ing solvents. The wax constituents were detected by spraying with 50% H_2SO_4 and charring at 100° for 40 min.

Liebermann–Burchard test. Triterpenoids turn red on the addition of concd H_2SO_4 followed by acetic anhydride.

MS were measured at 70 eV through a direct inlet system. At low resolution the source temp. was 140°; for high resolution the source temp. was 160°, with the trap at 300°.

IR were measured in KBr pellets (0.5 mg sample per 100 mg KBr).

Alkanes. The IR spectrum showed the absence of all oxygenated absorption bands. TLC gave $R_f = 0.83$ (C_6H_6), $R_f = 0.74$ ($CHCl_3$). These values correspond to those reported for hydrocarbons in *Triticum aestivum* [4].

Esters. The IR spectrum gave a strong band at 1150 (C—O) and at 1725 (C=O) cm^{-1}. TLC indicated $R_f = 0.66$ (C_6H_6), $R_f = 0.75$ ($CHCl_3$). These figures correspond to those reported for such compounds in many plants [4, 11–13].

Primary alcohols. IR revealed one strong band at 1100 (C—O) cm^{-1}. TLC gave $R_f = 0.07$ (C_6H_6), $R_f = 0.15$ ($CHCl_3$). These values are also very similar to those reported for such components in many plant waxes [4, 11–13].

Carboxylic acids. The IR spectrum showed a strong band at 1720 (C=O) cm^{-1} and TLC gave $R_f = 0$ (C_6H_6 and $CHCl_3$). These figures have been previously reported for fatty acids isolated from plant waxes [4, 11–13].

β-amyrin acetate. The IR spectrum gave very strong bands at 1720 (C=O) and 1235 (acetate) cm^{-1}. UV: $\lambda_{max}^{cyclopentane}$ nm: 2357. MS: *m/e* 468, 257, 249, 218 (base peak), 204, 203, 189, 175, 159, 147, 133, 119, 109, 94, 93, 81, 79, 69, 67, 65, 55, 43. (most intense peaks listed). This compound undergoes a retro Diels–Alder fragmentation which gives a characteristic ion *m/e* 218 [14]. This leaves a neutral fragment of 250 which is equal to 191 (unsubstituted fragment) + 59 (acetate). The MS figures correspond to those reported for this compound previously [13]. High resolution MS gave the empirical formula of $C_{32}H_{52}O_2$. PMR (99.8% $CDCl_3$) δ 0.76, 0.85, 0.9, 1.1, 1.2, *s* at 2.05 ($OCOCH_3$), *m* at 4.3–4.65 (CHOAc), multiplets at 5.1–5.25 (C=CH—). These values are in accordance with those reported earlier [15]. TLC gave $R_f = 0.32$ (C_6H_6) and $R_f = 0.48$ ($CHCl_3$). The mp was 231–234°. LB test: maroon red. An authentic sample of this compound gave the same IR spectrum, R_f values in both C_6H_6 and $CHCl_3$, and λ_{max}. The mmp was not depressed.

Taraxerone. The IR spectrum showed a strong wide carbonyl band at 1690–1700 cm^{-1}. UV [illegible] 2357. MS: *m/e* 424, 409, 300 (base peak), 285, [illegible] 205, 204, 189, 149, 133, 121, 119, 109, 107, 95, 93, 91, 67, 57, 43. (most intense peaks listed). This compound undergoes a retro Diels–Alder fragmentation to furnish *m/e* 300. High resolution MS indicated an empirical formula of $C_{30}H_{48}O$ and revealed that the *m/e* 300 fragment was $C_{21}H_{32}O_2$. This corresponds to the postulated structure for this fragment. The MS was identical to that published in ref. [16]. PMR (99.8% $CDCl_3$): δ 0.4, 0.75, 0.76, 0.85, 0.92, 0.96, 1.06, 1.1, 1.2, 1.4, 1.6, *m* at 1.8–1.95, 2.40–2.55, *s* at 3.5, 4.9, *m* at 5.5–5.7. TLC gave $R_f = 0.25$ (C_6H_6) and $R_f = 0.36$ ($CHCl_3$). The mp was 240–244°. LB test: maroon red. An authentic sample of taraxerone gave the same IR spectrum, R_f values in both solvents and λ_{max}. The mmp was not depressed.

