Pergamon

Tetrahedron, Vol. 52, No. 44, pp. 13981-13990, 1996

0040-4020/96 $15.00 + 0.00

PII: S0040-4020(96)00841-1

Bioconversion of 17β-Hydroxy-17α-methyl-androsta-1,4-dien-3-one and Androsta-1,4-diene-3,17-dione in Cultures of the Green Alga T76 *Scenedesmus quadricauda*

Marina DellaGreca and Lucio Previtera*

Department of Organic & Biological Chemistry, University Federico II, Via Mezzocannone 16, 80134 I-Napoli, Italy E-mail: previter@unina.it

Antonio Fiorentino

Institute of Biology, II University of Naples, Via Arena, I-81100 Caserta, Italy

Gabriele Pinto and Antonino Pollio

Department of Plant Biology, University Federico II, Via Foria 223, I-80139 Napoli, Italy

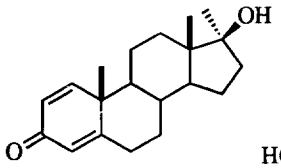
Abstract: Exogenus 17β-hydroxy-17α-methyl-androsta-1,4-dien-3-one and androsta-1,4-diene-3,17-dione are biotransformed by the green alga T76 *Scenedesmus quadricauda*. The bioproducts have been isolated by chromatographic processes and identified on the basis of their spectroscopic features. Hydration of the Δ^4 double bond may justify the presence of the epimeric 5-hydroxyderivatives while rather complex skeleton rearrangements are involved in the formation of the remaining products.

Steroids are widely used in pharmacology as drugs and hormones owing to their anti-inflammatory, anti-cancer, diuretic, progestational, contraceptive and anti-androgenic properties. Microbial transformations[1] represent a useful tool for the production of these compounds either to improve the yields of already known sterols or in the preparation of novel products. From the first patents in 1937,[2,3] bacteria and fungi are the microorganisms usually employed in biotransformations while microalgae have been utilized only occasionally.[4]

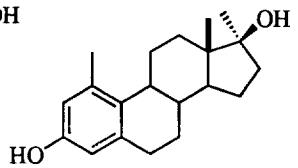
In preliminary studies on the potential use of these microoganisms we have found[5-8] that many algal strains bioconvert exogenus steroids causing regio- and stereoselective hydroxylations, regioselective reductions of carbonyl groups, introduction of double bonds and σ-bond cleavage. In continuing our studies we have recently reported that 17β-hydroxy-17α-methyl-androsta-1,4-dien-3-one (**1**) was partly converted into the rearranged compounds **2** and **3** when incubated for 10 days with the green alga T76 *Scenedesmus quadricauda*.[9]

In order to increase the bioproducts yields, we have tested different experimental conditions for the biotransformation changing the reaction times as well as the ratio between substrate and alga concentration. Under these different conditions, the formation of further products was observed and when the bioreaction was

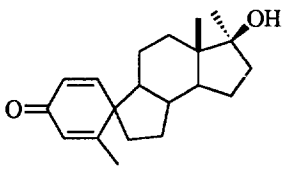
run for 20 days and at twice algal concentration three new bioproducts **4** - **6** were isolated from the culture in fair quantities along with **2** and **3.**



1

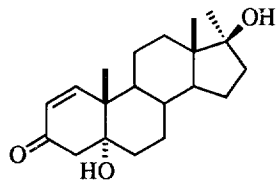


2

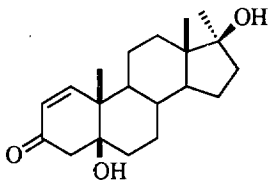


3

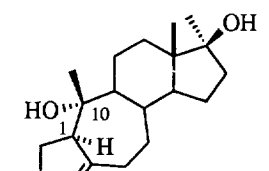
Sequential chromatographic processes on the bioreaction mixture gave the pure compounds which were identified on the basis of their physical features.



