754. *The Chemistry of the Bile Pigments. Part V.*[1] *The Stereoisomerism of Urobilins*

By W. J. Cole, C. H. Gray, and D. C. Nicholson

The possible sources of optical isomerism in urobilins are discussed in relation to asymmetry due to asymmetric carbon atoms, molecular asymmetry due to the dipyrrylmethene chromophore, and effects of the orientation of the end rings. The optical activity appears to be derived in part from molecular asymmetry outside the central dipyrrylmethene chromophore, and is accentuated by the Cotton effect, and also from asymmetry due to non-coplanarity of the central pyrrole nuclei.

This and the subsequent Paper describe investigations into the source of the high optical activity of the urobilins and the still higher activity of their hydrochlorides (Table 1).

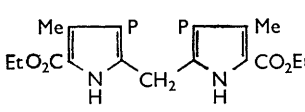
Table 1

Specific optical rotation in chloroform of the urobilins, their hydrochlorides, zinc complexes, and hydrogenated derivatives

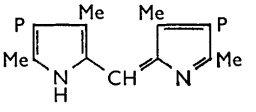
	Free pigment [2]	Hydrochloride [2,3]	Zinc [2] complexes *	Hydrogenated [4] derivatives
Stercobilin	−870	−4000	+467	−17
(+)-Urobilin †	+910	+5000	+740	+74
(+)-Urobilin IXα †	Not known	+5000	+1420	Not known

* Calculated for a pigment complex containing a ratio of one pigment molecule to one metal atom.
† The pigments previously called *d*-urobilin and *d*-urobilin IXα are here named (+)-urobilin and (+)-urobilin IXα since the absolute configuration of the molecules is unknown.

In the urobilins the main chromophore is the central dipyrrylmethene unit, which in the urobilinogens is reduced to a dipyrrylmethane unit. On classical views, the central dipyrrylmethene group of the urobilins, like simple dipyrrylmethenes, would be considered planar and well stabilised by resonance. No evidence has hitherto been provided of any structural factor in the urobilinogens which prevents the two middle rings assuming coplanarity. Atomic models of the urobilinogens and of the analogous simpler dipyrrylmethane (I) show that there is steric interaction between the central methane bridge and

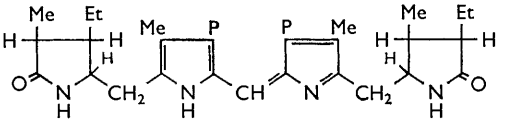


(I)



(II)

(P = $CH_2 \cdot CH_2 \cdot CO_2H$)



(III)

the proximal carbon atom in one β-propionic acid group. This effect is small in comparison with that between *o*-methyl substituents in diphenyl compounds, but the present work suggests that it is sufficiently large to cause non-coplanarity of the middle rings in urobilinogens. Free rotation of the two central pyrrole rings about the central methane bridge is hindered also by interaction of the methane group and the imino-group of the adjacent ring, but this effect is minimal when the rings are nearly coplanar. Similar effects probably occur also between each ring of the end pairs of pyrrole nuclei in the

[1] Part IV, C. H. Gray, A. Kulczycka, and D. C. Nicholson, *J.*, 1961, 2276.
[2] C. H. Gray, D. C. Nicholson, and W. J. Cole, *J.*, in preparation.
[3] C. H. Gray and D. C. Nicholson, *J.*, 1958, 3085.
[4] P. T. Lowry, R. Cardinal, S. Collins, and C. J. Watson, *J. Biol. Chem.*, 1956, **218**, 641.

urobilinogens. Consequently restricted rotation of these rings about the bonds joining them to the methylene bridge carbon atoms would cause the end rings to assume *cis*- and *trans*-configurations relative to one another and to the central dipyrrylmethane structure. The resulting asymmetry from these loci may also contribute to optical activity. Accurate atomic models of the urobilins cannot be constructued because of the hybrid nature of the carbon atoms taking part in the methene bridge. However, the bond-lengths of the methene bridge carbon atoms must be less than those in a methane bridge, and this ensures that the steric effects described above are enhanced.

