

THE BIOSYNTHESIS OF ERYTHRINA ALKALOIDS

a thesis presented by

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4th January, 1974.

To Elena whose courage and moral support lightened the bitter moments and sustained me to the end; and to Ruben, Romer and Ricardo whose affection and interruptions smoothed the difficult path.

"NADA IGUALA EN CALIDAD A LA
SATISFACCION DEL DEBER CUMPLIDO"

R.D.

" Nothing renders more satisfaction
than having fulfilled your duty"

ABSTRACT

Erysodienone was synthesised from *o*-benzylisovanillin in a new seven-step procedure in 12% overall yield.

The racemic dienone was resolved with 3-bromocamphor-9-sulfonic acid. The absolute stereochemistry of the enantiomers was established by relating them to natural dihydroerysodine and erythratidinone. Feeding experiments showed that (-)-erysodienone acted as an efficient precursor for erythraline and α -/ β -erythroidine in *E. crista galli* and *E. berteroana* respectively.

5,6,8,9-Tetrahydro-2,12-dimethoxy-7H-dibenz[d,f]azonine-3,11-diol was established as potentially the symmetrised intermediate in the inversion of chirality from *N*-norprotosinomenine to (5*S*)-erysodienone.

ACKNOWLEDGEMENTS

The author wishes to express his grateful thanks to Professor Sir Derek Barton, F.R.S., for the privilege and opportunity of working under his supervision and for the invaluable benefit and experience obtained from his guidance.

Dr. D.A. Widdowson is thanked for his help, advise and encouragement given throughout this work and also for reading the proofs of this thesis.

To all my colleagues, past and present in the Hofmann Laboratory, especially Dr. John Corrie, Dr. J.N. Shah and Mr. A.A.L. Gunatilaka, for their useful discussions and their congenial company.

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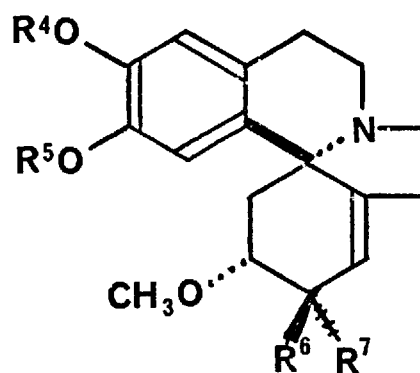
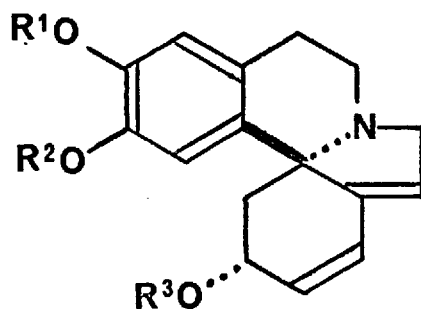
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INTRODUCTION

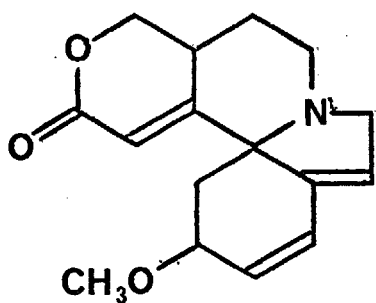
Erythrina alkaloids have been extensively studied for almost four decades¹⁻⁷. The aims of these studies have not always been the same. Thus during the first ten years (1937-1947), the principal target was the isolation and characterization of the largest possible number of alkaloids from the different species of the genus Erythrina. The reason for this approach lay in the curare-like physiological action of the compounds⁸⁻¹⁰.

In the course of the next decade, the isolation and characterization of the members of this family was essentially complete⁷. After 1949, the chemistry of the Erythrina alkaloids was mainly concerned with the elucidation of the structures of the alkaloids encountered¹¹⁻¹⁴. The extensive studies on degradation which were carried out led to structures for all the Erythrina alkaloids, which are now generally accepted (Table 1). The third stage was mainly characterized by the intensive work in the field of synthesis, as final confirmation of the Erythrina structures proposed. In the course of the last decade, due recognition has been given to conformational analysis and the assignment of absolute configuration in the natural products field and their relevance to biosynthetic considerations. Thus, recent Erythrina studies have been devoted mainly to stereochemical problems and to the establishment of biosynthetic pathways²⁴⁻²⁶.

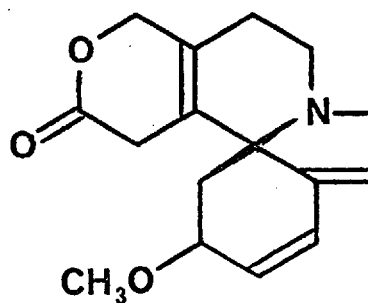
TABLE I
ERYTHRINA ALKALOIDS



NAME	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇
Erythraline ¹⁵ (1)	-CH ₂ -		CH ₃				
Erysodine ¹⁵ (2)	H	CH ₃	CH ₃				
Erysovine ¹⁵ (4)	CH ₃	H	CH ₃				
Erysopine ¹⁵ (5)	H	H	CH ₃				
Erysonine ¹⁶ (6)	H	CH ₃	H				
Erythramine ¹⁷ (7)				-CH ₂ -		H	H
Dihydroerysodine ¹⁸ (8)				H	CH ₃	H	H
Erythratine ^{19,20} (9)				-CH ₂ -		OH	H
Erythratinone ²⁰ (10)				-CH ₂ -		-O-	
Erythratidine ²¹ (11)				CH ₃	CH ₃	H	OH
α-Erythroidine ^{22,23} (12)							
β-Erythroidine ^{22,23} (13)							



(12)



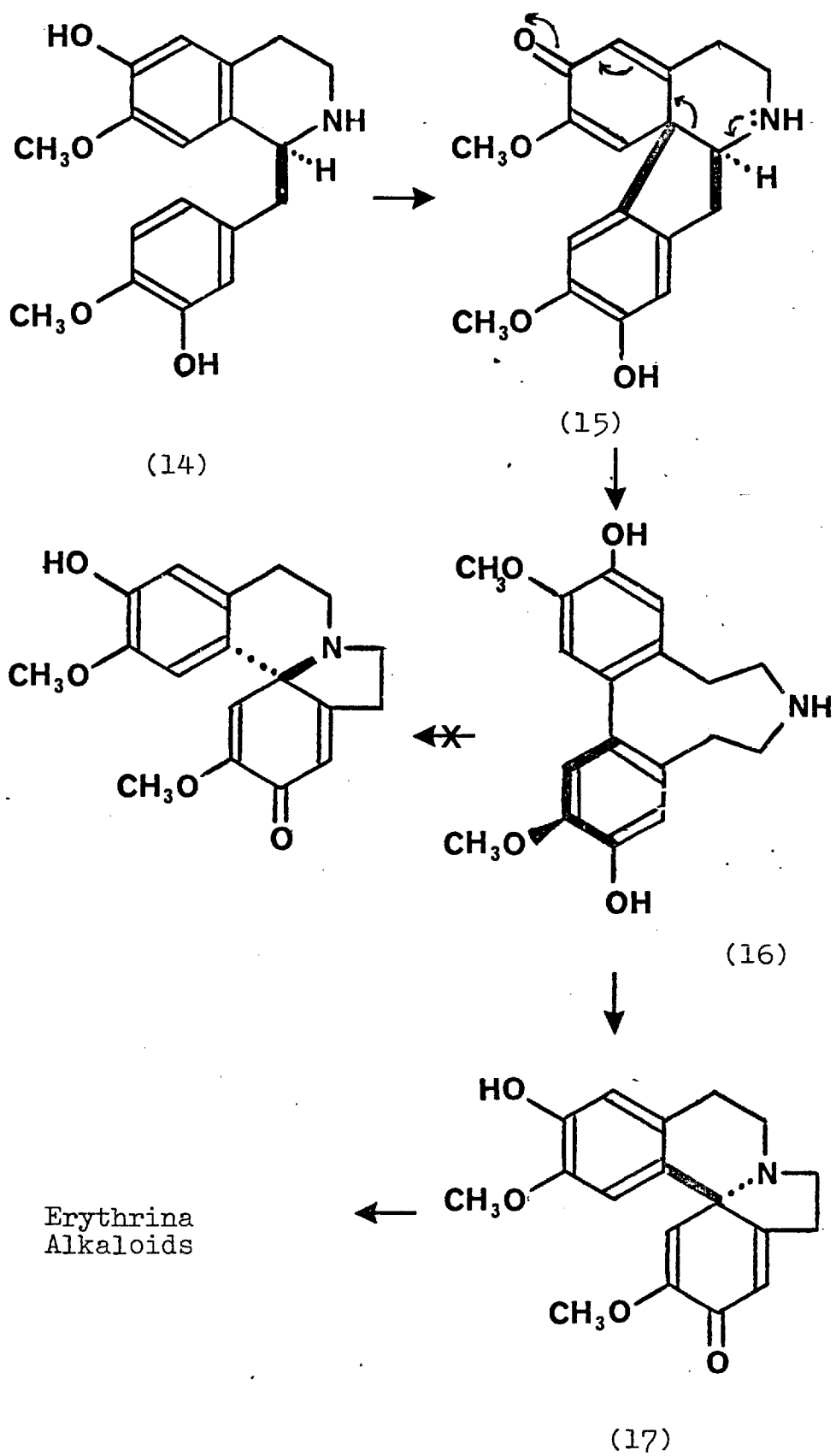
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BIOSYNTHESIS OF THE AROMATIC ERYTHRINA ALKALOIDS

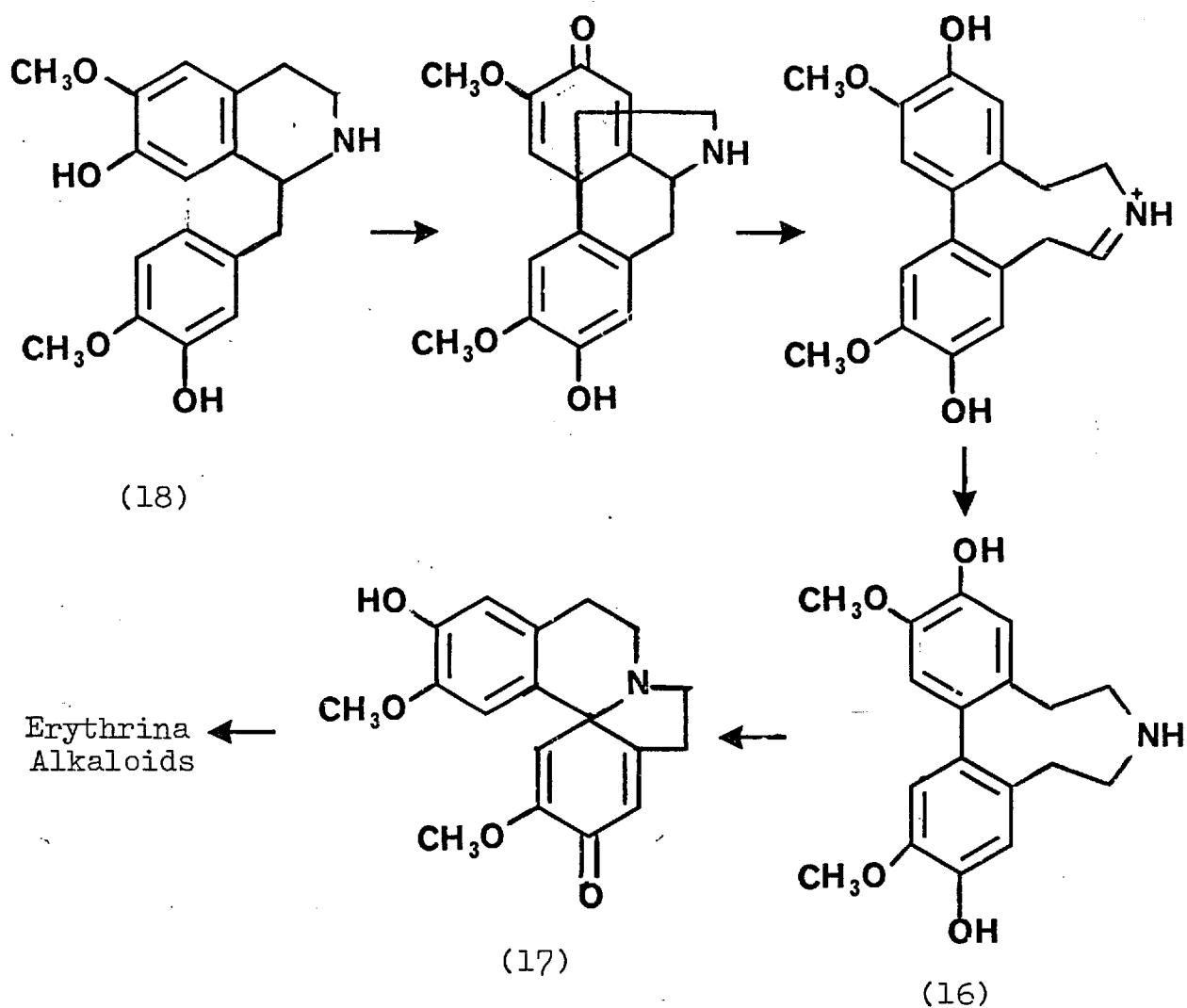
Taking into consideration only the most recent developments in this field, it should be mentioned that two different biosynthetic pathways have been proposed. The first biogenesis has been presented by Barton and his coworkers²⁶⁻²⁸, and the second by Franck and coworkers²⁹.

According to Barton's theory, (+)-N-norprotosinomenine (14) is the key precursor of the aromatic Erythrina alkaloids. (Scheme I).

The Franck alternative has N-norreticuline (18) as the key precursor (Scheme II).



SCHEME I

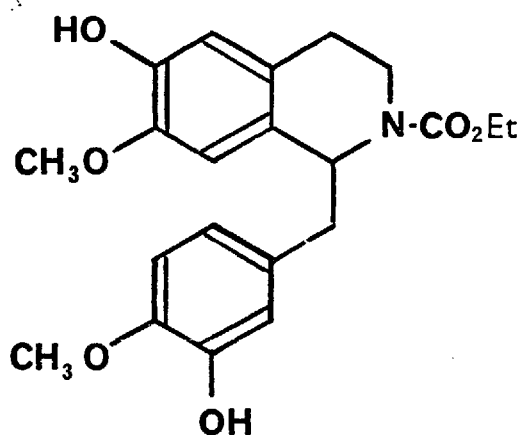


SCHEME II

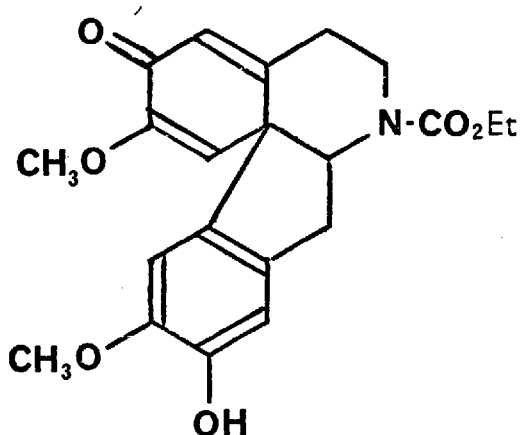
Labelling experiments in Erythrina crista galli have shown that (+)-N-norprotosinomenine (1)²⁶, (+)-5,6,8,9-tetrahydro-2,12-dimethoxy-7H-dibenz [d,f]azonine-3,11-diol (16)^{27,28}, and (+)-erysodienone (14)²⁶⁻²⁸ were incorporated into erythraline (1) whilst (+)-N-norreticuline²⁸ was not. Consequently, the biogenetic scheme proposed by Frank, which had N-norreticuline as intermediate was disproved in the case of E. crista galli

An in vitro reaction analogous to part of the proposed Barton biogenesis, has been performed by Kametani³⁰, who by a phenolic oxidative coupling reaction of the norprotosinomenine derivative (19) with potassium ferricyanide in the presence of ammonium acetate and ammonia, obtained the dienone (20) in 2% yield. Later, by photolytic reactions³¹, he was able to improve the yield of the same "proerythrinadienone" (20) to 12%.

Unfortunately, the realization of the next proposed biosynthetic step, the conversion of the "proerythrinadienone" into erysodienone (17) has not yet been accomplished.

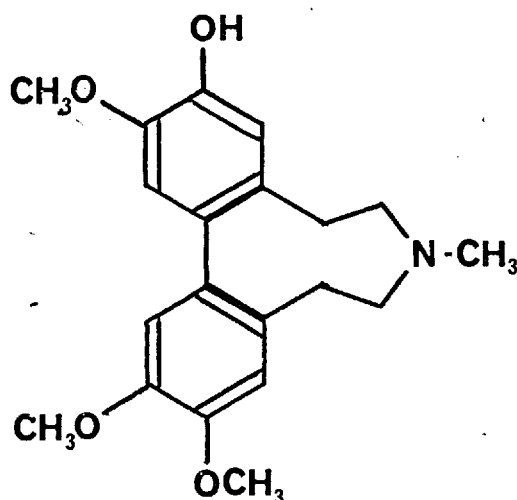


(19)



(20)

Recently, erysodienone (17) and (+)-N-norprotosinomenine (14) have been isolated from E. lithosperma³² and one N-methyl analogue (21) of the azcnine, (16), has been extracted from E. xbidwilli³³.



