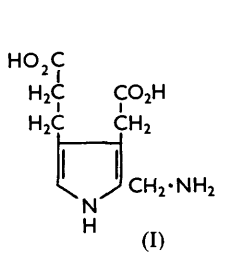
287. *A Synthesis of Coproporphyrin III.*

By E. Bullock, A. W. Johnson, E. Markham, and K. B. Shaw.

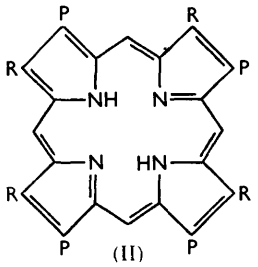
Coproporphyrin III tetraethyl ester has been obtained by the action of glacial acetic acid on 5-acetoxymethyl-4-2′-ethoxycarbonylethyl-3-methylpyrrole-2-carboxylic acid, followed by aerial oxidation. A mechanism is suggested for the polymerisations of 2-(α-substituted methyl)pyrroles to III-type porphyrins, which includes the biological polymerisations of porphobilinogen. A modified Knorr synthesis is employed for the preparation of certain of the pyrrole intermediates.

Biogenetic studies in the porphyrin series have established[1] that the immediate precursor of the porphyrin ring is porphobilinogen (I), itself obtained by the self-condensation of δ-aminolævulic acid. Porphobilinogen, which is the biological precursor of both hæmin and chlorophyll, can be converted into uroporphyrin III (II; R = $CH_2{\cdot}CO_2H$; P = $CH_2{\cdot}CH_2{\cdot}CO_2H$) both *in vitro* and *in vivo*.[2] In considering the precise mechanism of this polymerisation, we have now shown that coproporphyrin III (II; R = Me) may also be synthesised by the polymerisation of a suitably substituted pyrrole, formally related to porphobilinogen.

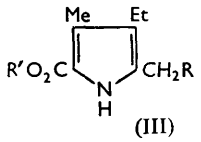
A relatively mild synthesis of porphyrins *in vitro* was described in 1943 by Siedel and Winkler[3] who claimed that 4-ethyl-5-hydroxymethyl-3-methylpyrrole-2-carboxylic acid (III; R = OH, R′ = H) gave a mixture of ætioporphyrins I and II when heated with



(I)



(II)



(III)

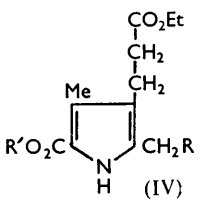
dilute hydrochloric acid. The 5-hydroxymethyl group was obtained by elaboration of the corresponding 5-methyl compound; it was claimed that the action of lead tetra-acetate on the ethyl ester gave the 5-hydroxymethyl derivative (III; R = OH, R′ = Et), m. p. 126—128°, which was acetylated to the 5-acetoxymethyl compound (III; R = OAc, R′ = Et), m. p. 135—136°, and hydrolysed with alcoholic potassium hydroxide to the corresponding acid (III; R = OH, R′ = H). In common with Professor G. W. Kenner (personal communication) we have observed that the product from the lead tetra-acetate

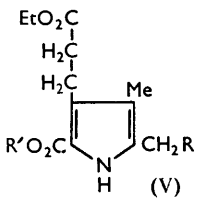
[1] Shemin and Russell, *J. Amer. Chem. Soc.*, 1953, **75**, 4873; Cookson and Rimington, *Nature*, 1953, **171**, 875; *Biochem. J.*, 1954, **57**, 476; Westall, *Nature*, 1952, **170**, 614.

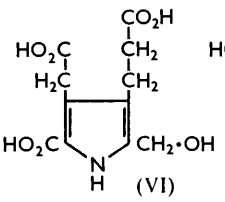
[2] Dresel and Falk, *Biochem. J.*, 1956, **63**, 80.

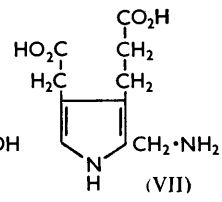
reaction is in fact the 5-acetoxymethyl compound (III; R = OAc, R′ = Et) as would be expected. It is unchanged by acetic anhydride but is readily converted into the 5-ethoxymethyl analogue (III; R = OEt, R′ = Et) when heated in ethanol for a short time. It is thus most unlikely that Siedel and Winkler's starting product had the structure claimed for it. The German authors also carried out similar reactions in the coproporphyrin series, but their product was not purified although it was thought to contain coproporphyrin I; however, by analogy with the experiments in the ætioporphyrin series it is probable that once again their starting material did not have the structure (IV; R = OH, R′ = H) claimed for it.

As a reliable method for distinguishing the ætioporphyrin isomers is lacking, we selected the coproporphyrin series, as the simplest in which the various isomers can be recognised,

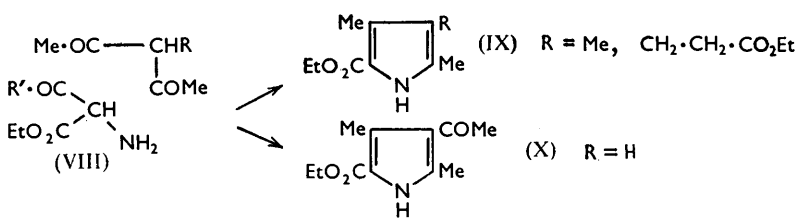

(IV)


(V)


(VI)


(VII)

in order to define the experimental conditions necessary to bring about the apparent "reversal" of ring D leading to the formation of III-type porphyrins. The requisite pyrrole intermediates for coproporphyrin were (IV; R = OAc, R′ = CH_2Ph) and (V; R = OAc, R′ = CH_2Ph), the ester groups being used to facilitate the synthesis of the intermediates and to prevent premature porphyrin formation. Moreover, the benzyl (or *tert.*-butyl) esters were preferred as conversion into the corresponding acids avoided the application of extremes of pH. There is evidence in the literature to suggest that the position of the α-acetoxymethyl group (or its equivalent) relative to the β-substituents is immaterial [as in (IV) and (V)] in this method of synthesis of III-type porphyrins; thus Treibs and


(VIII) (IX) R = Me, $CH_2 \cdot CH_2 \cdot CO_2Et$ (X) R = H

Ott [4] have prepared (although experimental details are still lacking) uroporphyrin III (since stated to be impure [5]) from the acid (VI) which is equivalent to *iso*porphobilinogen [6] (VII); and Fischer, Sturm, and Friedrich [7] have obtained coproporphyrin III (and I) from the ester (IV; R = Br, R′ = Et) by the action of hydrogen bromide under vigorous conditions.

