

MESOBILIRHODIN, AN ISOMERIDE OF *i*-UROBILIN
WITH THE SPECTRAL CHARACTERISTICS OF PHYCOERYTHROBILIN

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(Received in UK 7 September 1970; accepted for publication 17 September 1970)

A red trace contaminant often observed in crude preparations of *i*-urobilin and mesobiliviolin has been referred to by a number of workers over the past few years as 'rhodinoid pigment' or 'mesobilirhodin' (e.g. refs. 1,2,3,4) but it has never been fully characterized. We have now isolated this red pigment in quantities sufficient for the application of most of the techniques useful in the characterization and structural elucidation of bilins and we find, in agreement with the previous observations of Ó Carra, Ó hEocha and Carroll²), that the spectral properties of the pigment are practically identical with those of phycoerythrobilin (table 1) and bear no resemblance to those of the original pigment preparations for which Siedel⁵) coined the name mesobilirhodin and which were almost certainly mixtures of urobilin and mesobiliviolin²). It seems probable that the structure associated with the term 'mesobilirhodin' since its proposal by Siedel⁵) is merely a canonical form of mesobiliviolin (cf. ref. 6).

Although it might be better to abandon the confused terms 'rhodinoid', 'mesobilirhodin' and 'rhodinoid pigment' altogether, we continue for the present, in the absence of any agreed alternative, to refer to the above-mentioned red pigment as mesobilirhodin, and to pigments with similar spectral properties (table 1) as rhodinoid pigments.

We find that *i*-urobilin isomerizes readily in alkali to yield the same red pigment and this provides the most convenient method of preparing mesobilirhodin*.

Preparation of mesobilirhodin: *i*-Urobilin (2.5 mg) dissolved in methanol (2.5 ml) and 2N NaOH (0.5 ml) is refluxed under oxygen-free nitrogen for 15 min. Water (15 ml), acetic acid (2.5 ml) and saturated sodium acetate solution (1.25 ml) are added and the mixture is extracted with ether (20 ml x 5), which is in turn extracted with water (5 ml x 5). This aqueous solution is extracted with chloroform (5 ml x 5). The chloroform solution is evaporated to dryness, and the pigment is esterified with 14% boron trifluoride in methanol (1 ml) at 40° for 25 min. After addition of water (5 ml), the esterified pigment is extracted into chloroform (2 ml x 5) and this solution is dried and evaporated to dryness. TLC of this esterified material by double development with benzene-light petrol (100-120°)-methanol (50:10:6) on 0.25 mm layers of Silica gel G (cf. ref. 3) resolves a heavy, deep pink band of mesobilirhodin (Rf c. 0.2) preceded by a faint blue-green band (mesobiliverdin) and closely followed by a purple band (mesobiliviolin). Behind this come two faint yellow bands (residual *i*-urobilin) and some red material remains near the origin. The mesobilirhodin is eluted with methanol which is then evaporated off at 60° in a stream of nitrogen. The traces of mesobilirhodin present in crude preparations of *i*-urobilin may be separated out and purified by following a similar procedure.

Identification of bilins was carried out mainly by: a) application of a variety of TLC

* A similar reaction of *d*-urobilin had previously been reported by Gray and Nicholson⁷) but was presumed to depend on the presence of the vinyl side-chain which is considered to differentiate *d*-urobilin from *i*-urobilin.

