Structures of Oogoniol-1, -2, and -3, Steroidal Sex Hormones of the Water Mold, *Achlya*

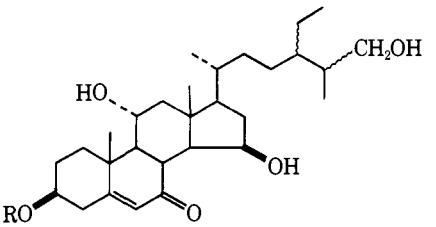
Sir:

Sexual reproduction in the water mold, *Achlya,* is initiated by secretion of the steroidal hormone, antheridiol, by the female strain.[1] Antheridiol induces the formation of antheridial branches in the male strain and also stimulates it to secrete a second hormone (hormone B) which acts on the female, causing the formation of oogonial branches.[2] Certain hermaphroditic strains of *Achlya* have been found to secrete hormone B without prior stimulation by exogenous antheridiol.[3] Extracts of culture liquids of one such strain of *A. heterosexualis* Whiffen-Barksdale, have yielded two crystalline compounds which possess hormone B activity. We wish to propose the structures **1** and **2** for these compounds, which have been named oogoniol-1 and -2. A third, closely related compound, **3,** oogoniol-3, was obtained as a noncrystalline mixture with another compound which has been identified as the C-15 ketone, **7.**

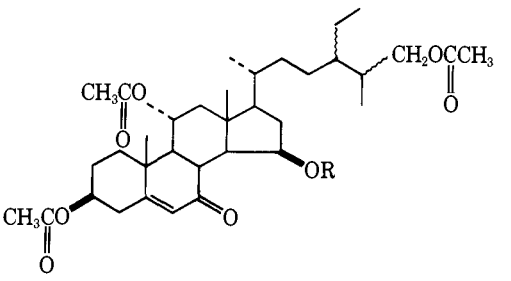
Crude active crystalline fractions from chromatography of methylene chloride extracts of the culture liquid[3] were further purified by multiple development preparative thin layer chromatography with ethyl acetate–petroleum ether (1:1), to give **1** (mp 165–167° (H_2O–MeOH); uv λ_{max}^{EtOH} 235 nm (ϵ 13,500); ir $\nu_{max}^{CHCl_3}$ 3625, 3525, 1732, 1662 cm^{-1}; NMR (220 MHz, $CDCl_3$, δ) 0.84 (t, J = 7 Hz, 29 H), 0.95 (d, J = 6 Hz, 27 H), 0.98 (18 H), 1.01 (d, J = 7 Hz, 21 H), 1.16 (d, J = 7 Hz, isobutyrate-CH_3), 1.36 (19 H), 3.65 (m, 26 H), 4.16 (m, 11β H, OH), 4.71 (m, 3α H, 15α H), 5.82 (s, 6 H); mass spectral *m/e* 546.3908 (M^+, 9), 458 (100), 301 (5), 299 (21), 283 (30), 161 (62), (calcd for $C_{33}H_{54}O_6$ 546.3920)), **2** (mp 161–163° (EtOAc–petroleum ether); uv λ_{max}^{EtOH} 235 nm (ϵ 14,300); ir $\nu_{max}^{CHCl_3}$ 3625, 3540, 1735, 1660 cm^{-1}; NMR ($CDCl_3$, δ), 0.84 (t, J = 7 Hz, 29 H), 0.95 (d, J = 7 Hz, 27 H), 0.98 (18 H), 1.05 (d, J = 7 Hz, 21 H), 1.10 (t, J = 6 Hz, propionate-CH_3), 1.36 (19 H), 2.32 (q, J = 6 Hz, propionate-CH_2), 3.65 (m, 26 H), 4.16 (m, 11β H, OH), 4.74 (m, 3α H, 15α H), 5.82 (s, 6 H); mass spectral *m/e* 532.3762 (M^+, 9), 458 (100), 440 (27), 301 (7), 299 (20), 283 (28), 161 (57), (calcd for $C_{32}H_{52}O_6$ 532.3764)), and two other components, a mixture of **3** and **7** present in a ratio of roughly 3:1 (ir $\nu_{max}^{CHCl_3}$ 3620, 1735, 1660 cm^{-1}; NMR ($CDCl_3$, δ), 0.84 (t, J = 7 Hz, 29 H), 0.95 (d, J = 6 Hz, 27 H), 0.98 (18 H), 1.03 (d, J = 7 Hz, 21 H), 1.36 (19 H), 2.04 (acetate), 3.66 (m, 26 H), 4.14 (m, 11β H, OH), 4.72 (m, 3α H, 15α H), 5.82 (s, 6 H of **3**), 5.86 (s, 6 H of **7**); mass spectral *m/e* 518.3588 (M^+ of **3**), 458 (100)).

