.23%). The material could not be crystallised.

Reaction of dibromopyruvic acid with tribenzyl phosphite (389)

Tribenzyl phosphite (13.2 g.) was stirred and treated dropwise with dibromopyruvic acid (4.92 g.) in ether (10 ml.) the temperature being kept between 0° and 10°. The reaction mixture was stirred at room temperature for 30 min. and then poured into an ice-cold solution of sodium bicarbonate (1.8 g.) in water (20 ml.). The product was then isolated as described above for the diethyl ester giving a colourless oil (1.32 g.). (Found: Br, 11.91; P, 7.89. Calc. for $C_{17}H_{16}BrO_6P$: Br, 18.70; P, 7.25). The material could not be crystallised.

Diethyl 2-bromo-1-methoxycarbonylvinyl phosphate (669)

Triethyl phosphite (2.49 g.) was stirred and treated dropwise with a solution of methyl dibromopyruvate (2.60 g.) in ether (10 ml.) the temperature being kept

between 0 and 10°. After completion of the addition, the reaction mixture was stirred at room temperature for 30 min., washed with 5% sodium bicarbonate solution and water, dried over anhydrous sodium sulphate, filtered and distilled under reduced pressure giving a colourless oil (21 g., 66%), identified as diethyl 2-bromo-1-methoxycarbonylvinyl phosphate, b.p. 107-110°/5.4 x 10^{-4} mm., η_D^{20} 1.4740 (Found: C, 29.88; H, 4.65; Br, 26.24; P, 8.71. $C_8H_{14}BrO_6P$ requires C, 30.30; H, 4.46; Br, 25.21; P, 9.77%), ν max. in chloroform 1035, 1280, 1322, 1625, 1740, 3470 $cm.^{-1}$. It has been observed previously[101] that analyses of halogen-containing phosphates are sometimes poor.

Reaction of methyl dibromopyruvate with tribenzyl phosphite (691)

Tribenzyl phosphite (2.33 g.) was treated dropwise, with stirring, with a solution of methyl dibromopyruvate (1.0 g.) in ether (5 ml.), the temperature being kept between 0 and 10°. After completion of the addition, stirring was continued, at room temperature, for 30 min. The reaction mixture was chromatographed on a column (25 x 2 cm.) of silica gel (B.D.H.). The chromatogram was developed with chloroform - light petroleum (b.p. 40-60°)

(1:4, 100 ml.) and the enol phosphate was then eluted with ethyl acetate. After removal of the solvent there remained a colourless, viscous oil (1.66 g., 98%), ν max. in chloroform 1030, 1288, 1620, 1730 $cm.^{-1}$. (Found: Br, 14.64. Calc. for $C_{18}H_{18}BrO_6P$: Br, 18.12%). The material could not be crystallised or distilled.

Some Other Halides of Biological Interest.

Ethyl α-bromophenylacetate (343)

This compound was prepared by bromination and esterification of phenylacetic acid, using the method of Rodionov *et al.*[129] It had b.p. 110-120°/0.45 mm., $n_D^{21.5}$ 1.5372; they quote b.p. 103-4°/2 mm., n_D^{20} 1.5395.

Ethyl α-bromophenylorthoacetate (347, 349)

Ethyl phenylorthoacetate was prepared from benzyl cyanide, by the action of ethanolic hydrochloric acid, using the method of McElvain and Stevens[103]. The crude, dry ester (70 g.) was dissolved in a mixture of pyridine (23.3 g.) and carbon tetrachloride (115 ml.), cooled to 0° and bromine (47 g.) was added dropwise, with stirring. On completion of the addition the reaction mixture was allowed to warm to room temperature. After filtration to remove pyridine hydrobromide, the solvent was evaporated off and the residue distilled under reduced pressure to give a colourless oil (60 g., 65%) identified as ethyl α-bromophenylorthoacetate, b.p. 94-5°/1.4 x 10^{-4}mm., n_D^{25} 1.5080. (Found: C, 52.05; H, 6.65. $C_{14}H_{21}BrO_3$ Requires C, 52.98; H, 6.68%). Further purification could not be effected by distillation; the compound would not crystallise.

