Tetrahedron Vol. 49, No. 28, pp. 6277-6284, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

New Diterpenes from *Salvia munzii*: Chemical and Biogenetic Aspects

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(*Received in UK* 17 *March* 1993)

Abstract: Three new natural diterpenes: 5,6-didehydro-7-hydroxy-taxodone (**1**), 17-hydroxycryptotanshinone (**2**) and salvicanaraldehyde (**4**) plus the known compounds: taxodione, taxodone, cryptotanshinone (**3**), 7α-hydroxyroyleanone, ferruginol, 6,7-didehydroferruginol (**6**), 6,7-didehydrosempervirol (**7**), demethylsalvicanol, salvicanaric acid (**5**) and the 11,12-dihydroxy-6,7-secoabieta-8,11,13-trien-6,7-dial-11,6-hemiacetals **9** and **10** were isolated from the roots of *Salvia munzii*. The structures of the new compounds were established from their spectroscopic data and by chemical correlations. The co-occurence of some of these compounds in the one species together with the results obtained of photochemical oxidation of 5,6-didehydro-7-hydroxytaxodone supports our earlier hypothesis of a biogenetic pathway to highly oxidized abietatriene diterpenes in which enzymatic dehydrogenation and singlet-state oxygen appear to play important roles.

The huge Salvia genus (Labiatae) with over 500 species is found throughout the world [1,2] and features in folk pharmacopoeias almost everywhere; as such, Salvia species are prime candidates for investigation.

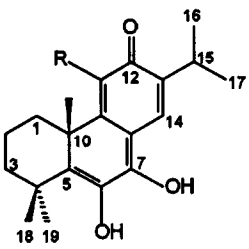
In previous papers [3,4], having isolated a large number of abietane diterpenes from Salvia species, we theorized that a biosynthetic pathway to highly oxidized abietatriene diterpenes might involve enzymatic dehydrogenation processes and the participation of singlet oxygen as a vital components. It would appear that this type of compound has a powerful antioxidant effect on free radicals and singlet oxygen injuries to the plant cells of Salvia species and, as many human ailments as well as aging are affected by the intervention of such chemical species in biological processes, some of these sustances may be interesting from the medicinal point of view. The results newly obtained from the study of *S. munzii* support the biosynthetic pathway outlined above.

Salvia munzii is a Mexican specie endemic to Baja California which grows on stony desert scrubland in the Rosario district near to the Sierra de Juárez and San Diego and is subjected to intense and protracted solar irradiation. The ground roots of the plant were extracted with distilled acetone at room temperature and the extract was fractionated as indicated in the Experimental section.

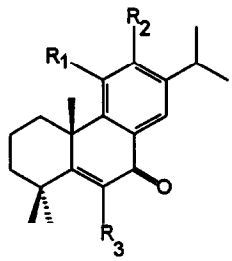
The known diterpenes, taxodione [5,6], taxodone [5,6], cryptotanshinone (**3**) [7], 7α-hydroxyroyleanone [8], ferruginol [9], 6,7-didehydroferruginol (**6**), 6,7-didehydrosempervirol (**7**), demethylsalvicanol, salvicanaric acid (**5**) and a mixture of 6-epimeric 11,12-dihydroxy-6,7-secoabieta-8,11,13-trien-6,7-dial-11,6-hemiacetals (**9+10**) [10] were obtained and identified by comparison with the spectral data of authentic samples and with those given in the literature. Three new abietanes were also separated and their structure and chemical behaviour determined.

The structure of **1** was established as 5,6-didehydro-7-hydroxy-taxodone as follows: the low resolution mass spectrum showed $[M]^+$ at *m/z* 330 ($C_{20}H_{26}O_4$ by HRMS). The IR spectrum had bands for phenols

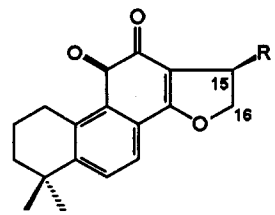
(3580 cm^{-1}) and for a methylene quinone grouping (1603 and 1567 cm^{-1}) which was confirmed by the UV spectrum (λ max 328, 287 and 255 nm). In the ^{1}HMR spectrum signals for an isopropyl group on an aromatic ring and three angular methyls were observed. In the low-field region of the spectrum one proton at δ 7.14 interchangeable with deuterium oxide could be assigned to the phenol hydroxy group on C-11 while only one proton of the quinone methide system, 14-H, was observed. Its low chemical shift (δ 7.73) indicated the presence of a coplanar hydroxy group on C-7 which was corroborated when the 14-H signals appeared at δ 8.25 when the spectrum was taken in Py-d_5 [11]. No signals were observed for the 5-H [12] or for protons allylic to an unsaturated group so the remaining oxygen atom must be part of a C5-C6 enol system. By means of homonuclear and ^{13}C-^{1}H heteronuclear double resonance and nOe difference experiments and a comparison of the ^{1}H NMR spectra of **1** taken in $CDCl_3$ and Py-d_5, the chemical shifts of all the protons and carbons could be ascertained. All the above data are in agreement with the structure given for compound **1**.



