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THE ISOLATION AND STRUCTURE OF 23-DEOXYANTHERIDIOL AND THE SYNTHESIS OF ITS C-22 EPIMER[1]

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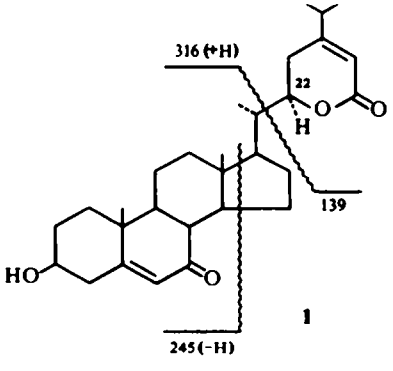
Abstract—The isolation and identification of a new steroid, 22*R*-3β, 22-dihydroxy-24-carboxymethyl-enecholest-5-en-7-one 24″, 22-lactone (**1**) (23-deoxyantheridiol) from the culture filtrates of the water mold *Achyla bisexualis* is described. A synthesis of the 22*S*-isomer of (**1**) is also reported. On the basis of these studies the 22*S*, 23*R*-stereochemistry is proposed for the fungal sex hormone antheridiol.

IN CONTINUATION of our studies of sexual reproduction in the water mold *Achlya* (at the New York Botanical Garden), we have examined another female strain (No. 369) of *A. bisexualis*, collected at Lago di Garde, Italy in 1958, for production of antheridiol (hormone A). In order to obtain crystalline hormone we found it necessary to modify the method of isolation which had been employed previously.[4]

Culture liquids of the mold (10 batches of 50 liters) were extracted with methylene chloride (10 × 12·5 liters) and the concentrated extract shaken briefly with dilute sodium hydroxide solution to remove fatty acid material. This treatment did not affect the activity of the extract. The solvent was then removed and the residue chromatographed on silica gel with ethyl acetate-petroleum (1 : 1). This gave 23-deoxyantheridiol (**1**, 3 mg, m.p. 265–270°) and the slightly more polar antheridiol (**2**, 2 mg, m.p. 245–252°). The two compounds appeared to be present in approximately equal amounts in other extracts we examined, including one which was prepared many years ago by Prof. J. Raper[4] and which he very kindly supplied to us. They were also present in an extract of another species, *A. ambisexualis* (No. 734).

The mass spectrum of (**1**) (M^+ 454) indicated the presence of one less O atom than in antheridiol ($C_{29}H_{42}O_5$, M^+ 470). The IR spectrum showed peaks at 1706, 1672 and 1633



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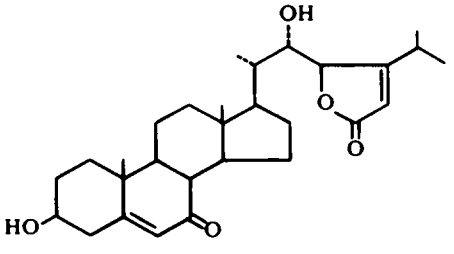

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cm^{-1} and the UV spectrum a maximum at 230 nm (ε 17,900). When the ethanolic solution was refluxed with hydrochloric acid the UV spectrum changed to λ_{max} 212, 278 nm. Since antheridiol (**2**) exhibits the same UV spectral changes on exposure to acid, this evidence suggests that the compound contains the 3β-hydroxy-Δ^5-7-ketone system present in (**2**).[6] The remaining two oxygens could be accommodated in an unsaturated Δ-lactone. The mass spectrum suggested the location of this structure on the side chain. Thus, important fragment ions are rationalized as shown in Structure 1.

Deoxyantheridiol (**1**) possessed an activity of about 1/1000 that of antheridiol but this was probably due to contamination with antheridiol.