Ethyl α-bromoorthoacetate

This compound was prepared by bromination of ethyl orthoacetate, in pyridine, employing the method of Beyerstedt and McElvain.[102]

p-(Bromomethyl)benzamide (361)

A mixture of p-bromomethylbenzoyl chloride and p-bromomethylbenzoyl bromide was prepared using the method of Titley[130]. Addition of an acetone solution of this mixture to an ice-cold solution of ammonia (20%) gave the amide in 80% yield. Crystallisation from methanol gave colourless plates of p-(bromomethyl)benzamide, m.p. 180-185° (Found: C, 45.67; H, 4.15. C_8H_8BrNO requires C, 44.85; H, 3.77 %). The compound could not be further purified.

p-(Bromomethyl)benzene sulphonamide (257)

p-Toluenesulphonyl chloride was brominated and converted to the amide by the method of Klarer.[131]

2-Bromo-4'-(chlorosulphonyl)acetophenone (259, 261, 263)

4'-Aminoacetophenone was diazotised and converted to 4,4'-diacetyldiphenyldisulphide. The disulphide was cleaved, by treatment with chlorine in aqueous acetic acid, to give 4'(chlorosulphonyl)acetophenone; the overall

method is that of Burton and Hu.[132] 4'-(Chlorosulphonyl)-acetophenone (2.0 g.) was dissolved in carbon tetrachloride (20 ml.). The solution was illuminated, and warmed by a 500W tungsten lamp whilst a solution of bromine (1.46 g.) in carbon tetrachloride (10 ml.) was added dropwise, with stirring. It was found necessary to add benzoyl peroxide (ca. 5 mg.) to initiate the reaction, which then proceeded to completion within 10 min. Removal of the solvent by evaporation gave a brown crystalline mass. Recrystallisation from chloroform - light petroleum (b.p. 60-80°) gave colourless needles (2.3 g., 85%) of 2-bromo-4'-(chlorosulphonyl)acetophenone, m.p. 98°. (Found: C, 32.46; H, 2.25; S, 11.1; total halogen 38.97. $C_8H_6BrClO_3S$ requires C, 32.28; H, 2.04; Cl, 11.92; Br, 26.85; S, 10.78%), ν max. in chloroform 1180, 1390, 1690 cm.$^{-1}$

Attempted preparation of 2-bromo-4'-sulphonamidoacetophenone (265, 267)

(a) 2-Bromo-4'-(chlorosulphonyl)acetophenone (0.6 g.) in chloroform (20 ml.) was shaken with a solution of ammonium carbonate (0.16 g.) in water (10 ml.) for 10 min. at room temperature. The layers were separated and the aqueous phase extracted with ether (2 x 10 ml.). The combined organic layers, after drying over sodium

sulphate, and filtration, were evaporated to dryness to give 2-bromo-4'-(chlorosulphonyl)acetophenone (0.59 g., 98% recovery). The experiment was repeated using a reaction time of 4 hr. Starting material (78%) was again recovered.

(b) A solution of ammonium carbonate (0.48 g.) in water (15 ml.) was treated dropwise, with stirring, with a solution of 2-bromo-4'-(chlorosulphonyl)acetophenone (0.6 g.) in acetone (10 ml.). The solution was then diluted with water and extracted with chloroform. The extract was dried over sodium sulphate, filtered and evaporated to yield a yellow gum (0.01 g.) which would not crystallise. The experiment was repeated keeping the reaction temperature at 0°; the result was unchanged.

4,4'-Di(diazoacetyl)diphenyldisulphide (653)

Diphenyldisulphide-4,4'-dicarboxylic acid was prepared from p-aminobenzoic acid by the method of Baker et al.[133]; it had m.p. 320-325°, Baker gives m.p. 320°. The diacid (3.06 g.) was boiled, under reflux, with redistilled thionyl chloride (10 ml.) for 1 hr. The excess thionyl chloride was then distilled off, under reduced pressure, to leave the crude diacid chloride. This material was taken up in dry ether (100 ml.) and added dropwise, with stirring, to a solution of diazomethane

(1.8 g.) in dry ether (120 ml.). The resulting solution was allowed to stand overnight. The pale yellow precipitate that formed was filtered off. Precipitation from chloroform with light petroleum (b.p. 40-60°) gave an amorphous yellow solid (1.35 g., 35%) that analysed fairly well for 4,4'-di(diazoacetyl)diphenyl disulphide, m.p. 147-153°. (Found: C, 55.35; H, 3.04; N, 15.35; S, 18.33 $C_{16}H_{10}N_4S_2O_2$ requires C, 54.24; H, 2.85; N, 15.73; S, 18.06%). The compound could not be further purified.