4



5



6

Structure 5α,17β-dihydroxy-17α-methyl-androst-1-en-3-one was attributed to bioproduct **4.** It had m.p. 238-241 °C and $[\alpha]_D$ +3.8°. The molecular formula $C_{20}H_{30}O_3$ was in agreement with the molecular peak at *m/z* 318 in the EIMS spectrum and with the presence of twenty carbon signals in the ^{13}C-NMR spectra. The UV spectrum showed a strong band of the enone group at 238 nm and in the IR spectrum were present the absorptions at 3606 and 1712 cm^{-1} of the hydroxyl and carbonyl groups. In the ^{1}H-NMR spectrum were identifiable the two doublets of the H-1 and H-2 protons at δ 7.96 and 6.28, the AB doublets of the H-4 protons at δ 3.21 and 2.09, the double triplet at δ 2.48 of one of the H-6 protons and the three singlets of the H-18, H-19 and H-20 methyls at δ 1.19, 1.11 and 1.47, while all the other protons were as overlapped signals in the 1.20 - 2.25 ppm range. The twenty carbon signals present in the ^{13}C-NMR spectrum were defined by a DEPT experiment as a carbonyl carbon, two olefinic methine carbons, three methyls, seven methylenes and four tetrasubstituted carbons, two of them bearing hydroxyl groups. The direct correlations among protons and carbons were based on ^{1}H-^{13}C one-bond COSY while ^{1}H-^{1}H one-bond COSY and ^{1}H-^{13}C long-range COSY allowed the full assignment of the nuclear signals to the molecule. (Table 1).

Noteworthy for placing the hydroxyl function at C-5 were the long-range correlations of the signal of the A ring: the H-1 proton, correlated to the C-3 carbonyl carbon, gave cross peaks with the C-5 and C-10 carbons; both these carbons were heterocorrelated to the H-2 and the H-19 protons while the first gave further correlations with the H-4 proton at δ 3.21 and the H-6 proton at δ 1.46. The 5α configuration was then

defined on the basis of a NOESY experiment: the H-19 methyl, which gave nOe with the H-8β proton centred at δ 1.28 along with the H-18 methyl, gave also nOe with the H-4β proton at δ 3.21 according to a *trans*-decalin junction.

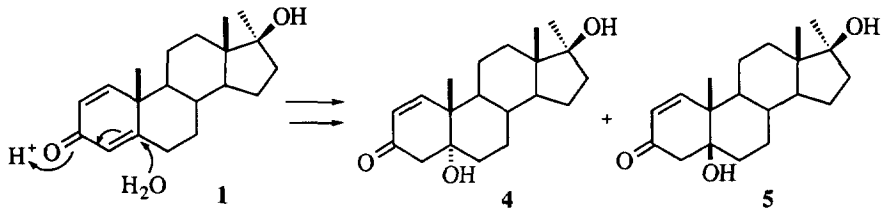
The epimeric 17α-methyl-5β,17β-dihydroxy-androst-1-en-3-one (**5**) had m.p. 208-210 °C and $[\alpha]_D$ -3°. The IR spectrum was identical to that of **4** while in the UV spectrum a shift at λ 283 nm was observed. **5** showed the same molecular peak at *m/z* 318 in the EIMS spectrum and twenty carbon signal in the ^{13}C NMR spectrum. In the ^1H-NMR spectrum the H-1 and H-2 olefinic doublets were at δ 7.66 and 6.30, the H-4 doublets at δ 3.21 and 2.19, the H-18, H-19 and H-20 methyls at δ 1.14, 1.41 and 1.46. Most of the ^{13}C-NMR resonances were comparable to those of **4**, the only significant shifts being those of C-4 and C-19 carbons. The ^1H-^1H one-bond COSY and ^1H-^{13}C long-range COSY evidenced the same correlations of **4** and the downfield shift of the H-19 methyl was consistant with the 5β configuration.[10] Accordingly were the nOe effects observed in a NOESY experiment of the H-4 proton onto the H-7 and H-9 protons.

Table 1. NMR Data of compound **4** in C_5D_5N.

