chloric acid secretion, but that the amount of carbonic anhydrase required is only a very small proportion of the total potential activity.

We wish to thank Prof. H. A. Krebs, F.R.S., for advice during the course of this work. One of us (J.E.) is indebted to the Agricultural Research Council for a grant.

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Isolation of Unequivocal Uroporphyrin III

A FURTHER STUDY OF TURACIN

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No authentic specimen of uroporphyrin III has until now been prepared either synthetically or from natural material.

The first uroporphyrin to be isolated was that from urine of the congenital porphyria patient Petry. Fischer (1915) originally considered it to be a heptacarboxylic porphyrin, but later (Fischer & Hilger, 1925) established that it was an octacarboxylic acid which could be partially decarboxylated, losing four acid groups and yielding coproporphyrin I, by heating with hydriodic acid in acetic acid or with 1% hydrochloric acid at 180–190° (Fischer, 1916; Fischer & Zerweck, 1924). This reaction established that it belonged to the isomeric series derivable from aetioporphyrin I, and Fischer concluded that it had the structure, porphin-1:3:5:7-tetra-acetic acid-2:4:6:8-tetrapropionic acid since comparison with a synthetic '*iso*uroporphyrin I' having four methyl and four methylmalonic acid β side chains and with a similar porphyrin having succinic acid instead of methylmalonic acid groups (Fischer & Zischler, 1937; Fischer & Hofmann, 1937) eliminated these structures as constitutional possibilities.

Uroporphyrin I, isolated from bones and urine of congenital porphyria patients and from pearl oyster shells, *Pteria vulgaris* (Fischer & Haarer, 1932), and the pigment from turacin, stated to be uroporphyrin I (Fischer & Hilger, 1924), was the only uroporphyrin known (although the melting points of the methyl esters of different preparations were widely variable from about 273 to 293°) when almost simultaneously, Waldenström, Fink & Hoerburger (1935; see also Waldenström, 1934, 1935, 1936) and Mertens (1936, 1937) claimed that the urine of sufferers from acute porphyria contained another uroporphyrin, the octamethyl ester of which melted at about 258°, and in the products of the decarboxylation of which

coproporphyrin III could be identified. It was thus regarded as uroporphyrin III belonging to the aetioporphyrin III series of position isomers.

About the same time, Rimington (1936) reported the study in South Africa of a living bovine case of congenital porphyria and the chromatographic separation upon alumina with dioxan as solvent of its urine and bone uroporphyrins into a fraction with m.p. 293° and another melting at 260–261°. It was suggested that the latter might be uroporphyrin III.

The homogeneity of the Waldenström ester from acute porphyria urines was disputed by Grinstein, Schwartz & Watson (1945) and Watson, Schwartz & Hawkinson (1945) who claimed that in spite of an apparently constant melting point of the crystalline material, it could in some cases (Type A Waldenström ester) be resolved by chromatography upon calcium carbonate into uroporphyrin I (ester m.p. 284°), yielding coproporphyrin I on decarboxylation, and a heptacarboxylic porphyrin (ester m.p. 208°) which apparently belonged to the III series, since on decarboxylation it yielded coproporphyrin III. Even in the case of esters which were not resolvable by chromatography (Type B Waldenström esters), it was claimed that decarboxylation indicated the same mixture or molecular association of I and III series porphyrins. Support for these claims has been proffered by Prunty (1946) and McSwiney, Nicholas & Prunty (1950). It followed that the porphyrin described by Waldenström and Mertens was not to be regarded as uroporphyrin III. Fischer & Hofmann (1937) applied adsorption chromatography, after Waldenström's publication, to a large quantity of 'uroporphyrin I' from Petry urine and claimed to have separated it into a uroporphyrin I ester melting at 302° (corr. 311°) and an ester of m.p. 261° which they regarded as identical with Waldenström's uroporphyrin III (mixed melting point determination). It was not further characterized.

The uroporphyrin copper complex 'turacin' (Church, 1869), which forms the red pigment in the flight feathers of several *Turacos* and related species of birds, was studied by Fischer & Hilger (1923, 1924), who concluded that it was a derivative of uroporphyrin I (comparison of melting point with that of uroporphyrin I; decarboxylation not performed). A re-investigation of turacin by one of the present writers (Rimington, 1939) produced unequivocal evidence that it yielded coproporphyrin III on decarboxylation and had therefore to be regarded as a series III isomer.

