Rearrangements of Exogenus 17*β*-Hydroxy-17α-methylandrosta-1,4-dien-3-one in Cultures of the Green Alga T76 *Scenedesmus quadricauda*

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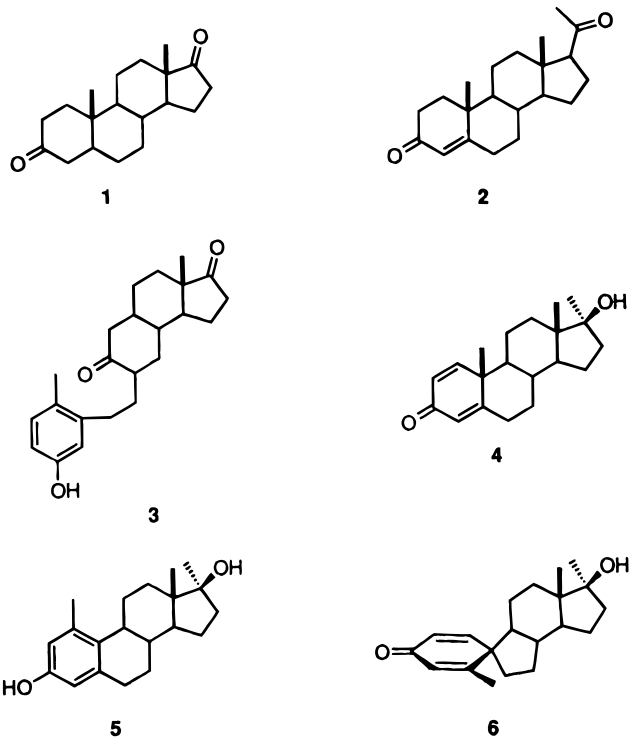
The transformation of exogenus substrates either by higher plant cells or by microorganisms has been reported in numerous studies,[1,2] while the use of microalgae in biotransformations has been only occasionally investigated.[3]

In a systematic study of the potential use of unicellular freshwater algae as bioreactors, we found that 5α-androsta-3,17-dione (**1**) and progesterone (**2**) were biotransformed by algae belonging to Rhodophyta and Chlorophyta.[4,5] The regio- and stereoselective reduction or hydroxylation of the substrates was the main reaction, and noteworthy in cultures of T76 *Scenedesmus quadricauda*, progesterone was transformed into the 9,10-seco derivative **3** in high yield.[5]

In an extension of our investigations, we have incubated 17*β*-hydroxy-17α-methylandrosta-1,4-dien-3-one (**4**) with *S. quadricauda*, and the main bioproducts isolated from the cultures were two new sterols identified as 1,17α-dimethyl-1,3,5(10)-estratriene-3,17*β*-diol (**5**) and (3aα,8aα,8b*β*)-dodecahydro-5a*β*,6α-dimethyl-6*β*-hydroxy-2′-methyl-*as*-indacene-3(*R*)-spiro-4′-(2′,5′-cyclohexadienone) (**6**),[6] respectively.

Compound **5**, mp 118 −121 °C, has a molecular formula $C_{20}H_{28}O_2$, as suggested by the HRMS and ^{13}C-NMR data. In the ^{1}H-NMR spectrum, the aromatic H-2 and H-4 protons appear as two *meta* coupled doublets (J = 2.5 Hz) at δ 6.49 and 6.42, the H-6α and H-6*β* protons as two multiplets at δ 2.86 and 2.66, respectively, and the H-18, H-19, and H-20 methyl protons as singlets at δ 0.95, 2.31 and 1.26, while the remaining protons are overlapped signals in the upfield region of the spectrum. In the ^{13}C-NMR spectrum the protonated carbons C-2 and C-4 are at δ 116.0 and 113.2, the other aromatic C-1, C-3, C-5, and C-10 carbons at δ 138.7, 152.8, 140.0, and 131.1, and the C-18, C-19, and C-20 carbons at δ 14.6, 22.3, and 26.0. The ^{1}H−^{1}H one-bond COSY and ^{1}H−^{13}C one-bond COSY experiments have also identified the H-8 and H-14 protons, overlapped at δ 1.56 and correlated to the carbons at δ 42.4 and 49.9; the H-9 proton at δ 2.31, correlated to the carbon at δ 46.4; and the H-11 methylene protons at δ 1.32 and 2.42, correlated to the carbon at δ 27.6.



In the ^{1}H−^{13}C long-range COSY, the H-2 proton is correlated to the C-1, C-3, C-4, C-10, and C-19 carbons while the H-4 proton is correlated to the C-2, C-3, C-5 and C-10 carbons. The correlations of the signal at δ 2.31 with the C-1, C-5, and C-10 carbons may be attributable to the H-19 as well as to the H-9 protons, owing to their overlapping, while the correlation with the C-2 carbon is confidentially attributable to the H-19 protons. Finally the C-10 carbon is heterocorrelated to the H-8 proton while the C-5 carbon gives cross peaks with the H-6 protons. In a NOESY experiment, NOE interactions are evidenced between the H-4 and the H-6*β* protons, the H-19 and the H-2 protons, and the H-19 and the H-11 protons.