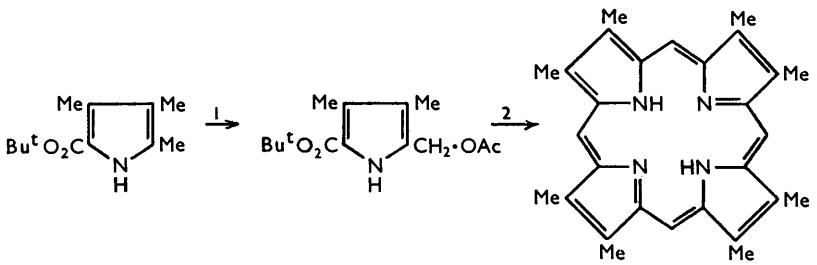
The pyrrole (IV; R = OAc, R′ = CH_2Ph) was prepared by the action of lead tetra-acetate on the ester (IV; R = H, R′ = CH_2Ph), itself obtained by a variation related to those of Kleinspehn [8] and Fischer and Fink [9] of the well-known Knorr synthesis. Condensation of α-aminoacetoacetic esters (VIII; R′ = Me) with 3-alkylpentane-2 : 4-diones is now

[3] Siedel and Winkler, *Annalen*, 1943, **554**, 165.
[4] Treibs and Ott, *Naturwiss.*, 1953, **40**, 476.
[5] MacDonald, quoted by Rimington, *Ann. Rev. Biochem.*, 1957, **26**, 562.
[6] Prasad and Raper, *Nature*, 1955, **175**, 629.
[7] Fischer, Sturm, and Friedrich, *Annalen*, 1928, **461**, 244.
[8] Kleinspehn, *J. Amer. Chem. Soc.*, 1955, **77**, 1546.
[9] Fischer and Fink, *Z. physiol. Chem.*, 1944, **280**, 123; 1948, **283**, 152.

shown to yield pyrroles of type (IX), although acetylacetone itself gives (X) by a normal Knorr condensation.

The presence of the 3-alkyl substituent in pentane-2 : 4-dione decreases the ease of carbanion formation at $C_{(3)}$ and thereby completely changes the course of the condensation. Pyrroles of type (IX), which are useful intermediates for porphyrin syntheses, may thus be obtained without difficulty. In establishing the course of the above condensation, diethyl 2-amino-3-oxoadipate (VIII; R′ = CH_2·CH_2·CO_2Et) was substituted for ethyl α-aminoacetoacetate, in the condensation with 3-methylpentane-2 : 4-dione: the compound (IX; R = Me) was obtained, the succinoyl residue having been eliminated from the amino-ester. Similarly, condensation of diethyl 2-amino-3-oxoglutarate (VIII; R′ = CH_2·CO_2Et) with ethyl 4-acetyl-5-oxohexanoate gave the same pyrrole (IX; R = CH_2·CH_2·CO_2Et) ≡ (IV; R = H, R′ = Et) as was obtained from ethyl α-aminoacetoacetate. When the 3- and the 5-substituent in the pyrroles (IX) are not identical an unsymmetrical β-diketone is required and the possible modes of condensation are appreciably increased.

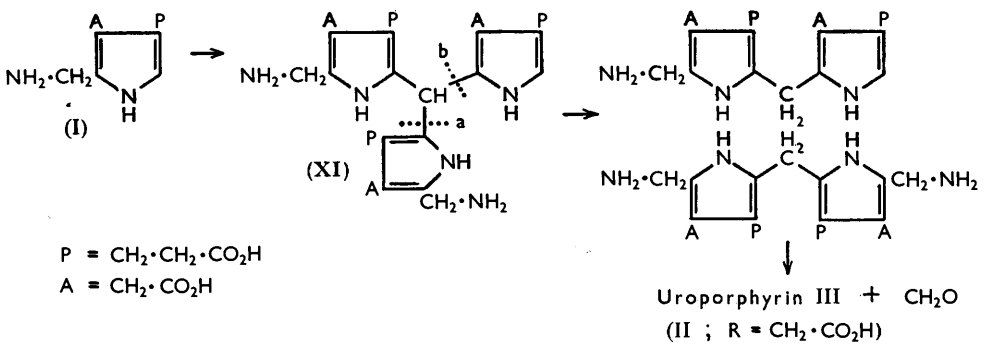
As an example of the simplified synthesis of porphyrins, *tert.*-butyl 3 : 4 : 5-trimethylpyrrole-2-carboxylate (obtained by condensation of *tert.*-butyl acetoacetate and 3-methylpentane-2 : 4-dione) was treated with lead tetra-acetate, to give the 5-acetoxymethyl



Reagents: 1, $Pb(OAc)_4$; 2, Heat.

derivative which, when heated in ethylene glycol, gave octamethylporphin in 22% yield.

For the preparation of coproporphyrin, the pyrrole (IV; R = H, R′ = CH_2Ph) was obtained by condensation of ethyl 4-acetyl-5-oxohexanoate (prepared by Michael addition of acetylacetone to ethyl acrylate) and benzyl α-aminoacetoacetate, and the corresponding acetoxymethyl compound (IV; R = OAc, R′ = CH_2Ph) was formed by the action of lead

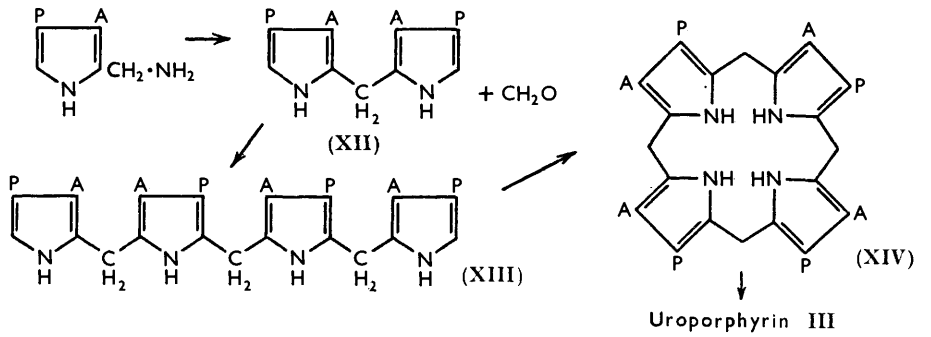


tetra-acetate. Hydrogenolysis of this ester gave the acid (IV; R = OAc, R′ = H) which, when heated under reflux with acetic acid, was rapidly transformed into a coloured condensation product from which crystalline coproporphyrin III tetraethyl ester,[10] m. p. 147—149°, was isolated. The identification of the porphyrin was confirmed by its ultraviolet

