Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 843–846. Pergamon Press. Printed in Great Britain

MICROBIAL DEHYDROGENATION OF TOMATIDINE*

I. BELIČ and H. SOČIČ†
Biochemical Institute of the Medical Faculty, University of Ljubljana,
Chemical Institute "Boris Kidric", Ljubljana, Yugoslavia

(*Received* 26 *February* 1972)

SUMMARY

Tomatidine (tomatanin-3β-ol) was dehydrogenated by *Nocardia restrictus* to tamatanin-3-one, 1-tomatenin-3-one, 4-tomatenin-3-one and 1,4-tomatidien-3-one. No degradation of 1,4-tomatadien-3-one by *Nocardia restrictus* could be achieved.

INTRODUCTION

MICROBIAL dehydrogenation of steroids is one of the rather thoroughly investigated pathways of the steroid metabolism [2]. Such dehydrogenation is scarcely known to occur with steroidal sapogenins and steroidal alkaloids [3]. The present study concerns the microbial dehydrogenation of tomatidine. As dehydrogenating agents two well known steroid transforming microorganisms, *Nocardia restrictus* and *Mycobacterium phlei*, were used and compared as to their ability to dehydrogenate tomatidine.

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. Infrared spectra were recorded with a Perkin–Elmer Model 521 spectrophotometer in KBr. Ultraviolet spectra were determined in ethanol with a Unicam spectrophotometer. 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°C. Electron energy 70 eV and ionising current 100 μA were used.

Thin-layer chromatography (t.l.c.) was done either on 0·25 or 1 mm thick silicagel GF_{254} chromatoplates using the solvent system cyclohexane–ethyl acetate (1:2, v/v) with two developments, and 50% sulphuric acid for detection as described previously [4].

Materials

Tomatidine was purchased from Koch-Light Laboratories Ltd.

Tomatanin-3-one was prepared by the Oppenauer oxidation of tomatidine according to Toldy [5]. The unchanged tomatidine (R_F: 0·57) was separated from tomatanin-3-one (R_F: 0·70) by t.l.c. on silicagel G. After recrystalisation from methanol, tomatanin-3-one had a m.p. 198–200°C and $(\alpha)_D + 21°(CH_3OH)$.

Toldy [5] reported: m.p. 195–197°C; $(\alpha)_D + 18°(CH_3OH)$.

4-Tomatenin-3-one was prepared by the Oppenauer oxidation of 5-tomatenin-

* For preliminary communication see Ref. [1].

† Taken in part from the doctoral thesis of H. Sočič.

3β-ol as above. T.l.c. failed to satisfactorily separate 4-tomatenin-3-one (R_F: 0·54) from 5-tomatenin-3β-ol (R_F: 0·57) if silicagel G was used. Distinction, however, was possible by spraying the chromatoplate with paraformaldehyde-phosphoric acid [6], 4-tomatenin-3-one giving a green, and 5-tomatenine-3β-ol a violet spot.

4-Tomatenin-3-one, recrystalised from methanol, had a m.p. 202–204°C and $(\alpha)_D + 53°$ ($CHCl_3$).

Incubation

Nocardia restrictus CBS 157·45 obtained from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands, was maintained at 28°C on potato-agar slants. For incubation 5–7 day old cultures were used. The cultures were grown in 500 ml flasks containing 100 ml of the medium composed of 0·6% cornsteep liquor (50% dry weight), 0·3% $NH_4H_2PO_4$, 0·25% $CaCO_3$, 0·25% yeast extract and 1% glucose. Before sterilisation, the pH was adjusted to 4·5. After 48–72 h of incubation at 28°C on a reciprocal shaker, 20 mg of the steroid, dissolved in 1 ml of acetone, was added to each flask and the incubation continued for 24 h.

Isolation of metabolites

The pooled broths (from 3 g tomatidine) were filtered through a layer of kieselguhr and the filtrate extracted with chloroform. The combined extracts were washed with 5% $NaHCO_3$, then with water, dried over sodium sulphate and evaporated under reduced pressure. The oily residue (1·76 g), dissolved in light petroleum (b.p. 40–60°) -benzene (3 : 1, v/v), was chromatographed on 85 g of neutral alumina (Merck grade II-III) and eluted (15 ml fractions) first with light petroleum–benzene (3 : 1, v/v) and then with light petroleum–benzene (2 : 1, v/v), light petroleum–benzene (1 : 1, v/v), benzene, benzene–ether (3 : 1, v/v) and chloroform–methanol (1 : 1, v/v), until a total of 1·35 g of the metabolites were recovered.

