

THE CHEMICAL POLYMERIZATION OF  
MONOPYRROLES TO PORPHYRINS

CENTRE FOR NEWFOUNDLAND STUDIES

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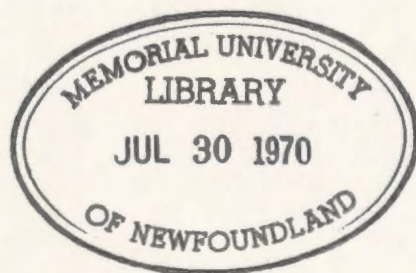
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The Chemical Polymerization of  
Monopyrroles to Porphyrins

A thesis

by

© Alvin E. Wells, B.Sc.

Submitted in partial fulfillment of the  
requirements for the degree of  
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..Mrs. S. Banfield who drew the diagrams for this thesis,

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..Miss M. Baggs who recorded the mass spectra.

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## ABSTRACT

The biogenesis of porphyrins and the intermediates in the biogenetic formation of porphyrins are briefly discussed. It is clear that the selective formation of porphyrin type isomers in an enzyme system can not, as yet, be duplicated by the chemical polymerization of monopyrroles. However, it has been shown that the type isomer composition may vary with the conditions under which the polymerization is done.

Trimethylaminomethylpyrrole salts were polymerized in an organic solvent in hopes of clarifying some aspects of the mechanism of porphyrin formation. Although a mixture of type isomers was obtained, the results indicated that the cyclization was not completely random. The polymerization appears to involve a number of different reactions in competition with each other.

The investigation of Raney nickel desulfurization of pyrrole thiolesters was continued and it was found that the thiolester residue can be converted exclusively to a methyl group in very good yield. A brief account of previous results is given.

When a pyrrole, containing both a thiolester residue and a methyl group, was treated with bromine under anhydrous conditions, it was found that the thiolester, rather than the methyl group, was being attacked. A very limited investigation of this reaction was carried out to determine if it would be a useful route in porphyrin syntheses.

Some new dipyrromethanes were synthesized and some of the problems encountered in dipyrromethane reactions are discussed.



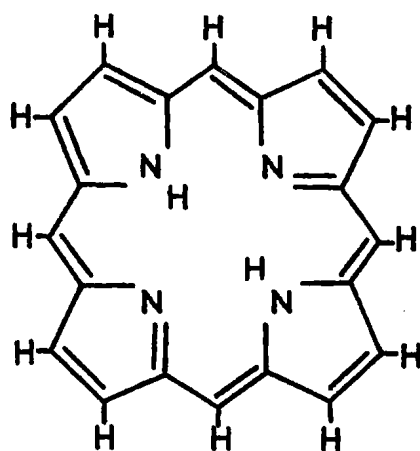
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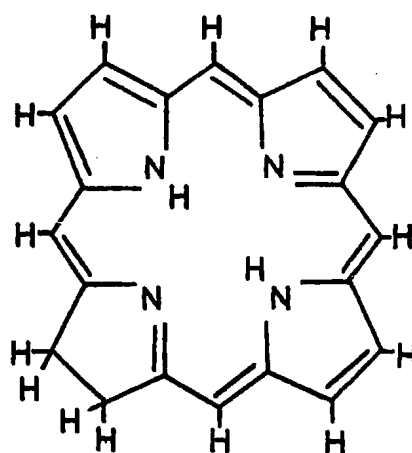
## INTRODUCTION

Tetrapyrrolic macrocyclic compounds have been a subject of chemical and biological investigation for over a century although most of the work concerning their biosynthetic pathway has been done in the past thirty years. These macrocycles consist of two main types (1). The porphins and dihydroporphins constitute the type which has four pyrrole rings joined by methine bridge linkages in their  $\alpha$ -positions, the basic ring system for each being porphin (I) and chlorin (II). The most famous dihydroporphins are the chlorophylls (for a recent review see ref. 2) which occur in every plant and alga whose existence relies on the conversion of radiant energy into chemical reactivity (3). The second type of tetrapyrrolic macrocycles consists of the corrins in which one of the methine bridge linkages is missing and instead, two pyrrole units are joined directly in their  $\alpha$ -positions. Included in the corrins are the cobalamins such as the vitamin B<sub>12</sub> coenzyme (4-6).

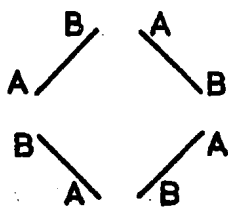
The porphins, which can occur in the free state or as metal complexes, may have several type isomers depending on the arrangement of their substituents. If each of the pyrrole units in the porphin ring contains two different substituents A and B in its  $\beta$ -positions, then a random arrangement of these pyrrole units will produce four different isomers of that porphyrin (III 1 - 1V). This is also true for the porphyrinogens where the pyrrole units are joined by methane bridge linkages instead of methine bridges as in the porphins. The only porphyrin



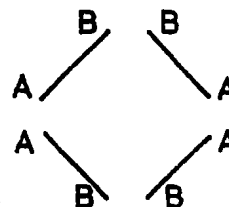
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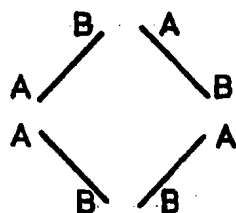
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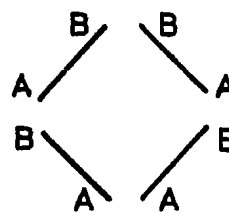
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III

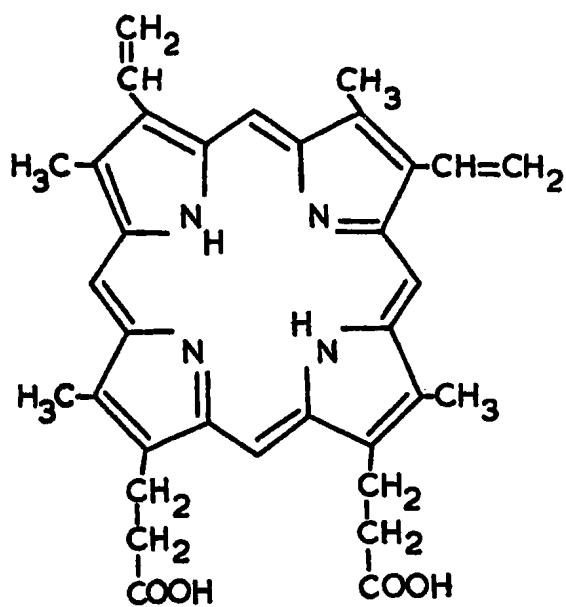


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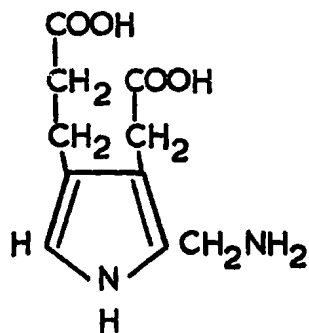
III

FOR COPROPORPHYRIN  $A = CH_3$ ,  $B = CH_2CH_2COOH$

FOR UROPORPHYRIN  $A = CH_2COOH$ ,  $B = CH_2CH_2COOH$



IV



V

isomer types known to occur naturally in the free state are protoporphyrin IX (IV) and the I and III isomers of uro- and copro- porphyrins (7).

The first big step in establishing the biosynthetic pathway of the tetrapyrroles was the demonstration by Shemin and Rittenberg (8) that the haeme of haemoglobin was labelled by  $N^{15}$ -glycine. Later it was shown, *in vitro*, that  $\delta$ -aminolevulinic acid was a more active precursor of haeme, porphobilinogen (V), and porphyrins than glycine (9-12). Purified preparations from erythrocytes of chickens have been used to show that  $\delta$ -aminolevulinic was formed from glycine and succinyl-CoA in the presence of pyridoxal phosphate (13-15) and ferrous ions (16).

The condensation of two molecules of  $\delta$ -aminolevulinic acid to porphobilinogen is said to involve, firstly, an aldol type condensation and, secondly, a Schiff base linkage, one or both of which are catalysed (17) by the enzyme aminolevulinic acid dehydrase (9, 10, 11).

One of the areas of mystery in the biosynthesis of porphyrins is the polymerization of porphobilinogen to form uroporphyrinogen both *in vivo* and *in vitro*. Both the I and III isomers occur in nature with most of the natural products being the III type. The II and IV series apparently do not occur naturally (18). It has been shown that two enzymes (19, 20) are responsible for the formation of uroporphyrinogen III. In the presence



of the first enzyme, uroporphyrinogen I - synthetase, porphobilinogen will be converted only to uroporphyrinogen I. However, in the presence of the second enzyme, uroporphyrinogen III - cosynthetase, porphobilinogen will be converted only to uroporphyrinogen III. Uroporphyrinogen I is not a substrate for the cosynthetase (12, 20, 21). Bullock (22) has suggested that the synthetase is a reversible de-amination enzyme which holds an equilibrium between porphobilinogen and a mixture of uroporphyrinogen isomers (plus ammonia in some form) containing mostly uroporphyrinogen I. The other isomers need only be present in minute amounts. The cosynthetase, because of the unusual stereochemistry of the III isomer, can pick this particular isomer out of the mixture, forcing the equilibrium to give entirely uroporphyrinogen III. This is in agreement with the work of Bogorad (19, 20, 23) whose kinetic studies suggest that the action of the synthetase on porphobilinogen is the rate determining process and the cosynthetase step, which also involves porphobilinogen as a substrate, is much faster.

Much of the work in recent years has been concerned with the chemical polymerization of monopyrroles to porphyrins, with a variety of methods used and results obtained, in hopes of elucidating the pathway by which Nature converts porphobilinogen to uroporphyrinogen III exclusively. The basic assumption was that the enzyme route is one that could be imitated *in vitro* if the right conditions were found. The formation of uroporphyrins from

porphobilinogen, available from the urine of patients with acute porphyria, was the subject of much of the earlier work but later more readily accessible synthetic pyrroles were used.

In 1943, Siedel and Winkler (24) claimed that they obtained a mixture of aetioporphyryns I and III when they heated 4-ethyl-5-hydroxymethyl-3-methylpyrrole-2-carboxylic acid (later shown to be the 5-acetoxymethyl compound (25)) in dilute hydrochloric acid. Westall (26) used acidic porphobilinogen solutions of varying pH to show that maximum yields of uroporphyrin were obtained using 0.3 - 0.5 N hydrochloric acid solutions. However, no attempts were made to separate any isomers formed.