[10] Fischer and Andersag, *Annalen*, 1927, **458**, 133.

and visible absorption spectrum and by the behaviour of the corresponding free acid on chromatography.[11] Smaller yields of coproporphyrin III tetraethyl ester were obtained by heating the acetate (IV; R = OAc, R′ = H) in ethanol with dilute hydrochloric acid or by keeping it in presence of either dilute hydrochloric acid or dilute ammonia at room temperature for several days. No evidence based on paper chromatography was obtained for more than traces of the other isomers of coproporphyrin in the products obtained from any of these reactions although it is possible that a certain amount of fractionation occurred during the purification. In view of this ambiguity and the difficulty we have experienced in obtaining reproducible paper-chromatographic results even in the presence of authentic markers, further experiments are in progress.

The almost exclusive formation of coproporphyrin III in this manner is thus in line with the biogenetic formation, from porphobilinogen (I), of III-type porphyrins as well as chlorophyll,[12] and vitamin B_{12},[13] and the basic mechanism of all of these reactions, *in vitro* as well as *in vivo*, is almost certainly the same. The decarboxylation of the pyrroles (IV; R = OAc, R′ = H) and (VI) during their self-condensation occurs quite readily and requires no fundamental modification of the mechanism. Various theories [14] have been proposed to account for the wide occurrence of the III-type porphyrins in Nature; the main experimental observations to be incorporated in any such theory being the elegant work, culminating in the discovery of porphobilinogen, of Shemin and his collaborators [15] on the biosynthesis of hæmin. Shemin's theory of the mode of self-condensation of porphobilinogen is based on the synthetic studies of Corwin *et al.*,[16] and postulates an intermediate tripyrromethane (XI) which, it is argued, could break in each



of the two modes (a and b) to give two dipyrromethanes which then combine to give the porphyrin with elimination of formaldehyde.

Several objections can be raised to such a scheme: (i) Pyrroles of the porphobilinogen type have never been found to yield a tripyrromethane by condensation. (ii) The fission of tripyrromethane yields dipyrromethenes,[17] not dipyrromethanes, and usually only one isomer; the condensation of dipyrromethenes to porphyrins requires comparatively vigorous chemical conditions. (iii) There is evidence [18] to suggest that all Ehrlich-reactive

[11] Dresel and Falk, *Biochem. J.*, 1956, **63**, 87. We are extremely grateful to Professor C. Rimington, F.R.S., and to Mrs. A. Latter for confirming this result on our synthetic sample.

[12] Granick, Ciba Foundation Symposium, " Porphyrin Biosynthesis and Metabolism," London, 1955, p. 143.

[13] Shemin, Corcoran, Rosenblum, and Miller, *Science*, 1956, **124**, 272; Smith, *Chem. and Ind.*, 1957, 572.

[14] Rimington, *Ann. Rev. Biochem.*, 1957, **26**, 561; Lemberg and Legge, " Haematin Compounds and Bile Pigments," Interscience Publ. Inc., New York and London, 1949, p. 632; Maitland, *Quart. Rev.*, 1950, **4**, 45.

[15] Shemin, Ciba Foundation Symposium, " Porphyrin Biosynthesis and Metabolism," London, 1955, p. 4.

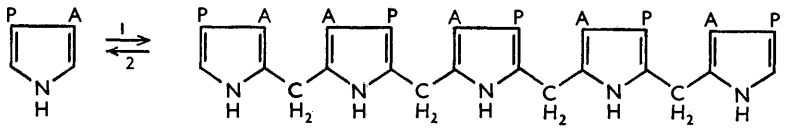
[16] Corwin, Andrews, *et al.*, *J. Amer. Chem. Soc.*, 1937, **59**, 1973; 1950, **72**, 491.

[17] Treibs, Herrmann, Meissner, and Kuhn, *Annalen*, 1957, **602**, 153.

[18] Bogorad, quoted by Granick, ref. 12, p. 146.

material (*i.e.*, α-free pyrroles) is removed in the reaction. (iv) Other possible modes of combination of the fragments are neglected.

Another theory due to Granick [12] meets certain of the objections but still involves

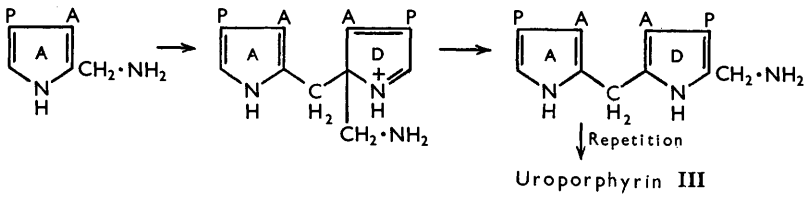


[1] + 4Porphobilinogen — $4NH_3$. [2] — Uroporphyrin III.

dipyrromethenes as break-down products. Cookson and Rimington,[19] on the other hand, avoid the tripyrromethane intermediate by postulating an initial condensation of two porphobilinogen molecules to give a symmetrical dipyrromethane (XII) and a molecule of formaldehyde by a mechanism involving carbonium ions. It is claimed that regular addition of porphobilinogen eventually gives a tetrapyrrane (XIII) which cyclises by condensation with formaldehyde (or the equivalent, *e.g.*, another molecule of porphobilinogen) to give uroporphyrinogen III (XIV) and this on oxidation yields the porphyrin.

The source of the final *meso*-bridge carbon atom is not clear although an experiment using [^{14}C]formaldehyde should determine whether in fact the condensation is intermolecular as claimed. If addition of the third and the fourth porphobilinogen unit does not proceed as illustrated, other isomers of the porphyrin will be produced and the theory offers no explanation of the very high yields of uroporphyrin III, apparently uncontaminated with other isomers, which can be formed from porphobilinogen, *e.g.*, 77% by the action of 0·5N-hydrochloric acid at 100° for 20 min.,[20] and approaching 90% under certain conditions *in vivo*.[2] A possible modification of Cookson and Rimington's scheme has been suggested by Jackson and MacDonald [21] and involves a penta- or higher poly-pyrrane: In this case, there is no evidence to support the suggestion that 4-2′-carboxyethyl-3-carboxymethylpyrrole is involved in the biogenesis or that it or the postulated pentapyrrane would react exclusively in the manner proposed.

