April, 1952. Robert Pascoe.

Experiments in the Synthesis of Folic Acid Analogues containing Modified Ring Systems.

The main object of the work described in this thesis is the preparation, for ultimate biological testing, of analogues of folic acid based on the 1:3:5- and 1:3:8-triazanaphthalene ring systems.

Owing to the scanty information available on the chemistry of these ring systems a study has been made of methods for their synthesis and of methods for the modification of substituents in these systems; a number of derivatives of both ring systems, including the parent compounds, have been prepared and characterised. Based on this work methods have been developed for the synthesis of 1:3:5- and 1:3:8-triazanaphthalenes containing substituents in the 8- position suitable for the introduction of the folic acid side chain and progress has been made in the synthesis, along these lines, of the required folic acid analogues.

Naphthalene analogues of folic and pteroic acid have been made.

EXPERIMENTS IN THE SYNTHESIS OF ANALOGUES OF FOLIC ACID CONTAINING MODIFIED RING SYSTEMS

A THESIS

PRESENTED BY

ROBERT PASCOE, B.Sc., A.R.C.S.

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October 1952.

Imperial College of
Science and Technology,
London, S.W.7.

To my father and mother.

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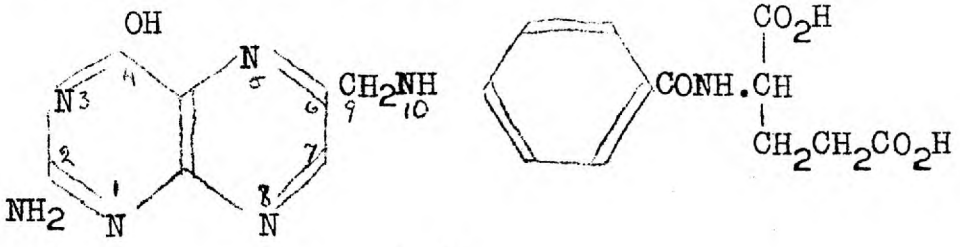
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The Folic Acid Group of Vitamins.

The isolation of the folic acid group of vitamins allowed a large number of earlier observations to be rationalised and correlated. The earliest observation of biological activity which can now be attributed to folic acid was that made in 1931 on the effect of a yeast extract in relieving a type of nutritional anaemia in pregnant women (Wills, Brit. Med. J., 1931, 1, 1059). This type of anaemia was later reproduced in monkeys and the factor responsible for alleviating the symptoms termed Vitamin M. (Day, Langston and Shukers, J. Nutrition, 1935, 9, 637.) Other early work includes that of Stokstad and Manning (J. Biol. Chem., 1938, 125, 687) who reported the necessity of a substance termed "Factor U" for the normal growth of chicks and that of Hogan and Parrott (J. Biol. Chem., 1940, 132, 507) who reported an active principle (Vitamin Bc) to be necessary for the prevention of nutritional anaemia in chicks. In 1939, Snell et al. (Snell and Peterson, J. Biol. Chem. 1939, 128, xciv; Mitchell, Snell and Williams, J. Amer. Chem. Soc., 1941, 63, 2284) described a "norite eluate factor" which was essential for the growth of Lactobacillus casei and Steptococcus faecalis R. The growth of these micro-organisms formed the basis of a convenient method of microbiological assay which was used to follow the final concentration of the folic acid group of vitamins from

natural sources. Folic acid is widely distributed in nature, the best sources being liver, yeast and fresh green leaves, particularly spinach. Mitchell, Snell and Williams suggested the name "Folic Acid" (Latin folium = leaf) after the last named source.

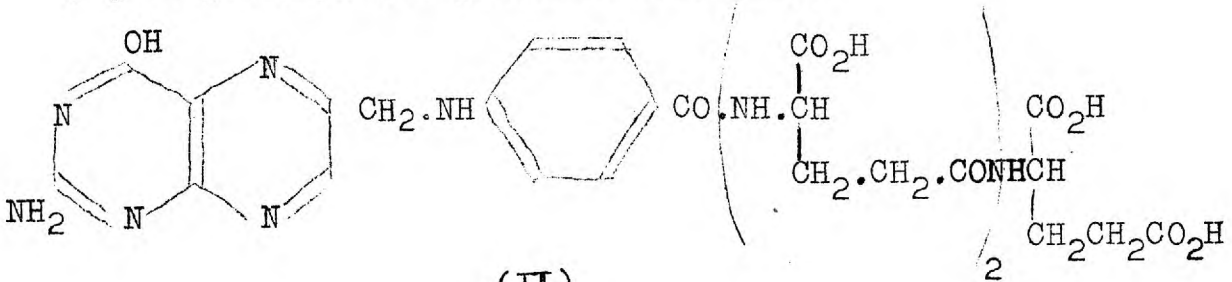
The first pure crystalline substance belonging to the folic acid group of B vitamins was isolated from liver by Pfiffner et al. (Pfiffner, Binkley, Bloom and O'Dell, J. Amer. Chem. Soc., 1947, 69, 1476) and shortly after Stokstad et al. (Stokstad, Hutchings and SubbaRow, J. Amer. Chem. Soc., 1948, 70, 3) isolated the same substance from liver. Mitchell, Snell and Williams (J. Amer. Chem. Soc., 1944, 66, 267) isolated the same substance in a substantially pure form from spinach. The structure of this substance which was called the "Liver L. casei factor" and is now usually referred to as "folic acid" was rapidly established by degradation and synthesis (Mowat et al., J. Amer. Chem. Soc., 1948, 70, 14, 19, 23, 25, 27) as N-pteroyl-L-glutamic acid (I).



(I)

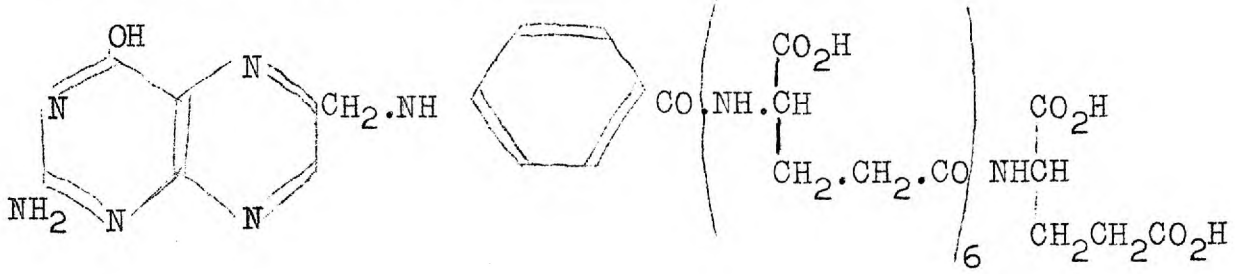
At the same time a second crystalline factor was

isolated from the culture medium of an unidentified organism belonging to the genus Corynebacterium (Stokstad, Hutchings, Bohonos, Sloane and SubbaRow, J. Amer. Chem. Soc., 1948, 70, 1). This factor was termed the "Fermentation L. casei factor" and is also known as "teropterin". Its structure was established by synthesis as N-pteroyl-di(-γ-L-glutamyl-)-L-glutamic acid (II).



(II)

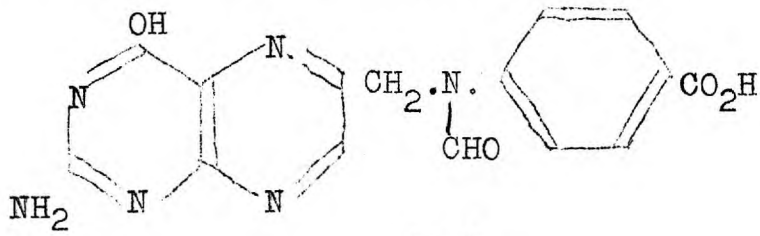
Another crystalline compound which prevents anaemia in chicks but is not appreciably active as a growth factor for L. casei or Str. faecalis R was isolated from yeast. (Pfiffner, Calkins, Bloom and O'Dell, J. Amer. Chem. Soc., 1946, 68, 1392). This substance, known as "Vitamin Bc conjugate", was shown to be N-pteroyl-hexa-(-γ-L-glutamyl)-L-glutamic acid (III).



(III)

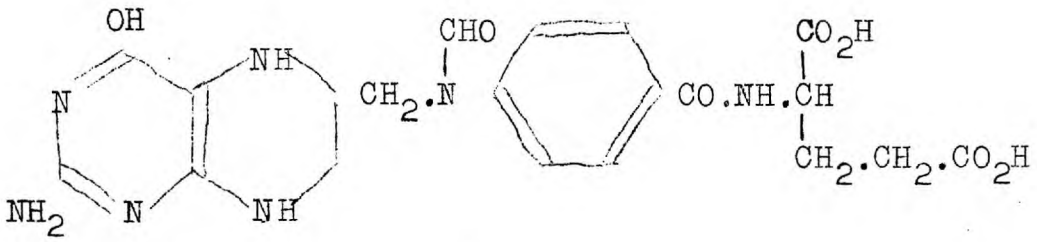
Vitamin Bc conjugate is readily hydrolysed to folic acid by an enzyme widely distributed in animal tissues.

A fourth substance, which was active in promoting the growth of Str. faecalis R but inactive for L. casei, was isolated from the fermentation liquors of Rhizopus nigricans and called "rhizopterin". On hydrolysis it gave pteroic acid and formic acid and synthesis showed it to be N^{10}-formyl-pteroic acid (IV).



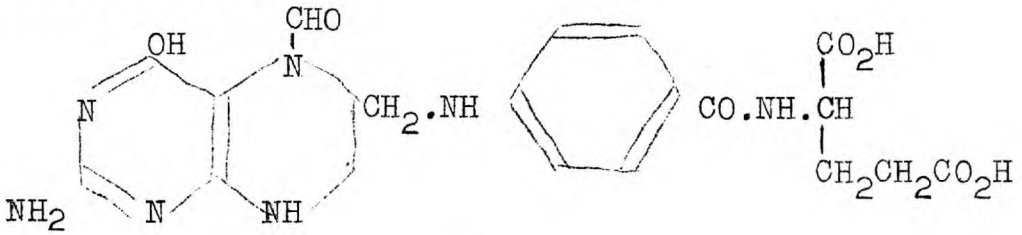
(IV)

A fifth crystalline member of the folic acid group was isolated from liver, peptone and yeast and called "folinic acid". (Sauberlich and Baurnam, J. Biol. Chem., 1948, 176, 165). This substance was synthesised by Stokstad et al. (Brockman, Roth, Broquist, Hultquist, Smith, Fahrenbach, Cosulich, Parker, Stockstad and Jukes, J. Amer. Chem. Soc., 1950, 72, 4325) by formylating pteroyl-glutamic acid and hydrogenating the product. On the basis of this synthesis the structure assigned to folinic acid was 5:6:7:8-tetrahydro-N^{10}-formyl-pteroyl-glutamic acid (V).



(V)

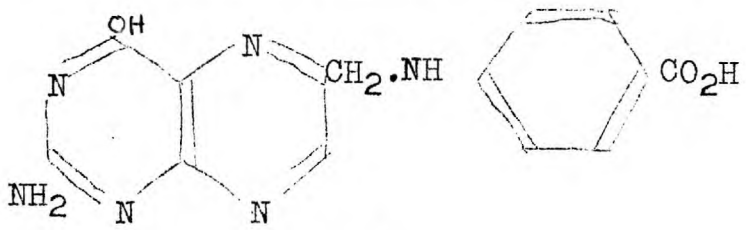
Later work on this substance (May, Bardos, Berger, Lunsford, Rewel, Sutherland and Shive, J. Amer. Chem. Soc., 1951, 73, 3067) suggests that the formyl group is on N^5, which accounts for the observation that reduction of N^{10} formyl-folic acid does not give folinic acid until the reaction mixture is heated. The accepted structure for folinic acid is 5:6:7:8-tetrahydro-N^5-formyl-pteroyl-glutamic acid (VI).



(VI)

Pteroic acid (VII) may be considered as a member of the folic acid group of vitamins although it is only known as a synthetic product and as a hydrolysis product of the more complex vitamins. It is prepared by the condensation of 2:4:5-triamino-6-hydroxy-pyrimidine, 1:2-dibromo-propionaldehyde and p-amino-benzoic acid. (Waller, Hutchings,

Mowat, Stokstad, Boothe, Angier, Semb, SubbaRow, Cosulich, Fahrenbach, Hultquist, Kuh, Northey, Seeger, Sickals and Smith, J. Amer. Chem. Soc., 1948, 70, 19).



(VII)

The gross biological effects of folic acid deficiency are fairly well known. Folic acid deficiency in man usually manifests itself in some form of anaemia which is readily relieved by the administration of folic acid. Chicks on a folic acid deficient diet fail to grow properly and become anaemic. Rats normally require no external source of folic acid but if intestinal bacteria are killed by feeding sulphaguanidine, then rats on a folic acid deficient diet become anaemic. Those bacteria which can grow on a chemically defined medium appear to require no external source of folic acid.

The relative biological activities of the various members of the folic acid group of vitamins are set out in Table I.

Very little is known about the fundamental biochemical reactions in which folic acid is involved but since all the B vitamins whose functions are known take part in vital

TABLE I

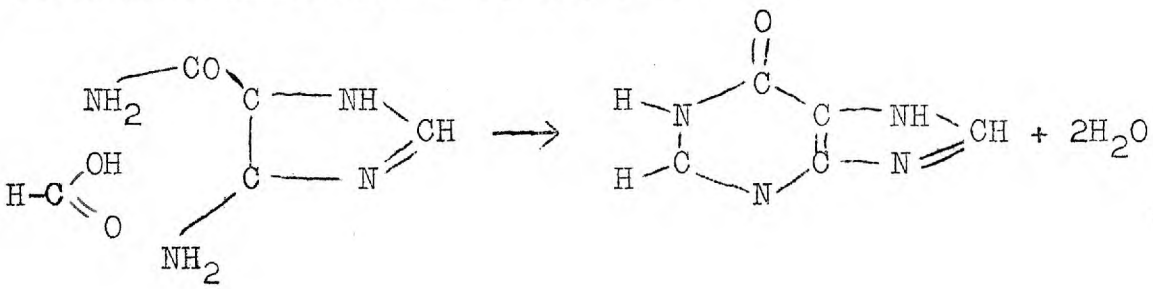
Relative Biological Activity of the Folic Acid Group of Vitamins

Vitamin	Man	Chick	L. casei	Str.faecalis R
Folic acid	100	100	100	100
Teropterin	+	100	101	3.8
Bc conjugate	+	60.65	0.8-1.6	0.55
Rhizopterin	0	0	0.009	100
Pteroic acid	0	0	0.08	30

enzyme systems as part of complex co-enzymes, it is reasonable to postulate that folic acid has a similar function although the enzyme system and the co-enzyme are not yet known.

Studies of the nutritional requirements of bacteria which require external supplies of p-aminobenzoic acid and folic acid demonstrated that the amount of vitamin required to produce growth responses was dependent on the presence or absence of purines, thymine and methionine (Lampen, Roepke and Jones, J. Biol. Chem., 1946, 164, 789). The hypothesis that these substances were the products of enzyme systems in which p-aminobenzoic acid was concerned was confirmed by the quantitative data. Whereas p-aminobenzoic acid/sulphonamide inhibition is competitive

the amounts of purines and amino-acids required to initiate growth in a completely inhibited culture are independent of the amount of inhibitor present which suggests that these substances are the products of the enzyme system in which p-aminobenzoic acid is involved (Shive and Roberts, J. Biol. Chem., 1946, 162, 463). Tracer studies and inhibition studies showed that these substances were all produced by reactions in which a single carbon unit intermediate was involved. The link between p-aminobenzoic acid and the single carbon unit was established when a substance which accumulated in a culture of Escherichia coli partially inhibited with sulphonamides was shown to be 4-amino-5 imidazole-carboxyamide (VIII) which only requires the addition of a single carbon unit to produce the naturally occurring purine, hypoxanthine (IX) (Shive, Ackerman, Gordon, Getzendoner and Eakin. J. Amer. Chem. Soc., 1947, 69, 725).

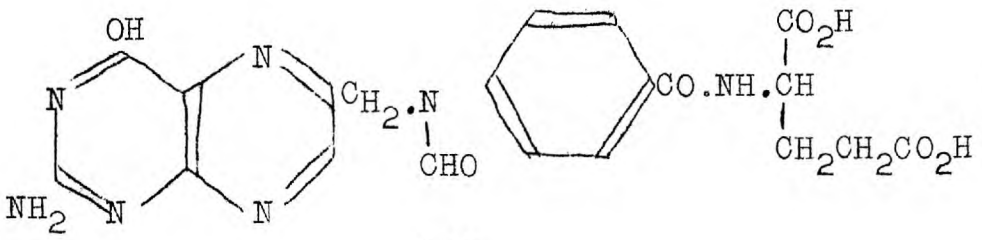


(VIII) (IX)

It was therefore postulated that the function of p-aminobenzoic acid was to catalyse a reaction by which a single carbon unit

was introduced into position 2 of the purine nucleus.

The part folic acid plays in the reaction was suggested when it was shown that the glutamic acid peptide of rhizopterin (X) was more effective than folic



(X)

acid itself in overcoming the inhibition of folic acid by antagonists of similar structure (Gordon, Ravel, Eakin and Shive, J. Amer. Chem. Soc., 1948, 70, 878). Since folic acid is biologically active whether it is formylated or not, it is suggested that it acts as a carrier of these single carbon units. As yet there is no direct evidence to prove or contradict this hypothesis.

Although specific reactions in which folic acid and p-amino-benzoic acid co-enzymes are involved cannot be formulated with any certainty, the following observations can be explained and integrated on the basis of the hypothesis that p-amino-benzoic acid and folic acid are involved in single carbon unit transfers (Williams, Eakin, Beerstecher and Shive, The Biochemistry of the B Vitamins, pp. 201-202, New York, 1950).

(1) Amino-acid Syntheses.

(a) Serine and Glycine.

A p-aminobenzoic acid co-enzyme is known to be involved in the reactions by which serine is formed from glycine and formic acid derivatives and in the reverse reaction by which glycine is obtained from serine, for the following reasons. The presence of serine increases the sulphonamide/p-amino-benzoic acid ratio needed to inhibit the growth of bacteria. Serine has been shown to be a precursor of glycine but the conversion cannot take place in the presence of sulphonamides. A biochemical synthesis of serine from formic acid and glycine has been demonstrated and folic acid has been shown to be involved in the interconversion of glycine and serine.

$$CH_2(NH_2) \cdot CO_2H + CH_2O \rightleftharpoons CH_2(OH) \cdot CH(NH_2) \cdot CO_2H$$

(b) Methionine.

The conversion of homocysteine into methionine involves a single carbon unit for the formation of the S-methyl group. A p-aminobenzoic acid co-enzyme is believed to be involved in these reactions for the following reasons. The presence of methionine increases the sulphonamide/p-aminobenzoic acid ratio required for bacterial inhibition

and decreases the requirements of bacteria which require an external supply of p-aminobenzoic acid. Homocysteine is ineffective in these situations

$$HS.(CH_2)_2.CH(NH_2)CO_2H + CH_2O \rightleftharpoons CH_3.S(CH_2)_2CH(NH_2)CO_2H$$

It is also known that when methionine, with the S-methyl group isotopically labelled, is present when serine is formed biosynthetically from glycine the labelled carbon appears in the β position in the serine.

(2) Purines and Pyrimidines.

Derivatives of folic acid and of p-aminobenzoic acid are believed to be co-enzymes in the reactions in which the single carbon unit is incorporated into the purine and pyrimidine nuclei for the following reasons. In the presence of amino acids the folic acid requirements of some micro-organisms can be replaced by thymine and purines. Similarly in the presence of amino acids the p-amino-benzoic acid requirements of other micro-organisms can be replaced by thymine and purines. In the presence of folic acid antagonists the inhibition produced can be prevented by thymine and purines. The inhibition of p-aminobenzoic acid by sulphonamides can be prevented by thymine and purines. Some of the functions of folic acid

in relieving macrocytic anaemia can be performed by thymine and nucleotides of purines and pyrimidines. In a medium containing amino acids and purines, thymine can be used to replace folic acid as a growth factor for L. casei, which suggests that these substances are the products of the reactions in which folic acid is involved.

In common with all B vitamins folic acid is universally distributed in all types of tissue. Extensive studies of the distribution of B vitamins in normal and cancerous tissues have indicated that folic acid is relatively the most abundant of the B vitamins in cancerous tissue. This relative abundance is observed in all types of cancerous tissue, regardless of the host, the site of the cancer or the method of induction, indicating that all cancer cells have a similar general metabolic pattern (Williams et al., University of Texas Publication, 1941, No.4137, 4237). Following the discovery by Woods of the antagonism between p-aminobenzoic acid and sulphonilamide (Brit. J. Expth. Path.,1940, 21, 74) it was suggested by Fildes (Lancet, 1940, i, 955) that chemotherapeutic research might be directed to preparing modifications of essential metabolites which would compete with those metabolites for enzymes. As folic acid is relatively the most abundant vitamin in cancerous tissue it is possible that folic acid antagonists will prove of value in the

chemotherapy of cancer. Further evidence to support this view is the fact that the Rous chick sarcoma can be controlled by regulating the amount of folic acid in the diet and by the administration of folic acid antagonists (Little, Sampath and SubbaRow, J. Lab. Clin. Med. 1949, 33, 1144). On this basis a considerable amount of work has been directed towards the synthesis of analogues of folic acid for use in the chemotherapy of cancer and to help to elucidate the mechanism by which folic acid is utilised.

Analogues of Folic Acid.

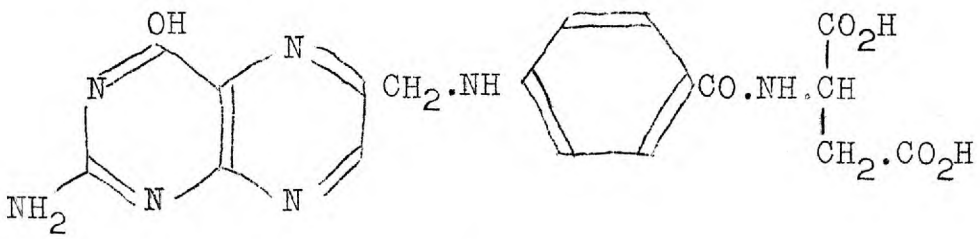
A considerable number of analogues of folic acid containing modified side chains have been prepared by variations in the original synthesis of folic acid. This method consists of reacting together 2:4:5-triamino-6-hydroxy-pyrimidine, 1:2-dibromopropionaldehyde and N-p-aminobenzoyl-L-glutamic acid. (Waller, Hutchings, Mowat, Semb, Stokstad, SubbaRow, Cosulich, Fahrenbach, Hultquist, Kuh, Northey, Seeger, Sickels and Smith, J. Amer. Chem. Soc., 1948, 70, 19.)

When DL-glutamic acid was used in this synthesis the product had only 50% of the activity of folic acid as a growth factor for L. casei and Str. faecalis R (Stockstad, Hutchings, Mowat, Boothe, Waller, Angier, Semb, and SubbaRow, J. Amer. Chem. Soc., 1948, 70, 5). From this result it can be deduced that D-folic acid is biologically inactive; this is confirmed by the fact that D-folic acid will not replace L-folic acid in stimulating the growth of the Rous chick sarcoma (Little, Sampath, Paganelli, Locke and SubbaRow, Trans. New York Acad. Sci., 1948, 10, II, 91).

During the determination of the structure of teropterin a number of N-pteroyl-L-glutamyl-L-glutamic acids and N-pteroyl-di-L-glutamyl-L-glutamic acids were prepared (Boothe, et al., J. Amer. Chem. Soc., 1949, 71, 2310). All these showed folic acid activity; those

with γ- peptide links were as active as folic acid for L. casei whereas those with α -peptide links had only 1-10% of the activity of folic acid for L. casei and Str. faecalis R.

When the glutamic acid in the above synthesis was replaced by aspartic acid N-pteroyl-L-aspartic acid (XI) was obtained. (Hutchings, Mowat, Oleson, Stokstad, Boothe, Waller, Angier, Semb, SubbaRow, J. Biol. Chem., 1947, 170, 323.)



(XI)

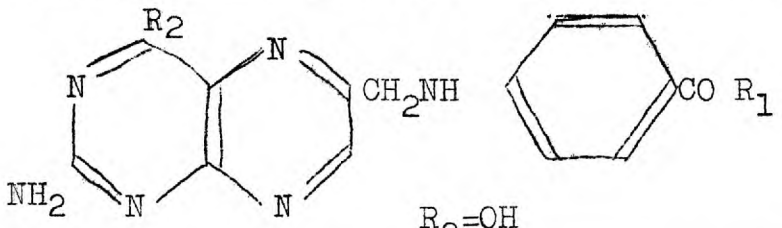
This substance was an active antagonist of folic acid having an inhibition index (i.e. ratio of concentrations of inhibitor and vitamin) for half maximum growth of 36-55 for Str. faecalis R, 1500-2200 for L. casei and about 500 for chicks.

Wright et al. (Wright, Cosulich, Fahrenbach, Waller, Smith and Hultquist, J. Amer. Chem. Soc.,1949, 71, 3014) prepared a comprehensive series of folic acid analogues in which glutamic acid was replaced by other amino acids. The method of preparation was the condensation of 2:4:5-triamino-6-hydroxy-pyrimidine, 1:2-dibromopropionaldehyde

and the requisite N-(p-amino-benzoyl)-amino acid. At the same time the corresponding 4-amino-4-desoxy-folic acid analogues were prepared by substituting 2:4:5:6-tetra-amino-pyrimidine in the above syntheses. The results are summarised in Table II. The folic acid activity is expressed as the % of the activity of pure folic acid and the antagonistic activity of the 4-amino-4-desoxy folic acid analogues as the % of the inhibitory activity of N^{10}-methyl-folic acid.

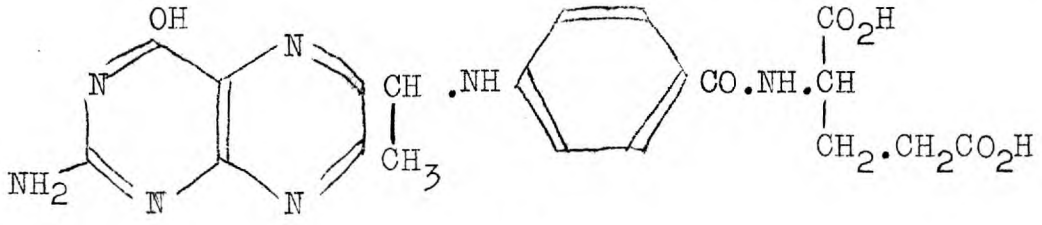
TABLE II

Pteroyl and 4-Amino-4-desoxy-pteroyl-Amino Acids.



	R_2=OH		R_2=NH_2	
R_1	Purity %	Biological activity	Purity %	Biological activity
dl-Alanine	100	0	-	-
dl-Alanine	20.7	0	14.5	0
β-Alanine	100	+ 2.46	-	-
Aminomalonic acid	100	+ 0.007	66.4	- 0.1
l-Cystine	22.5	0	-	-
dl-Isoleucine	8.7	-	5.4	-
Methionine	25.0	+	-	-
Phenylalanine	13	0	5.5	-
Sarcosine	20.4	+	18.3	-
dl-Serine	-	-	100	- 0.174
dl-Serine	18.3	0	22.8	-
Threonine.H_2O	19	-	22.4	-
Tryptophan	78	-	8.3	-
dl-Valine	13.8	0	11.0	0

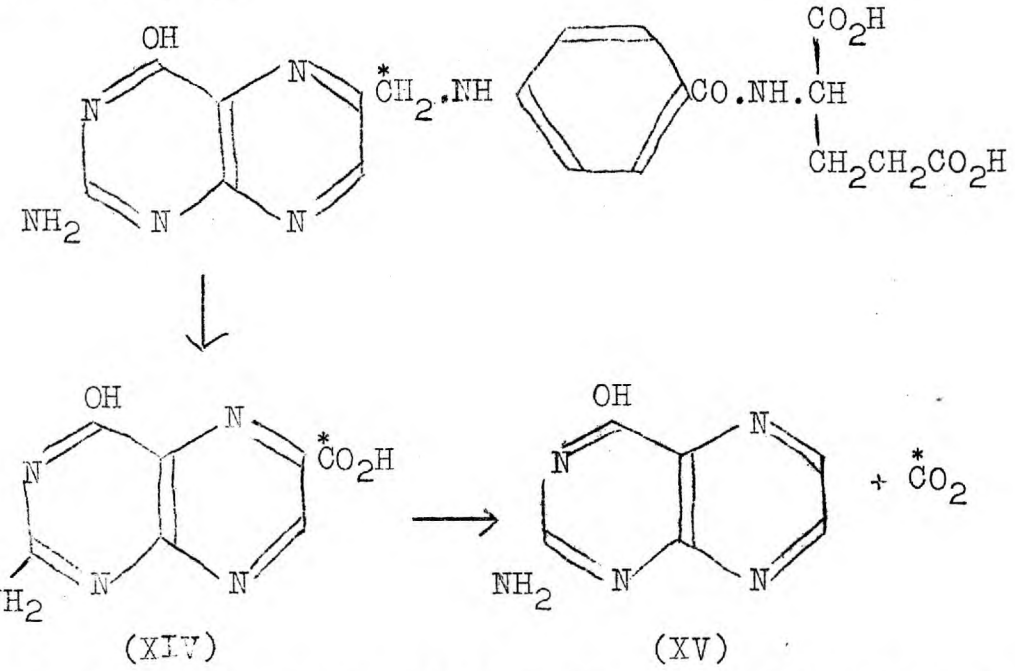
The first inhibitory analogue of folic acid was reported by Martin, Tolman and Moss (Arch. Biochem., 1947, 12, 318) who prepared a D-methyl-folic acid by reacting 2:4:5-triamino-6-hydroxy-pyrimidine, 1:2-dibromobutyraldehyde and N-(p-aminobenzoyl-)-D-glutamic acid. This substance had an inhibition index of 150 for Str. faecalis R. The corresponding L-methyl-folic acid was prepared (Franklin, Stokstad, Belt and Jukes, J. Biol. Chem., 1947, 169, 427) and had an inhibition index of 20-30 for Str. faecalis R. This inhibition index was determined using a crude reaction product and the quantitative results of these experiments are of doubtful value. Martin, Tolman and Moss, without proof, designated their product as 7-methyl-D-folic acid (XII)



(XIII)

but, in view of the work of Weygand and Schaefer, (Naturwiss,1951,18, 432) this D-methyl-folic acid is most probably the 9-methyl- compound (XIII).

Weygand and Schaefer (loc.cit.) synthesised folic acid by the usual method using 1:2-dibromopropionaldehyde in which carbon atom 1 was radioactive. On acid hydrolysis in the presence of air, this radioactive folic acid gave radioactive 2-amino-4-hydroxy-pteridine-6-carboxylic acid (XIV). Decarboxylation of this acid gave radioactive carbon dioxide and non-radioactive 2-amino-4-hydroxy-pteridine (XV).



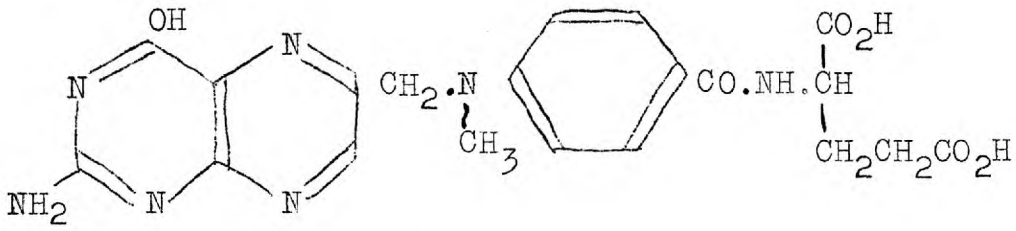
(XIV) (XV)

This work proves that carbon atom 1 of the 1:2-dibromo-propionaldehyde forms carbon atom 9 of folic acid. On the assumption that the same mechanism applies when 1:2-dibrombutyraldehyde is used it follows that the product of Martin, Tolman and Moss was actually 9-methyl-D-folic acid (XIII).

9-Methyl-L-folic acid was also prepared by Hultquist,

Smith, Seeger, Cosulich and Kuh (J. Amer. Chem. Soc., 1949, 71, 619) by the reaction between 2:4:5-triamino-6-hydroxy-pyrimidine hydrochloride, 2:2:3-trichloro-butyraldehyde and N-(p-aminobenzoyl-)-L-glutamic acid. This product was obtained analytically pure and its structure proved by oxidation with alkaline permanganate to the known 2-amino-4-hydroxy-pteridine-6-carboxylic acid. The identity of this product and that of Martin, Tolman and Moss cannot be inferred from the biological activity data since this was determined under different conditions.

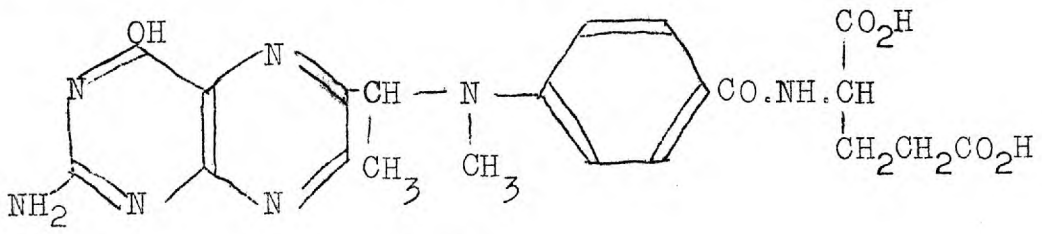
N^{10}-methyl-folic acid (XVI) was prepared (Cosulich and Smith, J. Amer. Chem. Soc.,1948, 70, 1922) from 2:4:5-triamino-6-hydroxy-pyrimidine, 1:2-dibromo-propionaldehyde and p-(methylamino)-benzoyl-L-glutamic acid.



(XVI)

A combination of the last two syntheses i.e. heating together 1:2-dibromobutyraldehyde, 2:4:5-triamino-6-hydroxy-pyrimidine and p-(methyl-amino)-benzoyl-L-glutamic acid gave 9:N^{10} dimethyl-folic acid. (XVII) (Hultquist, Smith, Seeger, Cosulich and Kuh,

J. Amer. Chem. Soc., 1949, 71, 619.)



(XVII)

The inhibition indices for these methyl-folic acids, determined for the pure substance and for half maximum rate of growth of Str. faecalis R are 9-methyl-folic acid, 1610, N^{10}-methyl-folic acid, 2 and 9:N^{10}-dimethyl-folic acid, 55.

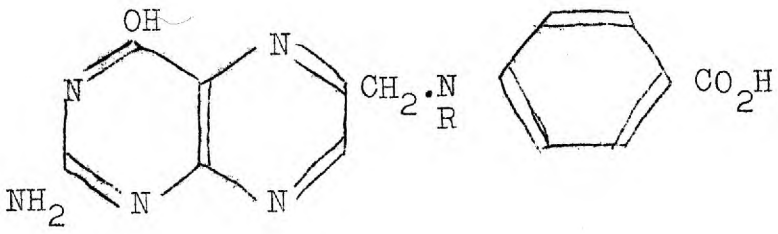
An interesting series of N^{10}-substituted pteroic acids was prepared by Cosulich and Smith (J. Amer. Chem. Soc., 1948, 70, 1922) from 2:4:5-triamino-6-hydroxy-pyrimidine, 1:2-dibromopropionaldehyde and the requisite N substituted p-aminobenzoic acid. The results of biological tests with these compounds, which indicate that the larger the N^{10} substituent the less effective the inhibitor, are summarised in Table III.