POSITION	DEPT	δ ^{13}C ⟷ δ ^1H ← long range → ^{13}C		
1	CH	169.1	7.96 d	C-3 C-5 C-10
2	CH	133.7	6.28 d	C-5 C-10
3	C	209.6		
4	CH_2	47.1	2.09 d 3.21 d	C-3 C-3 C-5
5	C	73.7		
6	CH_2	35.3	1.46* 2.48 dt	C-5 C-7 C-1 C-7 C-10
7	CH_2	27.9	1.22* 1.61*	C-6 C-6 C-8
8	CH	49.7	1.28*	C-9 C-10
9	CH	36.8	2.05*	C-10
10	C	55.3		
11	CH_2	21.9	1.58* 1.90*	C-13
12	CH_2	32.3	1.46* 1.75*	C-13 C-17
13	C	46.2		
14	CH	50.6	1.42*	C-15
15	CH_2	24.0	1.37* 1.61*	C-8 C-8 C-20
16	CH_2	39.7	1.85* 2.19*	C-17 C-13 C-17
17	C	80.8		
18	CH_3	14.9	1.19 s	C-12 C-13 C-14 C-17
19	CH_3	23.5	1.11 s	C-5 C-10
20	CH_3	26.9	1.47 s	C-13 C-16 C-17 C-18

* overlapped signals

The formation of **4** and **5** may be easily rationalized by a nucleophyle addition of water at the C-5 position of the enol intermediate as reported in Scheme 1.



Scheme 1. Proposed pathway for the conversion of **1** into **4** and **5**

The rearranged structure **6** was attributed to the latter bioproduct, m.p.112-115 °C and $[\alpha]_D$ + 21°.

Table 2. NMR Data of compound **6** in $CDCl_3$.

POSITION	DEPT	δ ^{13}C	δ ^{1}H	^{13}C
1	CH	54.9	3.28 d	C-3 C-5 C-10
2	CH_2	39.0	2.68 dd 2.52*	C-3 C-5 C-10 C-3 C-10
3	C	208.4		
4	CH	131.3	5.97 d	C-3 C-5
5	C	182.3		
6	CH_2	31.8	3.02dd 2.50*	C-5 C-3 C-10
7	CH_2	28.5	1.41* 1.80*	C-6 C-8 C-6 C-8
8	CH	4 2.1	1.30*	C-9 C-10
9	CH	59.0	1.35*	C-10
10	C	76.2		
11	CH_2	23.1	1.53* (β) 2.04*	C-13
12	CH_2	31.2	1.38* 1.59*	C-13 C-17
13	C	45.7		
14	CH	50.2	1.43*	C-15
15	CH_2	24.1	1.34* 1.73*	C-8 C-8 C-20
16	CH_2	38.4	1.78* 1.87* (β)	C-17 C-13 C-17
17	C	81.4		
18	CH_3	14.0	0.91 s	C-12 C-13 C-14 C-17
19	CH_3	17.3	0.96 s	C-5 C-10
20	CH_3	25.7	1.25 s	C-13 C-16 C-17 C-18

* overlapped signals

The UV spectrum showed a strong band at 266 nm and in the IR spectrum were present the absorptions of the hydroxyl and carbonyl groups at 3606 and 1685 cm^{-1}. The molecular formula $C_{20}H_{30}O_3$ was in agreement with the carbon signals in the ^{13}C-NMR spectrum (Table 2) and the molecular peak at *m/z* 318 in the EIMS spectrum. In the ^1H-NMR spectrum the H-1 proton appeared as a doublet at δ 3.28, the H-2 protons as a double doublet at δ 2.68 and an overlapped signal at δ 2.52, the H-4 proton as a doublet at δ 5.97. One of the H-6 protons was also detectable as a double doublet at δ 3.02 and the H-18, H-19 and H-20 methyls were at δ 0.91, 0.96 and 1.25. The remaining protons, overlapped in the upfield region of the spectrum, were identified on the basis of ^1H-^1H and ^1H-^{13}C one bond COSY experiments. In the ^1H-^{13}C long range COSY the C-3 carbon was correlated to the H-1, H-2 and H-4 protons, the C-5 carbon to the H-1, H-2 proton at δ 2.68, H-4, H-6 proton at δ 3.02 and H-19 protons, the C-10 to the H-2 and H-19 protons.