TABLE 1
Spectral Absorption Maxima (nm) of Bilins and Derivatives.
(Most intense maximum underlined; minor peaks in brackets)

	<u>FREE BASE</u> in chloroform	<u>HYDROCHLORIDE</u> in acid chloroform	in 0.1 N MeOH-HCl	<u>ZINC COMPLEX</u> in chloroform- pyridine (4:1)	pK of pyrrol- enine
Native Phycoc- erythrobilin*	304, <u>505</u>	312, <u>576</u>	307, <u>556</u>	320, (c540), <u>583</u>	6.4
Mesobilirhodin (from <i>i</i> -urobilin)	302, <u>504</u>	312, <u>576</u>	307, <u>555</u>	319, (543), <u>583</u>	6.2
<i>i</i> -Urobilin**	454	499	494	510	7.4
Mesobiliviolin**	<u>325</u> , 568	327, 602	<u>326</u> , 580	<u>337</u> , (583), 630	4.0

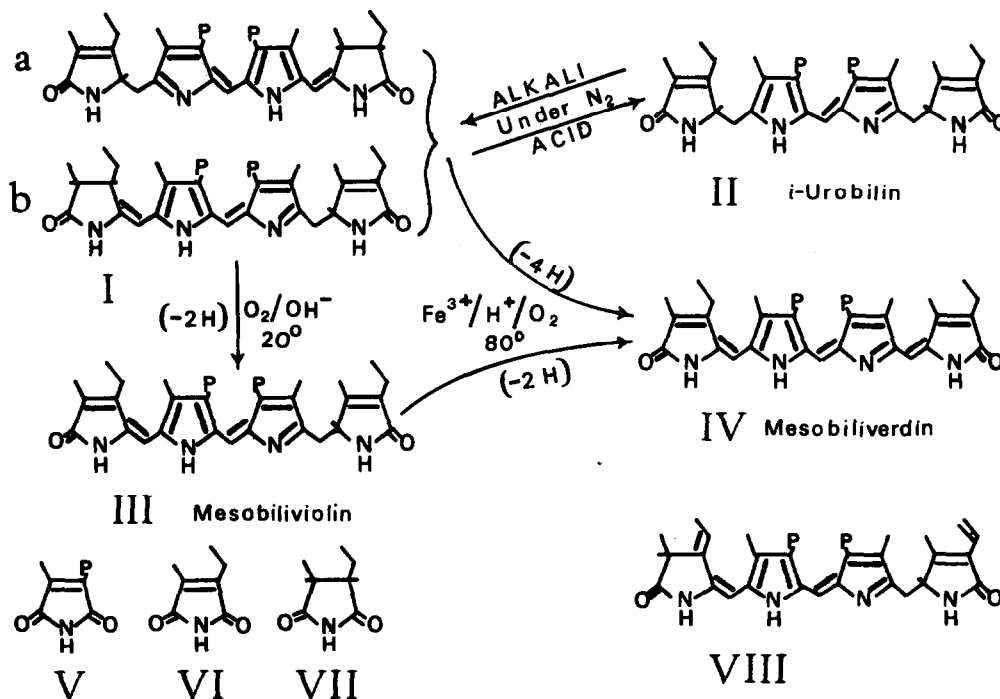
(Diffuse absorption in the 350-380 region is omitted).

*Refs. 2,9; **Personal observations and ref.8.

SCHEME 1

(Conjugated systems emphasized by thick double-bond lines)

P = $\text{CH}_2\text{CH}_2\text{COOH}$ or $\text{CH}_2\text{CH}_2\text{COOMe}$



methods by which most of the well-characterized bilins can now be identified (refs. 3,4,10); b) spectral characterization of the free base, hydrochloride and zinc complex derivatives (cf. refs. 6,8,9); c) identification of the products of chromic acid degradation.

Ferric chloride dehydrogenations were carried out as described by Watson *et al.*¹²⁾, chromic acid degradation of bilins and identification of the imide products as described by Rüdiger¹¹⁾, and spectrophotometric titration as described by Ó Carra and Ó hEocha¹³⁾.

All the data discussed below favour structure I for mesobilirhodin but do not distinguish between the two possible side-chain isomers represented by Ia and Ib. Extensive multiple development (X8) on Silica gel G in benzene-light petrol-methanol (50:10:6) eventually resolved our preparations into two approximately equal rhodin components, which have not so far been distinguishable on any other basis and may represent Ia and Ib.