A series of folic acid analogues in which the 4-hydroxy group is replaced by an amino-group has been prepared. This group is characterised by very high inhibitory activity which is usually either not reversed or only reversed to a limited extent, by folic acid. 4-Amino-4- desoxy-folic acid (XVIII) was prepared

TABLE III

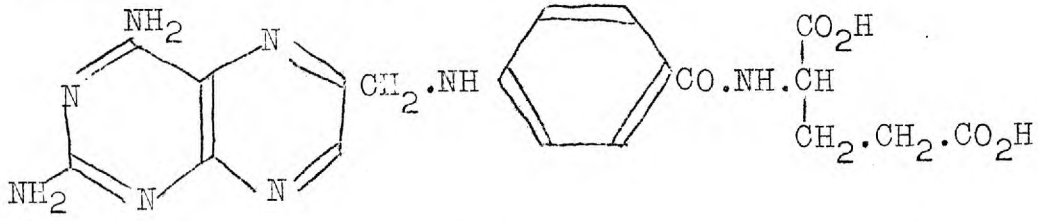
Inhibition Indices of N^{10} Substituted.

Pteroic Acids.



R-	Inhibition Index
CH_3-	18
C_2H_5-	24
n-C_4H_9-	1,770
CH_3.CO-	10,800
C_6H_5.CO-	20,500

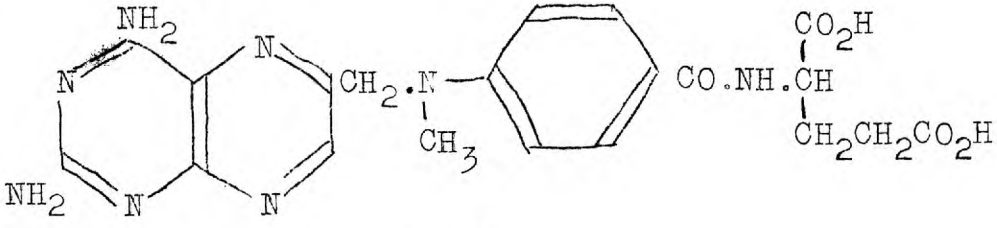
by substituting 2:4:5:6-tetra-amino-pyrimidine for 2:5:6-triamino-4-hydroxy-pyrimidine in the original folic acid synthesis (Oleson, Hutchings and SubbaRow, J. Biol. Chem. 1948, 175, 359).



(XVIII)

This substance markedly inhibits the growth of Str. faecalis R and the relative amount of folic acid required to overcome the inhibition depends on the concentration of the inhibitor, the inhibition index varying between about 3 and 0.01 according to the concentration of the inhibitor. This substance has been used in the treatment of leukaemia (Farber, Diamond, Mercer, Sylvester and Wolf, New England J. Medicine, 1948, 238, 787). It gives a temporary remission of the symptoms in some cases but is too toxic to be given in large doses or for prolonged periods.

4-Amino-4-desoxy-N^{10}-methyl folic acid (XIX) was prepared by a variation of the standard synthesis (Seeger, Cosulich, Smith and Hultquist, J. Amer. Chem. Soc., 1949, 71, 1753).

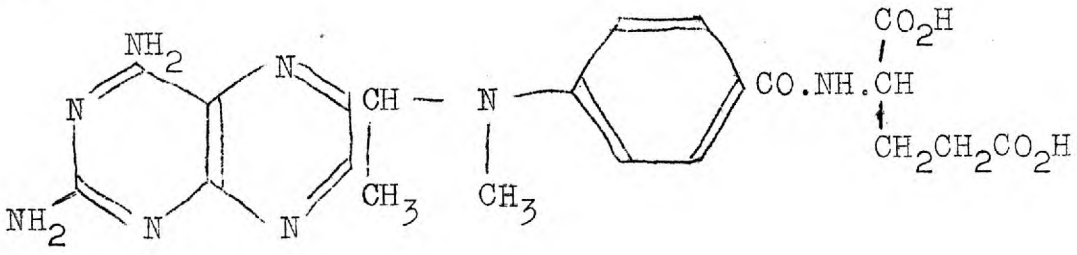


(XIX)

This substance inhibits folic acid competitively for Str. faecalis R, the inhibition index being 3; folic acid is, however, ineffective in overcoming the toxicity of this substance for rats.

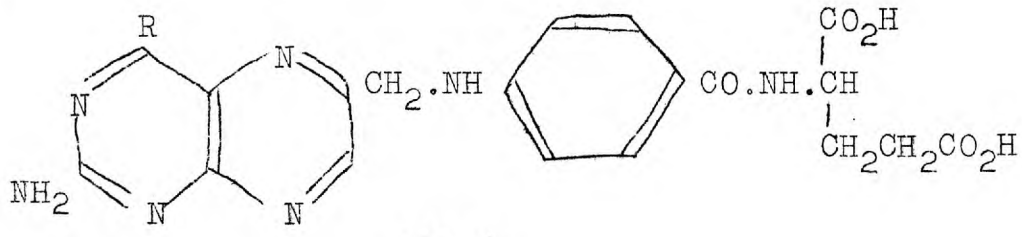
4-Amino-4-desoxy-9:N^{10}-dimethyl-folic acid (XX)

has an inhibition index of 0.13 (Hultquist, Smith, Seeger, Cosulich and Kuh. J. Amer. Chem. Soc., 1949, 71, 619).



(XX)

4-Amino-4-desoxy-pteroic acid, 4-amino-4-desoxy-N^{10}-methyl-pteroic acid, and 4-amino-4-desoxy-pteroyl-aspartic acid have all been prepared using variations of the syntheses previously mentioned. In an attempt to lower the toxicity of these potential chemotherapeutic agents, a series of analogues with modified 4-amino-groups were prepared. (Roth, Smith and Hultquist, J. Amer. Chem. Soc., 1950, 72, 1915.)

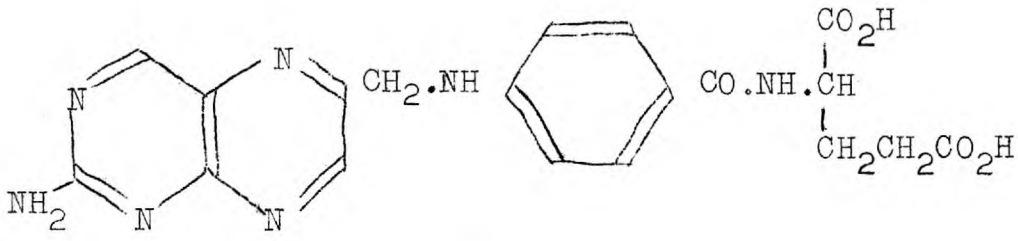


(XXI)

R in formula (XXI) was methylamino, dimethylamino and piperidylamino. 4-Dimethylamino-4-desoxy-N^{10}-methyl-folic acid was also prepared by these authors.

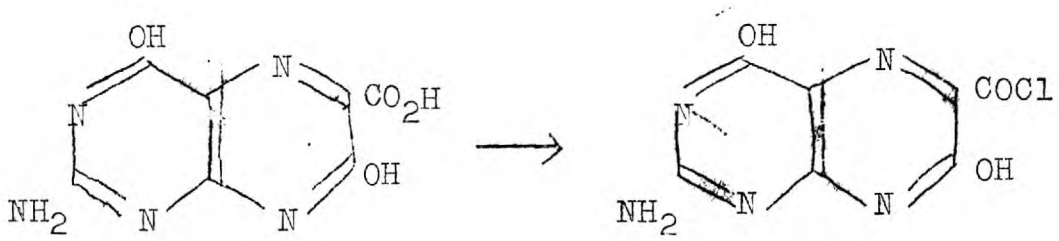
Preliminary results indicate that these substances are folic acid antagonists of lower activity than 4-amino-4-desoxy-folic acid.

4-Desoxy-folic acid (XXII) is reported (Swendseid, Wittle, Moersch, Bird and Brown, Fed. Proc.,1948, 7, 299) to be an antagonist of folic acid for rats.

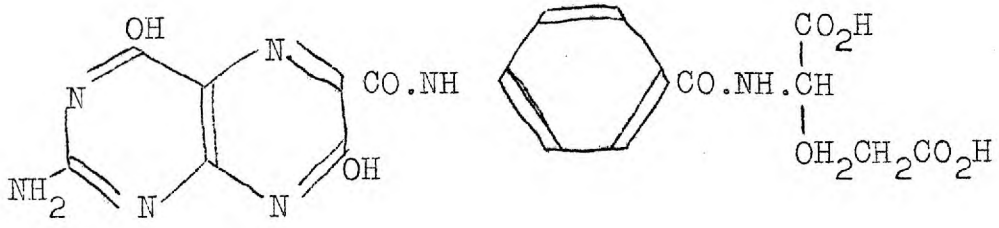


(XXII)

7-Hydroxy-9-keto-folic acid (XXIV) was prepared from iso-xanthopterin carboxylic acid (XXIII) by chlorination with phosphorus oxychloride and phosphorus pentachloride to give the acid chloride, which was condensed with p-amino-benzoyl-L-glutamic acid in sodium hydroxide solution (Woolley and Pringle, J. Biol. Chem.,1948, 174, 327):-

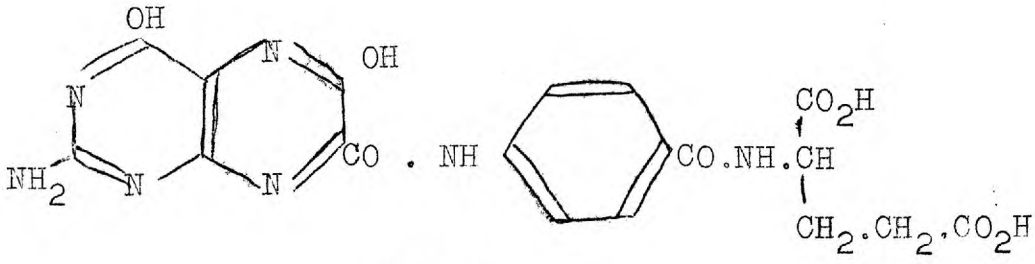


(XXIII)



(XXIV)

A similar substance (XXV) with the 6- and 7-substituents interchanged was prepared at the same time.

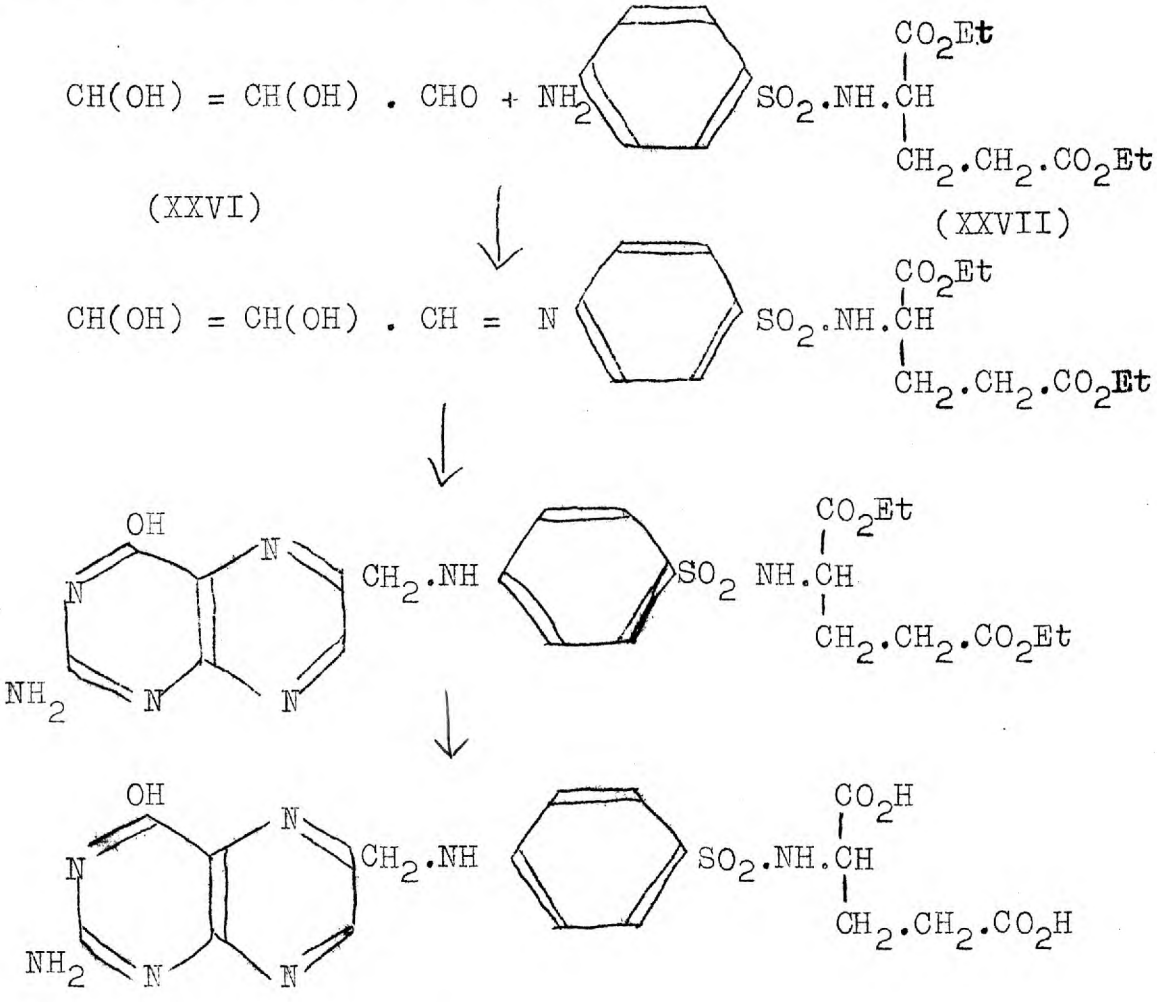


(XXV)

7-Hydroxy-9-keto-folic acid had an inhibition index of 10,300 for L. casei whereas the isomeric substance (XXV) was less than half as active (Inhibition index 23,400).

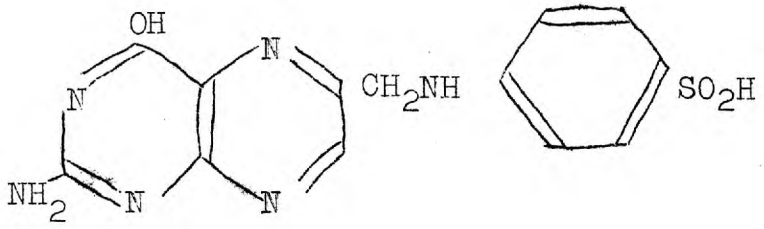
Viscontini and Meier (Helv. Chim. Acta, 1949, 32, 877) prepared an analogue of folic acid in which the p-aminobenzoic acid was replaced by p-aminobenzene-sulphonic acid (XXVIII). Reductone (XXVI) was condensed with the diethyl ester of N(p-aminobenzene-sulphonyl-)-L-glutamic (XXVII) acid to give the Schiffs base which was then condensed with 2:4:5-triamino-6-hydroxy-pyrimidine

to give the diethyl ester of the analogue. Alkaline hydrolysis gave the free acid, thus:-

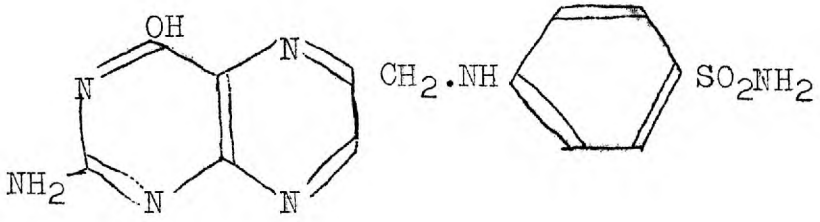


(XXVIII)

No biological results were reported but Martin, Brendel, Beiler, Moss, Avakian, Urist, Tolman and Alpert (Amer. J. Pharm., 1948, 120, 189), who prepared the corresponding pteroic acid analogue (XXIX) and its amide (XXX), state that they were both inactive as antagonists of folic acid for Str. faecalis R.



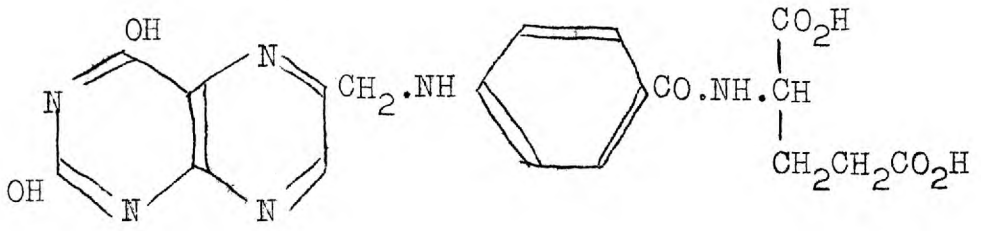
(XXIX)



(XXX)

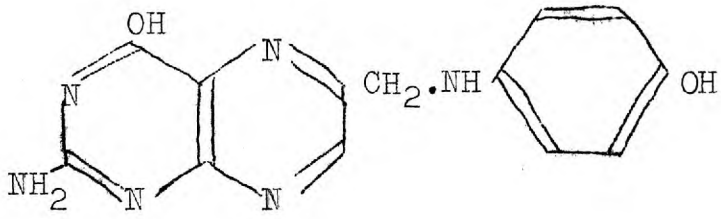
In the same paper Martin et al. reported briefly the preparation in an impure form of a large number of folic acid analogues and biological tests on these impure materials.

2-Hydroxy-2-desamino-folic acid (XXXI) was prepared from 5:6-diamino-uracil, 1:2-dibromopropionaldehyde and p-aminobenzoyl glutamic acid.

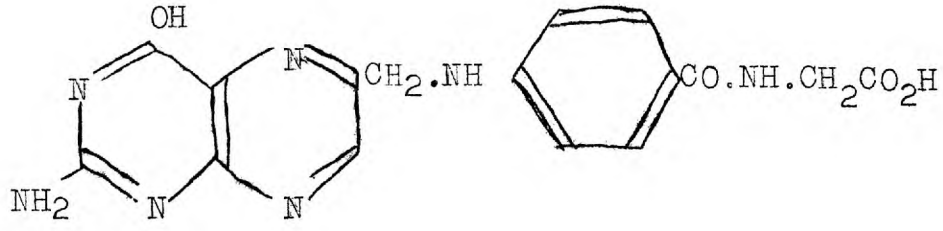


(XXXI)

This was inactive as an inhibitor of folic acid for Str. faecalis R. By variations in the original dibromopropionaldehyde synthesis these authors also prepared N(2-amino-4-hydroxy-6-pteridyl-methyl)p-aminophenol (XXXII) and N-(2-amino-4-hydroxy-6-pteridyl-methyl) hippuric acid (XXXIII)

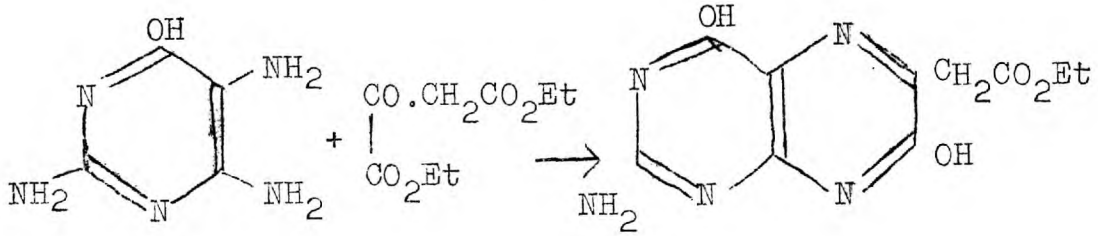


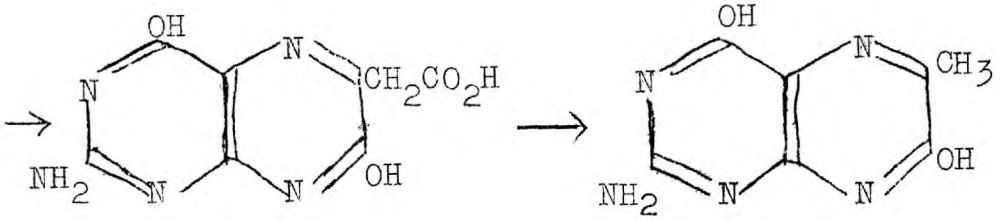
(XXXII)



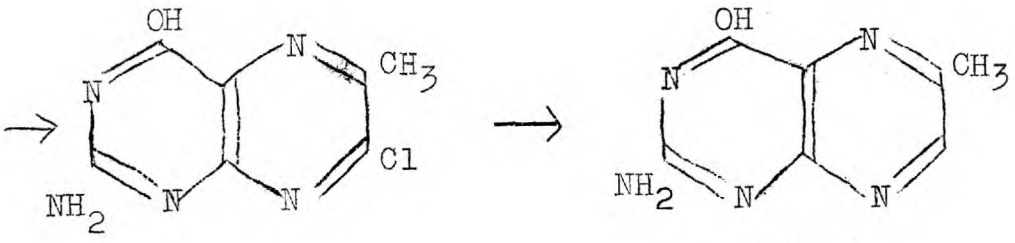
(XXXIII)

Tschesche, Köhncke and Korta, (Z. Naturforch 1950, 5b, 132) prepared folic acid by the following route:-

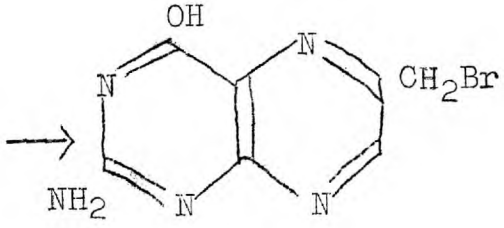


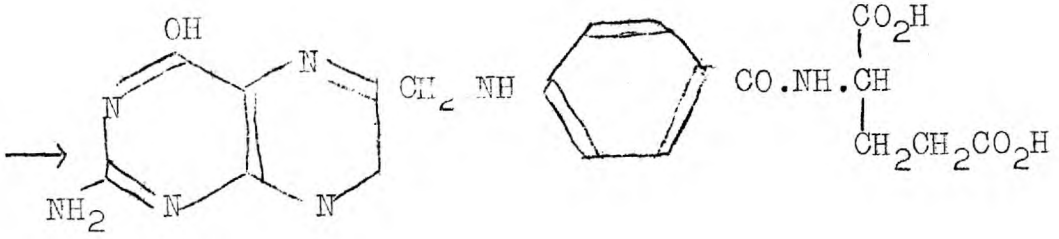


(XXXIV)

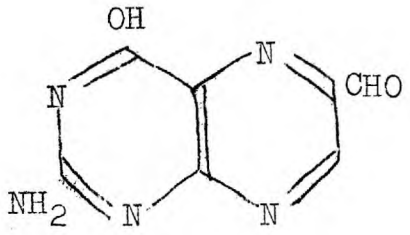


(XXXV)

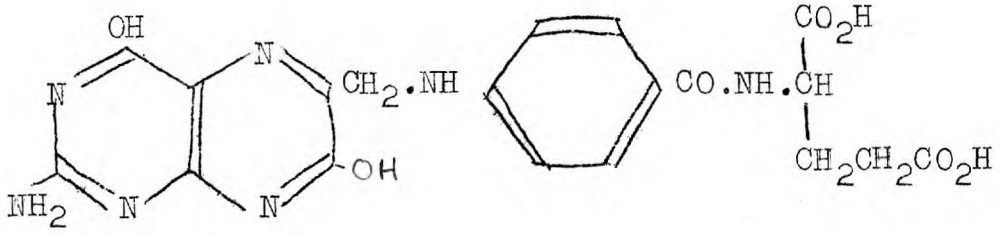




These workers reported that 2-amino-4-hydroxy-pteridine-6-aldehyde (XXXVI), prepared by selenium dioxide oxidation of 2-amino-4-hydroxy-6-methyl-pteridine (XXXV), was active in replacing folic acid for Str. faecalis R and that 7-hydroxy folic acid (XXXVII), prepared by brominating 2-amino-4:7-dihydroxy-6-methyl pteridine and condensing the bromo-compound with N(p-aminobenzoyl-)-L-glutamic acid, also had folic acid activity.



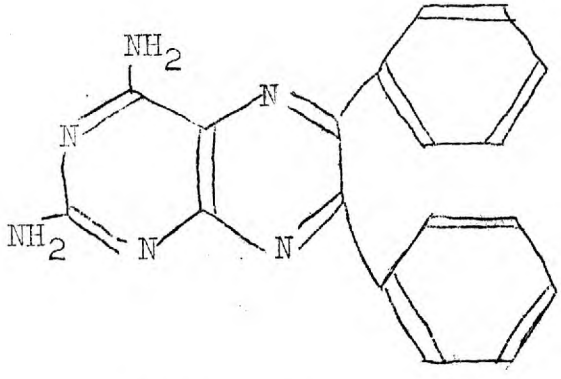
(XXXVI)



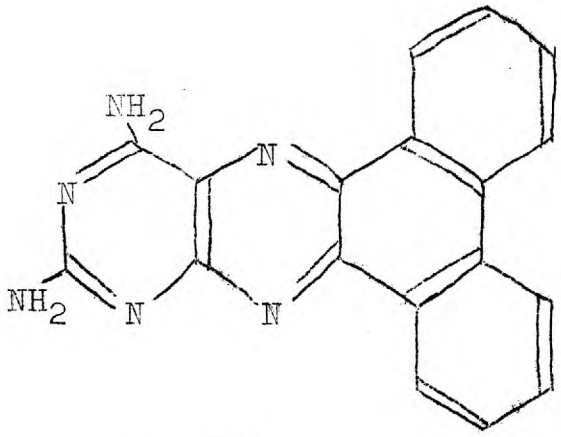
(XXXVII)

Daniel, Scotte, Norris and Heuser, (J. Biol.Chem., 1948, 173, 123) tested a number of simpler pterins as growth promotors for chicks fed on a diet containing a suboptimal amount of folic acid. Those with 2:4-dihydroxy-, 2-amino-4-hydroxy-, and 2-mercapto-4-hydroxy-substituents gave a significant growth response whilst those with 2:4-diamino substituents were inactive as growth factors.

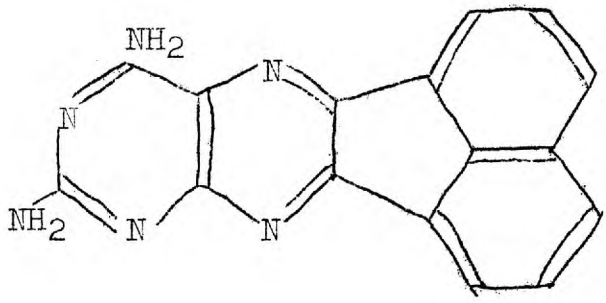
A number of simple 2:4-diamino-pterins, prepared by Malltte, Taylor and Cain (J. Amer. Chem. Soc.,1947, 69, 1814; 1948, 70, 3026) were tested as folic acid antagonists by Daniel, Norris, Scotte and Heuser (J. Biol. Chem., 1947, 169, 689). Those with aromatic substituents in the 6- and 7- positions were the most effective, e.g. 2:4-diamino-6-7-diphenyl pteridine (XXXVIII) (Inhibition index 8-10), 2:4-diamino-6:7-phenanthro-9:10-pteridine (XXXIX) (Inhibition index 2-4) and 2:4-diamino-acenaphtho-1:2-pteridine (XL) (Inhibition Index 20-100).



(XXXVIII)



(XXXIX)

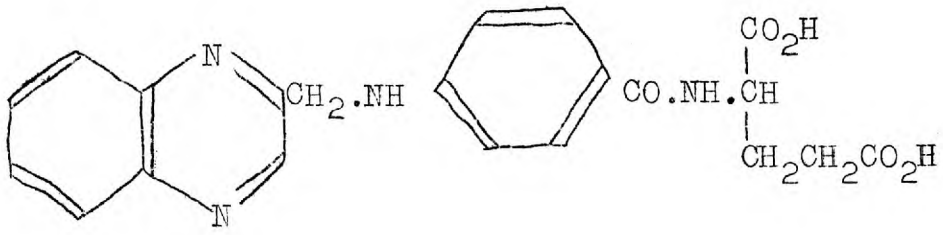


(XL)

Attempts to increase the solubility of these compounds by the introduction of substituents in the aromatic groups reduced their inhibitory activity.

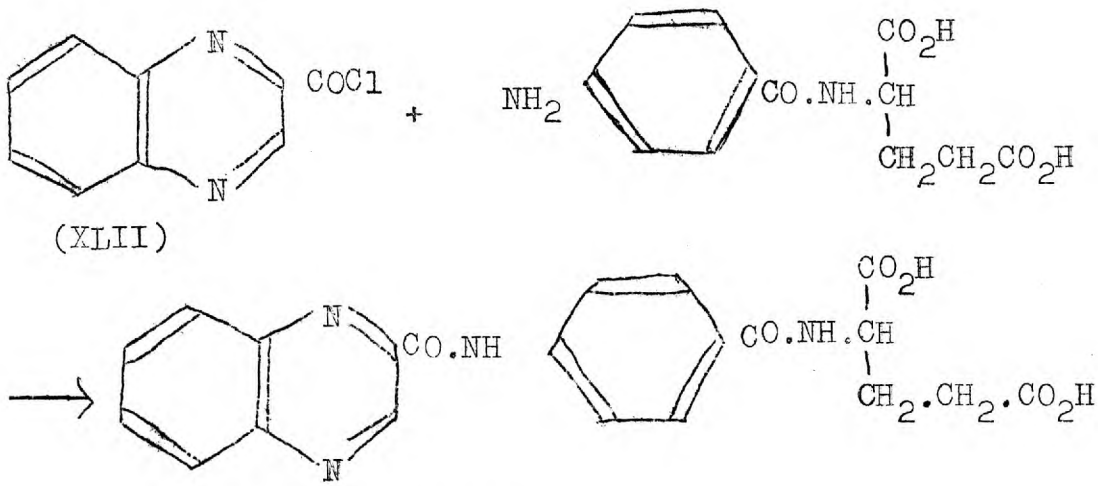
In the search for folic acid antagonists of chemotherapeutic value a number of analogues containing heterocyclic systems other than pteridine have been prepared. Woolley and Pringle (J. Biol. Chem., 1948,

174, 327) attempted to prepare a simple quinoxaline analogue (XLI) by condensation of o-phenylene diamine, 1:2-dibromopropionaldehyde and N(p-aminobenzoyl-)-L-glutamic acid.



(XLI)

Their product was not the expected substance but, nevertheless, was an inhibitor of folic acid for L.casei. These authors prepared quinoxaline-2-carboxyl-p-aminobenzoyl-glutamic acid (XLIII) by the condensation of quinoxaline-2-carboxylic acid chloride (XLII) and N(p-aminobenzoyl)-glutamic acid.

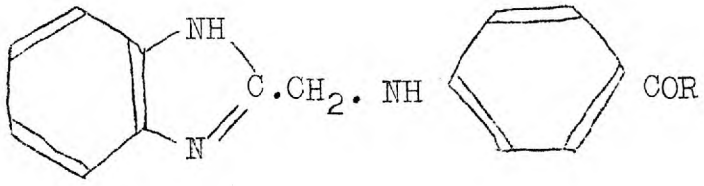


(XLII)

(XLIII)

It was also shown by these workers that quinoxaline itself had slight anti-folic acid activity.

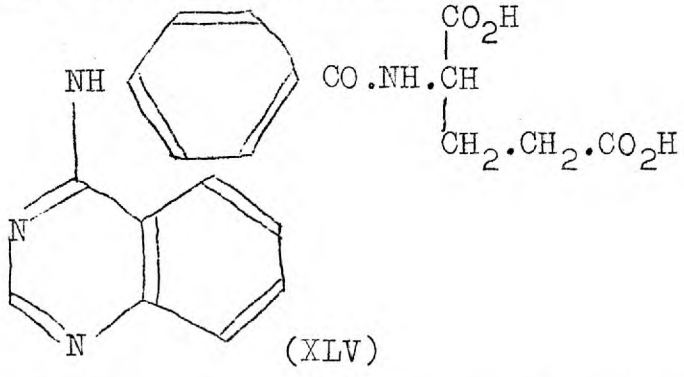
King, Acheson and Spensley, (J., 1949, 1401) prepared benziminazole analogues of folic and pteroic acids (XLIV) by condensing 2-chloromethyl-benziminazole with p-aminobenzoic ester and with the diethyl ester of N(p-amino-benzoyl)-L-glutamic acid and hydrolysing the resulting ester.



R = -OH and glutamic acid.

(XLIV)

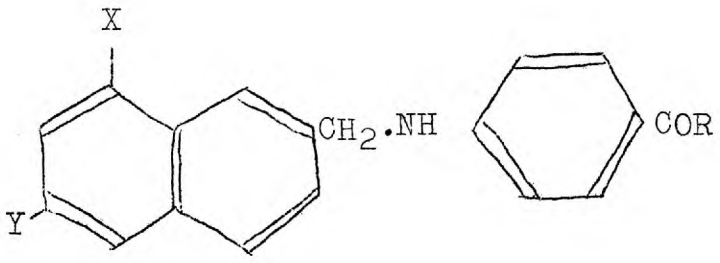
Martin, Moss and Avakian (J. Biol. Chem.,1947, 167, 737) condensed 4-chloro-quinazoline with N-(p-amino-benzoyl)-L-glutamic acid to give N-(4-(4-quinazolyl)-benzoyl)-L-glutamic acid (XLV)



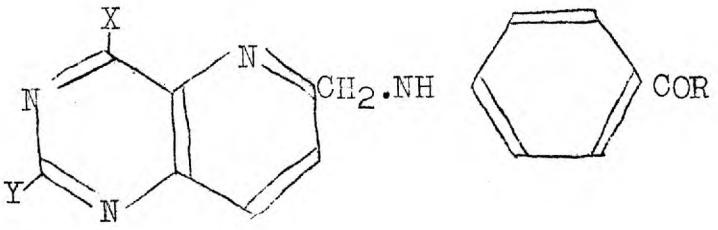
(XLV)

This substance was reported to be active in replacing folic acid for Str. faccalis R, being 1-10% as active as folic acid.

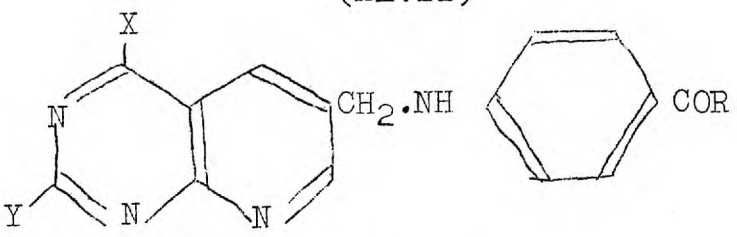
The present thesis describes attempts to synthesise analogues of folic and pteroic acids in which the pteridine nucleus was replaced by a naphthalene, (XLVI), or triazanaphthalene, (XLVII) and (XLVIII), nucleus.



(XLVI)



(XLVII)



(XLVIII)

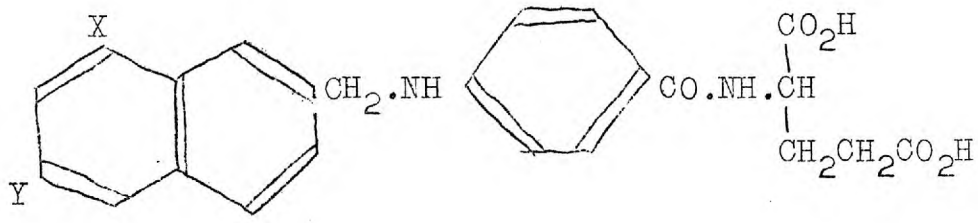
R = -OH and glutamic acid.

X =	- OH	-H	-OH	-H
Y =	- NH_2	-NH_2	-H	-H

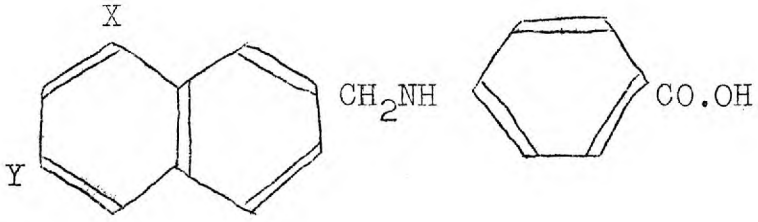
This work is part of a larger programme directed towards the synthesis of analogues of pteroic and folic acids in which the hetero-atoms are successively removed.

Naphthalene Analogues of Folic Acid.

This section of the work was concerned with attempts to synthesise analogues of folic and pteroic acids (XLIX and L) in which the pteridine nucleus was replaced by a naphthalene nucleus.