The ester of the uroporphyrin obtained by removal of copper from turacin was very difficult to crystallize, as Fischer & Hilger had found previously, but it is clear that if it could be properly purified it would provide for the first time a reference sample of authentic uroporphyrin III.

The present investigation was undertaken with two objects in view: (1) to obtain pure uroporphyrin III from turacin and to characterize it chemically and physically, and (2) to search for other porphyrins accompanying uroporphyrin III in turacin preparations.

Below, we present evidence that the main porphyrin derivable from turacin (mixed from several species of bird, but see Rimington, 1939) is indeed uroporphyrin III, melting point of octamethyl ester 264° and yielding coproporphyrin III on decarboxylation; it is accompanied by another porphyrin analysing as an 8 carboxyl porphyrin, but with octamethyl ester of melting point 210°, which we suggest may be a porphin octa-acetic acid. When decarboxylation of this pigment was attempted, employing the usual conditions, it proved to be unstable, the reaction mixture being colourless (except for some suspended material) and exhibiting only a trace of fluorescence.

The preparation contained, in addition, small quantities of a dichroic porphyrin, resembling spectroscopically a phylloporphyrin, and a chlorin-like material. These are regarded as chemical artifacts arising during the sodium-amalgam reduction and re-oxidation employed to remove the copper from turacin. Similar products have been obtained by subjecting a synthetically prepared copper uroporphyrin I complex to the same procedures.

MATERIALS AND METHODS

Feathers. These were obtained from the same material as used by Rimington (1939). Since all eleven species of birds had been shown to contain turacin, yielding coproporphyrin III on decarboxylation, the feathers from the different specimens were mixed.

Paper chromatography was performed according to Nicholas & Rimington (1949, 1951).

Adsorbents and solvents were prepared according to Nicholas (1951). Unless otherwise stated, the MgO used was grade III, and the Al_2O_3, grade IV.

Melting points were recorded upon an electrically heated micro-melting point stage using a calibrated thermometer.

Spectral absorption was measured with a Beckman photoelectric spectrophotometer, model DU, or on a Beck-Hartridge reversion spectroscope.

Decarboxylations were carried out in sealed Pyrex tubes in a thermostatically controlled, covered oil bath.

EXPERIMENTAL

Preparation of turacin. The red portions of the feathers were defatted by extraction with ether in a Soxhlet apparatus. Weight 6·68 g. They were steeped in successive changes of 1% (w/v) aqueous NH_3 until the extract was practically colourless. The deep-red turacin solution showed two bands only; there were no bands in the region of 635 mμ.

(see later). By addition of dilute HCl to pH 3·1, the pigment was precipitated. It was collected on the centrifuge and reprecipitated twice more under the same conditions. The last supernatant fluid was of pale straw colour.

Removal of copper. The turacin was dissolved in 0·1 N-NaOH and 200 g. of 2·5 % sodium amalgam added in small portions at a time, shaking on a machine between each addition. The colourless mixture was filtered through a folded paper and the porphyrinogen re-oxidized by drawing a stream of air through the liquid overnight. By adjustment to pH 3·1, the uroporphyrin was precipitated; it was purified by reprecipitating twice. The first and second supernatants were greenish brown in colour and showed a strong, broad absorption band at about 620 mμ. The third supernatant contained a trace only of this component.

Isolation of a chlorin-like pigment

The supernatant fluids, referred to above, from precipitation of the uroporphyrin were combined and shaken with kieselguhr which adsorbed the substance having the strong absorption band in the red. The kieselguhr was washed, then

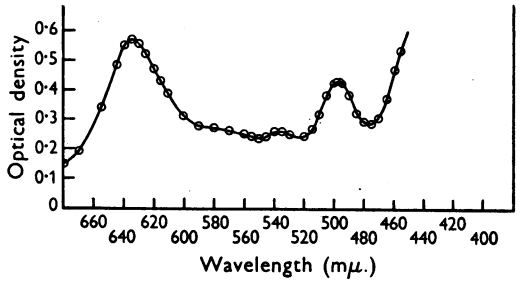

Fig. 1*a*.