The stereostructure (Figure 1) was defined on the basis of a NOESY experiment. The nOes of the H-18 methyl with the H-8, H-11β and H-16β protons and those of the H-20 methyl with the H-12α, H-14 and H-16α confirmed that no change occurred in the C and D rings during the bioconversion. According to the β-orientation of the H-19 methyl and the α-orientation of the H-1 proton in the spectrum were present the interactions of the H-19 methyl with the H-8 and H-11β protons and that of the H-1 with the H-9α proton. A further correlation between the H-19 methyl and the H-2β proton at δ 2.68 was in agreement with the structure.

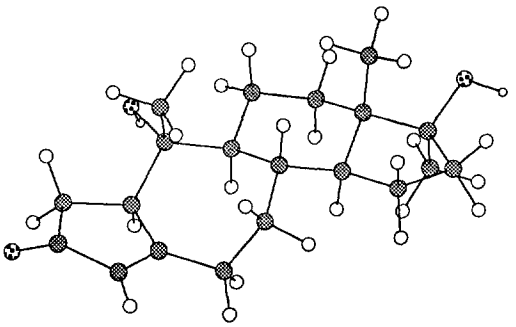
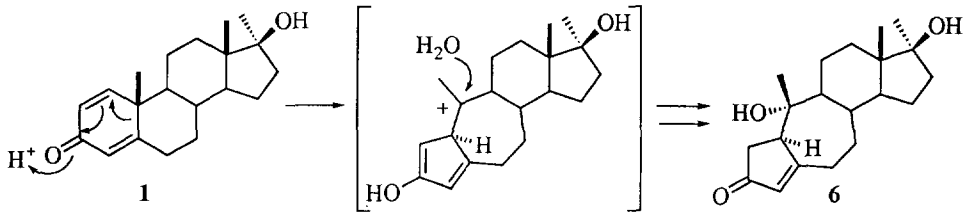


Figure 1. Minimum optimized structure of **6**.

As already proposed[8] for the formation of **2** and **3**, the acid enolization might be the driving force for the rearrangement of **1** into **6** (Scheme 2).



Scheme 2. Proposed pathway for the rearrangement of **1** into **6**.

The protonation of the C-3 carbonyl group, the migration of the Δ^1 π bond from C-1 to C-3 and the shift of the C-5 - C-10 σ bond from C-10 to C-1 give the carbenium-ion at C-10. The attack of water to the α-face of this intermediate followed by deprotonation and reinstatment of the carbonyl function afford **6**.

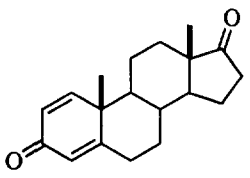
To verify if this biotransformation occurred on other substrates with the same functional group in the A ring, androsta-1,4-diene-3,17-dione (**7**) was cultured for 20 days with *S. quadricauda* in the same conditions of **1**.

Five main derivatives, which resembled compounds **2** - **6**, were isolated from the medium and the structures **8** - **12** were attributed to the compounds by comparison of their ^{1}H- (Table 3) and ^{13}C-NMR (Table 4) data with those of **7**[11,12] and **2** - **6**.

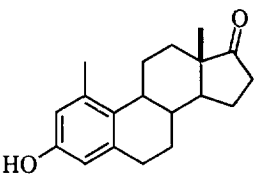
Table 3. ^{1}H-NMR Chemical Shifts of Sterols **7** - **12** in $CDCl_3$

H	7	8	9	10#	11#	12
1	7.03 d		6.86 d	7.92 d	7.66 d	3.30 d
2	6.24 dd	6.51 d	6.20 dd	6.28 d	6.08 d	2.67 dd 2.51 m
4	6.06 d	6.45 d	6.09 d	3.18 d 2.09 d	2.69 d 1.84 d	6.01 d
6		2.71 m 2.89 m				3.09 dd 2.50 m
18	0.92 s	0.95 s	0.88 s	0.85 s	0.83 s	0.93 s
19	1.24 s	2.31 s	1.98 s	1.06 s	1.45 s	0.97 s

