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MICROBIAL DEHYDROGENATION OF DIHYDROTOMATIDINES

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SUMMARY

(22S, 25S)-22, 26-Epimino-5α-cholestane-3β, 16β-diol and (22R, 25S)-22, 26-epimino-5α-cholestane-3β, 16β-diol were dehydrogenated by *Nocardia restrictus* to (22S, 25S)-22, 26-epimino-1,4-cholestadien-3-one and (22R, 25S)-22,26-epimino-1,4-cholestadien-3-one respectively. (20S,22ξ,25ξ)-26-Acetylamino-5α-furostan-3β-ol was dehydrogenated to (20S,22ξ,25ξ)-26-acetylamino-1,4-furostadien-3-one with low yield only; whereas (25ξ)-26-amino-5α-cholestane-3β,16β,22ξ-triol was acetylated to (25ξ)-26-acetylamino-5α-cholestane-3β, 16β, 22ξ-triol. No degradation of the 1, 4-diene-3-ones by *Nocardia restrictus* was observed.

INTRODUCTION

IN A PREVIOUS publication [1] we described the dehydrogenation of tomatidine by *Nocardia restrictus* to 1,4-tomatadiene-3-one. In contrast to other 1,4-dien-3-one steroids, no further transformation of 1,4-tomatadiene-3-one could be observed The purpose of the present study was to find out whether changes in the side chain of tomatidine affect the course of the microbial dehydrogenation of 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 [2].

Materials

(22S, 25S)-22, 26-Epimino-5α-cholestane-3β, 16β-diol (I) and *(22R, 25S)-22, 26-Epimino-5α-cholestane-3β, 16β-diol (II)* were prepared from tomatidine (2 g) according to Adam and Schreiber[3]. Their procedure for the isolation of the two isomers by column chromatography was modified by using alumina (Merck,

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grade II–III) and dichloromethane. 1·2 g of I, m.p. 191–194°C (cryst. from benzene-hexane) and $(\alpha)_D - 15°$ (chloroform); and 0·5 g of II, m.p. 223–230°C (cryst. from methanol) and $(\alpha)_D + 16°$ (methanol), all in agreement with the data reported by Adam and Schreiber, were thus obtained.

The mass spectra of I and II, which are almost identical, exhibit molecular ions of low intensity at m/e 417 (about 0·5% rel.int.), (M-1) peaks amounting to about 1% relative intensity and a cluster of ions in the (M-18) region. The base peaks at m/e 98 arise from the 20–22 bond cleavage as found earlier [4].

(20S, 22ξ, 25ξ)-26-Acetylamino-5α-furostan-3β-ol (III) was prepared from tomatidine diacetate (0·4 g) by the method of Sato and Latham [5]. The III obtained (0·26 g) had a m.p. 174–176°C (cryst. from acetone-hexane), $(\alpha)_D - 5°$ (chloroform) and I.R. bands at 1660 and 1540 cm^{-1} (NH–Ac) in agreement with the data reported by Sato and Latham.

The mass spectrum showed a molecular ion at m/e 459 corresponding to $C_{29}H_{49}O_3N$. Ring E fission (i.e. 17–20 and C–16–O bonds) leads to the base peak at m/e 185 and the steroidal part, gives the second most prominent ion at m/e 273 by losing a hydrogen atom. The structure given above is likewise supported by the fragment ions $(M-CH_3)^+$ and $(M-CH_3CO)^+$.

(25ξ)-26-Amino-5α-cholestane-3β-16β,22ξ-triol (IV) was prepared by refluxing III (26 mg) with 6 N HCl for 7 h and evaporating the HCl in vacuum. On t.l.c. the residue (15 mg) showed only one spot with the R_F value of 0·10. The upper part of the mass spectrum (Fig. 1a) showed a molecular ion at m/e 435 (calc. for $C_{27}H_{49}O_3N$: 435), and ions at 417 $(M-H_2O)^+$, 399 $(M-2H_2O)^+$ and 381 $(M-3H_2O)^+$. The base peak at m/e 143 is due to the subsequent transfer of the C–22 hydrogen and to the 17–20 bond fission. On the other hand, a simple 17–20 bond cleavage leads to an ion at m/e 291 arising from the steroidal part of the molecule. Thus the structure formulated above is in agreement with the mass spectrum presented in Fig. 1a.

