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MICROBIAL CLEAVAGE OF THE TOMATIDINE SPIROKETAL SIDECHAIN

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SUMMARY

Sidechain cleaving enzymes induced by cholesterol in *Arthrobacter symplex* are capable of cleaving the spiroketal sidechain of tomatidine. Yields of 2·5–4·0% of 1,4-androstadiene-3,17-dione are obtained in the presence of α,α-dipyridyl.

INTRODUCTION

We have found previously that tomatidine [1] and dihydrotomatidines [2] are dehydrogenated by *Nocardia restrictus* and *Mycobacterium phlei* to the corresponding 1,4-diene-3-oxo compounds. But no degradation of the steroid nucleus beyond the 1,4-diene-3-oxo stage and no sidechain cleavage could be observed. Two explanations can be envisaged for this specific behaviour of tomatidine and its derivatives: either they do not act as inducers for the degrading and sidechain splitting enzymes or they are not substrates at all for such enzymes. The aim of the present study was to elucidate this question.

EXPERIMENTAL

Materials

Tomatidine and cholesterol were purchased from Koch–Light Laboratories Ltd. as well as 1,4-androstadiene-3,17-dione, which was twice recrystallised from ethyl ether; tomatanin-3-one, 1-tomatenin-3-one, 4-tomatenin-3-one and 1,4-tomatadiene-3-one were obtained by incubation of tomatidine with *Nocardia restrictus* [1].

Incubation

Arthrobacter symplex IAM 1660, obtained from the Culture Collection of the University of Tokyo, Japan, was maintained on potato agar slants. Cultures were grown at 28°C in a medium [3] containing cornsteep liquor 1%, meat extract 0·2% and K_2HPO_4 0·05% in distilled water at pH 7. The medium (150 ml) was shaken in 500 ml flasks on a rotatory shaker (250 rev./min, 4–5 cm. amplitude).

After 72 h of incubation, cholesterol (1·5 mg), dissolved in 1 ml of ethanol, was added to each flask and the incubation continued for 24 h until cholesterol was completely degraded. Then tomatidine (30 mg) and α,α-dipyridyl (1·5 mg) was added and the incubation continued.

Separation of metabolites

An aliquot of the incubation broth was filtered and the filtrate extracted with chloroform three times. The combined extracts were washed with water, dried over sodium sulphate and evaporated under reduced pressure. The residue was dissolved in ethanol and the metabolites separated by t.l.c. using 0·25 mm thick silicagel GF_{254} chromatoplates and the solvent system dichloromethane–methanol (93:7, v/v). For spot detection spraying with 50% H_2SO_4 in ethanol was used as described previously [4].

The metabolite (R_F 0·63, λ_{max}^{EtOH} 244 nm) gave with sulphuric acid a red spot in daylight and an intense orange spot in U.V. light, and a mass spectrum (M^+ 284), all identical with those of an authentic sample of 1,4-androstadiene-3,17-dione.

Determination of 1,4-*androstadiene*-3,17-*dione*

The quantitative estimation of 1,4-androstadiene-3,17-dione in the broth was carried out by separating the metabolites by t.l.c. as described above. The band of 1,4-androstadiene-3,17-dione was then marked in U.V. light (R_F 0·63), scraped from the plate and eluted quantitatively with ethanol. The ethanolic eluate was evaporated, the residue dissolved in 4 ml of ethanol and the extinction was read against a reagent blank of silica gel eluate on a Beckmann DU spectrophotometer at 244 nm. In the range between 10–50 μg of 1,4-androstadiene-3,17-dione Beer's law was obeyed and the recovery of the added 1,4-androstadiene-3,17-dione averaged 95 ± 1·5%.

RESULTS AND DISCUSSION

When tomatidine was incubated with *Arthrobacter symplex* in the presence of α,α-dipyridyl, four metabolites were detected by t.l.c. and identified as tomatanin-3-one, 1-tomatenin-3-one, 4-tomatenin-3-one

Trivial name used: tomatidine = (22S,25S)-5α-tomatanin-3β-ol.

and 1,4-tomatadien-3-one as described previously [1]. No degradation of 1,4-tomatadien-3-one was achieved by changing the conditions of the incubation. These results are in agreement with our previous findings [1] that tomatidine is not a substrate for the microbial sidechain splitting enzymes. Microorganisms such as *Fusarium solani* and Mycobacteria which have been found capable of cleaving the sidechain of sapogenins [5, 6] possessing a similar structure to that of tomatidine were found ineffective with tomatidine.

When the sidechain cleaving enzymes were induced in *Arthrobacter symplex* by incubation in the presence of cholesterol, and tomatidine added to the incubation broth afterwards, sidechain cleavage of tomatidine occurred. Although the main products of the transformation of tomatidine were also, in this case, dehydrogenated derivatives of tomatidine, a conversion of tomatidine to 1,4-androstadiene-3,17-dione with yields ranging from 2·4 to 4·5% was obtained when the incubation was carried out for 72 to 150 h. This yield is lower than that (15%) obtained by Nagasawa *et al.*[3] from cholesterol with *Arthrobacter symplex* but similar to that (2·5 and 4·0%) obtained by Ambrus and Büki[6] from sapogenins with *Mycobacterium phlei.*

The above results suggest that the nitrogen atom in the sidechain of tomatidine prevents it from being an inducer for the sidechain cleaving enzymes, but not from serving as a substrate for such enzymes if the enzymes were induced by a steroid without a nitrogen atom in the sidechain.

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