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METABOLISM OF PROGESTERONE BY *ASPERGILLUS FUMIGATUS*

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SUMMARY

Metabolism of progesterone by a typical strain of *Aspergillus fumigatus* was studied. The four metabolites isolated were characterized as 5α-pregnane-3β-ol-20-one, 15β-hydroxy-1,4-pregnadiene-3,20-dione, 7β,15β-dihydroxy-4-pregnene-3,20-dione and 11α,15β-dihydroxy-4-pregnene-3,20-dione by the application of various spectrometric techniques.

INTRODUCTION

11α-Hydroxylation and 1-dehydrogenation of progesterone are the two important steps which are necessary for the preparation of important steroid drugs, e.g. prednisone, prednisolone, triamcinolone etc. Both these steps are conveniently carried out by microbiological methods. The reports on microbiological transformations of steroids are many [1–4]. However, industrially exploited processes are few and there is still scope for further improvement of the methods as well as combination of two or more steps into a single economically viable step. During our screening programme for isolation of microorganisms capable of producing 1-dehydrogenated and 11α-hydroxylated products of progesterone we isolated a typical strain of *Aspergillus fumigatus* [6] using progesterone as the sole source of carbon. The *Aspergillus* strain produced four metabolites which are reported in this communication. Although hydroxylation [1,4,5] at various sites in the progesterone molecule and reduction of the 3-keto group [7] by *Aspergillus* species have been reported it appears 1-ene-dehydrogenation and reduction of the 3-keto group cum hydrogenation of the 4-ene-double bond by any *Aspergillus* species have not so far been encountered. Evidently the production of metabolite A (**2**) and metabolite B (**3**) by the strain is of special interest.

MATERIALS AND METHODS

Culture method

Aspergillus fumigatus (AM-21) was isolated from soil by the enrichment culture technique using progesterone as a sole source of carbon. This was cultivated in flasks containing sterilized medium, composition (% w/v, sucrose 1, corn steep liquor 0.5, K_2HPO_4 0.05, pH 6.5). To each Erlenmeyer flask (250 ml) containing 50 ml of the medium, 25 mg of crystalline progesterone dissolved in acetone was added. Inoculation of the sterilized medium was made with a cell suspension obtained from a 3 day old culture maintained in Czapek Dox agar slant. A batch of 12 flasks thus inoculated was incubated at 30°C for 3 days.

Extraction and isolation of the transformed products

The culture solution in the flasks were pooled and filtered to separate the broth from the mycelia. The broth and the mycelia were then extracted with chloroform. The extracts were washed with water and dried over anhydrous Na_2SO_4. The two extracts were combined and the solvent evaporated when a semi-solid residue was obtained (0.2068 g).

The residue was chromatographed on a column of neutral alumina (4 g), elution being taken successively with benzene–chloroform (19:1, v/v) chloroform and chloroform–methanol (19:1, v/v). The fractions obtained were further purified by rechromatography and preparative t.l.c. followed by crystallisation. Thus, unconverted progesterone (96 mg), metabolite A (6 mg), metabolite B (15 mg), metabolite C (54 mg) and metabolite D (25 mg) were isolated. Thus, about two thirds of the progesterone was accounted for and the rest was lost.

Analytical methods

t.l.c. Was carried out on silica gel G (BDH) using chloroform–methanol (96:4, v/v) as solvent. Optical rotations were measured in chloroform on a Perkin-Elmer automatic polarimeter. i.r. Spectra were recorded on a Perkin-Elmer 177 spectrophotometer in Nujol mulls. Mass spectra were obtained on a Hitachi model RMU-6L mass-spectrometer at 70 eV by the direct insertion method. ^1H nmr and ^{13}C n.m.r. spectra were recorded at 100 MHz in $CDCl_3$ and C_5D_5N respectively with Tetra methyl silane as the reference compound on a JEOL FT-100 n.m.r. spectrometer. Melting Points were obtained in open capillary tubes in a sulphuric acid bath and are uncorrected.

RESULTS AND DISCUSSION

Metabolite A (**2**)

$C_{21}H_{34}O_2$ (M^+ 318) m.p. 197–198°C, $[\alpha]_D$ + 95° (*c*, 1,2 in $CHCl_3$) showed in its i.r. spectrum the hydroxyl band at 3400 cm^{-1} but the spectrum did not show any absorption band assignable to α-β unsaturated ketone. The mass spectrum displayed peaks at *m/z* (%) 318 (M^+, 100), 303 (M^+-CH_3, 17.8), 300 (M^+-H_2O, 43.8), 260 (24.6), 233 (32.8), 215 (53.4), 107 (50.6) and 84 (79.4) which indicated **2** to be a monohydroxy pregnane-20-one. The ^1H n.m.r. spectrum showed signals at δ 0.61 (3H, s, H-18), 0.81 (3H, s, H-19), 2.12 (3H, s, H-21) and 3.60 (1H, m, H-3), the pattern and positions of which suggested the identity of **2** as 5α-pregnane-3β-ol-20-one [8,9]. The physical data of **2** also agree well with those reported in the literature (Table 2).

