Journal of Steroid Biochemistry, 1977, Vol. 8, pp. 311-312. Pergamon Press. Printed in Great Britain

MICROBIAL DEHYDROGENATION OF STEROID ALKALOIDS WITH TERTIARY AMINO GROUPS

I. BELIČ, M. MERVIČ, T. KASTELIC-SUHADOLC
Biochemical Institute of the Medical Faculty, University of Ljubljana
and
V. KRAMER
Institute Jožef Stefan, Ljubljana, Yugoslavia

(Received 14 October 1976)

SUMMARY

N-methyl-5α-tomatanin-3β-ol, 5α-conanin-3β-ol and demissidine are dehydrogenated by *Nocardia restrictus* to the corresponding 1,4-diene-3-keto derivatives. N-acetyl-5α-tomatanin-3β-ol is dehydrogenated mainly to its 3-keto derivative, the 1,4-diene-3-keto derivative being obtained in only 1% yield.

INTRODUCTION

The microbial transformation of steroid alkaloids proceeds by dehydrogenations in ring A as far as the 1,4-diene-3-keto stage. No side-chain cleavage or further degradation of the steroid nucleus could be found [1]. This behaviour was observed only with steroid alkaloids having a secondary amino group; whereas alkaloids with a primary amino group underwent acetylation of the amino group. Therefore, the study of steroid alkaloids with tertiary amino groups seemed desirable.

EXPERIMENTAL

Methods. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were taken in a 1 dm cell at 20°C. Mass spectra were recorded with a CEC 21-110 C mass spectrometer by direct insertion of the sample into the ion source maintained at 150°. Electron energy 70 eV and ionising current 150 μA were used. N.M.R. Spectra were obtained on a JEOL 100 MHz spectrometer in $CDCl_3$ as solvent and TMS as internal standard. Thin layer chromatography (t.l.c.) was done on 0.25 mm thick silica gel or alumina chromatoplates. Visualisation was effected by spraying with 50% sulphuric acid in ethanol followed by heating at 110–120° for 15 min.

Materials. Tomatidine and demissidine were obtained from Koch-Light Laboratories Ltd.

N-methyl tomatidine was prepared from tomatidine by the method of Sato, Latham and Ikekawa [2], but the product obtained showed two spots on t.l.c. (silica gel G, *n*-butanol–acetic acid (4:1)), the R_F value of tomatidine after two developments being 0.35 and that of *N*-methyl tomatidine 0.15. From 600 mg of the crude product, 530 mg of *N*-methyl tomatidine were obtained by preparative t.l.c., m.p. 216–218° (Sato [2] 218–220°). Mass spectrum: $M^{+\cdot}$ 429, *m/e* 152, 128.

Trivial names used: Tomatidine = 5α-tomatanin-3β-ol; *N*-methyl tomatidine = *N*-methyl-5α-tomatanin-3β-ol; *N*-acetyl tomatidine = *N*-acetyl-5α-tomatanin-3β-ol.

N-acetyl tomatidine was prepared from tomatidine according to Sato and Latham [3]. Mass spectrum: $M^{+\cdot}$ 457, *m/e* 414 ($M^{+\cdot}$ —$COCH_3$), *m/e* 273, 180, 156, 138 (180—$COCH_2$), 114 (156—$COCH_2$).

5α-Conanin-3β-ol was prepared by hydrogenation of 4-conenin-3-one according to Khuong-Huu, Yassi and Goutarel [4]. Mass spectrum: $M^{+\cdot}$ 331, *m/e* 316 ($M^{+\cdot}$ —CH_3), 71 ($\dot{C}H_2$—$N^+(CH_3)$=CH—CH_3) [5].

4-Conenin-3-one was obtained by incubation of conessine with *Cryptoclines cyclaminis* CBS 202.59 as described by de Flines *et al.* [6].