The CD curve of **2,** [θ] nm +6772 (331), −91,900 (217) in MeOH was similar to that of 7-keto cholesterol, [θ] nm +4153 (323), −66,000 (214), but differed from that of Δ^4-cholesten-3-one, [θ] nm −6521 (313), +43,430 (220). This, together with uv and NMR evidence, established the Δ^5-7-ketone chromophore in **2.** The mass spectral data indicated the location of the ester group in relation to the unsaturated ketone (very ready loss of isobutyric, propionic, and acetic acids from the molecular ion of **1, 2,** and **3,** respectively, giving the intense ion at *m/e* (458).

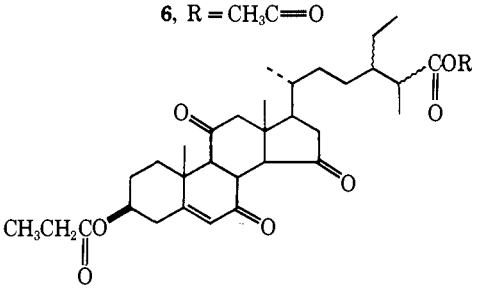
Catalytic hydrogenation (5% Pd:C–MeOH) of **2** afforded the dihydro derivative: mp 179–181°; ir $\nu_{max}^{CHCl_3}$ 1733, 1714 cm^{-1}; mass spectral *m/e* 534.3918 (M^+). Treatment of **1** with 1.1 equiv of aqueous K_2CO_3 in methanol overnight gave the tetrahydroxy ketone **4** (mp 181–183° (EtOAc); ir ν_{max}^{KBr} 3430, 1652 cm^{-1}; NMR ($CDCl_3$ + CD_3OD, δ) 0.84 (t, J = 7 Hz, 29 H), 1.02 (d, J = 7 Hz, 21 H), 0.96 (18 H), 1.34 (19 H), 4.14 (m, 11β H), 4.68 (m, 15α H), 5.80 (s, 6 H); mass spectral *m/e* 476 (M^+ 21), 458 (92), 440 (28), 301 (25), 161 (100)) and some elimination product, the $\Delta^{3,5}$-7-ketone; λ_{max}^{EtOH} 282 nm (ϵ 20,000).



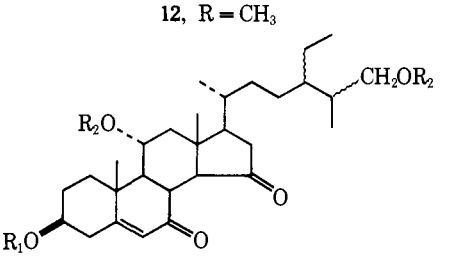
1, R = $(CH_3)_2CHC{=}O$
2, R = $CH_3CH_2C{=}O$
3, R = $CH_3C{=}O$
4, R = H



5, R = H
6, R = $CH_3C{=}O$



11, R = H
12, R = CH_3



7, R_1 = $CH_3CH_2C{=}O$; R_2 = H (14αH)
8, $R_1 = R_2 = CH_3C{=}O$ (14αH)
9, $R_1 = R_2$ = H (14βH)
10, $R_1 = R_2$ = H (14αH)
13, R_1 = $CH_3CH_2C{=}O$; R_2 = $CH_3C{=}O$

The water-soluble residue from the above reaction was treated with α-naphthylamine hydrochloride and *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride to form 1-isobutyramidonaphthalene, mp 149–150°.[4] Similar treatment of **2** gave the same tetrahydroxy ketone (**4**), the $\Delta^{3,5}$-7-ketone, and 1-propionamidonaphthalene, mp 117–119°.