4,4'-Di(chloroacetyl)diphenyl disulphide (337, 655)

4,4'-Di(diazoacetyl)diphenyl disulphide (1.0 g.) was suspended in ether (25 ml.). The well-stirred suspension was treated with concentrated hydrochloric acid (2 ml.) and stirring was then continued for 1 hour. The ether-insoluble product was extracted into chloroform (leaving a small amount of insoluble material), dried over calcium chloride, filtered and the chloroform removed by distillation to leave an oil which crystallised on cooling giving a pale yellow solid (0.8 g., 76%). Fractional precipitation of this material from chloroform with light petroleum (b.p. 40-60°) gave yellow crystalline granules of 4,4'-di(chloroacetyl)diphenyl disulphide, m.p. 131-134°. (Found: C, 51.52; H, 3.29; Cl, 19.16. $C_{16}H_{12}Cl_2O_2S_2$

requires C, 51.78; H, 3.26; Cl, 19.10 %).

2-Chloro-4'-(chlorosulphonyl)acetophenone (667)

4,4'-Di(chloroacetyl)diphenyl disulphide (0.2 g.) in chloroform (10 ml.) was treated with a solution of chlorine (0.2 g.) in carbon tetrachloride (10 ml.). Water (5 ml.) was then added and the mixture shaken vigorously, at room temperature, for 15 minutes. The organic phase was separated off, dried over calcium chloride, filtered and the solvent evaporated off to leave a yellow oil (0.27 g.) that crystallised on standing. Crystallisation from light petroleum (b.p. 60-80°) (in which the disulphide is insoluble) and then chloroform - light petroleum (b.p. 60-80°) (1:2) gave fine colourless needles of 2-chloro-4'-(chlorosulphonyl)acetophenone, m.p. 88°. (Found: Cl, 28.49; S, 13.19. $C_8H_6Cl_2O_3S$ requires Cl, 28.03; S, 12.67 %), ν max. in chloroform 1180, 1390, 1695 $cm.^{-1}$

2-Chloro-4'-sulphonamidoacetophenone (671)

A solution of 2-chloro-4'-chlorosulphonylacetophenone (0.31 g.) in acetone (1.5 ml.) was cooled to 0° and treated dropwise, with vigorous stirring, with a solution of ammonia (0.042 g.) in water (1 ml.). The resulting solution was stirred, at room temperature, until

it showed a neutral reaction to phenolphthalein (ca. 5 min.). The reaction mixture was diluted with water (2 ml.) and extracted into chloroform (50 ml.). The extract was dried over calcium chloride, filtered and the solvent evaporated to give a white, crystalline solid (0.09 g.) m.p. 120-140°. Precipitation of this material, from chloroform solution, with light petroleum (b.p. 60-80°) gave a slightly-yellow, crystalline powder m.p. 141-145°, which was mainly 2-chloro-4'-sulphonamidoacetophenone. (Found: C, 40.95; H, 3.14; Cl, 16.25; N, 4.87. $C_8H_8ClNSO_3$ requires C, 41.12; H, 3.46; Cl, 15.18; N, 6.00 %), ν max. in chloroform 1170, 1360, 3380, 3480 $cm.^{-1}$ The compound could not be further purified.

The Interaction of 2-Halo-2-phenylacetophenones with Pyridine.

(a) Using 90% ethanol - 10% water as solvent

(i) 2-Bromo-2-phenylacetophenone (469)

2-Bromo-2-phenylacetophenone (0.3438 g.) was treated with a solution of pyridine (0.25 M., 100 ml.) in 10% aqueous ethanol and the resulting solution was heated at 50° for 24 hr., under a blanket of nitrogen. Removal of the solvent by evaporation under reduced pressure gave a yellow syrup (0.3933 g.) which would not crystallise, but gave a positive test for ionic halogen. The syrup was dissolved in water (10 ml.) and extracted with ether (10 ml.). The ether extract was resolved into acidic, basic and neutral components, in the usual way, yielding benzoic acid (0.01 g.) m.p. and mixed m.p. 122° and traces of pyridine and ethyl benzoate respectively. The aqueous extract was evaporated to dryness, under reduced pressure to give a colourless syrup (0.309 g.) which would not crystallise even after extensive drying in vacuo. ν max. in chloroform 1580 (s), 1600 (s), 1630 (s), 1685 (s), 2450 (m) and 3400 $cm.^{-1}$ (m). Treatment of the syrup with a cold, saturated solution of picric acid in methanol gave a yellow crystalline derivative, m.p. 183° after recrystallisation from methanol. This compound was later identified as desyl pyridinium picrate (see below).