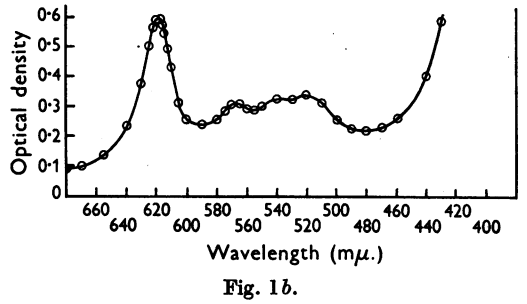

Fig. 1*b*.

Fig. 1. Spectral absorption of chlorin-like pigment isolated from mother liquors after isoelectric precipitation of turacin porphyrin. (*a*) In 0·1 N-NaOH; (*b*) in dilute HCl.

the pigment eluted with dilute ammonia, affording a greenish solution. Shaking with ethyl acetate removed the pigment from the acidified solution (pH 3·1) and from this solvent it passed back into dilute HCl. It was readsorbed upon a small quantity of kieselguhr (pH 3·1) and, after drying, this was treated with methanol saturated with HCl to esterify the pigment. The ester was extracted with $CHCl_3$ in the usual way and crystallized from $CHCl_3$-methanol. Recrystallization from benzene-light petroleum afforded greenish-brown needles. The spectral absorption of the free pigment was measured in 0·1 N-NaOH and in dilute HCl (see Fig. 1). The high band in the red in both acid (619 mμ.) and alkaline (635 mμ.) media suggests a chlorin structure for this material. Paper chromatography revealed the presence of material with 8 carboxyl groups. In addition to the two fluorescent spots characteristic of uroporphyrin (Nicholas & Rimington, 1949) this pigment and many of the others encountered in the present study of turacin exhibited a third faint spot lying below but very close to the uroporphyrin pair, i.e. there was a triple rather than a double spot system. The third spot was not sufficiently far down to correspond to a 7 carboxyl porphyrin.

Formation of a chlorin-like pigment by reduction of synthetic copper uroporphyrin

As it seemed likely that the chlorin could have been formed during the reduction of natural turacin, a similar experiment was carried out upon chemically prepared copper uroporphyrin. 1·50 g. of 2·5 % sodium amalgam was added to the pigment dissolved in 25 ml. of 0·1 N-NaOH. After filtration, air was drawn through the solution until reoxidation had occurred and the porphyrin was precipitated at pH 3·1. The supernatant fluid was greenish and contained the same chlorin-like material as previously encountered (red band at 635 mμ. in 0·1 N-NaOH) together with some unprecipitated uroporphyrin.

Esterification and preliminary purification of the crude porphyrin

The precipitated porphyrin was dried *in vacuo* and left for 12 hr. in contact with methanol saturated with HCl. An equal volume of $CHCl_3$ was then added, together with sufficient saturated aqueous sodium acetate (rapidly) to neutralize the mixture to congo red. Much water was then added, and after thorough shaking the $CHCl_3$ layer removed, washed with dilute ammonia, then water, filtered and evaporated to dryness. The residue of crude ester (475·3 mg., m.p. 253°) was chromatographed upon a column of MgO packed in $CHCl_3$, using $CHCl_3$-methanol (100:0·5) for development. A small, dark residue was left on the top of the column and discarded, the porphyrin passing through with an indication of separation into two zones. It was recovered from the solvent, and rechromatographed upon a large MgO column (1 ml. adsorbent/mg. porphyrin) again using 100:0·5 $CHCl_3$-methanol. Three zones appeared: I, a lower band containing the majority of the pigment; II, an intermediate, rather diffuse band; and, III, an upper zone surmounted by a small layer of non-porphyrin material on the surface of the adsorbent. Each of these fractions was further purified, as described below (see also Fig. 2).

Purification of zone I. Isolation of turacin porphyrin A

This material was rechromatographed three more times on MgO. The porphyrin moved as a compact, mobile band leaving in the first two columns a slightly greenish residue on the adsorbent. The porphyrin was then crystallized from hot $CHCl_3$-methanol, affording rather poorly formed hair-like needles, m.p. 257–262°. Successive recrystallizations from benzene-light petroleum afforded a product with constant m.p. 264° and good crystalline form (see Fig. 3), as follows: 1st recrystallization from benzene-light petroleum, m.p. 259–264°; 2nd recrystallization from benzene-light petroleum, m.p. 264°; 3rd recrystallization from benzene-

