the intermediate aldehyde[8,9] and by a similar set of arguments it is possible to derive the kinetic current of the carbonium ion at time zero ($\bar{i}_{ko}$) and time inifinity ($\bar{i}_{k\infty}$) as functions of the theoretical diffusion current ($\bar{i}_d$) and the 4 rate constants k_1, k_{-1}, k_2, k_{-2}(1,2.)

$$\bar{i}_{ko}/\bar{i}_d = 0.886\, t^{1/2} k_1/(k_{-1}+k_2)^{1/2} = 4.03\times10^{-2} \quad (1)$$

$$\bar{i}_{k\infty}/\bar{i}_d = 0.886\, t^{1/2} k_1 k_{-2}(k_{-1}+k_2)^{1/2}/(k_{-1}k_{-2}+k_1k_{-2}+k_1k_2) = 2.56\times10^{-2} \quad (2)$$

The overall reaction rate can be simplified to

$$k_{exp} = (k_1k_2+k_{-1}k_{-2})/(k_{-1}+k_2) = 9.65\times10^{-4} s^{-1} \quad (3)$$

The fourth equation needed to calculate all the 4 unknown rate constants is provided by the extrapolated extinction at 268 nm to time zero. This value yields the concentration of II at time zero and consequently it yields the equilibrium constant of the rapidly established equilibrium between II and III.

$$k_1/k_{-1} = 2.28\times10^{-3} \quad (4)$$

From equations (1), (2), (3) and (4) the 4 rate constants are as follows: $k_1 = 0.32\ s^{-1}$, $k_{-1} = 140\ s^{-1}$, $k_2 = 0.15\ s^{-1}$, $k_{-2} = 6.2\times10^{-4}\ s^{-1}$.

The value k_1 is in agreement with the rates of protonation of substituted styrenes in acetic acid sulphuric acid mixtures[10].

Polarographic kinetic currents prove to be a novel tool for the study of chemical reactions proceeding via carbonium ion intermediates.

Zusammenfassung. Mit Hilfe der Polarographie wird die Kinetik der Solvolyse von *p*-Methoxy-isopropalakohol bestimmt.

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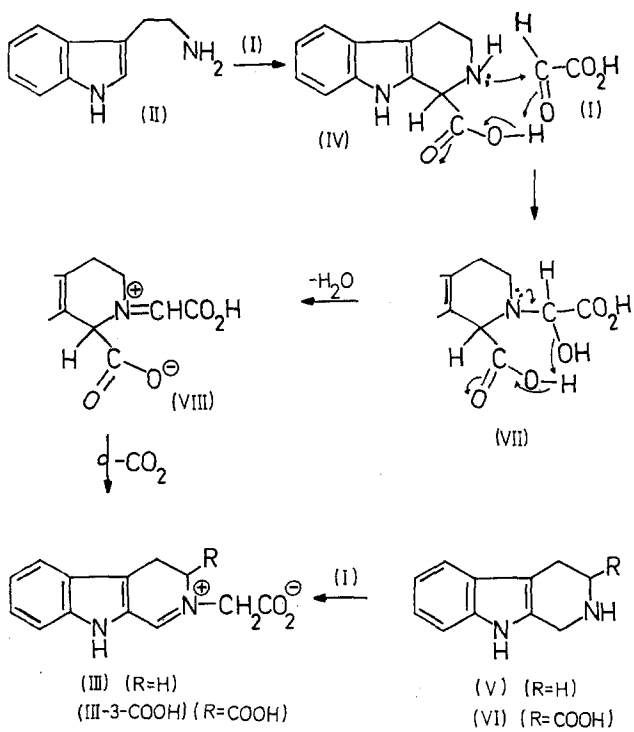
A New Reaction in Histochemistry: Structure and Mechanism of Formation of Fluorescent Compounds in the Reaction of Tryptamine and Carboxyl Substituted Tetrahydro-β-Carbolines with Glyoxylic Acid

Glyoxylic acid (I) has been found to react with biogenic amines (e.g. indoleamines, catecholamines), both in tissues and in solution, with the formation of highly fluorescent compounds. This reaction seems to offer a new very sensitive method for the histochemical demonstration of such amines in tissues[1].