In his Weizmann lectures,[22] Robinson suggested an intramolecular mechanism for the formation of type-III porphyrins. He suggested that the initial step involved attack of the α-substituent of one porphobilinogen molecule (corresponding to ring D of the porphyrin) at $C_{(2)}$ of a second molecule (corresponding to the porphyrin ring A) in the manner suggested by Cookson and Rimington.[19] Instead of elimination of formaldehyde, however, Robinson

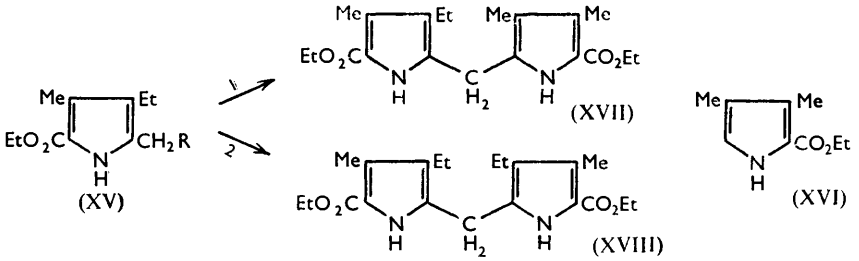


preferred to regard the aminomethyl group as migrating to the 5-position of the ring " possibly *via* an *N*-substituted intermediate." Repetition of the process gave a tetrapyrrane; the " reversal " of β-substituents thus involved rings A, B, and C rather than ring D. A final cyclisation, presumed to occur readily, then gave the macrocycle. In any such scheme involving intramolecular rearrangement, the reported isolation of formaldehyde [15] must be considered as the result of a side-reaction.

We have assumed that the polymerisation of porphobilinogen is a reaction involving carbonium ions and that it is an intramolecular process. The first assumption is at least

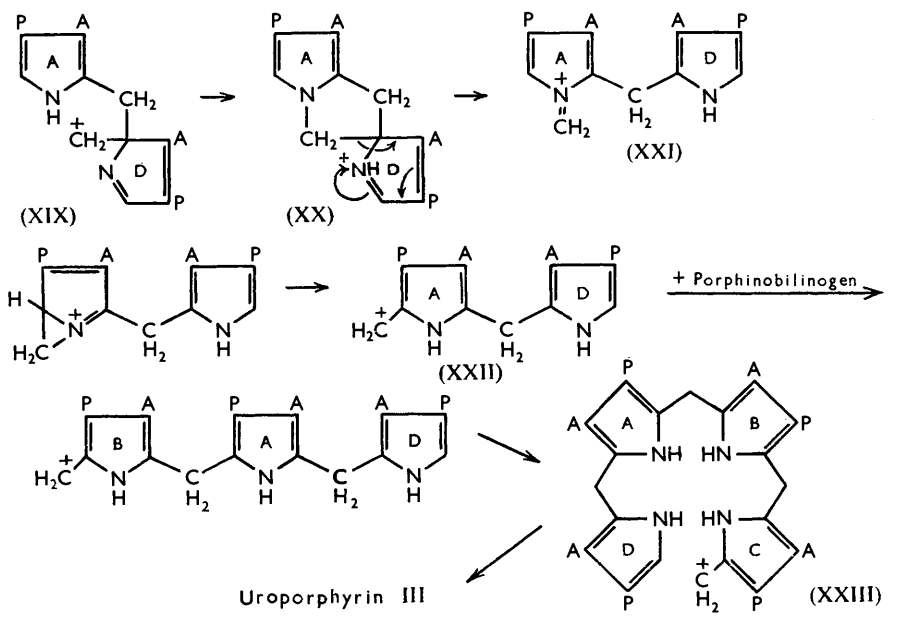
[19] Cookson and Rimington, *Biochem. J.*, 1954, **57**, 476.
[20] Rimington, *Ann. Reports*, 1954, **51**, 317.
[21] Jackson and MacDonald, *Canad. J. Chem.*, 1957, **35**, 715.
[22] Robinson, " Structural Relations of Natural Products," Oxford Univ. Press, 1955, p. 25.

partly justified by the fact that variation of pH seems to have little effect on the product, as judged by chromatographic analysis, and the second is supported by the preponderance



of III-type porphyrin in the product. The formation of I-type porphyrins is the consequence of an independent reaction involving the regular addition of porphobilinogen molecules involving attack by the carbonium ion at the 5- rather than the 2-position of the nucleus. The relative extent of these competing reactions is governed by electronic and steric factors; for example, it has been found that a blocking group such as ethoxycarbonyl in the 5-position has a very profound influence on the coupling reaction. The pyrroles (XV; R = OEt or hexamine salt) react with the α-free pyrrole (XVI) in dilute hydrochloric acid at 100° to give the mixed product (XVII) exclusively; the symmetrical dipyrromethane (XVIII) is formed only in the absence of (XVI).

For the conversion of porphobilinogen into uroporphyrin III we visualise an initial reaction similar to that already postulated by Cookson and Rimington [19] and by Robinson.[22] It is assumed that the intermediate (XIX) is formed from an attack of ring A on ring D, the subsequent stage being migration of the methylene group to the 5-position of ring A, rather than D. This migration involves the formation of a tricyclic intermediate and comprises the following stages:



The essential features of the present theory are: (i) reaction of the carbonium ion (XIX) at the nitrogen atom of ring A to form the tricyclic intermediate (XX); (ii) the fission of

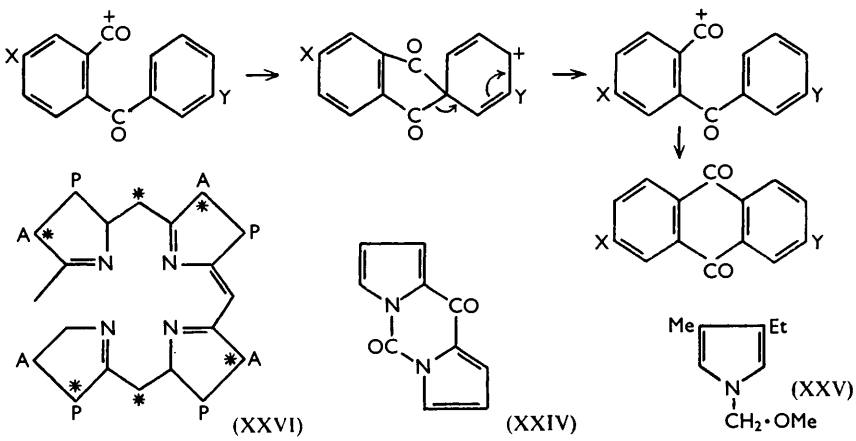
the new ring in the manner shown to yield the dipyrromethane (XXI); (iii) migration of the *N*-methylene substituent to the α-carbon as in (XXII), probably with the intermediate formation of a three-membered ring; (iv) repetition of the process (C ⟶ B ⟶ A ⟶ D) to give the tetrapyrrane (XXIII), and a final cyclisation and oxidation to uroporphyrin III. It will be observed that the methylene group originally attached to ring D migrates successively to rings A, B, and C and finally forms the link between rings C and D.