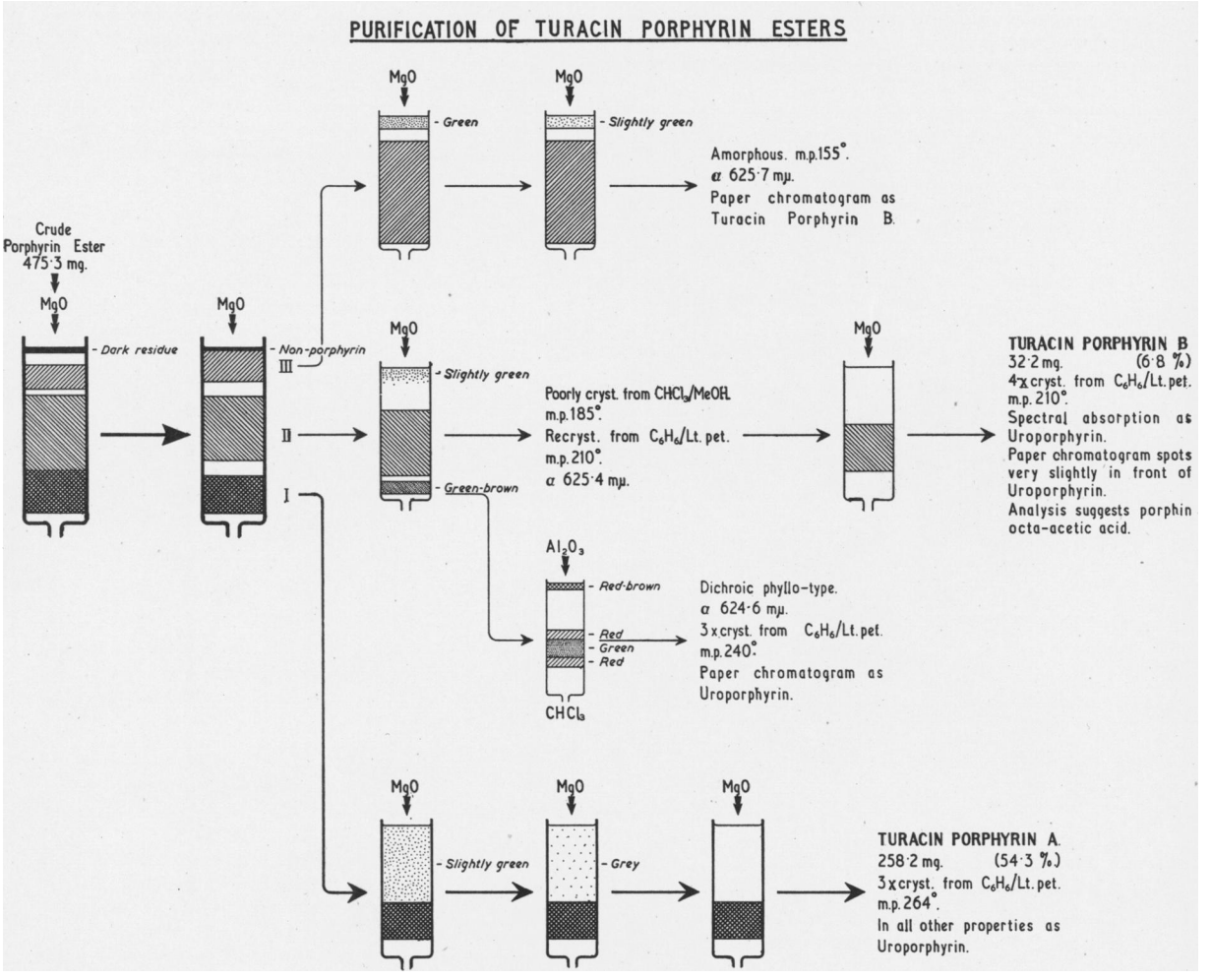

Fig. 2. Schematic representation of purification of turacin porphyrin.

Table 1. *Absorption maxima and relative intensities of turacin porphyrin esters A and B compared with those of uroporphyrin I ester and a Waldenström ester*

	Band I		Band II		Band III		Band IV		Soret band	
	mμ.	Relative intensity	mμ.	Relative intensity	mμ.	Relative intensity	mμ.	Relative intensity	mμ.	Relative intensity
Turacin porphyrin A ester, m.p. 264° (uroporphyrin ester III)	625	0·275	570	0·431	535	0·587	502	1·000	406	13·72
Uroporphyrin ester I, m.p. 293°, from bone	625	0·258	570	0·445	535	0·606	500	1·000	406	13·74
Waldenström ester, m.p. 260°, from acute porphyria urine	625	0·252	570	0·445	535	0·594	501	1·000	406	13·55
Turacin porphyrin B ester, m.p. 210°	625	0·268	570	0·491	535	0·625	502	1·000	406	13·72

light petroleum, m.p. 264°. This, the main constituent of the crude turacin porphyrin ester, weighed 258·2 mg. (54·3 % of the total crude porphyrin ester), and after hydrolysis behaved on paper chromatography as a single 8 carboxyl porphyrin indistinguishable from uroporphyrin I (see Fig. 4).