The urobilins and urobilinogens have asymmetric carbon atoms at the two innermost α-positions of the terminal pyrrolenone rings. Stercobilin and stercobilinogen have additional asymmetric carbon atoms at the four β-positions of the terminal rings. The high optical activities of the urobilins compared with those of the corresponding urobilinogens suggests that the dipyrrylmethene chromophore is responsible for much of the optical activity of the urobilins. Since the dipyrrylmethene chromophore absorbs light at a wavelength close to that of the *D*-line of sodium, much of its contribution to optical activity is due to an induced Cotton effect.[5]

The molecular asymmetry in the urobilins and urobilinogens may, therefore, arise from non-coplanarity of the two middle rings, geometric isomerism due to a variable orientation of the end rings relative to each other and to the central dipyrryl structure, and from the presence of asymmetric carbon atoms. Each of these factors may contribute to the optical activity, their total effect being greatly enhanced in the urobilins by optical absorption due to the dipyrrylmethene chromophore. The much lower activity of the urobilinogens arises because this Cotton effect is missing.

Optical Activity in Formally Symmetrical Dipyrryl Compounds.—To obtain evidence of contributions to optical activity by the central dipyrryl unit of urobilins and urobilinogens, simple dipyrrylmethene and methane without asymmetric carbon atoms have been synthesised, and their resolution attempted.

The dipyrrylmethane (I) was synthesised by known routes [6] and was resolved as the quinidinium salt. The solutions obtained on addition of the methane to hot ethanolic (+)-quinidine failed to crystallise on cooling. Partial evaporation gave a thick, viscous oil which was rendered granular by trituration with ether and this was crystallised in successive fractions from butyl acetate. The less soluble salt was recrystallised to a constant rotation, $[\alpha]_D$ +121° in butyl acetate, as was the more soluble salt $[\alpha]_D$ +128° (in butyl acetate). Elemental analyses showed these presumed diastereoisomers to be composed of one molecular part of alkaloid to one of dipyrrylmethane. Decomposition of these salts, removal of free quinidine, and repeated recrystallisations then gave enantiomorphic methanes, $[\alpha]_D$ −2·3 and +2·5°, respectively, both in ethanol. The mother-liquors from the recrystallisations of the methanes underwent spontaneous oxidation to the corresponding methenes, which showed considerably greater optical activity. The observed rotations for solutions of the methane never exceeded ±0·05° but those for the methene were −0·30 and +0·50°. A preparative oxidation of the dipyrrylmethane to dipyrrylmethene was not achieved and a quantitative assessment of the optical activity of the latter could not therefore be made.

Only limited optical rotatory dispersion studies of the enantiomorphic methanes were possible (the Figure). Each isomer retained its original sign of rotation between 400 and 600 mμ, and, although not of the same magnitude, the molecular rotation increased with decreasing wavelength.

Resolution of the dipyrrylmethene (II) was attempted but the brucine and quinidine salts were obtained always as highly insoluble, brick-red precipitates accompanied by very soluble green gums. Elementary analyses of the pure salts indicated a composition of one

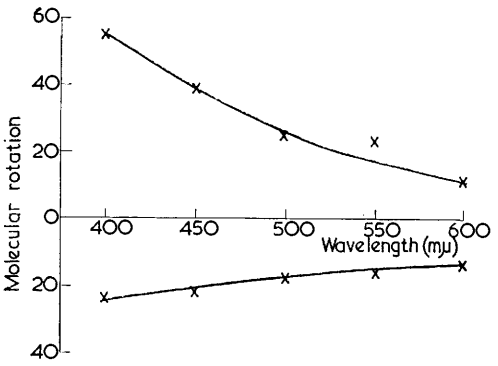
[5] C. H. Gray, P. M. Jones, W. Klyne, and D. C. Nicholson, *Nature*, 1959, **184**, 41.
[6] H. Fischer and H. Andersag, *Annalen*, 1926, **450**, 217.

molecular part of alkaloid to one of methene, the original hydration of the alkaloid being retained in each case.