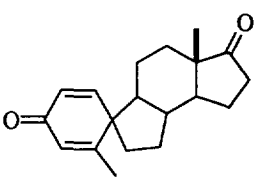
in pyridine d_5



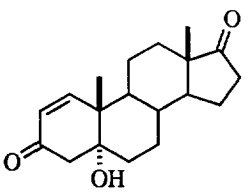
7



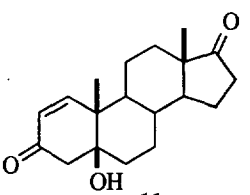
8



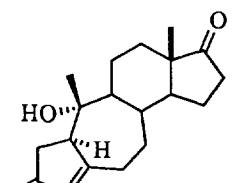
9



10



11



12

Table 4. ^{13}C-NMR Chemical Shifts of Sterols **7** - **12** in $CDCl_3$

C	7	8	9	10#	11#	12
1	155.3	138.7	154.5	168.9	169.5	55.0
2	127.7	116.2	126.9	133.8	133.9	39.0
3	186.2	153.0	186.1	209.0	209.1	207.8
4	124.1	113.4	128.7	46.9	44.1	131.5
5	168.3	139.7	163.1	73.5	74.2	181.2
6	32.3	32.2	34.7	35.0	34.2	31.6
7	31.2	24.8	29.8	26.8	27.5	27.7
8	52.3	41.3	56.2	49.6	51.3	40.9
9	35.1	46.4	43.1	35.3	37.0	59.2
10	43.4	130.5	52.6	55.1	56.2	76.0
11	22.1	27.4	21.9	22.0	21.9	22.8
12	32.6	32.3	32.0	32.1	32.1	31.2
13	47.7	48.5	47.9	47.7	47.3	48.1
14	50.4	50.6	50.5	50.9	51.0	51.0
15	21.9	21.6	21.8	21.2	20.7	22.6
16	35.6	35.8	36.9	36.0	35.9	35.6
17	219.9	215.5	216.8	217.8	219.1	214.9
18	13.8	14.6	14.0	13.8	13.9	13.9
19	18.7	22.5	19.5	23.2	18.8	17.3

in pyridine d_5

Both **1** and **7** are bioconverted by *S. quadricauda* to give the same pattern of bioproducts but GC analysis evidences some differences in the transformations. In the first place, in the same conditions, the bioconversion of **7** is lower and it is recovered in about twice amounts in comparison with **1** (Table 5). Further more while the amounts of the hydroxyderivatives **4**, **10** and **5**,**11** as well as those of **6**,**12** are comparable, the spirocompound is the most abundant product from **1** and it is present in small amounts among the derivatives of **7**.

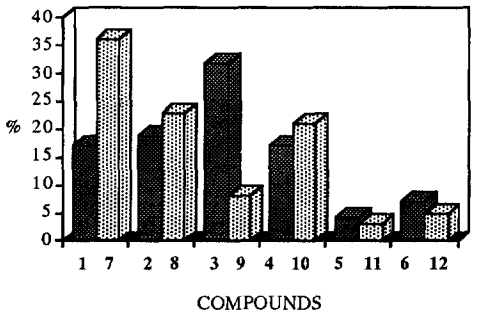


Table 5. % of bioproducts in the culture media by GC analysis.

As can be seen the microalgae possess unusual ability to trasform exogenus steroids. The reaction types and stereochemistry depends on the functional group but structural differences in the substrates play an important role in the ratio of bioproducts.

EXPERIMENTAL

^1H (400 Mz) and ^{13}C (100 MHz) NMR spectra were recorded in $CDCl_3$ or C_5H_5N. One-bond and long-range H-C COSY experiments were performed with the XHCORR microprogramme using delays corresponding to $J_{C,H}$ 140 Hz and 12 Hz respectively. 2D NOESY experiments were performed with a mixing time $\tau_m = 0.5$ s. GC analysis was run on OV-1 capillary column (285 °C, flow 1 mL/min) using 21-hydroxyprogesterone as internal standard. The strains of T76 *S. quadricauda* were supplied by The Algal Collection of Texas at Austin, USA.