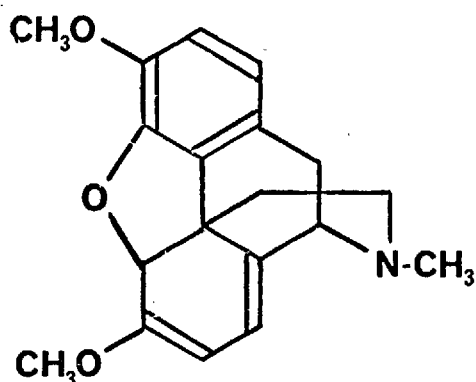
(21)

Erysodienone has been assumed to have the same absolute stereochemistry at C-5 as the other Erythrina alkaloids³⁴, since the steps required for the conversion of erysodienone into the respective alkaloids do not involve C-5. Thus, the 5 S-isomer of erysodienone should be the natural precursor.

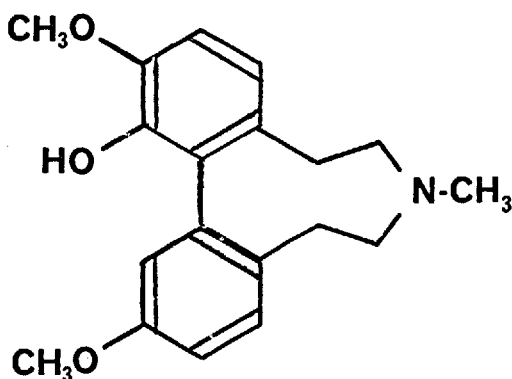
The stereochemistry of the conversion of S-(+)-N-norprotosinomenine (14) into 5 S-erysodienone (17) has been proven by Barton²⁷ to require an inversion of chirality during the process, (Scheme I).

On the basis of the experimental evidence up to that moment available, it was not possible to specify at which point in the sequence the system became symmetrised or inverted since it was known that in the case of thebaine (22) it was possible to obtain the dibenzazonine (23) which was optically stable at room temperature³⁵. According to that result, the biphenyl (16) does not have necessarily to lose its asymmetry during the biosynthesis of Erythrina alkaloids. It should be noted however that the biphenyl (23) does have an additional o-hydroxyl-substituent which increases its optical

stability (see below).



(22)



(23)

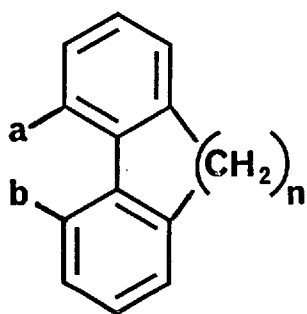
Only few aspects of the biosynthesis of Erythrina alkaloids still remain unproven. These are the formation of proerysodienone (15) from N-norprotosinomenine (14), the optical stability of the biphenyl (16) and its possible racemisation in vivo, the intermediacy of the diphenoquinone and the establishment of the absolute stereochemistry of natural erysodienone (17).

OPTICAL STABILITY OF BRIDGED 2,2'-BIPHENYLS

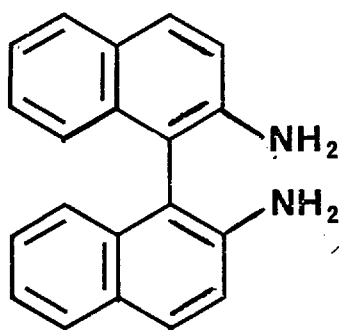
(1) 2,2'-Bridged biphenyls without 6,6'-Substitution.

The effect of bridging the 2,2'-positions in a potentially active biphenyl has been studied, e.g. in the system (24), since 1929. Kuhn and Goldfinger³⁶ had found that, when the resolved diamine (25) was condensed with benzil,

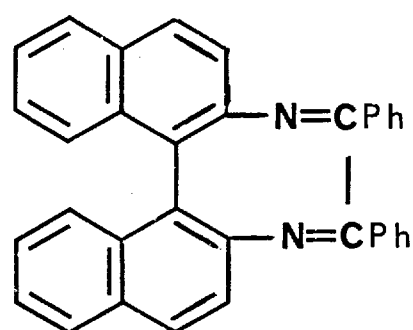
the eight-membered ring compound obtained (26) was optically active. Two years later, Sako³⁷ prepared the optically active compound (28) from the (-) form of (27). This showed that, when the 2- and 2'- positions in a resolvable biphenyl derivative are linked, in a strainless ring, the two aromatic nuclei can still be noncoplanar and the chirality can remain^{38,39}.



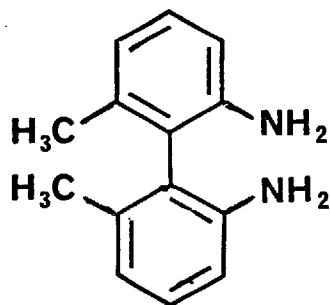
(24)



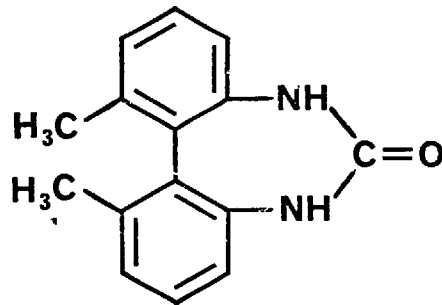
(25)



(26)

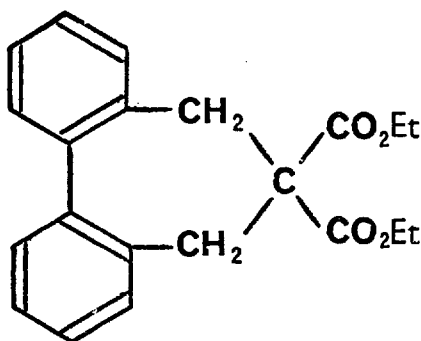


(27)



(28)

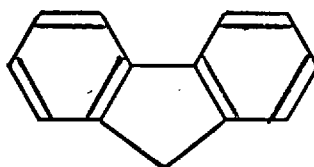
From the preceding discussion the occurrence of noncoplanar bridged biphenyls without the additional hindrance by the 6,6'-substituents would be predicted. Several investigators attacked this problem simultaneously but all attempts to demonstrate this failed until 1956 when Iffland and Siegel^{40,41} isolated the optically active ester (29).



(29)

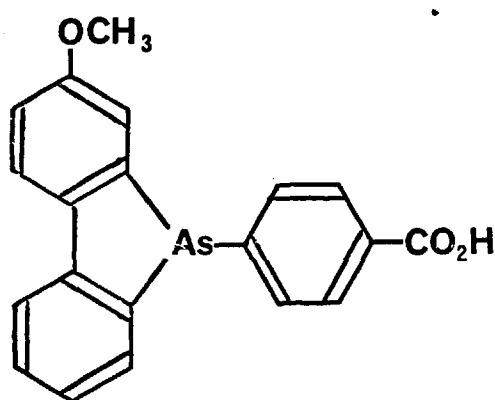
From the time of these pioneer studies, 2,2'-bridged biphenyls have been extensively studied^{38,42-44}. They constitute a large group of organic compounds which can exist in two configurations by virtue of the restricted rotation around the pivotal bond. The activation energies for racemization of the two configurations are generally large enough to be reliably measurable⁴⁵.

The first member of the series of 2,2'-bridged biphenyls, fluorene (30), is a molecule with near-planar geometry⁴⁶.

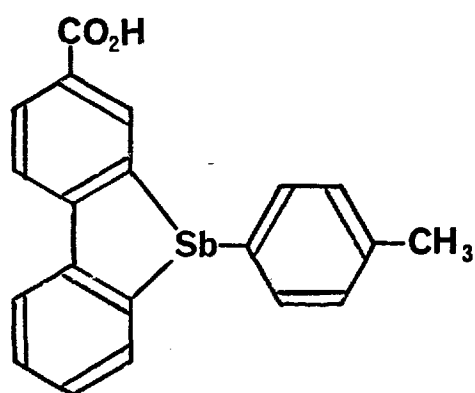


(30)

Nevertheless, replacement of the carbon of the methylene group by a much larger atom could produce a non-planar structure. The optical resolution of several 9-arsafluorenes and 9-stibiafluorenes⁴⁷, such as (31) and (32), were taken to support this, but later it was shown that these compounds do have a planar tricyclic system and owe their stable chirality to the pyramidal arrangement of the three different groups round the hetero atom^{48,49}.

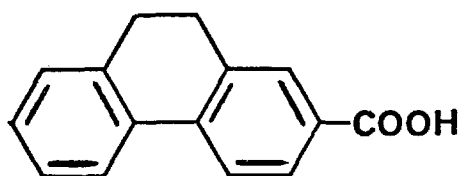


(31)



(32)

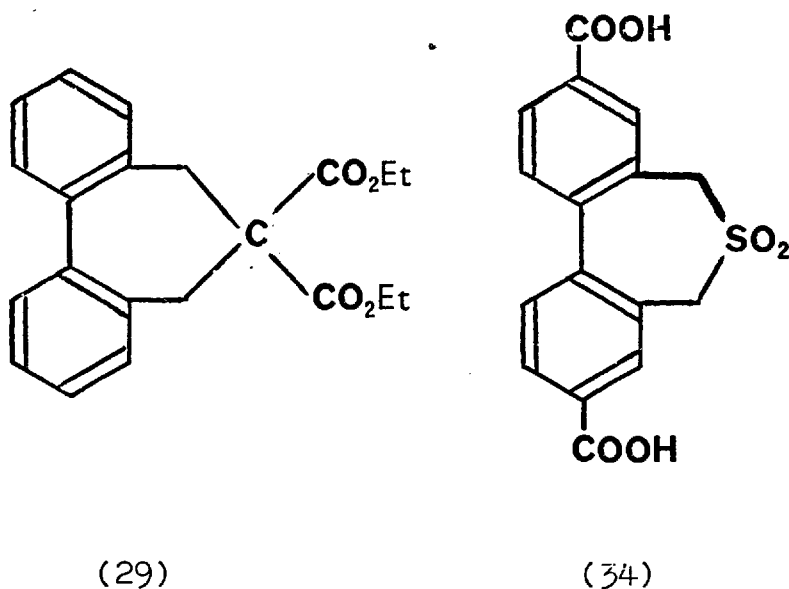
The optical resolution of several 9,10-dihydrophenanthrene derivatives (as 33) were attempted⁵⁰ on the basis that any compound in which the 2- and 2'- positions of biphenyl are bridged by a more than one carbon in the aliphatic chain, the two benzene rings are not coplanar, and with such compounds optical isomerism can occur irrespective of further substituents.



(33)

All the attempts made were unsuccessful and Howlett⁵¹ and Mislow⁵², by calculation and analogy indicated that the energy barrier to inversion would be very low.

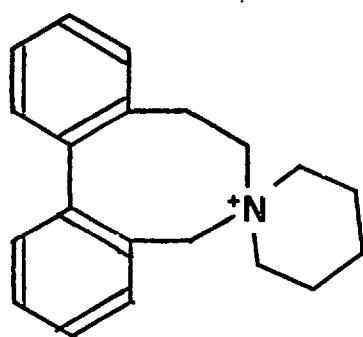
The next higher homologue, the seven-membered bridging ring, could also exhibit chirality. This was demonstrated by the preparation of (29) and (34) in optically active forms^{40,41,53}.



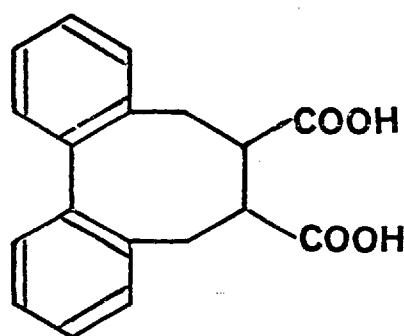
The compound (29) was optically labile. The racemisation in cyclohexane solution at 32.5° had a half-life of 80 min. but was optically stable for at least 200 hrs. in the crystalline state. The compound (34) was also obtained optically active in solution without observable racemization⁵³.

A bridge of four atoms was found in compound (35) which was resolved but was extremely optically labile. The racemisation at 23° showed a half-life of 1.4 min.⁵⁴. Another example of this group of compounds is the dicarboxylic acid (36)^{55, 56}. This was resolved and identified as trans and cis stereoisomers by the fact that the enantiomeric forms

of the former mutarotate (in ethanol solution at 31.5° , half-life of 12 min) while those of the latter, which are classically a meso form and therefore owe their optical activity solely to the twisted biphenyl system, racemize (in ethanol solution at 31.5° and half-life of 85 min.). Examination of molecular models of (36) revealed that the



(35)

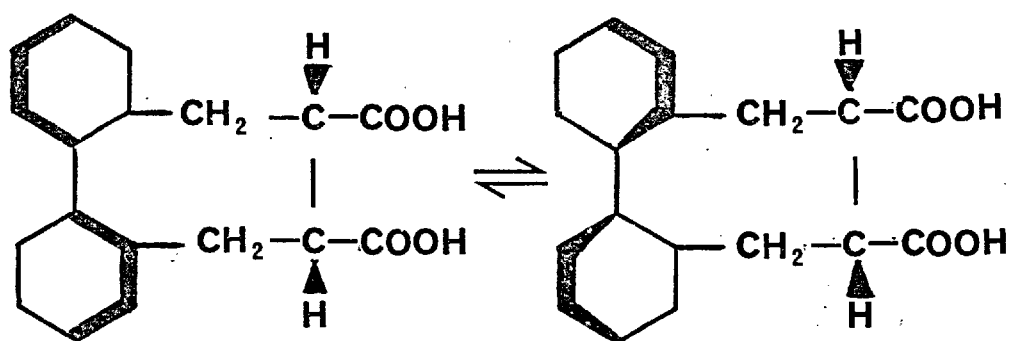


(36)

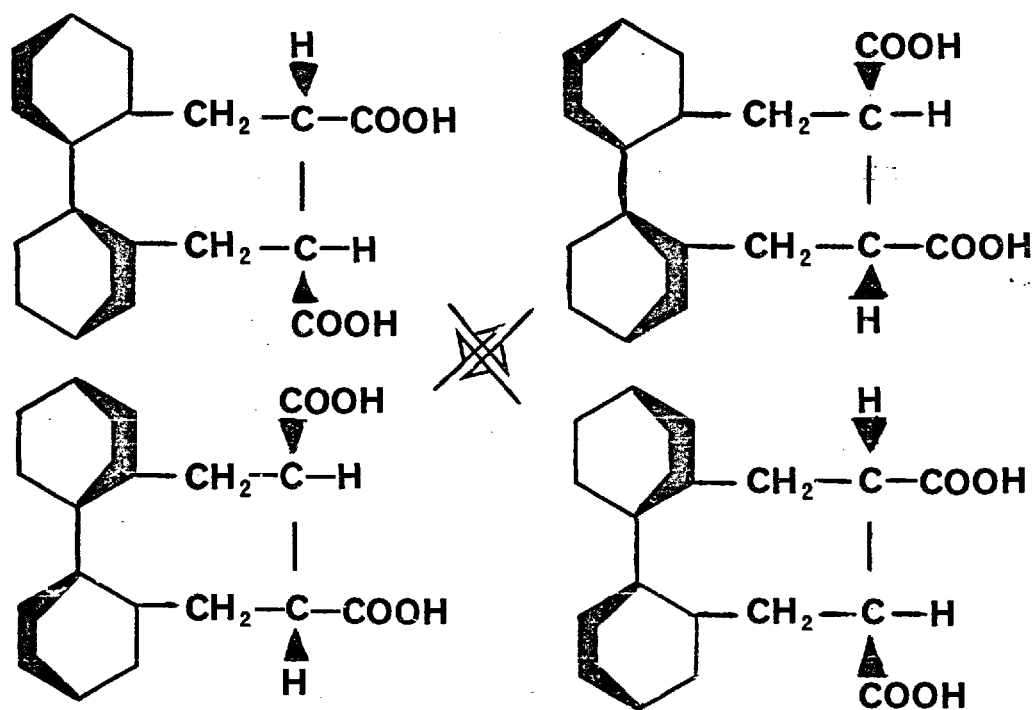
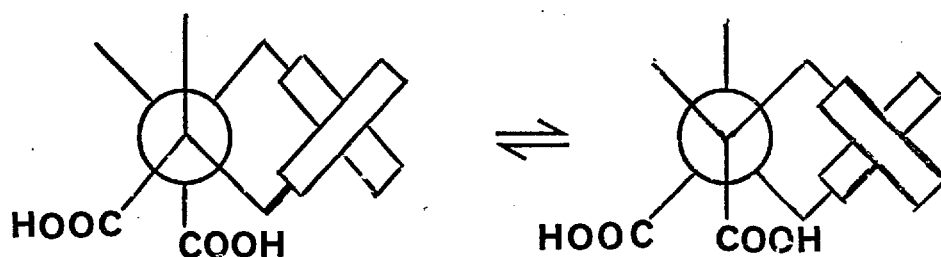
hydrogen atoms on the 6,7-positions of the bridge were disposed axially and equatorially in close analogy to the chair form of cyclohexane. Racemization of cis-(36) may then be viewed as a process involving interconversion of ae- and ea-6,7-dicarboxylic acid groupings with concomitant twisting of the biphenyl system (Scheme III). Mutarotation of trans-(36) involves interconversion of aa- and ee- 6,7-dicarboxylic acids accompanied by biphenyl twisting (Scheme III). Thus the mutarotation is due to the presence of a labile centre of chirality (biphenyl twisting) superimposed on the stable chirality of the trans-1,2-disubstituted cycloocta-4,6-diene system.