The brucine salts gave no optically active isomer of (II) on decomposition but the insoluble quinidine salt treated similarly provided an isomer of $[\alpha]_D$ +50° (in chloroform). Attempts to prepare a more highly active methene by further purification of the quinidine diastereoisomer were unsuccessful owing to its extreme insolubility in all solvents.

Stereochemistry of the Urobilins.—Stercobilin. The resolution of the dipyrrylmethane representative of the central unit of the urobilinogens, and the observation of optical activity in the corresponding methene, shows that these units may contribute to the optical activity of the urobilins.

In the bis-lactam structure of stercobilin (III) there exists, in addition to the dipyrrylmethene unit, no less than six obvious loci of molecular asymmetry. All the combinations of effects from these loci, with a resultant lævorotation, are possible. These combinations



Optical rotatory dispersion by the enantiomorphic methanes (I) (70 mg.) in ethanol (6 ml.), with a 2·5-cm. tube

may be associated with a lævorotatory, a dextrorotatory, or even a racemic dipyrrylmethene unit.

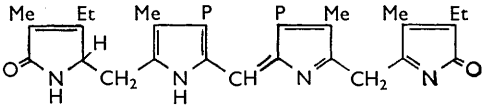
Little is known about the individual optical properties of the β-carbon atoms of the end rings. Earlier work [3] has shown the β-ethyl-β-methylsuccinimide, obtained by the degradation of stercobilin, to be weakly dextrorotatory. Whether this imide is a stereochemical individual, or a mixture of optical isomers, is unknown, and it is impossible to test the latter possibility as the compound bears no acidic or basic group which could be used for an attempted optical resolution. Thus the four β-asymmetric carbon atoms in all probability together contribute a dextrorotation to the optical activity of stercobilin, but the possibility of a lævo-contribution from one or more of these must be borne in mind.

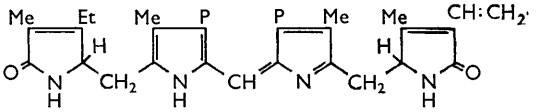
(+)-Urobilin and (+)-urobilin IXα. (+)-Urobilin and (+)-urobilin IXα are much simpler stereochemically than stercobilin. Structure (IV) for (+)-urobilin could display optical activity from the dipyrrylmethene chromophore and from the single asymmetric carbon atom present in one end ring. In the alternative structure (V) there is an additional asymmetric carbon atom.

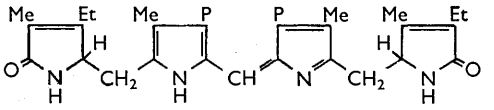
(+)-Urobilin IXα is derived from (+)-urobilin by catalytic hydrogenation; it has optical properties similar to those of (+)-urobilin and is probably an isomer of i-urobilin.[3] The specific rotation would be unaffected by the reduction of the vinyl group in structure (V), which already possesses two asymmetric carbon atoms. Reduction of structure (IV), which has only one asymmetric carbon atom, would introduce an additional asymmetric carbon atom which, however, would certainly be racemised. The identical optical rotation of (+)-urobilin and (+)-urobilin IXα is thus to be expected.

An interesting difference between stercobilin and (+)-urobilin is the ease of racemisation of the latter in alkali; the slight increase in optical activity [2] of stercobilin under similar conditions is probably due to racemisation of a dextrorotatory centre external to the chromophore. This observation is not accommodated by the simple theories given

above, for the dipyrrylmethene chromophores in the two pigments should be equally susceptible to racemisation. It suggests rather that the characteristic rotation of each pigment might arise largely from the α-asymmetric carbon atoms in the end rings, and that the contribution by the dipyrrylmethene group may be relatively small apart from its Cotton effect. The optical activity of the dipyrrylmethane group is even smaller than