(XLIX)

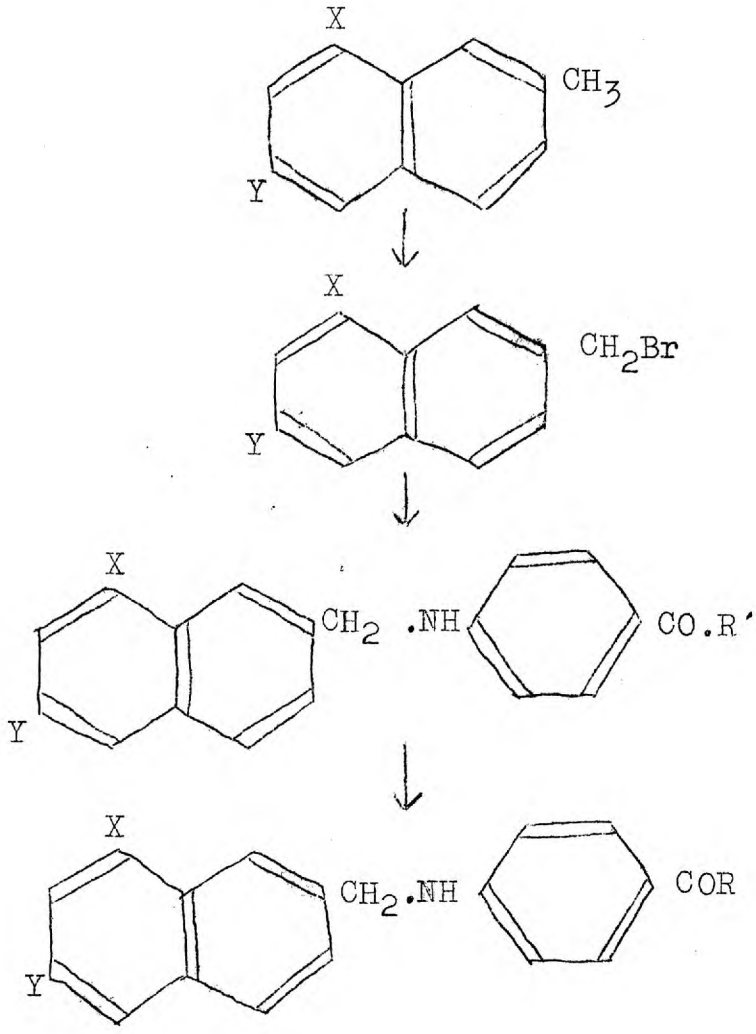


(L)

X :	-OH	-H	-OH	-H
Y :	$-NH_2$	$-NH_2$	-H	-H

The route chosen was the side chain bromination of the requisite 2-methyl-naphthalene followed by condensation with p-aminobenzoic acid or N(p-aminobenzoyl-)-L-glutamic

acid or their esters. The ester groups were to be removed by hydrolysis.

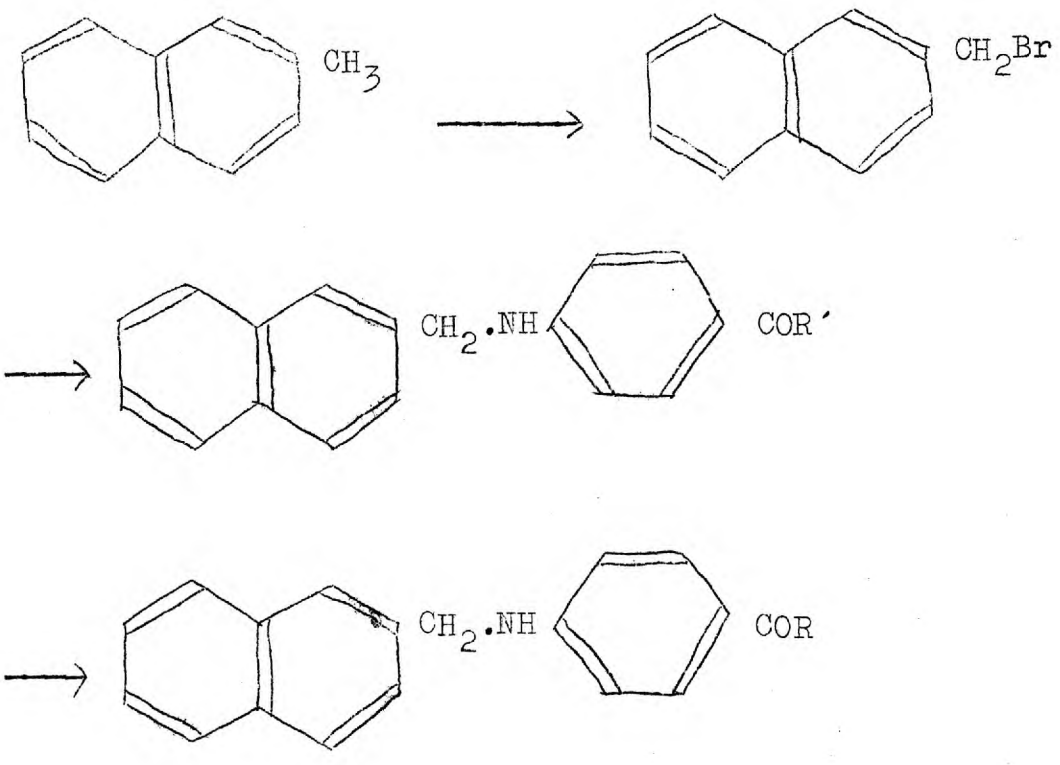


X :	-OH	-H	-OH	-H
Y :	$-NH_2$	$-NH_2$	-H	-H

R = OH or glutamic acid

R′ = -OMe or glutamic ester.

The unsubstituted naphthalene analogues, (XLIX and L; X = H; Y = H) were prepared by the following route:



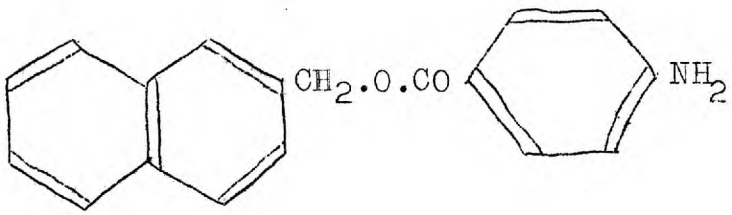
R' = OEt and glutamic ester

R = OH and glutamic acid

2-Bromo-methyl-naphthalene was prepared by brominating 2-methyl-naphthalene with N-bromosuccinimide (Buu-Hoï, Annalen, 1944, 556, 1). The 2-bromo-methyl-naphthalene was condensed with ethyl p-aminobenzoate in ethanol in the presence of the theoretical amount of sodium carbonate and the resulting ester hydrolysed to the naphthalene analogue of pteroic acid, N(2-naphthyl-methyl-)p-

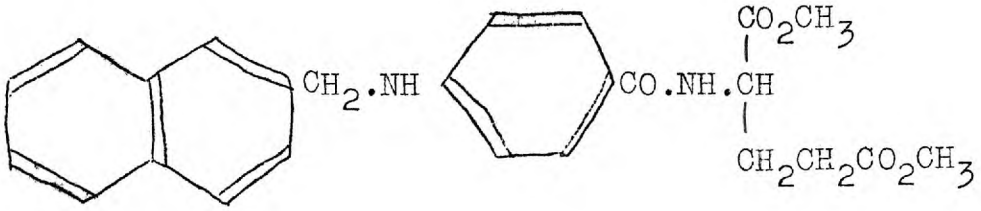
aminobenzoic acid (L ; X = H, Y = H) by refluxing with 10% alcoholic potassium hydroxide.

An attempt to prepare this compound by direct condensation of 2-bromo-methyl-naphthalene with p-aminobenzoic acid gave instead the 2-naphthyl-methyl ester of p-aminobenzoic acid (LI)



(LI)

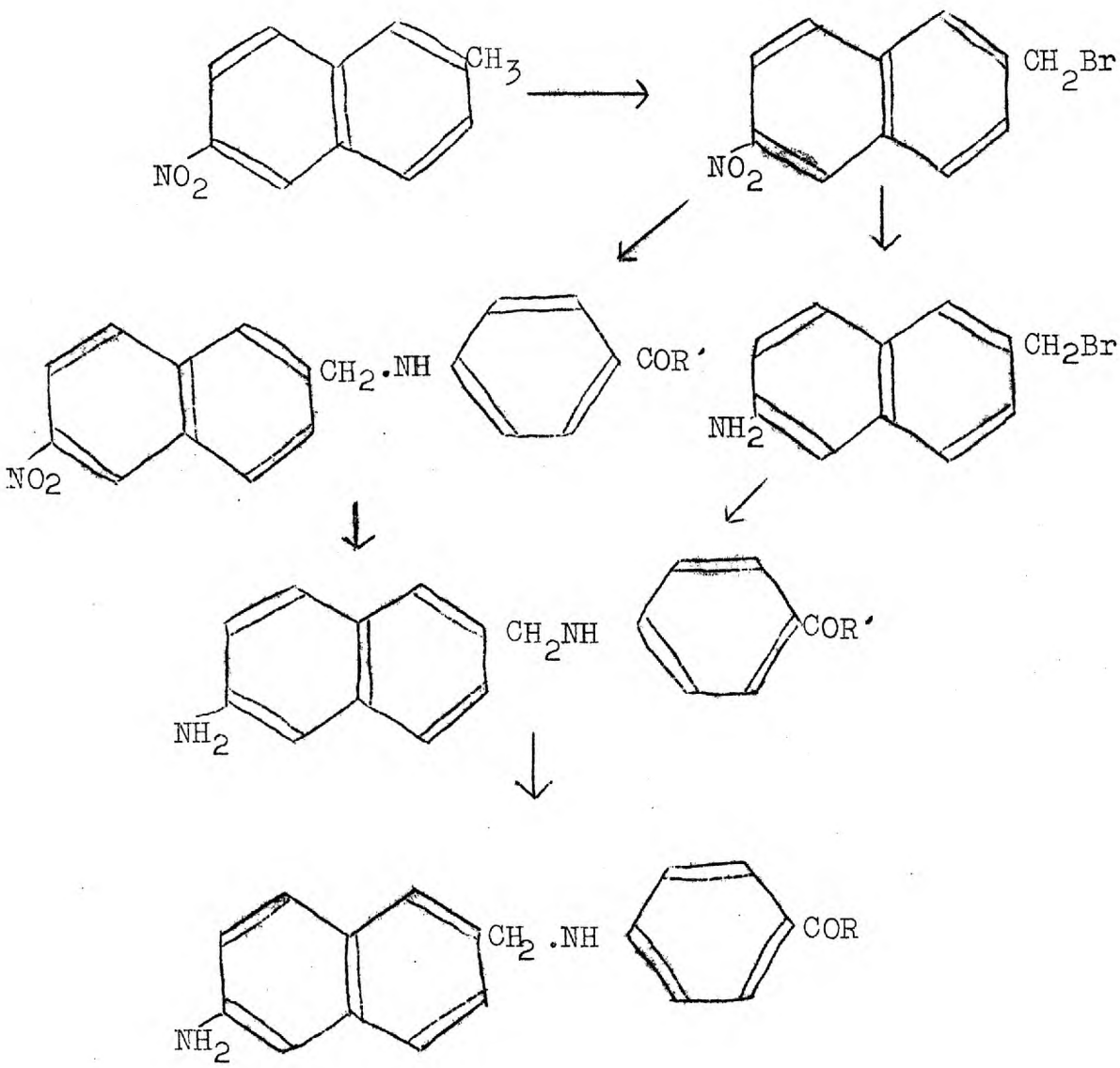
Condensation of 2-bromo-methyl-naphthalene with dimethyl N(p-amino-benzoyl)-L-glutamate was accomplished by refluxing these two substances in ethanol in the presence of the theoretical amount of potassium carbonate. This product, dimethyl N[(N'-2-methyl-naphthyl)-p-aminobenzoyl]-L-glutanate (LII) was the dimethyl ester of the required folic acid analogue.



(LII)

Attempts were made to hydrolyse this ester using (a) the theoretical amount of cold alcoholic potash, (b) an excess of cold alcoholic potash, (c) an excess of hot alcoholic potash, (d) the theoretical amount of cold aqueous potash, (e) an excess of cold aqueous potash, and (f) an excess of hot aqueous potash, but none were successful, the milder procedures (a, b, d and e) apparently causing no hydrolysis and the more drastic procedures (c and f) giving only the pteroic analogue (L; X = H, Y = H) owing to hydrolysis of the peptide linkage. An attempt to prepare the folic acid analogue by the condensation of the chloride of the pteroic analogue with glutamic acid in aqueous bicarbonate solution was also unsuccessful.

For the preparation of the amino-naphthalene analogues, (XLIX and L; X = H, Y = NH_2), the following synthesis appeared to be the most practicable.



R′ = -OEt or glutamic ester

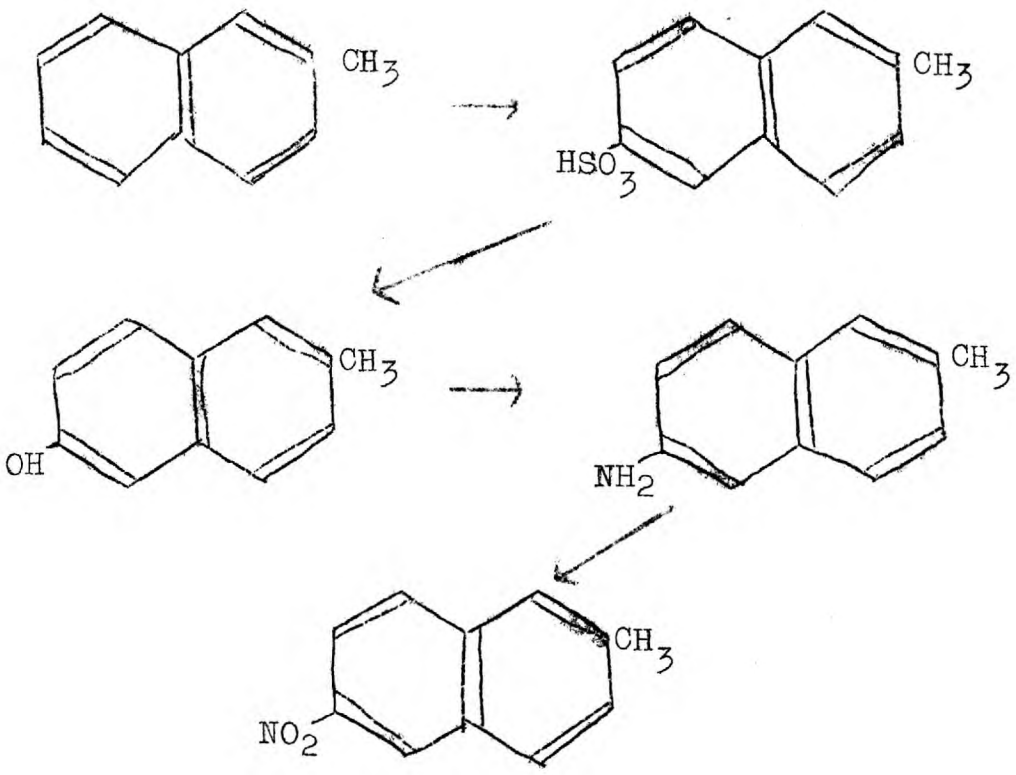
R = -OH or glutamic acid.

The required starting material, 2-methyl-6-nitronapthalene, had been shown by Wesely and Kapp, (Rec. Trav.

Chim., 1925, 44, 360) to be a minor product of the direct nitration of 2-methyl-naphthalene.

The yield was, however, too small for the method to be of practical value for the projected synthesis. Wesely and Pac (Coll. Czechoslovak.Chem. Comm., 1930, 2, 471) had isolated a very small quantity of 2-methyl-6-nitro-naphthalene as a minor product in a series of reactions. This also was of no practical value.

In order to obtain 2-methyl-6-nitro-naphthalene in suitable quantities for the above synthesis the following route was devised:-



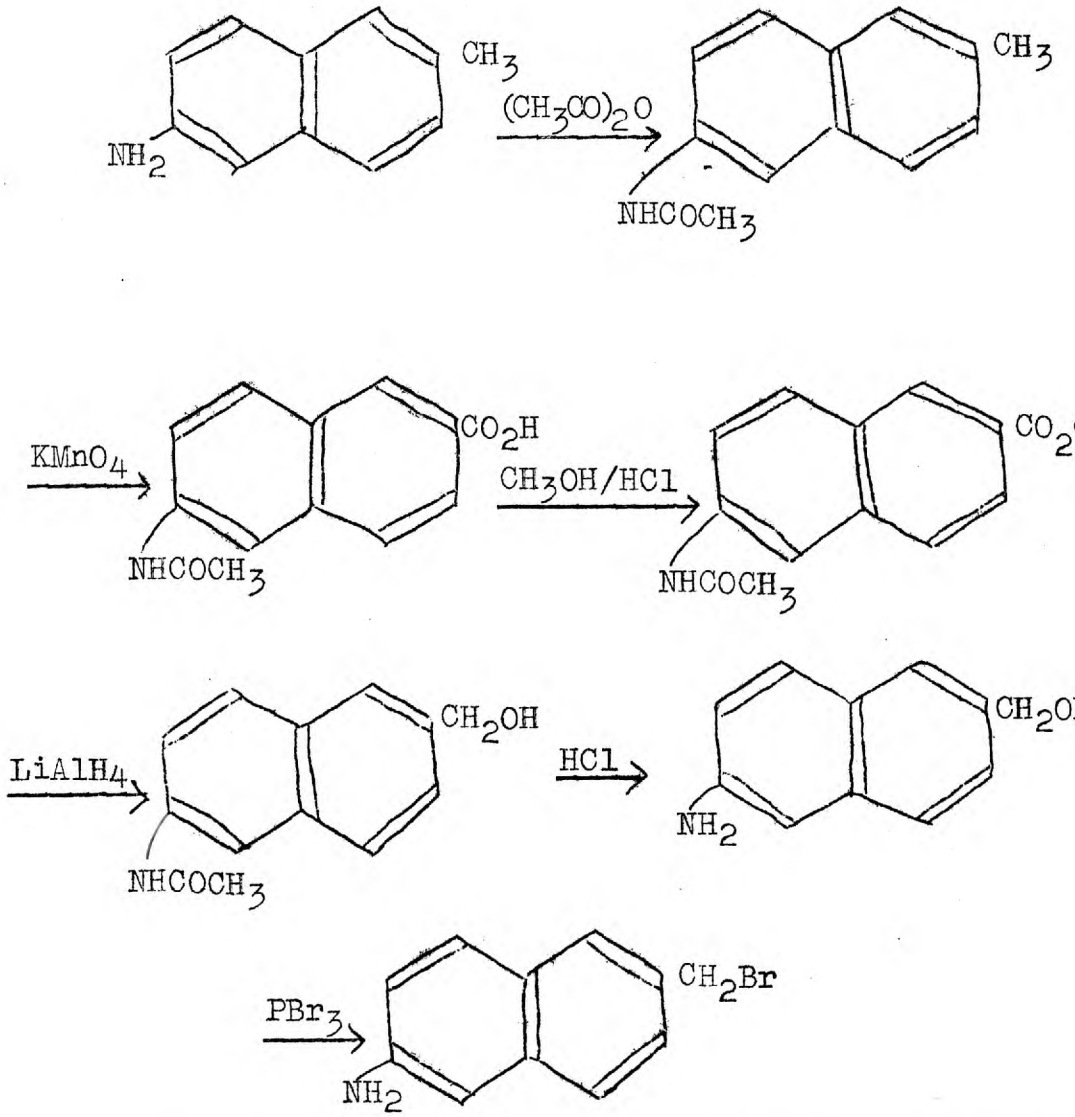
Roger (Ann. Chim., 1940, 71, 1, 395) had shown that 2-methyl-naphthalene could be sulphonated in the 6-position in 75% yield by heating on a steam bath with concentrated sulphuric acid and that fusion of this 2-methyl-naphthalene-6-sulphonic acid with potassium hydroxide gave 2-methyl-6-hydroxy-naphthalene in 40-45% yield. This work was repeated and by this means 2-methyl-6-hydroxy naphthalene was readily available in reasonable quantity.

A Bucherer reaction was then performed on this material and gave 2-methyl-6-amino-naphthalene in 77% yield.

Attempts were then made to convert 2-methyl-6-amino-naphthalene into 2-methyl-6-nitro-naphthalene by diazo-reactions. Sandmeyer (Ber., 1887, 20, 1484) and Hantzsch and Blagdon, (Ber., 1900, 33, 2544) had shown this was possible by diazotising the amine and decomposing the resultant diazo-compound with a mixture of sodium nitrite, copper sulphate and cuprous oxide. Yields by this procedure were too low (2-5%) for the method to be of much practical value. Hodgson (J, 1944, 22; 1947, 127 and 1392) claimed to have improved this reaction; he prepared diazo-cobaltinitrites and decomposed these either at room temperature with a mixture of sodium nitrite, copper sulphate and cuprous oxide or by stirring at 0^{o} for 24 hours. Hodgson (loc. cit.) stated that, using this technique, he had converted β-naphthylamine into β-nitro-naphthalene in 65% yield.

This procedure thus appeared to be a very promising route for the preparation of the required 2-methyl-6-nitro-naphthalene; however experiment showed the yield to be only 6%. Repetition of the above work of Hodgson using β-naphthylamine gave a yield of 8-10% instead of the 65% claimed, the yield could be increased to 15-17% by increasing the quantity of sodium nitrite in the mixture used to decompose the diazonium cobaltinitrite. In view of the very low yield in this reaction and the relative inaccessibility of the starting material this route was abandoned.

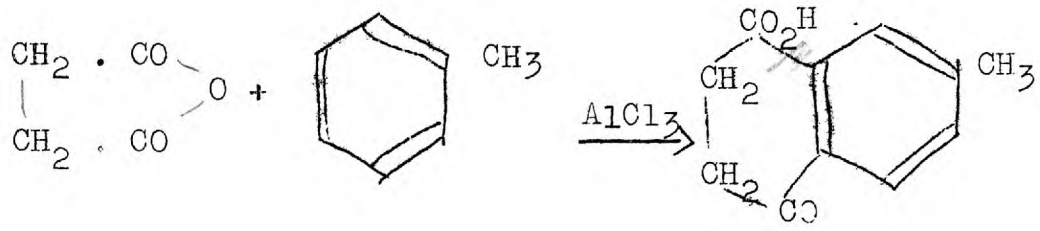
A second route for the preparation of a suitable intermediate for the synthesis of the amino-naphthalene analogues was then devised and is outlined below:-



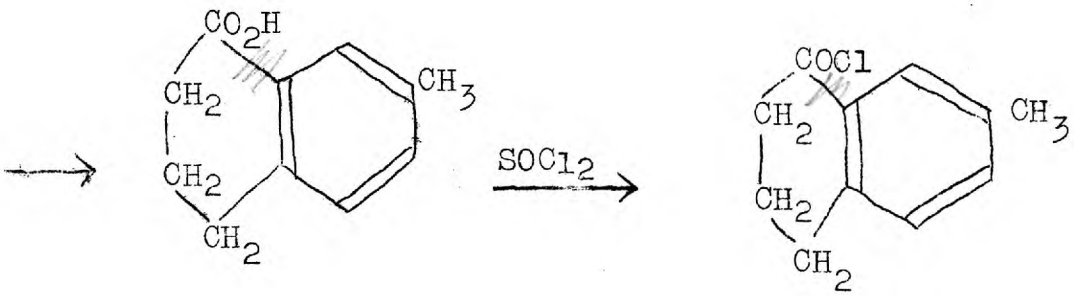
The oxidation of 2-methyl-6-acetamido-naphthalene to 6-acetamido-2-naphthoic-acid had been accomplished by Benson and King (J, 1880, 37, 752) using very dilute neutral permanganate as oxidising agent. No yield was given in the original paper.

The author prepared 2-methyl-6-acetamido-naphthalene by acetylation of the previously prepared 2-methyl-6-amino-naphthalene and oxidised it with dilute ~~alkaline~~ neutral permanganate to 6-acetamido-2-naphthoic acid. The yield on the oxidation was only 7% which was too low to be of practical value. The projected synthesis of amino-naphthalene analogues of folic and pteroic acids was then abandoned.

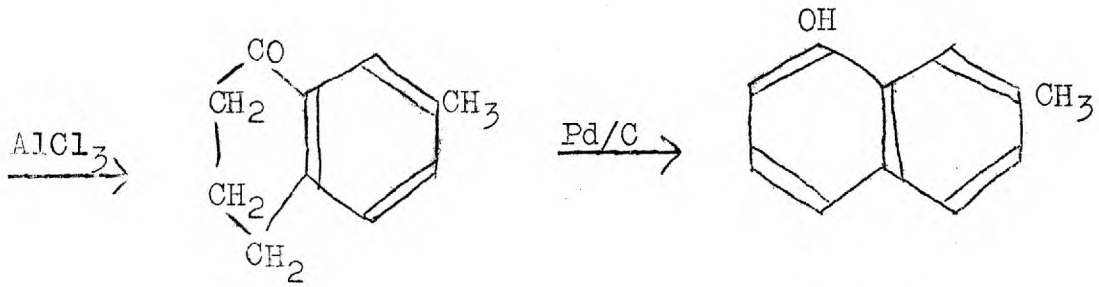
The necessary starting material for the preparation of the hydroxy-naphthalene analogues of folic and pteroic acids, ((XLIX) and (L); X = -OH, Y = H), was 2-methyl-8-hydroxy-naphthalene which had been prepared by Ruzicka and Morgelli (Helv. Chim. Acta, 1936, 19, 382) as follows:-



(LIII)



(LV)



(LVI) (LVII)

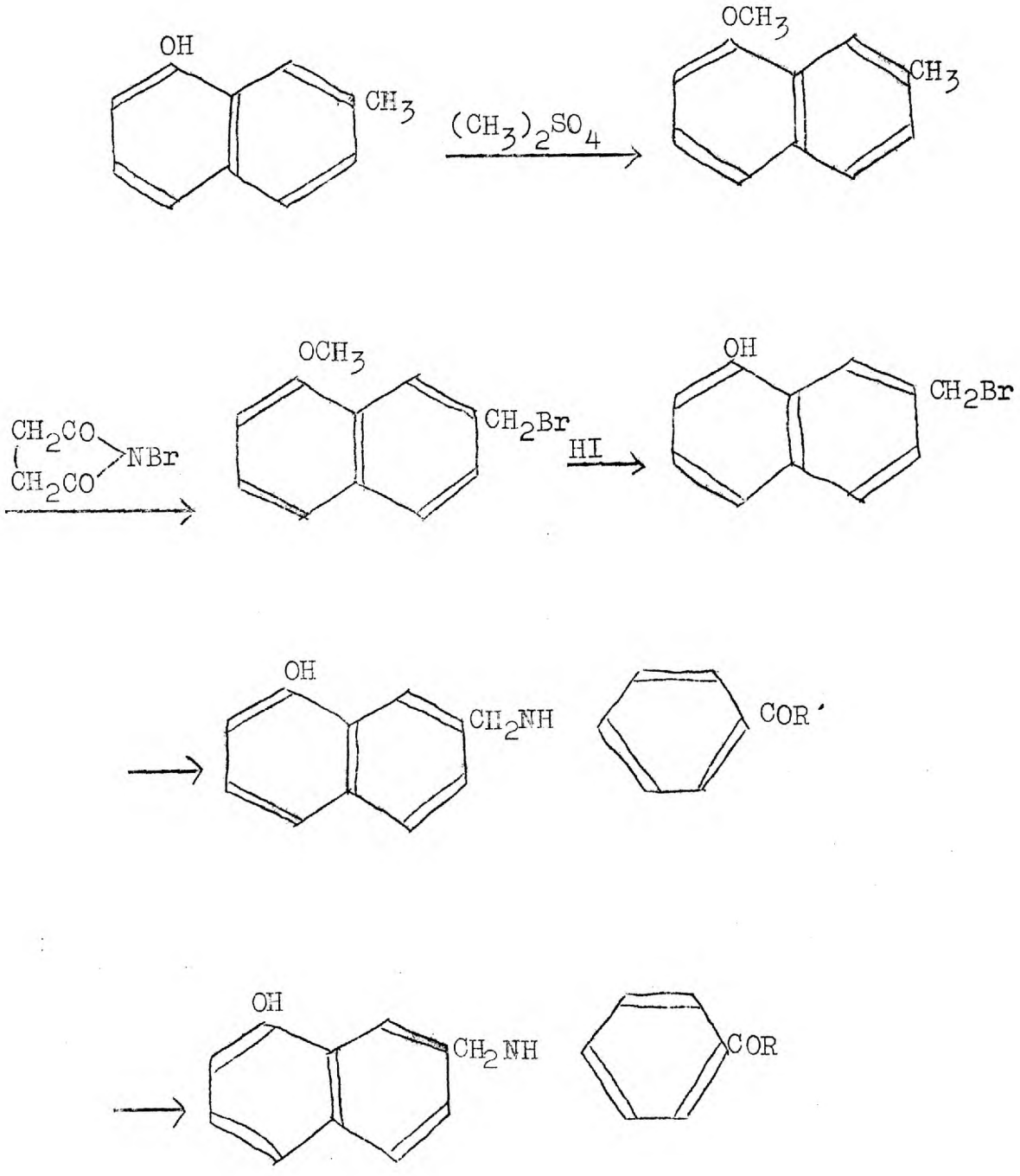
2-methyl-8-hydroxy-naphthalene was prepared by the above route using in the main the technique described in Organic Syntheses (Org. Syntheses. Coll Vol.II, 81, 499), for the preparation of α-tetralone. Several batches of 2-methyl-8-hydroxy-naphthalene were made and in the

course of this work several modifications of the above procedure were made. In the original synthesis the cyclisation of γ-(p-tolyl)-n-butyric acid chloride (LV) was accomplished by adding aluminum chloride to a solution of the acyl chloride in carbon-disulphide. The yield at this stage was improved from 60% to 80% and the quality of the product improved by using the inverse Friedel-Crafts technique of Johnson and Glenn (J. Amer. Chem. Soc., 1949, 71, 1092) in which the acid chloride, diluted with benzene, is added to a suspension of aluminium chloride in benzene below 10°C.

The preparation of γ-(p-tolyl)-n-butyric acid chloride by the action of thionyl chloride on the acid is rather unsatisfactory as considerable quantities of tar are produced. Eventually it was found to be best to cyclise the γ-(p-tolyl-)-n-butyric acid (LIV) directly using concentrated sulphuric acid as condensing agent.

Dehydrogenation of 7-methyl-1-tetralone (LVI) using palladised charcoal gave the required 2-methyl-8-hydroxy-naphthalene.

It was proposed to synthesise the hydroxy-naphthalene analogues of folic and pteroic acids from this 2-methyl-8-hydroxy-naphthalene by the following route.



R′ = -OEt or glutamic ester

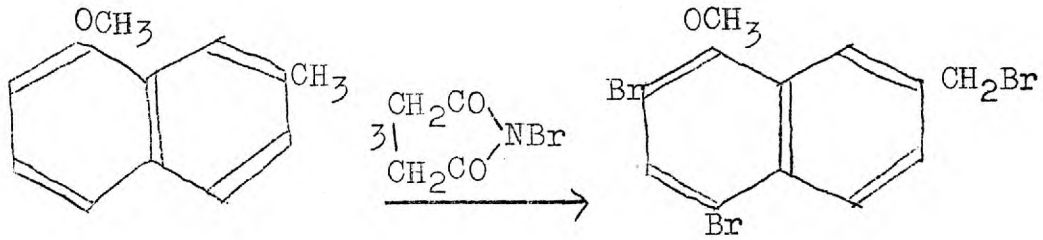
R = -OH or glutamic acid.

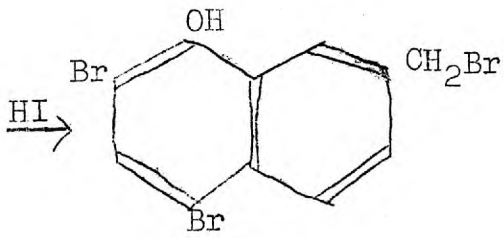
2-Methyl-8-hydroxy naphthalene was readily methylated using dimethyl sulphate. The 2-methyl-8-methoxy-naphthalene was reacted with one equivalent of N-bromo-succinimide and gave a white crystalline mono-bromide, m.p. 73°, analysing for $C_{11}H_9OBr$. This mono-bromide was resistant to hydrolysis with aqueous and alcoholic potash and gave no precipitate with alcoholic silver nitrate. These observations indicated that the product was a nuclear brominated compound. Attempts to prove this by oxidation of the methyl group were unsuccessful. When oxidations were attempted with hot alkaline permanganate or 10% nitric acid only unchanged starting materials were obtained. This tends to support the view that the mono-bromide was a nuclear brominated compound as it is known that 2-chloro-methyl-naphthalene is very readily oxidised by cold alkaline permanganate (Schulze, Ber., 1884, 17, 1529).

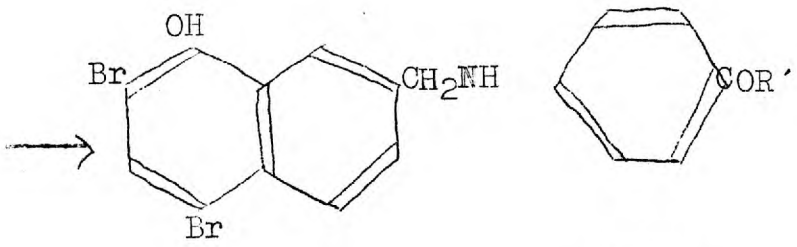
6-Methyl-2-methoxy-naphthalene also gives a nuclear bromo-compound on treatment with N-bromo-succinimide.

