and IV compared to what is observed for the reaction of I with V.

If this is the case, I and tetrahydro-β-carboline-3-carboxylic acid[3] (VI) ought to react much faster than I and V. This assumption has been verified, and formation of III-3-COOH from I and VI has been found to be more than 40 times as rapid as formation of III from I and V.

In analogy with the different pK_a-values found for indole-2-carboxylic acid[4] (5.28) and indole-3-carboxylic acid[5] (7.00), IV may be somewhat stronger an acid than VI, which should increase the catalytic efficiency of the carboxyl group in IV over that in VI. This is also reflected in the different rates for formation of III and III-3-COOH in the reactions of I with IV respectively VI.

Thus, the rapid formation of III from I and IV may be interpreted in terms of a mechanism involving intramolecular acid catalysis of the formation of the immonium compound VIII (Scheme). Concerted decarboxylation of VIII via the *zwitterion* and tautomeric rearrangement to gain resonance stabilization results in the formation of III (Scheme).

The reaction of I with VI can be described by a similar mechanism, but, in this case decarboxylation does not take place.

Also other carbonyl compounds, e.g. formaldehyde, benzaldehyde, acetone etc., react with IV to form yellow, highly fluorescent compounds. Moreover, β-phenethylamines have also been found to undergo reaction with I yielding fluorescent compounds, presumably by a similar mechanism.

Further studies on the histochemical application of the reaction described in this paper are in progress[1].

Zusammenfassung. Die Identifizierung und der Bildungsmechanismus des fluoreszierenden Produktes aus Glyoxylsäure und Tryptamin wird beschrieben. Eine intramolekulare säure-katalysierte Reaktion wird für die Bildung des fluoreszierenden Produktes vorgeschlagen.

L. Å. Svensson

Research and Development Department, AB DRACO, Fack, S–221 01 Lund (Sweden), 21 February 1972.

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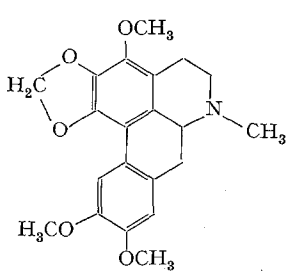
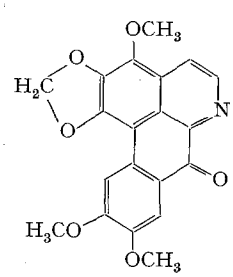
Dehydroocoteine and Didehydroocoteine from *Ocotea puberula*

Ocoteine (I) has been isolated from *Ocotea puberula* (Nees et Mart.) Nees[1] and from *Thalictrum minus* L.[2], *T. isopyroides* C. A. Mey[3], *T. fendleri* Engelm. ex A. Gray[4] and *Phoebe porphyria* (Gris.) Mez.[5]. Iacobucci[6] reported the presence of a second alkaloid in the benzene extract of *Ocotea puberula*.

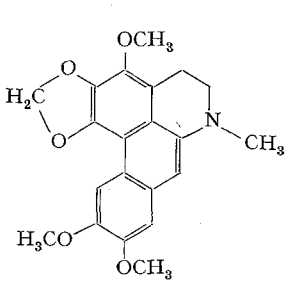
While the methanolic extract of the bark afforded only ocoteine (I) and the oxoaporphine II[7], from the light petroleum extract it was possible to isolate in 0.8‰ yield another basic substance which, purified by preparative TLC and crystallized from ethyl acetate, melted at 203–204°C and analyzed for $C_{21}H_{21}NO_5$. It was homogeneous on several TLC systems and unstable to light.



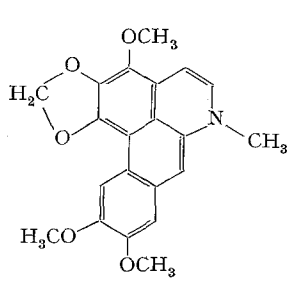
I



II



III



IV

The UV-spectrum suggests structure III for the compound, with a conjugated chromophore of the dehydroaporphine type[8–10], with maxima at 220, 263 and 335 nm (log ε 4.56, 4.80 and 4.06). Besides, the IR band at 1590 cm^{-1} due to skeletal C=C in-plane vibrations, is more intense than in ocoteine (I).

The NMR spectrum shows an N-methyl group (δ 3.10, s, 3H), 3 methoxy groups (δ 4.08, s, 6H, and δ 4.12, s, 3H), a methylenedioxy group (δ 6.12, s, 2H), and aromatic protons (δ 6.60, s, 1H; δ 7.10, s, 1H, and δ 8.45, d(?), 1H). These values are typical of the dehydroaporphine alkaloids[8–10]: the N-methyl group is shifted from δ 2.53 in ocoteine to δ 3.10, and the 2 hydrogen atoms of the methylene dioxy ring are now magnetically equivalent due to the planarity of the phenanthrene system. The signal at δ 8.45 which is typical of the C-11 proton appears as an assymmetric doublet, suggesting that the isolated base

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could be impurified by a minor component of very similar structure.