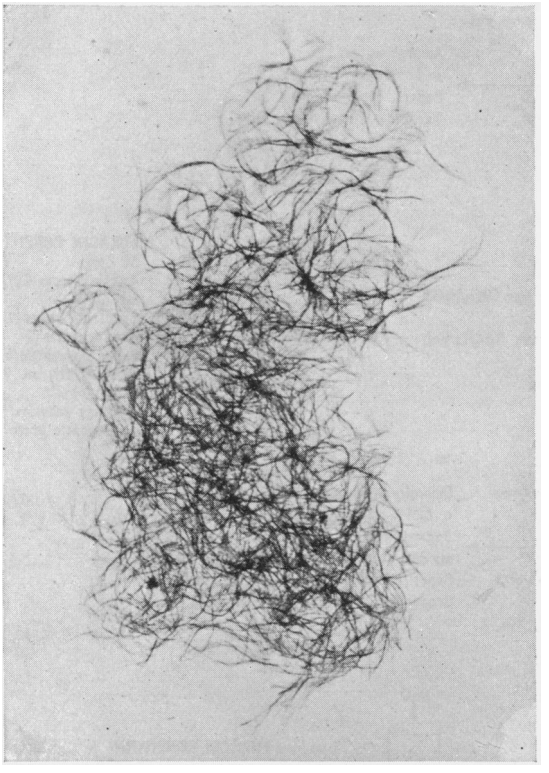

Fig. 3. Crystals from benzene-light petroleum of ester of turacin porphyrin A (uroporphyrin III octamethyl ester) m.p. 264°. Magnification × 250.

In elementary and methoxyl analysis it agreed with uroporphyrin octamethyl ester (Table 2). A spectrophotometric absorption curve of the ester in $CHCl_3$ (Fig. 5) showed no significant difference from either that (see Fig. 6) of uroporphyrin I octamethyl ester (m.p. 293°) from bones of a bovine case of congenital porphyria (Rimington, 1936) or that of a Waldenström ester (m.p. 260°) from acute porphyria urine (Rimington & Sveinsson, 1950). The ratios of the band intensities were as shown in Table 1.

Purification of zone II (see Fig. 2). Isolation of turacin porphyrin B and a dichroic pigment

Zone II, the middle zone in the second chromatogram of the crude turacin porphyrin (see Fig. 2), was rechromatographed upon MgO. It separated into a small, greenish-brown lower band which eventually yielded the dichroic pigment (see below) and a main, rather broad band. The upper part of the MgO retained a small quantity of greenish material, possibly similar to the chlorin already described, which was obtained from the precipitation mother liquors of the total crude porphyrin.

Turacin porphyrin B. The material from the main band crystallized poorly from hot $CHCl_3$-methanol. In $CHCl_3$ it had α-band at 625·4 mμ. It was rechromatographed once more upon MgO, on which it behaved as a single band,

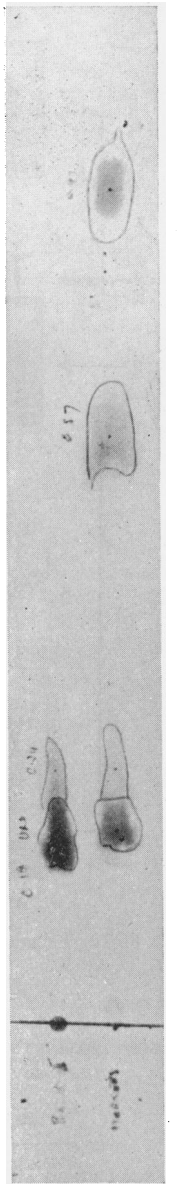

Fig. 4. Filter paper chromatogram (upper half) of turacin porphyrin A compared with (lower half) uroporphyrin I, coproporphyrin I and deutero-porphyrin IX as markers.