In 1953, Cookson and Rimington (27), prepared uroporphyrin under "neutral" (pH = 6.5), acidic and basic conditions and concluded that the composition of the isomeric mixture might vary with the pH of the solution and that uroporphyrin III could not be the sole product (Table 1). Paper chromatography, using the method of Falk and Benson (28), could only distinguish between I and III isomers and could not show if II and IV isomers were present. However, conversion of porphobilinogen to uroporphyrin in 0.5 N hydrochloric acid revealed, after esterification, only one spot in the uroporphyrin III position. Having established the structure of porphobilinogen, Cookson and Remington proposed mechanisms for its acid catalysed polymerization to give a random mixture of the four isomers.

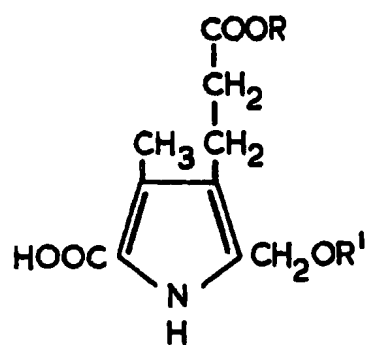
TABLE I

Conversion of porphobilinogen into uroporphyrins in various solutions at 20°(27).

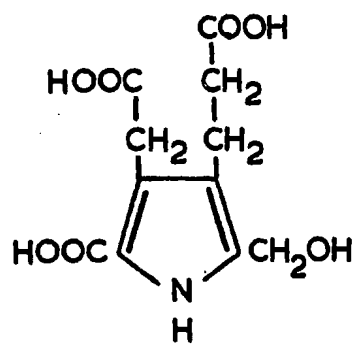
	<u>Composition</u>	pH	uroporphyrin yield in (μg/mg.Pbg*)	unchanged Pbg* (mg.)	apparent composition of uropor- phyrin mixture (%)	
					"I"	"III"
A.	2.4mg.Na <sub>2</sub> CO <sub>3</sub> , 0.5 ml water, 4.5 ml normal urine	10	65	0	60	40
B.	2.3mg.Pbg. 8.1ml HOAc 4.75ml normal urine 1.2ml 2N-NaOH	6.5	160	0	0	100
C.	2.0mg.Pbg 5.0ml 0.2N- NH <sub>3</sub>	10	80	0	50	50
D.	2.2mg.Pbg 5.0ml 0.5N- HCl	1	0.35	1.0	not enough material	

\*Pbg = porphobilinogen

In 1958, Bullock et al (25) claimed the acid polymerization of VI(a) yielded entirely coproporphyrin III tetraethyl ester. In the same year, Treibs and Ott (29) obtained uroporphyrin III as the main product when VII was heated in acid solution. In 1960, Falk and Dresel (30) described the exclusive formation of uroporphyrin III under acidic conditions. The papers of Bullock and Treibs were subjects of criticism by Mauzerall (31) whose work (Table 2) led him to the conclusion that uroporphyrinogens will isomerize in hot acid solutions leading to a random mixture of isomers. However, Mauzerall's experiments had been carried out under anaerobic conditions while those of Bullock et al had involved aerating of the acid solutions while refluxing. If the porphyrinogen of type III were the kinetically preferred product, then this might rapidly oxidize to the porphyrin before randomization of the isomers could occur. To test this hypothesis, Kay (32) did work similar to that of Mauzerall under conditions used by Bullock et al. He also polymerized VI(b) under several conditions and his results are given in Table 3. Note that the random formation of the coproporphyrin isomers would give 12.5% I, 12.5% II, and 75% III and IV. An interesting aspect of this work was the demonstration that little isomerization of coproporphyrinogen I took place in hot acid solution if cupric acetate were present. Also, the I isomer of coproporphyrin was favoured if VI(b) were acid polymerized in the presence of cupric (but not zinc) ions.



VI



VII

VI (a)  $R = C_2H_5$ ,  $R' = CH_3CO$

(b)  $R = CH_3$ ,  $R' = CH_3$



TABLE 2

Uroporphyrinogen Isomerization and Incorporation of  $\text{HC}^{14}\text{HO}$  (31).

Compound	Conditions	%URO Preoxidized	%URO Yield	Isomer I II		Ratio molar activ- ities URO: HCHO
1. URO'gen I* + $\text{HC}^{14}\text{HO}$	Acid	51	25	1/8	1/8	$1.00 \pm 0.05$
2. URO'gen I + $\text{HC}^{14}\text{HO}$	Neutral	1.3	83	1	...	$0.0093 \pm 0.001$
3. URO'gen I + $\text{HC}^{14}\text{HO}$	Alkaline	2.5	61	1	...	$0.0021 \pm 0.0004$
4. URO I† + $\text{HC}^{14}\text{HO}$	Acid	....	100	1	0	$0.0004 \pm 0.0004$
5. URO'gen I	Acid	1.0	87	1/8	1/8	
6. URO'gen III	Acid	0.2	81	1/8	1/8	
7. COPRO'gen I§	Acid	24	29	1/8	1/8	

\* URO'gen I = Uroporphyrinogen I

† URO I = Uroporphyrin I

§ COPRO'gen I = Coproporphyrinogen I

TABLE 3

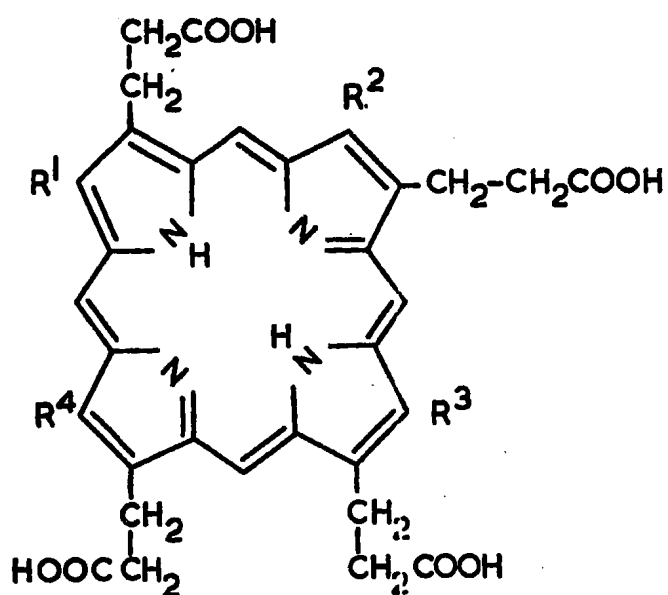
Paper chromatographic resolution of mixtures of coproporphyrin isomers formed under various conditions (32).

Compound	Conditions	Yield	% Isomers		
			I	II	III and IV
1. COPRO'gen I	pH = 4, 20° for 24 hours	67%	80	+	20
2. COPRO'gen I	pH = 4, 100° for 30 min.	75%	75	+	25
3. COPRO'gen I	pH = 4, Cu <sup>++</sup> , 20° for 48 hours	82%	92	?+	8
4. Pyrrole V(b)	pH = 4, 100° for 30 min.	52%	16	16	68
5. Pyrrole V(b)	pH = 4, 20° for 24 hours	34%	19	14	67
6. Pyrrole V(b)	pH = 4, Cu <sup>++</sup> , 20° for 48 hours	28%	57	6	37
7. Pyrrole V(b)	pH = 4, Zn <sup>++</sup> , 20° for 48 hours	38%	18	17	65

## DISCUSSION

### (A) Preparation of Dipyrromethanes

Part of this study was devoted to the preparation of pentacarboxylic acid porphyrins of type III. In these porphyrins, one of the methyl groups of coproporphyrin III is replaced by an acetic acid function. On examination, one finds there are four different isomers of these porphyrins depending on which of the methyl groups is replaced (VIII(a)-(d)). Pentacarboxylic acid porphyrins of type III have been prepared in 5 - 10 % yield by incubating uroporphyrinogen III with supernatant of chicken red blood cells haemolysates (33) and consistent results have demonstrated that the corresponding porphyrinogens are likely intermediates between uroporphyrinogen III and coproporphyrinogen III (33-37). However, using the four different isomers of these porphyrinogens individually, testing could be done which might elucidate an order (if any) of decarboxylation. If, in an enzyme system, one of these isomers were more readily decarboxylated than the others, it would seem quite likely that this particular isomer was the last intermediate between uroporphyrinogen III and coproporphyrinogen III. Similar testing with hexa- and hepta- carboxylic acid porphyrinogens could possibly determine an order of decarboxylation which, probably more noteworthy, would illustrate the importance of stereochemistry in this enzyme system.



