BIOLOGICAL TRANSFORMATION OF 17α,21-DIHYDROXY-4-PREGNENE-3,20-DIONE WITH FRUIT-BODY SLICES OF SOME MUSHROOMS*

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17α,21-Dihydroxy-4-pregnene-3,20-dione was submitted to preparative enzymatic transformation with fruit-body slices of three *Basidiomycetes*. The transformation products formed were isolated and identified.

In a previous communication[1] it was demonstrated that the testing of *Basidiomycetes* for their ability to hydroxylate or otherwise transform steroids may be carried out very conveniently with slices of naturally grown fruit-bodies. However, in the mentioned paper only a single preparative experiment was described. The results obtained with some 20 species were only qualitative, and the characterisation of the transformation products formed from 17α,21-dihydroxy-4-pregnene-3,20-dione (cortexolone, Reichstein's compound S) or 3β-hydroxy-5-androsten-17-one (dehydroepiandrosterone) was carried out only by paper and thin-layer chromatography.

In this paper we describe the results of transformation of 17α,21-dihydroxy-4-pregnene-3,20-dione with three as yet untested species. In all three instances the transformation was carried out on a preparative scale and the main isolated products of the transformation were characterised not only by chromatographic means but other conventional methods as well.

From our data it follows that the described method of transformation of steroids with tissue slices of mushroom fruit bodies, although not very efficient, may still be useful not only for screening purposes but for identification purposes as well. From the results obtained with *Lactarius necator* and the data described in the first paper on this method[1] it would seem that 2β-hydroxylation is a typical feature of the Lactarius genus which might be utilisable in systematics.

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EXPERIMENTAL

Material and Methods

17α,21-dihydroxy-4-pregnene-3,20-dione was of commercial origin, containing traces of 3β,17α, 21-trihydroxy-5-pregnen-20-one as impurity.

Solvent system: S_1 (Silufol 254) chloroform–isobutyl alcohol (5 : 1), S_2 (silica gel G plates) chloroform–methanol (98 : 2 to 90 : 10); S_3 (Whatman No 1) formamide/chloroform; S_4 (Whatman No 3) formamide/chloroform. Detection under the UV light (254 nm) or by spraying with alkaline Blue tetrazolium solution[1].

Fruit bodies of the investigated mushrooms were collected in the forests of Central Bohemia (near Prague) and worked up immediately. The following species were tested: *1. Lactarius necator* (PERS. ex FR.) KARST.; *2. Naematoloma fascicular* (HUDS. ex FR.) KARST.; *3. Russula ochroleuca* (PERS.) FR.

Transformation Procedure

The collected fruit-bodies were cut to small approximate cubes of about 1—8 mm size and suspended in a 3—4 fold amount of tap water. 17α,21-Dihydroxy-4-pregnene-3,20-dione (1 g per 1·7—1·9 kg mushrooms, dissolved in a 100 fold amount of methanol) was added to the suspension under stirring. Air was then blow through the suspension overnight at room temperature, ensuring both aeration and stirring. After filtration of the suspension through a gauze the filtrate was extracted twice with ethyl acetate (*N. fascicular*) or chloroform (*L. necator* and *R. ochroleuca*). The volume of the solvent for each extraction was equal to that of the filtrate. The extract was dried and evaporated to about 50 ml volume and washed free of acidic material with two 20 ml portions of 5% sodium hydrogen carbonate solution. After drying over sodium sulfate the organic layer was evaporated to dryness. The residue was submitted to countercurrent distribution between light petroleum and 90% methanol in three funnels using the double-withdrawal procedure. The combined methanolic fractions were evaporated to dryness and analysed by thin-layer chromatography. The residue was fractionated by dry-column chromatography on silica gel containing 10% of water, using first chloroform and then a chloroform–methanol mixture (with a content of methanol increasing up to 10%). In the case of *L. necator* ethyl acetate in chloroform was used instead.

Identification of the Isolated Compounds

1) *L. necator* transformed 17α,21-dihydroxy-4-pregnene-3,20-dione to two products. From 1·4 g of starting compound 171 mg were recovered and two transformation products isolated. The first product (85 mg) had R_F 0·72 in system S_3 and 0·47 in S_2 (90 : 10); the second product had R_F 0·41 and 0·52 in the same systems, respectively. The first product did not react with blue tetrazolium but quenched the fluorescence of the Silufol 254 plates. Its R_F values was identical with 17α,20β,21-trihydroxy-4-pregnene-3-one (*I*). The substance would not crystallise. Therefore it was acetylated by the pyridine method at room temperature, affording a product (diacetate) which, when crystallised from aqueous methanol had a double melting point (95—100°C and 181—188°C) close to that of an authentic sample crystallised from the same mixture. Literature[2] gives m.p. 189—191°C (acetone). The mixture melting point of the acetylated transformation product and of the authentic sample was undepressed and the IR spectra of the diacetate and the free triol were identical with the spectra of authentic samples.

According to chromatographic data the second product corresponded to 2β,17α,21-trihydroxy-4-pregnene-3,20-dione. This product also would not crystallise and the isolated amount was too small for further preparative purification. Therefore the whole fraction was purified by chromatography in system S_4. The zone containing the required product was cut out, dried in a desiccator over conc. sulfutic acid, and eluted with chloroform. The eluate was washed with water in three microtest tubes using a countercurrent distribution technique, dried and evaporated. The residue was submitted to IR spectrography showing that the isolated product (less than one milligramme) was identical with authentic 2β,17α,21-trihydroxy-4-pregnene-3,20-dione.

2) The main product of transformation with *N. fascicular* obtained from 500 mg of 17α,21-dihydroxy-4-pregnene-3,20-dione weighed 84 mg. Some starting material (73 mg) was also recovered. After crystallisation from acetone–light petroleum its m.p. was 203–205°C, $[\alpha]_D$ +61° ±4° (*c* 0·8, chloroform), R_F 0·32 in S_1; the product absorbed on Silufol 254 under the UV light of 254 nm wavelength and gave a positive reaction with blue tetrazolium. For $C_{21}H_{30}O_5$ (362·45) calculated: 69·58% C, 8·34% H; found: 69·31% C, 8·24% H. These data suggested that the product was 6β,17α,21-trihydroxy-4-pregnene-3,20-dione (*II*). The identity was corroborated on the basis of its mixture melting point with an authentic specimen which was undepressed, and the IR spectrum which was identical with the spectrum of authentic 6β,17α,21-trihydroxy-4-pregnene-3,2-dione.

3) *R. ochroleuca* transformed the added 17α,21-dihydroxy-4-pregnene-3,20-dione (700 mg) in low yield. The main fraction (60 mg) contained according to thin-layer chromatography analysis (R_F and detection) a product which corresponded to derivative *I*. As it would not crystallise it was acetylated to a diacetate the melting point (187–191°C), $[\alpha]_D$ 152·1° ± 4° (*c* 0·8, chloroform), IR spectrum and analysis of which corresponded to the data for diacetate of *I* (lit.[2] gives m.p. 189–191°C, $[\alpha]_D$ +150°). For $C_{25}H_{36}O_6$ (432·54) calculated: 69·42% C, 8·39% H; found: 69·22% C, 8·31% H.

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