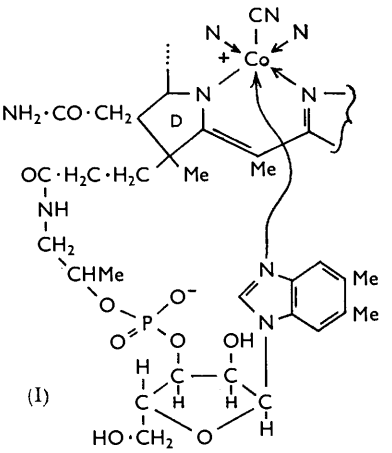
## 493. *The Preparation of a Cobaltic Porphyrin–Nucleotide Complex.*

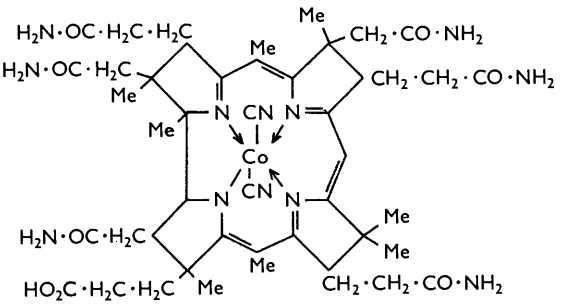
By A. W. Johnson, N. Shaw, and J. W. F. Wasley.

Three porphyrinmonocarboxylic acids have been prepared. The cobaltous derivative of the 2-hydroxypropylamide of 1-2′-carboxyethyl-3,4-diethyl-2,5,6,7,8-pentamethylporphyrin is readily converted by potassium cyanide into the cyano-hydroxy-derivative of the corresponding cobaltic complex which was treated with α-ribazole 2′,3′-cyclic phosphate. The porphyrin derivative so obtained gave ribose and 1-aminopropan-2-ol on hydrolysis and, like vitamin $B_{12}$, contained the large ring formed by the aminopropanol and nucleotide fragments.

A feature of the structure of vitamin $B_{12}$ is the large loop (I) formed by the 1-aminopropan-2-ol and nucleotide fragments which link the cobalt atom and the propionic acid substituent of ring D of the chromophore. In an attempt to simulate this part of the structure, it was decided to investigate the synthesis of such a grouping from a porphyrin containing a single carboxylic acid group as part of a propionic acid substituent.




(I)




(II)

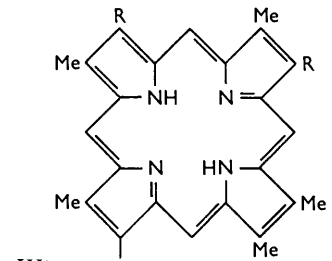
While this work was in progress, Bernhauer and his collaborators [1] described the isolation from sewage sludge of the so-called factor $V_{1a}$ and showed [2] that it was the monocarboxylic acid (II; dicyano-form) (cobyrinic acid *abcdeg*-hexa-amide) on the basis of its

[1] Bernhauer, Dellweg, Friedrich, Gross, Wagner, and Zeller, *Helv. Chim. Acta*, 1960, **43**, 693; *Z. Naturforsch.*, 1960, **15**b, 336.

[2] Bernhauer, Wagner, and Zeller, *Helv. Chim. Acta*, 1960, **43**, 696; Bernhauer, Wagner, and Wahl, *Biochem. Z.*, 1961, **334**, 279.

conversion[3] into factor B on condensation with 1-aminopropan-2-ol. Factor $V_{1a}$ is obviously the ideal intermediate with which to investigate the synthesis of the " loop " of vitamin $B_{12}$ and in further experiments [4] it was condensed with the monosodium salt of the *O*-phosphate of 1-aminopropan-2-ol to yield the phosphate of factor B which has also been isolated from natural sources.[5] Even more important, however, was the condensation [6] with the phosphoric acid diester obtained from 1-aminopropan-2-ol and the 2′,3′-cyclic phosphate of 1-α-D-ribofuranosyl-5,6-dimethylbenziminazole [7] which gave vitamin $B_{12}$ itself (identified by paper chromatography). This elegant reaction thus constitutes a partial synthesis of the vitamin and in later papers [8] similar reactions have been used to prepare a series of vitamin $B_{12}$ antagonists.