On GLC a sample previously purified by preparative TLC showed a certain amount of another component, with a slightly higher retention time on a SE-30%-Chromosorb-W-AW column than the major one. The same mixture, but with substantially lesser amounts of the minor component, was formed during permanganate oxidation of ocoteine (I) in acetone at room temperature[11] (26% yield, m.p. 201–202°), thus confirming structure III for the major component, which was accordingly named dehydroocoteine.

The heterogeneous character of the isolated product was confirmed by mass spectrometry, where 2 series of peaks appear, with relative intensities depending on the operating temperature. Spectra run at normal temperatures show peaks corresponding to II (m/e 367 (M^+), 352 (M-15), 337, 322, 183.5 (M^{++})), together with peaks due to a lower molecular weight component (M^+ 365). Runs made at higher temperature, and when most of the sample had volatilized, exhibit only the peaks corresponding to the minor component (m/e 365 (M^+), 350 (M-15), 335, 320, 182.5 (M^{++})). The similarity of both fragmentation patterns, although – as in the aporphine field[12–14] – of no diagnostic value, indicates a close structural similarity. Taking into account the NMR data of the isolated material, which favors a similar substituent orientation, formula IV can be advanced for the second component. Didehydroocoteine (IV) is a representative of a new kind of aporphine-type alkaloids.

Zusammenfassung. Zwei neue Alkaloide des Aporphintypus, Dehydroocotein (III) und Didehydroocotein (IV), wurden aus *Ocotea puberula* (Nees et Mart.) Nees isoliert, und ihre Strukturen aufgeklärt.

F. BARALLE, N. SCHVARZBERG, M. VERNENGO and J. COMIN

Instituto Nacional de Farmacología y Bromatología, Caseros 2161, Buenos Aires, Argentina, and Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Pabellón 2, Ciudad Universitaria, Buenos Aires (Argentina), 3 January 1972.

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The Structure of Mesobilirhodin

Mesobilirhodin was recorded as a minor product during the synthesis of mesobiliviolin[1]. SEIDEL and MÖLLER[2] proposed a biladiene b, c structure isomeric with mesobiliviolin (biladiene a, b) and containing the same side chain substituents. Mesobiliviolin and mesobilirhodin have recently been prepared[3] from mesobilirubinogen. A structure was proposed[3] for mesobilirhodin but certain alternate possibilities were not eliminated. The structure proposed is different to that[4,5] for mesobilirhodin prepared by alkaline isomerization of i-Urobilin.

We have prepared a rhodinoid pigment from mesobilirubinogen[6,7] which is presumably the same as mesobilirhodin prepared by STOLL and GRAY[3]. Analysis of the mass spectrum and NMR-spectrum supports the proposed structure I. This establishes that the two preparative methods yield identical products, and not different as is currently indicated.

Crude mesobilirubinogen was prepared by the sodium amalgam reduction[7] of two 300 mg lots of bilirubin (Nutritional Biochemicals). The crude mesobilirubinogen was dissolved in methanol and heated for 7 min with 1/10 volume of 20% $FeCl_3$ in HCl[6]. The products were phased into $CHCl_3$ and washed free of acid. Complete esterification was assured by the addition of diazomethane. Mesobilirhodin ester was purified to chromatographic homogeneity by preparative thin layer chromatography[8,9] on silica gel with CCl_4:CH_3COOCH_3 (1:2 v/v). Analytical chromatography of the red presumptive mesobilirhodin dimethyl ester on 2 additional systems[9] revealed only 1 pigment zone. The electronic absorption spectra of mesobilirhodin dimethyl ester showed absorption maxima at 557 and 306 nm in 5% HCl-CH_3OH w/v; 578,541 and 316 nm in ethanol saturated with zinc acetate.

The mass spectrum of the pure precipitated dimethyl ester was recorded in an AEI-MS 9 instrument. Direct inlet probe was employed, with a source temperature about 220° at 70 eV.

Principal fragment ions from mesobilirhodin dimethyl ester with their intensities in parentheses. 494 *m/e* (100) is taken as the base peak, and only peaks with intensity greater than 5% are given. Below 300 *m/e* only peaks with intensity greater than 10% are given. Peaks below 170 *m/e* are not given.

618 (5) M+	333 (5)	303 (10)	211 (12)
494 (100)	334 (5)	302 (11)	208 (12)
480 (3)	319 (5)	301 (9)	194 (27)
420 (5)	318 (12)	300 (5)	185 (18)
417 (8)	317 (15)	299 (10)	183 (19)
373 (8)	316 (31)	287 (11)	182 (10)
372 (18)	315 (13)	244 (11)	181 (31)
371 (27)	314 (5)	243 (26)	180 (78)
348 (7)	305 (7)	229 (17)	170 (18)
346 (6)	304 (19)	213 (11)	

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