VIII

VIII (a)  $R^1 = R^2 = R^3 = CH_3$ ,  $R^4 = CH_2COOH$

(b)  $R^1 = R^2 = R^4 = CH_3$ ,  $R^3 = CH_2COOH$

(c)  $R^1 = R^3 = R^4 = CH_3$ ,  $R^2 = CH_2COOH$

(d)  $R^2 = R^3 = R^4 = CH_3$ ,  $R^1 = CH_2COOH$

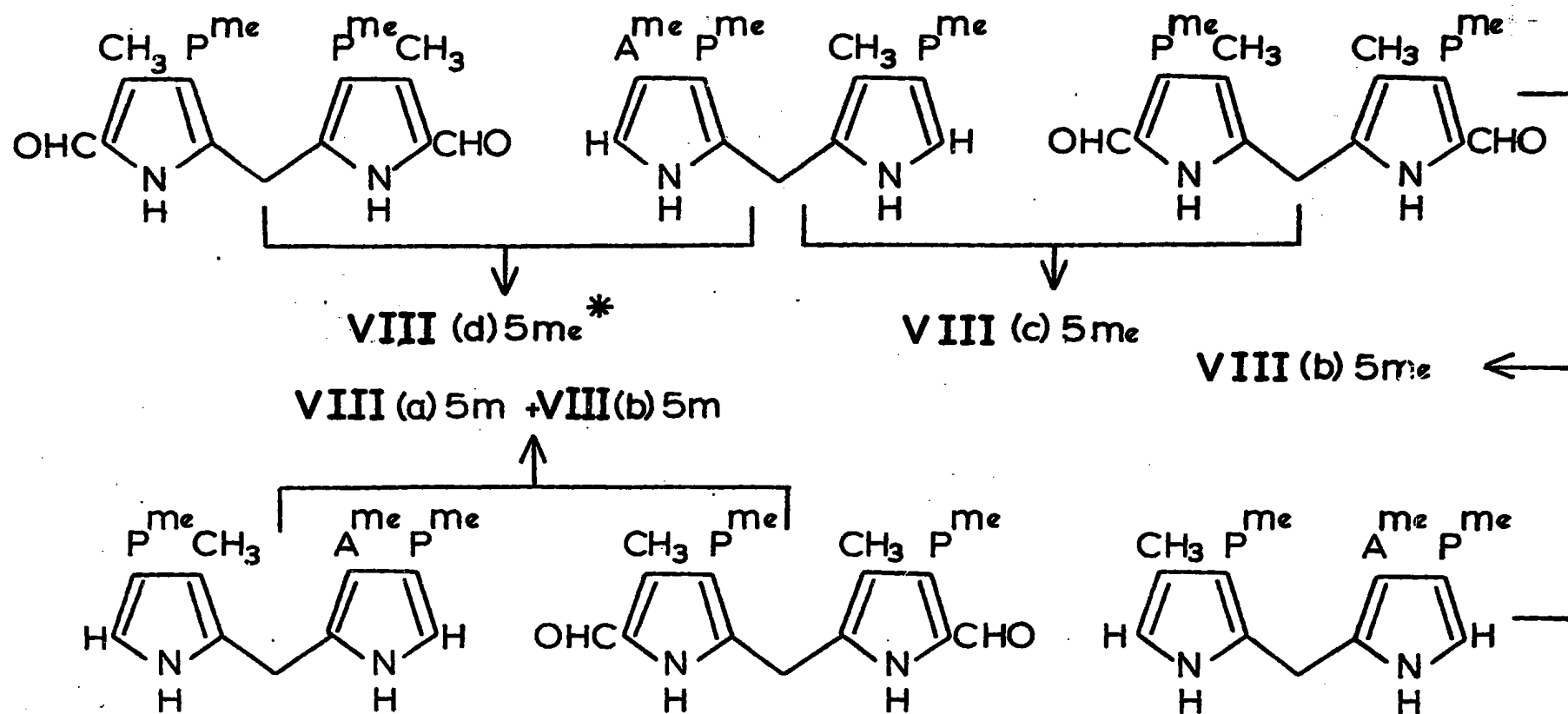
It was hoped to synthesize the porphyrins from dipyrromethanes as in Chart 1 since the porphyrin yield from dipyrromethanes is much higher than from the corresponding dipyrromethenes (38-40). Since VIII(a) is completely lacking in symmetry there is no way, using dipyrromethanes, to obtain it as the sole product. The dipyrromethane X(a) was prepared from XX(b) and XVIII(c). Similarly, XI(a) was formed from XVII(c) and XX(b), and XII(a) from XVIII(b) and XIX(c). The dipyrromethanes IX(a), XIII and XIV were prepared according to the methods of Morsingh and MacDonald (38).

It was first attempted to use XX(b) and XVII(b) in a 1:5 molar ratio which, when refluxed in ethanol, should yield mostly the dipyrromethanes XI(a) and IX(a) with a small amount of XVI being formed. This was based on the assumption that the rate of reaction of XX(b) with XVII(b) is approximately equal to the rates of these pyrroles undergoing self-condensation. However, IX(a) was the only dipyrromethane isolated from the reaction mixture.

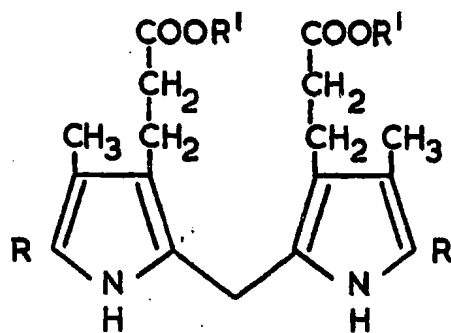
Difficulties arose in the attempt to synthesize IX(e). The dipyrromethane IX(a) was heated with aqueous sodium hydroxide according to the method of Arsenault et al (39) but IX(c) could not be isolated from the acidified aqueous layer. Similar results were obtained by Chong et al (41) when they tried the decarboxylation of IX(g) using a variety of conditions including those of Kleinspehn and Briod (42). Attempts by the Australian workers to formylate IX(g) by the Vilsmeier-Haack method



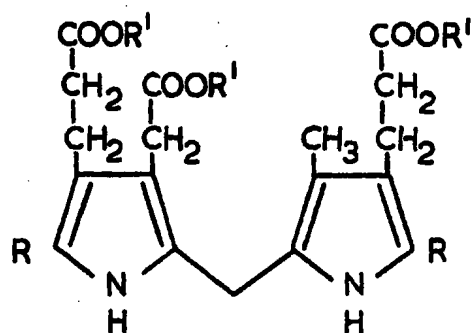
# CHART I



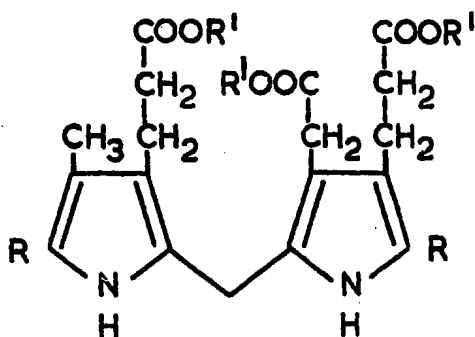
\* (d) 5me = (d) pentamethyl ester  
P<sup>me</sup> = CH<sub>2</sub> CH<sub>2</sub> COOCH<sub>3</sub>  
A<sup>me</sup> = CH<sub>2</sub> COOCH<sub>3</sub>



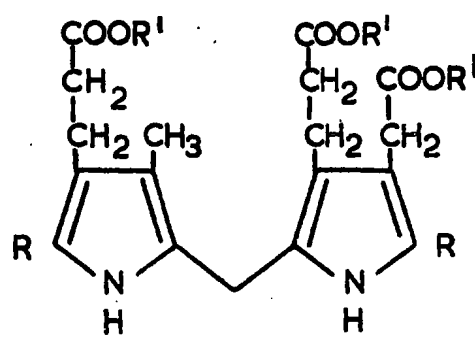
IX



X



XI



XII

(a)  $R = \text{COOC}_2\text{H}_5$ ,  $R' = \text{C}_2\text{H}_5$

(b)  $R = \text{COOH}$ ,  $R' = \text{H}$

(c)  $R = R' = \text{H}$

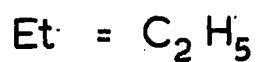
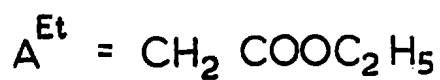
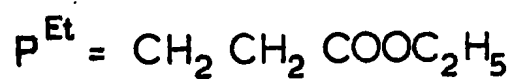
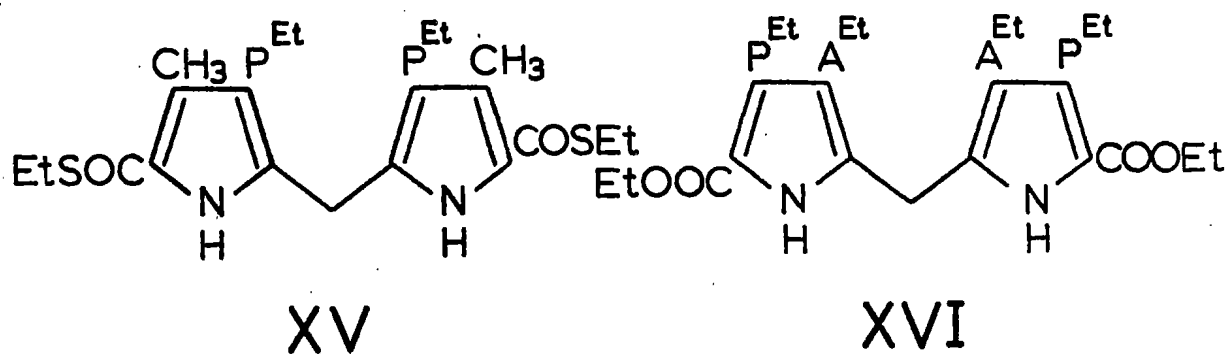
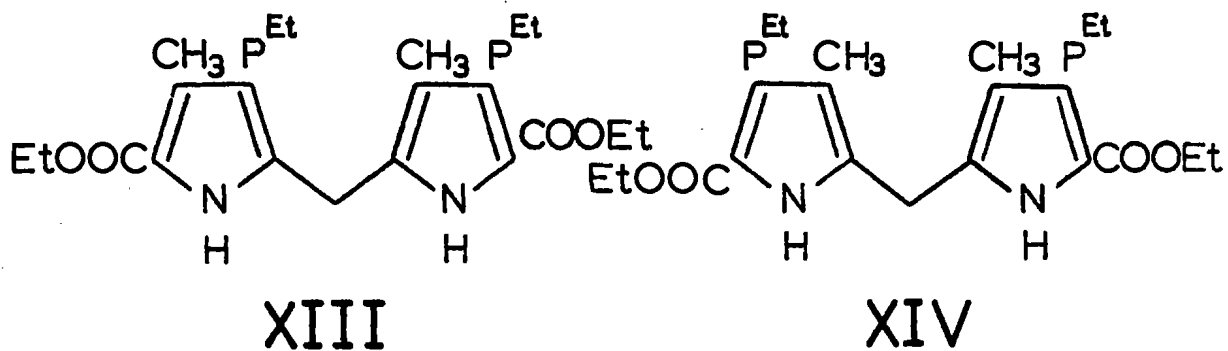
(d)  $R = \text{H}$ ,  $R' = \text{CH}_3$

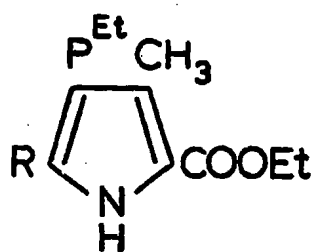
(e)  $R = \text{CHO}$ ,  $R' = \text{CH}_3$

(f)  $R = \text{CHO}$ ,  $R' = \text{H}$

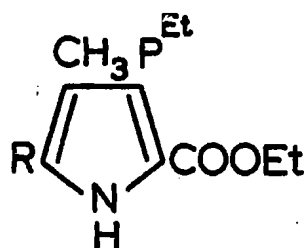
(g)  $R = \text{COOH}$ ,  $R' = \text{C}_2\text{H}_5$