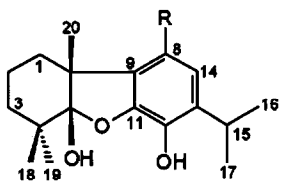
1 R=OH
1d R=OAc



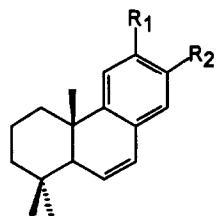
1a R_1=OAc ; R_2=OAc ; R_3=OAc
1b R_1=OAc ; R_2=OAc ; R_3=OH
1c R_1=OH ; R_2=OAc ; R_3=OAc



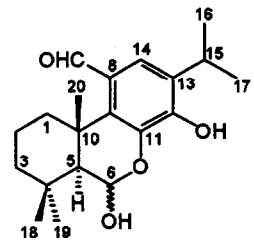
2 R=CH_2OH
3 R=CH_3



4 R=CHO
5 R=COOH



6 R_1=OH ; R_2=$CH(CH_3)_2$
7 R_1=$CH(CH_3)_2$; R_2=OH



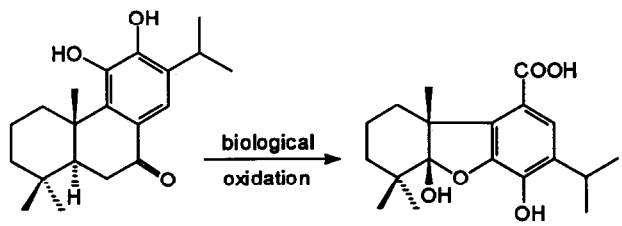
9+10

Chemical proof for structure **1** for this new compound was provided when treatment of **1** with acetic anhydride in pyridine gave a mixture of four products, **1a-1d**, which were separated by preparative TLC on silica gel. The major product was **1a**: low resolution MS showed the molecular ion $[M]^+$ at *m/z* 456. In its ^{1}H NMR spectrum signals appeared for one enolic and two aromatic acetates and the 14-H aromatic proton at δ 8.16, consonant with a periplanar ketone group on C-7. Compound **1b** had the molecular ion $[M]^+$ at *m/z* 414; ^{1}H NMR signals were recorded for two aromatic acetates as a six-proton singlet at δ 2.32, the 14-H aromatic proton at δ 8.03 and a proton singlet interchangeable with D_2O at δ 7.25 assignable to the 6-OH proton; **1c** also had ^{1}H NMR signals for the 14-H aromatic proton at δ 8.14 indicating the carbonyl structure of C-7, two vinyl acetates, and a proton singlet interchangeable with D_2O at a δ 7.05 attributable to 11-OH. The minor

product, **1d**, ([M]$^+$ at *m/z* 372) showed the 14-H proton at δ 7.69 and signals for only one aromatic acetate group. UV bands were very similar to those of the starting material, indicating the presence of an analogous methide quinone chromophoric group. All these reaction products were evidently formed by tautomerization of the vinyl hydroxy group on C-7 in the reaction medium with aromatization and subsequent total or partial acetylation and are in accordance with the proposed structure, 5,6-didehydro-7-hydroxytaxodone, for **1**.

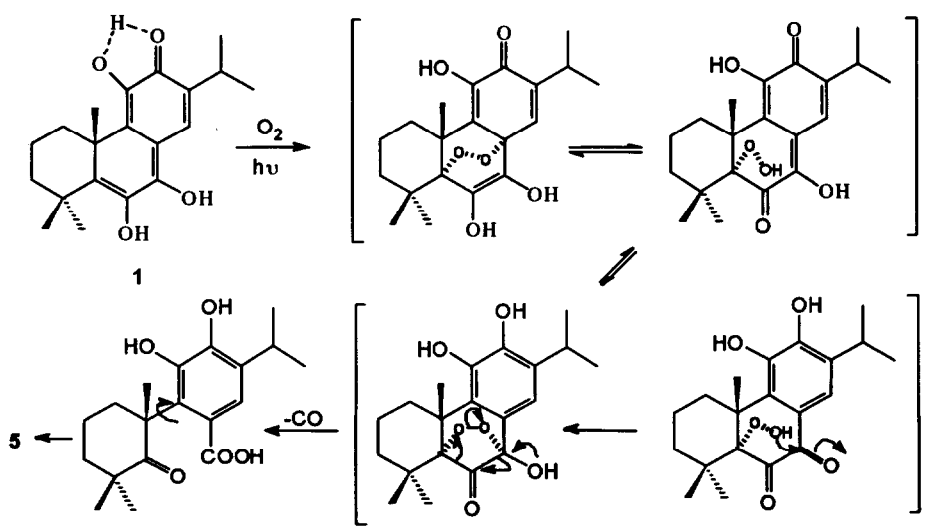
The biogenetic origin of salvicanaric acid (**5**) has been suggested [13] to proceed from the biosynthetic oxidation of demethylcryptojaponol (Scheme 1) and when, later, [3,4] a great number of highly oxidized abietatriene diterpenes were isolated, their chemical behaviour led us to propouse a similar biosynthetic pathway to highly oxidized diterpenes involving enzymatic dehydrogenation and the participation of singlet-state oxygen. 5,6-didehydro-7-hydroxytaxodone (**1**) represents a further stage in the possible oxidation of demethylcryptojaponol to salvicanaric acid and its co-appearance now with this latter in *S. munzii* led us to attempt to prove our hypothesis in the laboratory.