When III was refluxed with 6 N HCl for only 2 h, an additional spot with the R_F value of 0·46 was detected on t.l.c. The mass spectrum of this compound (Fig. 1b) showed a molecular ion at m/e 477 (calc. for $C_{29}H_{51}O_4N$: 477). Fragment ions at m/e 459, 441 and 423 indicate again that one, two and three molecules of H_2O, respectively, were lost from the M^+ ion. The principal fragment at m/e 185 is derived (analogous to III) from the McLafferty rearangement and the

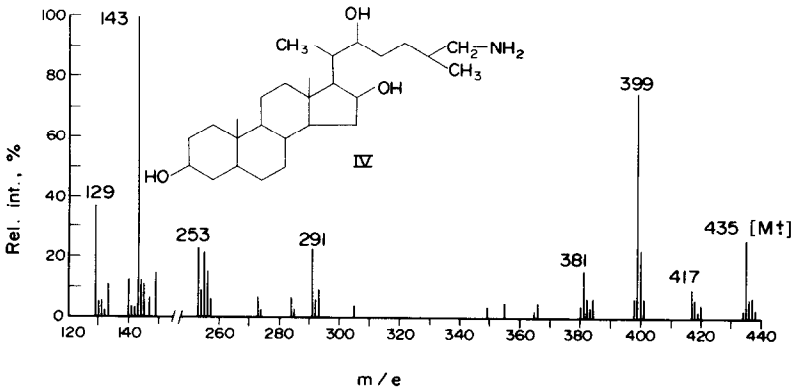

Fig. 1a. Mass spectrum of compound IV.

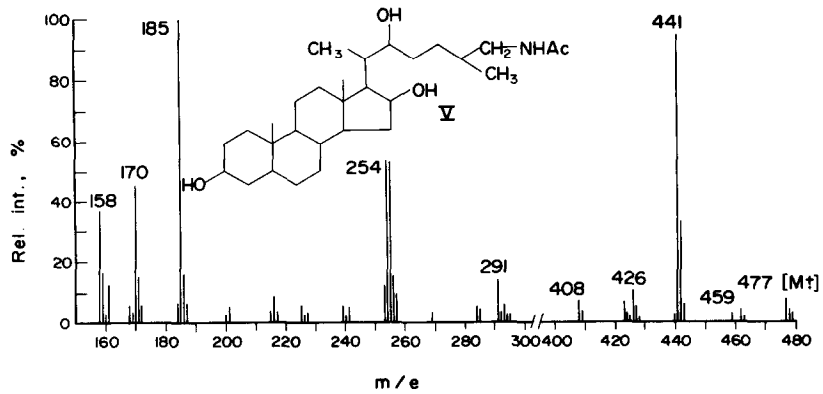

Fig. 1b. Mass spectrum of compound V.

17–20 bond cleavage. Ions at m/e 291, 255 (291–$2H_2O$) and 254 (255–H) confirm that the steroidal skeleton remains intact during the reaction. All the fragment ions arising from the side chain, as well as the M^+ ion, are shifted by 42 mass units (acetyl-group) towards the side of higher mass supporting the structure of *(25ξ)-26-acetylamino-5α-cholestane-3β,16β,22ξ-triol*, (V).

Incubation and isolation of metabolites

Incubation with *Nocardia restrictus* CBS 157·45 and the isolation and purification of the metabolites were carried out as described previously [1].