In order to reach a definite decision concerning the structure and activity of deoxyantheridiol a synthesis of this substance was attempted at Syntex Research from the previously described α, β-unsaturated lactone (**3a**),[7] an early intermediate in the synthesis of antheridiol. Exposure of (**3a**) to dilute hydrochloric acid in methanol yielded the hydroxy lactone (**3b**) which was photooxygenated in pyridine solution containing hematoporphyrin as sensitizer.[8] The resulting Δ^6-5α-hydroperoxide (**4**) was treated without purification with copper acetate to generate the Δ^5-7-ketone chromophore.[9] This afforded in 50% yield (after prep TLC) a keto lactone (**5**), m.p. 271–273°, which was not identical with deoxyantheridiol on the basis of spectral and TLC comparisons. However, the NMR spectra of the synthetic and natural products have essentially the same patterns

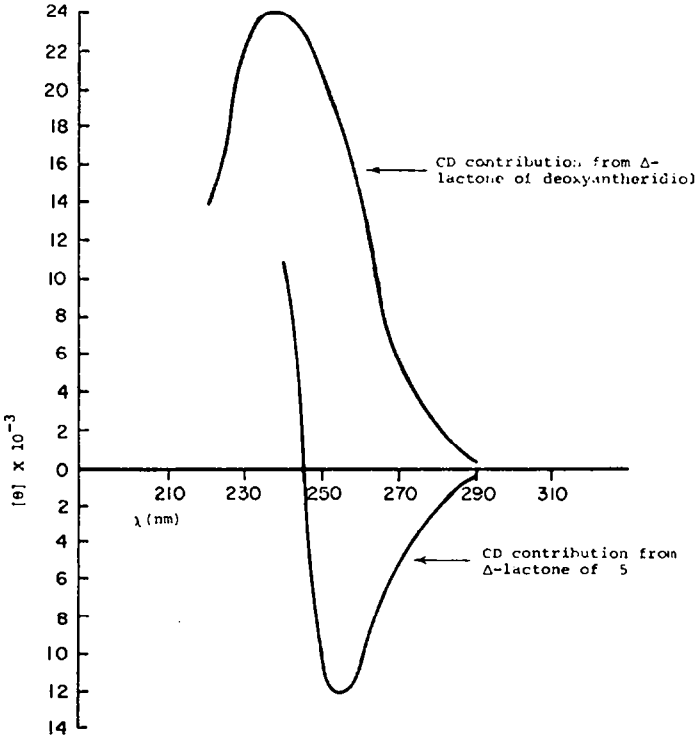
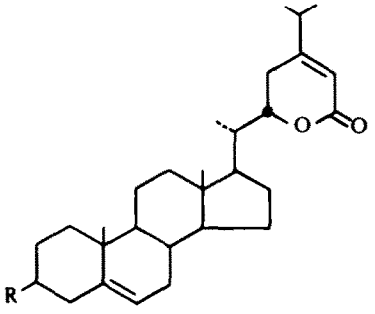
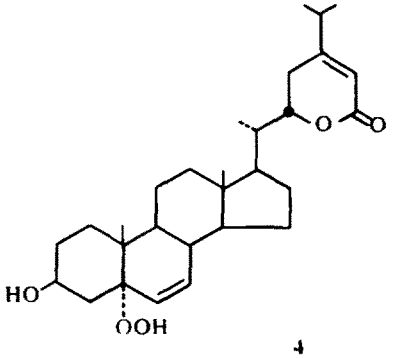


FIG. 1.



3a: R = tetrahydropyran-2'-yloxy
3b: R = OH



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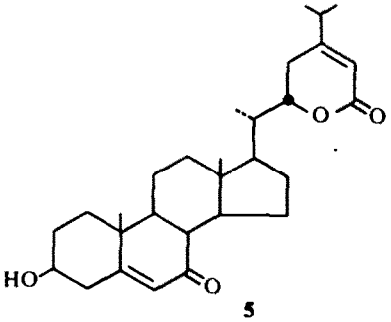

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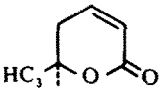

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and differ only in the chemical shifts of specific signals. Thus, the 21-H doublet is fully visible at 1·04 ppm, J=6 Hz, in the spectrum of the synthetic sample, whereas only the high field arm of this doublet is observed in the spectrum of deoxyantheridiol. The low field portion of the latter doublet falls under one of the isopropyl-H signals at 1·06 ppm. The 22-H signals appear as pairs of poorly resolved triplets centered at *ca* 4·33 and 4·41 ppm in the case of the synthetic lactone (**5**) and at *ca* 4·30 and 4·42 ppm for the natural product. Moreover, the mass spectrum of the synthetic lactone shows the same fragmentation pattern as deoxyantheridiol, the cleavage of the C_{20}—C_{22} bond and the D ring being principal pathways of fragmentation for (**5**). On the basis of this evidence it is concluded that deoxyantheridiol (**1**) and the synthetic product (**5**) are isomeric at the C_{22} centers.