The spirocompound **6**, mp 138−140 °C, has the molecular formula $C_{20}H_{28}O_2$, in agreement with the HRMS and ^{13}C-NMR data. The ^{1}H-NMR spectrum showes the H-1 and H-2 protons as a doublet (J = 10.2 Hz) and a double doublet (J = 10.2 and 1.9 Hz) at δ 6.88 and 6.20, the H-4 proton as a doublet (J = 1.9 Hz) at δ 6.18, and the H-18, H-19, and H-20 methyls as singlets at δ 0.85, 1.97, and 1.23, respectively. In the ^{13}C-NMR spectrum, the olefinic C-1, C-2, C-4, and C-5 carbons are at δ 154.7, 126.8, 128.7, and 162.8, and the C-3 carbonyl carbon is at δ 186.3, the quaternary C-10 carbon at δ 52.2, and the C-19 methyl carbon at δ 19.6. On the basis of the one-bond homo- and heterocorrelations, the H-6 protons at δ 2.03 and 1.35 are correlated to the carbon at δ 34.9, the H-8 and H-9 protons at δ 1.51 and 1.91 are correlated to the carbons at δ 56.4 and 43.3, respectively, and the H-11 protons at δ 1.27 are correlated to the the methylene carbon at δ 21.6. In the ^{1}H−^{13}C long-range COSY, the H-1 proton gives cross peaks with the C-2, C-3, C-5, C-9, and C-10 carbons, the H-2 proton is heterocorrelated to the C-1, C-3, C-4, and C-10 carbon, the H-4 proton to the C-2, C-3, C-5, C-10, and C-19 carbons, and finally the H-19 protons give cross peaks with C-1, C-4, C-5, and C-10. In the NOESY spectrum, NOE effects are present between the H-1 and the H-9 protons and among the

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(1) Suga, T.; Hirata, T. *Phytochemistry* **1990**, *29*, 2393−2406.
(2) Mahato, S. B.; Majumdar, I. *Phytochemistry* **1993**, *34*, 883−898.
(3) Abdul-Hajj, Y. J., Qian, X. *J. Nat. Prod.* **1986**, *49*, 244−248.
(4) Fiorentino, A., Pinto, G., Pollio, A., Previtera, L. *Biomed. Chem. Lett.* **1991**, *1*, 673−674.
(5) Pollio, A., Pinto, G., Della Greca, M., De Maio, A., Fiorentino, A., Previtera, L. *Phytochemistry* **1994**, *37*, 1269−1272.
(6) The numbering of structure **6** in the text is referred to the parent steroid **4**.

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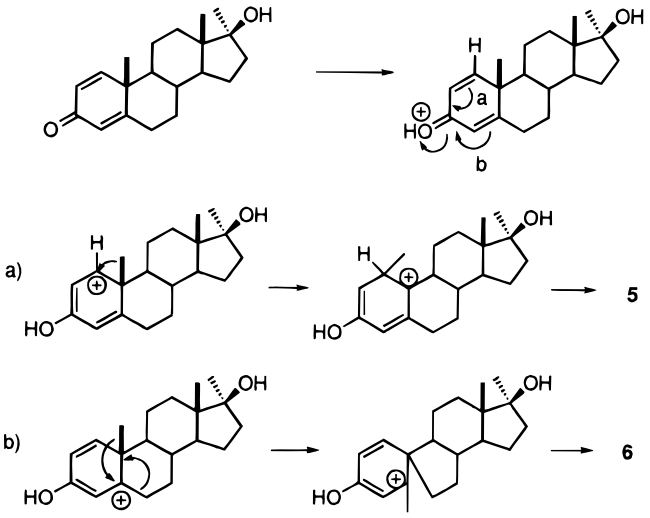
Scheme 1



H-19 protons and the H-4, H-8, H-11, and H-18 protons. All these interactions are in agreement with the *R* configuration at C-10.

The acid enolization of the carbonyl group with subsequent 1,2 shifts of the H-19 methyl group might be the driving force for the rearrangement of **4** into **5** and **6** (Scheme 1). In the first case, the possible sequence consists in the protonation of the carbonyl group, the shift of the Δ^1 π bond to give a carbenium ion at C-1, the shift of the methyl group from C-10 to C-1, and the elimination of the H-1 proton. In the latter case, the protonation of the carbonyl group is followed from the shift of the Δ^4 π bond with formation of the carbenium ion at C-5; the shift of the methyl group from C-10 to C-5 and consequent shift of the C-5−C-6 σ bond from C-5 to C-10 with reinstatement of the enonic function gives **6**.