(h)  $R = \text{CHO}$ ,  $R' = \text{C}_2\text{H}_5$

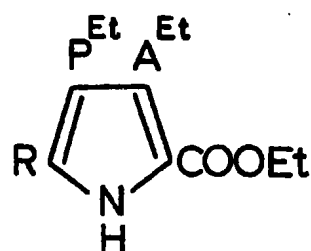




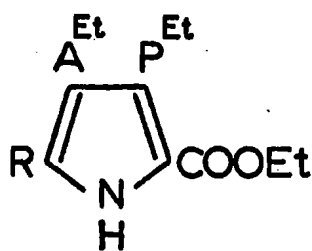
XVII



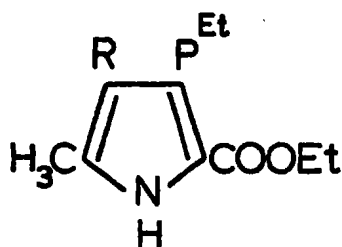
XVIII



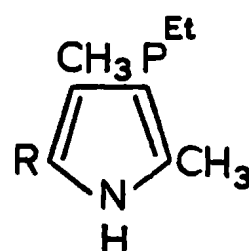
XIX



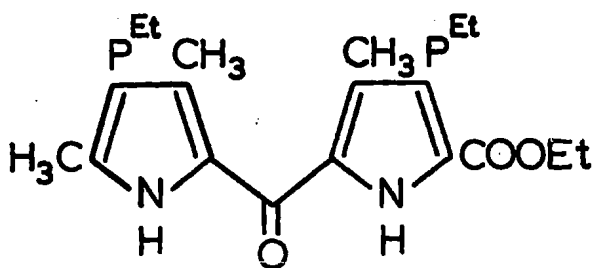
XX



XXI



XXII



XXIII

- (a) R = CH<sub>3</sub>  
 (b) R = CH<sub>2</sub>Br  
 (c) R = H  
 (d) R = COSEt

- (e) R = COOEt  
 (f) R = CH<sub>2</sub>OH  
 (g) R = CONHC<sub>6</sub>H<sub>5</sub>  
 (h) R = CHO

were also unsuccessful. However, using benzoyl chloride and N, N-dimethylformamide they reported a 56% yield of IX(h) from IX(g). An attempt to adapt their method to formylate IX(b) ended in failure. Apparently, it is necessary to have the acid functions in the  $\beta$ -positions protected by ester groups. This would mean, if the dipyrromethane IX(a) were used, selective hydrolysis of the 5,5'-esters. Although no attempt was made to find conditions for this partial hydrolysis, speculation would predict this not to be a useful pursuit. Hydrogenolysis of the 5,5'-benzyl esters would appear to be a much easier route (43).

#### B. Raney nickel reductions of pyrrole thiolesters

Chen (44) has discussed thiolesters as very useful intermediates in the syntheses of pyrroles having methyl and propionic acid or acetic acid and propionic acid functions in their  $\beta$ -positions. It has been shown that the pyrrole thiolester XXI(d) can be selectively reduced to the corresponding formyl, hydroxymethyl, methyl pyrroles (44, 45) and recently it has been shown that the thiolester residue can even be replaced by hydrogen (46). In previous experiments (44) reduction of XXI(d) with Raney nickel led to a mixture of XXI(a) and XXI(f) with the yield of XXI(a) being less than 50%. However, it has now been demonstrated that at higher temperature and pressure XXI(a) can be obtained as the sole product in fairly good yield.



It is also known that the pyrrole aldehyde can be reduced to the corresponding hydroxymethyl and methyl compounds (38,47) and XXI(f) has been converted to XXI(a) under conditions similar to the reduction of XXI(d) to XXI(a). Therefore, not only can the thiolester be reduced to any of the known intermediates but a sequential reduction of each intermediate to the next is also possible.

A progressive reduction of XXI(d) was carried out in the presence of W5 Raney nickel and the results are given in Table 4. The fact that over half of the methyl pyrrole XXI(a) had formed in the first half hour and that its rate of formation steadily decreased with time tends to suggest a duality of reaction path. It seems unlikely that this considerable decrease in reaction rate resulted from sulfur poisoning of the Raney nickel catalyst since there is such a large excess of catalyst present. Experimental evidence tends to suggest, in agreement with Chen's observations (44), that the methyl pyrrole XXI(a) can be formed either directly from the thiolester XXI(d) or from any of the other known intermediates.

#### C. Bromination of pyrrole thiolesters

In a search for easier methods of preparing dipyrromethanes with formyl groups in the 5,5'-positions, the idea of using pyrroles with thiolesters as intermediates seemed a useful pursuit. If a pyrrole such as XXII(d) could be converted to the corresponding

dipyrromethane XV according to the methods of Morsingh and MacDonald (38), subsequent reduction of the 5,5'-thiolester residues with Raney nickel should give the corresponding diformyldipyrromethane IX(h) in just two steps from the starting pyrrole.

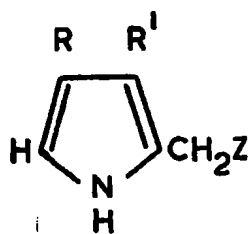
However, bromination of XXII(d) and the refluxing of this product in ethanol led to the formation of XVII(a). When bromination was carried out in the presence of excess bromine, a crystalline compound was isolated which was not identified but which appeared to be non-pyrrolic. When XXI(d) was treated with an equimolar amount of bromine and this compound was refluxed in ethanol, a compound was isolated with molecular weight (determined by mass spectrum) 325 and its proton magnetic resonance spectrum could only be interpreted as being that of XXI(e). This very interesting result demonstrates that the thiolester, rather than the methyl group, is undergoing bromination to give, most likely, the corresponding acyl bromide. The pyrrole XXII(d) was treated with a equimolar amount of bromine and, when this product was treated with aniline under anhydrous conditions, the corresponding anilide XXII(g) was formed in fairly good yield. The possibilities of this reaction seem quite promising. For instance, bromination of XXII(d) and treatment of this product with XVIII(c) could give the unsymmetrical dipyrroketone XXIII. The preparation of other pyrroles with thiolesters in their  $\alpha$ -positions

could lead to the syntheses of a whole new series of unsymmetrical dipyrroketones which would be useful intermediates in porphyrin syntheses.

D. The chemical polymerization of monopyrroles to porphyrins

Many pyrroles of the general form XXIV produce porphyrins when refluxed in acid or base solution. In general, if R and R' are alkyl groups and Z is a good leaving group, greater than 50% conversion to the porphyrin is observed. Several attempts have been made to rationalize the mechanism of cyclization. Some of these (26,48,49) may be ruled out because of later experimental evidence against them (20,50-52) while others have not been tested (25,53,54). Acid and base catalysed polymerizations apparently bring about different isomer compositions (26,32). The basic assumption in these mechanisms is that the Z group is dissociated from the pyrrole leaving a "benzyl-type" carbonium ion. Electrophilic attack on the pyrrole ring is well known (55). Therefore, mechanisms involving displacement of the ring hydrogen by the carbonium ion are very favourable.

In the pyrrole series, however, there is another complication. Substituents of the required type ( $-\text{CH}_2\text{Z}$ ) are known to be lost during the course of a reaction. When XVIII(b) is refluxed in ethanol the corresponding dipyrromethane XIV is produced. In fact, this is a standard method of preparing symmetrical dipyrromethanes (38). Therefore, in porphyrin syntheses



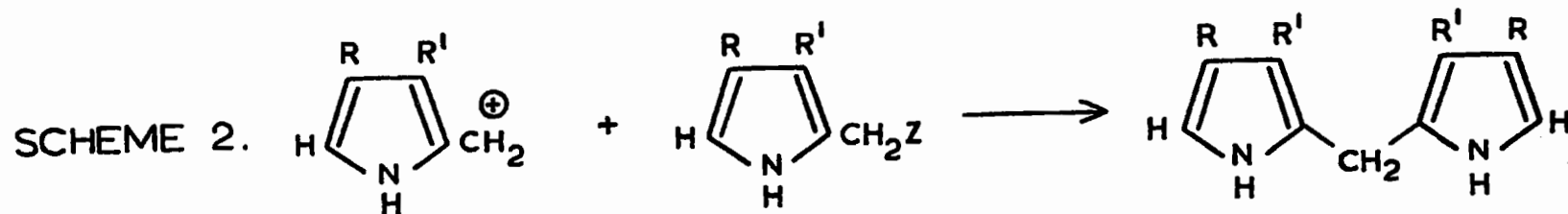
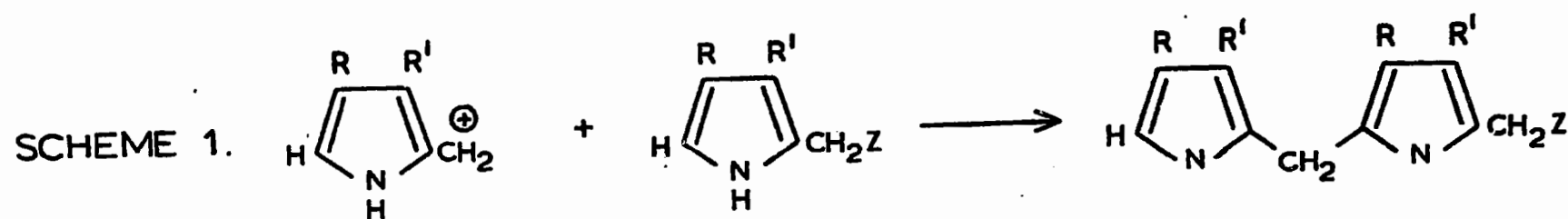
XXIV

- (a)  $R = R' = \text{CH}_3$ ,  $Z = \text{N}^+(\text{CH}_3)_3 \text{I}^-$
- (b)  $R = \text{CH}_2\text{CH}_2\text{COOCH}_3$ ,  $R' = \text{CH}_3$ ,  $Z = \text{N}^+(\text{CH}_3)_3 \text{I}^-$
- (c)  $R = \text{CH}_2\text{CH}_2\text{COOCH}_3$ ,  $R' = \text{CH}_3$ ,  $Z = \text{OCH}_3$

*in vitro* there are at least two possible ways of attack by one pyrrole unit on another (Fig. 1). If these reactions are in competition with each other, then polymerization of a pyrrole with R. different from R' (XXIV) will result in a mixture of type isomers. The problem is further complicated by the fact that different routes are also possible after this initial reaction. For example, if the dipyrromethane is formed as in Scheme I (Fig 1), further attack by another pyrrole unit could possibly be at the free position or, analogous to Scheme 2 (Fig. 1), at the methane bridge linkage with the elimination of one pyrrole from the bridge. In view of the poor analytical procedures available for the separation of aetio-, copro- and uro- porphyrins, many of the claims and counterclaims regarding this type of porphyrin formation must be treated with reserve. Any attempts to elucidate the mechanism by which the natural system produces a single isomer must take into consideration the possible influence of other factors such as stereochemistry. A really precise study of the uroporphyrins system should be made, provided methods of analysing the type isomers can be established.