The absolute configuration of the lactone rings of deoxyantheridiol (**1**) and (**5**) was determined from CD measurements. The curves shown in Fig. 1 were obtained by subtracting the CD curve of 7-ketocholesterol from the CD curves of deoxyantheridiol and (**5**). This gives the contribution of the Δ-lactone chromophore which is a positive curve for the natural product and a negative curve for the synthetic lactone (**5**). Since parasorbic acid (**6**) exhibits a positive CD curve[10] it follows that deoxyantheridiol has the same absolute configuration at C_{22} as (**6**). Thus, deoxyantheridiol is a 22R($22\beta_F$) lactone, whereas (**5**) belongs to the 22S($22\alpha_F$) series. The former assignment agrees with the C_{22} stereochemistry of the naturally occurring Δ-lactones withaferin A[11] and jaborosolactone A[12] which was deterimined by X-ray and CD measurements.

The foregoing conclusions provide an additional clue to the stereochemistry of the hydroxy butenolide system of antheridiol (2). It seems reasonable to assume that antheridiol and 23-deoxyantheridiol have the same absolute configuration at C_{22} since these substances are biosynthesized by the same strain of *A. bisexualis*. On this basis, antheridiol has the 22*S*($22\beta_F$) configuration. Since the method used for elaboration of the hydroxy butenolide system in the synthesis of antheridiol leads to the erythro configuration at the C_{22} and C_{23} centers* this requires that antheridiol possess the 22*S*,23*R* ($22\beta_F$,$23\beta_F$) stereochemistry.

The synthetic lactone (5) was inactive in the biological assay for hormone A at a concentration of 28 nanogram/ml (antheridiol is active at a concentration as low as 7 picogram/ml).

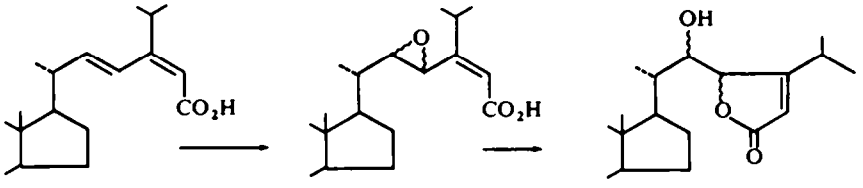
EXPERIMENTAL

Culture conditions for *A. bisexulais* No. 369 were the same as described in an earlier paper.[4] Fifty liters of culture liquid were extracted twice with CH_2Cl_2 (7·5 and 5 l.). The combined extract was concentrated to about 100 ml under reduced pressure at 50°. About 1 l. of concentrate was shaken briefly with 100 ml 10% NaOH, washed with water, dried (Na_2SO_4) and the solvent removed, leaving a brown gum (300 mg). This was chromatographed on silica gel (50 g, 0·05–0·2 mm) with EtOAc–light petroleum (b.p. 60–80°) 1:1, fractions of 20 ml being collected. Fractions 17–20 were almost pure 1 (3 mg, m.p. 265–270°) while 22 and 23 gave 2 (2 mg, m.p. 245–252°). Fractions 24–25 gave 70 mg of crystalline amide ($C_{11}H_{18}O_2N_2$, m.p. 158–161°) which had been obtained previously (though incorrectly reported as being eluted before antheridiol).[4]

On silica gel F-254 plates (Merck), 1 and 2 gave fluorescent spots R_f 0·58 and 0·43, respectively, with $CHCl_3$–MeOH 15:1. The NaOH aq from above, on acidification (dil HCl) and extraction with EtOAc, gave a brown gum (600 mg). TLC showed this to contain one major acidic component and chromatography on silica gel (60 g) with EtOAc–light petroleum 1:5 gave a colorless waxy solid 150 mg, m.p. 43–45°; ν_{max} (KBr) 3425, 2670, 1712 cm^{-1} (Found: C, 75·15; H, 11·91; O, 12·88; M.W. 382 by measurement of vapor pressure. $C_{24}H_{46}O_3$ requires: C, 75·34; H, 12·12; O, 12·54%; M.W. 382·61).