(ii) 2-Chloro-2-phenylacetophenone (473)

2-Chloro-2-phenylacetophenone (0.5768 g.) was treated with a solution of pyridine (0.5 M., 50 ml.) in 10% aqueous ethanol. The resulting solution was heated, in sealed tubes, at 100° for 16 hrs. The products were worked up in the same way as those of the bromo-compound (see above). Small amounts of benzoic acid and ethyl benzate were again obtained. The main product was a water-soluble, colourless syrup (0.54 g.) which could not be crystallised. ν max. in chloroform 1580 (s), 1595 (s), 1630 (s), 1685 (s), 2450 (s), 3370 $cm.^{-1}$ (s). Treatment of the syrup with picric acid (see above) again gave desyl pyridinium picrate, m.p. 183° (see below).

(b) Using anhydrous benzene as solvent

(i) 2-Bromo-2-phenylacetophenone (505, 529, 535, 549)

2-Bromo-2-phenylacetophenone (5.5 g.), pyridine (1.54 g.) and anhydrous benzene (25 ml.) were heated together at 50° for 24 hr. The white, crystalline precipitate (6.8 g., 97%) was filtered off; it had m.p. 120 - 123° and analysed correctly for desyl pyridinium bromide having one molecule of benzene of crystallisation. (Found: C, 70.06; H, 4.35; Br, 18.49. $C_{25}H_{22}BrNO$ requires C, 69.73; H, 4.69; Br, 18.58%) ν max., in chloroform,

1580 (s), 1600 (s), 1630 (s), 1685 (s), 2450 (m) and 3400 $cm.^{-1}$. τ, in deuterochloroform, 0.31, 0.49, 1.30, 1.84, 2.23. The mass spectrum showed no bromine-containing peaks. The compound was water soluble giving a solution containing ionic bromide; treatment of this solution with sodium hydroxide gave an orange-yellow colouration, whilst treatment with a saturated solution of picric acid in methanol gave a yellow, crystalline solid which, on recrystallisation from methanol, gave short yellow prisms of desyl pyridinium picrate, m.p. 183°. (Found: C, 59.91; H, 3.68; N, 11.45. $C_{25}H_{18}N_4O_8$ requires C, 59.74; H, 3.62; N, 11.16 %).

(ii) 2-Chloro-2-phenylacetophenone (499, 503)

2-Chloro-2-phenylacetophenone (1.15 g.) was dissolved in a solution of pyridine (0.5 M., 10 ml.) in anhydrous benzene and the resulting solution was heated at 100°, in a sealed tube, for 24 hours. On cooling a yellow glass (1.10 g.) was obtained. Trituration with dry ether gave a colourless, crystalline solid which was extremely hygroscopic and could not be recrystallised. It had a diffuse melting point, 80-100°, but analysis showed it to be essentially desyl pyridinium chloride. (Found: C, 72.02; H, 5.86; $C_{19}H_{16}ClNO$ requires C, 73.65; H, 5.22%), ν max.

in chloroform, 1580 (s), 1585 (s), 1630 (s), 1685 (s), 2450 (s) 3370 (s) $cm.^{-1}$; ν max. in nujol 604 (m), 621 (s), 680 (s), 741 (s), 704 (s), 758 (s), 769 (s) $cm.^{-1}$; τ in deuterochloroform 0.25, 0.48, 1.44, 1.86, 2.30. The compound gave an intense orange colour with aqueous sodium hydroxide and was water soluble, giving a solution containing ionic chloride. Treatment with a cold, saturated solution of picric acid in methanol gave desyl pyridinium picrate, m.p. 183° (see above).

Interaction of 2-Aryl-2-haloacetophenones with Pyridine and Aniline - Kinetic Results

Two stock solutions were prepared and aliquots of these solutions were used throughout the following series of experiments (unless otherwise stated).

Solution 'A' 90% "super dry" alcohol, 10% distilled water } by volume

Solution 'B' a 0.5 M. solution of dried, redistilled, "Analar" pyridine in the above solvent.

The glassware employed was semi-micro grade 'B' and was not re-calibrated; the same glassware was used throughout to minimise errors.