Incubation. Nocardia restrictus CBS 157.45 was maintained at 28°C on potato agar slants. The cultures were grown in 500 ml flasks containing 100 ml of the medium composed according to Wix *et al.* [8] of 2% cornsteep liquor (50% dry weight), 0.01% meat extract, 1% glycerol and water. Before sterilization the pH was adjusted to 6.8–7.0. After 72 h of incubation at 28°C on a rotatory shaker (280 rev./min), the alkaloid (10–20 mg), dissolved in 1 ml of ethanol, was added and the incubation continued.

RESULTS

Incubation of N*-methyl tomatidine*

N-methyl tomatidine (500 mg) was incubated with *Nocardia restrictus* until no *N*-methyl tomatidine could be detected by t.l.c., which took place in 48–72 h. The five metabolites detectable by t.l.c. were first separated on a column of alumina in chloroform solution, and the metabolite with the R_F value of 0.32 was additionally purified three times by preparative t.l.c., yielding 41 mg of metabolite, m.p. 180–182° and mol. wt. 423.3145 (calc. for $C_{28}H_{41}O_2N$: 423.3137).

The molecular ion at *m/e* 423 corresponds to the loss of six hydrogen atoms from *N*-methyl tomatidine. The presence of the ions at *m/e* 128 and 152 confirm the existence of unchanged E and F rings. Fragment ions at *m/e* 395 ($M^{+\cdot}$ –CO) and *m/e* 121 generated by ring B cleavage and subsequent hydrogen transfer [7], indicate the 1,4-diene-3-keto structure of

ring A. The absorption maximum λ_{max}^{EtOH} 243 nm (ϵ: 9,600) and the I.R. spectrum showing absorption bands at 1660, 1620 and 1600 cm^{-1} (1,4-diene-3-oxo-steroid) confirm the above structure. Therefore, the structure of the metabolite is *N*-methyl-1,4-tomatadien-3-one.

The remaining metabolites, presumed to be intermediate dehydrogenation products on the basis of their R_F values and absorption maxima, were not investigated in detail.

Incubation of N-*acetyl tomatidine*

When incubated for 7 days, *N*-acetyl tomatidine (1.1 g) was almost completely metabolized. The main metabolite (288 mg after separation on a column, R_F 0.65) shows a $M^{+\cdot}$ of 455, ions at *m/e* 412 ($M^{+\cdot}$—$COCH_3$), 271 (273—2 H), and ions at *m/e* 180, 156, 155, 138 and 114, which are the same as those of N-acetyl tomatidine, or two mass units less than *N*-acetyl tomatidine, all in agreement with the structure of *N*-acetyl-5α-tomatanin-3-one.

The metabolite (9 mg) with the R_F value of 0.43 had a m.p. 271–274°, λ_{max}^{EtOH} 244 nm (ϵ: 17,200), I.R. absorption bands at 1660, 1620 and 1600 cm^{-1}, all in agreement with a 1,4-diene-3-keto partial structure. Since the mass spectrum shows a $M^{+\cdot}$ of 451, six mass units less than the $M^{+\cdot}$ of *N*-acetyl tomatidine and the ion at *m/e* 121, the above partial structure is confirmed. Unchanged ions at *m/e* 180, 156, 155, 138 and 114, together with the $M^{+\cdot}$, exclude the possibility of other changes in the molecule, so that the structure of this metabolite is *N*-acetyl-1,4-tomatadien-3-one.

Incubation of 5α-conanin-3β-ol

5α-Conanin-3β-ol (300 mg) was incubated with *Nocardia restrictus* until it was completely metabolized, that is for 7 days. Two metabolites were detected by t.l.c./alumina GF_{254} with the solvent system cyclohexane–ethyl acetate (1:2, v/v). The metabolite with the R_F value of 0.48 was isolated by preparative t.l.c. yielding 76 mg of a metabolite having $[\alpha]_D$ + 61° ($CHCl_3$) and an absorption maximum $\lambda_{max}^{CHCl_3}$ 244 nm (ϵ: 14,600).