and then recrystallized four times from benzene-light petroleum when the melting point became constant at 210°. This material weighed 32 mg. (6·8 % of the total crude porphyrin ester), thus corresponding to about one-tenth of the weight of turacin porphyrin A. After hydrolysis its R_F value by paper chromatography was consistently (nine determinations) slightly, but significantly, lower than that of the uroporphyrin marker and turacin porphyrin A. Micro-analysis (see Table 2) agreed better with the requirements of a porphin

Table 2. *Micro-analysis of turacin porphyrin esters A and B*

	C (%)	H (%)	N (%)	MeO (%)
Turacin porphyrin A ester (uroporphyrin III ester)	61·9	6·4	5·8	26·5
Required for uroporphyrin octamethyl ester $C_{48}H_{54}O_{16}N_4$	61·1	5·8	5·9	26·3
Turacin porphyrin B ester	59·5	6·1	6·1	28·1
Required for a porphin octa-acetic acid methyl ester $C_{44}H_{46}O_{16}N_4$	59·6	5·2	6·3	28·0

octa-acetic acid ester than with that of a uroporphyrin ester (porphin-tetra-acetic acid tetrapropionic acid ester). The behaviour of this material on decarboxylation (see below) affords some support for this suggested structure. The spectral absorption of the methyl ester (see Fig. 7) was not significantly different from that of uroporphyrin ester

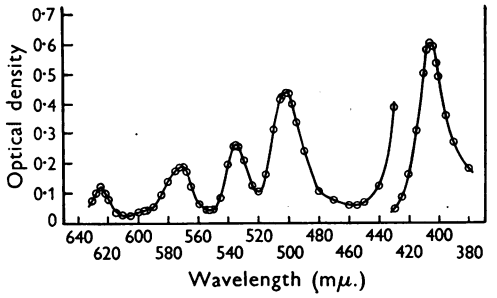

Fig. 5. Spectral absorption of ester of turacin porphyrin A (uroporphyrin III octamethyl ester). Solvent, chloroform. Concentration 28·13 mg./l. for range 630–430 mμ., 2·81 mg./l. for Soret band.

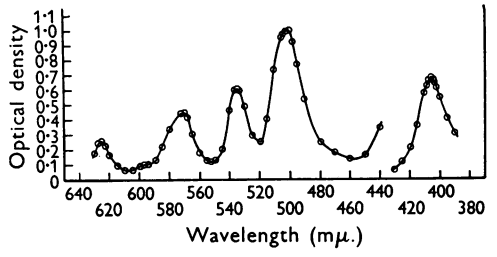

Fig. 6. Spectral absorption of uroporphyrin I octamethyl ester, m.p. 293° (see Rimington & Sveinsson, 1950). Solvent, chloroform. Concentration 65·8 mg./l. for range 630–440 mμ., 3·29 mg./l. for Soret band.

(Beckman spectrophotometer). The band ratios are recorded in Table 1. Comparison of the infrared absorption spectrum with those of uroporphyrin esters I and III (Falk & Willis, 1951), the latter provided by this work, supports the suggestion that turacin porphyrin B is closely related to, but not identical with, either of these uroporphyrins.

Dichroic pigment

The greenish-brown band obtained in the purification of zone II was rechromatographed upon Al_2O_3 packed in benzene. Development only began with pure $CHCl_3$ when a green band with red upper and lower edges moved down, leaving a small red-brown residue on the surface of the adsorbent. This residue was discarded and the green band eluted with methanol. Transferred again to $CHCl_3$, it had an α-band at 624·6 mμ. and was distinctly dichroic (red-green). It crystallized from $CHCl_3$-methanol in feathery greenish-

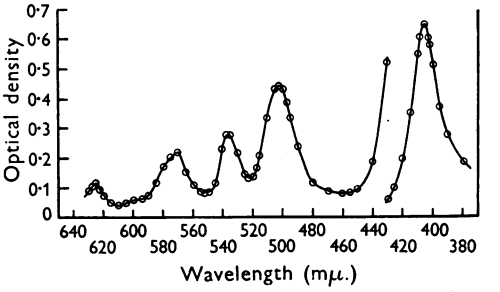

Fig. 7. Spectral absorption of octamethyl ester of turacin porphyrin B. Solvent, chloroform. Concentration approx. 30 mg./l. for range 630–430 mμ., 3 mg./l. for Soret band.