A known synthetic porphyrin containing a single carboxyl substituent as part of a propionic acid side-chain was the compound (III; R = Me) [9] containing seven methyl substituents, but it proved to be too insoluble for further reactions. Accordingly, a related porphyrin (III; R = Et) was synthesised which contained two ethyl and only five methyl substituents in addition to the acid group. Ethyl 5-formyl-4-2′-methoxycarbonylethyl-3-methylpyrrole-2-carboxylate (IV; R = CHO) was prepared by oxidation of the corresponding 5-bromomethyl compound (IV; R = $CH_2Br$) with chromic acid and then condensed with 2,3,4-trimethylpyrrole [10] to give the dipyrromethene salt (V; R = $CO_2Et$). Hydrolysis of the 5-ethoxycarbonyl group and replacement of the carboxyl by bromine gave the substance (V; R = Br) which was condensed with 5-bromo-5′-bromomethyl-3,4′-diethyl-4,3′-dimethyldipyrromethene [11] in a succinic acid melt to yield the porphyrin (III; R = Et).

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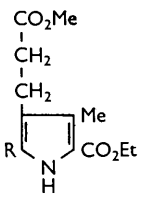


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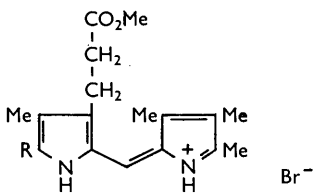
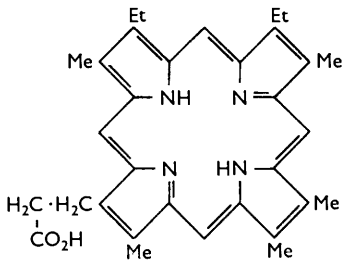



(V)

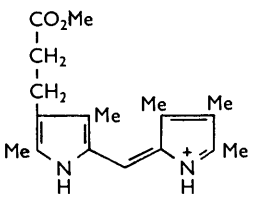
Preliminary condensations were carried out with the porphyrin (III; R = Et) but it was later found that the isomeric porphyrin (VI) could be synthesised more easily and the main experiments were carried out with this as the starting material. Condensing 2-formyl-3,4,5-trimethylpyrrole [12] with 3-2′-carboxyethyl-2,4-dimethylpyrrole [13] in methanolic hydrogen bromide gave the hydrobromide of 4-2′-carboxyethyl-3,5,3′,4′,5′-pentamethyldipyrromethene [14] (VII), which was further condensed with 5,5′-dibromo-3,3′-diethyl-4,4′-dimethyldipyrromethene hydrobromide [15] (VIII) in molten succinic acid, to give the porphyrin (VI).

[3] Armitage, Cannon, Johnson, Parker, Smith, Stafford, and Todd, *J.*, 1953, 3849.
[4] Bernhauer, Wagner, Dellweg, and Zeller, *Helv. Chim. Acta*, 1960, **43**, 700.
[5] Barchielli, Bovetti, di Marco, Julita, Migliacci, Minghetti, and Spalla, *Biochem. J.*, 1960, **74**, 382.
[6] Friedrich, Gross, Bernhauer, and Zeller, *Helv. Chim. Acta*, 1960, **43**, 704.
[7] Bonnett, Buchanan, Johnson, and Todd, *J.*, 1957, 1168.
[8] Bernhauer *et al.*, *Biochem. Z.*, 1960, **322**, 184, 190, 194; Friedrich, Heinrich, *et al.*, *ibid.*, 1961, **333**, 550, 554; **334**, 284.
[9] Fischer and Heirneis, *Annalen*, 1931, **492**, 21.
[10] Johnson, Markham, Price, and Shaw, *J.*, 1958, 4254.
[11] Fischer, Baumann, and Riedl, *Annalen*, 1929, **475**, 205.
[12] Fischer and Walach, *Annalen*, 1926, **450**, 109.
[13] Fischer and Andersag, *Annalen*, 1927, **458**, 117.
[14] Fischer and Berg, *Annalen*, 1930, **482**, 189.
[15] Fischer, Halbig, and Walach, *Annalen*, 1927, **452**, 283.