Whereas it is usual for electrophilic attack on the pyrrole nucleus to occur on carbon, it is nevertheless possible to obtain *N*-substitution, especially when the conditions are sterically favourable as in (XIX). The formation of the so-called pyrrocols (*e.g.*, XXIV) from pyrrole-α-carboxylic acids with acetic anhydride [23] is relevant in this connection, and the presence of alkyl substituents, known [17,24] to enhance the basic properties of the ring, favours *N*-substitution.

An attempt to obtain *N*-substituted pyrroles by direct condensation of either pyrrole itself [25] or kryptopyrrole with triphenylmethanol in glacial acetic acid was unsuccessful, only *C*-substitution being observed [strong infrared band at about 3450 cm.$^{-1}$ (>NH)]. From a preparative point of view, the condensation of alkyl or acyl halides with *N*-potassio-pyrroles in anhydrous media is the method of choice to achieve substitution on the nitrogen atom in the pyrrole series.

The mechanism suggested for the conversion of (XIX) into (XXI) is analogous to that proposed [26] for the Hayashi rearrangement of unsymmetrical benzoylbenzoic acids to anthraquinones,[27] and in both cases the juxtaposition of the reactive centres brings about the ready formation of the cyclic intermediate.

Migration of alkyl substituents from nitrogen to carbon is common in pyrrole chemistry but usually necessitates vigorous experimental conditions. However, it was expected that an *N*-methylenepyrrole cation (*e.g.*, XXI) would rearrange readily to the carbonium ion (XXII). In order to test this, *N*-methoxymethylopsopyrrole (XXV) was prepared from *N*-potassio-opsopyrrole and chloromethyl ether and it has been shown that the



(XXVI) (XXIV) (XXV)

product could be transformed into ætioporphyrin by the action of dilute hydrochloric acid at 100°, a reaction involving a migration of the type postulated.

According to the present suggestion for the biosynthesis of uroporphyrin, the first *meso*-bridge to be formed is that between rings A and D which corresponds to the direct linkage

[23] Fischer and Orth, " Die Chemie des Pyrroles," Leipzig, 1934, Vol. I, p. 236; Corwin *et al.*, *J. Amer. Chem. Soc.*, 1944, **66**, 1151; 1956, **78**, 3135.
[24] Stedman and MacDonald, *Canad. J. Chem.*, 1955, **33**, 468.
[25] Khotinsky and Patzewitch, *Ber.*, 1909, **42**, 3104.
[26] Sandin, Melby, Crawford, and McGreer, *J. Amer. Chem. Soc.*, 1956, **78**, 3817.
[27] Hayashi *et al.*, *J.*, 1927, 2516; 1930, 1513, 1520, 1524; *Bull. Chem. Soc. Japan*, 1936, **11**, 184.

of two five-membered rings in vitamin B_{12}.[28] This suggests that the vitamin B_{12} macrocycle is formed from the appropriate porphyrinogen by fission and recyclisation, with a methylation step at some intermediate stage. In a preliminary communication [29] describing the structure of vitamin B_{12} it was pointed out that, if porphobilinogen could be converted into a tetracyclic, partially reduced intermediate such as (XXVI), there would be six positions (*) which would be susceptible to *C*-alkylation; and that these were the positions in which methyl groups occurred in the vitamin. We are at present using this biogenetic scheme as the basis of a synthesis of the vitamin B_{12} chromophore *in vitro* although it is no longer necessary to postulate an intermediate such as (XXVI).

Experimental

Ethyl 4-Acetyl-5-oxohexanoate.—Sodium (0·22 g.) was dissolved in absolute ethanol (200 c.c.) and acetylacetone (50 g.) was added, followed by ethyl acrylate (50 g.). The mixture was heated under reflux for 2 hr. on the water-bath, then kept overnight and neutralised with glacial acetic acid (0·33 g.). The ethanol was removed under reduced pressure. Distillation of the residue gave ethyl 4-acetyl-5-oxohexanoate, b. p. 146—150°/17 mm. (70 g., 70%) (Found: C, 59·7; H, 8·0. Calc. for $C_{10}H_{16}O_4$: C, 60·0; H, 8·05%). March [30] gives b. p. 154—155°/15 mm.

Ethyl 3 : 4 : 5-Trimethylpyrrole-2-carboxylate (IX; R = Me).—(i) A solution of sodium nitrite (7·4 g.) in water (25 c.c.) was added to an ice-cooled, well-stirred solution of ethyl acetoacetate (13 g.) in glacial acetic acid (40 c.c.) at such a rate that the temperature remained <14°. After stirring of the cooled solution for a further 3 hr. it was kept overnight at room temperature and next day 3-methylpentane-2 : 4-dione [31] (11·4 g.) was added, followed by zinc dust (14 g.) at a rate which maintained the temperature of the mixture at 60°. The mixture was heated on the water-bath for 1 hr., poured into water, and then placed in the refrigerator for 2 hr. The suspended product was separated from excess of zinc by decantation and the pyrrole ester (7·5 g., 42%) removed by filtration and air-dried. After crystallisation from ethanol it formed colourless plates, m. p. 128° (lit.,[32] 128°) (Found: C, 66·1; H, 8·2; N, 8·0. Calc. for $C_{10}H_{15}O_2N$: C, 66·3; H, 8·35; N, 7·7%).

(ii) Pentyl nitrite (6·5 g.) was added to a stirred mixture of diethyl 3-oxoadipate [33] (10·8 g.) and concentrated hydrochloric acid (0·2 c.c.) at such a rate that the temperature remained <25°. After being kept overnight the product was added to a mixture of 3-methylpentane-2 : 4-dione (6·7 g.), glacial acetic acid (50 c.c.), ammonium acetate (8 g.), and zinc dust (2·5 g.) with vigorous stirring. A further quantity of zinc dust (5 g.) was added gradually while the temperature was kept at 60—65°. Then the product was heated on the water-bath for 2 hr., poured into water, and cooled in the refrigerator for several hr. The precipitated solid was separated and dissolved in hot ethanol (25 c.c.), and any remaining zinc separated by filtration. Water was added to the warm filtrate until a turbidity was produced and, after cooling, the pyrrole ester (2·25 g., 40%) was obtained as colourless plates, m. p. 128° (Found: C, 66·4; H, 8·3; N, 7·9%), identical with the product prepared as above.