1. Interaction of 2-aryl-2-bromoacetophenones with pyridine. General Method (437)

The bromo-ketone (0.00125 mole) was dissolved in solution 'A' (25 ml.) contained in a 100 ml., three-necked flask fitted with a mechanical stirrer and reflux condenser and immersed in a thermostat maintained at 50 ± 0.2°. Solution 'B' (25 ml.), preheated to the thermostat temperature, was then added and a stop watch started. At regular intervals a 5 ml. aliquot of the reaction mixture was pipetted into a solution of silver nitrate (0.025 N, 5 or

10 ml.) to which had been added benzene (2 ml., to dissolve organic materials), saturated ferric alum solution (0.5 ml.) and nitric acid (2 N., 3 ml.). The excess of silver nitrate present was then determined by titration with ammonium thiocyanate solution (0.025 N.).

Results.

The pseudo-unimolecular rate constant, k, was calculated from the equation:

$$k = \frac{1}{t} \ln \frac{a}{a-x}$$

where t = time in minutes

a = ml. of 0.025 N. silver nitrate for total ionic halide

x = ml. of 0.025 N. silver nitrate for ionic halide at time t.

With the exception of 2-bromo-2-(m-chlorophenyl)-acetophenone all the ketones investigated were analytically pure. "Infinity" values were obtained by titration when the reaction time reached a value equivalent to ten times the half-life; they agreed with the calculated value (5.00 ml. of silver nitrate) to within about ± 2%. Only the results for 2-bromo-2-phenylacetophenone are set out in full in the table below. The mean rate constants for the

remaining compounds are summarised in the table which follows this.

2-Bromo-2-phenylacetophenone (443)

Weight of 2-bromo-2-phenylacetophenone used = 0.3438 g.

Time (min.)	ml. $AgNO_3$ for Br^-	(a-x)	$10^4 k$ ($min.^{-1}$)
15	1.40	3.70	204
30	2.43	2.67	215
51	3.39	1.71	214
60	3.67	1.43	212
76	4.10	1.00	214
90	4.34	0.76	211
00	5.10	-	-

mean $k = 212 \times 10^{-4}$ $min.^{-1}$ at 50°

Summary of the mean rate constants at 50° for compounds of the type R.C_6H_4.CHBr.CO.Ph. (443, 445, 447, 487, 527, 561, 565)

R	$10^4 k$ ($min.^{-1}$)
H	212
o-Cl	60
m-Cl	88
p-Cl	158
o-NO_2	17
m-NO_2	98
p-NO_2	117

2. Interaction of 2-aryl-2-chloroacetophenones with pyridine. General Method (477)

The chloro-ketone (0.00125 mole) was dissolved in a mixture of solution A (25 ml.) and solution B (25 ml.). 5 ml. aliquots of this solution were sealed into "Pyrex" ampoules and heated, in a thermostatic oil bath at 100 ± 0.25°. At regular intervals an ampoule was withdrawn, cooled rapidly and the ionic halide was then determined as outlined above for the bromo-compounds.

Results

The pseudo-unimolecular rate constants were calculated as for the bromo-compounds (see above). In all cases the ketones investigated were analytically pure. "Infinity" values were obtained by titration when the reaction time reached a value equivalent to ten times the half-life; in some cases this value differed considerably from the calculated value (5.00 ml. of silver nitrate) and for this reason the results are set out in full below.

2-Chloro-2-phenylacetophenone (477)

Weight of 2-chloro-2-phenylacetophenone used = 0.2897 g.

Time (min.)	ml. $AgNO_3$ for Cl^-	(a-x)	$10^4 k$ ($min.^{-1}$)
15	0.47	4.60	64.7
30	0.88	4.19	63.5
60	1.65	3.42	65.5
90	2.27	2.80	65.9
120	2.78	2.29	66.2
180	3.59	1.48	68.4
242	4.20	0.87	72.8
∞	5.07	-	-

mean k = 67×10^{-4} $min.^{-1}$ at 100°

2-Chloro-2-(o-chlorophenyl)acetophenone (559)

Weight of 2-chloro-2-(o-chlorophenyl)acetophenone used = 0.3315 g.

Time (min)	ml. $AgNO_3$ for Cl^-	(a-x)	$10^4 k$ ($min.^{-1}$)
30	0.52	4.10	39.8
60	1.06	3.56	43.5
90	1.43	3.19	41.1
135	2.00	2.62	42.0
195	2.53	2.09	40.7
255	3.10	1.52	43.6
∞	4.62	-	-

mean k = 42×10^{-4} $min.^{-1}$ at 100°

2-Chloro-2-(m-chlorophenyl)acetophenone (485)

Weight of 2-chloro-2-(m-chlorophenyl)acetophenone used = 0.3315 g.