The collected fractions were tested by t.l.c. whereupon appropriate fractions were combined, and further purified by preparative t.l.c. and recrystallisation.

RESULTS

Since preliminary experiments showed that *Mycobacterium phlei* produces essentially the same metabolites as *Nocardia restrictus*, only the latter was used for large scale incubation. The optimum pH 6·8–7·2 for the growth of *Nocardia restrictus* having been found inexpedient for the recovery of all metabolites of tomatidine, pH 4·5 was used.

When tomatidine (3 g) was incubated with *Nocardia restrictus* for 24 h, four metabolites and some unchanged tomatidine were isolated and identified. The main metabolite (640 mg) had a m.p. 242–245°C (cryst. from chloroform–methanol) and $(\alpha)_D + 16°$ ($CHCl_3$). On t.l.c. (R_F: 0·43) it gave with sulphuric acid a brown spot in daylight and an intense reddish-brown spot in U.V. light. The mass spectrum showed a molecular ion 409, corresponding to a loss of 6 hydrogen atoms from the tomatidine molecule (calc. for $C_{27}H_{39}O_2N$: 409), and intense peaks at m/e 138 and m/e 114, typical of the unchanged tomatidine rings E and F. The presence of the peak at m/e 288, corresponding to M-121, points to the 1,4-diene-3-one structure of ring A [7]. The absorption maximum in ethanol at 244 nm (ε: 15,400) [8] and the IR spectrum showing absorption bands at 1660 cm^{-1} (3 C=O), 1622 cm^{-1} (1 : 2 C=C) and 1605 cm^{-1} (4 : 5 C=C) [9], confirm

the above structure. Therefore, the structure of the metabolite is that of 1,4-*tomatadien*-3-*one*.

The metabolite (80 mg) with the R_F value of 0·70, whose spot with sulphuric acid was pale brown in daylight and intense yellow in U.V. light, had a m.p. 198–200°C (cryst. from methanol), $(\alpha)_D + 21°$ (CH_3OH), mass spectrum (M^+ 413, m/e 385, 138, 114), IR spectrum (3 C=O 1710 cm^{-1}), that is, identical with those of synthesised *tomatanin*-3-*one*.

The metabolite (20 mg) with the R_F value of 0·62 whose spot with sulphuric acid was yellow in daylight and intense blue in U.V. light, had a m.p. 179–182°C (cryst. from methanol) and $(\alpha)_D + 25°$ ($CHCl_3$). The mass spectrum showed a molecular ion 411, corresponding to a loss of 4 hydrogen atoms from the tomatidine molecule (calc. for $C_{27}H_{41}O_2N$:411), and intense peaks at m/e 138 and m/e 114. The IR spectrum, showing absorption bands at 1675 cm^{-1} (3 C=O) and 1622 cm^{-1} (1:2 C=C)[9], and the absorption maximum in ethanol at 231 nm (ϵ: 9·000)[8] are in agreement with the structure of 1-*tomatenin*-3-*one*.

The metabolite (52 mg) with the R_F value of 0·50, whose spot with sulphuric acid was orange–yellow in daylight and intense yellow in U.V. light, had a m.p. 200–204°C (cryst. from chloroform–methanol), $(\alpha)_D + 53°$ ($CHCl_3$), mass spectrum (M^+ 411, m/e 138, m/e 114), IR spectrum (3 C=O 1665 cm^{-1}, 4:5 C=C 1610 cm^{-1}), and an absorption maximum in ethanol at 241 nm (ϵ: 16,200), that is, identical with those of the synthesised 4-*tomatenin*-3-*one*.

Finally, a part of tomatidine (180 mg) was recovered unchanged. It did not differ from the original tomatidine in its R_F value (0·57), the spots with sulphuric acid of both were green in daylight and pale grey in U.V. light and had identical mass and IR spectra.

DISCUSSION

The above findings show that it is possible to dehydrogenate tomatidine by both *Mycobacterium phlei* and *Nocardia restrictus* to tamatanin-3-one, 1-tomatenin-3-one, 4-tomatenin-3-one and, finally, 1,4-tomatadien-3-one as illustrated in Fig. 1. Such a pathway of dehydrogenation is common to steroids and does not stop at the 1,4-diene-3-one compound, but continues to lower molecular weight compounds as shown in the review by Hörhold, Böhme and Schubert[2].