Acetylation of **4** (acetic anhydride–pyridine, overnight at room temperature) gave a mixture of triacetate (**5**) (mp 135–138° (acetone–petroleum ether); ir $\nu_{max}^{CHCl_3}$ 3570, 1740, 1670 cm^{-1}; NMR ($CDCl_3$, δ), 0.84 (t, J = 7 Hz, 29 H), 0.91 (d, J = 7 Hz, 27 H), 1.01 (18 H), 1.32 (19 H, 2.02, 2.05, 2.06 (3 × acetate), 4.07 (m, 26 H), 4.73 (m, 3α H, 15α H), 5.32 (m, 11β H), 5.84 (s, 6 H); mass spectral *m/e* 602 (M^+), 542, 482, 283) and tetraacetate (**6**) (mp 142–148° (EtOAc–petroleum ether); ir $\nu_{max}^{CCl_4}$ 1740, 1690

cm^{-1}; NMR ($CDCl_3$, δ) 0.82 (t, J = 7 Hz, 29 H), 0.92 (d, J = 7 Hz, 27 H), 0.94 (18 H), 1.00 (d, J = 7 Hz, 21 H), 1.36 (19 H), 1.91 (15β acetate), 2.03, 2.04 (3 × acetate), 4.04 (m, 26 H), 4.70 (m, 3α H), 5.36 (m 11β H), 5.66 (m, 15α H), 5.77 (s, 6 H); mass spectral m/e 601 ($M^+ - 43$), 541, 481).

The location of the free hydroxyl in **5** was found in the following way. Jones oxidation gave the diketone (**8**): ir $\nu_{max}^{CHCl_3}$ 1746, 1703 cm^{-1}; NMR ($CDCl_3$, δ), 0.79 (18 H), 0.85 (t, J = 7 Hz, 29 H), 0.99 (d, J = 7 Hz, 21 H), 1.34 (19 H), 2.05 (3 × acetate), 4.07 (m, 26 H), 4.64 (m, 3α H), 5.33 (m, 11β H), 5.88 (s, 6 H); mass spectral m/e 600 (M^+), 585, 540, 525, 480, 465, 341, 313, 281. Hydrolysis of **8** (aqueous K_2CO_3–methanol) gave a mixture of epimeric trihydroxy diketones, (**9**) (major component) (ir $\nu_{max}^{CHCl_3}$ 1742 (cyclopentanone), 1668 cm^{-1}) and (**10**) (ir $\nu_{max}^{CHCl_3}$ 1748 (cyclopentanone), 1686 cm^{-1}).

Mass spectral evidence showed that **8** contained a C-15 rather than a C-16 ketone. For example, the fragment m/e 341 ($C_{21}H_{25}O_4$) results from cleavage of the C-17–C-20 bond (and also loss of acetic acid) which is consistent with the presence of a C-15 ketone.[5] A C-16 ketone would be expected to give a prominent ion at m/e 327 resulting from loss of the side chain (McLafferty rearrangement), the C-18 methyl, and acetic acid.[6] No such ion was observed.

The curve obtained by subtraction of the CD curve of **2** from that of **8** had [θ] nm +19,700 (298). This contribution is expected for a 15-keto steroid (a 14α H, 16-keto steroid gives a negative CD curve).[7] The CD curve of **10** was similar to that of **8**, while the difference between the curve of **9** and that of **2** gave [θ] nm −17,000 (299).