Bioconversion of 17β-hydroxy-17α-methyl-androsta-1,4-dien-3-one (**1**) *and androsta-1,4-diene-3,17-dione* (**7**). Steroid **1** [7] (500 mg sterilized at 100 °C for 1 hr) dissolved in dioxane (4 mL) was added to an Erlenmeyer flask (4 L) containing the axenic culture of S. quadricauda in Bold basal medium[13] (2 L) during the exponential phase of growth of the strain with algal concentration 3×10^6 cells/mL. The suspension was stirred at 24 °C for 20 days irradiating with a photoperiod of 16 hr light : 8 hr dark and then extracted with ethyl acetate ($2 \times$ 250 mL).

Bioproducts **2** - **6**. The ethyl acetate extract of culture medium of **1** (497 mg) was chromatographed on silica gel column (50 g, chloroform - ethyl acetate mixtures) to give fractions A -E. Fraction A (19 : 1) consisted of compound **2** (94 mg). **2**, purified by reverse phase HPLC (RP-8, MeOH - H_2O - MeCN 5 : 3 : 2) had: m.p. 118 -121 °C (hexane); $[\alpha]_D$ + 93° (c 0.36 in $CHCl_3$); EIMS *m/z* 300; UV λ_{max} 281 nm (EtOH); IR ($CHCl_3$) 3338 cm^{-1}; ^1H-NMR ($CDCl_3$) 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 ($CDCl_3$) 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). Fraction B (9 : 1) consisted of unreacted **1** (83 mg). Fraction C (4 : 1) consisted of the spirocompound **3** (157 mg) which after purification by HPLC (RP-8, MeOH - H_2O - MeCN 5 : 3 : 2) had: mp 138-140 °C (hexane); $[\alpha]_D$ - 3° (*c* = 0.22, $CHCl_3$); EIMS *m/z* 300; UV λ_{max} 269 nm (EtOH); IR ($CHCl_3$) ν 1660 cm^{-1}; ^1H-NMR ($CDCl_3$) 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 ($CDCl_3$) 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). Fraction D consisted of a mixture (102 mg) of **4** and **5** which was risolved by HPLC (Sigel, $CHCl_3$ - EtOAc 9 : 1). **4** had m.p. 238-241 °C (hexane); $[\alpha]_D$ +3.8° (c 0.41, $CHCl_3$); EIMS *m/z* 318, UV λ_{max} 238 nm (EtOH); IR ($CHCl_3$) 3606 and 1712 cm^{-1}; ^1H-NMR (C_5D_5N) δ 7.96 (d, J = 5.9 Hz, H-1), 6.28 (d, J = 5.9 Hz, H-2), 2.09 and 3.21 (d, J = 19.5 Hz, H-4), 2.48 (dt, J= 3.2 and 14.4 Hz, H-6), 1.19 (s, H-18), 1.11 (s, H-19), 1.47 (s, H-20); ^1H-NMR ($CDCl_3$) δ 7.78 (d, J = 5.8 Hz, H-1), 6.15 (d, J = 5.8 Hz, H-2), 1.90 and 2.80 (d, J = 19.4 Hz, H-4), 2.13 (dt, J = 3.1 and 14.0 Hz, H-6), 0.91 (s, H-18), 0.97 (s, H-19), 1.24 (s, H-20); ^{13}C-NMR ($CDCl_3$) δ 168.8 (C-1), 133.7 (C-2), 210.2 (C-3), 46.1 (C-4), 73.7 (C-5), 34.5 (C-6), 27.3 (C-7), 49.1 (C-8), 36.4 (C-9), 54.5 (C-10), 21.1 (C-11), 31.5 (C-12), 45.2 (C-13), 50.1 (C-14), 23.2 (C-15), 38.8 (C-16), 81.6 (C-17), 13.9 (C -18), 23.2 (C-19), 25.7 (C-20). **5** had m.p. 208-210 °C (hexane); $[\alpha]_D$ -3° (c 0.7, $CHCl_3$); EIMS *m/z* 318; UV λ_{max} 283 nm (EtOH); IR ($CHCl_3$) 3600 and 1710 cm^{-1}; ^1H-NMR (C_5D_5N) δ 7.66 (d, J = 5.8 Hz, H-1), 6.30 (d, J = 5.8 Hz, H-2), 2.19 and 3.21 (d, J = 18.7 Hz, H-4), 1.14 (s, H-18), 1.41 (s, H-19), 1.46 (s, H-20); ^1H-NMR ($CDCl_3$) δ