(IV)


(V)


(VI)

that of the dipyrrylmethene chromophore, and this, with the absence of a Cotton effect, accounts for the low optical rotation of the urobilinogens. In (+)-urobilin the α-hydrogen atom attached to the unsaturated system of one end ring is acidic; the ready formation of a resonating anion then causes racemisation due to planarisation of this part of the molecule. In stercobilin the corresponding carbon atoms are attached to saturated groups, and planarisation and racemisation due to a similar mechanism are impossible. However, the loss of optical activity on metal-complex formation [2] is explicable only on the basis of a substantial contribution of the dipyrrylmethene chromophore to the optical activity. The cause of the difference in the ease of racemisation as between (+)-urobilin and stercobilin therefore remains unknown.

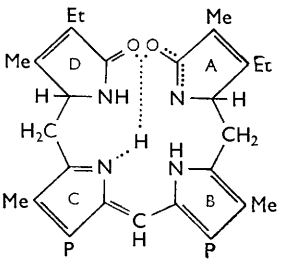
I-urobilin. The stereoisomeric status of i-urobilin is obscure. The bishydroxypyrrole structure postulated by Fischer and seemingly confirmed by Siedel [7] does not allow for optical activity except that due to the dipyrrylmethene chromophore. The modified bislactam structure (VI) contains two asymmetric carbon atoms, which also might impart some optical activity to this pigment. Since (+)-urobilin racemises so readily in alkali,[3] and since i-urobilin has been obtained only by a process involving the preliminary reduction of bilirubin in alkaline solution, it is possible that the pigment is a racemate. To test this it was prepared by the catalytic reduction of bilirubin in neutral solution, followed by oxidation with ethereal iodine. The product was optically inactive at 589 and 546 mμ. Molecular asymmetry is therefore lacking in bilirubin, which is optically inactive, and so the production of optically active urobilins would seem to depend upon the asymmetric activities of enzymes.

The hydrochlorides of the optically active urobilins show much higher specific rotation in chloroform than in methanol; [8] the values for the free pigments are much smaller than for the hydrochlorides, but again they are greater in chloroform than in methanol. In the hydrochloride, co-ordination of a proton or protons at one or both of the oxo-groups in the end rings might lead to monolactim or bislactim structure. The monolactim could result in a hydrogen-bonded entity such as (VII). Since ring A probably has a staggered configuration relative to the rest of the molecule it might, *via* the hydrogen bond in the hydrochloride, enhance the asymmetry in the molecule. Mutual repulsion of the two protons in a bislactim structure might also increase asymmetry. This effect would be

[7] W. Siedel and S. Meier, *Z. physiol. Chem.*, 1936, **242**, 101.
[8] C. H. Gray, " The Bile Pigments," Methuen Monographs, London, 1953.

greater in chloroform than in methanol owing to competition by the latter solvent for the proton in hydroxonium-ion formation.

Possible Stereochemistry of Bile Pigments Other than Urobilins.—Bile pigments other



(VII)

than the urobilins may possess, or be capable of possessing, optical activity. The α-asymmetric carbon atoms present in the end rings of urobilins have been credited, in part, with causing the optical activity of these pigments. However, violins and rhodins, when structurally represented by bislactam formulæ, possess α-asymmetric carbon atoms in one terminal ring.[3] Normally these pigments are derived from i-urobilin and would be expected to be inactive; these should, however, be resolvable. Alternatively, if they are produced from an optically active urobilin, such as (+)-urobilin IXα, one α-asymmetric carbon would remain intact; they should therefore exhibit optical activity. We have been unable to observe this, because of the broad absorption band for these pigments in the region of the wavelength of the light used.