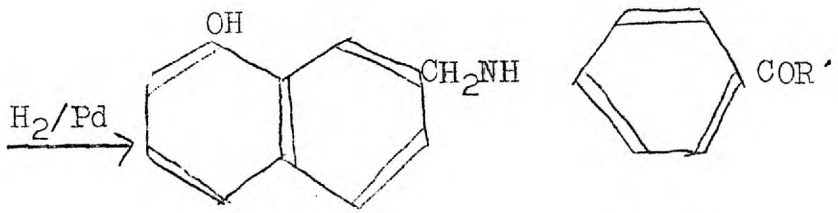
It appeared very likely that the bromine had entered the nucleus in a position either ortho- or para- to the methoxy group. It was hoped that on further bromination with N-bromosuccinimide the reactive ortho and para positions could be blocked and that a third bromine atom would then enter the side chain in the

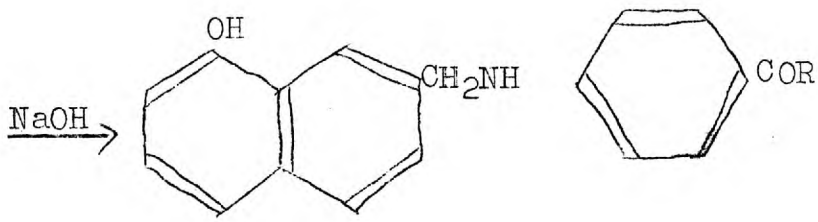
required position. This tribromo-compound could be used for the synthesis of the required hydroxy-analogues and the nuclear bromine removed at a later stage by hydrogenation, thus:-









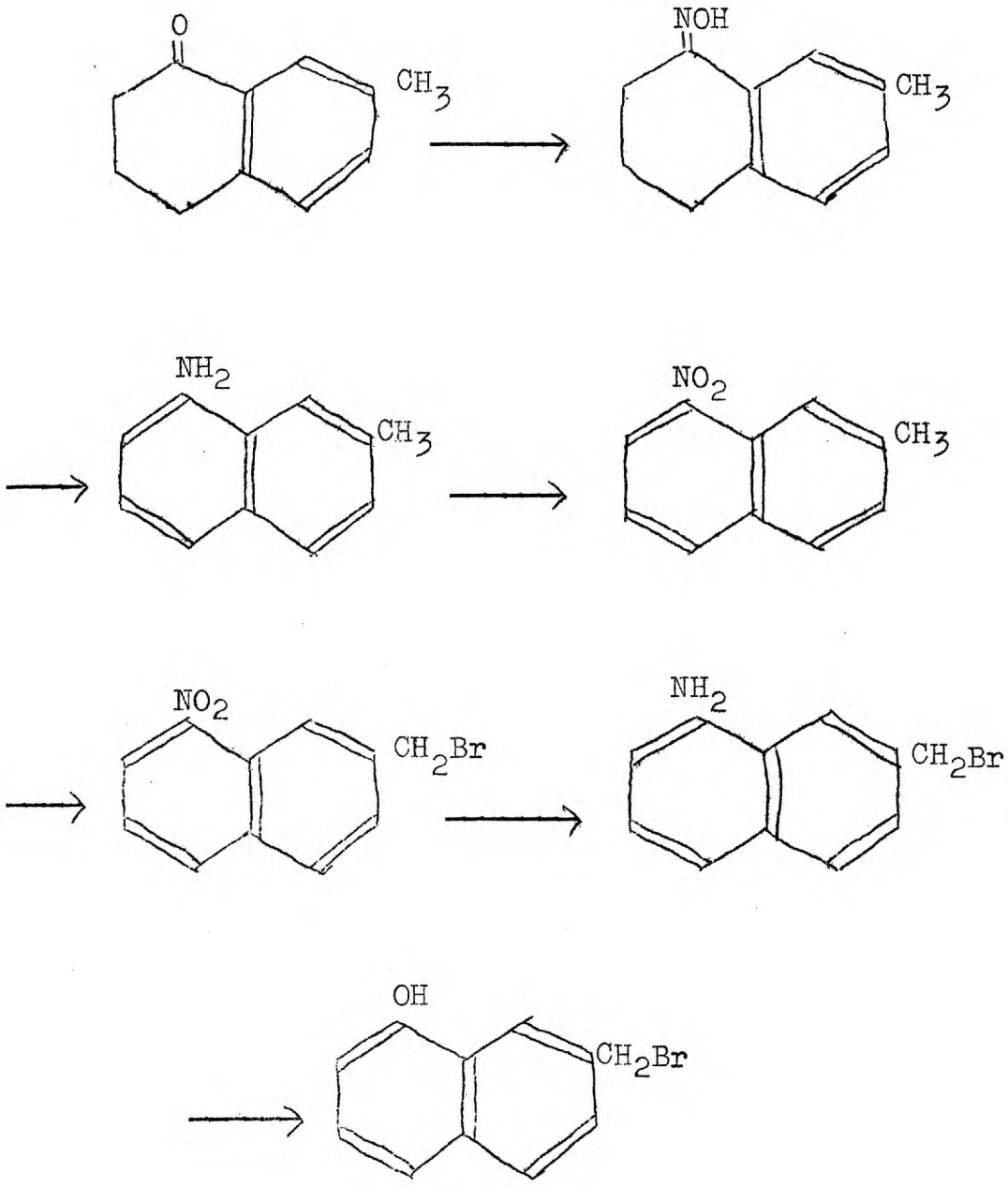


R′ = -OEt or glutamic ester

R = -OH or glutamic acid.

The previously prepared 2 methyl-8-methoxy-?-bromo-naphthalene was treated with a further equivalent of N-bromo-succinimide and gave a dibromo-compound. This dibromo-compound was also resistant to hydrolysis and oxidation and gave no precipitate with alcoholic silver nitrate and appeared to be the expected 2-methyl-8-methoxy-5:7-dibromo-naphthalene. A third equivalent of N-bromo-succinimide failed to react and this projected synthesis also had to be abandoned.

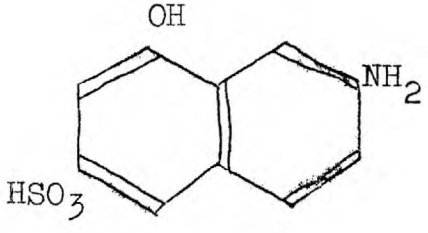
The next route studied was the following:-



Gluschke, Götzky, Huang, Irmisch, Laves, Schrader and Stier (Ber., 1930, 63, 1308) had shown that α-tetralone oxime could be rearranged to α-naphthylamine by heating in acetic acid solution containing acetic anhydride in a

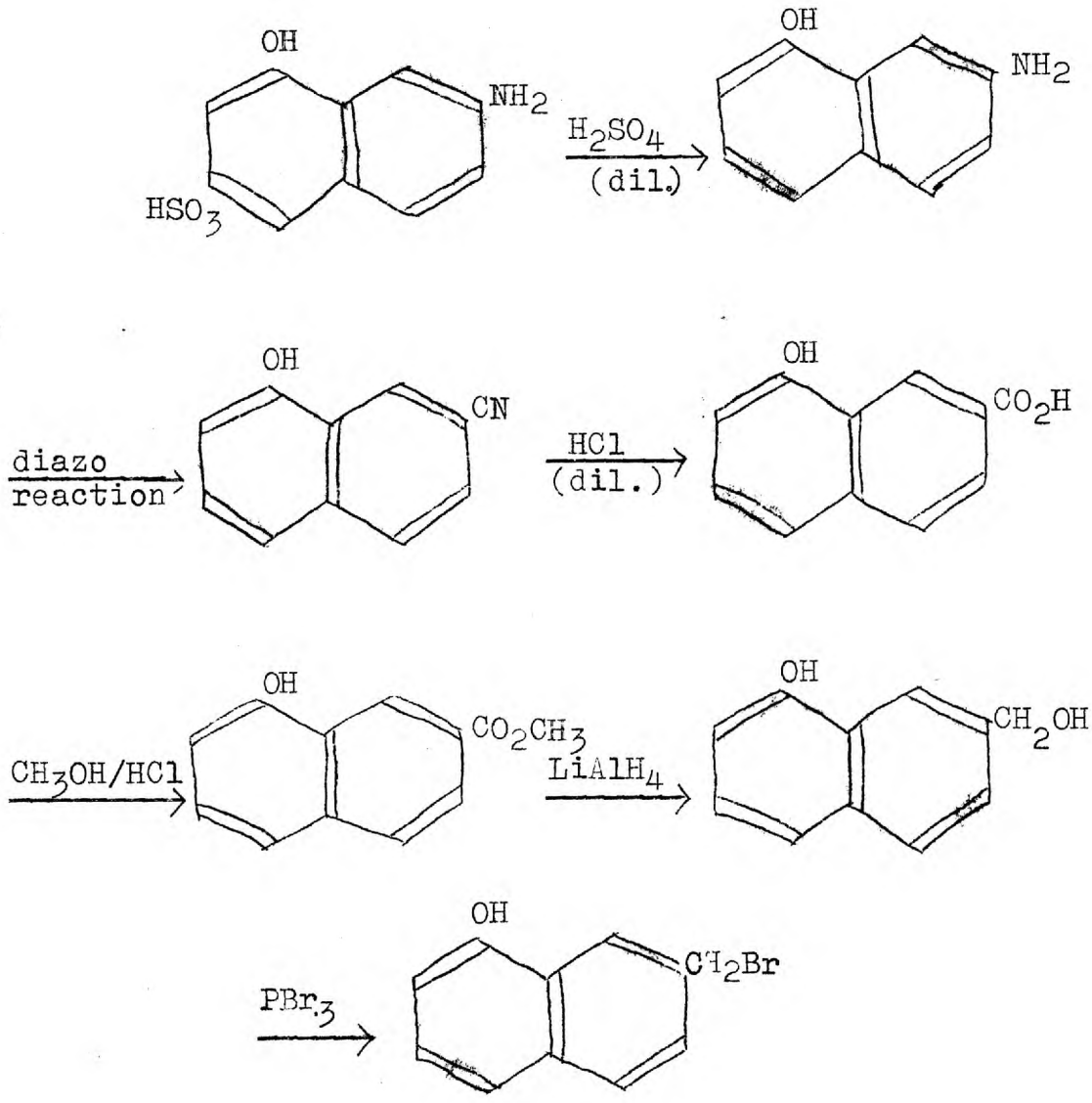
stream of dry hydrogen chloride. Application of this process to 7-methyl-α-tetralone oxime gave the acetyl-derivative of 2-methyl-8-amino-naphthalene. This was then to be converted into 2-methyl-8,9-nitro-naphthalene but, as previously stated in connection with the preparation of the amino-naphthalene analogues, this reaction does not give a practicable yield and for this reason this route had to be abandoned.

The final attempt to prepare the hydroxy-analogues used the commercially available γ-acid, 2-amino-8-naphthol-6-sulphonic acid (LVIII) as starting material.



(LVII)

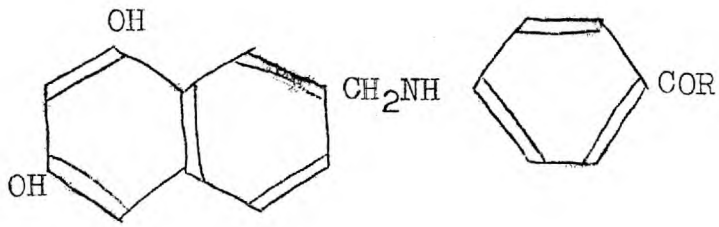
The route contemplated is as follows:-



Attempts to hydrolyse the γ-acid to 2-amino-8-naphthol failed, however, and this synthesis, too, was abandoned.

The preparation of a dihydroxy naphthalene (LIX)

analogue was considered.



R = OH or glutamic acid

(LIX)

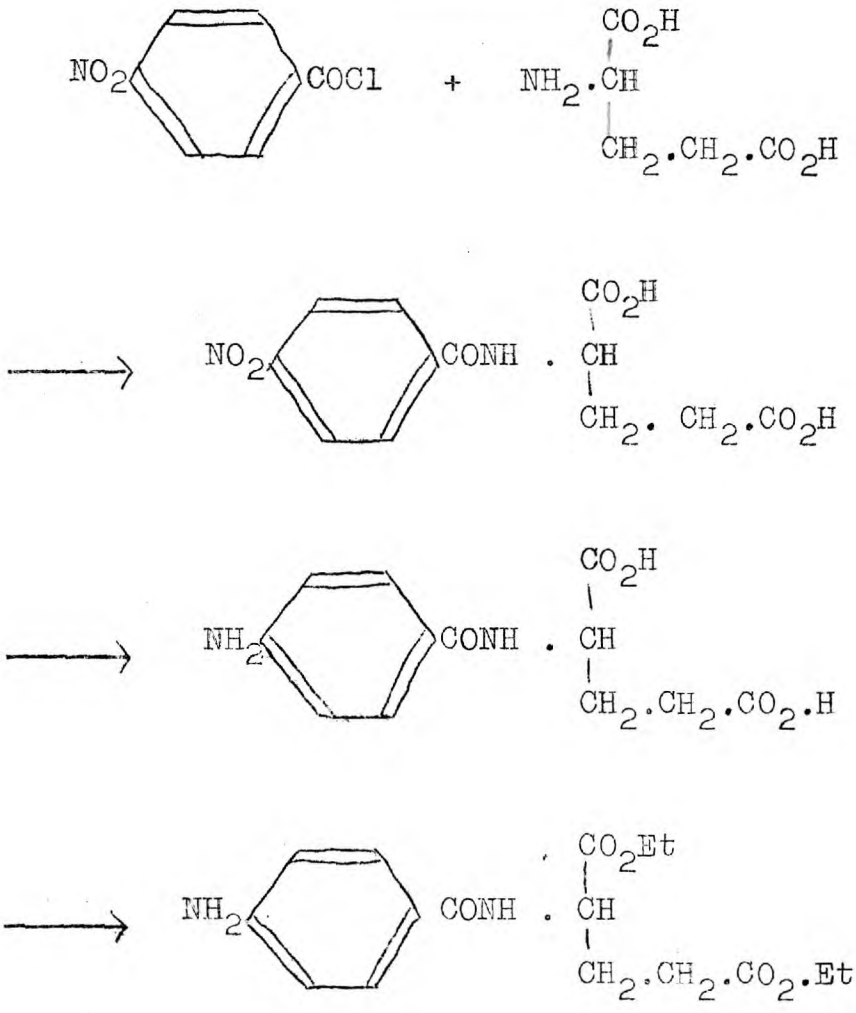
The proposed route was to fuse γ- acid with alkali to give 2-amino-6:8-naphthalenediol and then follow a similar series of reactions to those above. γ -Acid was, however, found to be very resistant to fusion and no 2-amino-6:8-naphthalenediol could be isolated from the fusion product; hence this route also had to be abandoned.

The Folic Acid Side Chain

A quantity of N(p-aminobenzoyl)-L-glutamic acid and of its diester were required for the preparation of analogues of folic acid.

A literature survey showed that two general methods were available for the preparation of these substances. Method I consists of p-nitrobenzoylating L-glutamic acid, reducing the N(p-nitrobenzoyl)-L-glutamic acid to N(p-aminobenzoyl)-L-glutamic acid and then esterifying it:-

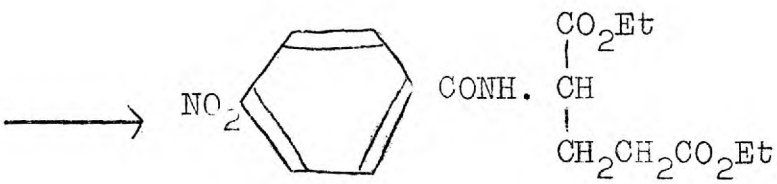
Method I

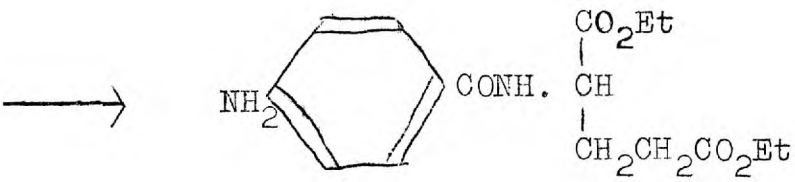


In method II the L-glutamic acid is first esterified, then p-nitro-benzoylated to give N(p-nitro-benzoyl)-L-glutamic ester which is hydrogenated to give the required N(p-aminobenzoyl)-L-glutamic ester:-

Method II







Method I has been employed by King, Acheson and Spensley (J., 1949, 1401) and by Call and Rydon (Private Communication).

King, Acheson and Spensley performed the p-nitrobenzoylation by the simultaneous addition of a solution of p-nitrobenzoyl chloride in dioxan and an equivalent amount of sodium hydroxide solution to a well stirred solution of sodium glutamate in water and obtained N(p-nitrobenzoyl)-L-glutamic acid in 60% yield. This was hydrogenated using palladised charcoal to N(p-aminobenzoyl)-L-glutamic acid which was esterified in 50% yield using cold ethanolic hydrogen chloride. Waller et al.(J. Amer. Chem. Soc., 1948, 70, 19) claim a yield of 75% for the esterification.

Call and Rydon performed the p-nitrobenzoylation by adding a large excess of p-nitrobenzoyl chloride in chloroform to a solution of glutamic acid in excess of 10% sodium bicarbonate solution and obtained N(p-nitrobenzoyl)-L-glutamic acid in 51% yield. This was then hydrogenated to N(p-aminobenzoyl)-L-glutamic acid using Adam's catalyst.

The author prepared N(p-aminobenzoyl)-L-glutamic acid and its ester using both these techniques. The method of King, Acheson and Spensley, for the preparation of N(p-nitrobenzoyl)-L-glutamic acid, gave a slightly higher yield than that of Call and Rydon and the product

was of better quality. Some difficulty was experienced in the hydrogenation of N(p-nitrobenzoyl-)-L-glutamic acid to N(p-aminobenzoyl)-L-glutamic acid when palladised charcoal was used, the product being dark and rather gummy. When Adams catalyst was used (cf. Call and Rydon) the hydrogenation went smoothly and gave a white crystalline product.

The esterfication of N(p-aminobenzoyl)-L-glutamic acid using cold ethanolic hydrogen chloride gave only a 35% yield.

Method II for the preparation of N(p-aminobenzoyl)-L-glutamic acid was also employed by King, Acheson and Spensley (J., 1949, 1401). Glutamic acid was esterified with hot ethanolic hydrogen chloride, the excess alcohol removed and the residual ethyl glutamate treated with a solution of p-nitrobenzoyl chloride in benzene. The N(p-nitrobenzoyl)-L-glutamic ester was isolated from this in 70% yield and hydrogenated to the required N(p-aminobenzoyl)-L-glutamic ester using palladised charcoal as catalyst.

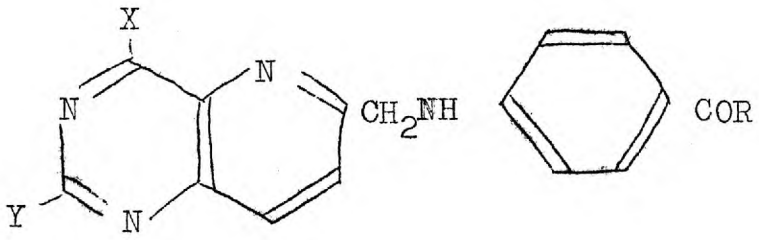
The author repeated this work and obtained diethyl N(p-nitrobenzoyl) glutamate in 50% yield. When methyl alcohol was substituted for ethyl alcohol the dimethyl N(p-nitrobenzoyl)-L-glutamate was obtained in 85% yield. This could be readily hydrogenated using Adams catalyst.

Small quantities of diethyl N(p-aminobenzoyl)-L-glutamate and dimethyl-N(p-aminobenzoyl)-L-glutamate were hydrolysed to the free acid. Neither the yield nor the quality of the product was very good.

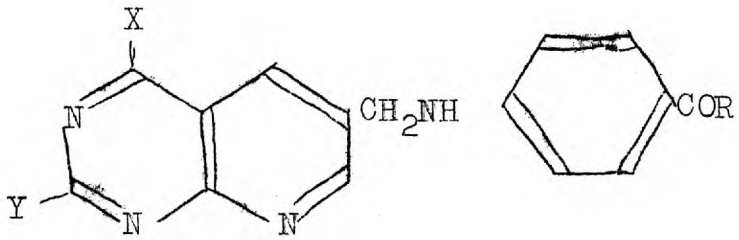
This work may be summarised by stating that Method I is the best route to the free acid and that Method II is the best for the preparation of the ester.

Triazanaphthalenes

The major part of the work described in this thesis was concerned with attempts to synthesise analogues of folic and pteroic acids based on the 1:3:5- and 1:3:8-triazanaphthalene (pyrido-pyrimidine) ring systems, the ultimate objectives having the general formulae (LX) and (LXI)



(LX)



(LXI)

R = -OH or glutamic acid.

X =	-OH	-H	-OH	-H	
Y =	-NH	$-NH_2$	-H	-H	

Such compounds may be synthesised either by starting with a preformed pyrimidine ring and building a pyridine ring onto this or conversely from pyridine derivatives by building on the pyrimidine nucleus. The first of these alternatives is closely analogous to all the published syntheses of folic acid itself; nevertheless it has not been used in this present work since the available methods for adding fused pyridine rings seemed much less adaptable than those for adding a fused pyrimidine ring. The general scheme, therefore, has been to prepare suitably substituted pyridines, having a methyl or carboxyl group in the position required for eventual introduction of the folic acid side chain, and to build the pyrimidine ring onto these. Owing to the small amount of information available on the chemistry of 1:3:5- and 1:3:8-triazanaphthalenes it has been necessary to carry out a good deal of general exploratory work in these two series.

As Lythoe (Quart. Rev., 1949, 3, 181) has pointed out the available methods for the synthesis of pyrimidines may be classified into three main types, thus:-

Method I



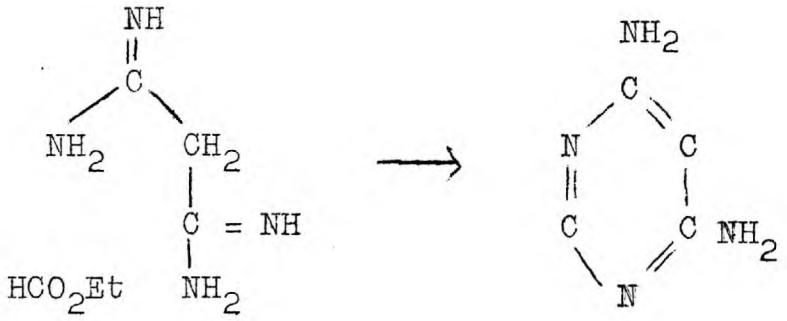
Method II



Method III

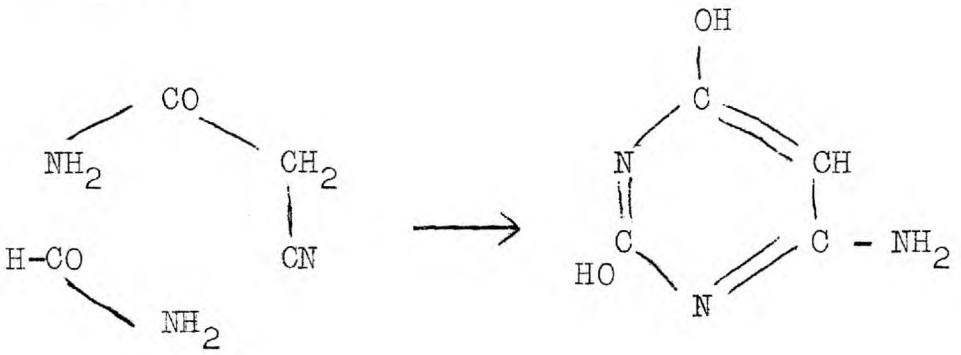


Type I may be illustrated by the condensation of malondiamidine and ethyl formate to give 4:6-diamino-pyrimidine:-

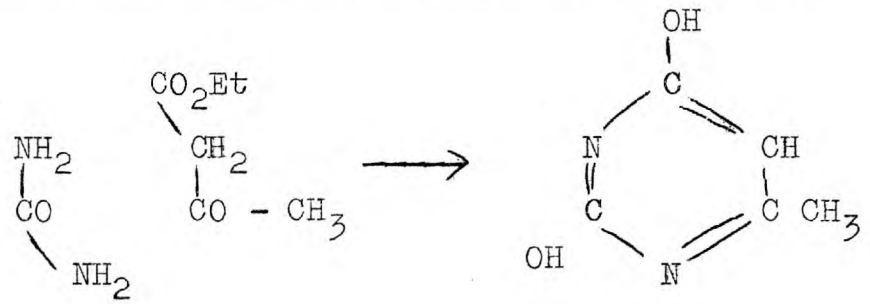


An example of Method II is the condensation of cyano-acetamide and formamide to give 6-amino-2:4-dihydroxy-

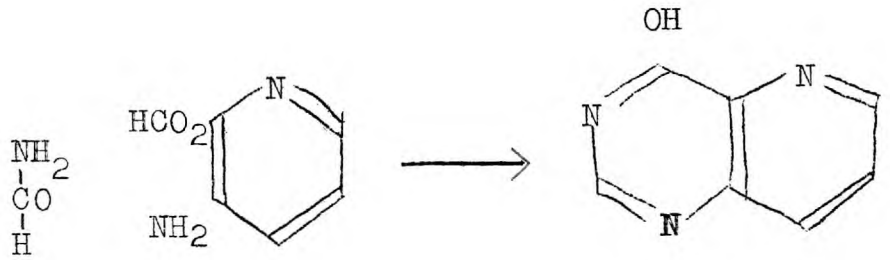
pyrimidine:-



Method III is that most widely used for the preparation of simple substituted pyrimidines, e.g. the condensation of urea and aceto-acetic ester to give 6-methyl-uracil:-



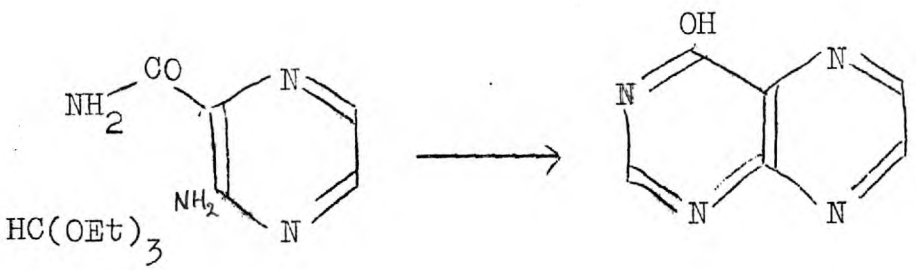
Of these general methods only the first two are readily applicable to the synthesis of triazanaphthalenes containing a pyrimidine ring fused in the 5:6 positions. Method II was used by Price and Curtis (J. Amer. Chem. Soc., 1946, 68, 914) who describe in a brief note the preparation of 4-hydroxy-1:3:5-triazanaphthalene (LXII) in 31% yield by the condensation of formamide and 3-amino-picolinic acid:-



(LXII)

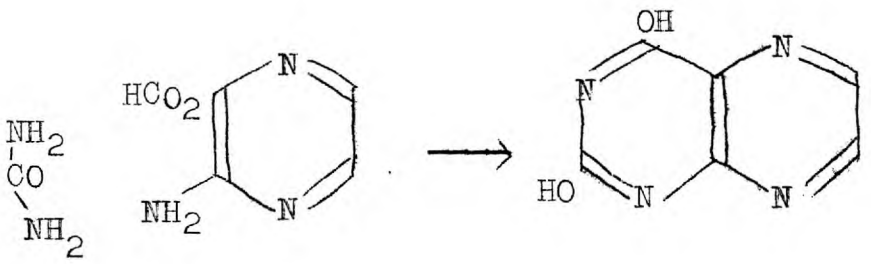
Clearly this method was worthy of a close study for the projected syntheses, the required starting materials being 3-amino-picolinic acid for the 1:3:5-triazanaphthalenes and 2-amino-nicotinic acid for the 1:3:8-triazanaphthalenes.

After this work had started Albert, Brown and Cheeseman (J., 1951, 474) published the synthesis of several pterins from pyrazine intermediates which is a very similar problem to the synthesis of triazanaphthalenes from pyridine intermediates; these authors used Method I. An example is the synthesis of 4-hydroxy-pteridine (LXIV) in 75% yield by refluxing 2-amino-pyrazine-3-carboxyamide (LXIII) with ethyl ortho-formate and acetic anhydride:-



(LXIII) (LXIV)

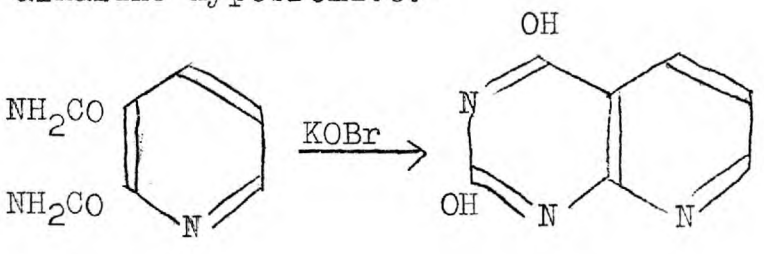
These authors also report unsuccessful attempts to use Method II in this field, e.g. heating urea with 3-amino-pyrazine-3-carboxylic acid failed to give the expected 2:4-dihydroxy-pteridine (LXV):-



(LXV)

As several of these unsuccessful syntheses were shown to be applicable to the synthesis of triazanaphthalenes it is clear that the analogy between the synthesis of pterins from pyrazine intermediates and of triazanaphthalenes from pyridine intermediates cannot be pressed too closely.

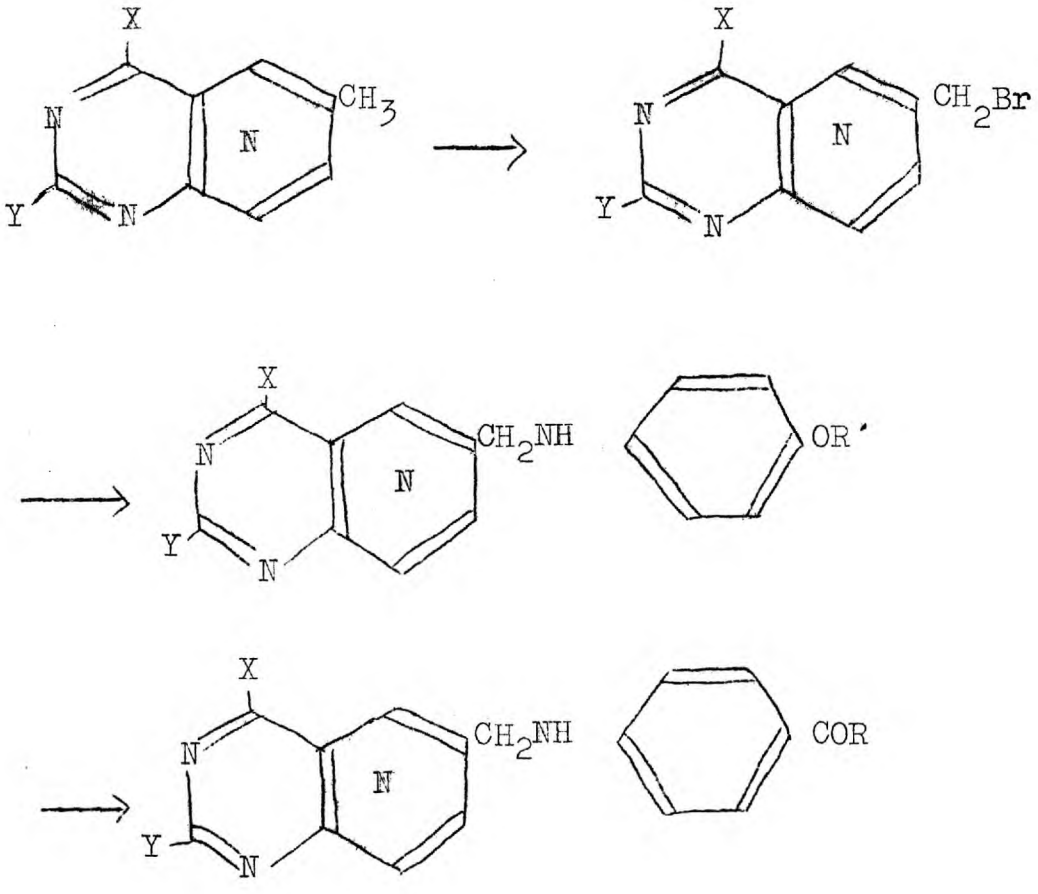
McClean and Spring (J., 1949, 2582), in the only publication on the preparation of 1:3:8-triazanaphthalenes, used a method which does not strictly fall into Lythgoe's classification. These authors prepared 2:4-dihydroxy-1:3:8-triazanaphthalene (LXVI) by treating quinolinamide with cold alkaline hypobromite:-



(LXVI)

On the basis of this work it was decided to investigate the synthesis of triazanaphthalenes, first the simpler compounds and later those with a suitable substituent in the 6-position for introduction of the folic acid side chain.

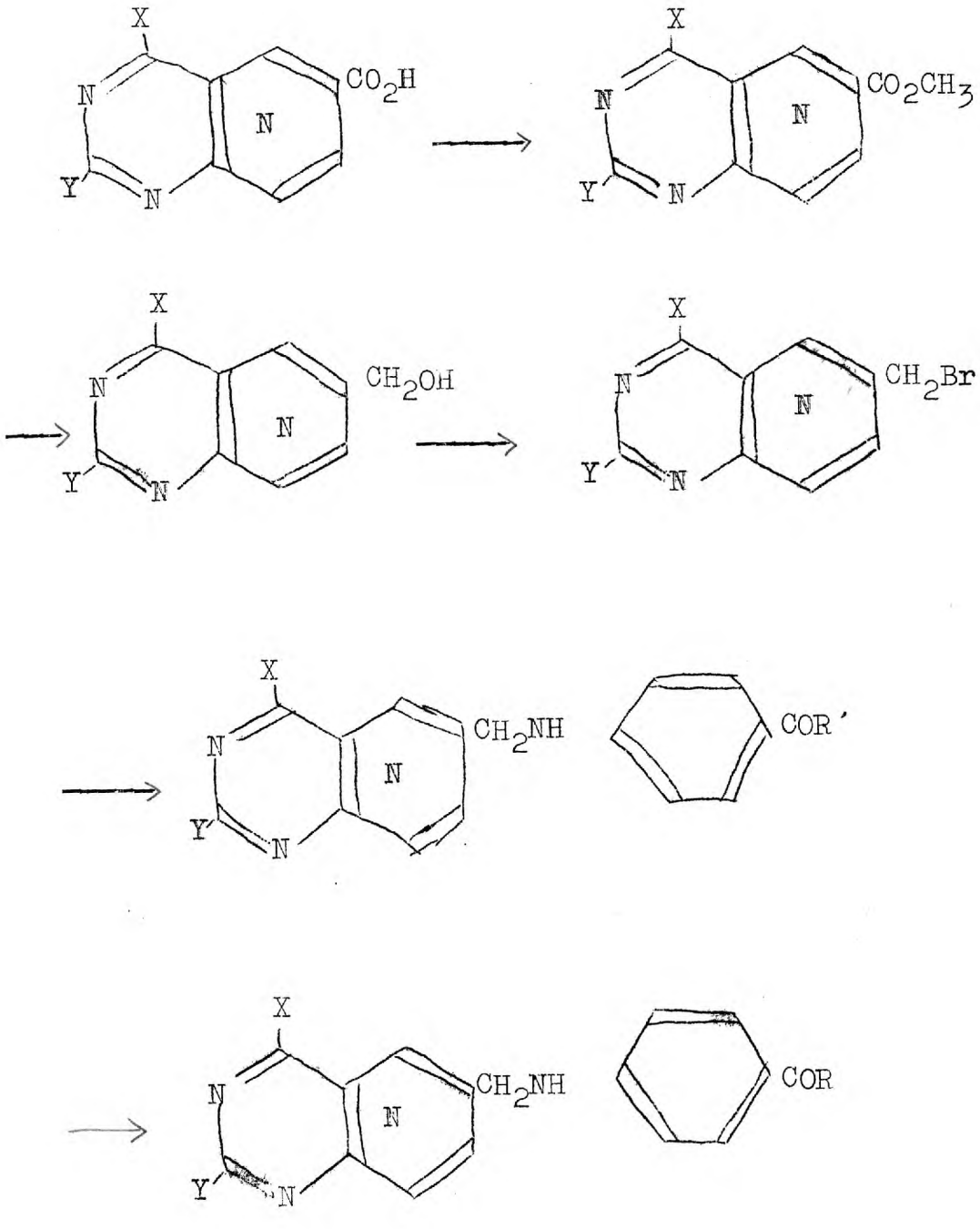
Suitable substituents for the introduction of the side chain are a methyl group or a carboxyl group. The route when a methyl group is present consists of side chain bromination (the use of N-bromo-succinimide was envisaged) followed by condensation with ethyl p-aminobenzoate or with dimethyl N(p-aminobenzoyl)-L-glutamate followed by hydrolysis of the resulting esters, thus:-



R′ = -OEt or glutamic ester

R = -OH or glutamic acid.

When a carboxyl group is present in the 6-position the side chain could be introduced by the following route. The carboxyl group is esterified and reduced with lithium aluminium hydride to a hydroxymethyl group. This side-chain hydroxyl group is then converted to a bromo-derivative which may then be condensed, as above, with ethyl p-aminobenzoate or with dimethyl N-(p-aminobenzoyl)-L-glutamate and the resulting ester hydrolysed:-

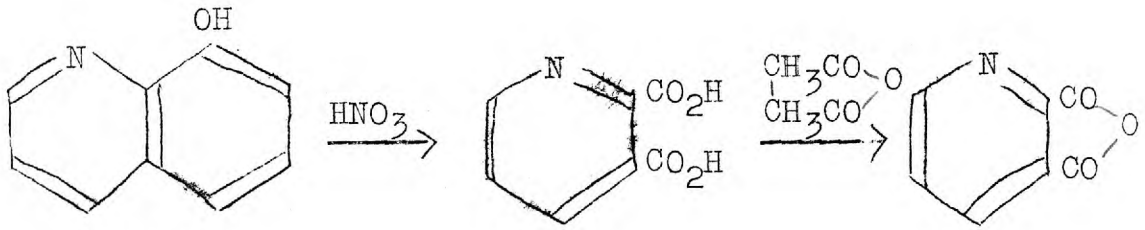


R′ = -OEt or glutamic ester.