Deoxyantheridiol 1 had the following spectral properties: CD† (c *ca* 0·001, MeOH) $[\theta]_{400}$ −25°, $[\theta]_{380}$ −90°, $[\theta]_{374}$ −90°, $[\theta]_{371}$ ±0°, $[\theta]_{360}$ +600°, $[\theta]_{350}$ +1550°, $[\theta]_{340}$ +2760°, $[\theta]_{330}$ +3400°, $[\theta]_{326}$ +3410°, $[\theta]_{320}$ +3160°, $[\theta]_{310}$ +2130°, $[\theta]_{300}$ +1230°, $[\theta]_{290}$ +850°, $[\theta]_{280}$ +2280°, $[\theta]_{270}$ +5740°, $[\theta]_{260}$ +7830°, $[\theta]_{250}$ +5360°, $[\theta]_{240}$ −9780°, $[\theta]_{230}$ −22,830°, $[\theta]_{220}$ −42,270°; ν_{max} (KBr) 3470, 1706, 1672, 1633 cm^{-1}; λ_{max} (EtOH) 230 nm (*ε* 17,900); NMR‡§ 0·71 (18-H), 1·02 (d., J = 6 Hz,

* The hydroxy butenolide system (*iii*) of antheridiol was elaborated from the 22,23(*t*)-dienoic acid (*i*) by epoxidation followed by lactonization. Stereospecific opening of the mixed epoxides (*ii*) yields a mixture of the 22,23-erythro butenolides (*iii*).[7]



i *ii* *iii*

†Circular dichroism spectra were recorded on a Jasco ORD/UV-5 spectrometer adapted for CD measurements. We are indebted to Dr. L. Throop, Syntex Research, for assistance with these measurements.

‡NMR spectra were determined on a Varian HA 100 spectrometer in deuterochloroform using tetramethylsilane as internal reference, chemical shifts are reported as parts per million (ppm) on the δ scale.

§This spectrum was time-averaged using an IBM 1800 computer. We are indebted to Dr. M. Maddox, Syntex Research, for determining this spectrum.

21-H), 1·10 (d, J = 7 Hz, isopropyl-CH_3), 1·19 (19-H), 3·3–3·8 (m, 3α-H), 4·3, 4·4 (pair of broad t., 22-H), 5·69 (6-H), 5·75 ppm (lactone olefinic-H). Mass spectrum (determined by Morgan–Schaffer Corp. on a Hitachi Perkin–Elmer RMU-6D spectrometer equipped with a direct inlet system, at 190°, ionizing potential of 70 eV and accelerating voltage of 1·75 kV): *m/e* (relative intensity) 454 (28, M^+), 436 (23), 316 (41), 245 (100), 192 (33), 161 (30), 139 (45). The soln of deoxyantheridiol which had been used for the UV spectrum (0·36 mg in 10 ml EtOH) was treated with a few drops of 6N HCl and refluxed for 4 hr. The UV spectrum now gave maxima at 278 and 212 nm.

Acid hydrolysis of tetrahydropyranyl ether (**3a**). A soln of 1·55 g of **3a** in 100 ml MeOH containing 2 ml conc HCl was allowed to stand at room temp for 30 min. The mixture was neutralized with dil NaOH aq and the bulk of the MeOH was evaporated. Water was added and the ppt was collected, dried and crystallized from aqueous MeOH to yield 1·17 g of **3b** m.p. 221–223°; $[\alpha]_D$ −76°; λ_{max} (MeOH) 205 nm (ε 13,100). (Found: C, 79·23; H, 10·14. $C_{29}H_{44}O_3$ requires: C, 79·04; H, 10·07%).