Experimental

Diethyl 4,4′-dimethyl-3,3′-di-(2-carboxyethyl)dipyrrylmethane-5,5′-dicarboxylate (I) and 3,5,3′,5′-tetramethyl-4,4′-di-(2-carboxyethyl)dipyrrylmethane hydrochloride (II) were prepared by Fischer's methods.[6,9]

Optical Resolutions of Dipyrrolic Compounds.—Resolution of diethyl 4,4′-dimethyl-3,3′-di-(2-carboxyethyl)dipyrrylmethane-5,5′-dicarboxylate (I). Quinidine sulphate (20 g.) was suspended in water (1500 ml.), the mixture being warmed to effect complete solution. The solution was made alkaline with 4N-sodium hydroxide, and the resulting precipitate of free base was crystallised from ethanol (yield 96%); it had m. p. 168—169°, $[\alpha]_D$ +187° (in ethanol).

The dipyrrylmethane (3·89 g.) was added in portions to a solution of quinidine (12·46 g.) dissolved in stirred boiling ethanol. Crystallisation in five stages of evaporation afforded a colourless compound (6·9 g.) composed almost entirely of free quinidine, m. p. and mixed m. p. 167°. Trituration with ether of the viscous oily mother-liquor afforded crystals (4·07 g.) of the required quinidine salt. Fractional crystallisation from n-butyl acetate gave a less soluble crop (2·99 g.), and on complete evaporation of the mother-liquor, a more soluble crop (0·67 g.).

The less soluble crop, recrystallised from n-butyl acetate, attained a constant rotation, $[\alpha]_D$ +121° (in n-butyl acetate), and had m. p. 131—134° [Found: C, 65·8; H, 7·15; N, 7·0. Calc. for 1 quinidine : 1 methane($C_{43}H_{54}N_4O_{10}$): C, 65·6; H, 6·9; N, 7·1%].

The more soluble salt, recrystallised from n-butyl acetate to a constant rotation, $[\alpha]_D$ +128° (in n-butyl acetate), had m. p. 128—130° [Found: C, 66·5; H, 7·0; N, 7·15. Calc. for 1 quinidine : 1 methane($C_{43}H_{54}N_4O_{10}$): C, 65·6; H, 6·9; N, 7·1%].

The quinidine salts suspended in water (50 ml.) were decomposed by addition of concentrated hydrochloric acid (4 ml.). The precipitated enantiomorphic dipyrrylmethanes were filtered off and washed many times with water to remove free quinidine hydrochloride. The dipyrrylmethanes were crystallised from ethanol to constant rotation (both in ethanol) of $[\alpha]_D$ −2·3°, m. p. 198—199°, and +2·5°, m. p. 196—198°, respectively. For polarimetry a 10-cm. tube was used. During crystallisation of the optically active methanes, the mother-liquors

[9] H. Fischer and K. Morgenrath, *Annalen*, 1928, **466**, 165.

Attempted resolutions of 3,5,3′,5′-tetramethyl-4,4′-di-(2-carboxyethyl)dipyrrylmethene (IV) using (+)-quinidine

A. Quinidine (3 moles, 5·1 g.) in hot ethanol (110 ml.) + dipyrrylmethene HCl (1 mole, 2 g.) in hot ethanol (100 ml.)

Less soluble fraction (1·49 g.), m. p. 224—225° (Found: C, 64·6; H, 7·5. 1 Quinidine : 1 methene ($C_{39}H_{48}N_4O_6,2\frac{1}{2}H_2O$) requires: C, 65·6; H, 7·4%). Decomp. with 10% HCl; pigment extracted with (*a*) $CHCl_3$, (*b*) ethyl acetate. Evaporated extracts were precipitated in petrol (b. p. 80—100°) from n-butanol: 84·8 mg., m. p. 225°, $[\alpha]_D$ (in $CHCl_3$) +50°.

More soluble fraction evaporated to small volume.

Green gum with free quinidine given by evaporation of a petrol (b. p. 40—60°) solution of filtrate, successively triturated with $CHCl_3$, decomp. with HCl, neutralised with Na_2CO_3, extracted with $CHCl_3$.