Metabolite B (**3**)

$C_{21}H_{28}O_3$, m.p. 227–228°C, $[\alpha]_D$ + 137° (*c*, 0.92 in $CHCl_3$) showed in its MS the ion peaks at *m/z* (%) 328 (M^+, 12), 310 (M^+-H_2O, 52), 295 (M^+-H_2O-CH_3, 20), 267 (M^+-H_2O-CO-CH_3, 100), 253 (20), 228 (16), 161 (24) and 141 (26) indicating the presence of an additional double bond and a hydroxyl group in the progesterone skeleton. The characterization of **3** as 15β-hydroxy-pregna-1,4-diene-3,20-dione was accomplished by its ^1H n.m.r. spectrum which exhibited signals at δ 0.96 (3H, s, H-18), 1.12 (3H, s, H-19), 2.16 (3H, s, H-21), 4.48 (1H, m, H-15), 5.68 (1H, s, H-4) and 6.24 (2H, ABq, J = 12 Hz, H-1 and H-2) and also by comparison of its physical data (Table 2) with those of an authentic sample [10, 11]. The substituent effect [12] of hydroxyl function on C_{18} and C_{19} methyl protons in the steroid molecule indicated the location of the hydroxyl group at C_{15}.

Metabolite C (**4**)

$C_{21}H_{30}O_4$, m.p. 228–229°C, $[\alpha]_D$ + 131° (*c*, 1.2 in $CHCl_3$) showed in its mass spectrum ion peaks at *m/z*. 346 (M^+, 2.6), 328 (M^+-H_2O, 13.4), 310 (M^+-2H_2O, 21), 295 (M^+-2H_2O-CH_3, 5.7), 267 (M^+-2H_2O-CH_3-CO, 44.6), 247 (21), 229 (57), 163 (7.3), 124 (31.5), 100 (21) and 43 (100) indicating it to be a dihydroxy progesterone. The ^1H n.m.r. spectrum which suggested the positions and orientations of the two hydroxyl groups [12] displayed signals at δ 1.0 (3H, s, H-18), 1.28 (3H, s, H-19), 2.2 (3H, s, H-21), 3.52 (1H, m, H-7), 4.48 (1H, m, H-15) and 5.80 (1H, s, H-4). The positions and configuration of the hydroxyl groups as in **4** were ascertained from ^{13}C n.m.r. spectral data (Table 1) using substituent effects of the hydroxyl groups [13, 14] on different carbons. Finally, **4** was characterized as 7β,15β-dihydroxy-4-pregnene-3,20-dione by comparison of its m.p. and $[\alpha]_D$ with those reported in the literature [15] (Table 2).

Metabolite D (**5**)

$C_{21}H_{30}O_4$, m.p. 195–196°C, $[\alpha]_D$ + 133° (*c*, 0.82 in $CHCl_3$) turned out to be a dihydroxy progesterone from its MS which showed ion peaks at *m/z* 346 (M^+, 38), 328 (M^+-H_2O, 9.5), 310 (M^+-2H_2O, 31), 295 (M^+-2H_2O-CH_3, 9.5), 247 (69.4), 229 (19), 163 (57), 124 (95), 100 (8.5) and 43 (100). The ^1H n.m.r. spectrum exhibited signals at δ 1.02 (3H, s, H-18), 1.38 (3H, s, H-19), 2.2 (3H, s, H-21), 4.08 (1H, m, H-11), 4.37 (1H, m, H-15) and 5.78 (1H, s, H-4). The pattern

Table 1. ^{13}C chemical shifts (δ ppm) of 7β,15β-dihydroxy-4-pregnene-3,20-dione (**4**), 11α,15β-dihydroxy-4-pregnene-3,20-dione (**5**)