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BIOSYNTHETIC INTERMEDIATES IN THE CONVERSION OF FUCOSTEROL TO OOGONIOL

ROBERT H. WHITE and TREVOR C. MCMORRIS

Department of Chemistry, University of California, San Diego, La Jolla, CA 92093, U.S.A.

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Key Word Index—*Achlya heterosexualis*; [CD_3]-methionine; fucosterol; antheridiol; oogoniol; steroid biosynthesis.

Abstract—When grown in the presence of [CD_3]-methionine *Achlya heterosexualis* produces oogoniols containing two deuterium atoms which are located at C-28 and C-29. This is consistent with conversion of fucosterol to a C-29 aldehyde followed by reduction to the C-29 hydroxyl present in oogoniol.

The oogoniols (**1**) are a group of closely related steroids which induce the formation of oogonia or female sex organs in the water mould *Achlya* [1]. A study of the biosynthesis of these steroids has revealed that fucosterol, the major sterol present in *Achlya*, is an intermediate in their biosynthesis [2]. When added to a culture of the

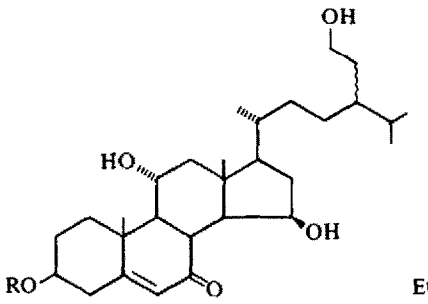
hermaphroditic strain *A. heterosexualis*, or of the male strain *A. ambisexualis* E 87 in the presence of the male activating hormone antheridiol, fucosterol-[3-^{3}H] was efficiently incorporated into the oogoniols.

This result has prompted us to investigate the metabolism of [CD_3]-methionine in *Achlya*. Our aim has been to obtain further evidence for the location of the primary hydroxyl in oogoniol. The C-26 position, which was proposed originally [1], was favored solely on evidence from NMR spectra (220 MHz) and so it was desirable to seek corroborating evidence from another source.

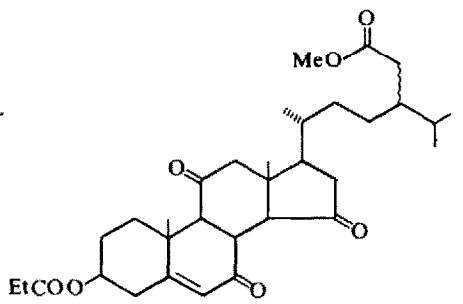
It is well established that the ethylidene group (C-28 and C-29) in fucosterol is derived biosynthetically from *S*-adenosylmethionine [3]. Thus fucosterol produced by *Achlya* when grown in the presence of [CD_3]-methionine, should contain up to four deuterium atoms in the molecule. Since oogoniol is biosynthesized from fucosterol, it too should contain up to four deuterium atoms provided the primary hydroxyl is located at C-26. However, if this hydroxyl were at C-29 instead of C-26, incorporation of up to three deuterium atoms would be expected. The experiment with [CD_3]-methionine could thus provide a way for distinguishing the two possibilities.

Table 1. Relative intensities of selected peaks in the mass spectra of (a) oogoniol and oogoniol-2, (b) four oogoniols biosynthesized in the presence of 100 μg/ml [CD_3]-methionine

m/e	458	459	460	461	462	463
(a) Oogoniol	100	32.1	7.3	1.2	0.19	0
Oogoniol-2	100	32.8	6.6	1.1	0.14	0
(b) Oogoniol	20.4	56.7	100	35.0	8.8	2.5
Oogoniol-1	17.2	56.2	100	35.4	7.6	2.1
Oogoniol-2	17.3	55.4	100	33.6	7.1	1.2
Oogoniol-3	18.5	53.8	100	33.7	8.9	1.7



1 (R = H, Ac, COEt and $COCHMe_2$)



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RESULTS AND DISCUSSION

Previous work has shown that *A. heterosexualis* B-14 is one of the best producers of the oogoniols [2] so this strain was used for all the experiments reported here. Four oogoniols were isolated from the culture liquids and these possessed very similar mass spectra which could be employed for determination of isotopic composition. The mass spectrum of oogoniol has an intense peak at *m/e* 458 (base peak) which is common to all the oogoniols, and corresponds to loss of the C-3 hydroxyl and hydrogen from the molecular ion. It can thus be used to estimate the incorporation of deuterium in the side chain.