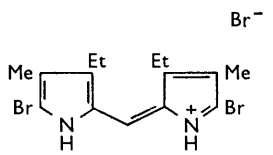
Condensation of the methyl esters of coproporphyrin I, mesoporphryin IX, and the porphyrins (III; R = Et) and (VI) with 1-aminopropan-2-ol gave the corresponding amides (-$CO_2Me$ ⟶ -CO·NH·$CH_2$·CHMe·OH) and treatment of the last two monoamides




(VI)

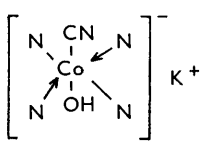



(VII)

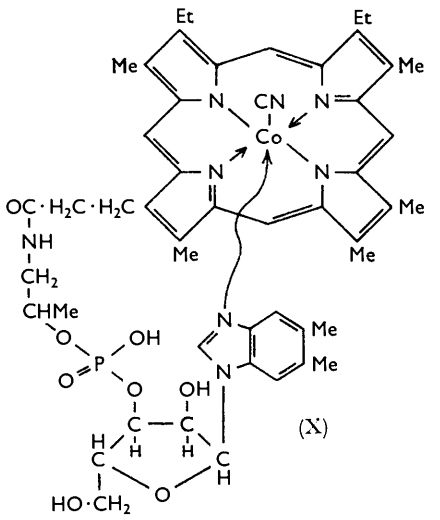



(VIII)

with methanolic cobaltous acetate in the presence of ammonia gave the corresponding cobaltous complexes. Subsequent reaction of these cobaltous compounds with potassium cyanide occurred as with the cobalt complex derived from coproporphyrin I, giving the cyano-hydroxy-cobaltic derivatives (partial structure IX) in contrast to the behaviour of ætio-porphyrin which gave a dicyano-complex under the same conditions.[16] The 2′,3′-cyclic phosphate of 1-α-D-ribofuranosido-5,6-dimethylbenziminazole [7] was then condensed [6] with the cyano-hydroxy-cobaltic complex of the porphyrin aminopropanol amide. The product, which formed reddish-purple needles, was obtained only in small amount, but it has been assigned the structure (X) (or the 2′-phosphate isomer) on the basis of nitrogen analysis and the detection of both ribose and 1-aminopropan-2-ol by chromatography of a hydrolysate furnished by concentrated hydrochloric acid at 100° (12 hours). Bernhauer and Friedrich [6,8] have described the isolation of both the 2′- and the 3′-phosphate from some reactions of the intermediate 2′,3′-cyclic phosphate, but with the small amount of final




(IX)




(X)

product available in the present instance it was not possible to separate the isomers. In view of the extensive German work with factor $V_{1a}$ it is not proposed to pursue this topic further.

[16] Johnson and Kay, *J.*, 1960, 2979.

## Experimental

Visible spectra were determined for chloroform solutions except where otherwise stated. Light petroleum refers to the fraction of b. p. 60—80°.

1-*Aminopropan*-2-*ol*.—Prepared (65%) by reaction of propylene oxide and ammonia according to Levene and Walti's directions,[17] the base had b. p. 158—160°. The N-*benzoyl derivative*, m. p. 92—93°, formed plates from benzene–light petroleum (Found: C, 67·2; H, 6·85; N, 7·55. $C_{10}H_{13}NO_2$ requires C, 67·0; H, 7·3; N, 7·8%). The N-*phenylacetyl*, m. p. 54·5—55·5° (Found: N, 7·3. $C_{11}H_{15}NO_2$ requires N, 7·25%), and N-β-*phenylpropionyl derivative*, m. p. 63·5—65° (Found: C, 69·3; H, 7·85; N, 6·4. $C_{12}H_{17}NO_2$ requires C, 69·55; H, 8·3; N, 6·75%), were also prepared, and crystallised from benzene–light petroleum.