NMR evidence (see below) supports the β-configuration assigned to the hydroxyl in ring D of **2**. Further, $NaBH_4$ reduction of **8**, followed by oxidation of the product with activated MnO_2 in $CHCl_3$, gave back **5** as the only isolable epimer as expected.

The mass spectrum of **8** was consistent with the presence of a hydroxyl on the side chain of **2**. Also, Jones oxidation of **2**, yielded the triketo acid, (**11**): mp 170–175°; ir ν_{max}^{KBr} 2800–2500, 1750–1700 cm^{-1}; NMR ($CDCl_3$ δ) 0.68 (18 H), 1.13 (t, J = 7 Hz, propionate-CH_3), 1.41 (19 H), 4.63 (m, 3α H), 5.79 (s, 6 H); mass spectral m/e 542.3244 (M^+, $C_{32}H_{46}O_7$), 540, 482, 468, 297 (100). This last peak, $C_{19}H_{21}O_3$, indicated that there were three oxygens associated with the tetracyclic moiety apart from the ester function in **11**, and it followed that the carboxyl group was on the side chain. The carboxyl was confirmed by making the crystalline methyl ester **12**, mass spectral, m/e 556.3425 (M^+), with diazomethane. The location of the primary hydroxyl at C-26 in **2** is favored from NMR data given above, e.g., triplet at δ ~0.84 for C-29 methyl in **2** and its derivatives. The other possibility, i.e., hydroxyl at C-21, is rendered unlikely by the NMR spectrum of **6** in which the triplet at δ 0.82, doublet at 0.92, and doublet at 1.00 are consistent only with the presence of a C-26 hydroxyl.

The chemical shifts for the 18 H and 19 H protons in **2** agree well only with values expected for 11α- and 15β-hydroxyl substituents (calcd δ 1.03, 18 H; 1.35, 19 H).[8] There is also good agreement between the observed and calculated values for all the other compounds above.

The noncrystalline fraction consisting of **3** and **7** gave on acetylation (acetic anhydride–pyridine, 2 hr at room temperature) the triacetate **5** and the ketone **13**, which were readily separated by TLC. The latter was identical with the compound obtained by partial acetylation, then oxidation of **2**.

Oogoniol-1 and -2 showed similar activity in biological tests for hormone **B**. The lowest concentration at which activity could be observed was 620 and 460 ng/ml, respectively. The tetrahydroxy ketone (**4**) was slightly more active.[9]

The identification of oogoniol-1, -2, and -3 demonstrates that sexual reproduction in *Achlya* closely parallels sexual reproduction in mammals, antheridiol and the oogoniols being plant counterparts to mammalian androgens and estrogens.[10] The oogoniols probably arise by hydroxylation of β- or γ-sitosterol,[11,12] which are abundant plant sterols. Microbiological hydroxylation of steroids at the 7, 11, and 15 positions is well known[13] and it is evident that the fungus can make use of such reactions for primary metabolic functions.

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(9) These activities are lower than that observed for antheridiol. The assay for hormone B requires a much longer time to perform, and factors such as poor solubility of oogoniol in the assay medium (aqueous MeOH) may be partly responsible for the lower values. The activity is increased considerably by the addition of Tween. It is significant that the oogoniols are accompanied by much larger amounts of nonsteroidal lipid material in extracts of culture liquids of *Achlya*.
(10) This may also prove to be true for certain other fungi. For a recent review on this subject, see G. W. Gooday, *Annu. Rev. Biochem.*, **43**, 35 (1974).
(11) The closely related sterol, fucosterol, has been shown to be a biosynthetic precursor of antheridiol: C. R. Popplestone and A. M. Unrau, *Can. J. Chem.*, **52**, 462 (1974).
(12) A significant difference between these fungal sex hormones and mammalian hormones is that no oxidative degradation of the steroid side chain is involved in the genesis of the former. They are thus closer to the ecdysones in this respect.
(13) See, for example, W. Charney and H. L. Herzog, "Microbial Transformations of Steroids", Academic Press, New York, N.Y., 1967, pp 26–32.

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