Experimental Section

General Methods. ^{1}H (400 Mz) and ^{13}C (100 MHz) NMR spectra were recorded in $CDCl_3$. H-C long-range COSY experiments were performed with XHCORR microprogram (Bruker) using delay corresponding to $J_{C,H} = 12$ Hz. 2D NOESY experiments were performed with a mixing time $\tau_m = 0.5$ s.

Biotransformation of 17β-hydroxy-17α-methylandrosta-1,4-diene-3-one (4). The strains of T76 *S. quadricauda* were supplied by The Algal Collection of Texas at Austin. In the semipreparative procedure, 17β-hydroxy-17α-methylandrosta-1,4-diene-3-one (**4**) (500 mg sterilized at 100 °C for 1 h) dissolved in dioxane (3.5 mL) was added to the axenic culture of *S. quadricauda* in BBM[7] (2 L) during the exponential phase of growth of the strain. The inoculum was 1.7×10^5 cells/mL and after 5 days the algal concentration was 6×10^5 cells/mL. The culture was stirred at 25 °C and irradiated with a photoperiod of 16 h light−8 h dark. After 10 days the suspension was extracted with ethyl acetate (2×200 mL) and the residue (495 mg) was chromatographed on a silica gel column (50 g; eluent chloroform−ethyl acetate mixtures) to give **5** (19:1, 55mg, 11%), starting **4** (9:1, 248 mg, 50%), and **6** (4:1, 103 mg, 21%).

1,17α-Dimethyl-1,3,5(10)-estratriene-3,17β-diol (5). Compound **5**, after purification by reverse phase HPLC (RP-8; eluent MeOH−H_2O−MeCN 5:3:2), had mp 118−121°C (hexane); $[\alpha]^{20}_D$ + 93° (c = 0.36, $CHCl_3$); HRMS *m/e* (M^+) calcd 300.209, obsd 300.218; UV λ_{max} 281 nm (EtOH); IR ($CHCl_3$) ν 3338 cm^{-1}; ^{1}H-NMR δ 6.49 (d, J = 2.5 Hz, H-2), 6.42 (d, J = 2.5 Hz, H-4), 2.86 (m, H-6α), 2.66 (m, H-6β), 2.31 (m, H-9), 0.95 (s, H-18), 2.31 (s, H-19), 1.26 (s, H-20); ^{13}C-NMR δ 138.7 (C-1), 116.0 (C-2), 152.8 (C-3), 113.2 (C-4), 140.0 (C-5), 32.5 (C-6), 25.6 (C-7), 42.4 (C-8), 46.4 (C-9), 131.1 (C-10), 27.6 (C-11), 32.5 (C-12), 46.6 (C-13), 49.9 (C-14), 23.0 (C-15), 39.2 (C-16), 81.7 (C-17), 14.7 (C-18), 22.3 (C-19), 26.0 (C-20).

(3aα,8aα,8bβ)-dodecahydro-5aβ,6α-dimethyl-6β-hydroxy-2′-methyl-*as*-indacene-3(*R*)-spiro-(2′,5′-cyclohexadienone) (6). Compound **6**, after purification by reverse phase HPLC (RP-8; eluent MeOH−H_2O−MeCN 5:3:2), had mp 138−140 °C (hexane); $[\alpha]^{20}_D$ −3° (c = 0.22, $CHCl_3$); HRMS *m/e* (M^+) calcd 300.209, obsd 300.213; UV λ_{max} 269 nm (EtOH); IR ($CHCl_3$) ν 1660 cm^{-1}; ^{1}H-NMR δ 6.88 (d, J = 10.2 Hz, H-1), 6.20 (dd, J = 1.9 and 10.2 Hz, H-2), 6.18 (d, J = 1.9 Hz, H-4), 1.51 (m, H-8), 0.85 (s, H-18), 1.97 (s, H-19), 1.23 (s, H-20). ^{13}C-NMR δ 154.7 (C-1), 126.8 (C-2), 186.3 (C-3), 128.7 (C-4), 162.8 (C-5), 34.9 (C-6), 30.4 (C-7), 56.4 (C-8), 43.3 (C-9), 52.2 (C-10), 21.6 (C-11), 31.6 (C-12), 46.4 (C-13), 50.9 (C-14), 23.8 (C-15), 39.2 (C-16), 81.2 (C-17), 14.2 (C-18), 19.6 (C-19), 26.1 (C-20).

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H−^{1}H COSY, ^{1}H−^{13}C COSY, NOESY, and long-range ^{1}H−^{13}C COSY of compounds **5** and **6** (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(7) Nichols, H. W., Bold, H. C. *J. Phycol.* **1965**, *1*, 34−38.