RESULTS

Incubation of I

When incubated with *Nocardia restrictus* for 18 h, I (150 mg) was completely metabolized. Only one metabolite (R_F value 0·58 on alumina in the solvent system benzene–methanol (95:5, v/v)), whose spot with sulphuric acid was brown in daylight and intense red in U.V. light, was observed. The metabolite (106 mg), m.p. 220–226°C and $(\alpha)_D - 11°$ (methanol), was purified by crystallisation from chloroform-ethylacetate.

The molecular ion at m/e 411 (calc. for $C_{27}H_{43}O_2N$:411) corresponds to the loss of six hydrogen atoms from compound I. The presence of the ion at m/e 98, resulting from the 20–22 bond fission [6] and by far the most abundant in both the spectrum of I and that of VI, confirms the existence of the unchanged ring F. Fragment ions at m/e 383 $(M-CO)^+$ 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 in ethanol at 244 nm (ε: 15,000) and the I.R. spectrum showing absorption bands at 1665 cm^{-1} (3 C=0), 1622 cm^{-1} (1:2 C=C) and 1605 cm^{-1} (4:5 C=C) confirm the above structure. Therefore, the structure of the metabolite is (22S, 25S)-22,26-*epimino-1,4-cholestadien-3-one* (VI).

Incubation of II

After 24 h of incubation, II (150 mg) was completely metabolized. Only one metabolite (R_F value 0·25 on alumina in the solvent system benzene–methanol (95:5, v/v)), whose spot with sulphuric acid was light brown in daylight and intense red in U.V. light, was observed. The metabolite (72 mg), m.p. 270–

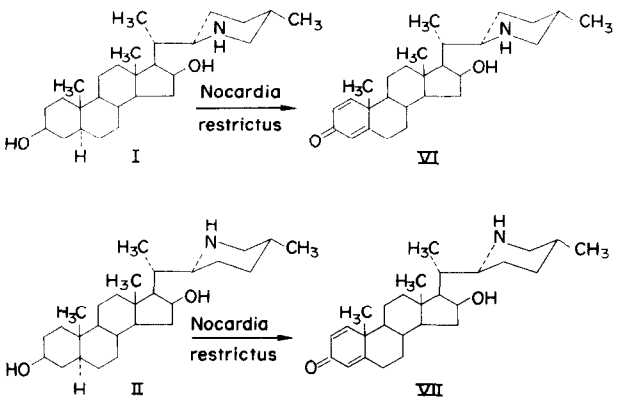

Fig. 2a. Dehydrogenation of dihydrotomatidines by *Nocardia restrictus*.

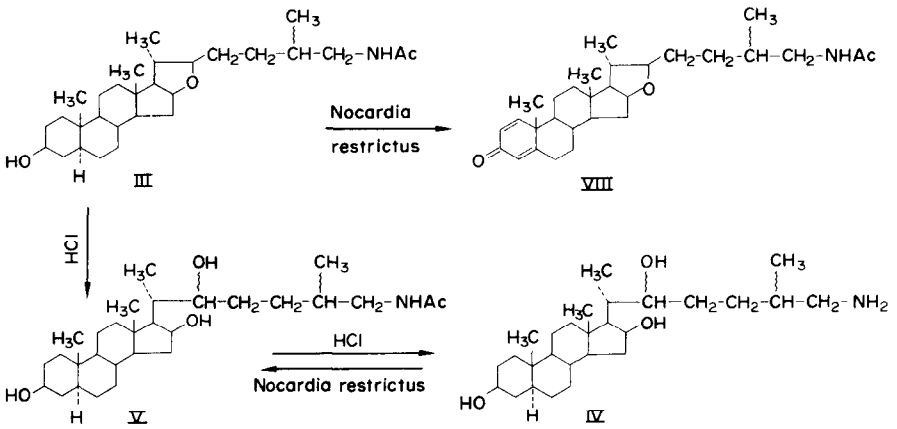

Fig. 2b. Dehydrogenation of dihydrotomatidines by *Nocardia restrictus*.