Ethyl 4-2′-Ethoxycarbonylethyl-3 : 5-dimethylpyrrole-2-carboxylate (IV; R = H, R′ = Et).—(i) Ethyl acetoacetate (13 g.) was nitrosated in the manner described in the first preparation of ethyl 3 : 4 : 5-trimethylpyrrole-2-carboxylate (above) and, the following day, ethyl 4-acetyl-5-oxohexanoate (20 g.) was added. Zinc dust (14 g.) was introduced into the stirred mixture at such a rate that the temperature was maintained at 65°. After the addition of the zinc, the product was heated on the water-bath for 1 hr., then poured on crushed ice. The suspension of the *product* was decanted from any excess of zinc, then separated by filtration and crystallised from ethanol: it formed colourless needles (11·7 g., 44%), m. p. 73° (lit.,[34] 73°) (Found: C, 62·9; H, 7·6; N, 5·2. Calc. for $C_{14}H_{21}O_4N$: C, 63·1; H, 7·85; N, 5·2%).

[28] Hodgkin, Pickworth, Robertson, Trueblood, Prosen, and White, *Nature*, 1955, **176**, 325; Bonnett, Cannon, Clark, Johnson, Parker, Smith, and Todd, *J.*, 1957, 1158.
[29] Bonnett, Cannon, Johnson, Sutherland, Todd, and Smith, *Nature*, 1955, **176**, 328.
[30] March, *Ann. Chim.* (*France*), 1902, **26**, 333.
[31] von Auwers and Jacobsen, *Annalen*, 1921, **426**, 227.
[32] Fischer and Walach, *ibid.*, 1926, **450**, 109.
[33] MacDonald and Stedman, *Canad. J. Chem.*, 1955, **33**, 458.
[34] Fischer and Süs, *Annalen*, 1930, **484**, 113.

(ii) Sodium nitrite (3·7 g.) in water (12·5 c.c.) was added dropwise with stirring and cooling to diethyl 3-oxoglutarate [35] (10·1 g.) in glacial acetic acid (20 c.c.) at <14°. Stirring was continued for a further 3 hr. and the mixture kept overnight at room temperature. After the addition of ethyl 4-acetyl-5-oxohexanoate (10 g.), zinc dust (7 g.) was added to the stirred mixture at 65°. Stirring was continued for another 30 min. and the product then heated on the water-bath for 1 hr., cooled, and poured on crushed ice. The precipitated oil slowly solidified at 0°. It crystallised from ethanol as colourless plates of the pyrrole ester (2·2 g., 16·5%), m. p. 73° after several crystallisations from ethanol (Found: C, 62·9; H, 7·5; N, 5·2%).

Benzyl 4-2′-Ethoxycarbonylethyl-3 : 5-dimethylpyrrole-2-carboxylate (IV; R = H, R′ = CH_2Ph).—Sodium nitrite (21·6 g.) in water (50 c.c.) was added with stirring to benzyl acetoacetate (50 g.) in glacial acetic acid (80 c.c.) at <10°. The cooled mixture was stirred for a further 4 hr., then kept overnight at room temperature and added to ethyl 4-acetyl-5-oxohexanoate (56·5 g.) in acetic acid (100 c.c.) in the presence of zinc dust (34 g.) with stirring, at about 70°. The mixture was heated under reflux for 30 min., then poured on crushed ice (*ca.* 1 kg.). The *ester* was separated, washed with water, and crystallised twice from aqueous ethanol, forming colourless needles (30 g., 35%), m. p. 75—76° (Found: C, 69·0; H, 6·85; N, 4·2. $C_{19}H_{23}O_4N$ requires C, 69·3; H, 7·05; N, 4·25%).

Benzyl 5-Acetoxymethyl-4-2′-ethoxycarbonylethyl-3-methylpyrrole-2-carboxylate (IV; R = OAc, R′ = CH_2Ph).—To benzyl 4-2′-ethoxycarbonylethyl-3 : 5-dimethylpyrrole-2-carboxylate (IV; R = H, R′ = CH_2Ph) (7 g.) in glacial acetic acid (600 c.c.), lead tetra-acetate (9·4 g.) was added with stirring during 30 min. Stirring was continued for a further 3 hr., after which most of the solvent was removed under reduced pressure. The residue was poured into ice-cold water (*ca.* 800 c.c.) and kept overnight. The colourless precipitate was separated, washed with water, and crystallised from acetone as colourless needles (5·5 g., 67%), m. p. 121—122° (Found: C, 65·0; H, 6·8; N, 4·0. $C_{21}H_{25}O_6N$ requires C, 65·1; H, 6·5; N, 3·6%).

Coproporphyrin III Tetraethyl Ester (II; R = Me, P = $CH_2{\cdot}CH_2{\cdot}CO_2Et$).—The above acetoxy-compound (IV; R = OAc, R′ = CH_2Ph; 2·5 g.) in ether (150 c.c.) was shaken with a little Raney nickel to remove any catalyst poisons. The nickel was separated and the filtrate hydrogenated in the presence of 5% palladium–charcoal (0·2 g.) and triethylamine (3 drops). Hydrogenolysis of the benzyl group was complete after about 6 hr., then the catalyst was separated and the solvent removed at room temperature and reduced pressure. The resulting pyrrole acid darkened very rapidly in air and could not be purified completely.