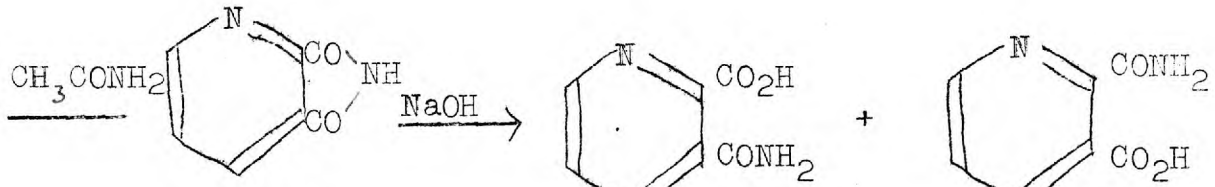
R = -OH or glutamic acid.

Synthesis of Simple Derivatives of 1:3:5-Triazanaphthalene.

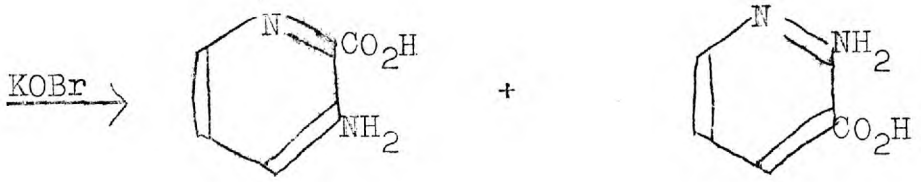
A suitable starting material for the synthesis of simple derivatives of 1:3:5-triazanaphthalenes of the type required is 3-amino-picolinic acid (LXIX) which was obtained, together with 2-amino-nicotinic acid (LXX), a suitable starting material for the preparation of 1:3:8-triazanaphthalenes, from 8-hydroxy-quinoline (LXVII) by way of quinolinic imide (LXVIII) following the procedure of Such arda (Ber., 1925, 58, 1727):-



(LXVII)



(LXVIII)

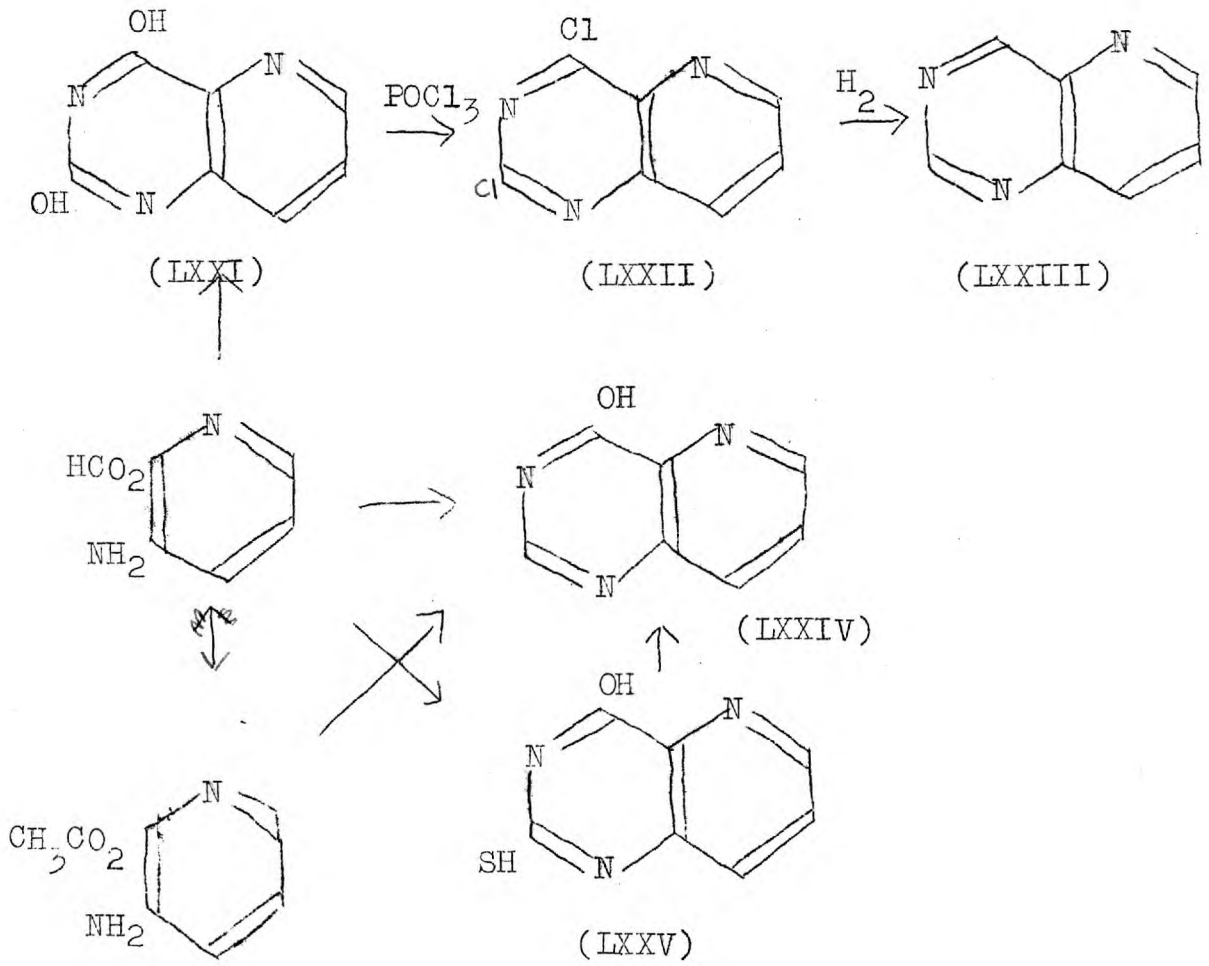


(LXIX) (LXX)

Sucharda's procedure was followed with only minor modifications which are detailed in the experimental section.

3-Amino-picolinic acid was also obtained in minor amounts as a by-product in the preparation of 2:4-dihydroxy-1:3:8-triazanaphthalene by the method of McClean and Spring (J., 1949, 2582) (see p.82).

Starting with 3-amino-picolinic acid, derivatives of 1:3:8-triazanaphthalene were prepared according to the following scheme:-



Heating 3-amino-picolinic acid with urea at 200° gave 2:4-dihydroxy-1:3:5-triazanaphthalene (LXXI) as a white, crystalline solid m.p. >360°. It is of interest to note that a similar reaction i.e. the fusion of urea and 3-amino-pyrazine-2-carboxylic acid to give 2:4-dihydroxy-pteridine, failed when attempted by Albert, Brown and Cheeseman (J., 1951, 474).

Attempts were made to chlorinate this 2:4-dihydroxy-1:3:5-triazanaphthalene using the technique used by McClean and Spring (J., 1949, 2582) for the chlorination of 2:4-dihydroxy-1:3:8-triazanaphthalene. This consists of refluxing the dihydroxy compound with excess phosphorus oxychloride until solution occurs, removing excess phosphorus oxychloride under reduced pressure, pouring the residue onto ice and extracting the dichloro-compound with chloroform. When 2:4-dihydroxy-1:3:5-triazanaphthalene was refluxed with excess phosphorus oxychloride it did not dissolve and a grey, insoluble solid was produced. The grey solid was filtered off and subjected to the rest of the procedure of McClean and Spring. This was unsuccessful, chloroform failing to extract any product from the mixture. The grey solid had no melting point, was deliquescent, was soluble in water and reacted with bicarbonate and was insoluble in organic solvents. Sodium fusion showed it to contain nitrogen, chlorine and

considerable quantities of phosphorus. A similar product was obtained when attempts were made to chlorinate 2:4-dihydroxy-1:3:5-triazanaphthalene using a mixture of phosphorus oxychloride and phosphorus pentachloride or .. by using phosphorus oxychloride in a sealed tube at 150°. This grey product could not be purified for analysis but is probably a phosphate.

Kenner, Lythgoe, Todd and Topham, (J., 1943, 574) had shown that the yield of 4:6-dichloro-pyrimidine, produced by the chlorination of 4:6-dihydroxy-pyrimidine with phosphorus oxychloride, was increased considerably and the reaction made much more reliable if a quantity of dimethylaniline was present. Application of this procedure to 2:4-dihydroxy-1:3:5-triazanaphthalene (LXXI) gave the required 2:4-dichloro-1:3:5-triazanaphthalene (LXXII). The reaction was not very reliable and could not be scaled up. The best yield ever obtained was only 46%.

It was hoped to dehalogenate this 2:4 dichloro-1:3:5-triazanaphthalene (LXXII) by hydrogenation to give the parent ring system 1:3:5-triazanaphthalene (LXXIII). Dehalogenation has been successfully used in the pyrimidine field by Marshall and Walker (J., 1951, 1013) who used a palladised strontium carbonate catalyst in ethanol, in the presence of magnesium oxide for the preparation of

2-chloro-4-methyl-pyrimidine from 2:4-dichloro-6-methyl pyrimidine.

Dehalogenation was also employed by Leese (Ph.D. Thesis, London, 1951, p.39) who used palladised strontium carbonate in N-sodium hydroxide for the hydrogenation of 2:3-diamino-5-bromo-pyridine to 2:3-diamino-pyridine.

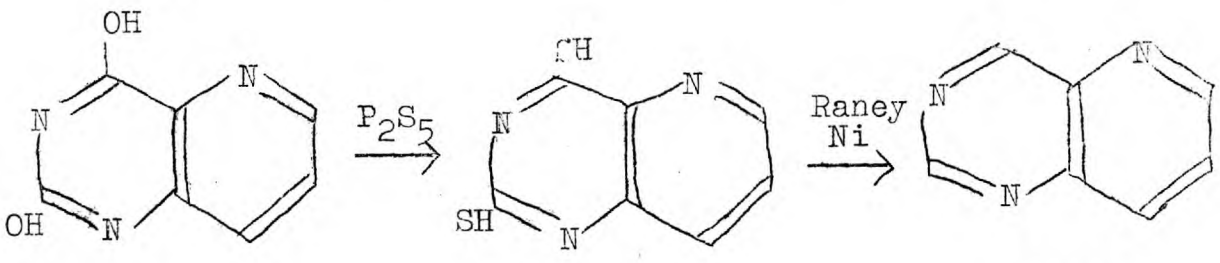
When 2:4-dichloro-1:3:5-triazanaphthalene (LXXII) was hydrogenated in methanol solution using a palladised strontium carbonate catalyst in the presence of magnesium oxide the product was clearly not the expected 1:3:5-triazanaphthalene (LXXIII). It was a white crystalline solid, m.p. 98° and was purified by sublimation. Analyses gave the following results:- C, 69.16, 70.16 and 69.92; H, 6.58, 7.00 and 7.11; N, 23.84. . Treatment of this compound with picric acid gave a picrate, m.p. 171°, found by analysis to contain C, 51.99% and H, 4.41%. These analyses agree with the formula $C_{14}H_{16}N_4$ (Calculated:- C, 69.9; H, 6.67; N, 23.4) and picrate $C_{14}H_{16}N_4.C_7H_3O_7N_3$ (Calculated: C, 52.3; H, 3.95). The structure of this product is not clear and further work is required for its elucidation.

4-Hydroxy-1:3:5-triazanaphthalene (LXIV) was prepared by heating 3-amino-picolinic acid with formamide. This gives the same product as prepared by Price and Curtis (J. Amer. Chem. Soc., 1946, 68, 914) and merely serves

to confirm their results. Heating the methyl ester of 3-amino-picolinic acid with formamide also gave 4-hydroxy-1:3:5-triananaphthalene (LXXIV). The identity of these two products was confirmed by m.p. and mixed m.p. and by comparison of their light-absorption curves.

The reaction between thiourea and 3-amino-picolinic acid gave 4-hydroxy-2-mercapto-1:3:5 triazanaphthalene (LXXV), which, on catalytic desulphurisation, with Raney nickel gave the previously prepared 4-hydroxy-1:3:5-triazanaphthalene. The yield on the catalytic desulphurisation stage was very low (only 21%). These results may be compared with those of Albert, Brown and Cheeseman (J., 1951, 474) who found that 4-amino-2-mercapto-pteridine did not react when refluxed in aqueous ammonia with Raney nickel and that 2-mercapto-pteridine and 2-mercapto-4-hydroxy-pteridine were destroyed under these conditions. The reaction, however, is known to proceed quite smoothly in the pyrimidine field (Brown, J. Soc. Chem. Ind., 1950, 69, 353).

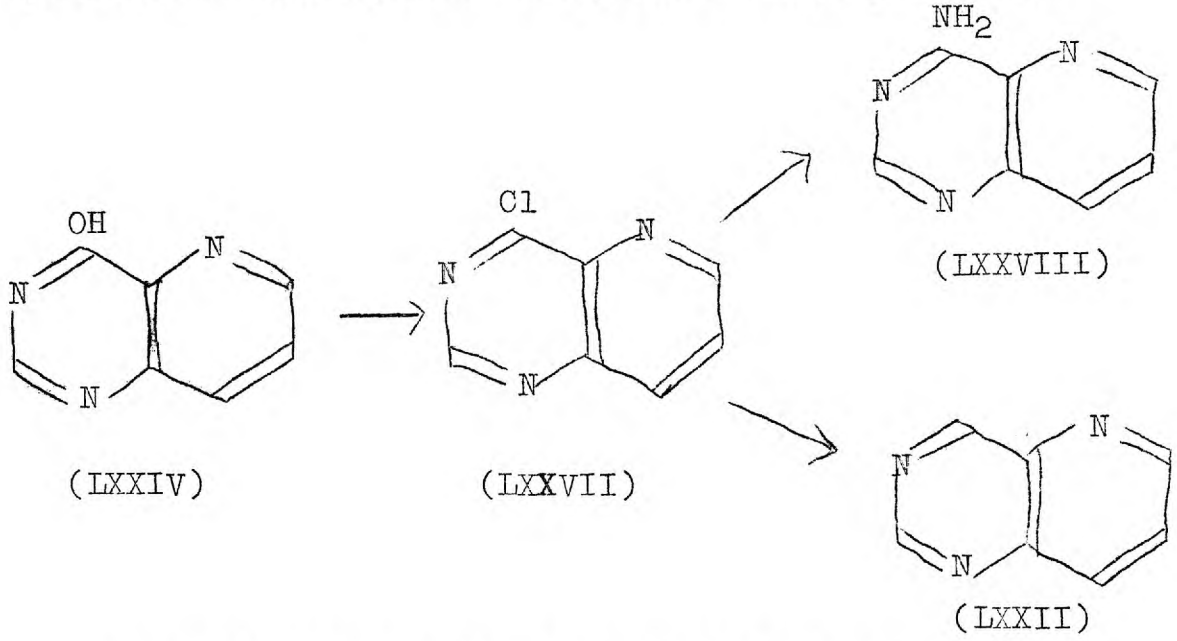
An attempt was also made to prepare 1:3:5-~~triazanapt~~ triazanaphthalene by the following route:-



(LXXI) (LXXVI) (LXXIII)

Elion and Hitchings, (J. Amer. Chem. Soc.,1947, 69, 2138) had prepared several dimercapto-uracils by heating the uracil with phosphorus pertasulphide in tetralin at 160°-180°. When this procedure was applied to 2:4-dihydroxy-1:3:5-triazanaphthalene, however, none of the expected 2:4-dimercapto-1:3:5-triazanaphthalene (LXXVI) was obtained.

The preparation of 4-chloro-1:3:5-triazanaphthalene (LXVII) and its conversion into 4-amino-1:3:5-triazanaphthalene (LXXVIII) and 1:3:5 triazanaphthalene (LXXII) was contemplated using the following routes:-

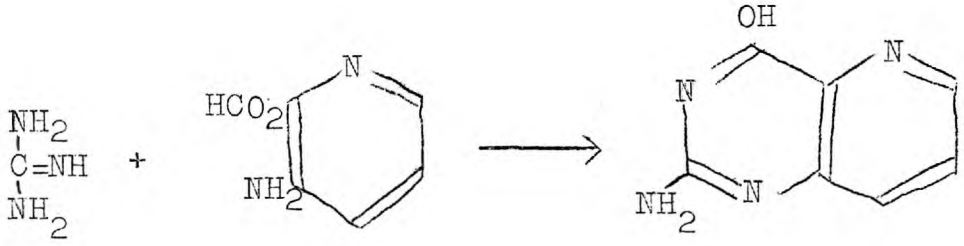


(LXXIV) (LXXVII) (LXXVIII) (LXXII)

Owing to the failure to chlorinate 4-hydroxy-1:3:5 triazanaphthalene (LXXIV) this group of reactions could not be performed. Unsuccessful attempts were made to chlorinate 4-hydroxy-triazanaphthalene (LXXIV) using

phosphorus oxychloride, and a mixture of phosphorus oxychloride and dimethyl aniline. Price and Curtis (J. Amer. Chem. Soc., 1946, 68, 914) reported that they could not chlorinate 4-hydroxy-1:3:5-triazanaphthalene "by any of the usual procedures" but gave no practical details.

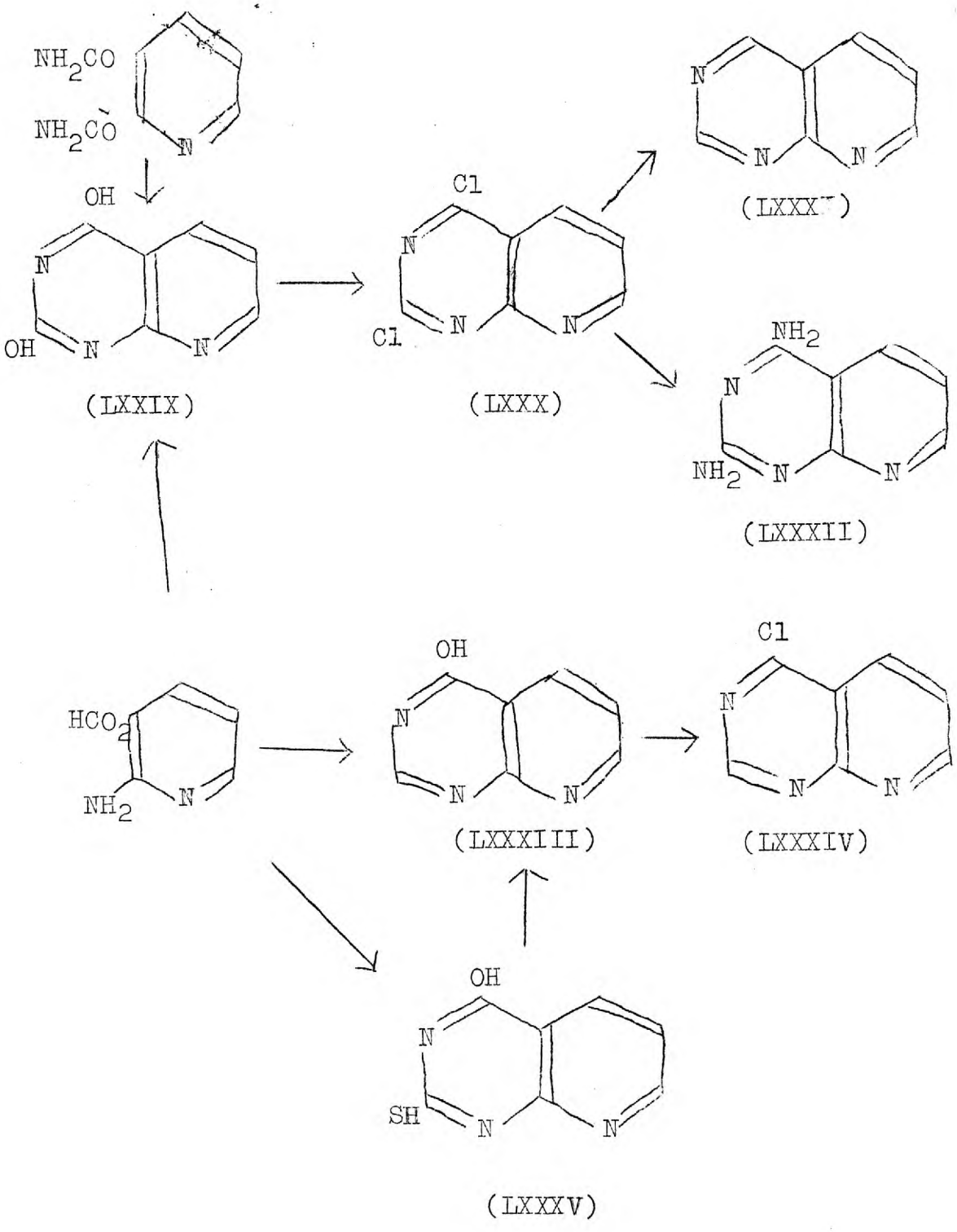
Attempts were also made to prepare 2-amino-4-hydroxy-1:3:5-triazanaphthalene by the condensation of 3-amino-picolinic acid with guanidine hydrochloride or carbonate.



The expected product was never isolated mainly, it is thought, owing to difficulties in working up the reaction mixture. This synthesis is worthy of further investigation.

Synthesis of Simple Derivatives of 1:3:8-Triazanaphthalene.

There are two routes for the preparation of 1:3:8-triazanaphthalenes. The first is that based on the work of Price and Curtis (J. Amer. Chem. Soc., 1946, 68, 914) which uses 2-amino-nicolinic acid as starting material and the second is the method of McClean and Spring (J., 1949, 2582) which uses quinolinamide as starting material. As previously mentioned in connection with the 1:3:5-triazanaphthalenes, 2-amino-nicotinic acid was obtained from 8-hydroxy-quinoline using the method of Sucharda (Ber., 1925, 58^{II}, 1727) a small quantity of 2-amino-nicotinic acid was also obtained as a by-product of the McClean and Spring reaction. 8-Hydroxy-quinoline was oxidised by the method of Suchorda (loc.cit.) and the resulting quinolinic acid converted into its diamide using the technique of Linstead, Noble and Wright (J., 1937, 911). Using 2-amino-nicotinic acid and quinolinamide as starting materials derivatives of 1:3:8-triazanaphthalene were prepared according to the following scheme:-


NH2CO
NH2CO
OH
N
OH
N
N
(LXXIX)
Cl
N
Cl
N
N
(LXXX)
N
N
N
(LXXXI)
NH2
N
NH2
N
N
(LXXXII)
HCO
NH2
N
OH
N
N
N
(LXXXIII)
Cl
N
N
N
(LXXXIV)
OH
N
SH
N
N
(LXXXV)

The action of alkaline hypobromite on quinolinamide gave 2:4-dihydroxy-1:3:8-triazanaphthalene (LXXIX). This is a repetition of the work of McClean and Spring (J., 1949, 2582). Heating urea and 2-amino-nicotinic acid gave the same compound, 2:4-dihydroxy-1:3:8-triazanaphthalene (LXXIX). The identity of these two products was confirmed by m.p. and mixed m.p. and by comparison of the light absorption.

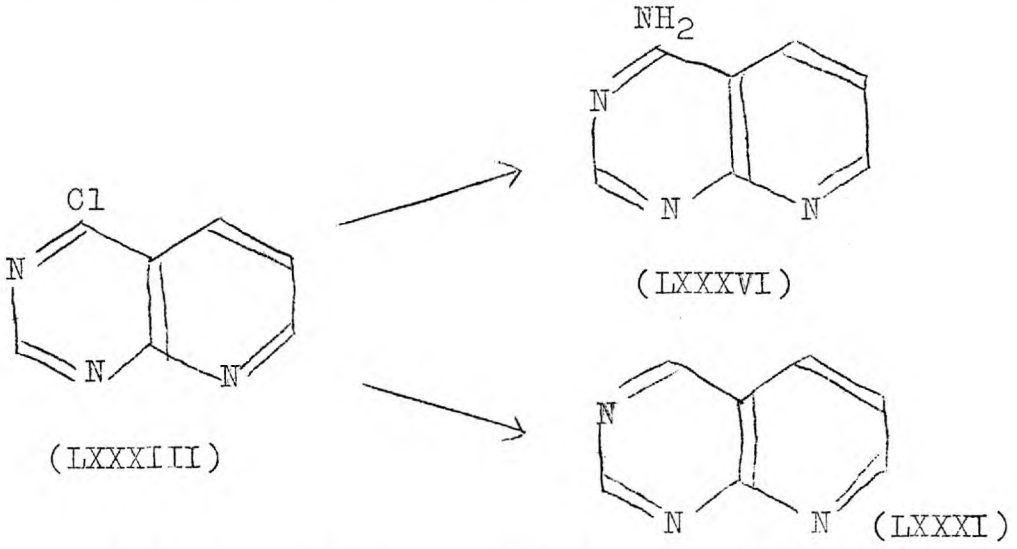
McClean and Spring chlorinated this 2:4-dihydroxy-1:3:8 triazanaphthalene by the following procedure; the dihydroxy compound was refluxed with excess phosphorus oxychloride until solution occurred, most of the excess phosphorus oxychloride was distilled off under reduced pressure and the residue was poured onto cold water and the 2:4-dichloro-1:3:8-triazanaphthalene (LXXX) extracted with chloroform. An attempt was made by the author to prepare 2:4-dichloro-1:3:8-triazanaphthalene (LXXX) using this same technique but no product was obtained. The use of a mixture of dimethylaniline and phosphorus oxychloride as chlorinating agent was also unsuccessful. The technique by which the required 2:4-dichloro-1:3:8-triazanaphthalene (LXXX) was finally obtained is as follows:- the 2:4-dihydroxy-1:3:8-triazanaphthalene (LXXIX) was refluxed with excess phosphorus oxychloride until solution occurred and then all traces of phosphorus oxychloride were removed and the residue treated with a

little ice-cold water. The required 2:4-dichloro-1:3:8-triazanaphthalene (LXXX) could then be extracted in 71% yield compared with McClean and Spring's 35%.

When 2:4-dichloro-1:3:8-triazanaphthalene (LXXX) was hydrogenated in methanol solution, using a palladised charcoal catalyst in the presence of magnesium oxide, the product was not the expected 1:3:8 triazanaphthalene (LXXXI); it was a white, crystalline solid m.p. 140° giving the following analyses:- Found: C, 55.42% and 55.04%; H, 6.94% and 6.70%; N, 21.87% (Dumas) and 19.39% (Kjeldahl). 1:3:8-Triazanaphthalene requires C, 64.1%, H, 8.84% and N, 32.05%. This unknown product gave a picrate, m.p. 211° (Found: C, 42.62% and 42.55%; H, 4.07% and 4.13%). These analyses agree with the empirical formulae $C_9H_{13}O_2N_3$ for the product (Required: C, 55.4%; H, 6.7%; N, 21.6%) and $C_9H_{13}O_2N_3 \cdot C_7H_3O_7N_3$ for the picrate (Required: C, 42.4% and H, 3.8%). These figures agree with the addition of two molecules of methanol to the expected 1:3:8-triazanaphthalene i.e $C_7H_5N_3.2CH_3OH$, but it is difficult to formulate such a product and further work will be necessary before any structure can be assigned.

2:4-Dichloro-1:3:8-triazanaphthalene (LXXX) was readily aminated by heating with alcoholic ammonia in a sealed tube at 150° to give 2:4-diamino-1:3:8-triazanaphthalene (LXXXII).

4-Hydroxy-1:3:8-triazanaphthalene (LXXXIII) was prepared from 2-amino-nicotinic acid and formamide. Attempts to chlorinate this to 4-chloro-1:3:8-triazanaphthalene using the technique of McClean and Spring or with phosphorus oxychloride and dimethylaniline were unsuccessful. The 4-hydroxy-1:3:8-triazanaphthalene was finally chlorinated in poor yield using the technique which was successful for the chlorination of 2:4-dihydroxy-1:3:8-triazanaphthalene (see p.82). It was intended to prepare 4-amino-1:3:8-triazanaphthalene (LXXXVI) by amination of 4-chloro-1:3:8-triazanaphthalene (LXXXIII) and to hydrogenate 4-chloro-1:3:8-triazanaphthalene to 1:3:8-triazanaphthalene (LXXXI) but lack of material frustrated these plans.



Heating thiourea with 2-amino-nicotinic acid gave 2-mercapto-4-hydroxy-1:3:8-triazanaphthalene (LXXXV) which was desulphurised using Raney nickel to the previously prepared 4-hydroxy-1:3:8-triazanaphthalene (LXXXIII).

Properties of Triazanaphthalenes

All the trianaphthalenes which contained hydrogen-bonding groups i.e. -OH, $-NH_2$ and -SH, were high melting solids, most of which did not melt below 360° , and were very slightly soluble in water and insoluble in the common organic solvents. All could be sublimed in a high vacuum (10^{-4}mm.) and at moderate temperatures (130° - 170°).

The hydroxy- and dihydroxy- triazanaphthalenes were soluble in sodium hydroxide and insoluble in sodium bicarbonate. They were readily purified by dissolving in 10% sodium hydroxide and precipitating with carbon dioxide.

The chloro- triazanaphthalenes were soluble in all the common organic solvents and insoluble in water and had melting points in the 100° - 200° range. They could be readily sublimed at 100° - 150° at 10^{-1}mm.

All the triazanaphthalenes when treated with ultraviolet light emitted a bluish fluorescence the intensity being the greater with the disubstituted ones. The ultraviolet spectra provides a useful method of identifying and estimating the purity of the triazanaphthalenes and Table IV gives the characteristic spectra of the trianaphthelene prepared.

TABLE IV

Light Absorption of Triazanaphthalenes

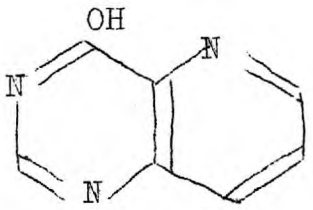
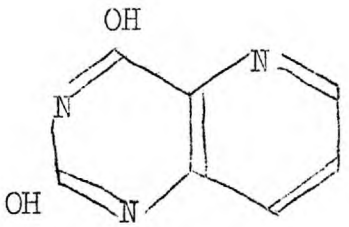
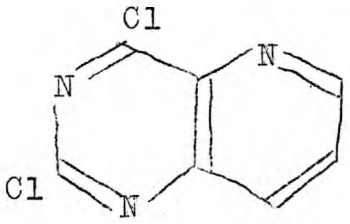
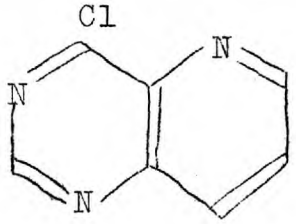
Substance	Solvent			$E^{1\%}_{1cm.}$	
	MeOH	265	M	470	6,900
		280	M	380	5,580
		291	M	290	4,260
		302	M	290	4,260
	0.1N NaOH	258	M	220	3,580
		265	M	250	4,070
		310	I	120	1,960
		328	M	140	2,380
		330	I	120	1,960
	$CHCl_3$	243	M	470	9,400
		248	M	470	9,400
		332	M	250	5,000
		294	M	210	4,200
	EtOH	265	M	430	7,110
		280	M	260	4,300
		304	M	260	4,300

TABLE IV (contd)

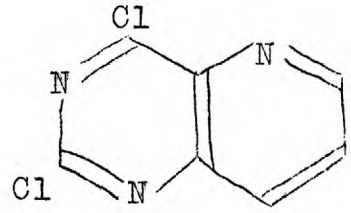
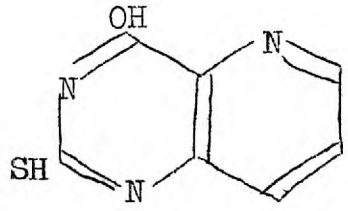
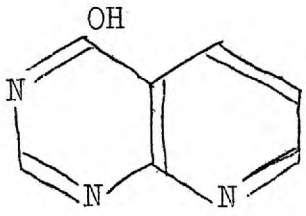
Substance	Solvent			$E^{1\%}_{1cm.}$	
Product of Hydrogenation of 	$CHCl_3$	257	M	380	4,980
		280	M	210	2,750
		333	I	210	2,750
		343	M	250	3,280
	O.IN NaOH	237	M	350	6,250
		248	M	240	4,290
		251	M	240	4,290
		270	M	420	7,500
		280	M	420	7,500
		328	M	150	2,680
		340	M	150	2,680
	MeOH	264	M	360	5,300
		280	M	300	4,400
		298	M	400	5,870

TABLE IV (contd)

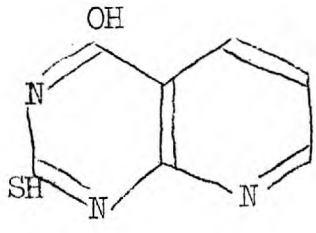
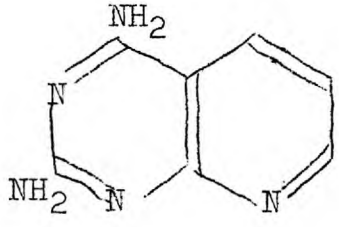
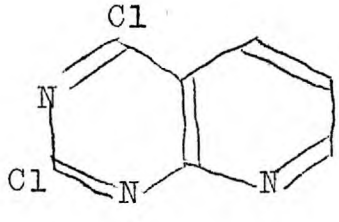
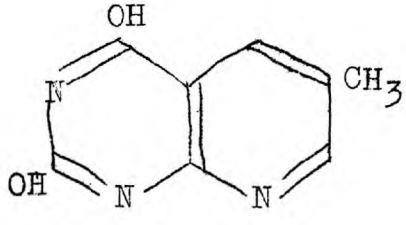
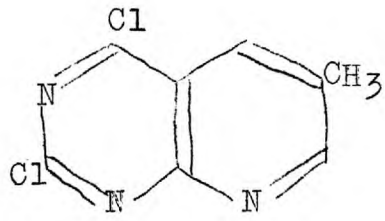
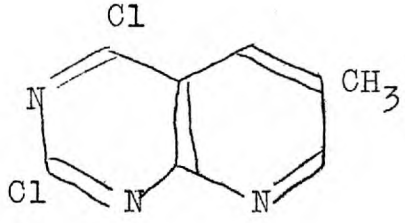
Substance	Solvent			$E^{1\%}_{1cm.}$	
	0.1N NaOH	335 347 271 280 287	M M M M I	420 420 900 900 770	7,500 7,500 16,100 16,100 13,800
	H_2O	265 320	M M	360 360	5,800 5,800
Product of Hydrogenation of 	$CHCl_3$	264 270	I M	450 510	5,890 6,670
	0.1N NaOH	236 259 266 326	M M M M	570 480 480 270	10,200 8,600 8,600 4,840

TABLE IV (contd)

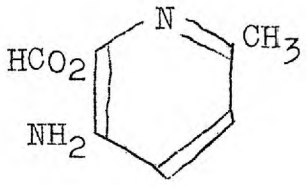
Substance	Solvent			$E^{1\%}_{1cm.}$	
	$CHCl_3$	251 321	M M	260 260	5,560 5,560
Product of Hydrogenation of 	EtOH	313 326	M M	260 240	3,760 3,460

I = Inflexion.

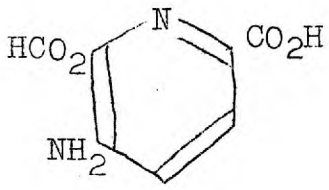
M = Maximum.

Synthesis of 1:3:5-Triazanaphthalene Analogues of Folic Acid

In order to prepare 1:3:5-triazanaphthalene analogues of folic acid by reactions similar to those used to prepare simple 1:3:5-triazanaphthalenes the starting material can be either 3-amino-6-methyl-pyridine-2-carboxylic acid (LXXXVII) or 3-amino-pyridine-2:6-dicarboxylic acid (LXXXVIII)



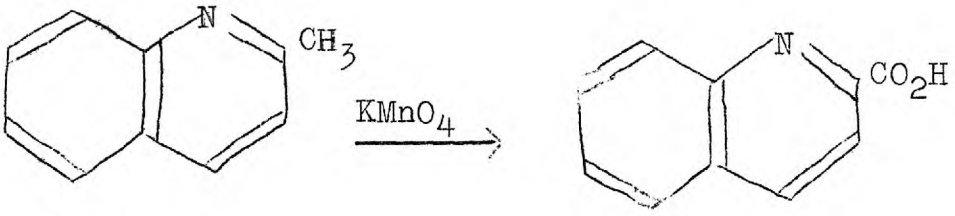
(LXXXVII)



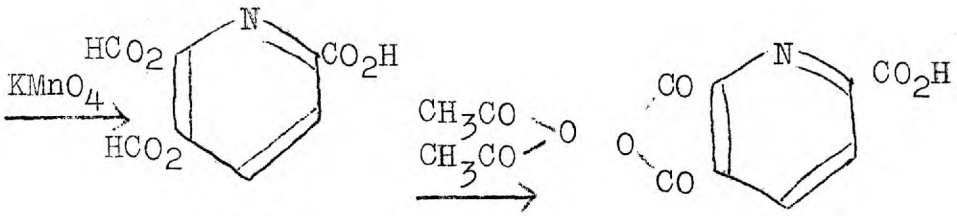
(LXXXVIII)

No convenient synthesis could be devised for the preparation of (LXXXVII) and the dicarboxylic acid (LXXXVIII) was selected as the atarting material for this work.