In the mass spectrum of oogoniol isolated from a culture grown in the presence of 100 μg/ml [CD_3]-methionine a strong peak appears at *m/e* 460 (79% of base peak, *m/e* 161) indicating that two deuterium atoms have been incorporated. These must be on the side chain since the position of fragment ions resulting from loss of side chain and water (*m/e* 265, 283, 301) is unchanged.

Table 1 gives the relative intensities of peaks in the region of *m/e* 458 for oogoniols isolated from the experiments with [CD_3]-methionine, and for unlabelled oogoniols. Taking into account natural isotope abundances it can be calculated for oogoniol-2, that 12% of the molecules contain no deuterium, 33% contain one deuterium and 55% contain two deuterium atoms. No molecules contain more than two deuterium labels. The same values are found for the other oogoniols. When the experiment was repeated with a higher concentration of [CD_3]-methionine (138 μg/ml), 65% of the molecules were found to contain two deuterium atoms, 28% one deuterium and 7% were unlabelled.

Fucosterol, 24-methylenecholesterol and cholesterol were isolated from the mycelium of *Achlya* in the first experiment. Examination of the mass spectra showed that 85% of the fucosterol contained four deuterium atoms, 24-methylenecholesterol contained up to two deuterium atoms and cholesterol was unlabelled. Therefore, if fucosterol is the precursor of oogoniol as was indicated in our earlier study, two deuterium atoms are lost in the transformation.

In order to rationalize this loss, it was necessary to determine the location of the two deuterium atoms remaining in labelled oogoniol. A large-scale feeding experiment was therefore carried out so as to isolate sufficient amounts of oogoniols for chemical modification. A lower concentration of [CD_3]-methionine was used and this resulted in lower isotopic enrichment of the oogoniols. The isotopic distribution corresponded to 60% of the molecules containing no deuterium, 31% containing one deuterium and 9% containing two deuterium atoms. The oogoniol-2 isolated from this experiment was oxidized with Jones' reagent to a triketocarboxylic acid and the latter converted to the methyl ester (2) with diazomethane [1]. In the mass spectrum of the ester the intensity of the peak at *m/e* 483 (which is 68% that of *m/e* 482) shows that one deuterium label is present in the methyl ester. More accurately, 78% of the molecules are unlabelled and 22% contain one deuterium atom. This is almost exactly the amount of deuterium to be expected on the C-28 carbon if C-28 and C-29 in oogoniol-2 are equally labelled in the feeding experiment.

One deuterium label is lost in the oxidation of primary hydroxyl to carboxyl which indicates that the methoxy carbonyl group is at C-29 rather than C-26 as proposed

earlier [1]. Cleavage of the C-24, C-28 bond gives the fragment ion at *m/e* 409 which does not contain a deuterium label (the intensity of peak at *m/e* 410 is 30% that of *m/e* 409). This result is fully consistent with the presence of one deuterium label at C-28 and the other at C-29 in the oogoniols.

Mass spectral data thus indicates that the primary hydroxyl is located at C-29. We have recently obtained evidence from ^{13}C NMR spectra of oogoniol-1 and model 3β,26- and 3β,29-dihydroxystigmast-5-enes, which also supports the presence of a C-29 hydroxyl in the oogoniols [4].

