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MICROBIOLOGICAL TRANSFORMATIONS. IV.
HYDROXYLATION OF CONESSINE*

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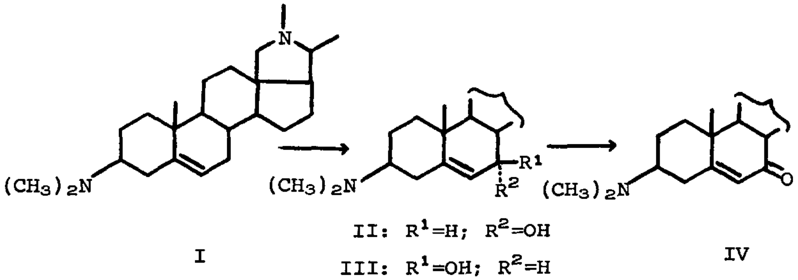
THE recent report of the microbiological conversion of conessine (I) to Δ^4-conenine-3-one (1) prompts us to record our observations concerning the microbiological hydroxylation of conessine. Incubation of conessine with Aspergillus ochraceus yielded three new hydroxyconessine derivatives, two of which have been shown to be 7α-hydroxyconessine (II) and 7β-hydroxyconessine (III).



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[*] Part III in the series: C. J. Sih, S. M. Kupchan, N. Katsui and O. El Tayeb, J. Org. Chem., 28, 854 (1963).

In a typical experiment a strain of Aspergillus ochraceus (kindly supplied by Professor K. B. Raper, Department of Bacteriology, University of Wisconsin) was grown in 9.6 l. (in twenty-four 2 l. erlenmeyer flasks) of a medium consisting of corn steep liquor, 0.6%; $NH_4H_2PO_4$, 0.3%; calcium carbonate, 0.25%; corn oil, 0.27%; yeast extract, 0.25%; and glucose, 1%. After 24 hr. of incubation at 27° on a rotary shaker, 5.0 g. of conessine in 24 ml. of dimethylformamide was distributed equally among the flasks. The fermentation was allowed to continue for 96 hr. The culture broth was filtered, adjusted to pH 9.5 with ammonium hydroxide, and extracted with chloroform. The combined chloroform extracts were evaporated to dryness under reduced pressure and the residue was extracted with hot isopropyl ether. Preparative paper chromatography of the isopropyl ether-extractable product [on Whatman filter paper (No. 4) which was treated with phosphate-citric acid buffer (pH 3.5), using as eluant the solution of 1 part of formic acid in the organic phase of a mixture of n-butanol : n-butyl acetate : water (25:10:10 by volume)] gave three bands. The band of highest R_f consisted of unchanged conessine. The band of lowest R_f, upon chromatography on neutral alumina and crystallization from acetone, yielded II (11%), $C_{24}H_{40}N_2O$ (Found: C, 77.44; H, 10.78; N, 7.49), m.p. 175-176°, $[\alpha]_D^{25}$ - 60° (c 0.75, $CHCl_3$), $\lambda_{max}^{CHCl_3}$ 2.80 μ, n.m.r. peaks at 7.03, 7.70, 7.80, 8.97, 9.10 (as for conessine), 4.39 (1H, doublet, J 6 c./s., $C{=}C\underline{H}{-}CHOH$), 6.08 τ (1H, $C\underline{H}OH$); and III (4%), $C_{24}H_{40}N_2O$ (Found: C, 77.14; H, 10.95; N, 7.77), m.p. 208-210°, $[\alpha]_D^{27}$ + 42° (c 0.91, $CHCl_3$), $\lambda_{max}^{CHCl_3}$ 2.80 μ, n.m.r. peaks at

7.03, 7.71, 7.82, 8.97, 9.15 (as for conessine), 4.70 (1H, doublet, J 1 c./s., C=C$\underline{H}$-CHOH), 6.15 τ (1H, C$\underline{H}$OH). The band of intermediate R_f yielded a third new compound, $C_{24}H_{40}N_2O$ (Found: C, 77.62; H, 11.05; N, 7.42), m.p. 193-196°, $[\alpha]_D^{23}$ + 9° (c 0.80, $CHCl_3$), $\lambda_{max.}^{CHCl_3}$ 2.76 μ.

Oxidation of II or III with chromic acid in aqueous acetic acid yielded ketone IV, $C_{24}H_{38}N_2O$ (Found: C, 77.81; H, 10.27; N, 7.71), m.p. 160-162°, $[\alpha]_D^{23}$ - 57° (c 1.01, $CHCl_3$), $\lambda_{max.}^{EtOH}$ 236 mμ (ε 10,000) (2); $\lambda_{max.}^{CHCl_3}$ 6.00, 6.12 μ (enone). Reduction of IV with lithium aluminum hydride gave III, indicating that III was the 7β-hydroxy epimer. (3,4). The molecular rotation differences with respect to conessine, ΔM_D - 219° for II, and ΔM_D + 160° for III, support assignment of the 7α-hydroxyconessine structure to II and 7β-hydroxyconessine structure to III (4).

In control experiments, incubation of conessine with numerous other organisms,under otherwise identical conditions, was followed by recovery of unchanged starting material. The latter observations indicated that the observed hydroxylation reactions were not merely autoxidations. Furthermore, after incubation of 7α-hydroxyconessine with <u>A. ochraceus</u> under the same conditions, the reaction product consisted of unchanged starting material. Hence, the possibility was precluded that III may have been formed by epimerization of II under the fermentation or work-up conditions.

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(2) Cf., e.g., 3β-acetoxy-5-cholestene-7-one, $\lambda_{\text{max.}}^{\text{EtOH}}$ 235 mμ (ε 12,050), O. Wintersteiner and M. Moore, J. Am. Chem. Soc., 72, 1923 (1950).

(3) Cf. L. F. Fieser, M. Fieser and R. N. Chakravarti, ibid., 71, 2226 (1949).

(4) Cf. H. J. Ringold, G. Rosenkranz and C. Djerassi, ibid., 74, 3318 (1952).