Structure I is consistent with the spectral properties of mesobilirhodin (table 1) which indicate a conjugated system intermediate between those of urobilins (e.g. II) and violins (e.g. III) as also does the spectral titration (table 1) which indicates that the conjugated system of mesobilirhodin contains one pyrrolenine (=N-) grouping with a pK intermediate between those of the urobilins and mesobiliviolin (cf. ref. 8 for general interpretation of such data).

Dehydrogenation of mesobilirhodin with acid ferric chloride yielded a verdin which was unambiguously identified by the method of Ó Carra and Colleran¹⁰⁾ as mesobiliverdin IX α (IV), establishing the carbon skeleton and the absence of oxygenated bridge positions (cf. ref. 6).

The conversion of *j*-urobilin to mesobilirhodin in alkali could be reversed by refluxing the rhodin in 10N HCl for 15 min under nitrogen and the *j*-urobilin thus regenerated could be reconverted to mesobilirhodin by repeating the alkaline treatment. These interconversions are readily explainable as isomerizations on the basis of structures I as depicted in scheme 1.

On standing aerobically in N methanolic KOH at 20° for 24 hours, mesobilirhodin auto-oxidized to mesobiliviolin (III), which could be further dehydrogenated with acid ferric chloride to mesobiliverdin as shown in scheme 1.

Chromic acid degradation of mesobilirhodin (as the dimethyl ester) yielded haematinic acid methyl ester (V) (derived from the two internal rings of IX α bilins), ethylmethylmaleimide (VI) and ethylmethylsuccinimide (VII), the latter indicating the presence of a reduced outer ring in mesobilirhodin. The connection of this reduced ring to the conjugated system, as depicted in structures Ia and Ib, would explain the facile auto-oxidation of mesobilirhodin to mesobiliviolin. Chromic acid degradation of the mesobiliviolin product gave only the imides V and VI in approximately equal proportions, confirming that the auto-oxidation involves dehydrogenation of the reduced ring, as indicated in scheme 1, rather than of a bridge position.

The mass spectrum of mesobilirhodin dimethyl ester had the base peak in the tripyrrolic region with weak peaks in the tetrapyrrolic and dipyrrolic regions. Since fragmentation of bilins occurs much more readily at reduced bridge positions than at ones included in the conjugated system (cf. ref. 4), this type of fragmentation pattern, with a relatively stable tripyrrolic fragment, indicates a conjugated system connecting three pyrrole rings. A similar type of pattern is observed, for example, with mesobiliviolin dimethyl ester (III)⁴⁾, but while the tripyrrolic fragment there has m/e 492⁴⁾, that from mesobilirhodin dimethyl ester has m/e 494.

representing two hydrogen atoms more. This confirms that in mesobilirhodin the reduced end ring is part of the stable tripyrrolic fragment and is connected to the conjugated system as in I a or b. In the tetrapyrrolic molecular ion region the strongest line occurs at m/e 618 as expected for I a and b. Weaker lines appearing at m/e 614, 616 and 620 are attributable to redox disproportionations in the mass spectrometer, a phenomenon well known in this field.

Since mesobilirhodin has spectral properties which are practically identical with those of native phycoerythrobilin, the two pigments presumably have identical, or very similar conjugated systems. However, structure VIII, which has been proposed for phycoerythrobilin^{3,14,15}, has a longer conjugated system than that of structure I, containing an additional double bond - that of the ethylidene grouping. As pointed out by Rüdiger and Ó Carra³, the pigment on which most of the structural work was performed is an artifact of true native phycoerythrobilin with different spectral properties suggesting a slightly longer conjugated system. It seems probable therefore that the ethylidene grouping is either shifted outside the conjugated system in native phycoerythrobilin, or that it is an artifact arising from some other type of grouping initially present.

Acknowledgements: We thank Professor C. Ó hEocha for helpful comments, Dr. M.S. Stoll for assistance in obtaining mass spectra, and the Irish Department of Education for a Research Maintenance Grant to S.D.K.

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