Time (min.)	ml. $AgNO_3$ for Cl^-	(a-x)	10^4k ($min.^{-1}$)
30	0.68	3.51	59.2
60	1.25	2.94	59.0
80	1.60	2.59	60.6
102	1.89	2.30	59.9
130	2.35	1.84	63.3
155	2.57	1.62	61.3
183	2.80	1.39	60.3
∞	4.19	-	-

mean k = 61 x 10^{-4} $min.^{-1}$ at 100°

2-Chloro-2-(p-chlorophenyl)acetophenone (563)

Weight of 2-chloro-2-(p-chlorophenyl)acetophenone used = 0.3315 g.

Time (min.)	ml. $AgNO_3$ for Cl^-	(a-x)	10^4k ($min.^{-1}$)
25	1.23	3.67	115
45	1.97	2.93	115
70	2.68	2.22	113
95	3.92	0.98	115
125	4.29	0.61	113
∞	4.90	-	-

mean k = 114 x 10^{-4} $min.^{-1}$ at 100°

3. Interaction of 2-aryl-2-bromoacetophenones with aniline

General method (517)

The method used was essentially the same as that for the interaction of these compounds with pyridine (see (i) above); the pyridine solution (solution B) was replaced with a solution of aniline (0.25 M.) in solution A. The aniline was initially purified by repeated distillation, at atmospheric pressure, from zinc dust. The reactions were carried out at 50°.

Results.

The pseudo-unimolecular rate constants were calculated in the usual way (see above). All of the bromoketones used were analytically pure. The rate constants showed some variation within each run and consequently, the results are recorded in full below. All reactions went to completion.

2-Bromo-2-phenylacetophenone (517)

Weight of 2-bromo-2-phenylacetophenone used = 0.3441 g.

Time (mins)	ml. $AgNO_3$ for Br^-	(a-x)	10^4k ($min.^{-1}$)
10	1.68	3.38	404
20	2.72	2.34	386
30	3.38	1.68	368
45	4.00	1.06	347
60	4.36	0.70	330
75	4.58	0.48	314
105	4.74	0.32	263
∞	5.06	-	-

2-Bromo-2-(o-chlorophenyl)acetophenone (519)

Weight of 2-bromo-2-(o-chlorophenyl)acetophenone used = 0.3871 g.

Time (mins)	ml. $AgNO_3$ for Br^-	(a-x)	10^4k ($min.^{-1}$)
15	1.00	4.18	143
45	2.38	2.80	137
75	3.26	1.92	132
105	3.86	1.32	130
135	4.22	0.96	125
165	4.50	0.68	123
∞	5.18	-	-

2-Bromo-2-(m-chlorophenyl)acetophenone (523)

Weight of 2-bromo-2-(m-chlorophenyl)acetophenone used = 0.3871 g.

Time (mins)	ml. $AgNO_3$ for Br^-	(a-x)	10^4k ($min.^{-1}$)
6	1.22	3.78	465
12	2.04	2.96	438
18	2.68	2.32	428
24	3.18	1.82	421
30	3.52	1.48	406
40	3.96	1.04	393
50	4.30	0.70	393
∞	5.00	-	-

2-Bromo-2-(p-chlorophenyl)acetophenone (521)

Weight of 2-bromo-2-(p-chlorophenyl)acetophenone used = 0.3871 g.

Time (mins)	ml. $AgNO_3$ for Br^-	(a-x)	10^4k ($min.^{-1}$)
9	2.70	2.28	868
12	2.98	2.00	760
19	3.76	1.22	740
25	4.02	0.96	658
35	4.38	0.60	605
45	4.64	0.34	596
∞	4.98	-	-

2-Bromo-2-(p-nitrophenyl)acetophenone (525)

Weight of 2-bromo-2-(p-nitrophenyl)acetophenone used = 0.4003 g.

Time (mins)	ml. $AgNO_3$ for Br^-	(a-x)	10^4k ($min.^{-1}$)
15	2.30	2.70	411
25	3.10	1.90	388
35	3.62	1.38	367
45	4.00	1.00	357
60	4.26	0.74	318
75	4.46	0.54	297
∞	5.00	-	-

Interaction of α-Acetoxy-α'-halostilbene with Pyridine.