*Coproporphyrin I Tetra*-(2-*hydroxypropylamide*).—Coproporphyrin I tetramethyl ester (100 mg.) and 1-aminopropan-2-ol (3 c.c.) were heated together under reflux for 3 hr., the ester dissolving completely. Crystals separated after cooling and were collected; they crystallised from methanol as very small brick-red needles (85 mg.), m. p. >300° (Found: N, 12·5. $C_{48}H_{66}N_8O_8$ requires N, 12·7%), $\lambda_{max.}$ 270, 395, 497, 531, 567, and 620 mμ (log ε 3·80, 5·26, 4·12, 3·94, 3·79, and 3·58, respectively). Acetylation of this *amide* (30 mg.) by acetic anhydride and pyridine under reflux yielded the *tetra*-O-*acetyl derivative* which was purified by chromatography of a chloroform solution on alumina; crystallisation from chloroform–methanol gave red needles (18 mg.), m. p. 276—278° (decomp.) (Found: C, 63·8; H, 6·8; N, 10·5. $C_{56}H_{74}N_8O_{12}$ requires C, 64·0; H, 7·1; N, 10·55%), $\lambda_{max.}$ 270, 399, 498, 534, 568, and 620 mμ (log ε 3·88, 5·21, 4·13, 3·99, 3·82, and 3·65, respectively).

*Mesoporphyrin* IX *Di*-(2-*hydroxypropylamide*).—Mesoporphyrin IX (from hæmin; 50 mg.) was treated with 1-aminopropan-2-ol as above. The *product* formed purple plates (45 mg.), m. p. >300° (from methanol) (Found: C, 70·6; H, 7·5; N, 11·8. $C_{40}H_{52}N_6O_4$ requires C, 70·55; H, 7·7; N, 12·3%), $\lambda_{max.}$ 269, 394, 495, 529, 566 and 620 mμ (log ε 3·84, 5·17, 4·06, 3·91, 3·74, and 3·55, respectively).

2-*Formyl*-3,4,5-*trimethylpyrrole*.—*NN*-Dimethylformamide (37 c.c.) was added to a solution of 2,3,4-trimethylpyrrole [10] (37 g.) in dry ether (200 c.c.), and the mixture was added gradually to a cooled (acetone–carbon dioxide) solution of phosphorus oxychloride (60 c.c.) in dry ether (200 c.c.). The yellow precipitate of the imine hydrochloride was separated, washed with dry ether (100 c.c.), dissolved in water (500 c.c.), and treated with aqueous sodium hydroxide until the aldehyde was precipitated. This was removed by filtration, and washed; it crystallised from aqueous ethanol as neeedles (35 g.), m. p. 145—146° (lit.,[12] 147°) (Found: N, 10·4. Calc. for $C_8H_{11}NO$: N, 10·2%).

1-2′-*Methoxycarbonylethyl*-2,3,4,5,6,7,8-*heptamethylporphyrin*.—2-Formyl-3,4,5-trimethylpyrrole (820 mg.) and 3-2′-carboxyethyl-2,4-dimethylpyrrole [13] (1 g.), in methanol (5 c.c.), were treated with 48% hydrobromic acid (1·5 c.c.). After a few minutes the hydrobromide of 4-2′-carboxyethyl-3,5,3′,4′,5′-pentamethyldipyrromethene crystallised from the dark red solution and was separated and recrystallised from ethanol as yellowish-red needles (1·7 g.), m. p. 205—206° (lit.,[14] 207°).

This hydrobromide (1·9 g.) was fused with 5,5′-dibromo-3,4,3′,4′-tetramethyldipyrromethene hydrobromide [12] (2·5 g.) and succinic acid (25 g.) at 180° for 1 hr. After removal of the succinic acid and esterification with methanolic hydrochloric acid, the mixture was worked up as in the previous porphyrin preparation, to yield the product [9] as purple needles (150 mg.), m. p. >300° (Found: C, 75·3; H, 7·0; N, 11·6. Calc. for $C_{31}H_{34}N_4O_2$: C, 75·25; H, 6·9; N, 11·3%), $\lambda_{max.}$ 269, 398, 498, 534, 568, 595, 620, and 645 mμ (log ε 3·91, 5·19, 4·10, 3·97, 3·79, 3·15, 3·66, and 3·03, respectively).