An example of an eight-membered bridged ring which shows high optical stability is the compound (37) obtained

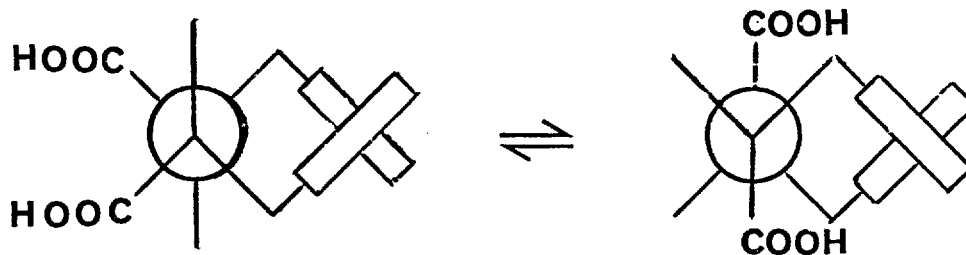
SCHEME III



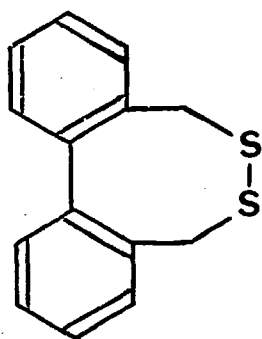
A



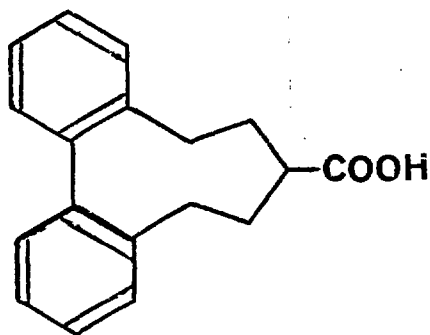
B



by Luttringhaus⁵⁷ in 1967. The stability is a consequence of the large size of the two sulphur atoms that confer remarkable rigidity on the molecule.



(37)

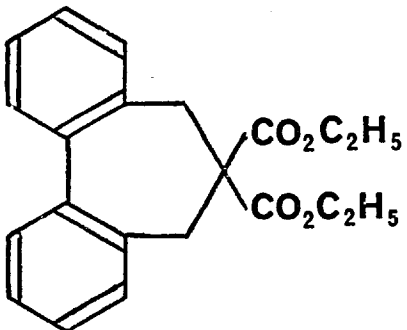
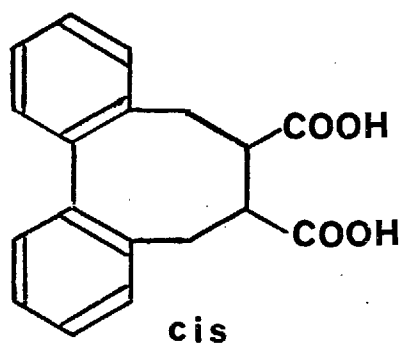
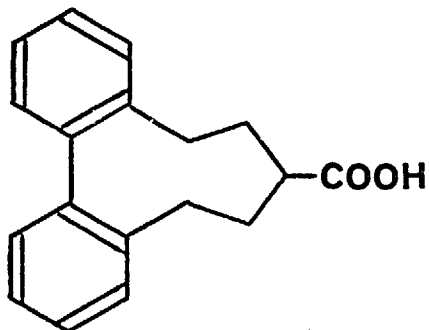


(38)

After several unsuccessful attempts to prepare a compound with a nine-membered bridging ring^{55,58}, Mislow and his associates⁵⁹ were able to synthesize compound (38) which was found to have somewhat greater optical stability than the lower homologues. From a study of the activation energies for racemization of bridged biphenyls, it was concluded that the barrier to racemization is to a first approximation independent or increases only very slightly with ring size in the series of seven-, eight- and nine-ring bridged biphenyls (Table 2). This is based on the assumption that the energy of activation is roughly independent of solvent and of substitution on the bridge. The reliability of estimates of the activation energy for racemisation depends on the geometry of the transition state and on the function relating molecular deformation and intramolecular non-bonded interactions to corresponding potential energies. Consequently, the approximate constancy of this parameter is the outcome of two counterbalancing factors.

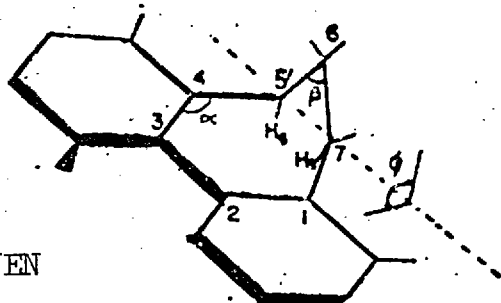
TABLE 2

RACEMIZATION DATA FOR SOME BRIDGED BIPHENYLS WITHOUT
ADDITIONAL ORTHO SUBSTITUENTS.

COMPOUND	No. of Atoms in bridging ring	E. KJ mol ⁻¹ .	t _{1/2} min.	Solvent	Ref.
	7	87	80 (32.5°)	Cyclo- hexane	41
	8	95.4	85 (31.5°)	ethanol	56
	9	100.4	1440 (25°)	o-xylene	59

PRESUMED TRANSITION STATE CONFORMATIONS IN RACEMIZATION OF:

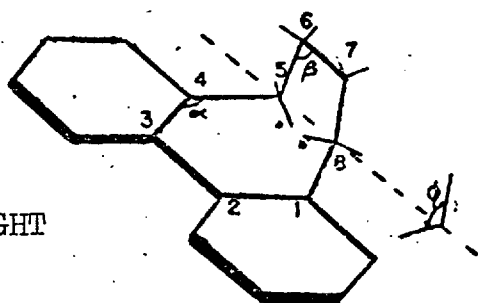
SEVEN



(39)

$$d \cdot C_5 \text{ to } C_7 = 2.3 \text{ \AA}$$

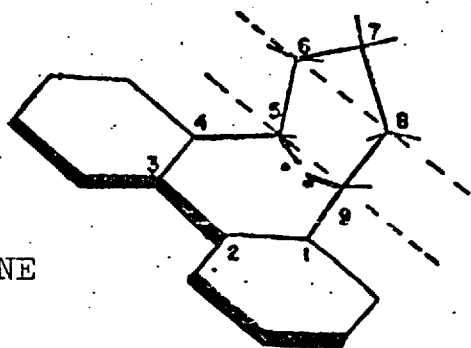
EIGHT



(40)

$$d \cdot C_5 \text{ to } C_8 = 2 \text{ \AA}$$

NINE



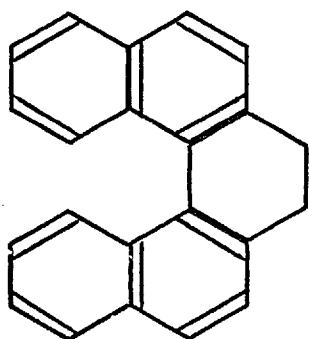
(41)

$$d \cdot C_5 \text{ to } C_9 = 1.4 \text{ \AA}$$

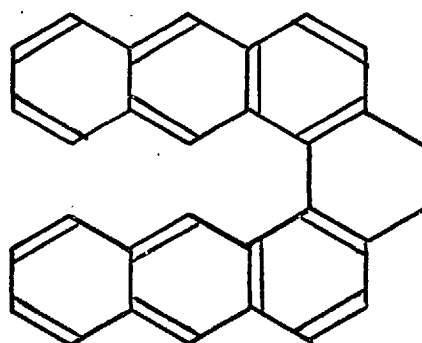
As the bridge length increases from seven, to eight, to nine-ring, the angle strain in the transition state decreases only at the expense of increased non-bonded interaction of opposed α -hydrogens (39, 40, 41); the two factors approximately compensate each other. In the nine membered ring, the barrier arises largely from interaction of non-bonded hydrogens oriented in direct opposition.

(2) 2,2'-Bridged biphenyl compounds with 6,6' substitution

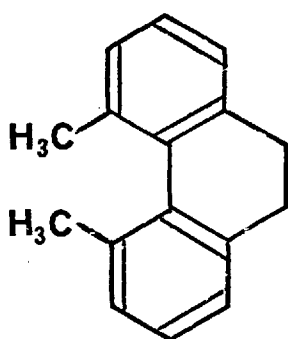
Up to this point all the compounds described have been bridged biphenyls without additional ortho substituents, nevertheless it was well known from the work on unbridged biphenyls⁶⁰ that when there were two bulky ortho substituents in one ring, they would interfere with the free rotation of the second ring, arising from the collisions between them and the third ortho substituent. The same criterion has been applied to the bridged biphenyls and it has been possible to prepare optically active analogues of compounds on which attempts to demonstrate optical activity had been unsuccessful. Such was the situation for 9,10-dihydrophenanthrenes. The compounds (42) and (43) have been prepared in optically active forms by synthesis from resolved intermediates^{39, 61}. In the same way Mislow and Hopps⁶² were able to prepare 9,10-dihydro-4,5-dimethylphenanthrene (44) which as expected, presented an optical stability of the same order as that of (45)^{63, 64}.



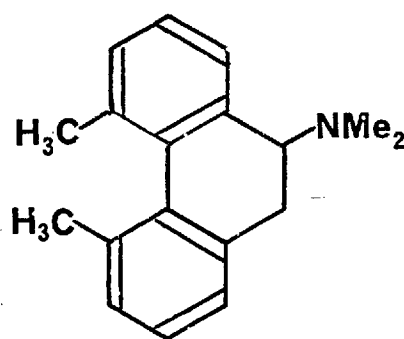
(42)



(43)

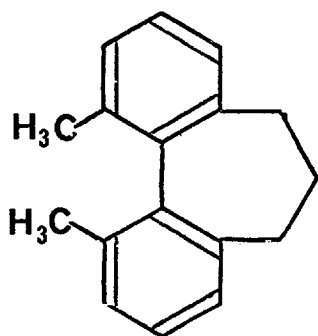


(44)

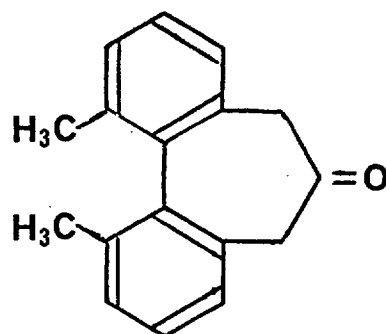


(45)

Among the seven-membered bridged biphenyls the hydrocarbon (46) and the ketone (47) can be mentioned, which were prepared by Mislow and Gordon in 1963⁶⁵. In the dark, these compounds have activation energies for the thermal racemizations of 163.1 and 150.6 KJ.mol⁻¹ but can racemize at room temperature when they are irradiated. This is due to the fact that the triplet state of the biphenyl itself seems to be planar⁶⁶.

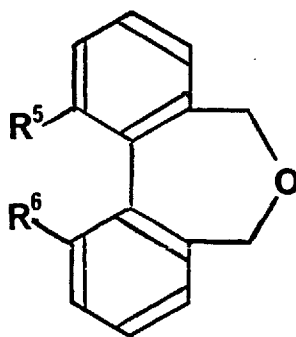
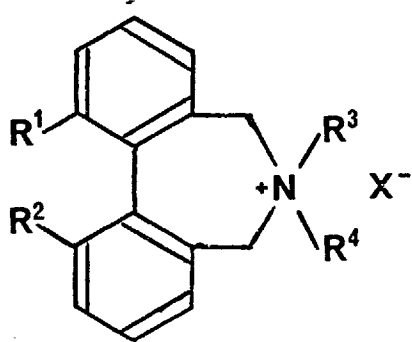


(46)



(47)

Several dibenzazepines and dibenzoxepines with additional ortho substituent have been studied. All of them (48-54) are optically stable. The energy barrier to racemization in these compounds depends on the effectiveness of the steric interaction produced by the additional ortho substituents.



(48)⁶⁷ $R_1 = H; R_2 = NO_2; R_3 = R_4 = Me; X = C_6H_2N_3O_7^-$

(49)⁶⁸ $R_1 = R_2 = F; R_3 = Me; R_4 = H; X = Cl^-$

(50)⁶⁸ $R_1 = R_2 = NO_2; R_3, R_4 = Me$

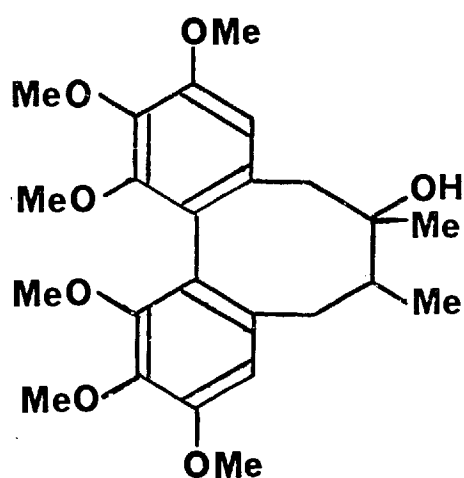
(51)⁴⁵ $R_1 = R_2 = OMe; R_3-R_4 = C_6H_{10}; X = I^-$

(52)⁴⁵ $R_1 = R_2 = Cl; R_3 = R_4 = Me; X = Br^-$

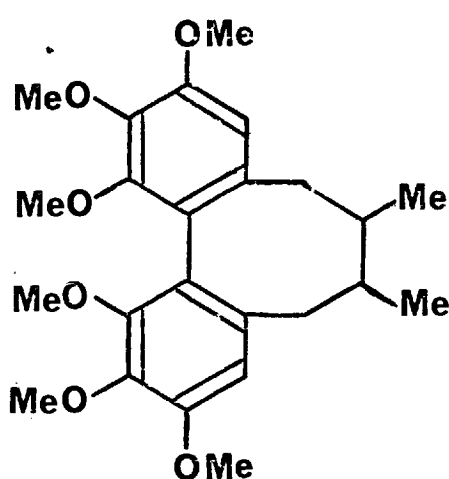
(53)⁴⁵ $R_5 = R_6 = OMe$

(54)⁴⁵ $R_5 = R_6 = Cl$

Two natural occurring optically active compounds have been found belonging to the eight-membered bridged ring series⁶⁹. These compounds (55) and (56) present extra optical stability conferred on the twisted biphenyl by two ortho methoxyl groups.

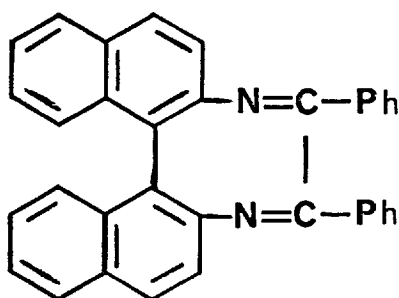


(55)



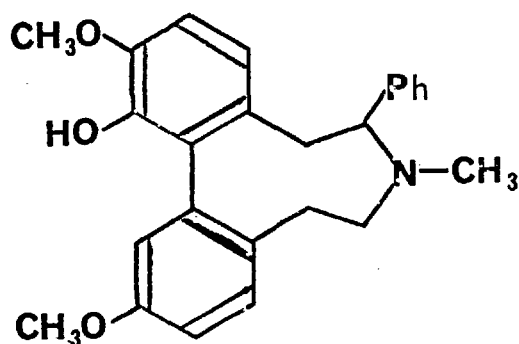
(56)

Another interesting example of this group is the highly active (26) obtained by the condensation of d-2,2'-diamino-1,1'-binaphthyl with benzil^{36, 70}.

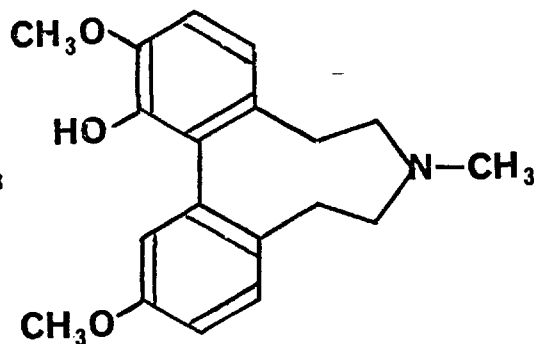


(26)

Of the nine-membered bridged systems, the first optically active compound reported was phenyldihydrothebaine (57)⁷¹. Later a similar compound without the asymmetric carbon atom, was obtained (58)⁵⁴. This compound showed to have a higher optical stability than the unsubstituted 9-membered ring compound (38), probably due to the presence of the hydroxyl group.