General method (539)

The method outlined above for the 2-aryl-2-chloroacetophenones was used for both α-acetoxy-α'-bromostilbene and α-acetoxy-α'-chlorostilbene. The "infinity" values were not measured experimentally because of the slowness of the reaction; the values given were obtained by calculation. The temperature used was 100°. The results are given in full below.

α-Acetoxy-α'-bromostilbene (541)

Weight of α-acetoxy-α'-bromostilbene used = 0.4365 g.

Time (mins)	ml. $AgNO_3$ for Br^-	(a-x)	$10^4 k$ ($min.^{-1}$)
285	0.29	4.71	2.1
1315	1.03	3.97	1.7
4055	2.63	2.37	1.8
5760	3.16	1.84	1.7
8590	4.07	0.93	2.0
∞ (calc.)	5.00	-	-

mean k = 1.9×10^{-4} $min.^{-1}$ at 100^0.

α-Acetoxy-α'-chlorostilbene (539)

Weight of α-acetoxy-α'-chlorostilbene used = 0.3810 g.

Time (mins)	ml. $AgNO_3$ for Cl^-	(a-x)	$10^4 k$ ($min.^{-1}$)
360	0.19	5.41	0.95
1260	0.68	4.92	1.03
5580	2.78	2.82	1.23
10140	3.06	2.54	0.78
∞ (calc.)	5.60	-	-

mean k = 1.0×10^{-4} $min.^{-1}$ at 100^0

BIOLOGICAL RESULTS

BIOLOGICAL RESULTS

Some of the compounds described above were tested for toxicity and anti-tumour activity by Professor J.F. Danielli and his associates at New York State University, Buffalo, U.S.A.

Test Conditions

Toxicity determinations were carried out using male Holtzmann rats and male Swiss mice. All deaths within a 21 day period were recorded and approximate LD_{50} values were estimated graphically from per cent mortality / log dose plots.

Tumour inhibition studies were made on male Holtzmann rats carrying a Walker 256 carcinosarcoma. The ratio of the mean weight of treated tumours to the mean weight of control tumours (T/C) was determined as a measure of the cytotoxicity of the drug. The results for the various compounds tested are summarised in Table I below.

Table I

L.I.C.No.	Compound	Approx. LD_{50}*		T/C at LD_{50}
		Mouse	Rat	
41	2-chloro-2-phenyl-acetophenone	>1000	>400	not act.
42	α-acetoxy-α'-chlorostilbene	>1000	>400	not act.
43	2-bromo-2-phenyl-acetophenone	50	15	0.9
44	α-acetoxy-α'-bromostilbene	150	90	0.95
45	2-bromo-2-(p-nitrophenyl)acetophenone	>1000	180	0.5
46	α'-acetoxy-α-bromo-4-nitrostilbene	750	250	0.6
47	2-bromo-2-(o-chlorophenyl)-acetophenone	125	50	1.05
48	α'-acetoxy-α-bromo-2-chlorostilbene	225	>50	not act.
51	2-bromo-2-(p-chlorophenyl)-acetophenone	150	50	0.19
52	α'-acetoxy-α-bromo-4-chlorostilbene	150	ca.80	0.72
49	1,2-dichloro-1,2-dibenzoylethane	120	25	0.4
50	1,2-dibromo-1,2-dibenzoylethane	1350	>400	0.92
55	p-(bromomethyl)-benzenesulphonamide	40	25	0.28

continued ....

Table I (cont.)

L.I.C.No.	Compound	Approx. LD_{50}*		T/C at LD_{50}
		Mouse	Rat	
62	2-chloro-4'-sulphonamidoacetophenone	20	> 8	0.3**
60	p-(bromomethyl)-benzamide	20	9.5	0.7***
56	ethyl α-bromophenylacetate	280	75	0.83
57	ethyl α-bromophenylorthoacetate	>1000	450	0.7
58	ethyl bromoacetate	40	30	0.2
59	ethyl bromoorthoacetate	350	-	-
63	ethyl 2-bromo-1-phenylvinyl phosphate	320	> 100	not act.
64	ethyl 2-bromo-1-methoxycarbonylvinyl phosphate	75	80	0.7

*expressed as mg. per Kg. body weight.

**measured at 8 mg./Kg.

***measured at 4.75 mg./Kg.

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