	Chemical Shifts		
Carbon	Progesterone **1**	**4** (Δ4–1)	**5** (Δ5–1)
1	35.8	35.9 (+0.1)	38.0 (+3.0)
2	34.2	34.3 (+0.1)	33.8 (−0.4)
3	197.8	198.0 (+0.2)	199.1 (+1.4)
4	124.0	124.7 (+0.7)	124.5 (+0.5)
5	170.1	167.1 (−3.1)	171.4 (+1.3)
6	36.7	44.0 (+7.3)	36.8 (+0.1)
7	32.1	73.6 (+41.5)	31.8 (−0.3)
8	35.4	39.8 (+4.4)	34.7 (−0.7)
9	53.7	51.1 (−2.6)	59.4 (+5.7)
10	38.6	38.6 (+0.0)	40.5 (+1.9)
11	21.1	21.3 (+0.2)	69.0 (+47.9)
12	38.6	39.9 (+1.3)	52.1 (+13.5)
13	43.8	43.1 (−0.7)	44.0 (+0.2)
14	55.3	60.8 (+5.5)	60.0 (+4.7)
15	24.4	71.3 (+46.9)	68.0 (+43.6)
16	23.0	35.9 (+12.9)	36.8 (+13.8)
17	63.3	63.6 (+0.3)	63.7 (+0.4)
18	13.3	15.8 (+2.5)	18.3 (+5.0)
19	17.1	17.1 (+0.0)	17.4 (+0.3)
20	207.3	207.6 (+0.3)	207.4 (+0.1)
21	31.3	31.3 (+0.0)	31.4 (+0.1)

Table 2. Comparison of melting points and specific rotations of metabolites A, B, C and D with the literature values

Compound	Present work		Literature values		
	m.p. (°C)	$[\alpha]_D$	m.p. (°C)	$[\alpha]_D$	References
Metabolite A	197–198	+95°($CHCl_3$)	194–195	+95°($CHCl_3$)	[8]
Metabolite B	227–228	+137°($CHCl_3$)	230–233	—	[10,11]
Metabolite C	228–229	+131°($CHCl_3$)	231–233	+136°($CHCl_3$)	[15]
Metabolite D	195–196	+133°C($CHCl_3$)	202–203	+134°($CHCl_3$)	[16,17]

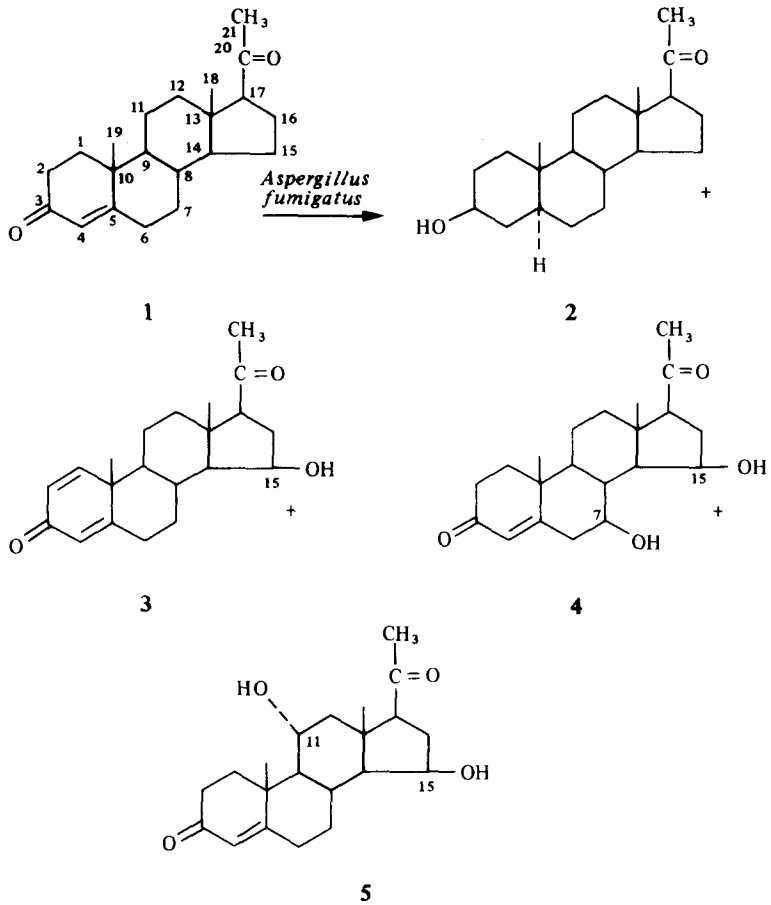

Fig. 1. Progesterone and its metabolites formed by *Aspergillus fumigatus*.

and positions of different signals suggested **5** to be 11α,15β-dihydroxy-4-pregnene-3,20-dione. The identity was further confirmed from its ^{13}C n.m.r. spectral data (Table 1) and by comparison of its m.p. and $[\alpha]_D$ with those of the literature values [16, 17] (Table 2).

The ^{13}C signals of **4** and **5** were assigned by known chemical shift rules [18], substituent effect of hydroxyl function on different carbons and multiplicity information obtained from single frequency off resonance as well as by comparison of the shift data of progesterone and its hydroxy derivatives [13, 14].

The four metabolites formed from progesterone by the *Aspergillus fumigatus* strain are shown in Fig. 1. The combination of the types of reactions involved in the transformation is unique.

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