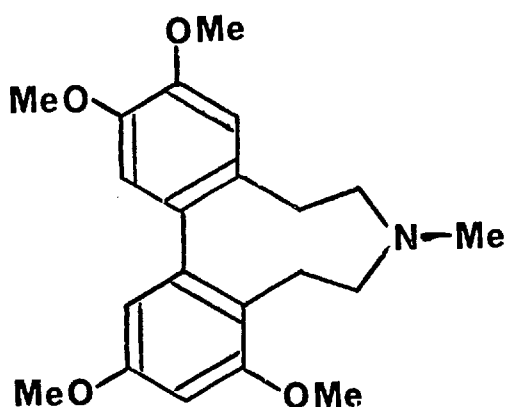


(57)

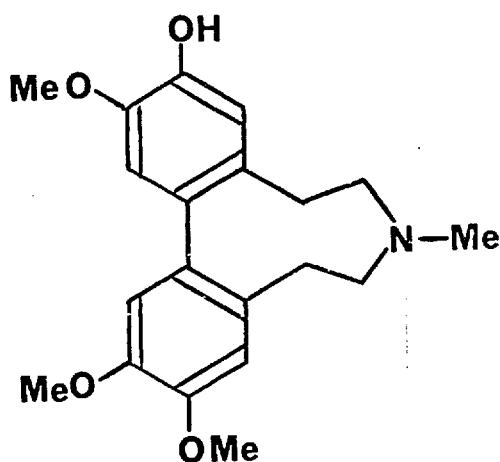


(58)

It is interesting that the ring structure of (58) is found in two different alkaloids, protostephanine (59), isolated from Stephania japonica Miers⁷², and erybidine (60) isolated from Erythrina xbidwilli Lindl.⁷³. The optical activity (if any) of these alkaloids has not been reported.



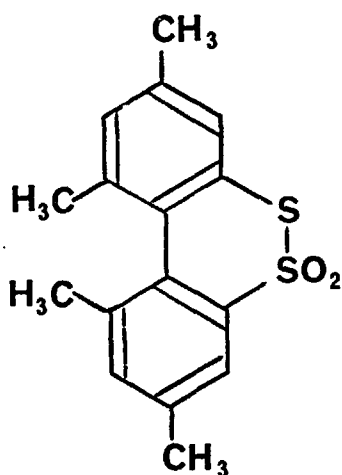
(59)



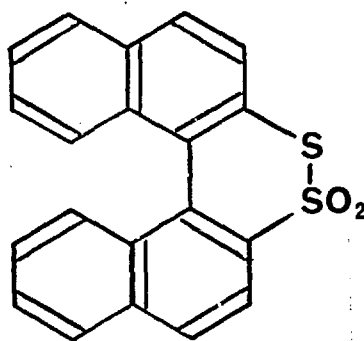
(60)

From the foregoing it is apparent that optical stability is not only favoured by an increase of the number of substituents in the bridged diphenyl. An increase in the size of the atoms forming the bridge and the presence of one or more double bond in the bridging ring, greatly increases the optical stability.

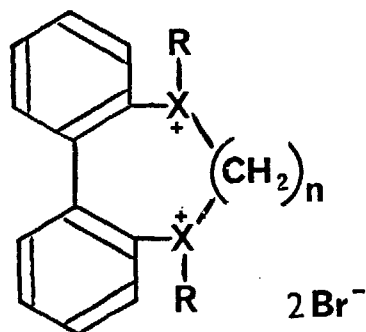
Examples of bridged biphenyls containing heteroatoms are the thiol sulphonates (61) and (62) which are optically stable at more than 150° ⁷², also the series of compounds prepared by Mann and his co-workers using two atoms of arsenic,⁷⁵ phosphorus⁷⁶ or sulphur⁷⁷.



(61)

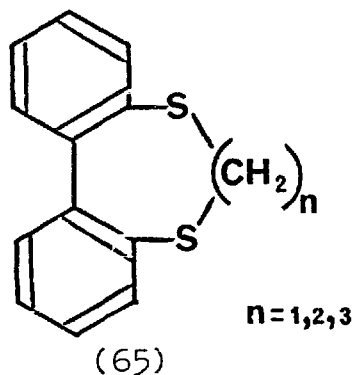


(62)



(63) X = As; R = Me; n = 1,2,3

(64) X = P; R = Et; n = 1,2,3,4

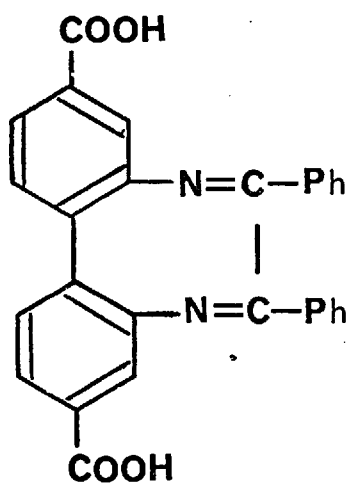


(65)

All the arsenic compounds are optically stable at room temperature. The increase of the ring size produces a marked increase in the optical stability. The optical resolution of the compounds having phosphorus and sulphur atoms is being investigated⁷⁶.

For the unsaturated bridging rings the dibenzodiazocine (66) which is optically stable up to 240° in ethyl benzoate⁷⁸

serves as an example.



(66)

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DISCUSSION

THE SYNTHESIS OF ERYSDIENONE

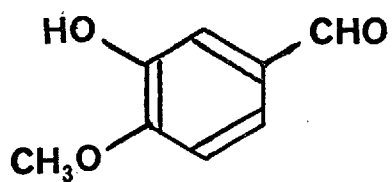
Erysodienone (4) has been established as an important intermediate in both the synthesis¹ and biosynthesis² of the Erythrina alkaloids. The first aim of this work, therefore, was to carry out the synthesis of this compound by the shortest and most efficient method available, since a large amount of the dienone compound was required..

The literature methods for the synthesis of this alkaloid are those of Scott² and Mondon¹ and Franck³. These gave low overall yields.

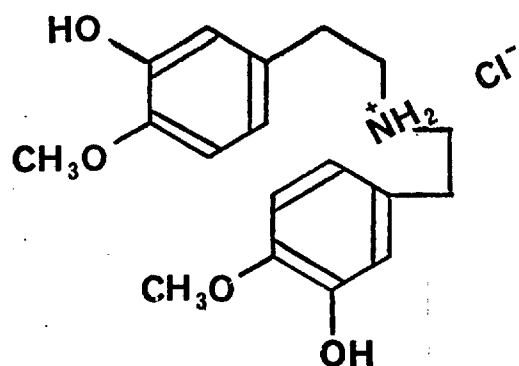
Barton and co-workers synthesized erysodienone (4) by a reported procedure⁴ in ten steps from isovanillin (1) in a total yield of 12%⁴. The route included the phenolic oxidation of bis-[2-(3-hydroxy-4-methoxyphenyl)ethyl]amine (2) to give erysodienone (4)^{1,2,4,5} in 35% yield. This last step is believed to occur through the formation of the biphenyl intermediate (3)^{5,6}.

Franck employed a more direct method via the conversion of the benzyloquinoline (5) into the dienone (8) through morphinanedienone (6) and biphenyl (7), in which again, phenolic oxidations were used in key steps. The overall yield of the dienone (8) was only 7.5%.

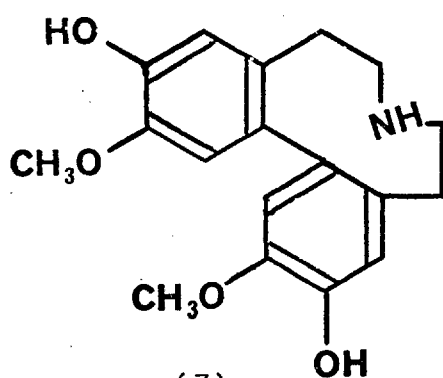
These results led to the search for a more efficient method



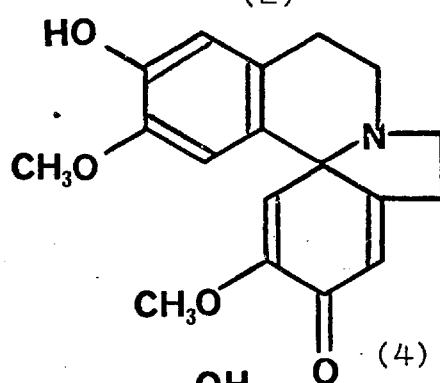
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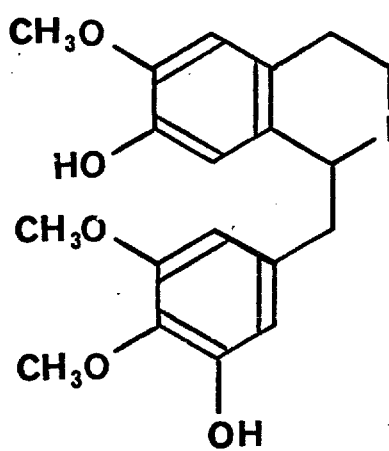
(2)



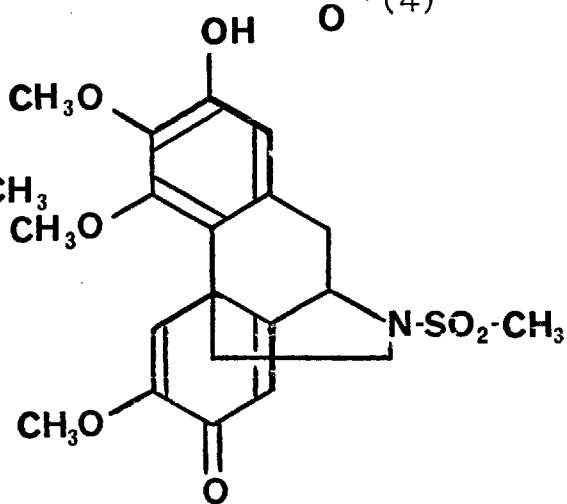
(3)



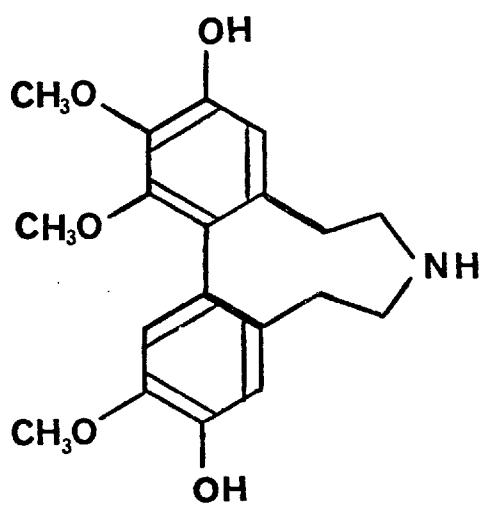
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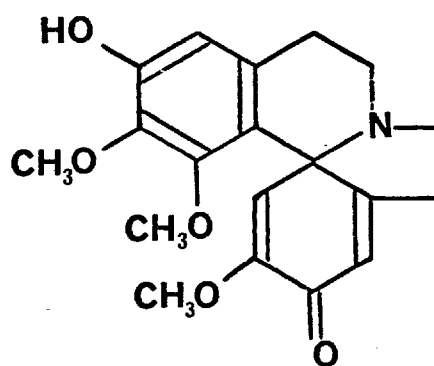
(5)



(6)



(7)



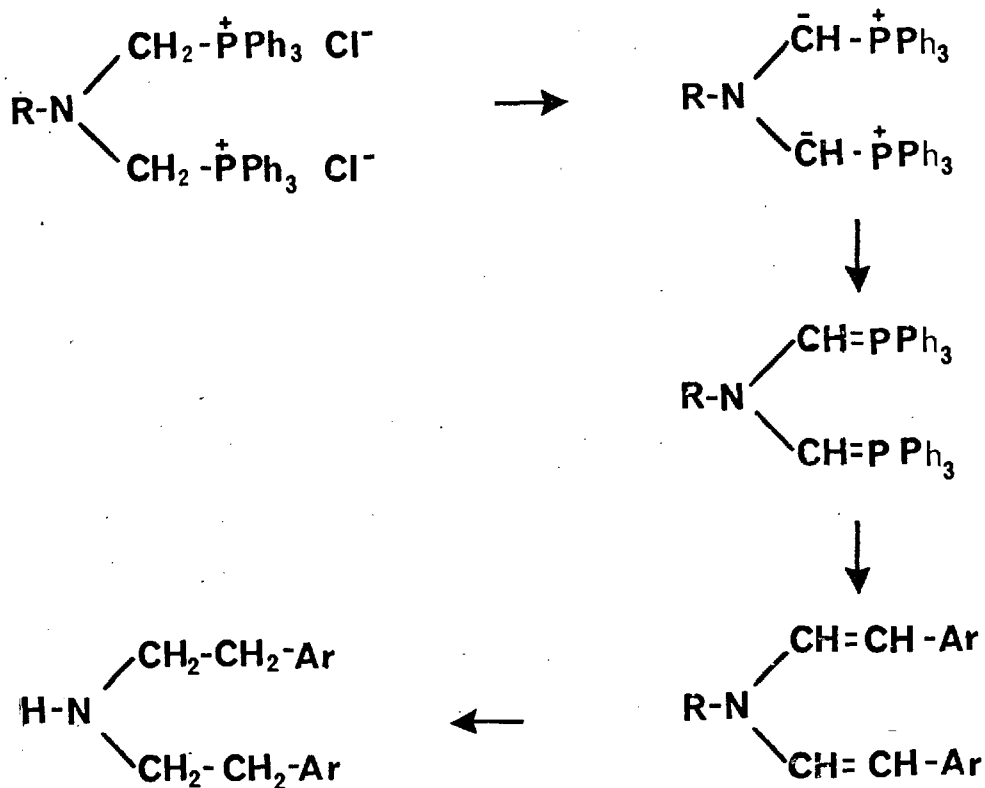
(8)

of synthesis of bis-2-phenylethylamine (2) and hence truly provide an efficient way to synthesise erysodienone.

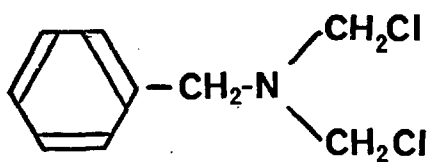
The molecule of bis-2-arylethylamine (2) is symmetrical and this was the foundation for the initial planning of its synthesis. It was thought, that if in any way one reactive unit of the type C-N-C could be prepared, C-C bond formation with an aryl-C₁ unit would give the desirable skeleton of a bis-2-arylethylamine.

The first C-N-C unit considered was the N-di(chloromethyl) amine (9). Chloromethylamine compounds would be expected to be highly reactive⁷⁻¹⁰ and the literature cited examples of compounds [(10) and (11)^{12,13}], that suggested the feasibility of formation of (9). Elaboration of this via the bisphosphonium salt and a Wittig process (Scheme 1) would produce the desired carbon skeleton.

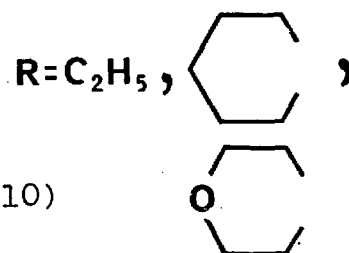
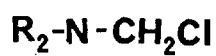
The reaction of formaldehyde with amines, reportedly gives hydroxymethylamines, RNHCH₂OH, and di(hydroxymethyl) amines¹⁰. The latter have been described as unstable oils¹⁴ which very easily undergo further self-condensation to give cyclic compounds such as (12)^{15,16}, (13)¹⁷ and (14)¹⁷⁻¹⁹ among others.



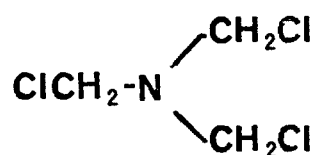
SCHEME I



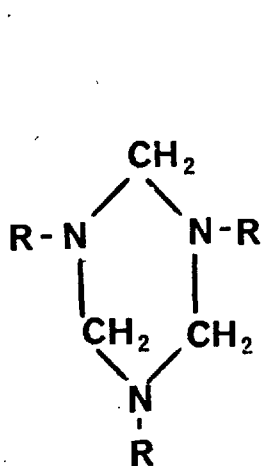
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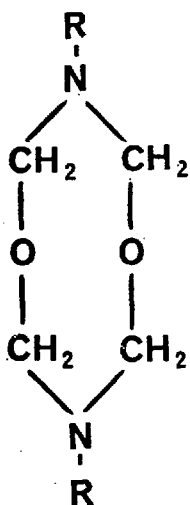
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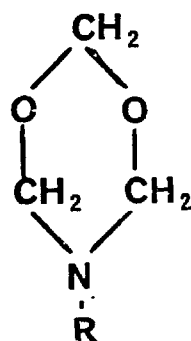
(11)



(12)



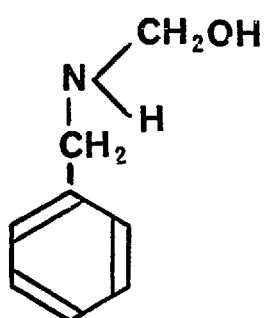
(13)



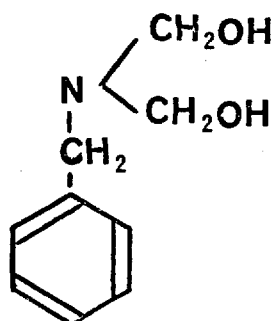
(14)

N-Hydroxymethyl-benzylamine (15) was obtained from the reaction of benzylamine with formaldehyde according to the literature method²⁰. In an attempt to prepare the di(hydroxymethyl)amine (16) further reaction was attempted under the conditions employed on the synthesis of the monohydroxymethylamine (15). The crystalline product showed a five-proton singlet in the aromatic region (τ 2.66) of the n.m.r. spectrum and three more singlets at τ 4.76, 5.33 and 5.72 integrating for 2:4:2 protons respectively. The spectrum was unaffected of D₂O exchange and this was confirmed by the i.r. spectrum which did not show any hydroxyl bands but instead showed bands at 925 and 720 cm⁻¹ characteristic of a methylenedioxy group²¹.