The residue after removal of the ether was heated in ethanol (40 c.c.) and glacial acetic acid (10 c.c.) on the water-bath for 90 min. The colour changed from brown to deep red and the resulting solution was then aerated for *ca.* 10 hr. Chloroform (100 c.c.) was next added and the solution washed with aqueous sodium carbonate, then water, and dried (Na_2SO_4). The solution was concentrated to 20 c.c. and chromatographed on an alumina column (Spence type H; 30 × 1·5 cm.). Elution of the product was followed by means of a hand spectroscope. Removal of the solvent from the main porphyrin fraction gave coproporphyrin III tetraethyl ester (185 mg., 15% based on the pyrrole benzyl ester) which crystallised from chloroform–methanol in thin reddish-brown needles, m. p. 147—149° (micro hot-stage) (lit.,[10] 124°, not sharp) (Found: C, 68·8; H, 6·95; N, 7·05. Calc. for $C_{44}H_{54}O_8N_4$: C, 68·9; H, 7·1; N, 7·3%), $\lambda_{max.}$ in $CHCl_3$: 400, 499, 534, 569, and 620 mμ (log ε 5·22, 4·12, 3·95, 3·76, and 3·60 respectively).

tert.-*Butyl 3 : 4 : 5-Trimethylpyrrole-2-carboxylate*.—Redistilled *tert.*-butyl acetoacetate (31·6 g.) in glacial acetic acid (80 c.c.) was treated with aqueous sodium nitrite (14·8 g. in 50 c.c). with stirring during 20 min., at 10—12° (ice-cooling). The product was kept for a further 2 hr. at 4°, then overnight at room temperature. The following day, 3-methylpentane-2 : 4-dione (22·8 g.) was added with stirring, followed by zinc dust (28 g.) at such a rate as to keep the reaction temperature at 80—85°. Next, the mixture was stirred for a further 30 min., then heated to 100° for 2 hr. The hot solution was poured, with stirring, into cold water (1 l.), and the precipitated solid separated and dissolved in hot ethanol (500 c.c.). The clarified solution was diluted with water to produce a faint turbidity and then kept, the *ester* being obtained as colourless needles (13 g.), m. p. 137—138° (Found: C, 69·0; H, 8·85; N, 6·7. $C_{12}H_{19}O_2N$ requires C, 68·85; H, 9·15; N, 6·7%).

tert.-*Butyl 5-Acetoxymethyl-3 : 4-dimethylpyrrole-2-carboxylate*.—The foregoing ester (5 g.) in glacial acetic acid (150 c.c.) was treated with lead tetra-acetate (10·6 g.) in ½ hr. at room temperature with stirring. After a further 2 hours' stirring, most (80 c.c.) of the acetic acid

[35] MacDonald and MacDonald, *Canad. J. Chem.*, 1955, **33**, 573.

was removed on the water-bath under reduced pressure and the residue poured into water (1 l.) with stirring. The colourless precipitate was separated, washed, dried (5·85 g.), and crystallised from aqueous acetone, forming colourless needles, m. p. 127—128° (Found: C, 63·3; H, 7·95; N, 5·0. $C_{14}H_{21}O_4N$ requires C, 62·9; H, 7·9; N, 5·25%).

Octamethylporphin.—tert.-Butyl 5-acetoxymethyl-3 : 4-dimethylpyrrole-2-carboxylate (1 g.) was suspended in ethylene glycol (10 c.c.) and heated under reflux for 75 min. The dark solution was cooled, diluted with methanol (40 c.c.), and aerated for 10 hr., further methanol being added when necessary to maintain the volume. The very dark precipitated solid (0·13 g.) was separated and extracted with methanol, and the insoluble residue continuously extracted from a thimble with chloroform (10 c.c.). From the extract there was obtained octamethylporphin (87 mg., 22%), $\lambda_{max.}$ in *o*-dichlorobenzene at 404, 501, 535, 573, and 603 mμ (log ε 5·11, 4·10, 3·93, 3·76, and 3·68 respectively).

Ethyl 5-Acetoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate.—Lead tetra-acetate (17·1 g.) was added gradually, during ½ hr., to a solution of ethyl 4-ethyl-3 : 5-dimethylpyrrole-2-carboxylate (5 g.) in glacial acetic acid (150 c.c.) at room temperature. The solution was stirred for a further 3 hr., then poured into water (500 c.c.). The precipitated white solid was separated, washed, and dried (4·4 g., 68%). After crystallisation from aqueous acetone it formed colourless needles, m. p. 128°, unchanged on being heated in acetic anhydride (Found: C, 61·6; H, 7·6; N, 5·5. $C_{13}H_{19}O_4N$ requires C, 61·65; H, 7·55; N, 5·5%).

Ethyl 5-Ethoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate.—The above acetoxymethyl compound (4·4 g.) was heated in ethanol (50 c.c.) under reflux for 30 min. The solution was cooled and the *product* crystallised as colourless needles (3·0 g., 72%), m. p. 56—58° (Found, on a sample sublimed at 50°/0·1 mm.: C, 64·8; H, 8·85; N, 5·8. $C_{13}H_{21}O_3N$ requires C, 65·2; H, 8·85; N, 5·85%).

Ethyl 4-Ethyl-3-methyl-5-(N-methyleneaminomethyl)pyrrole-2-carboxylate (XV; R = N:CH_2). —Ethyl 5-chloromethyl-4-ethyl-3-methylpyrrole-2-carboxylate [7] (0·5 g.) and hexamine (0·33 g.) were heated in chloroform (15 c.c.) under reflux for 1 hr. The solution was cooled, acetone (15 c.c.) was added, and the white crystalline salt (0·7 g.) so obtained was separated and washed with acetone. It was then dissolved in water (15 c.c.) and heated on the steam-bath, the solution rapidly becoming cloudy, and after 1 hr. a yellow gummy solid separated. The precipitated solid was dissolved in warm aqueous ethanol and, after cooling, a white crystalline *compound* (0·2 g.) was obtained which was insoluble in water but soluble in 3N-hydrochloric acid. It recrystallised from ethyl acetate–acetone as plates, m. p. 145—147° (Found: C, 64·45; H, 8·2; N, 12·8. $C_{12}H_{18}O_2N_2$ requires C, 64·85; H, 8·15; N, 12·6%).

The same product was obtained by a similar method from ethyl 5-bromomethyl-4-ethyl-3-methylpyrrole-2-carboxylate.[36]

Diethyl 3 : 3′-Diethyl-4 : 4′-dimethyldipyrromethane-5 : 5′-dicarboxylate (XVIII) (i).—Ethyl 5-ethoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate (2 g.) was heated in ethanol (30 c.c.) containing hydrochloric acid (5 c.c. of concentrated) under reflux for 30 min. After cooling, the crystalline solid (1·3 g., 83%) was separated, washed, dried, and crystallised from ethanol, forming colourless needles, m. p. 126° (lit.,[37] 126°). The formaldehyde also formed in the reaction was identified by bubbling the gaseous products from the reaction in a stream of nitrogen through a solution of 2 : 4-dinitrophenylhydrazine. The derivative (corresponding to 0·75 mol.) had m. p. and mixed m. p. 165—167°.