Biosynthesis from [CD_3]-methionine gives oogoniol with one deuterium label at C-28 and one at C-29 so that two deuterium atoms are lost from C-29 in the conversion of the fucosterol to oogoniol. A plausible way in which this could occur is for fucosterol to be oxidized to an alcohol then to an aldehyde. A similar oxidation takes place in the biosynthesis of antheridiol, for a C-29 carboxylic acid derived from fucosterol has been shown to be an intermediate [5]. However, there is some indication that formation of a double bond at C-22, C-23 precedes oxidation of methyl to carboxyl at C-29. Oogoniol does not possess oxygen functions at C-22 or C-23 so its biosynthesis may not involve such an intermediate. The possibility has been considered that oogoniol may be derived from antheridiol. This does not appear likely because oogoniol possesses a deuterium label at C-29 which would have been lost on oxidation of fucosterol to a C-29 carboxylic acid. If 29-oxo-fucosterol is indeed an intermediate then it may be converted to oogoniol by reduction of the C-24, C-28 double bond, reduction of aldehyde to alcohol, hydroxylation at C-11 and C-15 and oxidation at C-7. We are planning further experiments to define the sequence in which these reactions occur.

EXPERIMENTAL

Feeding experiments were carried out in a similar way to those described previously [2]. Production medium contained appropriate amounts of [CD_3]-methionine as indicated in the text. Steroids were isolated and purified by chromatography before determination of mass spectra. For oxidation of oogoniol-2, the purified sample was treated with Jones' reagent for 1 hr at room temp. H_2O was added and the resulting ppt. extracted with $CHCl_3$ and the extract purified by TLC. The acid was methylated by adding a few drops of ethereal CH_2N_2 and allowing the soln to stand for 15 min before removing excess reagent.

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ISOLATION OF 3β-HYDROXY-5α-PREGNAN-16-ONE FROM *SOLANUM HAINANENSE*

G. ADAM*, H. TH. HUONG* and N. H. KHOI†

*Institute for Plant Biochemistry, Halle/Saale, of the Academy of Sciences of the GDR, German Democratic Republic; †Institute for Sciences, Hanoi, Socialist Republic Vietnam

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Key Word Index—*Solanum hainanense*; Solanaceae; plant steroids; pregnanes; 3β-hydroxy-5α-pregnan-16-one; biosynthesis.

A variety of neutral pregane derivatives occur in higher plants [1–3], while more recently the isolation of pregnane glycosides has been reported [4]. We now wish to report the isolation and structure of another pregnane derivative, isolated from the Vietnamese Solanacea *Solanum hainanense* Hance, which we have identified as 3β-hydroxy-5α-pregnan-16-one.

Al_2O_3 chromatography of the $CHCl_3$ extracts of dried roots yielded 0.025% of the neutral compound $C_{21}H_{34}O_2$ (M^+ *m/e* 318), mp 153°, which showed a deep blue colour upon detection with iodine on the TLC plate. The IR (nujol) spectrum indicated a hydroxyl absorption at 3200 cm^{-1} (broad) and a 5 membered carbonyl function at 1741 cm^{-1}, the latter also established by UV absorption at λ_{max} (ε) 299 (58). The 60 MHz ^1H NMR spectrum was in agreement with a steroidal character giving diagnostic signals at δ 0.64 (s, C-18), 0.78 (s, C-19) and 3.53 ppm (m, 3α-H). The MS showed fragment ion at *m/e* 303 (M^+-CH_3), 300 (M^+-H_2O), 285 (M^+-CH_3—H_2O), the base peak at *m/e* 234 (M^+-84) was due to a loss of ring D by fission between C-13, C-17 and C-14, C-15 as found for other steroidal 16-ketones [5]. The ORD curve gave a strong negative Cotton effect ($a = -180.3$) at 298 nm, typical of a C-16 carbonyl steroid of the 14α-series [6]. These data suggested that the isolated plant steroid was 3β-hydroxy-5α-pregnan-16-one whose structure was finally supported by direct comparison with an authentic sample synthesized as described earlier [7].

The 16-keto pregnane could not be detected in the leaf extracts of *S. hainanense*. The isolation of the new spirosolane alkaloid solasodenone from the $CHCl_3$ extracts of roots and leaves of this plant has been reported recently [8] whereas the glycosidic fractions of the same plant contain solasodine as the main aglycone (unpublished results).

The occurrence of 3β-hydroxy-5α-pregnan-16-one in this plant is of particular interest with regard to its biogenesis. All the other neutral pregnanes isolated from higher plants show an oxygen function at C-20