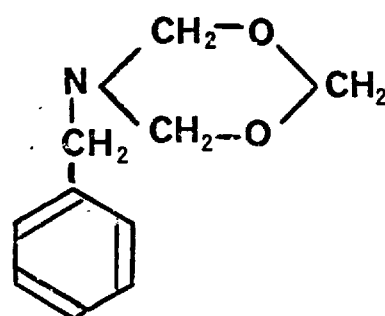
The mass spectrum and elemental analysis confirmed the compound as 5-benzyl-1,3,5-dioxazine (17). The product is clearly the condensation product of N-di(hydroxymethyl)benzylamine (16) analogous to (14)¹⁷⁻¹⁹.



(15)



(16)



(17)

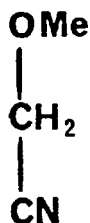
With this compound (17) in hand the formation of the di-chloromethyl-amine (9) by direct reaction with thionyl chloride was considered²².

The 1,3,5-dioxazine (17) was treated with excess of acid free thionyl chloride. After evaporation of the excess acid chloride, treatment with triphenyl phosphine, did not give the required phosphonium salt. The phosphine remained unreacted. It is probable that the salt of the amine (9) was generated rather than the required fission of the dioxazine ring,

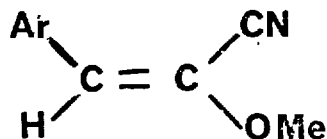
At this time a new method for the synthesis of phenylacetic acid derivative was reported ²³. This utilized a Knoevenagel-type condensation of benzaldehyde, or a substituted benzaldehyde, with a compound having a carbon bearing active hydrogen, to afford 2-arylethylenes, which by decomposition with acids gave ethylarylacetates in high yields.

Application of this type of route would provide a simpler and more efficient way to obtain an 1-carbon extension of the available isovanillin unit. Thus, the synthetic procedure followed above was discontinued in favour of the alternative method described below.

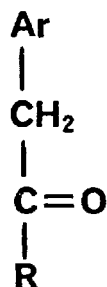
The compound chosen to provide the carbon unit was methoxyacetonitrile (18). This compound would allow the formation of the carbanion and hence condensation with the aldehyde to give the β -arylethylene (19). In addition, once the vinyl ether was obtained, the methoxy group could be expected to demethylate with mercaptide anion ²⁴ to give the corresponding homoacid derivative (20).



(18)



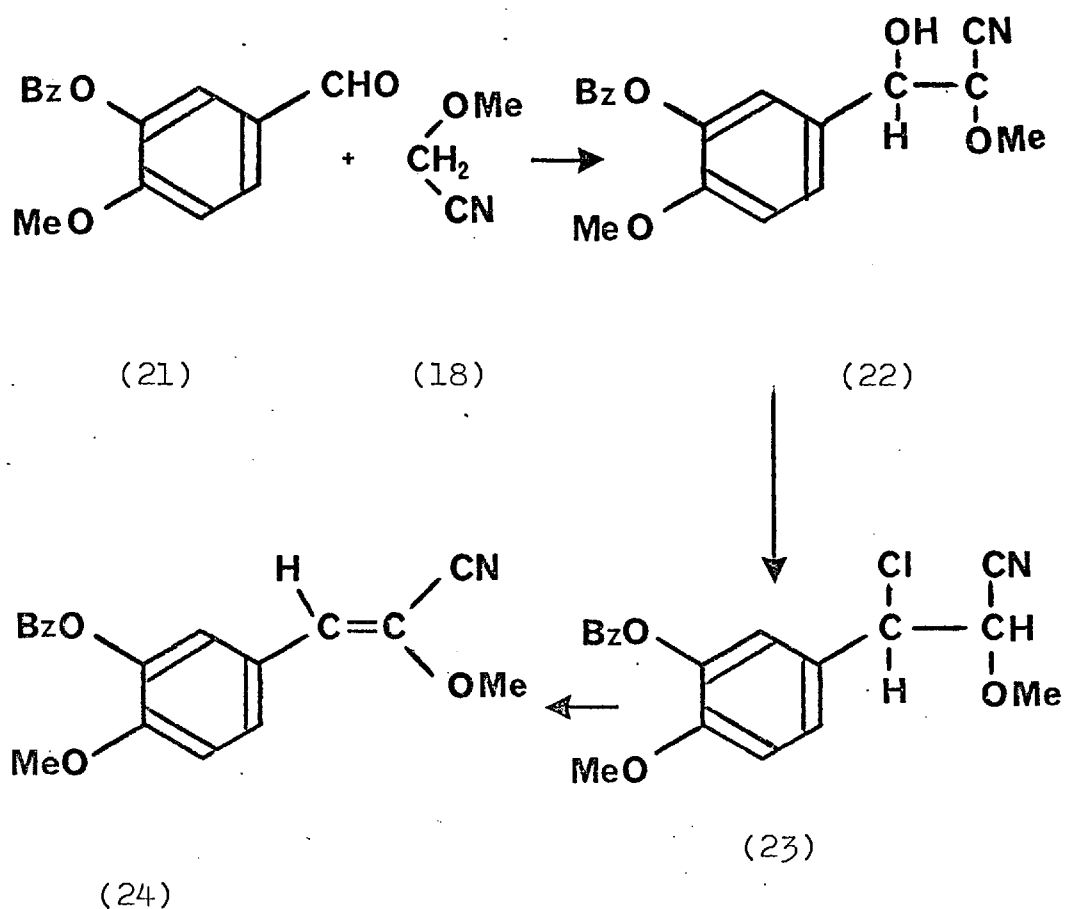
(19)



(20)

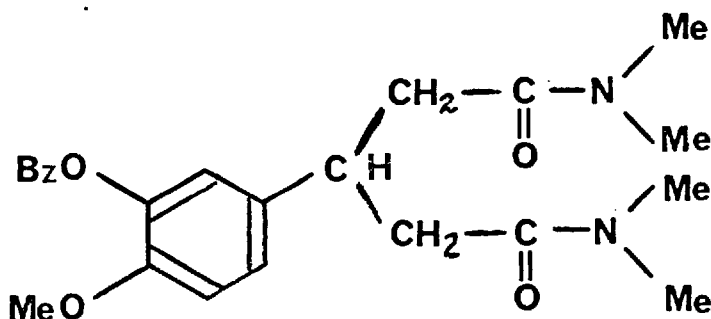
The condensation reaction was first achieved by stirring a solution of benzylisovanillin (21) in dimethylformamide with excess of methoxyacetonitrile (18) and one equivalent of sodium hydride²⁵, at room temperature. When the reaction of the aldehyde was complete, the mixture was poured into water and extracted with benzene/light petroleum ether. Examination of the crude reaction product by t.l.c. showed the presence of two compounds of which the more polar was in a ratio of 9 to 1 with respect to the less polar. The major compound was separated by preparative t.l.c. and extracted with diethylether. The n.m.r. spectrum of the new compound showed the presence of two methoxy groups (τ 6.26 and 6.63), one benzyloxy group (τ 2.60 and 4.98, 5H and 2H respectively), three aromatic protons and one D₂O exchangeable proton (τ 2.21). In addition, the i.r. spectrum showed absorptions at 3,600 and 2,250 cm⁻¹ which could correspond to hydroxyl and nitrile groups. This spectroscopic information led to the formulation of the compound isolated as (22). This alcohol material (22) reacted with thionyl chloride in pyridine at room temperature giving a solid m.p. 109-111^o, of which the i.r. spectrum indicated no hydroxyl group absorption and the n.m.r. showed the absence of olefinic protons. This suggested the presence of the corresponding chloride (23). This compound (23) was refluxed with triethylamine in benzene to give a new compound which corresponded to the less polar compound originally found together with the alcohol (22). This new compound was obtained directly when the mixture of methoxyacetonitrile (18), benzylisovanillin (21), sodium hydride and dimethylformamide was heated at about 110^o until all the aldehyde (21) had reacted. The yield of the

reaction was later improved (81%) when the reaction was carried out under an atmosphere of nitrogen and a few drops of triethylamine were added. The elemental analysis and all the spectroscopic information (see Experimental) left no doubt that the compound obtained was the expected α -methoxy-cinnamionitrile (24) (Scheme II). The configuration about the double bond was not determined.

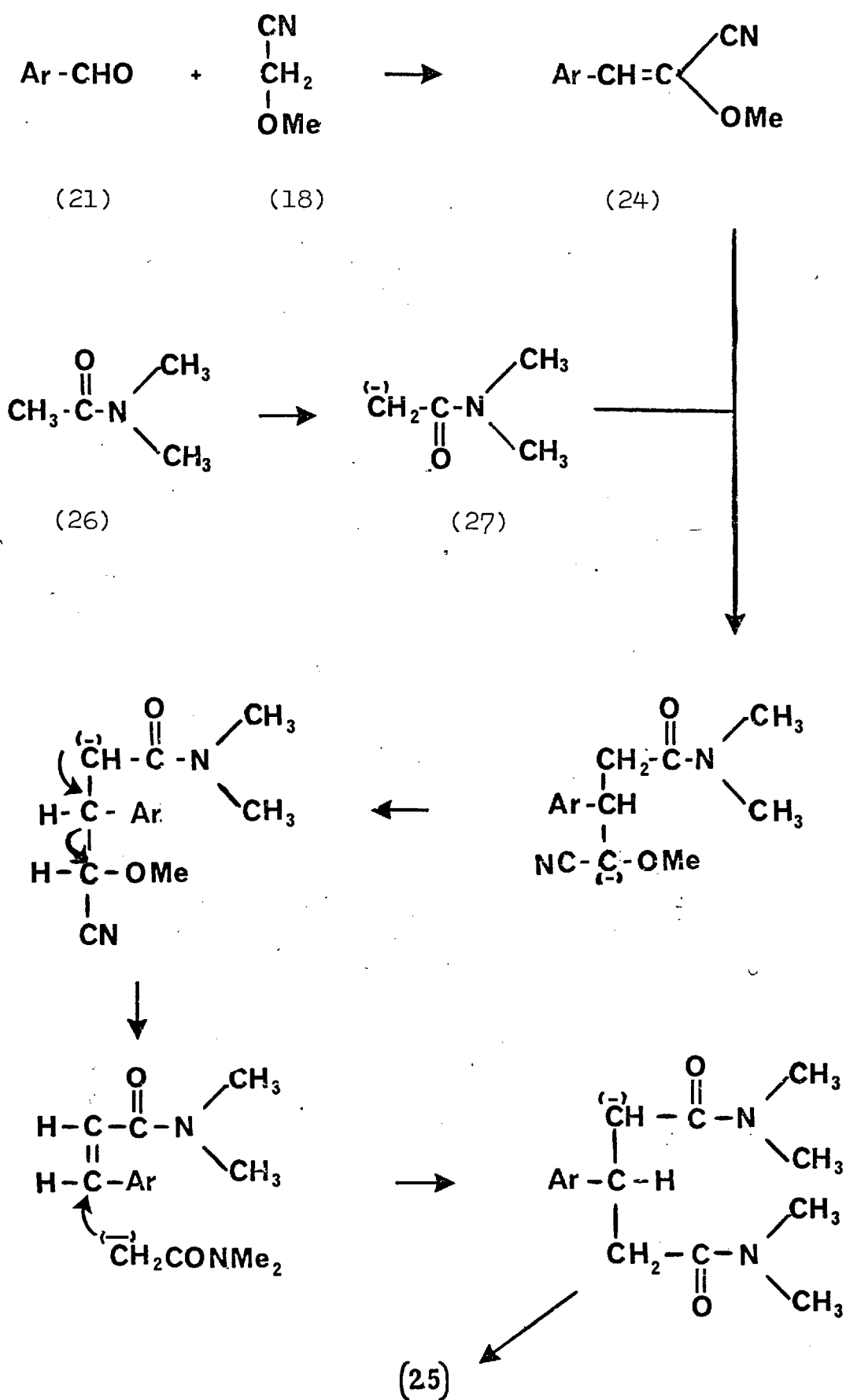


SCHEME II

It is pertinent to mention that during the preparation of the cinnamionitrile (24) different combinations of solvents and bases were tested, (D.M.A. - H.M.P.T. / NaH; T.H.F. - H.M.P.T. / NaH; t BuOH - T.H.F. / K^t BuO), but all were inferior than the above system. Nevertheless, when the reaction was carried out in D.M.A. - H.M.P.T. / NaH at room temperature and under an atmosphere of nitrogen, the reaction proceeded cleanly and in relatively good yield (55%). When the reaction mixture was heated no traces of the cyano-methoxyethylene compound (24) were found. Instead a new compound was obtained which showed in the n.m.r. spectrum eight aromatic protons (τ 2.54 - 3.38), two o-benzylic protons (τ 4.82), one methoxy group (τ 6.17), twelve N-methyl protons (τ 7.1, s) and four methylenic protons (τ 7.23 - 7.7). The i.r. spectrum had a very strong absorption at $1,640\text{ cm}^{-1}$ characteristic of amide compounds. The u.v. spectrum had absorptions at 280 nm (ϵ 380), 230 nm (ϵ 850) and 215 nm (ϵ 1,750), consistent with a styrene chromophore. The mass spectrum gave a molecular ion at m/e 398 and taken with the elemental analysis, corresponded to a molecular formula of $C_{23}H_{30}N_2O_4$. All this information allowed the formulation of the compound as (25).



(25)



SCHEME III

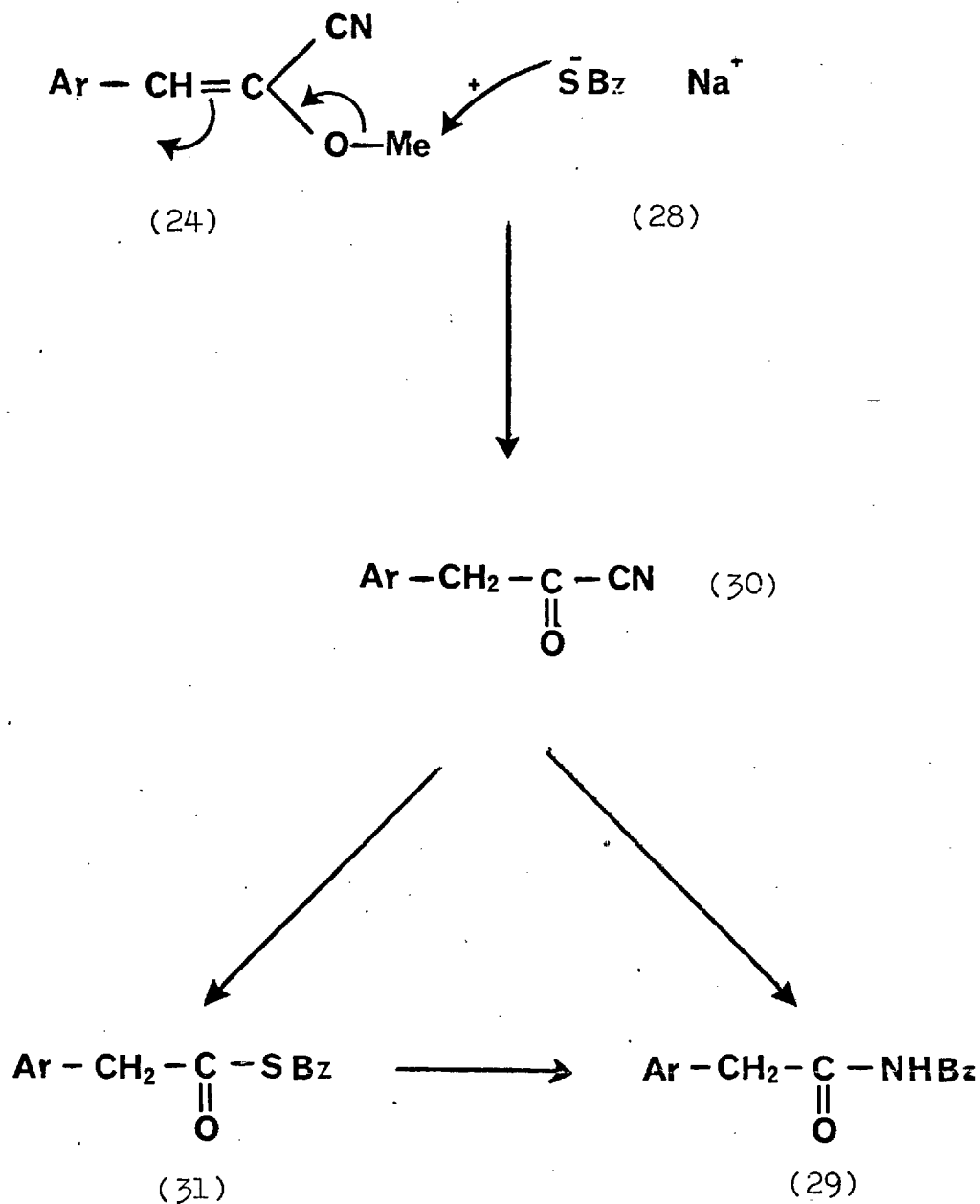
The formation of the glutaric acid diamide (25) appears to be via the formation of the anion of dimethylacetamide (27) followed by Michael addition of this to the acrylonitrile function (Scheme III). Elimination of methoxyacetonitrile anion followed by a second Michael addition would complete the process.