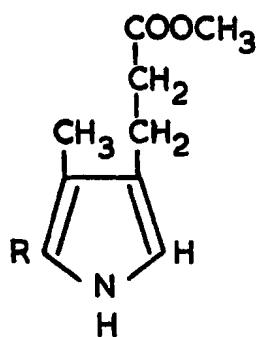
In the system studied here, trimethylaminomethylpyrrole salts were polymerized in organic solvents in the hope that displacement of the substituent would be minimized and a unique type isomer produced. It is known that, in hot acid (but not neutral) solution, considerable isomerization of the uroporphyrinogens occurs (31). Therefore, if one

FIG. 1



assumes that, in neutral solution, there is no isomerization of the porphyrinogens formed, then the production of a mixture of type isomers must be due to competition of the two reactions given in Fig. 1. In the case where the substituent ( $-\text{CH}_2\text{Z}$ ) in XXIV is uncharged, electrophilic attack would be relatively easy as in the syntheses of dipyrromethanes. However, with the Mannich base-methiodide salts there would probably be a considerable amount of dissociation of the salt in a polar solvent such as methanol. This would mean that the substituent would be positively charged, thus making electrophilic attack at this position rather difficult. Even without dissociation, displacement of the ring hydrogen should be much more facile.

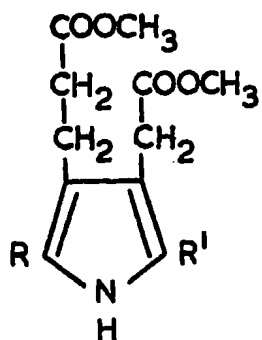
Octamethylporphin was prepared from XXIV(a) and, to study the mechanism, coproporphyrin was prepared from XXIV(b). Surprisingly, the latter was a complex mixture of the type isomers. The proton magnetic resonance spectra recorded in chloroform and deuterated trifluoroacetic acid solutions are shown in Fig. 2, 3 and 4. Interpretation suggests that the mixture consisted mostly of the III and IV isomers. According to Abraham et al (56) the proton magnetic resonance spectra of coproporphyrins I, II, III and IV in trifluoroacetic acid show a singlet, doublet, triplet and triplet resonance, respectively, for the *meso*-protons. The spectrum of the isomer mixture obtained, showed in deuterated trifluoroacetic acid (Fig. 4)



(a)  $R = H$

(b)  $R = CH_2N(CH_3)_2$

XXV



(a)  $R = R' = H$

(b)  $R = H \quad R' = CH_2N(CH_3)_2$

(c)  $R' = H \quad R = CH_2N(CH_3)_2$

XXVI



FIG. 2

COPROPORPHYRIN

0.2M (CDCl<sub>3</sub>)

100 MHz

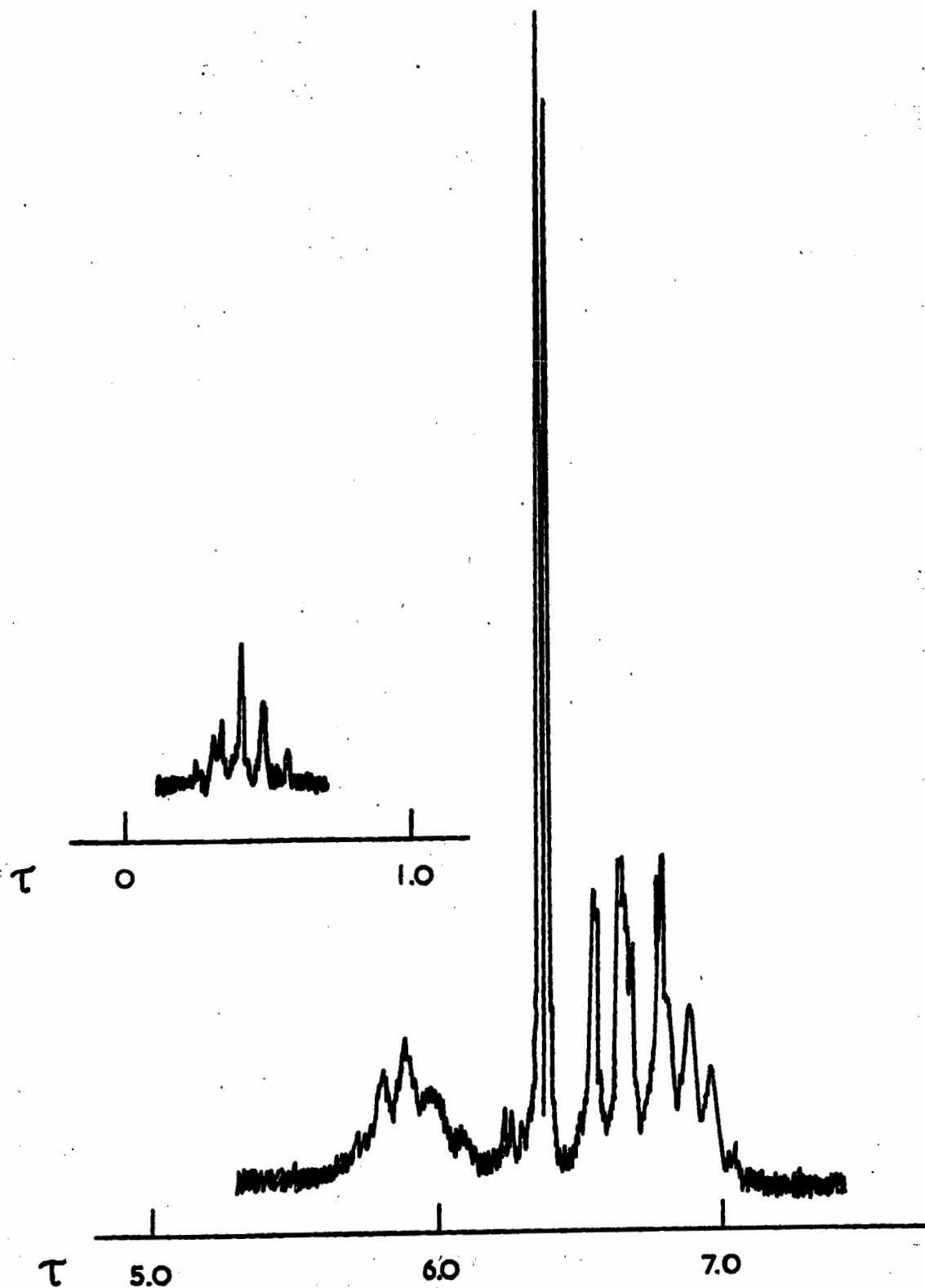


FIG. 3

COPROPORPHYRIN  
0.03M (CDCl<sub>3</sub>)  
100 MHz

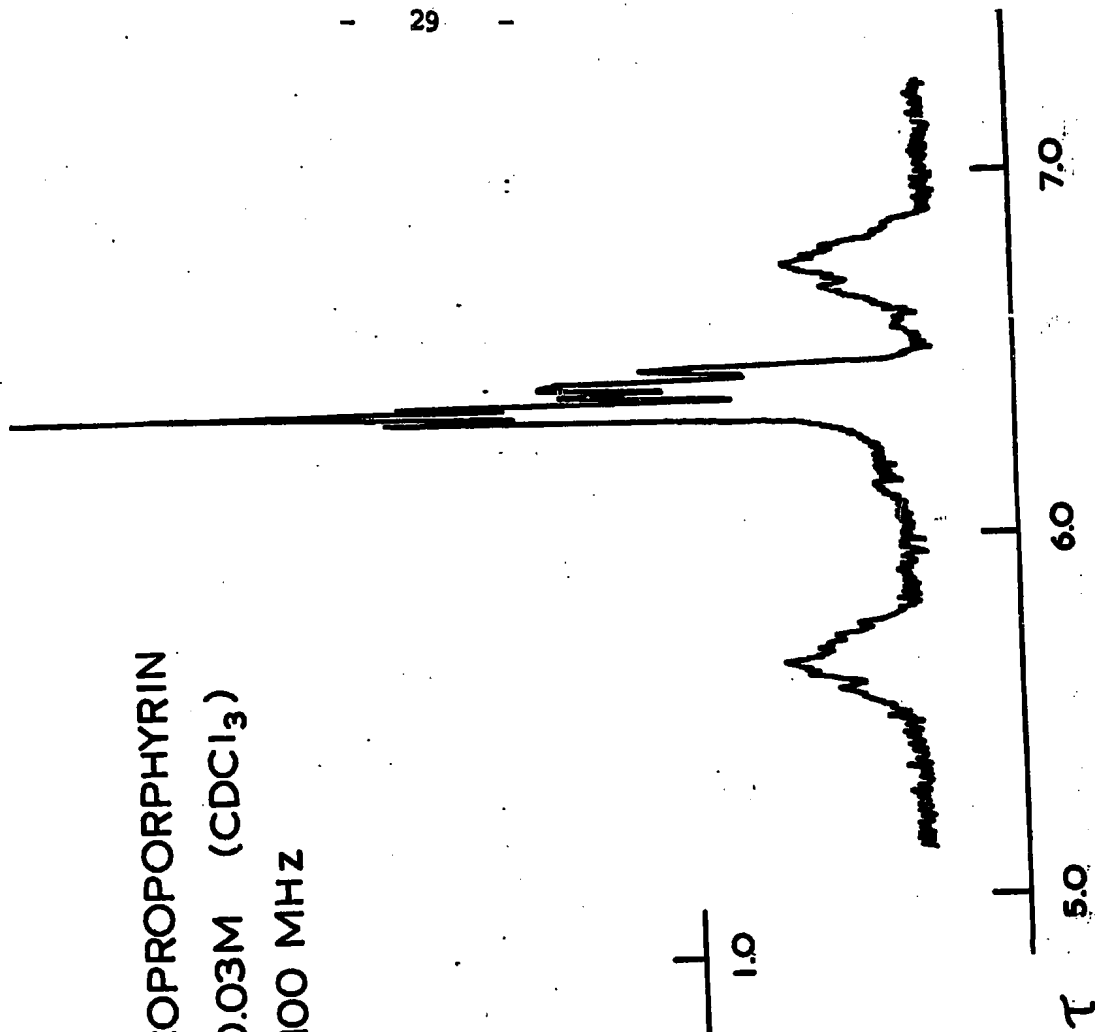
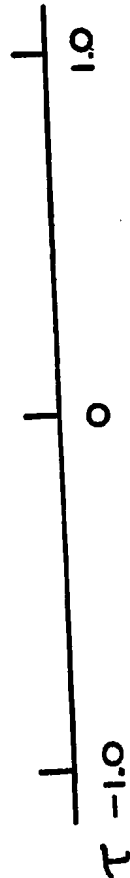
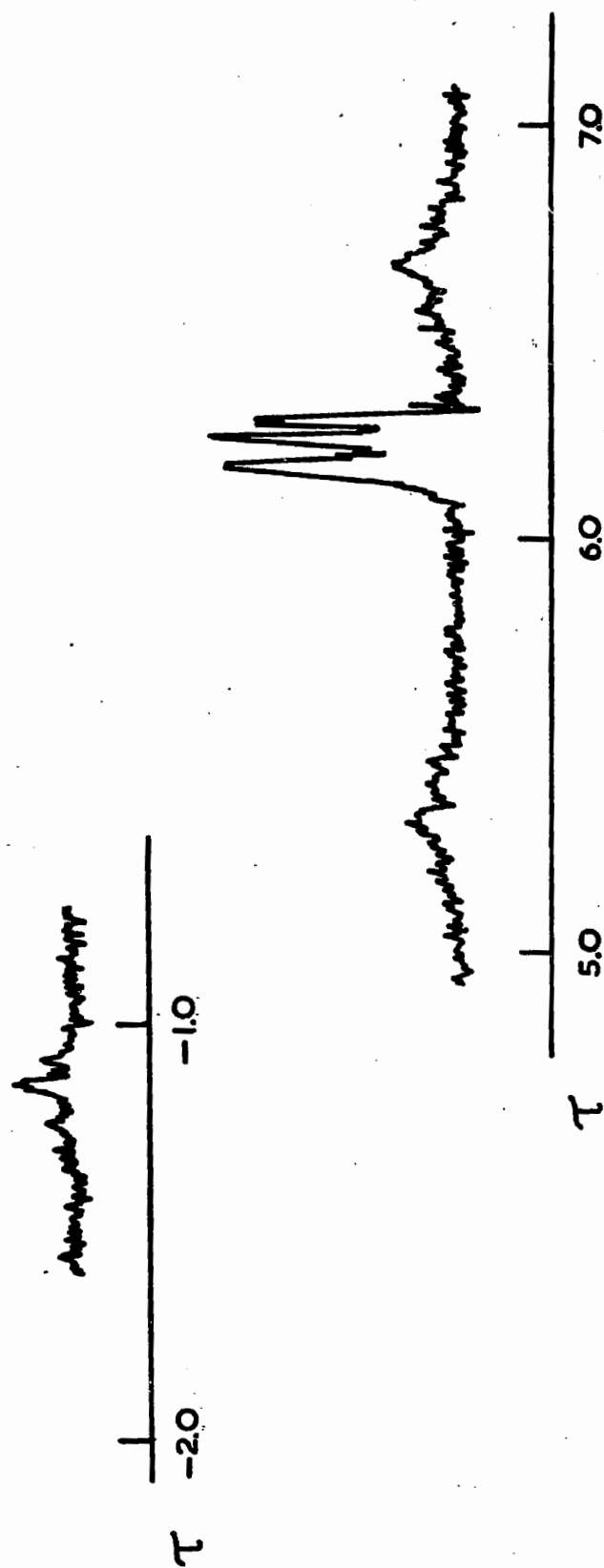