brown crystals, m.p. 212°. On recrystallization (three times) from benzene-light petroleum, the melting point became constant at 240°. The paper chromatogram of the ester after hydrolysis was similar to that of a uroporphyrin, but the second spot had a greater relative intensity. Spectral

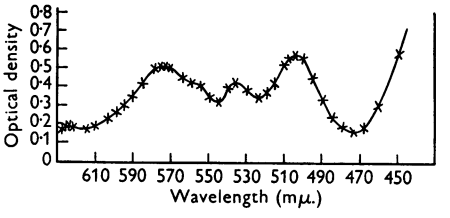

Fig. 8. Spectral absorption of dichroic pigment (phylloporphyrin type) separated during purification of turacin porphyrins. Solvent, chloroform.

absorption in $CHCl_3$, measured on the Beckman spectrophotometer, revealed a curve of phyllo-type (Fischer & Orth, 1937), band II being broad and intense; order of intensity IV, II, III, I (see Fig. 8). The α-band maximum lay at 625 mμ. We consider that this pigment is probably a phylloporphyrin with 8 carboxyl groups which may have arisen from the turacin porphyrin during the chemical manipulations employed to remove copper from the feather pigment.

Purification of zone III

This zone on the second MgO chromatogram of the total crude porphyrin was eluted by methanol containing 1% (v/v) of H_2SO_4. It was transferred to $CHCl_3$ and purified by two successive chromatograms upon MgO. The main porphyrin band was rather diffuse and left a small greenish zone upon the adsorbent. An unsuccessful attempt was made to secure crystalline material from benzene-light petroleum. From these solvents, the melting point rose continuously from 155°. A paper chromatogram showed similarity with turacin porphyrin B. In $CHCl_3$ it had α-band at 625·7 mμ. The amount was small.

Decarboxylation of turacin porphyrins

The acetic acid residue as a substituent on the pyrrole ring is unstable. The same is true to a less marked extent in the macrocyclic porphyrin system. Exploiting this property, Fischer & Zerweck (1924) showed that the uroporphyrin from Petry urine, when heated with 1% HCl in a sealed tube at 180–190° for 3 hr., lost 4 mol. of CO_2 and yielded coproporphyrin I. Since all four coproporphyrins have been synthesized, this reaction affords an excellent means of establishing to which aetioporphyrin series the parent substance belongs; uroporphyrin III would yield coproporphyrin III.

Turacin porphyrin A. The uroporphyrin ester from turacin porphyrin A (m.p. 264°) (3·8 mg.) described above, was decarboxylated by heating with 10 ml. of 1% HCl under the prescribed conditions. It was found most convenient to weigh out the ester, leave it to hydrolyse in 0·4 ml. of 25% HCl for 24 hr. and then to transfer the solution to the combustion tube with 9·6 ml. of water.

The reaction mixture contained some dark material which was filtered off and treated with glacial acetic acid to remove any adsorbed porphyrin, this extract being added to the main bulk from which the porphyrin was extracted with ether in the usual way, then from the latter into HCl. After precipitation and esterification, the total porphyrin in $CHCl_3$ was passed through a column of MgO and the purified coproporphyrin crystallized from a very small volume of $CHCl_3$ by addition of hot methanol. The crystals which separated had the habit of coproporphyrin III and m.p. 153°, remelt 179°. Recrystallization did not alter these melting points. By concentration of the first mother liquor, a further crop of similar crystals was obtained also melting at 153°, remelt 179°. Paper chromatography after hydrolysis showed only a 4 carboxyl porphyrin. The solution thus contained only coproporphyrin III, indicating that the parent uroporphyrin was the series III isomer alone. The yield of coproporphyrin III isolated was 68% of theory.

Turacin porphyrin B. On account of the small quantity of pigment available, only 2·5 mg. was taken for decarboxylation under the conditions described above. Examination at the end of the experiment showed that the substance had been degraded beyond the porphyrin stage, there being only a trace of fluorescence in the colourless solution.

DISCUSSION

The work reported here shows that by the removal of copper from turacin a mixture of porphyrins and porphyrin-like materials is obtained. These, by the aid of chromatography, have been separated. The ester of the main constituent, turacin porphyrin A, has m.p. 264°, remaining constant upon recrystallization. In elementary analysis, methoxyl content, spectral absorption and (after hydrolysis) behaviour on paper chromatography, it corresponds to the octamethyl ester of a uroporphyrin.