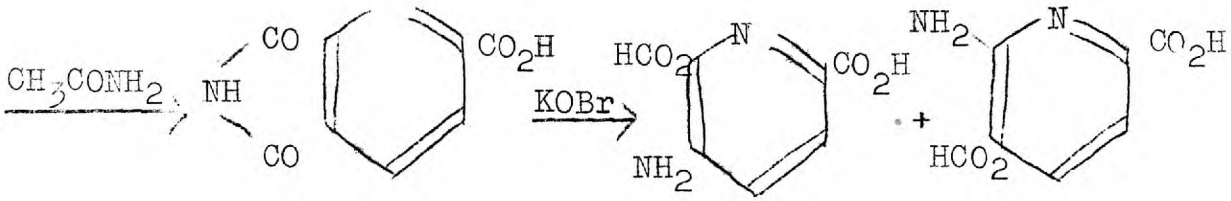
The first synthesis of 3-amino-pyridine-2:6-dicarboxylic acid (LXXXVIII) to be attempted is based on the preparation of 3-amino-picolinic acid and 2-amino-nicotinic acid by the method of Sucharda (Ber., 1925, $\underline{58^{II}}$, 1727) (see p.72) and is summarised in the following equations:-



(LXXXIX) (XC)



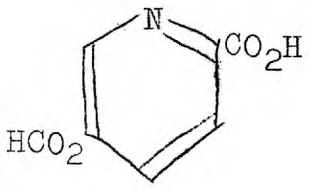
(XCI)



(LXXXVIII)

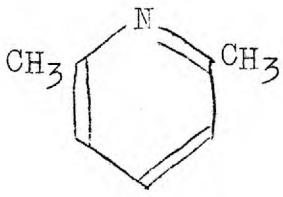
Quinaldine (LXXXIX) was oxidised to quinaldinic acid (XC) using dilute alkaline permanganate by Doebner and Miller (Ber., 1883, 16, 2472).

Kramer, (Ber., 1891, 24, 1916) reported the further oxidation of quinaldinic acid (XC) to pyridine-2:3:6-tricarboxylic acid (XCI) also by using dilute alkaline permanganate. The author prepared quinaldinic acid (XC) by this method but the main product of further oxidation was not the expected pyridine-2:3:6-tricarboxylic acid (XCI) but its decarboxylation product, pyridine 2:5-dicarboxylic acid (XCII).

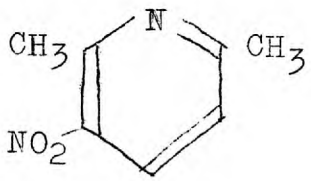


(XCII)

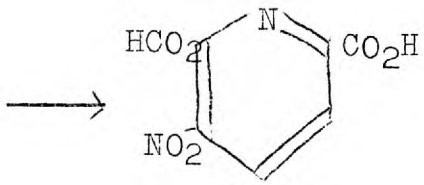
Owing to the very great ease with which pyridine-2:3:6-tricarboxylic acid decarboxylates this route was abandoned in favour of the following:-



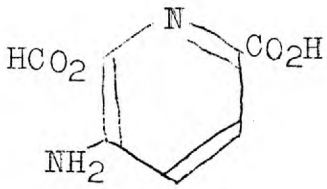
(XCIII)



(XCIV)



(XCV)

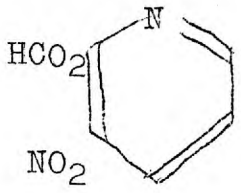


(XCVI)

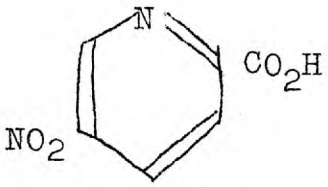
Lutidine (XCIII) had been nitrated by Plazek (Ber., 1939, 72B, 577-81) using potassium nitrate in 30% oleum at 100° and a quantity of 3-nitro-2:6 lutidine (XCIV) was prepared by this method. Oxidation of this 3-nitro-2:6-lutidine (XCIV) to 3-nitro-pyridine-2:6-dicarboxylic acid (XCV) proved to be extraordinarily difficult. The first successful oxidation was performed using alkaline permanganate. The required product, 3-nitro-pyridine-2:6-dicarboxylic acid (XCV) could not be obtained free from inorganic material by acidification and concentration of the oxidation mixture in the usual way, but was finally isolated using the following technique:- the crude oxidation mixture was filtered free from manganese mud and passed through an ion-exchange resin (Zeo-Carb) which removed all metal ions. The liquid was then evaporated to dryness under reduced pressure at a temperature not exceeding 35°. The residue consisted of two acids; the first, m.p. 105°, was soluble in ether and the second, m.p. 207-208°, insoluble in this solvent. Analysis showed that the ether soluble acid, m.p. 105°, was the required 3-nitro-pyridine-2:6-dicarboxylic acid.

Analysis showed that the second acid m.p. 207°-208° was a nitro-pyridine carboxylic acid. 3-Nitro-pyridine-2:6-dicarboxylic acid could be readily decarboxylated by heating its aqueous solution to 90° for five minutes

to give the same nitro-pyridine-carboxylic acid. This nitro-pyridine-carboxylic acid can thus only be either 3-nitro-pyridine-2-carboxylic acid (XCVII) or 3-nitro-pyridene-6-carboxylic acid (XCVIII).



(XCVII)



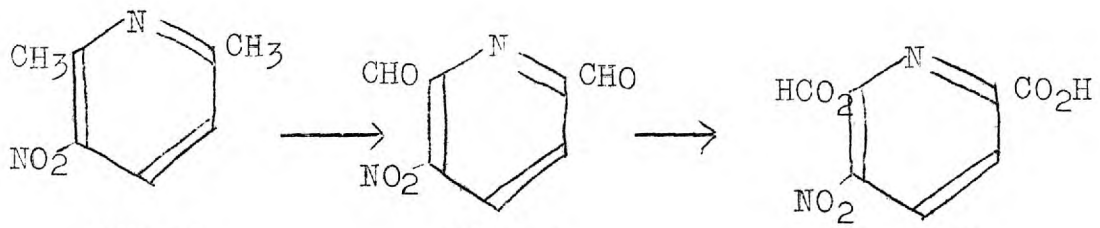
(XCVIII)

The last-named structure was shown to be the correct one by hydrogenation to the corresponding amino-pyridine-carboxylic acid which was shown by m.p. and mixed m.p. to be different from the known 3-amino-picolinic acid (3-amino-pyridine-2-carboxylic acid).

The ether solubility of 3-nitro-pyridine-2:6-dicarboxylic acid (XCV) allowed a fairly simple isolation procedure to be developed. The 3-nitro-2:6-lutidine (XCIV) was oxidised with alkaline permanganate and the oxidation mixture filtered and then acidified to Congo Red; the 3-nitro-pyridine-2:6-dicarboxylic acid (XCV) was then obtained by continuous ether extraction. Unfortunately the direct oxidation of 3-nitro-2:6-lutidine with alkaline permanganate proved to be very unreliable,

the yield varying without obvious cause from 0-53%, and had to be abandoned.

In an attempt to improve the yield and the reliability of the reaction experiments were performed using a variety of oxidising agents. Alkaline potassium ferricyanide was used without any success although this oxidising agent had been used successfully in the oxidation of methyl- and nitro-methyl-furans (Brown, Iowa State Coll. J. Sci., 1937, 11, 227-9). The use of a mixture of potassium permanganate and sodium hypochlorite as an oxidising agent has been developed by McGinty (McGinty and I.C.I. B.P. 642, 459) but oxidation of 3-nitro-2:6-lutidine (XCIV) with this reagent gave the required 3-nitro-pyridine-2:6-dicarboxylic acid (XCV in only 8% yield which is too low to be of much practical value. An attempt was made to carry out the oxidation in two stages. The first stage was oxidation to the aldehyde using selenium dioxide which was to be followed by permanganate oxidation to the required 3-nitro-pyridine-2:6-dicarboxylic acid thus:

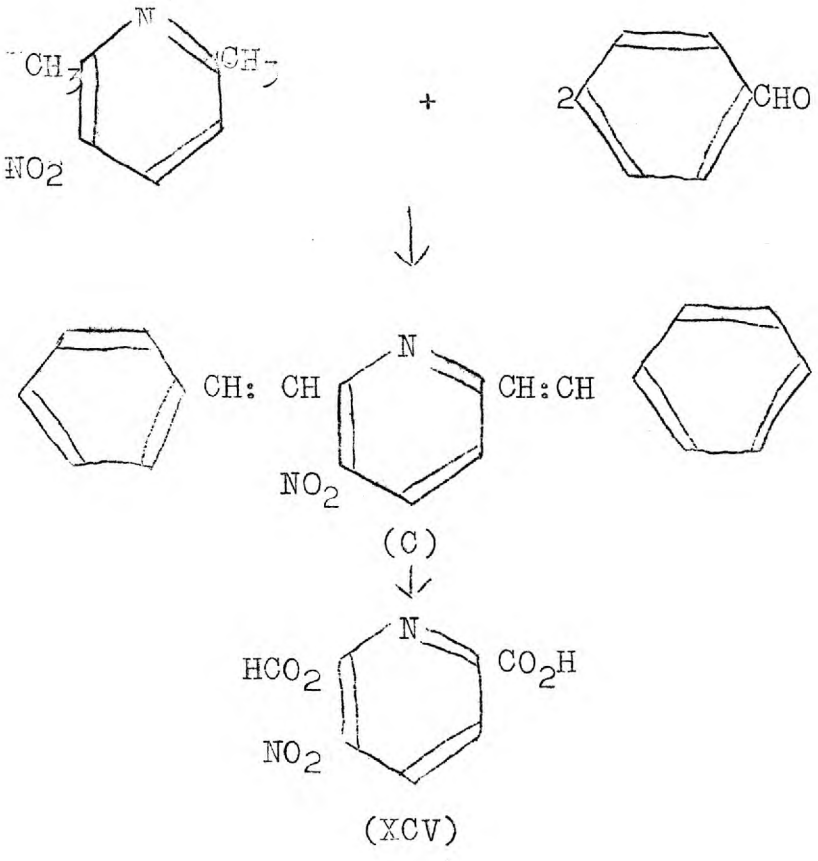


(XCIV) (XCIX) (XCV)

Oxidation of 3-nitro-2:6-lutidine (XCIV) with selenium dioxide in boiling xylene, however, gave none of the expected 3-nitro-pyridine-2:6-dialdehyde and this route was abandoned.

It is known that halogenated methyl groups are very much more readily oxidised than methyl groups. (Schulze, Ber., 1884, 17, 1529.) An attempt to side chain brominate 3-nitro-2:6-lutidine, using N-bromo-succinimide, in order to make it more readily oxidisable failed.

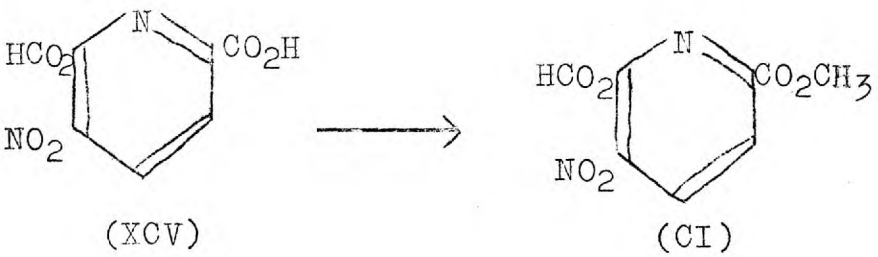
The route finally chosen was to prepare the dibenzylidine derivative of 3-nitro-2:6-lutidine (C) and to oxidise this with alkaline permanganate:-



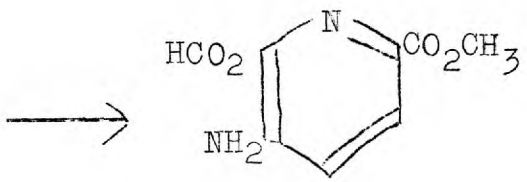
Condensation of 3-nitro-2:6-lutidine and benzaldehyde in the presence of zinc chloride proceded quite smoothly and gave the dibenzylidene derivative of 3-nitro-2:6-lutidine in good yield. This dibenzylidine derivative was readily oxidised to the required 3-nitro-pyridine-2:6-dicarboxylic acid.

In all the above work the 3-nitro-pyridine-2:6-dicarboxylic acid was isolated by continuous ether extraction. Later, when a small quantity of 3-amino-pyridine-2:6-dicarboxylic acid (XCVI) had been prepared, it was found that this amino-acid was much more stable than the nitro-acid and was much less soluble in water. It was therefore decided to attempt to prepare it by direct reduction of the crude oxidation mixture from permanganate oxidation of the dibenzylidene derivative of 3-nitro-2:6-lutidine. Experiments were performed using stannous chloride and sodium dithionite as reducing agents. The method met with some success and in one case of dithionite reduction a 23% over all yield for the oxidation and reduction was obtained. This method is, however, not too reliable and the final choice of method for the preparation of the required starting material was the permanganate oxidation of the dibenzylidine derivative of nitro lutidine (XCV) and isolation of the product by continuous ether extraction.

At this stage it was realised that 3-amino-pyridine-2:6-dicarboxylic acid was too easily decarboxylated to be of much value as a starting material for preparation of 1:3:5-triazanaphthalenes by reactions involving heating to relatively high temperatures. The 6-methyl ester (CII) would, however, be a convenient starting material and it was decided to modify the programme in order to prepare this by partial esterification followed by reduction, thus:-



(XCV) (CI)

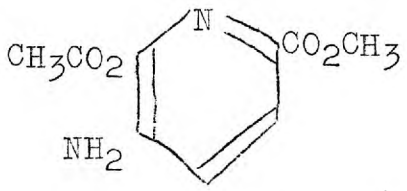


(CII)

Attempts to prepare the 6-methyl ester of 3-nitro-pyridine-2:6-dicarboxylic acid (CI) by the use of the theoretical quantity of diazo-methane gave only the

dimethyl ester in low yield. A later literature survey showed that this might have been expected as it is known that carboxyl-groups ortho to a nitro-group exhibit anomalous behaviour when treated with diazomethane (Arndt, Eistert and Patale, Ber., 1927, 60B, 1364). Treatment of the acid with cold methanolic hydrogen chloride for a short time gave only the dimethyl ester.

It was at this stage that the previously mentioned experiment (see p. 77) in which it was shown that esters could be used successfully in the preparation of 1:3:5-triazanaphthalenes was performed. As a result a suitable starting material was now dimethyl 3-amino-pyridine-2:6-dicarboxylate (CIII).



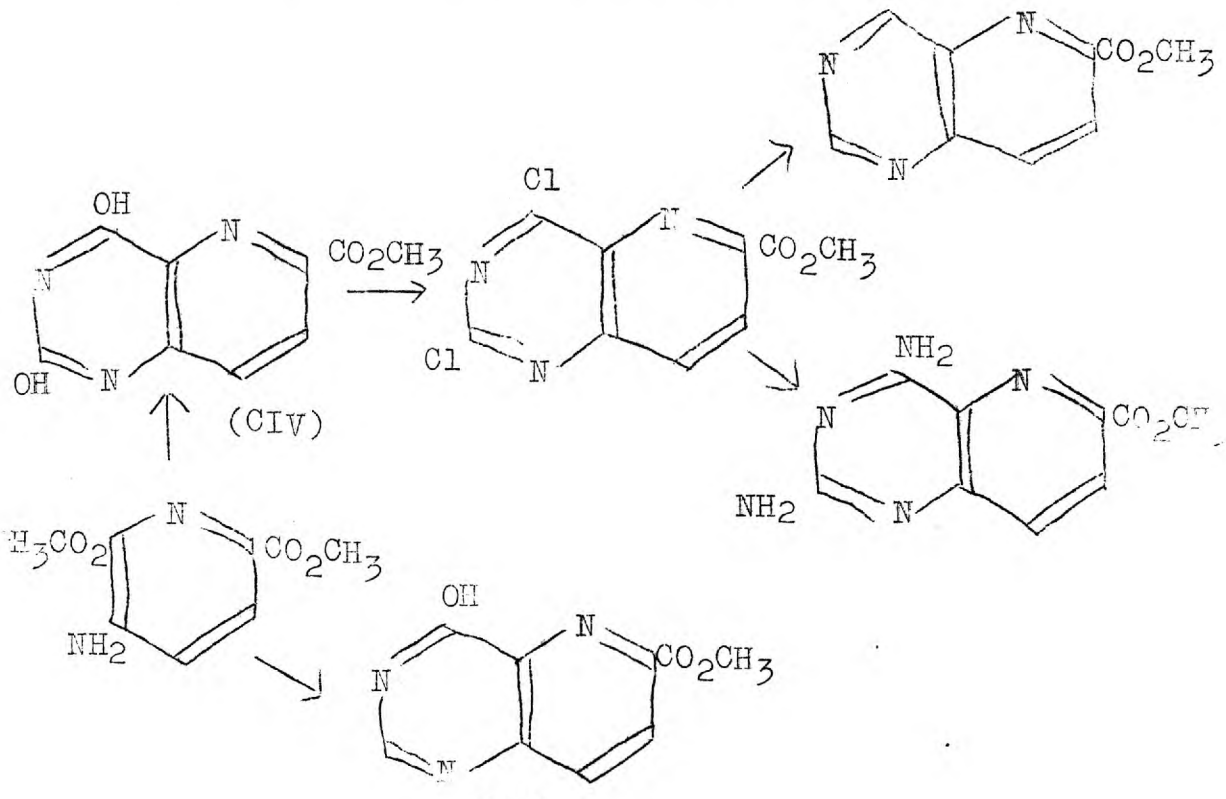
(CIII)

The previously prepared 3-nitro-pyridine 2:6-dicarboxylic acid was estrified with cold methanolic hydrogen chloride. Hydrogenation of this nitro dimethyl ester using Adam's catalyst gave the required dimethyl 3-amino-pyridine-2:6-dicarboxylate (CIII). The small

quantity of 3-amino-pyridine-2:6-dicarboxylic acid which had been prepared by dithionite reduction was esterified using hot methanolic hydrogen chloride.

Attempts to prepare 3-amino-pyridine-2:6-dicarboxylic acid by permanganate oxidation of 3-amino-2:6-lutidine or of its acetyl derivative were unsuccessful, the whole molecule being destroyed by the oxidation.

By the methods outlined above a quantity of dimethyl 3-amino-pyridine-2:6-dicarboxylate was obtained and the following scheme was contemplated:-



(CV)

The above 1:3:5-triazanaphthalenes were to be prepared and the carbomethoxy group used for the introduction of the

folic acid side chain by the method outlined on p. 71.

In the time available the only part of this project to be studied was the preparation of 2:4-dihydroxy-1:3:5-triazanaphthalene-6-carboxylic-ester (CIV) and 4-hydroxy-1:3:5-triazanaphthalene-6-carboxylic acid (CV). Two attempts were made to prepare the dihydroxy compound (CIV), by heating dimethyl 3-amino-pyridine-2:6-dicarboxylate with urea; at 170° no reaction occurred while at 220° there was too much charring to permit the isolation of any product. It is very likely that the reaction will proceed in a satisfactory manner at some temperature between these two and the reaction is well worth further investigation.

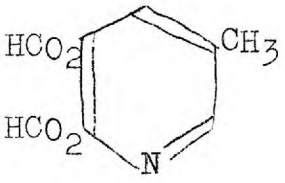
Similarly, an attempt to prepare the mono-hydroxy-compound (CV) by heating formamide and dimethyl 3-amino-pyridine-2:6-dicarboxylate at 170° failed owing to the absence of a reaction.

In the author's opinion the reactions represented in the above scheme are quite practical and well worth further investigation.

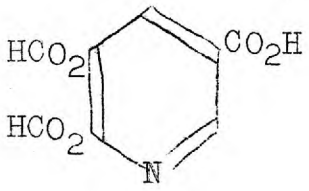
Synthesis of 1:3:8-Triazanaphthalene

Analogues of Folic Acid

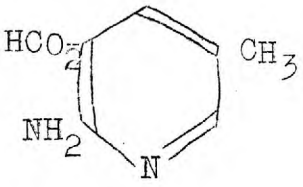
For the preparation of 1:3:8-triazanaphthalene analogues of folic acid by one of the routes previously discussed in relation to the preparation of simple 1:3:8-triazanaphthalenes the following substances may be used as starting materials:- 5-methyl-quinolinic acid (CXI), pyridine-2:3:5-tricarboxylic acid (CXII), 2-amino-5-methyl-pyridine-3-carboxylic acid (CXIII) or 2-amino-pyridine-3:5-dicarboxylic acid (CIX)



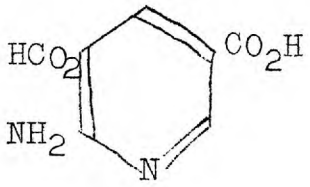
(CVI)



(CVII)



(CVIII)

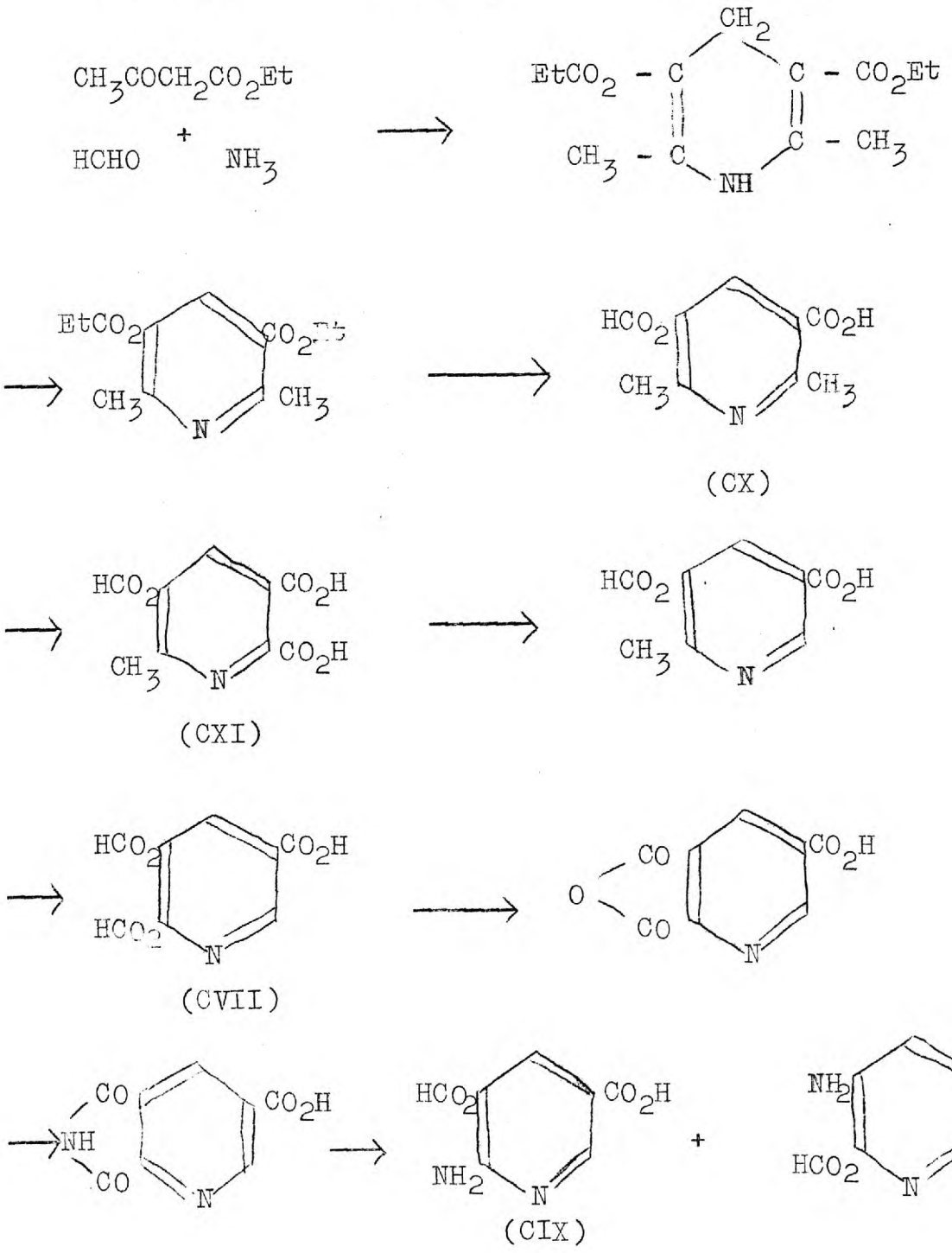


(CIX)

Compounds (CXI) and (CXII) were to be used in the McClean and Spring (J., 1949, 2582) type of synthesis and compounds (CVIII) and (CIX) in the Price and Curtis

synthesis (J. Amer. Chem. Soc., 1946, 68, 914).

An attempt was made to prepare pyridine 2:3:5-tricarboxylic acid (CVII) and 2-amino-pyridine-3:5-dicarboxylic acid (CIX) by the following route:-



(CX)

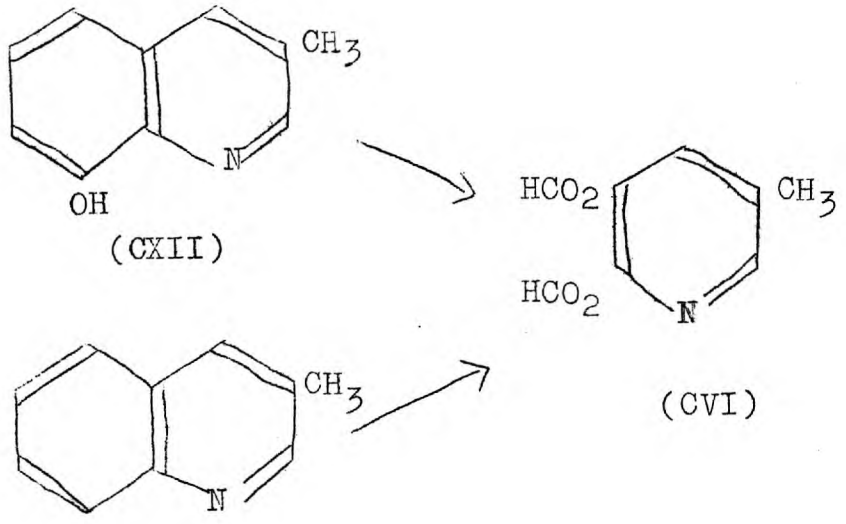
(CXI)

(CVII)

(CIX)

2:6-Dimethyl-pyridine-3:5-dicarboxylic acid (CX) was prepared by the above route using the method of Organic Syntheses, Coll. Vol. II, p.215. Oxidation of 2:6-dimethyl-pyridine-3:5-dicarboxylic acid (CX) to 2-methyl-pyridine-3:5:6-tricarboxylic acid (CXI) had been accomplished by Weber (Annalen., 1882, 214, 9) by the use of dilute alkaline permanganate. Attempts by the author to repeat this work were unsuccessful and the route was abandoned.

It was hoped to prepare 5-methyl-quinolinic acid (CVI) by oxidation of 3-methyl-8-hydroxy-quinoline (CXII) or 3-methyl-quinoline (CXIII)



(CXII)

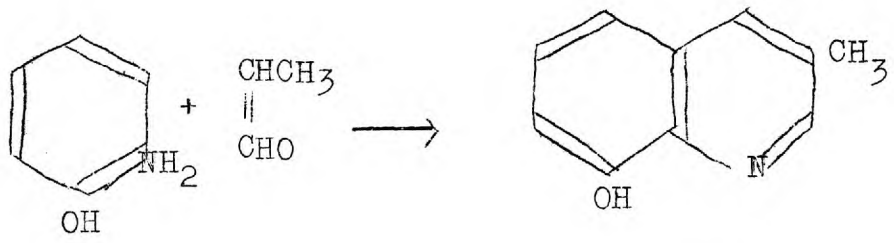
(CVI)

(CXIII)

Model experiments using quinoline, 8-hydroxy-quinoline and quinaldine showed that alkaline permanganate was not a suitable oxidising agent. The technique used

in these model experiments was that employed by Maggiolo, Phillips and Hitchings (J. Amer. Chem. Soc., 1951, 73, 107) for the preparation of pyridine-3:5-dicarboxylic acid from isoquinoline. These authors oxidised isoquinoline to a mixture of phthalic acid and pyridine-3:5-dicarboxylic acid using hot alkaline permanganate, the crude oxidation mixture being filtered and evaporated to dryness and the residue esterified with methanolic hydrogen chloride to give a mixture of esters. The esters were readily separated by dissolving in dry ether and precipitating the hydrochloride of pyridine-3:5-dicarboxylic ester with dry hydrogen chloride. Application of this procedure to the oxidation of quinoline, quinaldine and 8-hydroxy-quinoline gave none of the expected products.

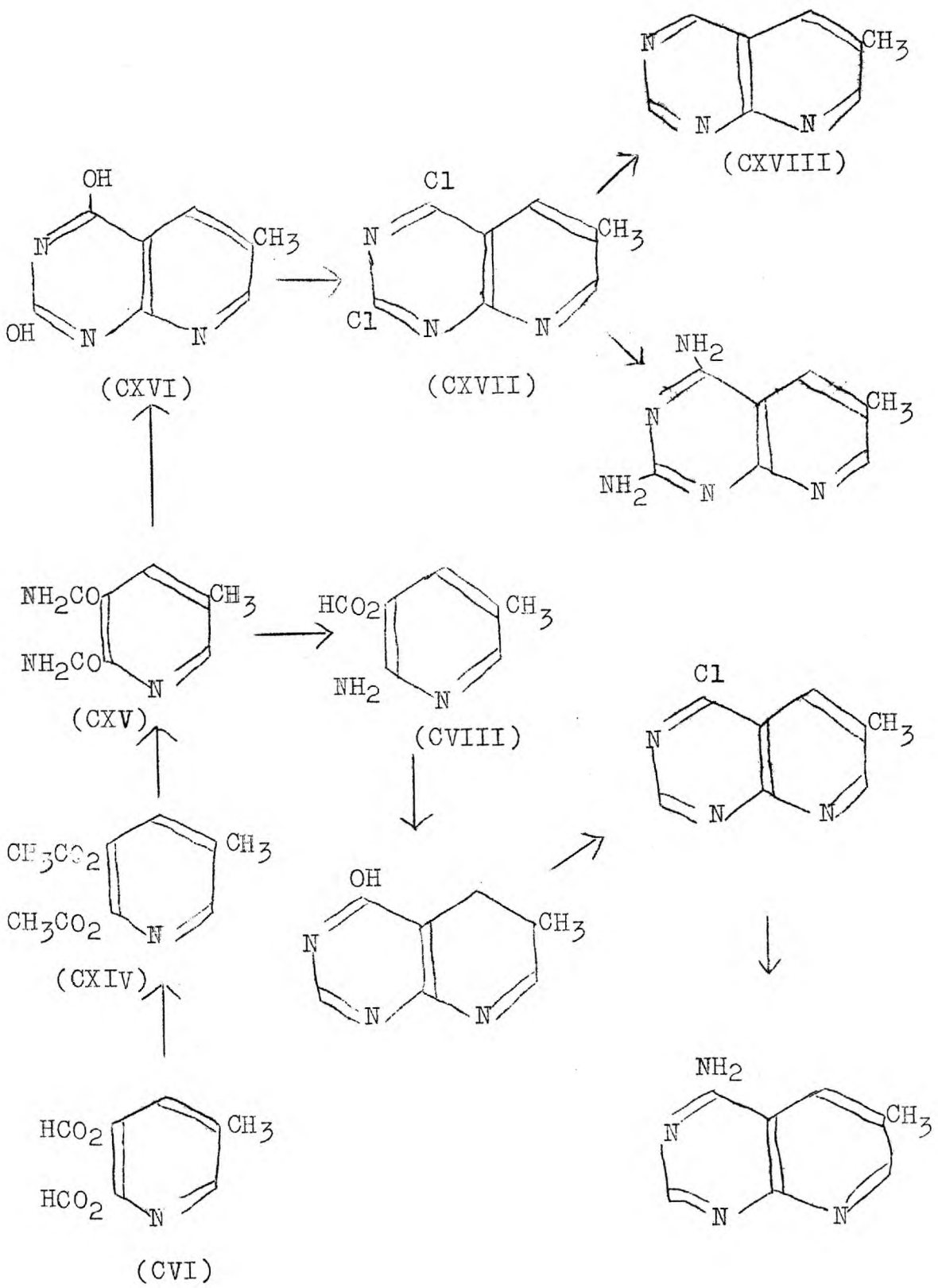
It is known that concentrated nitric acid will oxidise 8-hydroxy-quinoline satisfactorily to quinolinic acid (Sucharda, Ber., 1925, 58^{II}, 1727) and it was decided to attempt to extend this reaction to the preparation of 5-methyl-quinolinic acid (CVI) by the nitric acid oxidation of 3-methyl-8-hydroxy-quinoline (CXII). 3-Methyl-8-hydroxy-quinoline was prepared by the Skraup reaction between o-amino-phenol, o-nitro-phenol and α-methyl-acrolein:-



(CXII)

Nitric acid oxidation of this 3-methyl-8-hydroxy-quinoline (CXII) gave the required 5-methyl-quinolinic acid (CXI).

This 5-methyl-quinolinic acid was to be used to prepare 1:3:8-triazanaphthalenes analogues of folic acid as outlined below. As was previously shown in connection with the preparation of 2:4-dihydroxy-1:3:8-triazanaphthalene by the method of McClean and Spring, small quantities of amino-pyridine carboxylic acids are produced as by-products. It is therefore to be expected that, when preparing 6-methyl-2:4-dihydroxy-1:3:8-triazanaphthalene by the same method, some of the required 2-amino-5-methyl-pyridine-3-carboxylic acid (CXIII) would be obtained. On this assumption the following reaction scheme was devised:-


(CXVIII)
(CXVI)
(CXVII)
(CXV)
(CVIII)
(CXIV)
(CVI)

When the above scheme was completed a series of 6-methyl-1:3:8-triazanaphthalenes would be available which could then be converted into analogues of folic acid using the method outlined on p.69.

The dimethyl 5-methyl-quinolinate (CXIV) was prepared using methanolic hydrogen chloride. The yield in this reaction was very low (29%) and diazomethane was used for the preparation of a second batch. Unfortunately no comparison of yield is possible owing to considerable accidental loss in the second preparation. 5-Methyl-quinolinamide (CXV) was prepared from this ester (CXIV) in good yield using the method of Linstead, Noble and Wright, (J., 1937, 911).

Treatment of 5-methyl-quinolinamide (CXV) with alkaline hypobromite gave 6-methyl-2:4-dihydroxy-1:3:8-triazanaphthalene (CXVI) and a small quantity of an amino-methyl-pyridine-carboxylic acid. Time was not available for the determination of the structure of this acid but, if the reaction proceeds in a similar manner to the analagous one used for the preparation of 2:4-dihydroxy 1:3:8-triazanaphthalene, this acid should be the required 2-amino-6-methyl-pyridine-3-carboxylic acid.

Some difficulty was encountered in the chlorination of 6-methyl-2:4-dihydroxy-1:3:8-triazanaphthalene to give (CXVII). Chlorination with phosphorus oxychloride and

dimethylaniline gave only a phosphate. The use of phosphorus oxybromide and dimethylaniline led to a very vigorous reaction and no bromo- compound could be isolated from the tarry product. 6-Methyl-2:4-dichloro-1:3:8-triazanaphthalene was finally obtained by the method detailed on p.82 and in the experimental section.