FIG. 4

COPROPORPHYRIN  
d-trifluoroacetic acid  
60 MHz



a very well defined triplet at  $\tau$  -1.25, -1.16, and -1.08. This is in good agreement with the results of Abraham et al for the positions of the triplets for the coproporphyrin III and IV isomers ( $\tau$  -1.24, -1.16, -1.08 and -1.23, -1.15, -1.09 respectively). The spectrum recorded in deuterated chloroform of a 0.2 M solution (Fig. 2) showed two very well defined singlets for the methoxyl groups, suggesting that there were two major isomers. Also, the *meso*-protons, although seven peaks were discernible, showed a very probable triplet which, according to Abraham et al, is indicative of the III isomer. The spectrum recorded of a 0.03 M deuterated chloroform solution (Fig. 3) showed the drastic change in spectrum with concentration. Because of this large dependance on concentration, proton chemical shifts of type isomer mixtures recorded in chloroform solution are of little use, since the individual concentrations are unknown and the effects of isomer interaction can not be predicted. Also, the closeness of the peaks in dilute solution makes interpretation of isomer composition difficult.

The conversion of the methiodide salt (XXIV(b)) to coproporphyrin by refluxing in methanol was very high but, clearly, displacement had occurred at both the substituted and unsubstituted positions. One problem that might be suspected is the possibility of the methiodide salt reacting with the solvent, eliminating trimethylamine and forming the corresponding pyrrole ether (XXIV (c)).

One would suspect attack at the substituted position would then be easier than if the methiodide salt were present. However, this type of displacement requires treatment with sodium methoxide in methanol (personal communication with Dr. E. Bullock) and these drastic conditions are surely not duplicated in this reaction. This hypothesis could be tested by carrying out the polymerization in a non-hydroxylic solvent.

It is interesting to note that the Mannich reaction on opsopyrrole monocarboxylic acid methyl ester (XXV(a)) gave only a single compound. This compound, although there are two possible structures, is most likely XXV(b), since, according to Fischer (57), treatment of opsopyrrole monocarboxylic acid with hydrocyanic acid and subsequent hydrolysis gave 3-methyl-4-propionic acid-2-aldehyde.

The Mannich base formed from opsopyrrole dicarboxylic acid dimethyl ester (XXVI(a)) was found to be a mixture of XXVI(b) and XXVI(c). The proton magnetic resonance spectrum showed two aromatic protons as well as two peaks corresponding to the dimethylamino groups. The ratio of these peaks was approximately 2:1 although it was not possible to identify which isomer was present in the larger amount. Separation can probably be done and polymerization of these methiodides separately may give further clues to the mechanism involved.

EXPERIMENTAL

Melting points (uncorrected) were determined on a Fisher - Johns melting point apparatus, unless otherwise stated. Infrared spectra were recorded by a Perkin - Elmer 237B grating spectrophotometer in chloroform solution. Nuclear magnetic resonance spectra were recorded on a Varian A-60 analytical spectrometer and a Varian HA-100 spectrometer and resonance positions were reported on the  $\tau$  scale, using tetramethylsilane as an internal reference. Ultraviolet spectra were recorded by a Perkin - Elmer 202 Ultraviolet spectrophotometer. Mass spectra were recorded by a Hitachi-Perkin-Elmer RMU-6E mass spectrometer. Vapour phase chromatography was done on a Varian Aerograph 1520.

The following compounds were prepared in bulk as starting materials for the experimental work. Given with the name of each compound is the number of the literature reference.

<u>Compound</u>	<u>Literature</u>
Ethyl 4-acetyl-5-oxohexanoate	25
Ethyl acetothiolacetate	44
Ethyl 5-carbethoxy-4-carbethoxy-methyl-2-methylpyrrole-3-thiolcarboxylate	44
Ethyl 4-carbethoxyethyl-5-carbethoxy-2-methylpyrrole-3-thiolcarboxylate	44
Ethyl 3-carbethoxyethyl-2,4-dimethylpyrrole-5-carboxylate	25

<u>Compound</u>	<u>Literature</u>
Ethyl 3-carbethoxymethyl-4-carbethoxyethyl-5-methylpyrrole-2-carboxylate	44

2,3-Dimethyl-4-carbethoxyethyl-5-carbethoxypyrrole (XVIII(a)).

(a) A solution of ethyl 4-carbethoxyethyl-5-carbethoxy-2-methylpyrrole-3-thiolcarboxylate (XXI(d)) (5.00 g) in ethanol (150 ml) was placed in an autoclave with W5 Raney Ni (15 g) under hydrogen. During 70 min the temperature and hydrogen pressure were increased from the initial conditions of 25° and 1100 psi to 110° and 1400 psi. After another 1.5 h, during which time the temperature and pressure were dropped to 90° and 1100 psi respectively, the heat was shut off, the pressure released to 200 psi and the autoclave left to cool to room temperature. After filtering off the Raney Ni, (by means of a "Celite" Filter), a colourless solution remained. Removal of the solvent *in vacuo* left a colourless product which crystallized from aqueous ethanol to give colourless plates (3.40 g, 87%), m.p. (capillary) 89.5 - 91.0° (lit. m.p. (38) 88 - 89°). In all other respects, the product proved to be identical to an authentic sample.

(b) A solution of 2-methyl-3-hydroxymethyl-4-carbethoxyethyl-5-carbethoxypyrrole (XXI(f)) (0.752 g) in ethanol (150 ml) was treated with W5 Raney Ni (15 g) as in (a) to yield 0.515 g (72%), m.p. (capillary) 89.5 - 91.0°.

otherwise identical to an authentic sample.

Progressive Raney Ni reduction of ethyl 2-methyl-4-carbethoxyethyl-5-carbethoxypyrrole-3-thiolcarboxylate (XXI(d)).

A solution of the thiolester XXI(d) (2.00 g) in absolute ethanol (100 ml) containing W5 Raney Ni (10 g) was placed in an autoclave under 55 psi of hydrogen at 70<sup>6</sup>. It was possible to follow the production of the pyrrole aldehyde (XXI(h)), the hydroxymethyl pyrrole (XXI(f)), and the methyl pyrrole (XXI(a)) by means of vapour phase chromatography. The conditions used and results obtained are given in Table 4. Retention times for XXI(a), XXI(d) and XXI(f) had been previously determined using authentic samples. The fourth peak in the V. P. C. was assumed to be XXI(h), although there was no authentic sample available for comparison. Using the given V. P. C. conditions, the retention times for the pyrroles XXI are given below.

<u>Functional Group (R)</u>	<u>Retention Time (min)</u>
-COSC <sub>2</sub> H <sub>5</sub>	5.8
-CHO	5.1
-CH <sub>2</sub> OH	1.7
-CH <sub>3</sub>	2.2

Ethyl 3-carbethoxyethyl-2,4-dimethylpyrrole-5-thiolcarboxylate (XXII(d))

A solution of sodium nitrite (12.7 g) in water



TABLE 4

Progressive Raney Ni reduction of Ethyl 2-methyl-4-carbethoxyethyl-5-carbethoxypyrrole-3-thiolcarboxylate as followed by vapour phase chromatography\*

Time (min)	%COSC <sub>2</sub> H <sub>5</sub>	%CHO	%CH <sub>2</sub> OH	%CH <sub>3</sub>
0	100			
30	8	23.5	21	47.5
60	4	5.5	15.5	75
90	8**		14	78
120	<5		12	86
150	trace		12	88
180	trace		10	90
660	trace		10	90

\*V.P.C. Conditions:

Column temperature	230°
Detector temperature	305°
Injector temperature	275°
Carrier gas	He
Flow rate	0.4 ml sec <sup>-1</sup>
Column	Apiezon L on firebrick
Sample volume	3μl

\*\* The retention times (see p.35) are so close that differentiation between the two peaks is difficult at low concentration.