For the cleavage of the enol-ether (24) the use of one equivalent of sodium benzylthiolate (28) in dimethylformamide was tried^{26,27}. Thus, the cinnamonnitrile (24) was stirred with sodium hydroxide (powder) in dimethylformamide, under nitrogen and treated with the mercaptide (28). The mixture was heated until all the enol-ether had reacted, then the cooled mixture was poured into water. After treatment with gaseous carbon dioxide, the aqueous solution was separated, acidified with hydrochloric acid and extracted with ether, to give the expected benzylhomoisovanillic acid (20).

The formation of the acid confirmed that the oxygen-methyl cleavage of the enol-ether compound (24) could be accomplished by the use of sodium benzylthiolate (28) in dimethylformamide, therefore it could be anticipated that the formation of the amide (29) could be relatively straightforward. In fact treatment of α -methoxycinnamonnitrile (24) with benzylmercaptan, benzylamine and sodium hydride in dimethyl formamide, gave the benzylamide (29) in 95% yield, when the reaction was carried out under nitrogen at 100° for five minutes.

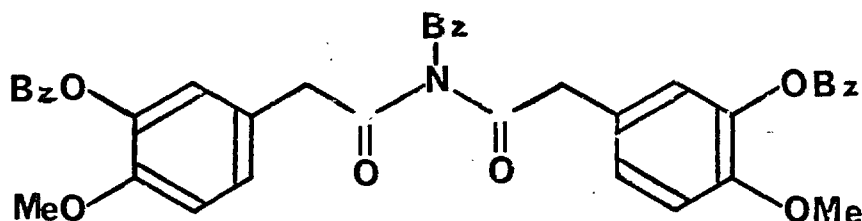
The mechanism of its formation has not been studied but it is conceivable that it takes place through the formation

of the aroyl cyanide (30). This would then react with benzylamine to give the amide (29) although the possibility of formation of the thiolester intermediate (31) has not been disproved. (Scheme IV).



SCHEME IV

Conceivably the aroyl cyanide (30) could be used to further alkylate the amide (29). However, all attempts to prepare the di-amide (32) failed. Consequently, the amide was first reduced to the amine. The amide (29) was refluxed with lithium aluminium hydride in tetrahydrofuran to give the benzylethylamine (33) in 75% yield.



(32)

The diphenyl ethylamine skeleton required for the synthesis of erysodienone was finally achieved when the secondary amine (33) was used to capture the aroyl cyanide (30) or the thiol ester (31), thus generating the required amide (34). The tertiary amide (34) has been synthesised before at Imperial College⁴ and all the spectroscopic data reported corresponded with those found in compound (34).

Finally, reduction and debenzylation of (34) following the method previously described⁴ gave bisphenylethylamine (2). Scheme V summarises all the results described above.

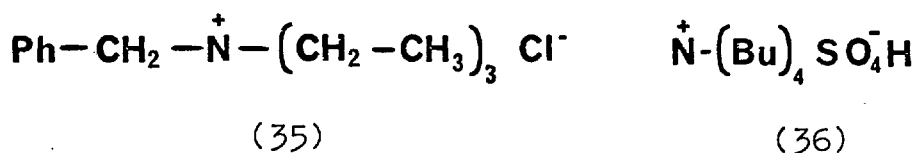
The last step of the synthesis was the phenolic oxidation of bis-[2-(3-hydroxy-4-methoxyphenyl)ethyl]amine (2) to erysodienone (4). The reaction has been done with potassium ferricyanide as the oxidizing agent, in a two phases chloroform-water system and in the presence of 5% sodium hydrogen carbonate, with a yield of about 35%. The method requires the use of high dilution technique so as to favour intramolecular over intermolecular coupling. The mechanism of this oxidative cyclisation is not yet fully understood and studies on various aspects of this phenomenon are still in progress.

In a search for a possible way of improving the yield of the phenol oxidation step, the use of two recently reported oxidizing agents, a new iron complex $[\text{Fe}(\text{D.M.F.})_3 \text{Cl}_2]^+ \text{FeCl}_4^-$ ²⁸ and palladium dichloride were tried²⁹. Both methods were unsuccessful and no erysodienone was ever detected among the products of the reaction.

The development of phase transfer catalysis³⁰ as a method for increasing the contact of organic and inorganic systems, suggested the possibility of applying this method to the two phase system necessary for the synthesis of erysodienone (4). In the event, when the oxidation reaction of the diphenol (2) was carried out under exactly the same conditions as previously reported⁴ but with the addition of few mg. of benzyltriethylammonium chloride³¹ (35), the yield of the reaction was increased to 44%.

The use of varying amounts of the phase transfer catalyst did not produce any alteration in the yield formerly obtained.

Increase or diminution of the amount of the oxidizing agent, variation in the temperature (0° or 60°) and reduction of volume of the organic solvent, always brought diminution of the yield of erysodienone. As no other improvement was obtained with benzyltriethylammonium salt (35) another salt, tetrabutylammonium bisulfate (36) was tried in an attempt to produce a greater dissolution of the ferric salt into the organic layer and perhaps facilitate reaction. The increase in solubility was confirmed when blank experiments were carried out with both salts. It was clear from a qualitative assessment of the colour, that the tetrabutyl-ammonium salt produced a larger dissolution of the ferricyanide into the chloroform layer.



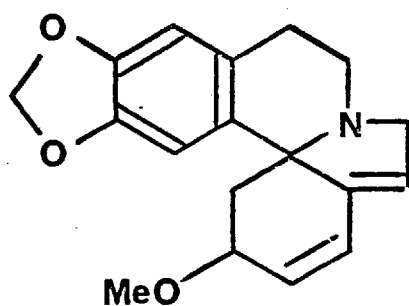
However, when the phenolic oxidation was repeated employing tetrabutylammonium bisulfate as the phase transfer catalyst, the yields obtained of erysodienone dropped to 27%.

These results seem to be in agreement with the fact that large increases in the concentration of oxidizing agent in the organic phase would produce increases in the number of oxidations products and consequently the yield for erysodienone would decrease.

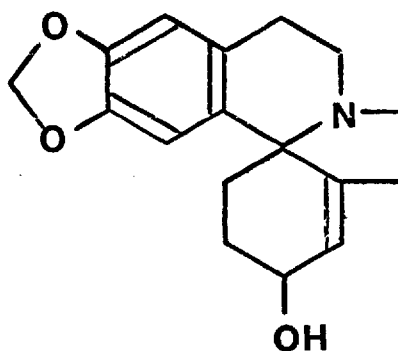
Under the conditions now established, erysodienone can be synthesised from O-benzylisovanillin in seven steps in 12% overall yield. The process is simpler than the reported ones and represents a real improvement where larger amounts of dienone are required (see below).

The Resolution of ($\pm$)-erysodienone

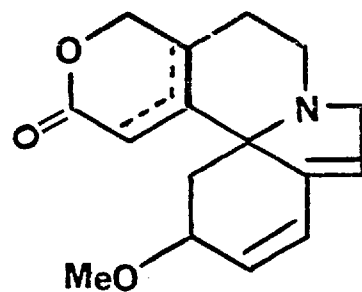
The incorporation of ($\pm$)-erysodienone (4) into erythraline (37)^{4,5}, erythratine (38)²¹ and α -and β -erythroidines (39)³⁰ have left no doubt of its importance as an intermediate in the biosynthesis of the Erythrina alkaloids. Consequently, it was of great interest to know which of the enantiomers was the precursor. The stereospecific incorporation of one optical form of erysodienone would confirm the essential correctness of the above conclusion³¹. The resolution of



(37)



(38)



(39)

erysodienone had been unsuccessfully attempted^{32, 33}.

Its failure to form salts with nitro-tartranillic acid or dibenzoyl-(±)-tartaric acid and its ability to do so with (+)-camphor-10-sulfonic acid suggested that it was possible that the dienone system reduces the basicity of the nitrogen atom causing erysodienone to form salts only with very strong acids such as sulfonic acids³³.

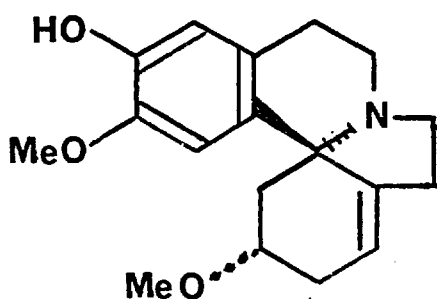
(±)-Erysodienone treated with (+)-camphor-10-sulfonic acid in chloroform, produced a crystalline salt. This was submitted to a fractional crystallisation procedure and gave crystals with optical rotations up to $[\alpha]_D + 27^\circ$ (c 1.2 in chloroform). The camphorsulphonic acid itself would only have contributed 10° to this so it was evident that resolution, at least in part, had been achieved. The salt with the highest value was basified with aqueous sodium carbonate and the solution extracted with chloroform to yield erysodienone of $[\alpha]_D + 8.2^\circ$ (c 0.92 in chloroform).

This encouraging result led to 3-bromocamphor-9-sulfonic acid³⁴ as the next choice of resolving agent.

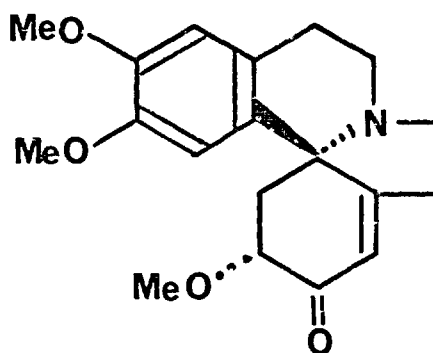
When (±)-erysodienone was refluxed in chloroform with this acid³⁵, evaporation of the solvent gave the salt which was crystallized from a large volume of ethanol in the form of white needles. Recrystallization of the salt to constant rotation finally gave $[\alpha]_D^{20} + 44.8^\circ$ (c 0.98) m.p. $203 - 204^\circ$ (dec.). The resolved erysodienone, generated from this dextro salt, using aqueous sodium hydrogen carbonate as base had $[\alpha]_D^{19} - 27.0^\circ$ (c 0.8). A corresponding treatment of (±)-erysodienone with (-)-3-bromocamphor-9-sulfonic acid³⁶ gave the sulfonate

salt of erysodienone, $[\alpha]_D^{20} - 43.5^\circ$ (c 1.4), m.p. 197-198° (dec.) and thence (+)-erysodienone, $[\alpha]_D^{21} + 25.1^\circ$ (c 0.9).

At this stage there was no guarantee of the optical purity of the compounds derived from this resolution nor any indication of the absolute configuration of the individual isomers. To obtain this information, erysodienone was converted into dihydroerysodine (40) a compound of known configuration and optical rotation³⁷, by means of a long but previously established method, and into erythratidinone (41), a recently isolated and characterised alkaloid³⁸.



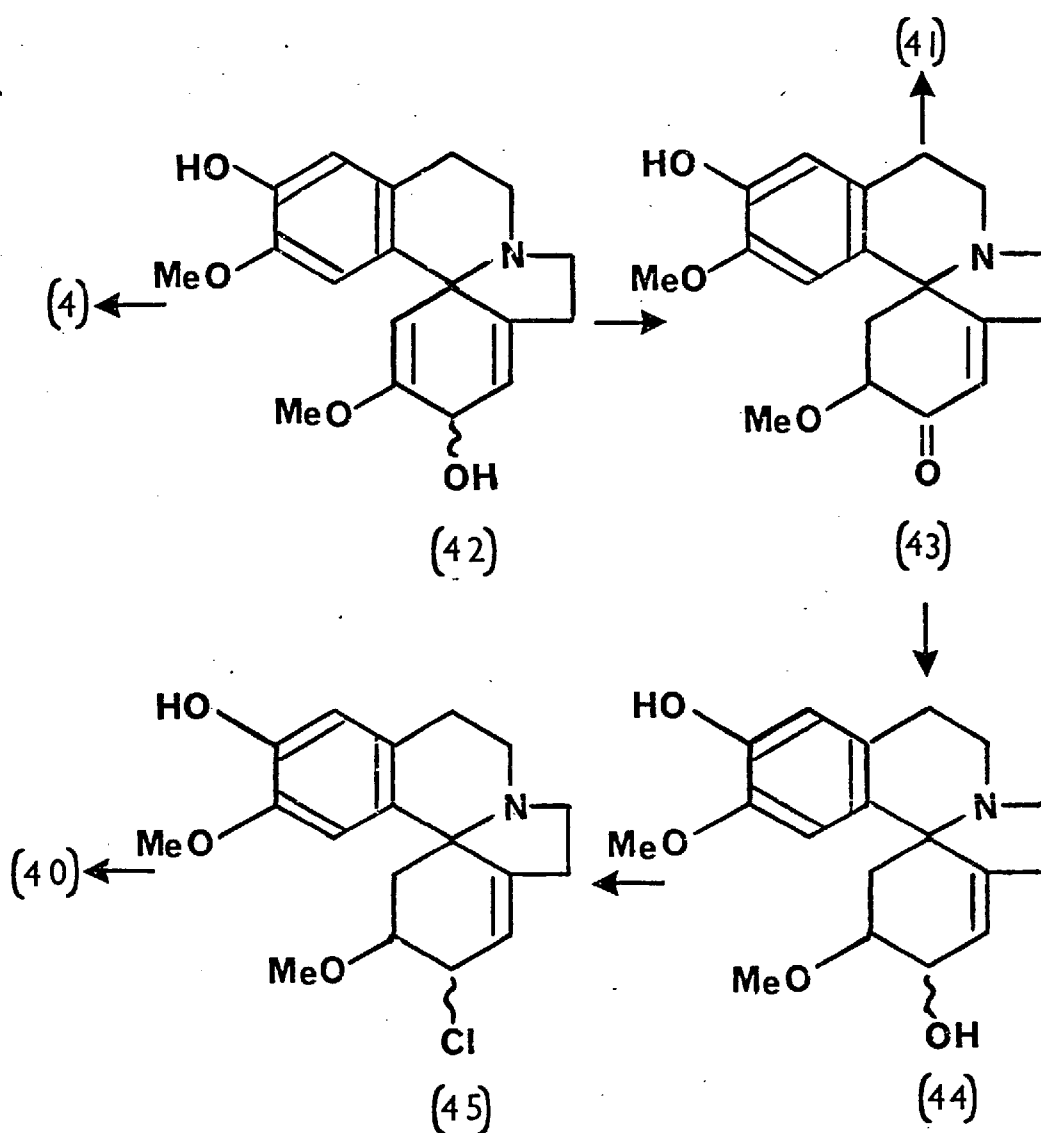
(40)



(41)

Treatment of (±)-erysodienone with sodium borohydride gave a mixture of two dienols (42) which were submitted to rearrangement³², by the use of gaseous hydrogen chloride in

anhydrous ether, to erysotinone (43). This material was divided in two parts. One was treated with diazomethane to give erythratidinone (41) and the second fraction was reduced with sodium borohydride to form erysotine (44). This hydroxyl compound was transformed into the corresponding chloride (45) by means of thionyl chloride. Finally, catalytic hydrogenation of (45) afforded dihydroerysodine (40). (See Scheme VI)¹.