*Ethyl* 5-*Formyl*-4,2′-*methoxycarbonylethyl*-3-*methylpyrrole*-2-*carboxylate*.—Ethyl 4-2′-methoxycarbonylethyl-3,5-dimethylpyrrole-2-carboxylate [18] (25 g.), m. p. 103—104° (lit., 104°) (Found: C, 61·8; H, 7·4; N, 5·7. Calc. for $C_{13}H_{19}NO_4$: C, 61·65; H, 7·55; N, 5·5%), in dry ether (500 c.c.) was treated with bromine (18 g.). The solvent was then removed under reduced pressure and the waxy product washed with more ether (200 c.c.) and used directly for the oxidation. Chromium trioxide (8 g.) in glacial acetic acid (250 c.c.) was added to it, and the mixture was heated to 80°, then poured into water (200 c.c.) and extracted with benzene (3 × 80 c.c.). The benzene extract was dried ($MgSO_4$), the solvent removed, and the residual

[17] Levene and Walti, *J. Biol. Chem.*, 1927, **71**, 461.
[18] Fischer, Süs, and Weilguny, *Annalen*, 1930, **481**, 169.

*aldehyde* crystallised from aqueous methanol, forming colourless needles (4·0 g.), m. p. 141—143° (Found: C, 58·0; H, 6·2; N, 5·7. $C_{13}H_{17}NO_5$ requires C, 58·4; H, 6·1; N, 5·2%).

*Ethyl 3-β-Methoxycarbonylethyl-3′,4,4′,5′-tetramethyldipyrromethene-5-carboxylate Hydrobromide.*—The foregoing aldehyde (200 mg.) and 2,3,4-trimethylpyrrole [10] (200 mg.) were suspended in methanol (3 c.c.), cooled to 0°, and treated with 48% hydrobromic acid (0·5 c.c.) in glacial acetic acid (1 c.c.). The solution immediately changed colour from yellow to deep red, and the product was obtained as orange-red prisms which crystallised from chloroform–light petroleum. This *hydrobromide* (300 mg.) had m. p. 174° (decomp.) (Found: C, 54·15; H, 6·2; N, 6·0. $C_{20}H_{27}BrN_2O_4$ requires C, 54·65; H, 6·2; N, 6·35%).

*4,6-Diethyl-1-2′-methoxycarbonylethyl-2,3,5,7,8-pentamethylporphyrin.*—The above dipyrromethene hydrobromide (10 g.) was heated in ethanol (100 c.c.) containing 10% aqueous sodium hydroxide (50 c.c.) under reflux for 3 hr. The ethanol was removed by distillation, and the solution cooled and acidified to Congo Red with acetic acid which precipitated the dicarboxydipyrromethene. The dry acid was used without further purification in the next stage: it was treated with bromine (3 c.c.) in glacial acetic acid (20 c.c.) in order to replace the nuclear carboxyl group by bromine. The mixture was kept for 1 hr., then most of the solvent was removed under reduced pressure, the final traces over solid potassium hydroxide in a desiccator. The bromodipyrromethene hydrobromide was thus obtained as a dark green residue (3 g.), m. p. >300°, which could not be crystallised.