(45 ml) was added to an ice-cooled, well-stirred solution of ethyl acetothiolacetate (25 g) in glacial acetic acid (70 ml) at such a rate that the temperature remained less than 14°. The mixture was stirred for 3 h at this temperature and then stirred overnight at room temperature. After adding ethyl 4-acetyl-5-oxohexanoate (34.4 g) to the solution, zinc dust (26.0 g) was added at a rate such that the temperature remained almost constant at 65°. Stirring continued for another 0.5 h. Following this, the product was heated on a steam bath for 1 h, then cooled and poured onto crushed ice. After 3 h, the product was collected and recrystallized from ethanol to give colourless prismatic needles (16.0 g, 33%) m.p. 72.0 - 73.0°.

Ultraviolet spectrum:  $\lambda_{\max}$  204, 248, and 315m $\mu$  (log  $\epsilon_{\max}$  3.80, 3.69 and 4.45) in 95% ethanol. Infrared spectrum:  $\nu_{\max}$  3435s (N-H), 3295w (broad, N-H bonded), 1725s (C=O, -CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Et), 1600s (C=O, COSEt), 1490m, 1450m, 1415s, 1375s, 1350w, 1335w, 1320w, 1295w, 1265m, 1130w, 1095w, 1060m, 1030w, 970w, 920s, 890w, 840-825w cm<sup>-1</sup> in chloroform.

N.M.R. spectrum (CDCl<sub>3</sub>):  $\tau$  0.61 (broad, NH), 5.87 (quartet, -OCH<sub>2</sub>), 6.93 (quartet, -SCH<sub>2</sub>), 7.43 (-CH<sub>2</sub>CH<sub>2</sub>CO A<sub>2</sub>B<sub>2</sub> multiplet), 7.67 (singlet, -CH<sub>3</sub>), 7.77 (singlet, -CH<sub>3</sub>), 8.68 (triplet, ester -CH<sub>3</sub>), 8.77 (triplet, ester -CH<sub>3</sub>). Anal. Calc'd.

for C<sub>14</sub>H<sub>21</sub>O<sub>3</sub>NS : C, 59.35; H, 7.47; N, 4.94; S 11.30.

Found: C, 59.48; H, 7.36; N, 4.92; S, 11.30.

3-Methyl-3'-carbethoxymethyl-4,4'-dicarbethoxyethyl-5,5'-dicarbethoxydipyrromethane (X(a)).

2-Carbethoxy-3-carbethoxyethyl-4-methylpyrrole (38) (1.55 g) and ethyl 2-bromomethyl-3-carbethoxymethyl-4-carbethoxyethyl-5-pyrrolicarboxylate (40) (2.56 g) were refluxed in benzene (70 ml distilled from sodium wire) for 1.5 h while protected from moisture. The benzene was removed *in vacuo* at 20° and the residue (dark red) dissolved in ethyl ether. After washing the ether layer, first with 1% sodium hydroxide solution (3 x 30 ml), then with water (2 x 50 ml), the ether layer was dried (MgSO<sub>4</sub>) and the ether removed *in vacuo*. This procedure produced a colourless solid which, when recrystallized from aqueous ethanol, yielded colourless feather-like needles (2.13 g, 55%) m.p. 146.0 - 147.0°. Ultraviolet spectrum:  $\lambda_{\max}$  272 and 284 m $\mu$  ( $\epsilon_{\max}$  4.36 and 4.42) in 95% ethanol. Infrared spectrum:  $\nu_{\max}$  3420w (N-H), 3310m (broad, N-H bonded), 1725s (broad, C=O, -CH<sub>2</sub>CH<sub>2</sub>COOEt and -CH<sub>2</sub>COOEt), 1690s (C=O, COOEt), 1575w, 1505m, 1475m, 1445s, 1405w, 1385w, 1370s, 1360m, 1300m, 1140m, 1095w, 1070m, 1020m, 935w, cm<sup>-1</sup> in chloroform. N.M.R. spectrum (CDCl<sub>3</sub>):  $\tau$  0.29 (broad, N-H), 0.69 (broad, N-H), 5.72 and 5.87 (overlapping quartets, OCH<sub>2</sub>) 6.10 (singlet, methane bridge -CH<sub>2</sub>-), 6.53 (singlet, CH<sub>2</sub>CO), 6.82-7.63 (A<sub>2</sub>B<sub>2</sub> multiplets, -CH<sub>2</sub>CH<sub>2</sub>CO), 7.39 (singlet, -CH<sub>3</sub>), 8.69, 8.75, 8.77 (overlapping triplets, ester CH<sub>3</sub>). Anal. Calc'd for C<sub>30</sub>H<sub>42</sub>O<sub>10</sub>N<sub>2</sub>: C, 61.00; H, 7.17; N, 4.74. Found: C, 60.86; H, 7.08; N, 4.73.

3-Carbethoxymethyl-4,3'-dicarbethoxyethyl-4-methyl-5,5'-dicarbethoxypyrromethane (XI(a)).

The pyrroles XVII(c) (38) (1.67 g) and XX(b) (40) (2.62 g) were refluxed in dry dichloromethane (40 ml) while protected from moisture. Ultraviolet spectra showed that the reaction had gone to completion in 3 h. The dichloromethane was removed *in vacuo* at 20° and the residue dissolved in ether. The ether layer was washed with 1% sodium hydroxide solution (20 ml) and then with water (2 x 50 ml). Drying of the ether layer (MgSO<sub>4</sub>) and removal of the ether *in vacuo* left a colourless residue. The product crystallized from ethanol to give colourless needles (3.00 g, 79%) m.p. 127-128°. Ultraviolet spectrum:  $\lambda_{\max}$  274 and 287m $\mu$  (log  $\epsilon_{\max}$  4.46 and 4.54) in 95% ethanol. Infrared spectrum:  $\nu_{\max}$  3420m (N-H), 3325s (broad, N-H bonded), 1720s (C=O, -CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Et and -CH<sub>2</sub>CO<sub>2</sub>Et), 1685s (C=O, CO<sub>2</sub>Et), 1575w, 1508m, 1475m, 1460m, 1445s, 1370s, 1300m, 1090m, 1080s, 1025m, 940w, 885w, 860w cm<sup>-1</sup> in chloroform. N.M.R. spectrum (CDCl<sub>3</sub>):  $\tau$  0.08 (broad, N-H), 0.15 (broad, N-H), 5.78, 5.83, 5.89, (overlapping quartets, -OCH<sub>2</sub>), 6.09 (singlet, methane bridge -CH<sub>2</sub>-), 6.47 (singlet, -CH<sub>2</sub>CO), 6.81-7.02 (A<sub>2</sub>B<sub>2</sub> multiplets, -CH<sub>2</sub>CH<sub>2</sub>CO), 7.73 (singlet, -CH<sub>3</sub>), 8.70, 8.78, 8.85 (overlapping triplets, ester CH<sub>3</sub>). Anal. Calc'd for C<sub>30</sub>H<sub>42</sub>O<sub>10</sub>N<sub>2</sub>: C, 61.00; H, 7.17; N, 4.74. Found: C, 61.09; H, 7.32; N, 4.76.

3,4'-Dicarbethoxyethyl-4-carbethoxymethyl-3'-methyl-5,5'-dicarbethoxydipyrromethane (XII(a)).

The pyrroles XVII(b) (38) (1.39 g) and XIX(c) (40) (1.30 g) were refluxed in dry dichloromethane (20 ml) for 1.5 h. Since ultraviolet spectra showed the reaction had gone to completion, the dichloromethane was removed *in vacuo* at 20° and the residue (dark red) dissolved in ether. The ether layer was washed with 1% sodium hydroxide solution (10 ml) and then with water (2 x 10 ml). The ether layer was dried (MgSO<sub>4</sub>) and the ether removed *in vacuo*. The orange coloured oil was then passed through a column of Sephadex LH20 (Pharmacia Ltd.). The Sephadex LH20 (40 g) was stirred in methanol for 24 h (by means of a magnetic stirrer). It was then packed into the column and eluted with methanol for 24 h prior to use. The appropriate fractions from the column (determined by ultraviolet spectra) were combined, the methanol removed and the oily residue recrystallized four times from aqueous ethanol to yield slightly pink needles, m.p. 39.0 - 40.5°. After passing through a neutral alumina column (2 x 30 cm) and eluting with chloroform, the product was recrystallized twice more from aqueous ethanol to yield colourless needles, (1.5. g, 65%) m.p. 45.5 - 47.5°. When the product was dried *in vacuo*, it turned to an oil and darkened. Recrystallization of this oil from aqueous ethanol would yield colourless crystals again. In view of this result, spectra and m.p. were determined on a sample which

had dried in air for 4 days. Analyses were done on both an air-dried sample and a sample that had been dried under vacuum. Ultraviolet spectrum:  $\lambda_{\max}$  275 and 292m $\mu$  ( $\log \epsilon_{\max}$  4.34 and 4.45) in 95% ethanol. Infrared spectrum:  $\nu_{\max}$  3425m (N-H), 3330w (broad, N-H, bonded), 1725s (C=O, -CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Et and -CH<sub>2</sub>CO<sub>2</sub>Et), 1685s (C=O, CO<sub>2</sub>Et), 1600w, 1580w, 1500w, 1475m, 1465m, 1445s, 1390w, 1370s, 1330m, 1300s, 1140m, 1095m, 1075w, 1050w, 1020w, 960 - 940w, 860w cm<sup>-1</sup> in chloroform. N.M.R. spectrum (CDCl<sub>3</sub>)  $\tau$  0.20 (singlet, broad, N-H), 5.75, 5.87 (overlapping quartets, -OCH<sub>2</sub>), 6.05 (singlet, methane bridge -CH<sub>2</sub>-), 6.23 (singlet, -CH<sub>2</sub>CO), 6.85 - 7.80 (A<sub>2</sub>B<sub>2</sub> multiplets, -CH<sub>2</sub>CH<sub>2</sub>CO), 8.00 (singlet, -CH<sub>3</sub>), 8.72, 8.75, 8.77 (overlapping triplets, ester -CH<sub>3</sub>). Anal Calc'd for C<sub>30</sub>H<sub>42</sub>O<sub>10</sub>N<sub>2</sub>: C, 61.00; H, 7.17; N, 4.74. Found (air-dried:) C, 57.91; H, 7.54; N, 4.51. Found (vacuum-dried): C, 60.84; H, 7.28; % drying loss, 4.29.

