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MICROBIOLOGICAL CONVERSION OF CONESSINE

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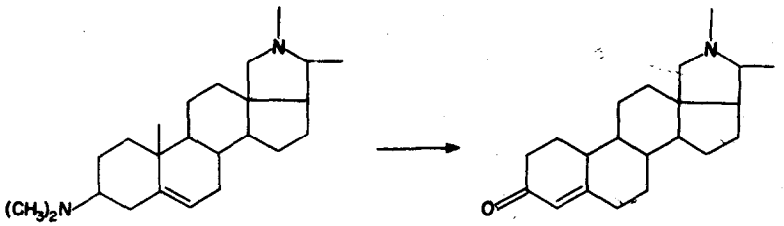
MICROBIOLOGICAL conversion of steroids has already become a well known procedure.[1] However, as far as we are aware, the transformation of steroidal alkaloids by means of micro-organisms has been described only once, namely by Sato and Hayakawa[2] who converted solasodine and tomatidine by means of Helicostylum piriforme to their respective hydroxylated derivatives.

We wish to report the microbiological removal of the 3-dimethylamino-group and displacement of the double bond of conessine (I) from



I II

[1] For a recent review see: E. Vischer and A. Wettstein, Advanc. in Enzymol. 20, 237 (1958).

[2] Y. Sato and S. Hayakawa, J. Org. Chem. 26, 4181 (1961).

the 5,6-position to the 4,5-position by Gloeosporium cyclaminis or Hypomyces haematococcus to yield Δ^4-conenine-3-one (II).*

Compound II has already been obtained chemically by Bertho[3], Pappo[4] and Johnson et al.[5]

In a typical experiment a strain of Gloeosporium cyclaminis v. Arx, obtained from the "Centraal bureau voor schimmelcultures" (Baarn, Holland), was innoculated from an oatmeal agar slant into an Erlenmeyer flask containing a 2% cornsteep solids - 2% glucose nutrient medium. After three days shaking at 26°C on a rotary shaking machine (stroke 2.5 cm; 250 rpm) this culture was used to innoculate (5%) flasks with a 0.5% cornsteep solids - 0.5 glucose medium. After twenty four hours incubation at 26°C 400 mg conessine (as the sulfuric acid salt dissolved in 10 ml water) were added per 1000 ml shake flask media. Paper chromatography of a sample 21 hours after addition of the substrate using the system butanol-acetic acid-water (4 : 1 : 5 by volume; upper phase) for 8 hours indicated a complete conversion of I to II. The content of ten 2-liter Erlenmeyer flasks (10 l culture) was filtered after acidification to pH 3; the filtrate was adjusted to pH 10, and then extracted three times with 2 liters of methylisobutyl-ketone. The combined organic solvent extracts were concentrated in vacuo to a small volume whereupon the compound was

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[3] A. Bertho and M. Goetz, Liebigs Ann. 619, 96 (1958).

[4] R. Pappo, U.S. Patent no. 2.913.455, Nov. 17, (1959).

[5] W.S. Johnson, V.J. Bauer, and R.W. Franck, Tetrahedron Letters No. 2, 72 (1961)

* Patents pending.

extracted with dilute sulfuric acid.

The combined acidic extracts were again extracted with methylisobutylketone after pH adjustment to 10. The final extract was thus concentrated in vacuo to yield 2.67 grams of crude crystalline II. An analytically pure sample could be prepared by recrystallisation respectively from ethanol-water, methanol-water and again from ethanol-water. Yield 1.30 grams of II. The compound has the following properties: melting point 108-110°C, λ_{max}^{MeOH}: 240 mμ (ε : 17000). Analysis: calc. for $C_{22}H_{33}NO$; C: 80.73%; H: 10.09%; N: 4.28% - found: C: 80.7%; H: 10.1%; N: 4.3%. Infrared spectrum: maxima in KBr-pellet: 1610 cm^{-1}, 1667 cm^{-1} (α, β-unsaturated ketone). Equivalent weight: 335. $(\alpha)_D^{20°}$: + 160.4° (c = 1; dioxane). R_F-value in butanol-acetic acid-water 0.68; R_F-value in methylisobutylketone-acetic acid-water (5 : 1 : 5; upper phase) 0.26. After spraying a chromatogram with a solution of sodium hydroxide a bright yellow fluorescent spot was observed in UV-light. This reaction is characteristic for a 3-keto-delta-4-structure.[6]

The same procedure was used and approximately the same yield was obtained with the conversion of I to II by Hypomyces haematococcus.

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[7] . Neher, J. of Chromatog. 1, 57 (1958).