Cryptopyrrole [10] (10 g.; freshly distilled) was treated with bromine (10 c.c.) in acetic acid (80 c.c.) as directed by Fischer *et al.*,[11] to give 5-bromo-5′-bromomethyl-3,4′-diethyl-4,3′-dimethyldipyrromethene hydrobromide as red prisms (5·5 g.), m. p. >350° (after crystallisation from chloroform–light petroleum). This dipyrromethene hydrobromide (4 g.) was heated with 5-bromo-3-2′′-carboxyethyl-3′,4,4′,5′-tetramethyldipyrromethene hydrobromide (1 g.; above) and succinic acid (60 g.) at 180° for 1 hr. The succinic acid was then removed by repeated extraction with hot water, and the residue was dried and extracted (Soxhlet) with chloroform to remove any ætioporphyrin I. The remaining porphyrin was esterified with methanolic hydrochloric acid, poured into a saturated solution of sodium acetate, and extracted with chloroform (4 × 100 c.c.). The extract was washed and dried, most of the solvent was removed, and the remaining solution chromatographed on deactivated alumina (Spence's type " H "). The column was eluted with chloroform, and the porphyrin fraction (hand-spectroscope) collected. Removal of the solvent gave the *product* as lilac needles (10 mg.) (Found: C, 76·0; H, 7·4; N, 10·5. $C_{33}H_{38}N_4O_2$ requires C, 75·8; H, 7·3; N, 10·5%), $\lambda_{max.}$ 269, 398, 498, 534, 567, 595, 620, and 650 mμ (log ε 3·89, 5·22, 4·11, 3·98, 3·81, 3·09, 3·65, and 2·34, respectively).

The porphyrin methyl ester (73 mg.) was treated with 1-aminopropan-2-ol in the manner described above for coproporphyrin I and mesoporphyrin IX. The amide, without purification, in chloroform (25 c.c.) was converted into the cobalt derivative by reaction with a saturated methanolic solution (10 c.c.) of cobaltous acetate containing a few drops of aqueous ammonia (*d* 0·88). The solution was heated under reflux until the visible spectrum (hand-spectroscope) showed complete conversion into the cobalt complex (10 min.). The product was purified by chromatography on alumina and elution with methanol. A saturated methanolic solution (10 c.c.) of potassium cyanide was added to the methanolic eluate, and the solution was heated to the b. p., kept for 1 hr. at room temperature, and then chromatographed on alumina with ethanol as solvent. The *cyanide complex* (partial structure IX) formed purple needles (10 mg.) from ethanol–light petroleum (Found: N, 12·0. $C_{36}H_{42}CoKN_6O_3$ requires N, 11·9%), $\lambda_{max.}$ 215, 234, 273, 346, 435, 550, and 579 mμ (log ε 4·71, 4·68, 3·91, 4·57, 5·12, 4·16, and 3·74, respectively).

*5,5′-Dibromo-3,3′-diethyl-4,4′-dimethyldipyrromethene Hydrobromide.*—This salt was prepared as directed by Fischer, Halbig, and Walach.[15] The corresponding *zinc complex* formed orange-red needles, m. p. 219° (decomp.) (from chloroform–methanol) (Found: C, 43·1; H, 4·45; N, 6·65; Br, 38·0. $C_{30}H_{34}Br_4N_4Zn$ requires C, 43·0; H, 4·1; N, 6·7; Br, 38·15%), $\lambda_{max.}$ 235, 370, and 515 mμ (log ε 4·42, 3·97, and 4·85, respectively).

*3,4-Diethyl-1-2′-methoxycarbonylethyl-2,5,6,7,8-pentamethylporphyrin.*—5,5′-Dibromo-3,3′-diethyl-4,4′-dimethyldipyrromethene hydrobromide (5 g.), 4-2′-carboxyethyl-3,5,3′,4′,5′-pentamethyldipyrromethene hydrobromide (above; 2·5 g.) and succinic acid (80 g.) were fused together at 200° for 1 hr. The succinic acid was removed from the product by extraction with hot water, and the residue was methylated with methanolic hydrochloric acid. The *porphyrin* was purified as above and formed purple needles (200 mg.), m. p. >300° (Found: C, 75·4; H,

7·2; N, 10·8. $C_{32}H_{38}N_4O_2$ requires C, 75·8; H, 7·3; N, 10·5%), $\lambda_{max.}$ 241, 270, 398, 498, 533, 567, and 620 mμ (log ε 2·97, 2·94, 4·25, 3·16, 3·03, 2·85, and 2·72, respectively).