An attempt was made to prepare 6-methyl-1:3:8-triazanaphthalene (CXVIII) by hydrogenation of 6-methyl-2:4-dichloro-1:3:8-triazanaphthalene. The product was neither the expected 6-methyl-1:3:8-triazanaphthalene nor judging from the analytical results, an analogue of either the compound obtained by hydrogenation of 2:4-dichloro-1:3:8-triazanaphthalene (see p. 83) or that prepared by hydrogenation of 2:4-dichloro-1:3:5-triazanaphthalene (see p.76). The analytical results led to an empirical formula $C_{16}H_{18}N_6O_5$ (Calculated:- C, 51.5; H, 4.8; N, 22.4. Found C, 51.9; H, 4.6; N, 22.5%). This further case of anomalous hydrogenation of a 2:4-dichloro-triazanaphthalene indicates the necessity for further work in this field.

Owing to lack of time no more of the reactions projected in the above scheme were accomplished. The scheme is, however, in the author's opinion, a practical one and it is well worth while attempting to carry it out.

Attempts were made to side-chain brominate 2:4-dihydroxy-6-methyl-1:3:8-triazanaphthalene in order to prepare an analogue of folic acid (LXI, X = OH, Y = OH) by the route outlined on p. 69.

The brominating agents used were bromine in acetic acid at 150° in a sealed tube and bromine in 45% hydrogen bromide at 90°. Both these methods, which had been used successfully by Boothe et al., (J. Amer. Chem. Soc., 1949, 70, 1) for the bromination of 2-amino-4-hydroxy-6-methyl-pteridine, failed, the starting material being recovered unchanged.

It is possible that the dichloro-compound (CXVII), being soluble in organic solvents, could be side-chain brominated satisfactorily leading to an obvious modification of the projected synthesis.

Experimental Section

NAPHTHALENE ANALOGUES OF FOLIC ACID.

Condensation of 2-bromomethyl-naphthalene and ethyl p-aminobenzoate.

2-Bromomethyl-naphthalene (5g.), ethyl p-aminobenzoate (4.5g.) and anhydrous sodium carbonate (2.5g.) were refluxed overnight in ethanol (50 c.c.). The resulting mixture was allowed to cool to room temperature and poured into cold water (200 c.c.). The precipitate of ethyl N-(2-naphthylmethyl-)-p-aminobenzoate was filtered off and recrystallised from ethanol/water. m.p. 130°-131°. (Yield: 3.5g. = 51%) (Found: C, 78.4; H, 6.5. $C_{20}H_{19}O_2N$ requires C, 78.7; H, 6.2%.)

Hydrolysis of ethyl N-(2 naphthyl methyl)-p-amino-benzoate.

Ethyl N-(2-naphthyl-methyl)-p-aminobenzoate (1g.) was dissolved in ethanol (90 c.c.) and an equivalent of 10% alcoholic potassium hydroxide solution (2c.c.) added. The mixture was allowed to stand at room temperature for 48 hours and was then poured into water (200c.c.) and the white precipitate (0.95g.) filtered off; m.p. 130°-131°

and mixed m.p. with ethyl N-(2-naphthyl-methyl)-p-aminobenzoate, 130°- 131° showed this to be unchanged ester. The filtrate was acidified to Congo Red but gave no precipitate of the expected N-(2-naphthyl-methyl)-p-aminobenzoic acid.

Ethyl N-(2-naphthyl-methyl-)-p-aminobenzoate (1.5g.) was refluxed with 10% alcoholic potassium hydroxide solution (50 c.c.) for 4 hours. A small sample of the hydrolysate gave no precipitate on dilution with water. The solution was cooled to room temperature, diluted with water to dissolve the potassium salt which separated and acidified to Congo Red. The white precipitate of N-(2-naphthyl-methyl)-p-aminobenzoic acid (L; X = H, Y = H) was filtered off and recrystallised twice from ethanol/water. (Yield 1g. = 74%)m.p. 199° - 200°. (Found: C, 78.3; H, 5.8. $C_{18}H_{15}O_2N$ requires C, 78.0; H, 5.4%).

Condensation of 2-bromomethyl-naphthalene and dimethyl N-(p-aminobenzoyl)-L-glutamate.

2-Bromo-methyl-naphthalene (6.5g.), dimethyl N-(p-aminobenzoyl)-L-glutamate (7.5g.) and anhydrous potassium carbonate (2g.) were dissolved in ethanol (120 c.c.) and

refluxed for 3 hours. On cooling to room temperature the mixture was poured into water (400c.c.). The resultant white precipitate of dimethyl N'-[-N-(2-naphthyl-methyl)-p-aminobenzoyl]-L-glutamate (LII) was filtered off and recrystallised from methanol. (Yield: 6g. = 47%) m.p. 141° (Found: C, 69.1; H, 6.0. $C_{25}H_{26}O_5N_2$ requires C, 69.1; H, 6.0%).

Attempts to hydrolyse dimethyl-N-[-N'-(-2-naphthyl-methyl)-p-aminobenzoyl]-L-glutamate (LII).

Dimethyl N-[N'-(2-naphthyl-methyl)-p-aminobenzoyl-]-L-glutamate (LII) (1.5g.) was dissolved in methanol (95c.c.) and 10% potassium hydroxide solution (4c.c.) added. The solution was left at room temperature for 48 hours and then poured into cold water (200 c.c.). This produced a precipitate of unchanged ester (1.4g.) m.p. 141° and mixed m.p. with authentic diester 141°. The filtrate was acidified to Congo Red and no precipitate was formed showing that no hydrolysis had taken place.

Dimethyl N-[N'-(2-naphthyl-methyl)-p-aminobenzoyl-]-L-glutamate (LII) (1.5g.) was dissolved in methanol (100c.c.) and potassium hydroxide (10g.) dissolved in a little water was added and the solution left at room temperature for

48 hours. A small sample (5 c.c.) was poured into cold water (25c.c.). This produced a precipitate of unchanged ester, m.p. 141° and mixed m.p. 141°. Acidification of the filtrate to Congo Red produced none of the expected N-[N'-(2-naphthyl-methyl)-p-aminobenzoyl]-L-glutamic acid (XLIX; X = H, Y = H).

The remainder of the hydrolysis mixture was refluxed for two hours and poured into cold water (200c.c.). There was no precipitate and the clear liquid was acidified to Congo Red which produced a precipitate of N(2-naphthyl-methyl-)-p-aminobenzoic acid (L, X = H, Y = H) (0.9g.), m.p. 130°- 131° and mixed m.p. 130° - 131°, showing that the peptide bond had been hydrolysed.

Dimethyl N-[N'-(2-naphthyl-methyl)-p-aminobenzoyl]-L-glutamate (LII) (1.5g.) was suspended in water (100c.c.) to which was added 10% potassium hydroxide solution (4c.c.), and the mixture was shaken for 48 hours at room temperature. The unchanged ester was filtered off (1.5g.) and the filtrate acidified to Congo Red. The absence of any precipitate showed that no hydrolysis had occurred.

Similarly shaking dimethyl N[N'(2-naphthyl-methyl)-p-aminobenzoyl-)-L-glutamate (1.5g.) (LII) with excess 10% potassium hydroxide (100c.c.) produced no hydrolysis.

Dimethyl N[N'-(2-naphthyl-methyl)p-aminobenzoyl]-L-glutamate (LII) (1.5) was refluxed with 10% potassium

hydroxide (100c.c.) until all had entered solution (3 hours). The solution was allowed to cool to room temperature and acidified to Congo Red. A white precipitate of N-(2-naphthyl-methyl)-p-aminobenzoic acid (L, X = H, Y = H), (0.8g.) m.p. 130-131°C and mixed m.p. 130 - 131°C, was produced showing that hydrolysis of the peptide linkage had occurred.

Attempted preparation of N-(2-naphthyl-methyl)-p-aminobenzoic acid (L, X = H, Y = H) by direct condensation of 2-bromomethyl-naphthalene and p-aminobenzoic acid.

2-Bromomethyl-naphthalene (5g.), p-aminobenzoic acid (3g.) and anhydrous potassium carbonate (1.5g.) were refluxed in ethanol (100c.c.) for 3 hours, the mixture allowed to cool to room temperature and poured into water (200c.c.). The white precipitate which formed was filtered off and recrystallised from ethanol/water. (Yield: 3.1g. = 49%) m.p. 123° (Found: C, 78.3; H, 5.59. $C_{18}H_{15}O_2N$ requires C, 78.0; H, 5.4%). The product was insoluble in bicarbonate and dilute sodium hydroxide and was the 2-naphthyl-methyl ester of p-aminobenzoic acid (LI).

Attempted condensation of N-2-naphthyl-methyl-p-aminobenzoic acid chloride and glutamic acid.

N-2-naphthyl-methyl-p-aminobenzoic acid (L, X = H, Y = H) (1g.) was refluxed for 1 hour with thionyl chloride (10c.c.). The excess thionyl chloride was distilled off under reduced pressure. The residual acid chloride was dissolved in dry chloroform (5c.c.) and added dropwise with stirring to an ice-cold solution of glutamic acid (0.7g.) and sodium bicarbonate (0.5g.) in water (50c.c.). After stirring for one hour the solution was acidified to Congo Red. This produced a white precipitate of N-2-naphthyl-methyl-p-aminobenzoic acid (L, X = H, Y = H), (0.4g.) m.p. 130 - 131°C and mixed m.p. 130 - 131°C.

Preparation of 2-methyl-6-amino-naphthalene.

2-Methyl-6-hydroxy-naphthalene was prepared by the method of Roger (Ann. Chim., 1940, 395). 2-Methyl-6-hydroxy-naphthalene (100g.), ammonium hydroxide solution (s.g. 0.880) (700c.c.) and ammonium sulphite (80g.) were placed in a stirred autoclave and heated to 160° - 170° for 6 hours. After allowing to cool overnight the contents of the autoclave were dissolved in hot water (6 l.) and hydrochloric acid (1 l.) and the hot solution treated with

charcoal and filtered. Neutralisation with 10% sodium hydroxide gave a precipitate of 2-methyl-6-amino-naphthalene which was filtered off and dried at 100°. (Yield: 77g. = 77.5%) m.p. 129°.

Preparation of 2-methyl-6-nitro-naphthalene

2-Methyl-6-amino-naphthalene (7.5g.) was dissolved in a hot mixture of concentrated hydrochloric acid (20c.c.) and water (30c.c.) and chilled rapidly to produce a finely divided precipitate of 2-methyl-6-amino-naphthalene hydrochloride. This was cooled to 0°C and a solution of sodium nitrite (4g.) in water (20c.c.) added dropwise with shaking. The temperature was maintained at 0° - 5° during the addition of the nitrite. The resulting solution was allowed to stand in an ice bath for 10 minutes and then neutralised by pouring onto a mixture of powdered chalk and ice containing a little ether to minimise frothing. The resulting mixture was filtered into a solution of sodium cobaltinitrite (8g.) in water (40c.c.) and allowed to stand for 10-15 minutes. The precipitate of diazo-cobaltinitrite was filtered off, washed with water and dried by washing with alcohol and ether.

The diazo-cobaltinitrite was decomposed by the following techniques:- (1) The diazocobaltinitrite was stirred with water (30c.c.) for 24 hours and the resulting mixture then steam distilled to give 2-methyl-6-nitro-naphthalene. (Yield: 0.4g. = 5%). (2) The diazo-cobaltinitnitrite was slurried with water (30c.c.) and slowly added to a well stirred mixture of sodium nitrite (15g.), copper sulphate (15g.), cuprous oxide (4g.) and water (60c.c.) at a temperature of 60° - 70°. The decomposition was complete in 15 minutes and the resulting mixture was then neutralised with sodium carbonate and steam distilled to give 2-methyl-6-nitro-naphthalene. m.p. 119°. (Yield: 0.5g. = 6%).

Both of the above procedures were repeated several times without any significant change in the yield.

Investigation of the conversion of β-naphthylanine in β-nitro-naphthalene.

Both of the above procedures were investigated using β-naphthylanine. The yield varied between 8 - 10% instead of the expected 65%.

The diazo-cobaltinitrite was decomposed at 60° - 70° using the following mixtures:- sodium nitrite (30g.),

copper sulphate (15g.), cuprous oxide (4g.) and water (60c.c.) (Yield: 15% - 17%.)

Sodium nitrite (15g.), copper sulphate (30g.), cuprous oxide (4g.) and water (60c.c.). (Yield: 8 -10%).

Sodium nitrite (15g.), copper sulphate (15g.), cuprous oxide (7g.) and water (60c.c.) (Yield: 9%).

Thus it appears that only the amount of sodium nitrite has any appreciable effect on the yield of nitro-compound.

Acetylation of 2-methyl-6-amino-naphthalene.

2-Methyl-6-amino-naphthalene (15g.) was heated on a steam bath for 1½ hours with a mixture of acetic anhydride (12c.c.) and acetic acid (75c.c.). The mixture was poured into water (250c.c.) and left overnight to crystallise. The acetyl derivative was filtered off and recrystallised from aqueous ethanol. m.p. 173° (Yield: 16g. = 84%).

Oxidation of 2-methyl-6-acetamido-naphthalene.

2-Methyl-6-acetamido-naphthalene (5g.) and magnesium sulphate (12g.) were suspended in distilled water (4 l.) and the suspension heated on a steam bath. Potassium

permanganate (12g.) was added to the well stirred suspension in small portions over a period of 8 hours and the mixture then stirred for a further 16 hours. The precipitate of manganese dioxide was filtered off and washed well with sodium bicarbonate solution and with ethanol. The combined filtrate and washings were extracted once with ether (250c.c.) to remove any unchanged acetyl derivative and then concentrated to small volume under reduced pressure. The resulting solution was acidified to Congo Red and the required 6-acetamido-2-naphthoic acid separated out. m.p. 228 - 230°. (Yield: 0.42g. = 7%).

Preparation of 7-methyl-α-tetralone (LVI)

(1) By normal Friedel-Crafts technique.

γ-(p-tolyl)-n-butyric acid (LV) chloride was cyclised by the technique described fully in Organic Syntheses (Org. Synth. Coll. Vol.II, p.499) Yield = 60%.

(2) Inverse Friedel-Crafts technique.

γ-(p-tolyl)-n-butyryl chloride (LV) (48g.) was dissolved in sodium-dried benzene (50c.c.) and added to

a suspension of aluminium chloride (50g.) in dry benzene (200c.c.), keeping the temperature below 10°C by external cooling with ice. The mixture was stirred at room temperature for 6 hours, diluted with ether (50c.c.) and cooled to ca. 0°C with an ice-salt freezing mixture. Hydrochloric acid (1:1) (250c.c.) was added dropwise keeping the temperature below 15°C. The benzene layer was separated and the aqueous layer extracted with ether (50c.c.). The combined ether and benzene solutions were dried over sodium sulphate and the solvents distilled. The residual 7-methyl-α-tetralone (LVI) distilled at 121°/1 mm. (Yield: 39g. = 80%).

(3) By direct cyclisation of γ-(p-tolyl-)-n-butyric acid (LIV).

γ-(p-tolyl-)-n-butyric acid (LIV) (15g.) was heated on a steam bath with concentrated sulphuric acid (40c.c.) for 2 hours. The resulting mixture was poured into cold water (500c.c.) and the 7-methyl-α-tetralone (LVI) extracted with ether (2 x 50c.c.). The ethereal solution was dried over calcium chloride and the residue distilled under reduced pressure. b.p. 121°/1 mm. (Yield 5.5g. = 41%.)

The above experiment was repeated several times, in each case varying the length of time during which the mixture was heated. The time could be reduced to 30 minutes without materially affecting the yield. The shorter heating period gave a better quality product.

Dehydrogenation of 7-methyl-α-tetralone (LVI)

7-methyl-α-tetralone (LVI) (5g.) was dissolved in ether (50c.c.) and left overnight in contact with a little Raney nickel. The Raney nickel was filtered off and the ether distilled. The residual 7-methyl-α-tetralone (LVI) was redistilled in vacuo in an all glass apparatus. (b.p. 121°/1mm.). The purified 7-methyl-α-tetralone (LVI) was heated for 6 hours at 280° - 310° with 30% palladised charcoal (2 g.) in a stream of nitrogen. The mixture was allowed to cool to room temperature in a stream of nitrogen and extracted with ether (75 c.c.). The catalyst was filtered off and the ether extracted with 2 N-NaOH (50c.c.). The aqueous layer was acidified to Congo Red and extracted with ether (50c.c.). Evaporation of the ether left the required 2-methyl-8-hydroxy-naphthalene (LVII) which was recrystallised from 60° -80° petrol. (Yield 3g. = 60%) m.p. 110°.

Methylation of 2-methyl-8-hydroxy-naphthalene (LVII).

2-Methyl-8-hydroxy-naphthalene (LVII) (3g.) was dissolved in a solution of sodium hydroxide (4g.) in water (20c.c.). The mixture was cooled in ice and dimethyl sulphate (4c.c.) added dropwise during about 30 minutes, the mixture was then heated on a steam bath for 1 hour, cooled to room temperature and extracted with ether (25c.c.). The ether was evaporated and the residue distilled to give 2-methyl-8-methoxy-naphthalene, b.p. 150°/7mm., m.p. 35° - 36°. (Yield: 1.1g. = 34%) (Found: C, 84.0; H, 7.3. $C_{12}H_{12}O$ requires C, 83.7; H, 7.0%).

A second preparation on a 12g. scale improved the yield to 45%.

Bromination of 2-methyl-8-methoxy-naphthalene.

2-Methyl-8-methoxy-naphthalene (2.5g.), N-bromosuccinimide (2.5g.) and carbon tetrachloride (15c.c.) were refluxed on a steam bath for 4 hours. The precipitate of succinimide was filtered off and the carbon tetrachloride distilled from the filtrate. The residue of bromo-2-methyl-8-methoxy-naphthalene was

recrystallised twice from ethanol/water. m.p. 72°
Yield: 2.4g. = 65%. (Found: C, 57.5; H, 4.6.
$C_{12}H_{11}OBr$ requires C, 57.4; H, 4.4%).

The above bromo-compound gave no precipitate with alcoholic or aqueous silver nitrate.

Attempted oxidation of bromo-2-methyl-8-methoxy-naphthalene.

Bromo-2-methyl-8-methoxy-naphthalene (0.3g.), potassium permanganate (0.3g.) and sodium hydroxide (0.1g.) were mixed with water (20c.c.) and refluxed for 8 to 9 hours. Sulphur dioxide was passed into the cold mixture until it was colourless and acid to Congo Red. This produced a small precipitate which had m.p. 72°C; mixed m.p. 72°C showed it to be unchanged bromo-2-methyl-8-methoxy naphthalene.

The above process was repeated substituting sodium carbonate for the sodium hydroxide with identical results.

Bromo-2-methyl-8-methoxy-naphthalene (550mg.), nitric acid (3.5c.c.) and water (40c.c.) were refluxed overnight. The mixture was evaporated to dryness and the residue treated with sodium bicarbonate solution. Nothing dissolved in the bicarbonate and the isoluble residue was unchanged

bromo-2-methyl-8-methoxy-naphthalene, m.p. and mixed m.p. 72°.

Further Bromination of bromo-2-methyl-8-methoxy-naphthalene.

Bromo-2-methyl-8-methoxy-naphthalene (1.35g.) and N-bromosuccinimide (0.9g.) were refluxed in carbon tetrachloride (10c.c.) for 5 hours. The precipitate of succinimide was filtered off and the carbon tetrachloride distilled off under reduced pressure. The dark, oily residue crystallised on standing and the crystals were pressed on a porous tile and then recrystallised twice from ethanol/water. The yield of dibromide was 350mg. (20%), m.p. 66°, mixed m.p. with monobromide, 40 - 50°. (Found: Br, 46.0 and 46.2. $C_{12}H_{10}OBr_2$ requires 48.5%.)

The dibromo-2-methyl-8-methoxy-naphthalene gave no precipitate with alcoholic or aqueous silver nitrate and was resistant to hydrolysis. Attempts to oxidise the dibromide were made using the same techniques as were used in attempts to oxidise the monobromide and met with similar lack of success.

Further bromination of dibromo-2-methyl-8-methoxy-naphthalene.

Dibromo-2-methyl-8-methoxy-naphthalene (300mg.), N-bromosuccinimide (250mg.) and carbon tetrachloride (20c.c.) were refluxed for several hours. The mixture was filtered and the carbon tetrachloride distilled off. The residue was recrystallised from ethanol water. (Yield: 100mg.) m.p. 65 - 66° and mixed m.p. 66°C showed the product to be unchanged dibromide.

Preparation of 7-methyl-α-tetralone oxime.

7-Methyl-α-tetralone (5g.) was dissolved in ethanol (100c.c.), hydroxylamine hydrochloride (5g.) and sodium carbonate (6g.) were added and the mixture refluxed for one hour. The resulting solution was cooled and poured into water (400c.c.). The 7-methyl-α-tetralone-oxime was extracted with ether (2 x 100c.c.), the ethereal solution dried over calcium chloride, the ether distilled off and the residue recrystallised from ether and from ethanol. (Yield: 5g. = 91%) m.p. 98°C. (Found: C, 75.4; H, 7.5. $C_{11}H_{13}ON$ requires C, 75.4, H, 7.4%.)

Rearrangement of 7-methyl-α-tetralone-oxime.

7-Methyl-α-tetralone oxime (7g.) was dissolved in acetic acid (50c.c.) containing acetic anhydride (10c.c.) and heated on a steam bath in a stream of dry hydrogen chloride for 3 hours. The mixture was then cooled to room temperature and poured into water (250c.c.). The product, 2-methyl-8-acetamido-naphthalene was extracted with ether (2 x 50c.c.). The ether was distilled off and the residue recrystallised from aqueous ethanol. m.p. 218°. (Yield: 3.2g. = 40%.)

Hydrolysis of 2-methyl-8-acetamido-naphthalene.

2-Methyl-8-acetamido-naphthalene (3g.) was refluxed with constant boiling hydrochloric acid (30c.c.) in an atmosphere of nitrogen for 3 hours. The product was allowed to cool and was then poured into water (200c.c.) and made alkaline with concentrated sodium hydroxide solution. The product 2-methyl-8-amino-naphthalene was extracted with ether, the ether distilled off and the product recrystallised from ethanol. m.p. 178°. (Yield: 1.8g. = 72%.)

Attempted preparation of 2-amino-8-naphthol

γ-Acid (LVIII) (10g.), water (40c.c.) and concentrated sulphuric acid (30c.c.) were refluxed in an atmosphere of nitrogen for 6 hours. The mixture was cooled to room temperature, diluted with water and filtered. The filtrate was neutralised to bicarbonate and extracted with chloroform. The chloroform extracted nothing. The residue which was filtered off consisted of unchanged γ-acid (9.1g.).

γ-Acid (LVIII) (9g.) was refluxed with water (30c.c.) and sulphuric acid (40c.c.) for 6 hours. No hydrolysis occurred.

γ-Acid (LVIII) (5g.) was refluxed with water (20c.c.) and sulphuric acid (45c.c.); the mixture charred badly. No γ-acid was recovered and no product was obtained.

Attempted Preparation of 2-amino-6:8-naphthalene-diol.

γ-Acid (LVIII) (10g.) was fused with sodium hydroxide (60g.) at 280° - 300° for 20 minutes. The resulting mass was dissolved in water (500c.c.) filtered and the filtrate acidified to Congo Red. The only product was unchanged γ-acid.

THE FOLIC ACID SIDE CHAIN

Preparation of N(p-nitro-benzoyl)-L-glutamic acid.

Method I.

L-glutamic acid (19.6g.) was dissolved in N-NaOH (270c.c.) and stirred at room temperature. To this solution was added dropwise at equal rates the following solutions:- (a) p-nitrobenzoyl chloride (25g.) in dioxan (66c.c.) and (b) 2N.NaOH (66c.c.). The mixture was stirred for 10 minutes after the addition was complete and was then acidified to Congo Red with (1:1) hydrochloric acid. The small quantity of p-nitro-benzoic acid which separated was filtered off and the filtrate concentrated under reduced pressure to 70c.c. and allowed to stand in a refrigerator for 24 hours. The white solid, N(p-nitrobenzoyl)-L-glutamic acid which separated was recrystallised from aqueous ethanol. m.p. 116°C (Yield 24g. = 62%.)

Method II.

p-Nitrobenzoyl chloride (12g.) was dissolved in chloroform (30c.c.) and added dropwise to a vigorously stirred and cooled solution of glutamic acid (2.95g.) in

10% sodium bicarbonate (100c.c.). Stirring was continued for 1 hour after the addition was complete. The aqueous layer was separated and acidified to Congo Red with concentrated hydrochloric acid. The precipitate of p-nitrobenzoic acid was filtered off and the aqueous solution evaporated to dryness under reduced pressure. The residue, which was a gum, solidified on keeping for some time in a vaccum desiccator. The crystalline N(p-nitrobenzoyl-)-L-glutamic acid was washed with ice cold water to remove sodium chloride and recrystallised from water. m.p. 115°. (Yield: 3g. = 51%.)

Preparation of N(p-aminobenzoyl)-L-glutamic acid.

Method I.

N(p-nitrobenzoyl)-L-glutamic acid 3g.) was dissolved in methanol (100c.c.), 10% palladised charcoal (0.25g.) was added and the mixture hydrogenated at atmospheric pressure. 850c.c. of hydrogen were taken up in one hour after which the hydrogenation stopped. The solution was filtered from catalyst and consentrated under reduced pressure. The residue was a dark gum which solidified on standing and was recrystallised from ethanol/water to give N(p-amino-benzoyl)-L-glutamic acid.

m.p. 168°. (Yield: 1.8g. = 67%.)

Method II.

N-(p-nitrobenzoyl)-L-glutamic acid (10g.) was dissolved in methanol 500c.c. and Adam's catalyst (0.3g.) added and the mixture hydrogenated at atmospheric pressure. 2,900c.c. of hydrogen were absorbed in 30 minutes. The solution was filtered from catalyst and concentrated under reduced pressure. Solid N-(p-aminobenzoyl)-L-glutamic acid separated. m.p. 169°. (Yield: 7g. = 77%.)

Esterification of N(p-aminobenzoyl)-L-glutamic acid.

N(p-aminobenzoic)-L-glutamic acid (5g.) was dissolved in ethanol (100 ml.) saturated with dry hydrogen chloride and left at room temperature for 48 hours. The excess hydrogen chloride and most of the ethanol were distilled off under reduced pressure at a temperature below 35°. The residue was poured into water and neutralised to pH8 with dilute ammonia. A brown solid separated which was recrystallised twice from aqueous ethanol. m.p. 138°C. (Yield: 2.3g. = 35%.)

Preparation of N-(p-nitrobenzoyl)-glutamic ester.

A suspension of glutamic acid (20g.) in ethanol (500c.c.) was saturated with dry hydrogen chloride at room temperature and then refluxed for 3 hours. The excess ethanol was distilled off under reduced pressure, the final traces being co-distilled with benzene. A solution of p-nitrobenzoyl chloride (27g.) in benzene was added to the ethyl glutamate and the mixture refluxed for 1 hour. The benzene solution was washed with bicarbonate, dried over calcium chloride and evaporated to dryness under reduced pressure. The residue of ethyl N(p-nitrobenzoyl)-L-glutamate was recrystallised from ethanol water. m.p. 94°. (Yield: 23.5g. = 50%.)

Preparation of N(p-aminobenzoyl)-L-glutamite ester.

Diethyl N-(p-aminobenzoyl)-L-glutamate (5g.) was dissolved in ethanol (100c.c.) and hydrogenated over 10% palladised charcoal (0.5g.) at atmospheric pressure. 2,200c.c. of hydrogen was absorbed in 1 hour. The solution was filtered free of catalyst and concentrated under reduced pressure. Diethyl N(p-aminobenzoyl)-L-glutamate separated. m.p. 170°. (Yield: = 4.1g. = 90%.)

A similar procedure gave dimethyl N(p-aminobenzoyl)-L-glutamate in 93% yield. m.p. 108°. (Found:- C, 57.5;

H, 6.2. $C_{14}H_{18}O_5N_2$ requires C, 57.2; H, 6.1%.)

Preparation of N(p-aminobenzoyl)-L-glutamic acid.

Diethyl N-(p-aminobenzoyl)-L-glutamate (2g.) was dissolved in ethanol (25c.c.), sodium hydroxide (3g.) added and the solution left for 48 hours at room temperature. The solution was then acidified to Congo Red, concentrated to about 5 ml. and allowed to stand. Crystalline N-(p-aminobenzoyl)-L-glutamic acid separated. m.p.166°C. (Yield: 0.8g. = 44%.)

A similar procedure using dimethyl N-(p-aminobenzoyl)-L-glutamate gave N(p-aminobenzoyl)-L-glutamic acid in 49% yield.

SIMPLE 1:3:5 TRIAZANAPHTHALENES.

Preparation of 3-amino-picolinic acid (LXIX) and 2-amino-nicotinic acid (LXX).

Quinolinimide (LXVIII) was prepared by the method of Sucharda (Ber., 1925, 58, 1727).

Finely powdered quinolinimide (LXVIII) (25g.) was dissolved in ice-cold 10% sodium hydroxide (500c.c.). A solution of potassium hypobromite was prepared by adding bromine (28g.) to ice-cold 10% potassium hydroxide (220c.c.). The two solutions were mixed and allowed to stand for 1 hour; the mixture was then heated to 80° and maintained at that temperature for 1 hour. The resulting solution was acidified to pH5 with 50% sulphuric acid and kept at 2° for 48 hours. The 2-amino-nicotinic acid (LXX) which separated was filtered off. m.p. 306°. (Yield: 4.5g. = 19%.) A solution of copper acetate (20g.) in water (200c.c.) containing acetic acid (10c.c.) was added to the filtrate. The precipitated copper salt of 3-amino-picolinic acid was filtered off, washed with water and suspended in water (60c.c.). The suspension was treated with hydrogen sulphide until no more was absorbed. The resultant mixture was heated to boiling and filtered free

from copper sulphide; the required 3-amino-picolinic acid (LXIX) separated on cooling. Concentration of the solution gave a further crop of crystals.m.p. 233°. (Yield: 13g. = 56%.)

Preparation of 2:4-dihydroxy-1:3:5-triazanaphthalene (LXXI).

3-amino-picolinic acid (LXIX) (7.5g.) and urea (3.5g.) were finely powdered and mixed together and the mixture slowly heated to 190° - 200° and maintained at that temperature for 30 minutes. The solid product was dissolved in 2N.NaOH (80c.c.) and a stream of carbon dioxide passed through the solution. 2:4-Dihydroxy-1:3:5-triazanaphthalene (LXXI) separated as a white crystalline solid. A specimen was recrystallised for analysis from a large volume of water. m.p. $>360°$. (Yield: 3g. = 34%.) (Found C, 51.9; H, 3.3. $C_7H_5O_2N_3$ requires C, 51.6; H, 3.1%.)

Preparation of 2:4-dichloro-1:3:5-triazanaphthalene.

Method I.

2:4-Dihydroxy-1:3:5-triazanaphthalene (LXXI) (2g.)

and phosphorus oxychloride (30c.c.) were refluxed for 3 hours. The resulting grey solid was filtered off and the filtrate evaporated under reduced pressure, leaving no residue. The grey solid was dissolved in water and the aqueous solution extracted with chloroform. The chloroform was separated, dried and evaporated under reduced pressure but left no residue. Sodium fusion showed the grey solid to contain nitrogen, chlorine and phosphorus.

A sample of the grey solid was refluxed for 2 hours with 2 N.NaOH; the solution was cooled to room temperature and a stream of carbon dioxide passed through. No 2:4-dihydroxy-1:3:5-triazanaphthalene (LXXI) separated.

A sample of the grey solid was refluxed for two hours with constant boiling hydrochloric acid and the mixture then filtered, cooled and saturated with sodium bicarbonate. No 2:4-dihydroxy-1:3:5-triazanaphthalene (LXXI) was obtained.

Attempts to prepare 2:4-dichloro-1:3:5-triazanaphthalene (LXXII) using phosphorus oxychloride at steam bath temperature and in a sealed tube at 150° gave similar results.

Method II.

2:4-dihydroxy-1:3:5-triazanaphthalene (LXXI) (2g.), phosphorus oxychloride (40c.c.) and phosphorus pentachloride

(2g.) were mixed and refluxed for 6 hours. The resulting mixture was filtered free from the grey solid produced and the filtrate evaporated to dryness; there was no residue. The grey solid was treated with cold water and extracted with chloroform. Evaporation of the chloroform left no residue. The grey solid had similar properties to that of the similar product obtained by Method I.

The above procedure was repeated several times using various proportions of phosphorus oxychloride and phosphorus pentachloride with similar results.

Method III.

2:4-Dihydroxy-1:3:5-triazanaphthalene (LXXI) (0.5g.), dimethylaniline (20c.c.) and phosphorus oxychloride (20c.c.) were refluxed until a clear solution was obtained (1-1½ hours). The liquid was cooled to room temperature and poured onto ice and the pH of the resulting solution adjusted to about 5 when 2:4-dichloro-1:3:5-triazanaphthalene (LXXII) separated. This product was filtered off, washed with water and purified by sublimation at 120 - 130°/10^{-1}mm. and recrystallisation from petroleum ether (b.p. 80-100°); (Yield: 0.28g. = 46%) (Found: Cl = 35.7; $C_7H_3N_3Cl_2$ requires 35.5%). m.p. 173°.

The above experiment was repeated on a 2g. scale when the yield fell to 21% and on a 3.4g. scale when the yield

was only 8%.

Hydrogenation of 2:4-dichloro-1:3:5-triazanaphthalene (LXXII).

2:4-Dichloro-1:3:5-triazanaphthalene (LXXII) (200mg.) was dissolved in methanol (200c.c.); 5% palladium hydroxide on strontium carbonate (200mg.) and magnesium oxide (400mg.) were added and the mixture was hydrogenated at atmospheric pressure; 55c.c. of hydrogen were absorbed in 30 minutes after which hydrogenation stopped. The solution was filtered from the catalyst and evaporated to dryness. The product was purified by sublimation. m.p. 98°. (Found: C, 69.2, 70.2 and 69.9; H, 6.6, 7.0 and 7.1; N, 23.8. $C_7H_5N_3$ requires C, 64.1; H, 3.8; N, 32.1. $C_{14}H_{16}N_4$ requires C, 69.9; H, 6.7; N, 23.4%.)

A small quantity of this product was dissolved in a little ethanol and added to a solution of picric acid in aqueous ethanol. The precipitated picrate was filtered off and recrystallised from aqueous ethanol. m.p. 171°. (Found: C, 52.0; H, 4.4. Calculated for $C_{14}H_{16}N_4.C_7H_3O_7N_3$ C, 52.3; H, 3.95%.)

Preparation of 4-hydroxy-1:3:5-triazanaphthalene (LXXIV)

Method I.

3-Amino-picolinic acid (LXIX) (8g.) and formamide (5c.c.) were mixed and heated slowly to 180°C and maintained at that temperature for 30 minutes. The mixture was allowed to cool to room temperature and then dissolved in 2N.NaOH (250c.c.). A stream of carbon dioxide was bubbled through the solution and the 4-hydroxy-1:3:5-triazanaphthalene (LXXIV) which separated was filtered off and dried at 100°. m.p. 346-7°. (Yield: 3.25g. = 38%.)

Method II.

Methyl 3-amino-picolinate (0.8g.) and formamide (0.5c.c.) were slowly heated to 180° and maintained at that temperature for 35 minutes. The mixture was allowed to cool to room temperature and then dissolved in 2N.NaOH (50c.c.) and a stream of carbon dioxide passed through the solution. A white precipitate of 4-hydroxy 1:3:5-triazanaphthalene (LXXIV) formed and was filtered off and dried; m.p. and mixed m.p. with previously prepared 4-hydroxy-1:3:5-triazanaphthalene, 346-7°.

Preparation of 4-hydroxy-2-mercapto-1:3:5-triazanaphthalene (LXXV).