280°C and $(\alpha)_D + 14°$ (methanol), was purified by crystallization from chloroform-methanol. Its mass spectrum and I.R. spectrum were the same as those of VI. The U.V. spectrum in ethanol showed an absorption maximum at 244 nm (ϵ 11,000); thus, the structure of this metabolite is: (22R, 25S)-22,26-*epimino-1,4-cholestadien-3-one* (VII).

Incubation of III

When III (500 mg) was incubated for 100 h, some unchanged III (R_F 0·28 after two developments in cyclohexane–ethylacetate (1:2, v/v), light brown in daylight and intensely yellow in U.V.) and a metabolite with the R_F value of 0·20 whose spot with sulphuric acid was red in daylight and intense red in U.V. light, were detected. Better separation was achieved with the solvent system chloroform–ethanol (97:3, v/v). After two developments, the R_F value of III and that of the metabolite VIII were 0·30 and 0·46, respectively. The metabolite (30 mg) was isolated by preparative t.l.c. in the above solvent system. The mass spectrum showed a molecular ion at m/e 453, corresponding to the loss of six hydrogen atoms from the molecule of III (calc. for $C_{29}H_{43}O_3N$: 453). The six mass units shift from m/e 273 to m/e 267 and the ion at m/e 121, as well as the

$(M-121)^+$ ion, suggest the 1,4-diene-3-keto structure of ring A. The absorption maximum in ethanol at 245 nm (ϵ 11,300) and the IR. spectrum showing absorption bands at 1665, 1625 and 1610 cm^{-1} confirm the structure: (20*S*, 22*ξ*, 25*ξ*)-*26-acetylamino-1,4-furostadien-3-one* (VIII).

Incubation of IV

When IV (10 mg) was incubated for 48 h, only one metabolite was detected on t.l.c., whose R_F value, color of the spot and mass spectrum were identical with those of V.

DISCUSSION

When I and II were incubated with *Nocardia restrictus* the same results as with tomatidine were obtained, that is, I and II were dehydrogenated in high yield to the corresponding 1,4-dien-3-ones and no further transformation was observed. The opening of the ring E of tomatidine, therefore, seems to have no effect on the course of dehydrogenation.

When III with the intact ring E and an acetylated aliphatic amino group was incubated, different results from those with tomatidine and the above dihydrotomatidines were obtained. Some incubations yielded the expected 1,4-dien-3-keto derivative VIII even though the yield was low while others yielded no VIII at all.

As this behaviour might have been due to the presence of the acetylated amino group in the side chain, incubation with the deacetylated compound seemed desirable. Since, however, the deacetylation procedure yielded IV which beside the desired free aliphatic amino group, had also an open ring E, an exact answer to this question cannot be given. But, considering the results of the incubation of tomatidine and dihydrotomatidines I and II with open ring E, it may be assumed that the pattern of dehydrogenation of IV with open ring E does not differ from that of the corresponding compound with ring E.

The incubation of IV produces V as the only detectable metabolite. The structure of V is easily determined since the same compound is formed during deacetylation by boiling with hydrochloric acid. The microbial acetylation of an amino group in the side chain of a steroid was first reported by Smith *et al.* [8].

They found that *Streptomyces roseochromogenus* acetylates 21-amino-9α-fluoro-11β, 17α-dihydroxy-4-pregnene-3, 20-dione to 21-acetylamino-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione. Thus it seems that such microbial acetylation of an amino group in the steroid side chain might be found to occur more frequently.

The results of the present study confirm, therefore, our previous observation [1] that the presence of a nitrogen atom in the side chain of a steroid alkaloid has a strong effect on the course of microbial transformation. The heterocyclic nitrogen in the side chain prevented, not only in the case of tomatidine but also in the case of dihydrotomatidines, a further degradation of the steroid nucleus beyond the 1,4-dien-3-one stage. If an aliphatic amino group is present in the side chain of a steroid, acetylation takes place and the resulting acetylated compounds do not seem to be good substrates for dehydrogenation by *Nocardia restrictus*.

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