The molecular ion at *m/e* 325 shows the loss of six hydrogen atoms from 5α-conanin-3β-ol. The ion *m/e* 121 generated by ring B cleavage and subsequent hydrogen transfer confirms the 1,4-diene-3-keto structure of ring A. The radical ion *m/e* 71 which is formed by cleavage of C17-C20 (or C13-C18) bonds is stabilized by resonance [5]. Therefore, the structure of the metabolite is 1,4-conadien-3-one.

The other metabolite isolated by preparative t.l.c. has an R_F value of 0.56. Its mass spectrum shows a $M^{+\cdot}$ of 327 (four mass units less than 5α-conanin-3β-ol), and the ions *m/e* 312 ($M^{+\cdot}$—CH_3) and *m/e* 71, which appear in the mass spectra of 5α-conanin-3β-ol and 1,4-conadiene-3-one. The absorption maximum $\lambda_{max}^{CH_3OH}$ at 240 nm is in agreement with the 4-ene-3-keto structure of the metabolite. Since the R_F value and other data of this metabolite are the same as that of the authentic 4-conenin-3-one, the structure of this metabolite is 4-conenin-3-one.

Incubation of demissidine

Demissidine (100 mg) was incubated for 4 h, when it was completely transformed. Two metabolites could be detected by t.l.c. (silica gel GF_{254} with the solvent system cyclohexane–ethyl acetate (1:2, v/v). Visualization of the spots was effected by spraying with $Ce(SO_4)_2$ in sulphuric acid. The metabolites were separated by preparative t.l.c. The metabolite with the R_F 0.41 shows a $M^{+\cdot}$ of 393, six mass units less than the $M^{+\cdot}$ of demissidine, and ions *m/e* 378 ($M^{+\cdot}$—CH_3) and 204. The ion *m/e* 150 is formed by cleavage of the C13—C17 bond [5]. The ions *m/e* 204 and 150 appear in the mass spectrum of demissidine too. The ion *m/e* 121 points to the 1,4-diene-3-keto structure. The absorption maximum $\lambda_{max}^{CHCl_3}$ 244 nm is in agreement with the 1,4-diene-3-keto structure. Therefore, the structure of this metabolite is 1,4-demissidien-3-one.

The molecular ion of the other metabolite (R_F 0.53) $M^{+\cdot}$ of 395 shows the loss of four hydrogen atoms from demissidine. In the mass spectrum of this metabolite, the ion *m/e* 380 ($M^{+\cdot}$—CH_3) and unchanged ions 204 and 150 appear. The absorption maximum $\lambda_{max}^{CHCl_3}$ 241 nm confirms the 4-ene-3-keto structure. The structure of this metabolite is 4-demissiden-3-one.

DISCUSSION

The results of the present study show that a tertiary amino group of a steroid alkaloid has the same effect on microbial transformation as that of a secondary amino group; that is, only products of dehydrogenations in ring A are observed. *N*-acetyl tomatidine represents another example of an acetylated steroid alkaloid, which appear to be rather poor substrates for the introduction of double bonds in ring A by *Nocardia restrictus* [1].

Acknowledgements—The financial support of the Boris Kidrič Fund is gratefully acknowledged.

REFERENCES

1. Belič I. and Sočič H.: *Acta microbiol. Hung.* **22** (1975) 389–395.
2. Sato Y., Latham H. G. and Ikekawa N.: *J. Org. Chem.* **25** (1960) 1962–1965.
3. Sato Y. and Latham H. G.: *J. Am. Chem. Soc.* **78** (1956) 3146–3150.
4. Khuong-Huu Q., Jassi J. and Goutarel R.: *Bull. Soc. Chim.* Fr. (1963) 2486–2489.
5. Budzikiewicz H.: *Tetrahedron* **20** (1964) 2267–2278.
6. de Flines J., Marx A. F., van der Waard W. F. and van der Sijde D.: *Tetrahedron Lett.* (1962) 1257–1259.
7. Kienitz H.: *Massenspektrometrie.* Verlag Chemie, Weinheim (1968) p. 546.
8. Wix G., Büki K. G., Tömörkeny E. and Ambrus G.: *Steroids* **11** (1968) 401–405.