3-Amino-picolinic acid (LXIX) (3g.) and thiourea (2g.) were ground together in a pestle and mortar to give a uniform mixture. The mixture was slowly heated to 180° and maintained at that temperature for 25 minutes. The mixture was cooled and triturated with water when crystalline 4-hydroxy-2-mercapto-1:3:5 triazanaphthalene (LXXV) separated. This was purified by subliming twice at $170°/10^{-4}$mm. m.p. $>360°$. (Yield: 600mg. = 15.5%.) (Found: S = 16.5. $C_7H_5ON_3S$ requires 17.9%)

Desulphurisation of 4-hydroxy-2-mercapto-1:3:5-triazanaphthalene (LXXV).

4-Hydroxy-2-mercapto-1:3:5-triazanaphthalene (350 mg.) was dissolved in 2 N.NaOH (50c.c.) and Raney nickel (500mg.) added and the mixture left with occasional shaking for about 8 hours. The Raney nickel was filtered off and carbon dioxide passed through the solution. A small quantity of 4-hydroxy-1:3:5-triazanaphthalene (LXXIV) separated. m.p. 340-342° mixed m.p. with authentic 4-hydroxy-1:3:5-triazanaphthalene (LXXIV) 345°. (Yield: 60mg. = 21%.)

Attempted preparation of 2:4-dimercapto-1:3:5-triazanaphthalene (LXXVI).

2:4-Dihydroxy-1:3:5-triazanaphthalene (LXXI) (1g.) was finely powdered and suspended in tetralin (100c.c.) and finely powdered phosphorus pentasulphide (4g.) added. The mixture was heated at 170 - 180° for 1.5 hours; 15% sodium hydroxide solution (50c.c.) added to the cooled product and the mixture stirred for 2 hours. The aqueous layer was separated and saturated with carbon dioxide when only a trace of a sticky solid separated. This was filtered off and the filtrate acidified to Congo Red. None of the expected 2:4-dimercapto-1:3:5-triazanaphthalene (LXXVI) separated.

Attempted preparation of 4-chloro-1:3:5-triazanaphthalene (LXXVII).

Method I.

4-Hydroxy-1:3:5-triazanaphthalene (LXXIV) (1g.) was refluxed with phosphorus oxychloride (30c.c.). The mixture turned blue and darkened as the heating proceeded. Finally after 30 minutes refluxing a blue-black liquid was obtained. The excess phosphorus oxychloride was

distilled off under reduced pressure. The residue was treated with cold water (25c.c.) and extracted with chloroform (20c.c.). The chloroform extracted none of the expected 4-chloro-1:3:5-triazanaphthalene (LXXVII).

Method II.

4-Hydroxy-1:3:5-triazanaphthalene (LXXIV) (1g.) was refluxed with phosphorus oxychloride (30c.c.) and dimethyl-aniline (20c.c.) until a clear solution was obtained (30 minutes). The solution was cooled to room temperature and added dropwise to ice water (200c.c.). The pH of the resulting solution was adjusted to 5 and the solution was stored in a refrigerator. No 4-chloro-1:3:3-triazanaphthalene (LXXVII) separated.

Attempted preparation of 2-amino-4-hydroxy-1:3:5-triazanaphthalene.

Method I.

3-amino-picolinic acid (LXIX) (4g.) and guanidine hydrochloride (5.5g.) were mixed and heated at 180°C for 30 minutes. Attempts to crystallise the product failed. The product was soluble in water, bicarbonate, dilute

acids and in alcohol. The only solid product isolated was guanidine.

Method II.

3-amino-picolinic acid (LXIX) (3g.) and guanidine carbonate (4.5g.) were heated slowly to 200° and maintained at that temperature for 20 minutes. On cooling to room temperature the product was obtained as a very gummy solid which could not be recrystallised from water. The product was soluble in dilute sodium hydroxide but was not precipitated from this solution by carbon dioxide. Sublimation at high vacuum was accompanied by considerable charring and the small amount of sublimate had m.p. $> 360^{\circ}$ and could not be further purified.

SIMPLE 1:3:8-TRIAZANAPHTHALENES.

Preparation of 2:4-dihydroxy-1:3:8-triazanaphthalene (LXXIX).

Method I.

Quinolinamide (25g.) was dissolved in a solution of potassium hypobromite prepared by adding bromine (31g.) to 10% potassium hydroxide (525c.c.). The solution was stirred for one hour at 0° and for a further hour at 80°. The solution was then cooled to room temperature and saturated with carbon dioxide. The precipitate of 2:4-dihydroxy-1:3:8-triazanaphthalene (LXXIX) m.p.361° was filtered off, washed with water and dried in a desiccator. (Yield: 4.6g. = 18.5%.)

The filtrate was acidified to pH5 and left in a refrigerator overnight; 2-amino-nicotinic acid (LXX) separated and was filtered off. m.p. 306°. (Yield; 2.5g. = 12%.)

The filtrate was treated with a solution of copper acetate (15g.) in water (150c.c.) and acetic acid (5c.c.). The precipitate of copper 3-aminopicolinate which separated was filtered off, washed and suspended in water (25c.c.) and treated with hydrogen sulphide. The resulting suspension was filtered whilst still hot and the filtrate

concentrated. 3-amino-picolinic acid (LXIX) separated. m.p.233°. (Yield: 1.9g. = 9%.)

Method II.

2-Amino-nicotinic acid (LXX) (2.4g.) was mixed with urea (2.0g.) and slowly heated to 190° and maintained at that temperature for 25 minutes. The product was allowed to cool to room temperature and then dissolved in 10% sodium hydroxide solution (60c.c.). This solution was saturated with carbon dioxide and the 2:4-dihydroxy-1:3:8-triazanaphthalene (LXXIX) which separated was filtered off and dried in a desiccator. m.p.361° undepressed on mixing with authentic 2:4-dihydroxy-1:3:8-triazanaphthalene (LXXIX). (Yield: 800mg. = 28%.)

Preparation of 2:4-dichloro-1:3:8-triazanaphthalene (LXXX).

2:4-Dihydroxy-1:3:8-triazanaphthalene (LXXIX) (1.4g.) was heated under reflux with phosphorus oxychloride (20c.c.) until a clear solution was obtained (2 hours). Most of the excess phosphorus oxychloride was distilled off under reduced pressure and the residual mixture was treated with ice water (20c.c.). The resulting solution

was extracted with chloroform (25c.c.), the chloroform on evaporation left none of the expected 2:4-dichloro-1:3:8-triazanaphthalene (LXXX).

Method II.

2:4-Dihydroxy-1:3:5-triazanaphthalene (LXXXIX) (2g.), phosphorus oxychloride (30c.c.) and dimethyl aniline (8c.c.) were refluxed until a clear solution was obtained, (4 hours). The solution was cooled to room temperature and poured into ice water (200c.c.). The pH of the resultant solution was adjusted to 5 and the solution stored at 2^{o}. None of the expected 2:4-dichloro-1:3:8-triazanaphthalene (LXXX) separated.

Method III.

2:4-Dihydroxy-1:3:8-triazanaphthalene (LXXIX) (2g.) and phosphorus oxychloride (60c.c.) were refluxed until homogeneous (4 hours). The resulting solution was evaporated to dryness, care being taken to remove all traces of phosphorus oxychloride and the residue was treated with ice water (10c.c.) and extracted with chloroform. Evaporation of the chloroform left a grey solid which on sublimation at $150^{o}/10^{-1}$mm. gave white, crystalline 2:4-dichloro-1:3:8-triazanaphthalene (LXXX).

m.p. 156 - 7°. (Yield: 1.7g. = 71%.)

Hydrogenation of 2:4-dichloro-1:3:8-triazanaphthalene (LXXX).

2:4-Dichloro-1:3:8-triazanaphthalene (500mg.) was dissolved in methanol (200c.c.), 5% palladium hydroxide on strontium carbonate (500mg.) and magnesium oxide (500mg.) were added and the mixture hydrogenated at atmospheric pressure. 135c.c. of hydrogen were absorbed and the hydrogenation then stopped. The methanol was filtered free from catalyst and distilled under reduced pressure. The residue was recrystallised from petroleum ether (b.p. 80-100°). This substance, m.p.140°, was not the expected 1:3:8-triazanaphthalene and had the following analyses:- (Found: C, 55.4 and 55.0; H, 6.9 and 6.7; N, 21.9 (Dumas) and 19.4 (Kjeldahl). $C_7H_5N_3$ requires C, 64.1; H, 8.8; N, 32.1; $C_9H_{13}O_2N_3$ requires C, 55.5; H, 6.7; N, 21.6%.)

A small quantity of this unknown product was dissolved in ethanol and a solution of picric acid in aqueous ethanol added. The precipitated picrate was filtered off and recrystallised from aqueous ethanol m.p. 211°. (Found: C, 42.6; H, 4.1. $C_9H_{13}O_2N_3.C_7H_3O_7N_3$ requires C, 42.4; H, 3.8%.)

Amination of 2:4-dichloro-1:3:8-triazanaphthalene (LXXX)

2:4-Dichloro-1:3:8-triazanaphthalene (LXXX) (250mg.) was dissolved in methanol (25c.c.) and liquid ammonia (1c.c.) added. The solution was sealed in a Carius tube and heated at 160° overnight. The tube was allowed to cool and was opened. The contents consisted of a white crystalline material suspended in methanol. The white crystals were filtered off and recrystallised from water. m.p. > 360°C. (Yield: 135mg. = 67%.) (Found: C, 44.7; H, 5.2; $C_7H_7N_5.^3/_2 H_2O$ requires C, 44.6; H, 5.3%.)

Preparation of 4-hydroxy-1:3:8-triazanaphthalene(LXXXIII)

2-Amino-nicotinic acid (LXX) (4g.) and formamide (2.6g.) were heated slowly to 180° and maintained at that temperature for 30 minutes. The product was allowed to cool and was dissolved in 2N.NaOH (100c.c.). The alkaline solution was saturated with carbon dioxide when 4-hydroxy-1:3:8-triazanaphthalene (LXXXIII) separated and was filtered off and recrystallised from aqueous methanol. m.p. 255 - 6°C. (Yield: 1.3g. = 31%.) (Found: C, 56.9; H, 3.6. $C_7H_5ON_3$ requires C, 57.2; H, 3.4%.)

Preparation of 4-chloro-1:3:8-triazanaphthalene (LXXXIV)

Method I.

4-Hydroxy-1:3:8-triazanaphthalene (LXXXIII) (1g.) and phosphorus oxychloride (20c.c.) were refluxed for 4 hours. The solution was evaporated to small volume and poured onto ice water (50c.c.) and the resulting solution extracted with chloroform. No 4-chloro-1:3:8-triazanaphthalene (LXXXIV) was extracted.

Method II.

4-Hydroxy-1:3:8-triazanaphthalene (LXXXIV) (1g.), phosphorus oxychloride (20c.c.) and dimethyl aniline (5c.c.) were refluxed for 4 hours. The resulting mixture was poured into ice water (200c.c.) and the pH adjusted to 5. No 4-chloro-1:3:8-triazanaphthalene (LXXXIV) separated.

Method III.

4-Hydroxy-1:3:8-triazanaphthalene (LXXXIII) (2g.) and phosphorus oxychloride (60c.c.) were refluxed until a clear solution was obtained (2 hours). The excess phosphorus oxychloride was distilled off under reduced pressure and care was taken to remove all traces of

phosphorus oxychloride. Ice water (20c.c.) was added to the residue and it was extracted with chloroform (2 x 100c.c.). The chloroform was evaporated and the residue sublimed at 130°/10^{-4}mm. and the sublimate recrystallised from ethyl acetate/60-80°petrol. m.p. 210°C. Yield 400mg. = 45%. (Found: Cl, 21.0. $C_7H_4N_3Cl$ requires 21.4%.)

Preparation of 2-mercapto-4-hydroxy-1:3:8-triazanaphthalene (LXXXV).

2-Amino-nicotinic acid (LXX) (3g.) and thiourea (1.85g.) were mixed and heated slowly to 180° and maintained at that temperature for 30 mins. The mixture was cooled to room temperature to give a brown solid. Attempts to purify this by crystallisation and by solution in dilute alkali followed by precipitation with carbon dioxide or dilute acid failed. The solid was finely powdered and washed with water and alcohol and sublimed at 170°/10^{-4}. m.p. >360°. (Yield: 250mg. = 10%.) (Found: S, 16.85. $C_7H_5ON_3S$ requires 17.85%.)

Desulphurisation of 4-hydroxy-2-mercapto-1:3:8-triazanaphthalene (LXXXV).

4-Hydroxy-2-mercapto-1:3:8-triazanaphthalene (LXXXV) (200mg.) was dissolved in 2N.NaOH (50c.c.) and Raney nickel (500mg.) added and the mixture left with occasional shaking for 6 hours. The Raney nickel was filtered off and carbon dioxide passed through the filtrate. 4-Hydroxy-1:3:8-triazanaphthalene (LXXXIII) separated. m.p. and mixed m.p. 255° - 6°. (Yield: 45mg. = 27%.)

Attempted oxidation of quinaldinic acid (XC) to pyridine-2:3:6-tricarboxylic acid (XCI).

Quinaldinic acid (XC) (10g.) was dissolved in water (500c.c.) containing potassium carbonate (4g.). The cold slightly alkaline solution was treated with a cold saturated aqueous solution of potassium permanganate (41g.). The resulting mixture was stirred overnight at room temperature and then at 90° for 1 hour. The precipitate of manganese dioxide was filtered off and the filtrate acidified with (1:3)sulphuric acid. The solution was cooled to 0° and dilute permanganate solution added until no longer decolourised. The solution was neutralised with diluted ammonia and concentrated to small volume under reduced pressure. The solution was acidified to Congo Red and poured into twice its own volume of alcohol. The precipitate of inorganic material was filtered off and the filtrate concentrated. Crystalline pyridine-2:5-dicarboxylic acid (XCII) separated on standing. m.p. 278°.

The above process was repeated at a temperature below 35° without altering the results.

Oxydation of 3-nitro-2:6-lutidine (XCIII).

Method I.

3-Nitro-2:6-lutidine (XCIII) (3g.) was suspended in water (100c.c.) at 50° containing potassium hydroxide (2g.) and a solution of potassium permanganate (15g.) in water (250c.c.) at 50° was added during a period of 2 hours. The reaction mixture was cooled to room temperature and filtered free from manganese dioxide. The filtrate (400c.c.) was passed through a freshly activated column of zeo-carb. The first 300c.c. through the column was evaporated to dryness under reduced pressure and left only a trace of solid residue which was discarded. The second 600c.c. through the column was concentrated to 40c.c. under reduced pressure and the residue to dryness in a vacuum desiccator. The product was a yellow solid m.p. 75-85°. The solid was extracted with ether when part dissolved.

The ether soluble portion was recrystallised from ether/petrol and ethyl acetate/petrol. m.p. 105°. (Found: C, 35.6; H, 3.0; N, 11.3. $C_7H_4N_2O_6 \cdot {}^3/_2 H_2O$ requires C, 35.3; H, 2.9; N, 11.7%.)

The ether insoluble portion was recrystallised from water. m.p. 207-208°. (Found: C, 42.7; H, 2.8. $C_6H_4O_4N_2$ requires C, 42.8; H, 2.4%.)

Decarboxylation of 3-nitro-pyridine-2:6-dicarboxylic acid (XCV).

3-Nitro-pyridine-2:6-dicarboxylic acid (XCV) (50mg.) was dissolved in water (1c.c.) and heated on a steam bath for 5 minutes. On cooling the solution deposited crystals. m.p. and mixed m.p. with the ether insoluble nitro-pyridine carboxylic acid 207° - 8°.

Structure of 3-nitro pyridine-?-carboxylic acid (XCVIII)

3-Nitro-pyridine-?-carboxylic acid (XCVIII) (200mg.) was dissolved in methanol (100c.c.) and Adam's catalyst (30mg.) added and the mixture hydrogenated at atmospheric pressure. The solution was filtered free from catalyst and concentrated under reduced pressure and the product crystallised from ethanol/water. m.p. 254°. Mixed m.p. with 3-amino-picolinic acid (LXIX) 170° - 185°. (Found: C, 51.8; H, 4.5. $C_6H_6O_2N_2$ requires C, 52.2; H, 4.35%.)

Oxidation of 3-nitro-2:6-lutidine (XCIII).

Method II.

3-Nitro-2:6-lutidine (XCIII) (6.7g.) was suspended in

a solution of sodium hydroxide (20g.) in water (4.5 l.) and potassium permanganate (300g.) added in small portions over a period of 8 hours. The solution was stirred overnight and then the excess permanganate removed with sulphur dioxide. The reaction mixture was filtered free from manganese mud, acidified to Congo Red and continuously ether extracted. The ether was evaporated and the product recrystallised from ethyl acetate/petrol m.p. 104°. Yield: 50g. = 53%.

The above procedure was repeated several times and the yield varied considerably.

Method III.

3-Nitro-2:6-lutidine (XCIII) (5g.) was suspended in a solution of potassium ferricyanide (135g.) and potassium hydroxide (50g.) in water (900c.c.). The mixture was stirred at room temperature and 5c.c. samples were withdrawn at intervals and the amount of potassium ferricyanide remaining determined.

After 24 hours all the ferricyanide had been consumed and the solution was acidified to Congo Red and continuously ether extracted. Evaporation of the ether left a small gummy residue and none of the expected 3-nitro-pyridine-2:6-dicarboxylic-acid (XCV).

Method IV.

3-Nitro-2:6-lutidine (XCIII) (15g.) was suspended in a mixture of sodium hypochlorite (425c.c.), water (2000c.c.) potassium permanganate (10g.) and sodium hydroxide (45g.). This mixture contains sufficient available oxygen to oxidise 3-nitro-2:6-lutidine (XCIII) to the required 3-nitro-pyridine-2:6-dicarboxylic acid (XCV). The mixture was stirred at room temperature for 3 days. Further quantities of potassium permanganate (10g.) were added at 24 hour intervals. The mixture was filtered, the filtrate acidified to Congo Red and continuously extracted with ether. Evaporation of the ether gave 3-nitro-pyridine-2:6-dicarboxylic acid (XCV). m.p. 105°. (Yield: 1.7g. = 8%.)

Method V.

3-Nitro-2:6-lutidine (XCIII) (5g.) and selenium dioxide (12g.) in xylene (100c.c.) were refluxed for 1 hour. On cooling the precipitate of selenium (0.3g.) was filtered off and the filtrate evaporated to dryness under reduced pressure. The residue did not react with sodium bicarbonate or with 2:4-dinitro-phenyl-hydrazine and was rejected.

Attempted Bromination of 3-nitro-2:6-lutidine (XCIII).

3-Nitro-2:6-lutidine (5g.) was dissolved in chloroform (50c.c.) and N-bromosuccinimide (12g.) added and the mixture refluxed for 6 hours. The reaction mixture was cooled and filtered and the filtrate evaporated to dryness. The residue was unchanged 3-nitro-2:6-lutidine (XCIII) (4.8g.).

Preparation of the dibenzilidene derivative of 3-nitro 2:6-lutidine (C).

3-Nitro-2:6-lutidine (XCIII) (55g.), freshly redistilled benzaldehyde (86g.) and zinc chloride (10g.) were mixed and heated to 170° - 180° for 3½ hours. The mixture was cooled and dissolved in benzene (1,000c.c.) and washed with bicarbonate and water and dried over sodium sulphate. The benzene solution was filtered through a pad of alumina about 3" thick and the pad well washed with benzene. The benzene was evaporated under reduced pressure and left the required dibenzilidine derivative (C). m.p. 134°C. (Yield: 80g. = 74%.) (Found: C, 76.5; H, 4.9. $C_{21}H_{16}O_2N_2$ requires C, 76.8; H, 4.9%.)

Oxydation of dibenzilidine derivative of 3-nitro-2:6-lutidine (C).

Dibenzilidene derivative (C) (65g.) was finely powdered and suspended in a solution of potassium hydroxide (30g.) in water (1.5 l.) and potassium permanganate (180g.) added in small portions over a period of 6 hours. The slight excess of permanganate was removed with sulphur dioxide and the manganese dioxide filtered off. The filtrate was acidified to Congo Red and cooled in ice; the precipitated benzoic acid was filtered off and the filtrate continuously ether extracted. Evaporation of the ether gave 3-nitro-pyridine-2:6-dicarboxylic acid (XCV). m.p. 105°. (Yield: 16.5g. = 39%.)

The yield on the above reaction varied between 15% and 45%.

Preparation of 3-amino-pyridine-2:6-dicarboxylic acid.

Dibenzilidine-derivative of 3-nitro-2:6-lutidine (50g.) was finely powdered and suspended in a solution of potassium hydroxide (20g.) in water (1200c.c.) and potassium permanganate (150g.) added in small portions.

The excess permanganate was removed with sulphur dioxide and the manganese dioxide filtered off. The filtrate was acidified to Congo Red, cooled to 0°C and the precipitated benzoic acid filtered off. The filtrate and washings (1800c.c.) were divided into two equal portions (900c.c.).

The first portion was neutralised with bicarbonate and sodium dithionite added (50g.) and the mixture heated to 60°C for 20 minutes and then concentrated to 500c.c. under reduced pressure, cooled to 0°C and acidified to Congo Red. 3-Amino-pyridine-2:6-dicarboxylic acid (CIII) separated and was recrystallised from water. m.p. 235°. (Yield, 3.4g. = 23%.) (Found: C, 43.6; H, 4.0. $C_7H_5O_4N_2 . \frac{1}{2}H_2O$ requires C, 43.9; H, 3.7%.)

The second portion of reaction mixture was mixed with a solution of stannous chloride (75g.) in (1:1) hydrochloric acid (200c.c.). After standing for 2 hours the pH of the solution was adjusted to 5. No 3-amino-pyridine-2:6-carboxylic acid separated.

Attempted Preparation of 6-methyl ester of 3-nitro-pyridine-2:6-dicarboxylic acid (CI).

Method I.

3-Nitro-pyridine-2:6-dicarboxylic acid (XCV) (3g.) was

dissolved in ether and treated with one equivalent of ethereal diazomethane. The mixture was allowed to stand at room temperature for 1 hour and the ether distilled off. The residue was insoluble in bicarbonate and was recrystallised from aqueous methanol. m.p. 86°. (Yield: 1g. = 27%.) (Found: C, 45.9; H, 3.5. Calculated for $C_9H_8O_6N_2$, C, 45.0; H, 3.3%.)

Method II.

3-Nitro-pyridine-2:6-dicarboxylic acid (XCV) (2.5g.) was dissolved in methanol saturated with dry hydrogen chloride (50c.c.) and allowed to stand at room temperature for 1 hour. The solution was then evaporated to dryness under reduced pressure at a temperature not more than 35° and the residue kept in a vacuum dessicator over potassium hydroxide. The product was a crystalline solid. m.p. 86° and mixed m.p. with authentic dimethyl 3-nitro-pyridine-2:6-dicarboxylate 86°. (Yield: 2g. = 59%.)

This method was used for the preparation of diester.

Hydrogenation of 3-nitro-pyridine-2:6-dicarboxylic ester.

3-Nitro-pyridine-2:6-dicarboxylic ester (2.6g.) was dissolved in methanol (100c.c.) and hydrogenated at

atmospheric pressure using Adam's catalyst. The product was filtered free from catalyst and concentrated under reduced pressure. The dimethyl 3-amino-pyridine 2:6-dicarboxylate (CIII) crystallised out and was recrystallised from methanol. m.p. 192 -3°. (Yield: 2g. = 88%.) (Found: C, 51.7; H, 4.8. $C_9H_{10}O_4N_2$ requires C, 51.5; H, 4.8%.)

Esterification of 3-amino-pyridine-2:6-dicarboxylic acid.

3-Amino-pyridine-2:6-dicarboxylic acid was dissolved in methanol (100c.c.) and the solution saturated at room temperature with dry hydrogen chloride. The mixture was left at room temperature for 48 hours, concentrated to small volume, poured into water and the dimethyl 3-amino-pyridine-2:6-dicarboxylate (CIII) extracted with ether. The ether was evaporated and the residual ester recrystallised from methanol. m.p. 192 - 3°. (Yield: 1.4g. = 42%.)

Preparation of 3-amino-2:6-lutidine.

3-Nitro-2:6-lutidine (5g.) was dissolved in methanol and hydrogenated at atmospheric pressure using Adam's

catalyst. The catalyst was filtered off, the filtrate evaporated to dryness under reduced pressure and the residue recrystallised from methanol/water. m.p. 124°. (Yield: 3.9g. = 97%.)

Oxydation 3-amino-2:6-lutidine.

3-Amino-2:6-lutidine (10g.) was suspended in a solution of potassium hydroxide (25g.) in water (1 l.) and potassium permanganate (60g.) was added in small portions. As each portion of potassium permanganate was added it was immediately consumed with the evolution of heat. The resulting mixture was a sticky liquid quite unlike the usual suspension of manganese dioxide. This was filtered and the filtrate acidified to Congo Red and seeded with 3-amino-pyridine-2:6-dicarboxylic acid. No 3-amino-pyridine-2:6-dicarboxylic acid separated.

Acetylation of 3-amino-2:6-lutidine.

3-Amino-2:6-lutidine (10g.) and acetic anhydride (20c.c.) were heated on a steam bath for 2 hours. The resulting mixture was poured onto ice and filtered free from a small dark residue. The filtrate was made alkaline

with sodium hydroxide solution and the acetyl derivative extracted with ether, the ether evaporated and the residual acetyl derivative recrystallised from ethanol/water. m.p. 137°. (Yield: 7.5g. = 56%.)

Oxydation of 3-acetamido-2:6-lutidine.

3-Acetamido-2:6-lutidine (5g.) was suspended in a solution of sodium hydroxide (2g.) in water (150c.c.) and permanganate (12g.) added. The permanganate was immediately consumed and the solution became hot. The reaction mixture was filtered free from precipitate and acidified to Congo Red. None of the expected 3-acetamido-pyridine-2:6-dicarboxylic acid separated.

Attempted Preparation of 2:4-dihydroxy -1:3:5-triazanaphthalene-6-carboxylic ester (CIV).

3-Amino-pyridine-2:6-dicarboxylic ester (4.5g.) and urea (1.5g.) were mixed and heated to 170° and maintained at that temperature for 30 minutes. On cooling the solid product was recrystallised from methanol. m.p. 192° and mixed m.p. with 3-amino-2:6-dicarboxylic ester (CIII) 192 -3°.

A repeat of the above experiment at 220° led to a product which was charred. No product or starting material was recovered.

Attempted preparation of 4-hydroxy-1:3:5-triazanaphthalene -6-carboxylic ester (CV).

3-Amino-pyridine-2:6-dicarboxylic ester (3g.) and formamide (1g.) were heated slowly to 170° and maintained at that temperature for 30 minutes. The product was cooled and recrystallised from methanol. m.p. 192-3°; mixed m.p. with 3-amino-2:6-dicarboxylic ester (CIII) 192 -3°.

SYNTHESIS OF 1:3:8-TRIAZANAPHTHALENE ANALOGUES OF FOLIC ACID.

Attempted preparation of 2-methyl-pyridine-3:5:6-tricarboxylic acid.

2 :6-Dimethyl-pyridine-3:5-dicarboxylic acid (10g.) was dissolved in a solution of sodium hydroxide (5g.) in water (1 litre) and potassium permanganate (25g.) added. The mixture was heated on a steam bath for 4½ hours by which time all the permanganate had been consumed. The mixture was filtered free from manganese dioxide and evaporated to small volume under reduced pressure. The solution was acidified to Congo Red with 50% sulphuric acid. None of the expected tricarboxylic acid separated. Addition of silver nitrate gave none of the expected precipitate of the silver salt of 2-methyl-pyridine-3:5:6-tricarboxylic acid (CVII).

Oxidation of Quinoline, Quinaldine and 8-hydroxy-quinoline.

Quinoline (10g.) was suspended in 5% sodium hydroxide (250c.c.) at room temperature and potassium permanganate (30g.) added in three portions at 1 hour intervals. After stirring overnight at room temperature the mixture was

filtered free from manganese dioxide and evaporated to dryness under reduced pressure. The dry residue was refluxed with methanol (250c.c.) in a stream of dry hydrogen chloride. The mixture was evaporated to dryness under reduced pressure, dissolved in water (60c.c.), made alkaline to bicarbonate and extracted with ether (100c.c.). The ether solution was dried over sodium sulphate and evaporated to about 50c.c. Dry hydrogen chloride was passed into the ethereal solution. None of the expected quinolinic ester hydrochloride separated.

Repetition of the above procedure with quinaldine (CXIII) and 8-hydroxy-quinoline (CXII) gave similar negative results.

Preparation of 3-methyl-8-hydroxy-quinoline (CXII).

o-Amino-phenol (55g.) and o-nitro-phenol (25g.) were dissolved in 12N hydrochloric acid (100c.c.) in a 3-necked flask. The mixture was heated on a steam bath and α-methyl-acrolein (40c.c.) was added dropwise during 45 minutes. The mixture was heated on a steam bath for a further hour. The excess o-nitro-phenol was steam distilled, the residual mixture saturated with sodium bicarbonate and again steam distilled. The second steam

distillate was extracted with ether, the ether dried and distilled leaving 3-methyl-8-hydroxy-quinoline which was recrystallised from methanol. m.p. 137°. (Yield: 3.5g. = 4.65%.) (Found: C, 80.4; H, 6.2. $C_{10}H_9ON$ requires C, 80.5; H, 6.0%.)

Several experiments were performed on the above scale in order to improve the yield. The results may be summarised as follows:-

Increasing the quantity of α-methyl-acrolein to 80c.c. increased the yield to 6.5%.

Increasing the temperature to 120°C improved the yield to 25%.

Increasing the temperature by refluxing the mixture over a bunsen gave a yield of 23%.

Oxydation of 3-methyl-8-hydroxy-quinoline (CXII)

3-Methyl-8-hydroxy-quinoline (95g.) was placed in a 5 litre flask and fuming nitric acid (200c.c.) added dropwise with external cooling. After standing at room temperature for 4 hours the mixture was heated on a steam bath until the vigorous evolution of oxides of nitrogen ceased. The mixture was cooled to room temperature and the above procedure repeated using first

300c.c. of fuming nitric acid and then 500c.c.

The mixture was then transferred to a large porcelain basin and evaporated to dryness. The residue, 5-methyl-quinolinic acid (CVI) was recrystallised from 40% acetic acid. m.p. 186°. (Yield: 60g. = 52%.) (Found: C, 53.7; H, 3.8; N, 8.0. $C_8H_7O_4N$ requires C, 53.1; H, 3.9; N, 7.7%.)

Esterification of 5-methyl-quinolinic acid (CVI).

5-Methyl quinolinic acid (CVI) (60g.) was dissolved in methanol (1 l.) and refluxed in a stream of dry hydrogen chloride for 4 hours. The mixture was concentrated under reduced pressure, poured into water (200c.c.), the aqueous solution made alkaline to bicarbonate and the ester extracted with ether (200ml.). The ether was evaporated and the residue recrystallised from methanol. m.p. 59-60°. (Yield: 20g. = 29%.) (Found: C, 56.8; H, 5.4; $C_{10}H_{11}O_4N$ requires C, 56.4; H, 5.3%.)

Preparation of 5-methyl-quinolinamide (CXV).

Dimethyl 5-methyl-quinolinate (CXIV) (18g.) was suspended in 0.880 ammonia (200c.c.) and the mixture was cooled in ice and a stream of ammonia gas bubbled through.

After 6 hours the precipitate of 5-methyl-quinolinamide was filtered off. An analysis specimen was recrystallised from warm water. m.p. 215°. (Yield: 10.5g. = 91%.) (Found: C, 52.9; H, 4.9. $C_8H_9O_2N_3$ requires C, 53.6; H, 5.0%.)

Preparation of 6-methyl-2:4-dihydroxy-1:3:8-triazanaphthalene (CXVI).

5-Methyl-quinolinamide (CXVI) (10g.) was dissolved in a solution of potassium hypobromite prepared by dissolving bromine (6c.c.) in 10% potassium hydroxide (200c.c.) at 0°. The solution was kept at 0° for 1 hour and then heated to 80° for a further hour. The solution was cooled to room temperature and saturated with carbon dioxide. The 6-methyl-2:4-dihydroxy-1:3:8-triazanaphthalene (CXVI) which separated was filtered off and dried in a vacuum desiccator. An analysis specimen was recrystallised from water. m.p. >360° (Yield: 6.1g. = 62%.) (Found: C, 53.8; H, 4.1. $C_8H_7O_2N_3$ requires C, 54.2; H, 3.9%.)

The filtrate from above was acidified to pH5 and left in a refrigerator for 48 hours. No acid separated. A solution of copper acetate (5g.) in 5% acetic acid (100c.c.) was added, the precipitate of copper salt filtered off,

suspended in water and treated hydrogen sulphide. The copper sulphide was filtered off and the solution concentrated and crystalline 2-amino-5-methyl-pyridine 3-carboxylic acid (CVIII) separated. m.p. 185°. (Yield: 300mg. = 3%.) (Found: C, 50.25, H, 6.3. $C_7H_8O_2N_2.H_2O$ requires C, 49.6; H, 5.9:.)

Chlorination of 6-methyl-2:4-dihydroxy-1:3:8-triazanaphthalene (CXVI).

Method I.

6-Methyl-2:4-dihydroxy-1:3:8-triazanaphthalene (5g.), phosphorus oxychloride (60c.c.) and dimethyl aniline (15c.c.) were refluxed until a clear solution was obtained (3 hours). The mixture was cooled, poured onto ice water (1 l.) and the pH of the resulting solution adjusted to 5. A solid separated and was filtered off. The solid was insoluble in organic solvents and could not be sublimed. Sodium fusion showed it contained large quantities of phosphorus.

Method II.

5-Methyl-2:4-dihydroxy-1:3:8-triazanaphthalene (CXVI) (600mg.) was refluxed with phosphorus oxychloride (20c.c.)

until a clear solution was obtained (3 hours). The excess phosphorus oxychloride was evaporated under reduced pressure care being taken to remove all traces of oxychloride. The residue was treated with water (5 c.c.) and extracted with chloroform (25c.c.). The chloroform was evaporated and the residue sublimed. m.p. 142°. (Yield: 300mg. = 41%.) (Found: Cl = 32.53. $C_8H_5N_3Cl_2$ requires 33.1%.)

Attempted bromination of 5-methyl-2:4-dihydroxy-1:3:8-triazanaphthalene (CXVI).

5-Methyl-2:4-dihydroxy-1:3:8-triazanaphthalene (CXVI), phosphorus oxybromide (5g.) and dimethyl aniline (5c.c.) were refluxed. The mixture reacted very violently and produced a black tar.

Hydrogenation of 5-methyl-2:4-dichloro-1:3:8-triazanaphthalene (CXVII)

5-Methyl-2:4-dichloro-1:3:8-triazanaphthalene (CXVII) (300 mg.), 5% palladium hydroxide on strontium carbonate (500mg.) and magnesium oxide (300mg.) were mixed in methanol

(100c.c.) and hydrogenated at atmospheric pressure. 85c.c. of hydrogen were absorbed. The methanol was filtered free from catalyst and evaporated to dryness. The residue was recrystallised from benzene petrol. m.p. 160°. (Found: C, 51.93; H, 4.59; N, 22.5. $C_{16}H_{18}N_6O_5$ requires C, 51.5; H, 4.8; N, 22.4%.)

Attempted side-chain bromination of 5-methyl-2:4-dihydroxy-1:3:8-triazanaphthalene (CXVI).

Method I.

5-Methyl-2:4-dihydroxy-1:3:8-triazanaphthalene (CXVI) (1g.), acetic acid (10c.c.) and bromine (0.4c.c.) were sealed in a carius tube and heated overnight at 150°. The tube was allowed to cool, opened and the crystalline deposit filtered off (1g.). These crystals were recrystallised from water. A sodium fusion showed they did not contain bromine.

Method II.

5-Methyl-2:4-dihydroxy-1:3:8-triazanaphthalene (CXVI) (1g.) was dissolved in 45% hydrogen bromide (50c.c.) containing bromine (1c.c.) and the mixture heated on a

steam bath for 4 hours. On cooling a white crystalline solid separated (0.8g.). This was recrystallised from water and a sodium fusion showed that the substance contained no bromine.

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