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1β,7β-DIHYDROXYHECOGENIN, A SPIROSTANE PRODUCED FROM HECOGENIN BY *CUNNINGHAMELLA ELEGANS*

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Key Word Index—*Cunninghamella elegans*, Phycomycetes, Mucorales, spirostanes, 1β,7β-dihydroxyhecogenin, hecogenin

Abstract—The major transformation product produced by incubation of hecogenin with *Cunninghamella elegans* is (25*R*)-1β,3β,7β-trihydroxy-5α-spirostan-12-one (1β,7β-dihydroxyhecogenin)

INTRODUCTION

Cunninghamella elegans has been shown to effect a wide range of hydroxylations of steroidal compounds [1-3] However, incubation of steroidal sapogenins with this fungus has received little attention and only few studies have been carried out on microbiological transformations of these compounds [4, 5]

RESULTS AND DISCUSSION

Compound A, $C_{27}H_{42}O_6$, (M^+ at m/z 462 298, calculated 462 298), mp (uncorr) 223-225°, $[\alpha]_D^{20}$ + 20 84° (MeOH, c 0 15) was the major product obtained after incubation of (25*R*)-3β-hydroxy-5α-spirostan-12-one (hecogenin) with *Cunninghamella elegans* Absorptions were observed in the IR spectrum of compound A at 3440 (hydroxyl), 1694 (carbonyl), and 976, 919, 902 and 862 cm^{-1} (spiroketal), with the absorption at 902 cm^{-1} being of greater intensity than that at 919 cm^{-1} (25*R*-spirostane) [6] The mass spectrum revealed that the compound was a trihydroxy keto spirostane with the mode of fragmentation being similar to that of hecogenin The base peak at m/z 139 and the intense fragment at m/z 126 confirmed that hydroxy groups were absent from ring F [7] Major fragments at m/z 390 and 348, and the absence of prominent ions at m/z 168 and 153 localized the hydroxy and carbonyl groups to rings A–C [8] The major fragment at m/z 273 in the mass spectrum of hecogenin, formed partly by the expulsion of carbon monoxide from ring C, with the protons of C-11 retained, was present at m/z 305 in the mass spectrum of compound A [7]

Two of the three hydroxy groups of compound A were readily located at C-1 and C-3 from the ^1H NMR spectrum (270 MHz TMS int standard) The C-3 hydroxy group present in hecogenin was shown to be unmodified in compound A from the resonance at δ 3 59 ($CDCl_3$, 1H, *m*, $W_{1/2} \simeq 25$ Hz, H-3α), and one of the introduced hydroxy groups can be assigned to the C-1β position by the presence of a doublet of doublets at δ 3 66 (C_5D_5N, 1H, $J_{1\alpha\,2\beta} \simeq 11\ 5$ Hz and $J_{1\alpha 2\alpha} \simeq 4\ 4$ Hz, H-1α) The second introduced hydroxy group gave rise to a multiplet overlapping with the downfield C-26α proton signals at δ 3 31 In the ^1H NMR spectrum of compound A triacetate (M^+ at m/z 588), mp 270–271 °, the signal for the proton associated with the second introduced hydroxy group was seen as a multiplet at δ 4 63 (C_5D_5N, 1H, $W_{1/2} \simeq 26$ Hz, H-7α), indicating equatorial substitution This observation together with the C-18 and C-19 methyl signals of both compound A (δ 1 06 and 0 95) and its triacetate (δ 1 05 and 1 10) are consistent also with either 6α or 7β hydroxylation [9, 10] A clear distinction between the two possible 6α and 7β hydroxylation sites was obtained from the ^{13}C NMR spectrum of compound A ($CDCl_3$, 22 54 MHz), in particular when values for C-5, C-8 and C-19 were compared (Table 1) From these data the second introduced hydroxy group was readily assignable to the 7β-position and thus compound A is (25*R*)-1β,3β,7β-trihydroxy-5α-spirostan-12-one

EXPERIMENTAL

Hecogenin (0 25 mg/ml) was incubated for 9 days with *Cunninghamella elegans* Ledner (CBS 167 53) using the liquid nutrient and general microbial procedure described by Crabb *et al* [13] The mixture was filtered and the filtrate satd with NaCl before being extracted with CH_2Cl_2 The extract was treated with Na_2SO_4, evaporated to dryness and the residue dissolved in a small vol of $CHCl_3$ On standing a white solid (compound A) was obtained floating on the surface, which was removed by filtration

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Table 1 ^{13}C NMR spectral data of hecogenin and 1β,7β-dihydroxyhecogenin*

Carbon No	Hecogenin [11]	1β,7β-Dihydroxy-hecogenin	1β,7β-Dihydroxy-hecogenin (calculated values) [11, 12]	1β,6α-Dihydroxy-hecogenin (calculated values) [11, 12]
1	36 5	76 2†	76 4	76 6
2	31 2	42 4	42 1	41 8
3	70 7	67 6	68 2	67 8
4	37 8	37 5	36 8	30 9
5	44 6	39 4	40 8	50 5
6	28 3	37 8	37 5	68 8
7	31 4	73 4	74 3	40 8
8	34 4	42 7	42 7	33 4
9	55 5	53 5	54 0	55 1
10	36 0	41 2	41 6	42 8
11	37 8	40 5	41 4	41 3
12	213 0	213 7†	213 2	213 0
13	55 0	55 2	55 4	54 4
14	55 8	54 5	54 9	55 3
15	31 5	34 4	34 6	31 8
16	79 1	79 4	79 4	78 9
17	53 5	52 7	52 3	53 3
18	16 0	16 2	15 8	15 7
19	12 0	6 6	6 5	7 5
20	42 2	42 4	42 2	42 2
21	13 2	13 3	13 2	13 2
22	109 0	109 3	109 0	109 0
23	31 2	31 4	31 2	31 2
24	28 8	28 8	28 8	28 8
25	30 2	30 2	30 2	30 2
26	66 8	67 0	66 8	66 8
27	17 1	17 1	17 1	17 1

* Values given in δ-values (ppm)

† Value obtained from solution in Me_2CO

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