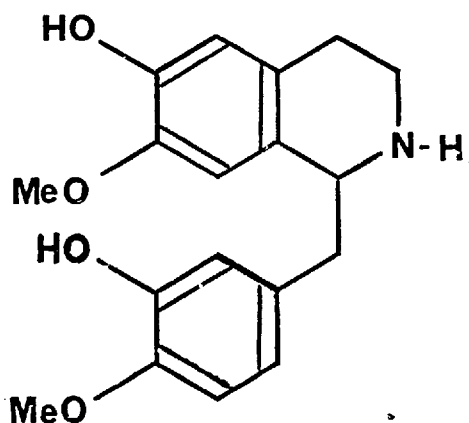


SCHEME VI

This reaction sequence was then repeated using (-)-erysodienone. This isomer gave (+)-erythratidinone, $[\alpha]_D^{21} + 334.7$ (c 1.2) [lit.⁴¹ $[\alpha]_D + 358$ (c 1.1) from natural material] and (+)-dehydroerysodine, $[\alpha]_D^{21} + 218$ (c 0.9 methanol), [lit.³⁷ $[\alpha]_D + 224^{\circ}$ (methanol)]. From these results could be concluded that the resolution of ($\pm$)-erysodienone had proceeded essentially to completion and that the (-)-isomer corresponded to the absolute stereochemistry of the natural series.

Synthesis and optical stability of 5,6,8,9-tetrahydro-2,12-dimethoxy-7H-dibenz[d,f]azonine-3,11-diol (3)

The in vivo incorporation of (+)-N-norprotosinomenine (46) into the Erythrina alkaloids has been successfully demonstrated several times^{4,30,39}. In this process an inversion of chirality of the system was required since a priori (+)-N-norprotosinomenine would be expected to give (5R)-erysodienone. It has been demonstrated however, that the absolute configuration of C-5 of all the natural Erythrina alkaloids is 5S³⁹. A symmetrical intermediate must be involved but it was not known if this was the azonine (3) (which may be optically unstable) or the corresponding diphenoquinone which has not been demonstrated as an in vivo intermediate.



(46)

In order to resolve at least one aspect of this problem, the dibenzazepine (3) was generated from optically active erysodienone and its optical stability established. Resolved erysodienone was reduced by treatment with chromous chloride⁴⁰ at 0°. The reaction was worked up as quickly as possible at low temperature and the optical rotation promptly determined. The magnitude of the rotation decayed rapidly with a half life of ca. 1.2 min. at 20°. As the reduction with chromous chloride at zero degrees took a relatively long time, titanous chloride was tried⁴¹. In a trial run on racemic material, this reducing agent reacted at 0°, faster than had chromous ion, and in improved yield (84%).

The reaction was repeated with optically active erysodienone and the temperature maintained at 0° until the rotation was taken. Again the dibenzazonine obtained racemised rapidly with $t_{\frac{1}{2}} \sim 8.2$ min.

That the dibenzazonine (3) was potentially the symmetrised intermediate was thus established. The possible intermediacy of a diphenoquinone species is still not proven.

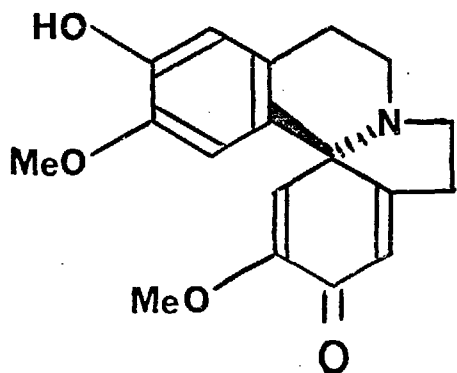
The Biosynthesis of the Erythrina Alkaloids

It has been well established that the radioactivity of ($\pm$)-erysodienone (4), equally labelled with tritium at C-1 and C-17 and equally labelled with carbon-14 in the two methoxy groups was specifically incorporated into erythraline, in young Erythrina crista galli plants³⁹. Similar experiments with Erythrina berteroana plants confirmed a specific incorporation into the erythroidine alkaloids³⁰. These results have left little doubt that erysodienone is a true precursor of the Erythrina alkaloids.

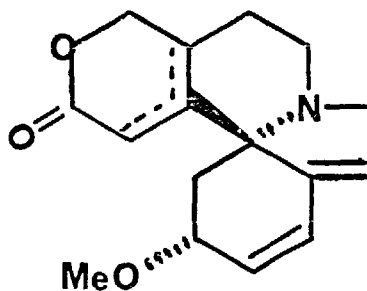
In order to confirm which of the enantiomers of erysodienone was the true precursor of the Erythrina alkaloids, the two enantiomers were labelled with tritium^{4,39}, and fed in parallel, under similar conditions, to E. crista galli and E. berteroana plants. With the exchange procedure used the relative activities in the positions labelled were not necessarily the same³², but from previous work, the tritiated product

was assigned as $[1,7,17-^3\text{H}_3]$ -erysodienone (47).

In E. crista galli, feeding experiments can be satisfactorily performed only during the months of June to September inclusive^{4,39}, and in E. berteroana incorporations of erysodienone have been only observed in first year plants during the end of May and the month of June³⁰. In addition it has been established that in E. crista galli incorporations into erythraline (37) are optimal with four or five days of feeding³², and in E. berteroana optimum incorporation into erythroidines (48) are obtained after feeding for eleven days³⁰. Accordingly, feeding was carried out under these conditions and in both cases the administration of the dienone (47) to the plants was carried out by the cotton wick method using aqueous solutions of the hydrochloride salt.



(47)



(48)

The two enantiomers and the racemic material, labelled with tritium at C-1, C-7 and C-17, were fed at the same time, under identical conditions to six months old E. crista galli and E. berteroana plants for six and fourteen days respectively. Three alkaloids were isolated, erythraline (37) from E. crista galli and an α/β -erythroidine mixture (48) which cocrystallises from E. berteroana. The results are given in Table II. These results indicated that the precursor was being utilised quite stereospecifically and only (-)-erysodienone was acting as an efficient precursor for both erythraline (37) and α/β erythroidine (48).

PRECURSOR	% incorporation	
	Erythraline	α/β -Erythroidine
(+)-[1,7,17- ³ H ₃]-erysodienone	0.0006	0.045
(-)-[1,7,17- ³ H ₃]-erysodienone	0.27	0.33
(±)-[1,7,17- ³ H ₃]-erysodienone	0.10	0.20

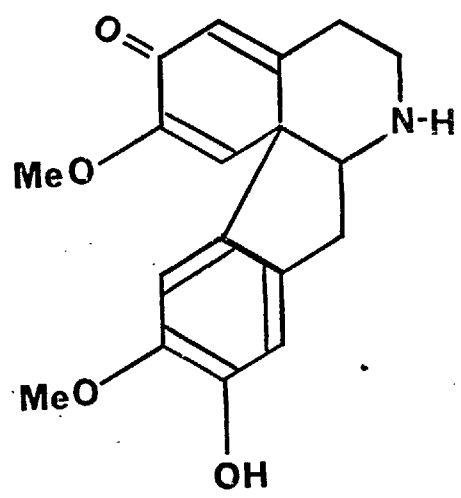
TABLE II

This work has finally resolved the one stereochemical ambiguity in the biogenetic pathway proposed by Barton⁴ for the Erythrina alkaloids. Provided that the dibenzazonine

has a free existence in the plant, the inversion of chirality required for the transformation of (+)-N-nor-protosinomenine into the alkaloids can occur at the dibenzazonine (3) stage, whether or not a diphenoquinone is involved. Certainly, as may have been expected, by the time the erysodienone is formed, the chirality has inverted and the stereochemistry is now shown to be locked in the natural 5-S configuration. The inversion is now simply explained either by racemisation and selection of the antipodal form of the azonine or by a non-stereospecific oxidation of the azonine followed by sterically controlled ring closure to the dienone.

The resolution of ($\pm$)-erysodienone (4) now provides the possibility of isolation of the correct(5 S)-erysodienols (42) which once separated could be fed to confirm if they really lie on the natural biosynthetic pathway to Erythrina alkaloids.

The only major facet which still remains unconfirmed in the proposed biogenesis of Erythrina alkaloids, is the formation of the proerysodienone (49). Here the synthetic work outlined above may be of use in the generation of the basic skeletal units required.



(49)

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus. Mass spectra were recorded on an A.E.I. MS-9 double focussing mass spectrometer, using an electron beam of 70 ev. The samples were introduced by direct probe insertion. The nuclear magnetic resonance (n.m.r.) spectra refer to deuteriochloroform solutions with tetramethylsilane as an internal standard and were recorded on a Varian T-60 spectrometer. Lines appearing as singlets in n.m.r. spectra are denoted by the symbol s. Unless otherwise stated infrared spectra and rotations refer to chloroform, and ultraviolet spectra to absolute ethanol solutions. Column chromatography was on neutral alumina of Brockmann activity III and thin layer chromatography (t.l.c.) on plates prepared using either Merck Aluminium Oxide G.F.₂₅₄ or Silica Gel G.F.₂₅₄. Light petroleum is the fraction boiling between 40-60°.

I. SYNTHESIS OF ERYSDIENONE

N-Hydroxymethylbenzylamine (15).--

Benzylamine (1.07 g.) and 40% aqueous formaldehyde (1 eq.) were shaken overnight. The aqueous layer was then removed and the remaining organic layer was kept in a desiccator over sulfuric acid and under vacuum. After two days the oily liquid was dissolved in ether, dried with anhydrous sodium sulfate and then the solution was left for spontaneous evaporation of the ether until crystalline N-hydroxylamine (15) was obtained (1.14 g., 83%), m.p. 46-47° (lit.²⁰ m.p. 47°), τ 2.69 (5H, s, aromatic protons), 6.53 (2H, s, N-CH₂-Ar) and 6.35 (2H, s, N-CH₂-O-).

5-Benzyl-1,3,5-dioxazine (17).--

N-hydroxymethylbenzylamine (15) (1.0 g.) and 40% aqueous formaldehyde (1 eq.) were shaken overnight. The organic layer was separated and dissolved in ether, dried (Na₂SO₄) and left for spontaneous evaporation of the ether. The dioxazine (17) was obtained as a crystalline material (0.78 g. 60%) m.p. 73-75°, τ 2.66 (5H, s, aromatic protons), 4.76 (2H, s, O-CH₂-O), 5.33 (4H, s, N-CH₂-O), 5.72 (2H, s, N-benzylic protons); $\nu_{\max}$. 925, 1030, 1155 cm⁻¹ mass spectrum 179 (M⁺), 178, 148, 119, 92, 91 (100%) [Found: C, 67.29; H, 7.05; N, 8.09. C₁₀H₁₃NO₂ requires C, 67.04; H, 7.26; N, 7.82%].

Attempted Preparation of N-di(chloromethyl)-benzylamine (9).-

The dioxazine compound (17) (180 mg.) was dissolved in acid-free thionyl chloride (15 ml.) with a trace of lithium chloride. The solution was stirred at room temperature until t.l.c. analysis showed the absence of starting material. The solvent was evaporated under vacuum at room temperature. The residue was extracted twice with dry tetrahydrofuran and the extract evaporated under vacuum. The residue was diluted with tetrahydrofuran (15 ml.) and two equivalents of triphenylphosphine were added. After two days of stirring at room temperature, the t.l.c. analysis showed that the phosphine compound had not reacted.

1-Cyano-1-methoxy-2-(3-benzyloxy-4-methoxyphenyl)ethylene (24).-

(a) To a cooled solution of sodium hydride (240 mg.) in dimethylformamide (5 ml.) and methoxyacetonitrile⁴² (710 mg.) was added a solution of benzylisovanillin (2.42 g.) and methoxyacetonitrile (2 ml.) in dimethylformamide (20 ml.). The mixture was stirred at room temperature for three hours, and poured into water. The aqueous mixture was extracted with ether and the ethereal extract was washed once with water, dried (Na_2SO_4) and finally evaporated under reduced pressure. The residue was separated by preparative t.l.c. to give the adduct (22) (2.03 g., 67%) τ 2.21 (1H, s, -OH), 2.60-3.33 (8H, complex, aromatic protons) 4.98

(2H, s, O-CH₂ Ar), 5.3 (1H, m, $\text{-}\overset{\text{CN}}{\underset{\text{OMe}}{\text{HC}}}$ and 6.0 (1H, m, $\text{-}\overset{\text{OH}}{\underset{\text{H}}{\text{C}}}\text{-Ar}$), 6.26 (3H, s, O-Me), 6.63 (3H, s, OMe) $\nu_{\text{max.}}$ 3600, 2850, 2250, 1600 cm⁻¹.

To a cooled mixture of the adduct (22) (0.471 g.) and pyridine (1.5 ml.), thionylchloride (0.4 ml.) was added and left overnight with stirring. The excess of thionyl chloride was removed under reduced pressure and the remaining black oil was diluted with water (50 ml.). The mixture was extracted with ether to give the chloride (23) (0.344 g., 70%) m.p. 109-110°, without further purification, this compound (250 mg.) was refluxed in benzene (2 ml.) with a few drops of triethylamine for one hour giving the cinnamionitrile (24) (200 mg., 90%).

(b) Methoxyacetonitrile (710 mg.) was added dropwise to a cooled solution of sodium hydride (240 mg.) on freshly distilled dimethylformamide (20 ml.) under an atmosphere of nitrogen. Once the sodium hydride had reacted, benzylisovanillin (2.42 g.) in methoxyacetonitrile (2 ml.) and

dimethylformamide (10 ml.) were added, together with some triethylamine (1 ml.) and the mixture heated at 110° until t.l.c. analysis showed that all the aldehyde had reacted. The mixture was poured into water (500 ml.) and extracted with benzene-light petroleum ether. The combined extracts were washed once with water (50 ml.) dried (Na₂SO₄), and evaporated under reduced pressure. The residue was chromatographed over alumina with benzene as eluent to give the cinnamionitrile (24) (24 g.), 81% m.p. 85-87° (from CHCl₃/pet. ether), τ 2.66-3.30 (8H, complex, aromatic protons), 3.96 (1H, s, olefinic proton), 4.82

(2H, s, O-CH₂ Ph), 6.10 (3H, s, Ar-O-Me), 6.20 (3H, s, O-Me);
 $\nu_{\text{max.}}$ 2,250, 1,520, 1275, 1218, 1150, 1020 cm^{-1} , $\lambda_{\text{max.}}$ 208 nm
 (ϵ 26800), 233 (ϵ 18,950), 293 (ϵ 21,200) and 315 nm (ϵ 19,600),
 mass spectrum M^+ 295 with the major peak at m/e 91 (100%)
 [Found: C, 73.07; H, 5.58; N, 4.46 $\text{C}_{18}\text{H}_{17}\text{NO}_3$ requires
 C, 73.22; H, 5.76; N, 4.74%].

3-(3-Benzoyloxy-4-methoxyphenyl)-Glutaric acid-N-N-dimethyl-
diamide (25).-

Benzylisovanillin (24.2 g.) in freshly distilled dimethyl acetamide (100 ml.) was added to a cooled solution of methoxyacetonitrile (21.0 g.) and sodium hydride (2.80 g.) in a mixture of dimethyl acetamide - hexamethylphosphoric triamide (5:1) (100 ml.) under an atmosphere of nitrogen. The reaction mixture was heated at the steam bath during 48 hours. The mixture was then poured into water and the diamide (25) precipitated (34.8 g., 88%) m.p. 152-3° (from CHCl_3 /pet. ether), λ 2.54-3.38 (8H, complex, aromatic protons), 4.82 (2H, s, O-CH₂ Ar), 6.17 (3H, s, O-Me), 7.10 (12 H, s, N-Me), 7.23-7.70 (4H, complex, -CH₂-), $\nu_{\text{max.}}$ 3000, 1640, 1250, 1150 cm^{-1} , $\lambda_{\text{max.}}$ 280 (ϵ 380), 230 (ϵ 850) and 215 nm (ϵ 1,750), mass spectrum M^+ 398 (100%) and significant peaks at m/e 312, 262, 234, 220, 91. [Found: C, 69.44; H, 7.46; N, 6.92. $\text{C}_{23}\text{H}_{30}\text{N}_2\text{O}_4$ requires C, 69.32; H, 7.59; N, 7.03%].

Benzyl homoisovanillic acid (20).-

The enol-ether (24) (200 mg.) was stirred with powdered sodium hydroxide in freshly distilled dimethylformamide and benzylmercaptan (84 mg.) and sodium hydride (20 mg.) was added under an atmosphere of nitrogen. The mixture was heated at 110° for two hours. The cooled reaction mixture was poured into water and treated with gaseous carbon dioxide. The aqueous phase was separated, cooled and acidified with 10% aq. HCl and then extracted with ether. The ether extraction was dried (Na_2SO_4) and concentrated to give the acid (143.8 mg., 78%) m.p. $125-8$ (lit.⁴³ $128-9^{\circ}$) τ 2.60 (5H, s, aryl H), 3.00-3.40 (3H, complex, aryl-H), 4.87 (2H, s, $\text{O}-\underline{\text{CH}_2}-\text{Ph}$), 6.12 (3H, s, $\text{O}-\underline{\text{Me}}$), 6.40 (3H, s, $-\underline{\text{CH}_2}-$), 6.47 (1H, s, $-\text{CO}_2\text{H}$), ν_{max} 3000, 2,500, 1700, 1600, 1440, 1,280, 920 cm^{-1} , mass spectrum M^+ 272 (100%) with significant peaks at m/e 227, 213, 181, 137, 106, 91.