(ii) The hexamine salt from ethyl 5-chloromethyl-4-ethyl-3-methylpyrrole-2-carboxylate (2 g.; preparation as above) was dissolved in ethanol (10 c.c.) and water (10 c.c.); 10N-hydrochloric acid (5 c.c.) was added and the mixture heated under reflux for 4 hr. On cooling, colourless needles (0·65 g.) were obtained which had m. p. 119°, raised to 126° on crystallisation from ethanol. The m. p. was undepressed on admixture with the product of the previous experiment.

Diethyl 3-Ethyl-4 : 3′ : 4′-trimethyldipyrromethane-5 : 5′-dicarboxylate (XVII).—(i) A mixture of ethyl 5-ethoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate (256 mg.) and ethyl 3 : 4-dimethylpyrrole-2-carboxylate (251 mg.; prepared by decarboxylation [38] of 5-ethoxycarbonyl-3 : 4-dimethylpyrrole-2-carboxylic acid [39]) was dissolved in ethanol (100 c.c.), concentrated

[36] Fischer and Ernst, *Annalen*, 1926, **447**, 139.
[37] Fischer and Halbig, *ibid.*, p. 123.
[38] Fischer and Höfelmann, *ibid.*, 1938, **533**, 216.
[39] Fischer and Hierneis, *ibid.*, 1931, **492**, 21.

sulphuric acid (2 c.c.) was added, and the mixture heated under reflux for 4 hr. After storage for 2 weeks a quantity of a colourless solid had crystallised from the dark red solution. This was separated and after crystallisation from light petroleum (b. p. 60—80°) formed colourless needles, m. p. 174—176°. A further quantity was obtained by dilution of the mother-liquors and after crystallisation from ethanol had m. p. 173—174° (Found: C, 66·6; H, 7·4; N, 8·1. $C_{20}H_{28}O_4N_2$ requires C, 66·6; H, 7·7; N, 7·7%).

(ii) The hexamine salt (370 mg.) from ethyl 5-bromomethyl-4-ethyl-3-methylpyrrole-2-carboxylate and ethyl 3 : 4-dimethylpyrrole-2-carboxylate were dissolved in ethanol (20 c.c.) and treated with concentrated hydrochloric acid (2 c.c.) in water (20 c.c.). The mixture was heated on the water-bath for 4 hr. although a precipitate was obtained after *ca.* 10 min. The resulting suspension was cooled and the solid product (295 mg.) separated, washed with aqueous ethanol, and dried; it had m. p. 173°, raised to 175° on crystallisation from aqueous ethanol. The m. p. was unchanged on admixture with the product from the previous experiment (Found: C, 66·9; H, 7·7; N, 7·9%).

3-*Ethyl*-1-*methoxymethyl*-4-*methylpyrrole*; 1-*Methoxymethylopsopyrrole* (XXV).—Freshly distilled opsopyrrole (3·3 g.; prepared by an adaptation of the method given by Eisner, Linstead, Parkes, and Stephen [40] for 3 : 4-dimethylpyrrole) was dissolved in light petroleum (b. p. 100—120°; 25 c.c.) and treated with potassium (1 g.) in small pieces. The mixture was heated under reflux for 12 hr., until all the metal had dissolved. Then the colourless potassium salt was separated, washed with dry ether, suspended in dry ether (150 c.c.), and treated with chloromethyl ether (13·2 g.; freshly distilled) in dry ether (40 c.c.) which caused the solvent to reflux. Next the product was heated under reflux on the water-bath for 15 min. and the solid material separated. Removal of the solvent under reduced pressure from the filtrate gave a yellow oil which rapidly darkened in contact with air. Attempted distillation under reduced pressure caused the formation of an intractable red gum. The yellow oil was therefore used in the following experiment without further purification.

Ætioporphyrin.—1-Methoxymethylopsopyrrole (1 g.) from the foregoing experiment was dissolved in ethanol (5 c.c.), treated with 2N-hydrochloric acid (3 c.c.), and heated under reflux for 1 hr. The resulting solution was aerated for 4 hr. and the red resinous precipitate separated and dissolved in a small volume of chloroform (3 c.c.). Addition of methanol (6 c.c.) caused precipitation of a dark red solid (0·25 g.) which was very soluble in chloroform to give a solution which showed the typical porphyrin spectrum (log $\varepsilon_{max.}$ 4·88 at 398 mμ, *i.e.*, approx. 50% pure). Purification was achieved by chromatography of the chloroform solution on alumina (15 × 1 cm.) and elution with chloroform: the porphyrin fraction was easily separated. Removal of the solvent gave ætioporphyrin (120 mg.) which was further purified by crystallisation from chloroform–methanol (1 : 2) and sublimation at 210°/0·1 mm. Its light absorption in $CHCl_3$ had max. at 398, 498, 533, 567, and 620 mμ (log ε 5·17, 4·05, 3·92, 3·75, and 3·57 respectively).

2-*Triphenylmethylpyrrole.*—Prepared according to Khotinsky and Patzewitch,[25] it had m. p. 252° after crystallisation from ethanol. The infrared spectrum showed a strong band at 3460 $cm.^{-1}$.

4-*Ethyl*-3 : 5-*dimethyl*-2-*triphenylmethylpyrrole.*—Kryptopyrrole (130 mg.) and triphenylmethanol (250 mg.) were heated with glacial acetic acid (1·5 c.c.) in a sealed tube at 100° for 2 hr. After cooling, the yellow crystalline product (200 mg.) was separated and washed with ether. After crystallisation from ethanol it had m. p. 156—158° (Found: C, 88·5; H, 7·55; N, 3·7. $C_{27}H_{27}N$ requires C, 88·7; H, 7·45; N, 3·8%). The infrared spectrum showed a strong band at 3446 $cm.^{-1}$.

Grateful acknowledgment is made to the Department of Scientific and Industrial Research for the award of Maintenance Grants (to E. B., E. M., and K. B. S.) and to several colleagues, especially Professor Melvin S. Newman, for helpful and stimulating discussions. We thank Professor C. Rimington, F.R.S., Professor A. Neuberger, F.R.S., Imperial Chemical Industries Limited (Paints and Dyestuffs Divisions), and British Industrial Solvents Ltd., for generous gifts of chemicals.

UNIVERSITY OF NOTTINGHAM. [*Received, November 12th*, 1957.]

[40] Eisner, Linstead, Parkes, and Stephen, *J.*, 1956, 1655.