7.55 (d, J = 5.7 Hz, H-1), 6.20 (d, J = 5.7 Hz, H-2), 2.04 and 2.78 (d, J = 19.0 Hz, H-4), 0.89 (s, H-18), 1.34 (s, H-19), 1.25 (s, H-20); ^{13}C-NMR (C_5D_5N) δ 169.5 (C-1), 133.8 (C-2), 209.5 (C-3), 44.1 (C-4), 74.3 (C-5), 34.5 (C-6), 28.6 (C-7), 51.4 (C-8), 38.6 (C-9), 56.3 (C-10), 21.2 (C-11), 32.2 (C-12), 46.3 (C-13), 50.7 (C-14), 23.8 (C-15), 39.6 (C-16), 80.5 (C-17), 15.0 (C -18), 18.7 (C-19), 26.7 (C-20). ^{13}C-NMR ($CDCl_3$) δ 167.8 (C-1), 134.3 (C-2), 209.3 (C-3), 43.3 (C-4), 74.8 (C-5), 34.1 (C-6), 28.0 (C-7), 50.0 (C-8), 38.3 (C-9), 55.3 (C-10), 20.3 (C-11), 31.3 (C-12), 45.5 (C-13), 50.5 (C-14), 23.1 (C-15), 38.9 (C-16), 81.5 (C-17), 14.0 (C -18), 19.0 (C-19), 25.9 (C-20). Fraction D (7 : 3) (37 mg) was purified by preparative TLC ($CHCl_3$ - EtOAc 11 : 9) to give **6** m.p. 112-115 °C (hexane); $[\alpha]_D$ + 21° (c 0.81, $CHCl_3$); EIMS *m/z* 318; UV λ_{max} 266 nm (EtOH); IR ($CHCl_3$) 3606 and 1685 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.28 (d, J = 5.6 Hz, H-1), 2.68 (dd, J = 2.3 and 19.8 Hz, H-2), 5.97 (d, J = 1.3 Hz, H-4), 3.02 (dd, J = 4.8 and 20.2 Hz, H-6), 0.91 (s, H-18), 0.96 (s, H-19), 1.25 (s, H-20).