N-Benzyl-N-[2-(3-benzyloxy-4-methoxyphenyl)]acetamide (29).-

The cinnamionitrile (24) (735 mg.) and benzylamine (266 mg.) in freshly distilled dimethylformamide (3 ml.) were treated with benzylmercaptan (309 mg.) and sodium hydride (78 mg.) in dimethylformamide (5 ml.), under an atmosphere of nitrogen. The mixture was heated for five minutes at 100° , cooled at room temperature and poured into water, to precipitate the amide (29) (854.4 mg., 95%) m.p. $136-138^{\circ}$ (CHCl_3 /pet. ether), τ 2.69-2.83 (10 H, complex, aromatic protons), 3.23 (3H, s,

aromatic protons), 4.13 (1H, m, exchangeable with D_2O , NH), 4.96 (2H, s, $O-CH_2-Ph$), 5.66 (2H, d, $-CH_2-NH$), 6.16 (3H, s, $O-Me$) 6.53 (2H, s, $Ar-CH_2-\overset{O}{\underset{||}{C}}-$); ν_{max} . 3,420, 2,850, 1660, 1530, 1270 cm^{-1} ; mass spectrum M^+ 361 (100%) with significant peaks at 270, 255, 227, 228, 167, 137, 106, and 91. [Found: C, 76.37; H, 6.19; N, 3.61. $C_{23}H_{23}NO_3$ requires C, 76.43; H, 6.41; N, 3.87%].

N-Benzyl-N-2(3-benzyloxy-4-methoxyphenyl)ethalamine Hydrochloride (33).

The amide (29) (1.2 g.) in dry tetrahydrofuran (50 ml.) was added dropwise to a slurry of lithium aluminium hydride (300 mg.) in T.H.F. (10 ml.), and refluxed overnight. The mixture was carefully poured into water (100 ml.) and extracted several times with ether (10 x 15 ml.). The combined ethereal solutions were dried (Na_2SO_4), the solvent evaporated under reduced pressure, and the residue, in ethanol, saturated with gaseous hydrogen chloride. Addition of ether precipitated the amine hydrochloride (33) (0.95 g., 75%) m.p. 208-210° ($CHCl_3$ /pet. ether) (lit.² m.p. 208-211°) τ 2.46-2.86 (10 H, complex, aromatic protons), 3.27 (3H, s, aromatic protons), 4.90 (2H, s, $O-CH_2-Ph$), 6.13 (3H, s, $O-Me$), 6.26 (2H, s, $N-CH_2-Ph$) and 7.27 (4H, m, $-CH_2CH_2-$), ν_{max} . (nujol) 3,400, 300 2600, 1610, 1595 cm^{-1} ; mass spectrum M^+ 345 and significant peaks at 345, 254, 243, 228, 137 (100%), 103, and 91 (100%).

N-Benzyl-N-[2-(3-benzyloxy-4-methoxyphenyl)ethyl]-(3-benzyloxy-4-methoxyphenyl)acetamide (34).-

The cinnamionitrile (24) (231 mg.) in freshly distilled dimethylformamide (5 ml.) was added dropwise to a solution of benzylmercaptan (97 mg.) and the ethylamine compound (33) (300 mg.) with sodium hydride (56 mg.) in dimethylformamide (2 ml.) under an atmosphere of nitrogen. The mixture was stirred until all the enol-ether had reacted (t.l.c. analysis), then poured into water and extracted with ether. The combined extracts were washed once with water, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was dissolved in chloroform and addition of a few ml. of light petroleum precipitated the amide (376 mg., 80%) m.p. 140-141°. τ 2.63-3.34 (2H, aromatic protons), 4.96 (2H, s, $-\text{O}-\text{CH}_2\text{Ph}$) 5.06 (2H, s, $\text{O}-\text{CH}_2\text{-Ph}$), 5.63 (2H, m, $\text{N}-\text{CH}_2\text{-Ph}$), 6.27 (6H, s, $\text{O}-\text{Me}$), 6.43-6.93 (4H, m, $-\text{CH}_2\text{CH}_2$) and 7.30 (2H, AB quartet $\text{J } 8\text{Hz}$ $-\text{CO}-\text{CH}_2\text{-Ar}$), mass spectrum M^+ 601 with significant peaks at m/e 361, 240, 227, 120 (100%) 91 (100%).

Erysodienone (4).-

The phenolic amine hydrochloride (2) (354 mg.) and benzyltriethyl ammonium chloride (35) (100 mg.) in chloroform (1 l.) were added to a rapidly stirred solution of potassium ferricyanide (1.32 g.) in 5% sodium hydrogen carbonate solution (100 ml.) under an atmosphere of nitrogen. After 30 minutes the chloroform layer was separated and the aqueous

phase was washed with chloroform. The combined chloroform extracts were washed once with water, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was chromatographed over alumina (10 g.) with chloroform as eluent, to give erysodienone (140 mg., 44%) m.p. $227-229^\circ$ (from CHCl_3 /pet. ether) (lit.² $226-229^\circ$).

THE RESOLUTION OF ERYSDIENONE

Racemic erysodienone (250 mg.) was refluxed with (+)- α -bromocamphor- π -sulfonic acid (250 mg.) in chloroform (75 ml.). Evaporation of the solvent and crystallization of the product from a large volume of ethanol (150 ml.) gave, after several days at 0° , a white crystalline salt of erysodienone-bromocamphorsulfonate. After repeated crystallization the salt gave m.p. $203-204^\circ$ (dec.), and $[\alpha]_D^{20}$ constant at $+44.8^\circ$ (c 0.98). The resolved erysodienone was recovered from this salt by treatment with aqueous sodium hydrogen carbonate and extraction with chloroform. Evaporation of the solvent gave material which had $[\alpha]_D^{19} - 27.0$ (c 0.8).

($\pm$)-Erysodienone, enriched with the (+)-enantiomer, recovered from the above mother liquors, was treated in an analogous manner with (-)- α -bromocamphor- π -sulfonic acid to give the salt, m.p. $197-198^\circ$ (dec.) $[\alpha]_D^{20} - 43.5^\circ$

(c 1.4) and thence (+)-erysodienone $[\alpha]_D^{21} + 25.1^\circ$ (c 0.9).

Conversion of (-)-erysodienone into (+)-erythratidine (41).--

(-)-Erysodienone (100 mg.) in ethanol (5 ml.) was treated with sodium borohydride (110 mg.) with stirring at room temperature until all the erysodienone had reacted (3 hrs.). The solution was evaporated to dryness and the residue treated with water and extracted with chloroform. The extracts were dried (Na_2SO_4) and evaporated to give a white foam (93.5 mg., 95%) m.p. $128-130^\circ$ (lit.¹ m.p. 131°) T.l.c. (silica) showed the product to be a mixture of the two epimeric dienols³².

The mixture of the erysodienols (42) (93 mg.) in absolute methanol (magnesium dried) was cooled on an ice bath and treated with dry ether saturated with gaseous hydrogen chloride (3 ml.). The solution was allowed to warm to room temperature and left overnight. Evaporation gave a white foam which was treated with sodium hydrogen carbonate solution and extracted with chloroform. The extracts were dried (Na_2SO_4) and evaporated at reduced pressure. The residue was separated by preparative t.l.c. (silica) to give erysotinone (43), which crystallized from acetone, m.p. $196-198^\circ$ (lit.¹ m.p. $196-197^\circ$) (600 mg., 64%).

Finally, erysotinone (30 mg.) in methanol (2 ml.) was treated with an ice-cooled ethereal solution of diazomethane

(6 ml.) and left overnight at 0°. The mixture was evaporated to ca. 1/3 of its volume and the residue separated by t.l.c. using benzene/ethylacetate (1/2) to give (+)-erythratinone as pale yellow needles (20.0 mg., 67%) m.p. 160-161° (120-121°), $[\alpha]_D^{25} + 334.7$ (c 1.2), (lit.³⁸ m.p. 119-120°, $[\alpha]_D + 358^\circ$).

Conversion of (-)-erysodienone into (+)-dihydroerysodine (40).-

Erysotinone (43) (29 mg., obtained as before from (-)-erysodienone), in ethanol (2 ml.) was treated with sodium borohydride (30 mg.) with stirring at room temperature for three hours. The solution was evaporated to dryness and the residue treated with water and extracted with chloroform. The extracts were dried (Na₂SO₄) and evaporated to give the alcohol (44) (23.3 mg., 80%) m.p. 189-190° (lit.¹ 188°). Erysotone (44) (23.0 mg.) in dry pyridine (1 ml.) was stirred overnight at 0°, then at room temperature for four hours. The mixture was diluted with water (40 ml.) neutralized with aqueous sodium carbonate and extracted with chloroform (10 x 10 ml.). The extract was dried (Na₂SO₄) and the solvent evaporated under reduced pressure. T.l.c. of the residue gave the chloride (45) (15.8 mg., 65%), m.p. 175-177° (lit.¹ m.p. 177°).

The chloride (45) (15.0 mg.) in methanol (2 ml.) containing 10% palladium on charcoal (5 mg.), was exhaustively hydrogenated (20 min.) at atmospheric pressure and room temperature. The solution was heated to boiling, filtered

hot, and the catalyst washed thoroughly with hot methanol. The combined filtrates were evaporated to dryness and the residue recrystallised from acetone to give dihydroerysodine (40) (13.0 mg., 96%) m.p. $206-208^{\circ}$, $[\alpha]_D^{21} + 218^{\circ}$ (c 0.9 MeOH) [lit.⁴⁰ m.p. $208-209^{\circ}$, $[\alpha]_D + 224^{\circ}$ (MeOH)].

PREPARATION OF THE AZONINE (3)

(a) Partially resolved erysodienone (30 mg.) was stirred with a 1M-solution of chromous chloride in 3% hydrochloric acid (6 ml.) under nitrogen at 0° (1.5 hrs.). Air was allowed to enter the system and the pH was adjusted to ca. 9 with sodium carbonate. The precipitated solid was filtered off and washed thoroughly with ethanol. The filtrate was extracted with chloroform and the combined chloroform and ethanolic extracts were evaporated. The residue was treated with water and re-extracted with chloroform. All manipulations were carried out in a cold room at 0° . Finally, the chloroform solution was dried (Na_2SO_4) and evaporated at reduced pressure at zero degrees to give the azonine m.p. $220-22^{\circ}$ (lit. m.p. $222-224^{\circ}$). At room temperature the compound racemised rapidly ($t_{\frac{1}{2}} \approx 1.2$ min.).

(b) A reduction with titanous chloride was made as for

the chromous chloride process described above. Polarimetric studies were made on this sample at 0° . Racemisation was again rapid ($t_{\frac{1}{2}} \approx 8.2$ min) and no estimate of the specific rotation of optically pure material could be made.

II THE SYNTHESIS AND BIOTRANSFORMATION OF LABELLED ERYSDIENONE

Counting of Radioactive Compounds.-

Radioactive assays were made, unless otherwise indicated, on a Nuclear Enterprises Ltd. N.E. 8310 Liquid Scintillation Counter using a solution of the active compound (ca. 0.5 mg. or 10 μ l of 0.005% solution depending on activity), in analar methanol (2 ml.) and Nuclear Enterprises Ltd. type 240 scintillation liquid (8 ml.). Internal standardisation with $[1 - ^{14}\text{C}]$ -n-hexadecane and $[1,2 - ^3\text{H}_2]$ -n-hexadecane allowed the counting efficiency to be determined for each sample.

(+), (-) and (+)- $[1,7,17 - ^3\text{H}_3]$ -Erysodienone.-

The optically active forms and the racemic material, (50 mg.) were dissolved separately, in redistilled N-dimethyl-

acetamide (1 ml.) and tritium oxide (0.1 ml. of activity 5.0 Ci/ml. or 0.3 ml. of activity 0.2 Ci/ml.) was added. The solution was sealed under nitrogen and kept at 118° for three days. The solvents were evaporated in vacuo. The residue was treated with 5% sodium hydrogen carbonate solution and extracted repeatedly with chloroform. The extracts were dried (Na_2SO_4) and evaporated, the residue was separated on t.l.c. to give the appropriate erysodienone ($[\alpha]_D - 27.0$ and $[\alpha]_D + 25$) m.p. $216-220^{\circ}$ (lit.² m.p. $226-230^{\circ}$). The activities were 2.16 and 3.28×10^6 ; 10.61 and 27.69×10^6 ; and 4.12 and 9.10×10^6 dps. mg^{-1} for the (+)-, (-)- and (+)-isomers fed to E. crista galli and E. berteroana respectively.

FEEDING EXPERIMENTS

General Method³⁵.-

The precursor as its hydrochloride salt was dissolved in water (2 ml.) and the solution was divided equally between two small sample tubes which were fixed to the stem of healthy first year (six months) E. crista galli and E. berteroana plants. Into each tube was placed the ends of a length of untreated cotton which had been threaded through the stem of the plants. Watering of the plant was stopped and the precursor allowed to pass via the cotton into the plant. After all the solution had passed, distilled water was added to the tubes to wash in the last traces of precursor.

This process was repeated several times. Watering was continued after four days or when the plants started wilting, whichever came sooner, and the plants harvested after six days for E. crista galli and fourteen days for E. berteriana.

General work up Procedure.-

The whole plant was macerated in 0.03 N-hydrochloric acid (300 ml.) and then stirred at room temperature for three days. The mixture was filtered through Celite and the residues reextracted with 0.03 N-hydrochloric acid (200 ml.) for two days. After filtration, the two filtrates were combined and adjusted to pH 8 with solid sodium hydrogen carbonate. The solution was then extracted with chloroform (10 x 50 ml.) and the extracts dried (Na_2SO_4). Evaporation of the solvent under reduced pressure gave a gum from which, after dilution with inactive material, the desired alkaloid (erythraline or α/β -erythroidine) was separated by preparative t.l.c. The alkaloids were converted into their hydrobromide or hydrochloride salts and recrystallised until constant specific radioactivity was obtained.

Extraction of inactive erythraline (37).-

Fifteen E. crista galli plants (> 1 year) (367 g.) were macerated and stirred with 0.03 N-hydrochloric acid

(2.5 l.) for three days at room temperature. The mixture was filtered through Celite, extracted with chloroform (10 x 250 ml.) and the combined chloroform solutions dried (Na_2SO_4) and evaporated under reduced pressure. The crude alkaloid which was obtained as a brown gum, (0.591 g., 0.16%), was separated on a column of alumina (10%) ethylacetate-benzene) and then on t.l.c. (SiO_2 , 50% ethylacetate-benzene) to give erythraline (37) as its hydrobromide salt (75 mg., 0.02%) m.p. (from ethanol) $245-248^\circ$ (dec.) (lit.² m.p. $240-243^\circ$). The salt was spectroscopically identical to authentic material.

Inactive α - and β -erythroidine (48).-

These compounds were obtained by recrystallisation of available commercial samples.

Erythraline from (+), (-) and ($\pm$)-[1,7,17 - ^3H]-erysodienone.-

The hydrochlorides of (-), (+) and ($\pm$)-erysodienone (1.03 mg., $10.61 \pm 0.2 \times 10^6$ dps; 3.35 mg., $2.15 \pm 0.1 \times 10^6$ d.p.s. and 3.34 mg., $4.12 \pm 0.1 \times 10^6$ d.p.s. respectively) were each fed to one E. crista galli plant on 31/5/73. After six days the whole plants (21, 20 and 18 g. respectively, wet weight) were worked up

as above.

The three samples of erythraline thus isolated were each diluted with inactive erythraline (31.7, 16.5 and 21.26 mg. respectively), and converted into their hydrobromide salts. Each sample was then recrystallised several times to a constant specific activity. From (-)-erysodienone the erythraline obtained showed an activity of 899 ± 6.5 d.p.s./mg., 0.27% incorporation. That from ($\pm$)-erysodienone showed a constant specific activity of 199.4 d.p.s./mg., 0.10% incorporation. Finally the activity of the erythraline obtained from (+)-erysodienone was too low to be accurately assayed, corresponding to less than $10^{-3}\%$ incorporation.

α/β -Erythroidines from (+), (-) and ($\pm$)-[1,7,17- $^3\text{H}_2$]-erysodienone

The hydrochlorides of (-), (+) and ($\pm$)-erysodienone (2.70 mg., $27.7 \pm 0.4 \times 10^6$ d.p.s., 5.11 mg., $3.28 \pm 0.3 \times 10^6$ d.p.s. and 7.35 mg., $9.10 \pm 0.1 \times 10^6$ d.p.s. respectively) were each fed to one E. berteriana plant on 31/5/73 and harvested on 13/6/73. Dilution of each sample (obtained as above) with inactive α -erythroidine (47.6, 40.3 and 40.8 mg. respectively) and conversion into their hydrochlorides salts gave the crude alkaloid. Each sample was then recrystallised several times. That from (-)-erysodienone showed a constant specific activity of 1913 ± 12 d.p.s., 0.33% incorporation. That from ($\pm$)-erysodienone

had 467.0 ± 6.5 d.p.s., 0.20% incorporation and that from (+)-erysodienone showed a low activity consistent with an incorporation of $\leq 0.045\%$.

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