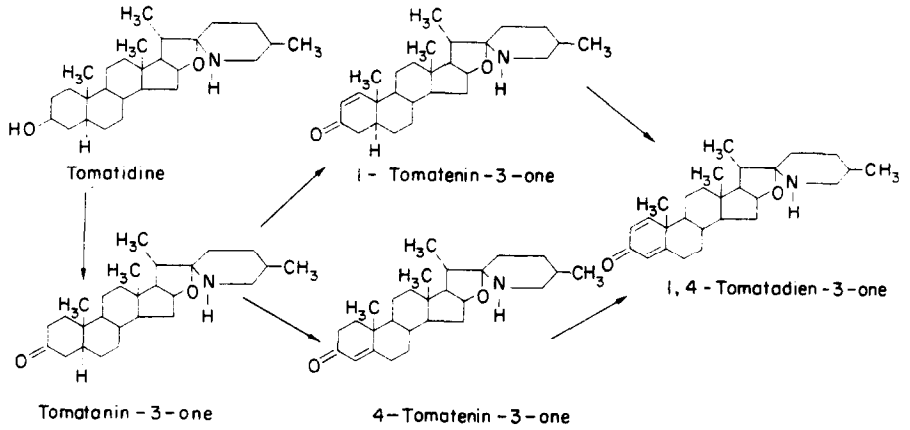

Fig. 1. Dehydrogenation of tomatidine by *Nocardia restrictus*.

In our case, however, it was impossible to observe a transformation of tomatidine beyond 1,4-tomatadien-3-one, even though experimental conditions had been varied, thus, for example, the incubation time was prolonged.

It is interesting to note that the rather efficient dehydrogenator *Fusarium solani*, which dehydrogenates over 90% of progesterone to 1,4-androstadiene-3,17-dione [10], and diosgenine to 1,4-androstadiene-3,16-dione, as shown by Kondo and Mitsugi [11], fails to dehydrogenate tomatidine.

Thus it seems that, at least in the case of some microbes, the nitrogen atom in the side chain of steroid alkaloids does not prevent the induction of enzymes which dehydrogenate steroid alkaloids to 1,4-dien-3-one compounds. The presence of the nitrogen atom, however, prevents both further degradation of the alkaloid and the splitting off of the side chain.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs. A. Cimerman for assistance with microbial incubations, Dr. J. Marsel and Eng. Chem. D. Milivojevic, Jozef Stefan Institute, Ljubljana for the determination of the mass spectra and B. P. Moll for translating the manuscript. We are also indebted to Dr. G. Willuhn for the generous gift of 5-tomatenin-3β-ol. The financial support of the Boris Kidrič Fund is gratefully acknowledged.

REFERENCES

1. Belič I. and Sočič H.: *Experientia* **27** (1971) 626.
2. Hörhold C., Böhme K. H. and Schubert K.: *Z. allg. Mikrobiol.* **9** (1969) 235.
3. Iizuka H. and Naito A.: *Microbial Transformation of Steroids and Alkaloids* University of Tokyo Press (1967) p. 228.
4. Sočič H. and Belič I.: *Z. Analyt. Chem.* **243** (1968) 291.
5. Toldy L.: *Acta Chim. Acad. Sci. Hung.* **16** (1958) 401.
6. Schreiber K., Aurich O. and Osske G.: *J. Chromat.* **12** (1963) 63.
7. Budzikiewicz H., Djerassi C. and Williams D. H.: *Structural Elucidation of Natural Products by Mass Spectrometry* Holden-Day, San Francisco (1964) pp. 21; 91.
8. Scott A. I.: *Interpretation of Ultraviolet Spectra of Natural Products* Pergamon Press, London (1964) pp. 64; 406.
9. Neudert W. and Röpke A.: *Atlas of Steroid Spectra* Springer, Berlin (1965) p. 293.
10. Belič I., Pertot E. and Sočič H.: *Mycopathol. Mycologia Appl.* **38** (1969) 225.
11. Kondo E. and Mitsugi T.: *J. Am. Chem. Soc.* **88** (1966) 4737.