Bioproducts **8 - 12.** The ethyl acetate extract of the culture medium of **7** (495 mg) was chromatographed on silica gel column (50 g, hexane - acetone mixtures) to give fractions A - D. Fraction A (9 : 1) was rechromatographed on preparative TLC (benzene - Et_2O 9 : 1) to give **8** (99 mg) m.p. 209-212 °C (hexane); $[\alpha]_D$ +173° (c 0.77, $CHCl_3$); EIMS *m/z* 284, UV λ_{max} 269 nm (EtOH); IR ($CHCl_3$) 3597 and 1734 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 6.51 (d, J = 2.3 Hz, H-2), 6.45 (d, J = 2.3 Hz, H-4), 0.95 (s, H-18), 2.31 (s, H-19). Fraction B (216 mg) consisted of starting **7** and spirocompound **9** which were separated by HPLC (Sigel, $CHCl_3$ - EtOAc 19 : 1). **9** had: m.p. 147-152 °C (hexane); $[\alpha]_D$ +82° (c 0.42, $CHCl_3$); EIMS *m/z* 284; UV λ_{max} 267 nm (EtOH); IR ($CHCl_3$) 1664 and 1735 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 6.86 (d, J = 10.0 Hz, H-1), 6.20 (dd, J = 2.1 and 10.0 Hz, H-2), 6.09 (d, J = 2.1 Hz, H-4), 0.88 (s, H-18), 1.98 (s, H-19). Fraction C (4 : 1) (120 mg) consisted of a mixture of **10** and **11** which were separated by HPLC (Sigel, $CHCl_3$ - EtOAc 9 : 1). **10** had: m.p. 187-190 °C (hexane); $[\alpha]_D$ +80° (c 0.61, $CHCl_3$); EIMS *m/z* 302, UV λ_{max} 229 (EtOH); IR ($CHCl_3$) 3620, 1734 and 1713 cm^{-1}; ^{1}H-NMR (C_5D_5N) δ 7.92 (d, J = 5.9 Hz, H-1), 6.28 (d, J = 5.9 Hz, H-2), 2.09 and 3.18 (d, J = 19.0 Hz, H-4), 0.85 (s, H-18), 1.06 (s, H-19). **11** had: m.p. 182-184 °C (hexane); $[\alpha]_D$ +74° (c 0.53, $CHCl_3$); EIMS *m/z* 302, UV λ_{max} 280 nm (EtOH); IR ($CHCl_3$) 3599, 1734 and 1716 cm^{-1}; ^{1}H-NMR (C_5D_5N) δ 7.66 (d, J = 6.0 Hz, H-1), 6.08 (d, J = 6.0 Hz, H-2), 1.84 and 2.69 (d, J = 18.7 Hz, H-4), 0.83 (s, H-18), 1.45 (s, H-19). Fraction D (7 : 3) (28 mg) consisted of **12** which, purified by preparative TLC (CH_2Cl_2 - acetone 17 : 3), had: m.p. 127-129 °C (hexane); $[\alpha]_D$ +178° (c 0.27, $CHCl_3$); EIMS *m/z* 302, UV λ_{max} 262 nm (EtOH); IR ($CHCl_3$) 3689, 1735 and 1687 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.30 (d, J = 5.5 Hz, H-1), 2.67 (dd, J = 2.4 and 20.0 Hz, H-2), 6.01 (d, J = 1.2 Hz, H-4), 3.09 (dd, J = 5.0 and 20.8 Hz, H-6) 0.93 (s, H-18), 0.97 (s, H-19).

Acknowledgements. Work supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST). NMR experiments were performed at Centro Interdipartimentale Metodologie Chimico-Fisiche of the University Federico II. The EIMS spectral data were provided by Servizio di Spettrometria di Massa of CNR, Naples.

REFERENCES

1. Mahato, S.B.; Majumdar, I. Phytochemistry, **1993**, *34*, 883.
2. Schoeller, W. *United States Patent 2*, **1937**, 184, 167
3. Koester, D.H. *United States Patent 2*, **1937**, 236, 754.
4. Abul-Hajj, Y.J.; Qian, X. *J. Nat. Products*, **1986**, *49*, 244.
5. Fiorentino, A.; Pinto, G.; Pollio, A.; Previtera, L. *Biomed. Chem. Letters*, **1991**, *1*, 673.
6. Pollio, A.; Pinto, G.; DellaGreca, M.; DeMaio, A.; Fiorentino, A.; Previtera, L. *Phytochemistry*, **1994**, *37*, 1269.
7. DellaGreca, M.; Fiorentino, A.; Pinto, G.; Pollio, A.; Previtera, L. *Phytochemistry*, **1996**, *41*, 1527.

8. DellaGreca, M.; Fiorentino, A.; Pinto, G.; Pollio, A.; Previtera, L. *Biotechnol. Lett.*, **1996**, *18*, 649.
9. DellaGreca, M.; Fiorentino, A.; Pinto, G.; Pollio, A.; Previtera, L. *J. Org. Chem.*, in press.
10. Tori, K.; Komeno, T.; Nakagawa, T. *J. Org. Chem.*, **1964**, *29*, 1136.
11. Kirk, D.N.; Toms, H.C.; Douglas, C.; White, K.A.; Smith, K.E.; Latif, S.; Hubbard, R.W.P. *J. Chem. Soc., Perkin Trans. 2*, **1990**, 1567.
12. Blunt, J.W.; Stothers, J.B. *Org. Magn Reson.*, **1977**, *9*, 439.
13. Nichols, H.W.; Bold, H.C. *J. Phycol.*, **1965**, *1*, 34.

(*Received in UK* 22 *July* 1996; *accepted* 19 *September* 1996)