*Amide from* 3,4-*Diethyl*-1,2′-*methoxycarbonylethyl*-2,5,6,7,8-*pentamethylporphyrin and* 1-*Aminopropan*-2-*ol.*—The porphyrin methyl ester was treated with 1-aminopropan-2-ol to form the *amide*, obtained from chloroform–methanol as red-purple needles (86%), m. p. >300° (Found: C, 74·2; H, 7·3; N, 12·2. $C_{35}H_{43}N_5O_2$ requires C, 74·3; H, 7·6; N, 12·4%). The O-*acetyl derivative*, prepared by use of acetic anhydride in pyridine, formed purple needles (from chloroform–light petroleum) (42%), m. p. >300° (Found: N, 11·3. $C_{37}H_{45}N_5O_3$ requires N, 11·5%), $\lambda_{max.}$ 266, 399, 500, 535, 570, and 620 mμ (log ε 2·89, 5·16, 2·95, 2·86, 2·69, and 2·47, respectively). The *cobalt complex* of the amide formed microcrystalline red needles, m. p. >300° (from chloroform–light petroleum) (Found: C, 67·1; H, 6·2; N, 11·25. $C_{35}H_{41}CoN_5O_2$ requires C, 67·5; H, 6·6; N, 11·3%), $\lambda_{max.}$ at 267, 326, 392, and 564 mμ (log ε 4·24, 4·32, 5·45, and 4·44, respectively).

Treatment of the cobalt complex with potassium cyanide in the manner described above gave the *cobaltic cyano-hydroxy-complex* (88%) as purple needles (from methanol), m. p. >300° (Found: N, 12·0. $C_{36}H_{42}CoKN_6O_3$ requires N, 11·9%), $\lambda_{max.}$ 233, 279, 339, 410, 528, and 560 mμ (log ε 4·65, 3·93, 4·42, 5·14, 4·04, and 4·17, respectively).

*Reaction of the above Cyano-hydroxy-cobaltic-porphyrin Complex with* α-*Ribazole Cyclic Phosphate.*—The calcium salt of α-ribazole cyclic phosphate [6,7] (60 mg.) and the foregoing cyano-hydroxy-cobaltic-porphyrin complex (75 mg.) were dissolved in *NN*-dimethylformamide (1·5 c.c.), sodium t-butoxide (10 mg.) was added, and the mixture shaken until all had dissolved. After 2 hr., water (3 c.c.) was added and the precipitate separated and dried. The product was dissolved in chloroform (5 c.c.) and chromatographed on alumina with chloroform as eluant. Two porphyrin bands were collected, the first being unchanged starting material and the second yielding reddish-purple needles (14 mg.), m. p. >300° (from chloroform–methanol) (Found: N, 10·8. $C_{50}H_{58}CoN_8O_8P$ requires N, 11·3%), $\lambda_{max.}$ 279, 342, 411, 529, and 560 mμ (log ε 4·16, 4·57, 5·26, 4·20, and 4·33, respectively).

This *product* (X) (2 mg.) was hydrolysed with concentrated hydrochloric acid (0·1 c.c.) at 100° for 12 hr. in a sealed tube. After cooling and evaporation to dryness over solid potassium hydroxide, the residue was dissolved in a little water and extracted with chloroform. The dried chloroform extract was shown to contain a porphyrin carboxylic acid by examination of its spectra. The aqueous layer was evaporated, re-dissolved in a drop of water, and applied to two paper chromatograms with butan-1-ol–acetic acid–water (4 : 1 : 5) as the developing solvent. The first chromatogram gave a single purple spot, $R_F$ 0·34 (authentic 1-aminopropan-2-ol, $R_F$ 0·34), after spraying with ninhydrin. The second chromatogram gave a black spot, $R_F$ 0·31 (authentic D-ribose, $R_F$ 0·31), after spraying with ammoniacal silver nitrate.

We gratefully acknowledge the award of Maintenance Grants from Parke, Davis and Company Ltd. (to N. S.) and from the Department of Scientific and Industrial Research (to J. W. F. W.).

Department of Chemistry, The University, Nottingham. [*Received, January 5th*, 1962.]