#### Octamethylporphin

A solution of 3,4-dimethylpyrrole (58) (5.80 g), dimethylamine hydrochloride (5.20 g) and sodium acetate (9.40 g) in methanol (25 ml) was stirred by a magnetic stirrer and cooled to 0° in an ice salt bath. 40% aqueous formaldehyde solution (5.00 g) in water (25 ml) was added dropwise to the solution with the temperature being maintained between 0° and 5° during addition (20 min). The mixture was then stirred for a further 2 h at this temperature. The

alkaline solution was extracted with ether (4 x 50 ml), the ether layer then washed with water (40 ml) and dried ( $K_2CO_3$ ). After adding a two-fold excess of methyl iodide to the filtered ether solution, the precipitated methiodide salt XXIV(a) was filtered. A solution of this salt in methanol (50 ml) was refluxed for 4 h and then left at  $10^\circ$  overnight. A filtering of the solution yielded octamethylporphin (1.20 g) which was purified by hot extraction, using o-dichlorobenzene. Measurement of accurate  $\log \epsilon_{\max}$  values proved difficult, since the product is very insoluble. Consequently, the visible spectrum was measured by using a saturated o-dichlorobenzene solution. Visible spectrum (o-dichlorobenzene):  $\lambda_{\max}$  406 (Soret), 503, 537, 572 and 628 in approximately the same ratios as recorded by Eisner et al (59).

2-N,N-Dimethylaminomethyl-4-carbomethoxyethyl-3-methylpyrrole (XXV(b)).

A solution of 2-carbomethoxyethyl-3-methylpyrrole (XXV(a)) (2.50 g) in methanol (15 ml) was placed in a three-necked flask equipped with a magnetic stirrer, dropping funnel and nitrogen inlet. The solution was then cooled to  $-15^\circ$  under nitrogen in a "dry-ice" - acetone bath. A solution of dimethylamine hydrochloride (1.25 g), potassium acetate (1.50 g) and 40% aqueous formaldehyde (1.25 g) in water (6 ml) was added dropwise with the pot temperature being maintained between  $-15^\circ$  and  $-10^\circ$  during addition.

The solution was then stirred at  $-10^{\circ}$  under nitrogen for another 2 h. Cold 5% hydrochloric acid (18 ml) was added slowly with stirring and the cold solution extracted with ether. The aqueous layer was then made basic by the slow addition of 2N sodium hydroxide while stirring and cooling in an ice bath. The solution was immediately extracted with ether and the ether layer immediately washed with water. The ether layer was dried ( $\text{MgSO}_4$ ) and the ether removed *in vacuo* to yield 1.76 g (55%) of an oil which was shown by N.M.R. to be substantially pure product. Treatment with Ehrlich reagent gave a deep red colour in the cold. N.M.R. spectrum ( $\text{CDCl}_3$ ):  $\tau$  0.80 (broad, N-H), 3.57 (doublet in  $\text{CDCl}_3$  and singlet in  $\text{CDCl}_3 + \text{D}_2\text{O}$ , aromatic H), 6.35 (singlet,  $-\text{OCH}_3$ ), 6.65 (singlet,  $-\text{CH}_2\text{N}$ ),  $\sim 7.35$  ( $\text{A}_2\text{B}_2$  multiplet,  $-\text{CH}_2\text{CH}_2\text{CO}$ ), 7.80 (singlet,  $\text{N}(\text{CH}_3)_2$ ), 8.03 (singlet,  $-\text{CH}_3$ ).

2-N,N-Dimethylaminomethyl-4-carbomethoxymethyl-3-methyl-pyrrole methiodide salt (XXIV(b)).

To the Mannich base XXV(b) (0.820 g) dissolved in absolute ether (50 ml) was added a two-fold excess of methyl iodide. After 3 h the precipitated methiodide salt was collected and dried in a vacuum desiccator for 3 h to yield 0.910 g (70%). Anal Calc'd. for  $\text{C}_{13}\text{H}_{23}\text{N}_2\text{O}_2\text{I}$ : C, 42.63; H, 6.33; N, 7.65; I, 34.65. Found: C, 42.69; H, 6.19; N, 7.53; I, 35.07.



Coproporphyrin tetramethyl ester

The methiodide salt XXIV(b) (0.910 g) was refluxed in methanol (45 ml) for 4h and, after cooling, was aerated for a further 2 h. Removal of the methanol

at 30° gave a solid residue which was passed through a column (Alumina, Spence type H) (2 x 30 cm) eluting with chloroform. Removal of the chloroform from the appropriate fractions (determined by ultraviolet spectra) and recrystallization from chloroform-methanol gave coproporphyrin (0.298 g, 67%) m.p. 134-319°. The N.M.R. spectra, as determined in  $\text{CDCl}_3$  (100  $\text{MH}_2$ ) and trifluoroacetic acid-d (60  $\text{MH}_2$ ), are given in figures 2,3 and 4. Visible spectrum ( $\text{CHCl}_3$ ):  $\lambda_{\text{max}}$  402 (soret), 501, 534, 570 and 620 m $\mu$  (log  $\epsilon_{\text{max}}$  5.19, 4.14, 3.99, 3.82 and 3.70 respectively).

2-N,N-Dimethylaminomethyl-3-carbomethoxymethyl-4-carbomethoxyethylpyrrole (XXVI(b)) and 2-N,N-Dimethylaminomethyl-3-carbomethoxyethyl-4-carbomethoxymethylpyrrole (XXVI(c)).

A solution of opsopyrrole dicarboxylic acid dimethyl ester (XXVI(a)) (0.260 g) in methanol (1.5 ml) was placed in a 10 ml three-necked round-bottom flask fitted with a dropping funnel, a nitrogen inlet tube and a magnetic stirrer. The solution was cooled to -15° under nitrogen in an ice-salt bath and a solution of dimethylamine hydrochloride (0.096 g), potassium acetate (0.115 g)

and 40% aqueous formaldehyde (0.096 g) in water (0.5 ml) was added dropwise, keeping the pot temperature between  $-15^{\circ}$  and  $-10^{\circ}$  during addition. The solution was stirred at  $-5^{\circ}$  under nitrogen for a further 0.75 h and then kept at  $0^{\circ}$  for 22 h. Cold 5% hydrochloric acid (1.4 ml) was added with stirring and the cold solution extracted with ether. The aqueous layer was made basic by the slow addition of 2N sodium hydroxide (1.70 ml) while stirring and cooling in an ice-salt bath. The basic solution was immediately extracted with ether (3 x 5 ml) and the ether layer immediately washed with water. The ether layer was dried ( $\text{MgSO}_4$ ) and the ether removed *in vacuo* at  $20^{\circ}$  to yield 0.088 g of an oil which was shown by N.M.R. to be a mixture of the two products in an approximate 2:1 ratio. Ehrlich reaction gave a deep red colour in the cold.

Attempts to separate the isomers failed because of the small amount of material available. The products showed possibilities of separation with thin-layer chromatography (silica gel G F254 on 5 x 20 cm glass plates). However, the  $R_f$  values were very low even with polar solvents.

Preparation of Ethyl 2,4-dimethyl-3-carbethoxyethyl-5-pyrrolicarboxylate (XVII(a)) from Ethyl 2,4-dimethyl-3-carbethoxyethyl-5-pyrroliothiolcarboxylate (XXII(d)).

The pyrrole thiolester XXII(d) (0.500 g) was

dissolved in carbon tetrachloride (20 ml) and treated dropwise under anhydrous conditions with a solution of bromine (0.300 g) in carbon tetrachloride (1.5 ml) at room temperature. After being stirred at room temperature for 0.75 h, the carbon tetrachloride was removed *in vacuo* at 20° and the residue refluxed in ethanol (30 ml) for 0.75 h. Removal of the ethanol *in vacuo* and recrystallization of the product from aqueous ethanol gave colourless needles (0.265 g, 56%). The product was identical with an authentic sample.

3-Carbethoxyethyl-2,4-dimethylpyrrole-5-carbanilide(XXII(g)).

The thiolester XXII(d) (0.500 g) was treated with bromine (0.300 g) as above. After being stirred for a further 0.75 h, the solution was concentrated *in vacuo* to half its original volume. To this solution was added a solution of aniline (0.206 g) in carbon tetrachloride (10 ml) followed immediately by trimethylamine (0.100 g). The solution was stirred at room temperature for 0.5 h and the carbon tetrachloride removed *in vacuo* at 20°. Ether (20 ml) was added to the residue and the solution filtered. The ether layer was washed consecutively with water (20 ml), 1N hydrochloric acid (10 ml), water (10 ml), 10% sodium bicarbonate solution (10 ml) and water (20 ml). The ether layer was dried (MgSO<sub>4</sub>) and the ether removed *in vacuo*.

The dark brown residue was passed through a silica gel column ( 2 x 20 cm) eluting with (9:1) benzene-acetone. Removal of the solvent from the first 250 ml of eluate gave the product (0.329 g, 71%) as an oil which was shown by its N.M.R. spectrum to be the desired product. Attempts to crystallize the compound were unsuccessful and lack of time prevented further work on it. N.M.R. spectrum ( $\text{CDCl}_3$ ):  $\tau$  0.00 (broad, N-H of pyrrole ring), 2.35 - 2.97 (multiplet, aromatic H and CONH), 5.88 (quartet,  $-\text{COCH}_2$ ), 7.45 (approximate center of  $A_2B_2$  multiplet,  $-\text{CH}_2\text{CH}_2\text{CO}$ ), 7.65 (singlet,  $-\text{CH}_3$ ), 7.87 (singlet,  $-\text{CH}_3$ ), 8.78 (triplet, ester  $-\text{CH}_3$ ).

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Addendum:

Explanatory notes, following referees comments.

1. The words 'porphin' and 'porphyrin' have been used interchangeably as a generic name.
2. The use of Roman numerals for structural formulae throughout this thesis has led to some confusion with respect to the names of porphyrin derivatives. When a type isomer is defined, e.g. coproporphyrin III, this is to be interpreted as the H. Fischer definition of the substitution pattern.