Irradiation of hydroxy lactone (**3b**). A soln of 0·3 g of **3b** in 30 ml pyridine containing 21 mg hematoporphyrin was irradiated in a water-cooled jacket with two 15 Watt fluorescent lamps (General Electric F 15 T 12) for 17 hr during which time O_2 was bubled through the soln. The mixture was diluted in the ether, treated with charcoal, filtered through celite and the ether evaporated. Cupric acetate·H_2O (0·15 g) was added to the pyridine soln and the resulting mixture was stirred for 5 hr and diluted with EtOAc. This soln was washed sequentially with dil phosphoric acid, dil NaHCO aq and water, dried and evaporated. The crude product was purified by preparative TLC (3% MeOH in $CHCl_3$) and crystallized from acetone MeOH to yield 0·15 g of **5**, m.p. 271–273°; $[\alpha]_D$ −138°; CD (*c*, 0·002, MeOH) $[\theta]_{400}$ ±0°, $[\theta]_{384}$ −30°, $[\theta]_{380}$ −65°, $[\theta]_{376}$ −77°, $[\theta]_{370}$ ±0, $[\theta]_{360}$ +330°, $[\theta]_{350}$ +1160°, $[\theta]_{340}$ +2170°, $[\theta]_{330}$ +3030°, $[\theta]_{320}$ +2870°, $[\theta]_{310}$ +2110°, $[\theta]_{300}$ +1040°, $[\theta]_{289}$ ±0°, $[\theta]_{280}$ −2180°, $[\theta]_{270}$ −6300°, $[\theta]_{260}$ −14,710°, $[\theta]_{250}$ −24,740°, $[\theta]_{240}$ −22,100°, $[\theta]_{230}$ −14,140°; λ_{max} (EtOH) 229 nm (ε 18,600); ν_{max} (KBr) 3450, 1690, 1675, 1630 cm^{-1}; NMR 0·69 (18-H), 1·04 (d, J = 6 Hz, 21-H), 1·09 (d, J = 7 Hz, isopropyl-CH_3), 1·16 (19-H), 3·4–3·8 (m, 3α-H), 4·3, 4·4 (pair of broad t., 22-H), 5·69 (6-H), 5·77 ppm (lactone olefinic-H). Mass spectrum (determined on an Atlaswerke CH-4 spectrometer equipped with a direct inlet system, an ionizing potential of 70 eV and an acceleration voltage of 3 kV): *m/e* (relative intensity) 454 (20, M^+), 436 (28), 316 (43), 245 (30), 192 (18), 161 (17), 139 (49), 47 (100). (Found: C, 75·85; H, 9·19. $C_{29}H_{42}O_4$·0·4 H_2O requires: C, 75·72; H, 9·33%).

7-Ketocholesterol. CD (*c*, 0·003, MeOH) $[\theta]_{400}$ −20°, $[\theta]_{390}$ −30°, $[\theta]_{385}$ −50°, $[\theta]_{380}$ −110°, $[\theta]_{377}$ −120°, $[\theta]_{374}$ −110°, $[\theta]_{370}$ ±0°, $[\theta]_{365}$ +100°, $[\theta]_{360}$ +320°, $[\theta]_{350}$ +730°, $[\theta]_{340}$ +3360°, $[\theta]_{330}$ +4320°, $[\theta]_{325}$ +4510°, $[\theta]_{320}$ +4300°, $[\theta]_{310}$ +3000°, $[\theta]_{300}$ +1620°, $[\theta]_{290}$ +470°, $[\theta]_{280}$ ±0°, $[\theta]_{270}$ −500°, $[\theta]_{260}$ −4700°, $[\theta]_{250}$ −15,530°, $[\theta]_{240}$ −33,570°, $[\theta]_{230}$ −45,120°, $[\theta]_{223}$ −56,240°.

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[3] New York Botanical Garden, The Bronx, New York, New York. We thank Barbara Bloswick, Helen McMorris and Robert Bruckman for technical assistance. This work was supported by grants HDO0850 and GM 12150 from the National Institutes of Health

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