Decarboxylation afforded a single coproporphyrin, identified as coproporphyrin III by paper chromatography, spectral absorption and the melting point behaviour of its tetramethyl ester. That only coproporphyrin III was present was shown by the fact that there was no difference in melting point between the first crop of crystals separating and those obtained by further concentration of the mother liquors; moreover, their melting points were not changed by recrystallization of the respective fractions.

The main constituent of turacin is thus uroporphyrin III copper complex (confirming the earlier result of Rimington, 1939) and the ester, m.p. 264°, we have prepared provides for the first time an authentic specimen of uroporphyrin III octamethyl ester.

Accompanying this porphyrin and separable from it by chromatography is a second porphyrin, turacin porphyrin B, the ester of which we have obtained with constant m.p. 210°. Although this material resembles uroporphyrin octamethyl ester in such characteristics as paper chromatographic behaviour and spectral absorption, its elementary and methoxyl analysis agrees more nearly with the requirements of a substance containing four CH_2 groups less than uroporphyrin ester, such as, for example, the octamethyl ester of porphin octa-acetic acid. Difference from uroporphyrin is shown by the infrared spectrum and by the greater instability of this porphyrin under the conditions usually employed for decarboxylation. Only a trace of material exhibiting red fluorescence in ultraviolet light was recoverable from the reaction mixture. An acetic acid grouping attached to a β-position in the pyrrole ring is known to be unstable. Fischer & Müller (1937) synthesized 1:4:5:8-tetramethylporphin-2:3:6:7-tetra-acetic acid which proved to be less stable than uroporphyrin. On decarboxylation under the usual conditions, octamethyl porphin was obtained, the yield of crude material being 20% of theory.

In the present instance, more complete degradation of the molecule appears to take place. Elucidation of the exact structure of turacin porphyrin B will be attempted by a synthetic approach. It is of interest to recall that from the urine of an atypical case of porphyria, Gray, Rimington & Thomson (1948) obtained an ether-insoluble porphyrin which suffered extensive degradation to colourless products when decarboxylation was attempted in acid solution. The presence of turacin porphyrin B,

together with uroporphyrin III in turacin from feathers, no doubt accounts for the extreme difficulty of crystallizing the methyl ester of the crude porphyrin obtained by the removal of copper from the natural pigment. In their first paper on turacin, Fischer & Hilger (1923) report that their porphyrin ester would not crystallize; in a later paper (Fischer & Hilger, 1924) they state that from 0·1 g. turacin they obtained a uroporphyrin ester in very small needles, but do not record the yield. The melting point was given as 295°, not depressed by admixture with uroporphyrin I octamethyl ester. This finding is difficult to understand.

During the purification of turacin porphyrins A and B, we also isolated a chlorin-like pigment and a dichroic pigment (ester m.p. 240°) with a phyllo-type spectrum; both possessed 8 carboxyl groups by paper chromatography. We regard these as chemical artifacts arising during the reduction necessary to remove the copper from turacin. A similar chlorin-like pigment was obtained as a by-product by subjecting some uroporphyrin I copper complex to the same treatment with sodium amalgam as was accorded to turacin.

Now that an authentic specimen of uroporphyrin III octamethyl ester is available as a reference standard, it is hoped that some of the uncertainty surrounding the nature of the Waldenström porphyrin may be experimentally resolved. This pigment is excreted by sufferers from acute porphyria and was regarded as uroporphyrin III (see Waldenström, 1937) until Watson *et al.* (1945) disputed its homogeneity. Experiments with such an object in view have already been commenced.

SUMMARY

1. The porphyrin mixture resulting from the removal of copper from turacin has been examined chromatographically.

2. The main constituent has been isolated (ester m.p. 264°) and identified as uroporphyrin III. Decarboxylation afforded only coproporphyrin III. This provides for the first time an authentic specimen of uroporphyrin III.

3. Another porphyrin, present in about one-tenth of the amount, has been isolated as the methyl ester (m.p. 210°). From the results of analysis and paper chromatography it is suggested that this material may be porphin octa-acetic acid. When decarboxylation was attempted, it proved to be less stable than uroporphyrin, degradation into non-porphyrin products taking place.

4. In addition to these porphyrins, two materials having chlorin-type and phyllo-type spectra respectively have been isolated in very small quantity. They are, however, regarded as chemical artifacts.

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