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ON THE EXISTENCE AND STRUCTURE OF A STABLE MESOBILIVIOLIN OF MOLECULAR WEIGHT 590*

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Mesobiliviolin (Mv, mol wt 588) was first described by Fischer and Niemann (1) as a product of the oxidation of mesobilirubinogen by $FeCl_3$ and 25% HCl (w/v). It was next encountered in human feces as a by-product during the isolation of stercobilin (2). It is the primary product of a simple dehydrogenation of dihydromesobilirubin (3). It has been prepared by alkaline isomerization of d-urobilin (d-U, mol wt 588) (4), and by $FeCl_3$ oxidation of various urobilins (5). Siedel (6) synthesized the Mv represented by structure I together with an accompanying red-violet pigment, mesobilirhodin, which he regarded as an isomer, differing only in the position of the methane bridge. Since the position of the bridge saturation is immaterial for present considerations, we shall use the name Mv for either structure.

Ferric chloride oxidation (5) of synthetic i-urobilin yields a violin as the first oxidation product (λ max. 560 mμ 1.5 N HCl). On continued heating in the $FeCl_3$ mixture a major proportion is converted to glaucobilin (Gl, λ max. 650 mμ 1.5 N HCl) (5, 7). A smaller but variable proportion is not oxidized to Gl but resists further heating with $FeCl_3$ and HCl even up to 45 min. In this paper we shall show that the stable component represents a violin of mol wt 590 having structure II, i.e., a pyrrolidone-pyrrolinone; thus it bears the same relation to half-stercobilin (ℓ-HS, mol wt 592) (8) as labile Mv, structure I (mol wt 588) does to dipyrrolinone urobilins (mol wt 590).

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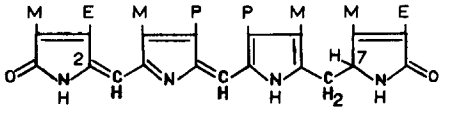
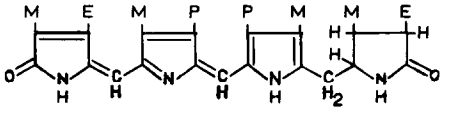
All of the methods have been described in recent publications (5, 7-10). Mv (588) was prepared from natural d-U (590) after heating for 1.5 min. with the standard $FeCl_3$ mixture (7). After chromatographic partial purification according to Siedel's method (6), the absorption spectrum was measured (DK Beckman) and the Mv was studied by means of thin layer chromatography (TLC) and CrO_3 oxidation followed by TLC of the products. The Mv was then subjected to $FeCl_3$ oxidation for 15 min., thus being converted to Gl. Oxidation of d-U (590) for 15 min. also yielded Gl. When *l*-HS (8) was oxidized for 15 min. with $FeCl_3$ relatively little Gl was observed, the main products being the stable Mv together with unchanged *l*-HS. This Mv was resistant to prolonged additional $FeCl_3$ oxidation.

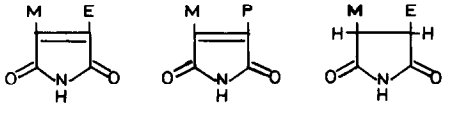
TLC on polyamide layer (Gallard-Schlesinger) in CH_3OH:H_2O, 3:1 v/v (10), differentiated the stable and labile forms of Mv. Stable Mv formed a spot with R_f = 0.14; labile Mv formed a spot with R_f = 0.43. A mixture of stable and labile forms was separated in this way.



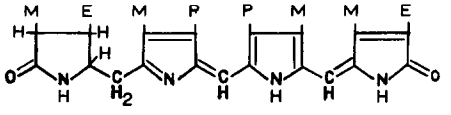
I



II, or



III IV V



M = CH_3; E = C_2H_5; P = CH_2CH_2COOH

Evidence for structure II was obtained from CrO_3 oxidation (11) of the two forms. Oxidation of labile Mv yielded only ethylmethylmaleimide III and hematinic acid IV, while that of the stable form gave III, IV and ethylmethylsuccinimide (V), the two imides in about equal proportions, as estimated from the size and density of the spots on TLC and by gas chromatography (12). Since TLC indicated homogeneity of the stable Mv and since the two imides appearing in equal amounts are derived from rings I and IV, structure II is fully compatible with the experimental observations.

Mass spectrometry provided additional evidence for structure II. While the molecular ion peak for labile Mv was at m/e 588, that for the stable form was at m/e 590 (Table I).

TABLE I
Mass spectral data in molecular region

	588	589	590	591	592
Stable Mv	3.5		10.0		4.0
Labile Mv	10.0	2.0		0.5	0.5

It is seen that the stable form exhibits peaks at m/e 588 and 592 as well as the parent ion peak m/e 590. Such satellite peaks are typical of urobilinoids and undoubtedly arise from thermally induced oxidation and reduction reactions in the melted pigment, as discussed elsewhere (8, 13).

The present results clarify the nature of the stable and labile forms of Mv previously encountered in the $FeCl_3$ oxidation method used for distinguishing urobilinoid types (5, 7).

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