Thus, the yellow, highly fluorescent compound formed after treatment of tryptamine hydrochloride (II) with a 4 molar excess of (I) (monohydrate) in *n*-BuOH at room temperature has been characterized and found to be the quaternary 2-carboxymethyl-3.4-dihydro-β-carbolinium chloride, III (Scheme); IR (KBr): 3100, 2750, 2500, 1720, 1630, 1540, 1340, 880 and 760 cm^{-1}; NMR (DMSO-d_6, 100 MHz): 3.32 (t, 2H, J = 9.0 Hz, $-CH_2-CH_2-$), 4.12 (t, 2H, J = 9.0 Hz, $-CH_2-CH_2-$), 4.96 (s, 2H, *pyr*-N–CH_2-), 7.5 (m, 5H, Ar-H and *ind*-NH), 9.21 (s, 1H, 1-H), 12.86 (broad s, 1H, –COOH); fluorescence: 375/500 nm; mass spectrum at 70 eV (*m/e* (relative intensity %) M^+ 229 (0.3), 212 (3), 186 (13), 185 (6), 172 (6), 169 (6), 144 (17), 143 (100), 142 (10), 128 (6), and 115 (17); after $NaBH_4$-reduction: M^+ 230 (5), 186 (10), 169 (3), 156 (5), 143 (100), 130 (7), 128 (7), 115 (23), 102 (7) and 89 (7).

Formation of III in the reaction of I with tetrahydro-β-carboline-1-carboxylic adic (IV) (intermediate in the formation of III form I and II), has been found to be more than 80 times as rapid as the analogous reaction of I with tetrahydro-β-carboline[2], (V) (Pseudo first-order conditions in *n*-BuOH at 84°C).

Owing to steric and inductive effects from the carboxyl group introduced in IV, the *pyr*-nitrogen in this compound ought to be less reactive against I than the *pyr*-nitrogen in V. However, since the molecular geometry in IV is so arranged that catalytic assistance from the carboxyl group may be possible, (both in the reaction of the *pyr*-nitrogen with the carbonyl carbon in I, as well as in the subsequent dehydration reaction of the carbinolamine, VII), this may be an explanation for the rapid formation of III from I

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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.

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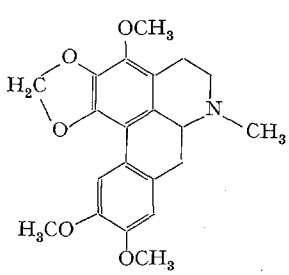
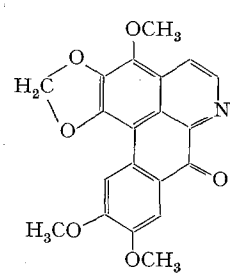
Dehydroocoteine and Didehydroocoteine from *Ocotea puberula*

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*.

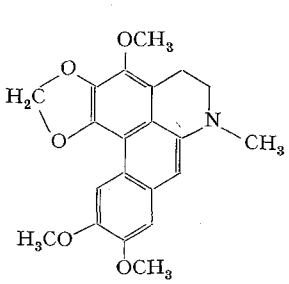
While the methanolic extract of the bark afforded only ocoteine (I) and the oxoaporphine II[7], from the light petroleum extract it was possible to isolate in 0.8‰ yield another basic substance which, purified by preparative TLC and crystallized from ethyl acetate, melted at 203–204°C and analyzed for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



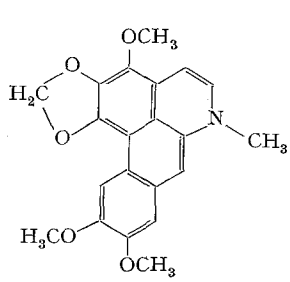
I



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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