$CHCl_3$ *extract*, re-extracted into Na_2CO_3 solution. Residual $CHCl_3$ solution inactive. The green aqueous layer turned yellow overnight. Quinidine removed in $CHCl_3$. Remaining product, after neutralisation (HCl), extracted into ethyl acetate; α in $CHCl_3$ (100 ml.) −0·07°.

Green aqueous layer made just alkaline (HCl) and extracted with ethyl acetate.

Residue from green ethyl acetate extract redissolved in $CHCl_3$ (200 ml.); 30 ml. diluted to 50 ml. gave α −0·22°.

Aqueous phase neutralised and extracted with ethyl acetate gave inactive red and yellow pigment fractions.

Crystals (1·87 g.), m. p. 158°, triturated with cold acetone gave free quinidine. Acetone-soluble material redissolved in ethanol (100 ml.) and diluted (×4) gave $[\alpha]_D$ +177°.

Chromatography: $CaCO_3$, elution with $CHCl_3$ gave incomplete separation. Effluent (50 ml.) decomposed with HCl, the aqueous phase then being neutralised with 2N-K_2CO_3. The green chloroform phase was now re-extracted with Na_2CO_3 solution, acidification (HCl), extraction into ethyl acetate, and evaporation gave a residue: α −0·07° (in ethanol, 50 ml.); α −0·09° (in $CHCl_3$, 25 ml.).

B. *d*-Quinidine (3 moles, 1·110 mg.) in hot ethanol (70 ml.). + dipyrrylmethene HCl (1 mole, 380 mg.) in ethanol (70 ml.).

Less soluble fraction: brown (260 mg.), m. p. 220° (decomp.) (Found: C, 64·6; H, 7·0; N, 8·1. 1 Quinidine : 1 methene ($C_{39}H_{48}N_4O_6,2\frac{1}{2}H_2O$) requires: C, 65·6; H, 7·4; N, 7·8%). Theoretical quantity of N-HCl (0·33 ml.) added to salt (177 mg.) suspended in ethanol (10 ml.) to remove quinidine. Further ethanol (10 ml.) added; yellow needles (167 mg.), m. p. >300°, precipitated in petrol (b. p. 80—100°; 150 ml.). Product in hot ethanol diluted with chloroform.

Insoluble material (23 mg.): in $CHCl_3$ was inactive.

Green filtrate evaporated in stages gave: (1) 1·7 mg., dissolved in $CHCl_3$ (15 ml.), α = −0·03°; (2) 3·3 mg., dissolved in $CHCl_3$ (15 ml.), α = −0·03°; (3) 45·1 mg., inactive in $CHCl_3$; (4) 41 mg., inactive in $CHCl_3$.

Aqueous extract, extracted with $CHCl_3$ and $CHCl_3$ extracted with (*a*) water (yellow), acidified with 10% HCl (50 ml.), extracted with $CHCl_3$, evaporated to 15 ml., α = −0·06°; (*b*) 10% HCl, yellow pigment extracted into $CHCl_3$ was inactive. The residual $CHCl_3$ solution evaporated to 15 ml. had α = −0·06°.

More soluble fraction: in small volume, decomposed by conc. HCl (20 ml.) diluted in 300 ml., extracted into n-butanol (green) and re-extracted into water.

n-Butanol solution: equal volume of ethanol added, diluted with much water, extracted into CHC_3, and re-extracted into water.

$CHCl_3$ *phase* (apple green): extracted with Na_2CO_3, aq. soln. being re-extracted into $CHCl_3$. Both $CHCl_3$ solns. united (yellow) (A, below).

Aqueous extract made slightly alkaline, extracted with (*a*) $CHCl_3$ (B, below), (*b*) n-butanol (C, below).

Solutions A, B, and C united, evaporated and chromatographed.

Chromatography (de-acidite G, acetate form: eluted with (*a*) ethanol, inactive; (*b*) 1 : 20 (v/v) acetic acid–ethanol; (*c*) 1 : 5 (v/v) acetic acid–ethanol (green), inactive.

A dextrorotatory isomer ($[\alpha]_D$ +50°, in chloroform) was obtained from the less-soluble diasterioisomer (Scheme A), and a lævo-rotatory isomer was detected in solutions obtained from the more soluble fraction. Extreme insolubility of (+)-(+)-diasterioisomer precluded further resolution of the (+)-methene, and extreme solubility of the (±)-diasterioisomer precluded isolation of the (−)-methene (Schemes A and B).

underwent spontaneous oxidation to the corresponding red methenes, the solutions then showing enhanced lævo- and dextro-rotations of $-0{\cdot}35$ and $+0{\cdot}55°$, respectively. Rotations observed for the colourless methanes were only about $\pm 0{\cdot}05°$.

Polarimetry.—This was carried out with a Hilger M.375 polarimeter. The zero rotation (an average of ten readings) for sodium light was checked before each experiment. The observed rotation for each solution examined was also obtained as an average of ten readings. Unless otherwise stated a 20-cm. polarimetry tube was used, and polarimetry was carried out between 20 and 25°.

Optical Rotatory Dispersion.—A Bellingham and Stanley spectropolarimeter was used with a tungsten source; reproducible readings were obtainable down to 0·002°. (−)-Dipyrrylmethane (68·9 mg.) and (+)-dipyrrylmethane (70 mg.) each dissolved in ethanol (6 ml.) were contained in a 10-cm. tube. Error for $[M]_D$ ±0·02°.

Optical Stability of Stercobilin and Stercobilinogen in Alkali.—(*a*) Stercobilin (9·7 mg.) in 5N-sodium hydroxide (50 ml.) was reduced with a stream of hydrogen, in the presence of Adams catalyst (20 mg.), the solution being diluted with methanol (5 ml.) to prevent frothing. Further catalyst (20 mg.) was added after 30 min. The solution was heated (50°) for 45 min. with continued passage of hydrogen. The colourless solution thus obtained was filtered, neutralised (Congo Red), and shaken with ethanolic iodine (10 mg./20 ml.), the stercobilin hydrochloride then being extracted into chloroform. The recovered pigment (1·89 mg.) in chloroform (100 ml.) had $[\alpha]_D$ −4500°.

(*b*) Stercobilin (12·8 mg.) was added slowly to molten sodium hydroxide (1 g.) containing a trace of water. The reactants were kept molten for 1½ min. and diluted with water to 70 ml.; concentrated hydrochloric acid (6 ml.) was added, and pigment extracted into chloroform (150 ml.). The recovered pigment (9 mg.) had $[\alpha]_D$ −4700°.

The Preparation of I-urobilin by Catalytic Reduction of Bilirubin.—Bilirubin (10 mg.) in chloroform (6 ml.) and ethanol (20 ml.) was reduced with a stream of hydrogen in the presence of Adams catalyst (25 mg.). Further catalyst (30 mg.) was added after 20 min., the solution then turning pale yellow in 45 min. Evaporation of the filtered solution gave colourless crystals of mesobilirubinogen, identified by their intense reaction with Ehrlich reagent and ready oxidation to urobilin. These in ether (50 ml.)–water (50 ml.) mixture were oxidised by shaking with ethanolic iodine (4·37 mg. in 14 ml.). The ether was evaporated without separation, leaving an aqueous solution and considerable solid, both containing urobilin. All pigment was converted into hydrochloride by addition of 33% hydrochloric acid (4·5 ml.) and extracted into chloroform. Crude i-urobilin hydrochloride thus obtained was crystallised from chloroform ($\lambda_{max.}$ in $CHCl_3$, 499 mμ). Absence of an absorption maximum at 540 mμ showed absence of violins. With a mercury vapour lamp, an E.T.L., N.P.L. automatic polarimeter optical unit, an E.T.L., N.P.L. control unit type 143A, and a "Honeywell," Brown Electronik automatic recorder, a chloroform solution of isourobilin was found by Dr. Capon of Birkbeck College to be inactive, analogous to readings with sodium light.

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