Scheme 1



5

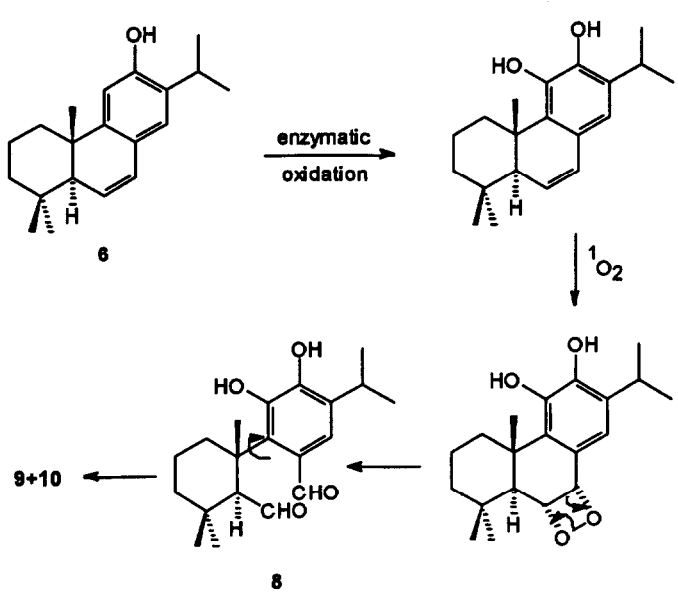
Scheme 2



When **1**, dissolved in n-hexane-EtOAc, was irradiated with UV light (240 nm) for 1/2 hour, TLC revealed two spots, the less polar being unreacted starting material. When the solution was left to stand, the more polar product rearranged to give **5**. Heating the solution accelerated the process. Scheme 2 outlines the proposed mechanism via singlet oxygen with the substrate as autosensitizer. Compound **1** was stable after 24 hours when a solution in n-hexane-EtOAc was stirred under oxygen atmosphere alone or with silica gel added. It was also stable under silica gel chromatography. These reactions suggest that **5** may have been formed by the biosynthetic oxidation of **1**.

6,7-Didehydroferruginol, identified by comparison of its spectral data with those of an authentic sample, was also obtained from this extract of *S. munzii* and provided an explanation for the presence of the hemiacetal mixture (**9+10**) as owing to biological oxidation with enzymatic dehydrogenation and singlet oxygen acting in the other way in which it can attack a double bond and form a dioxethane intermediate as shown in Scheme 3. The same mixture of hemiacetals had been obtained before from *Coleus barbatus* [10] as had a mixture of analogous hemiacetals hydroxylated at C-2 isolated in our laboratory from *S. texana* [14].

Scheme 3



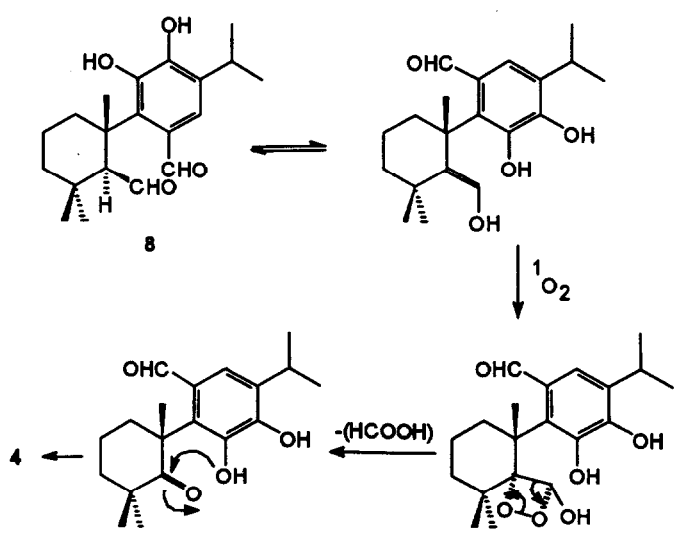
Product **3** was isolated as a solid which crystallized in orange-red prisms with the molecular ion at *m/z* 296 in MS. In its ^1H NMR spectra, signals were seen for two angular methyls and for a methyl coupled appeared as a sextet centred at δ 3.60 and further coupled to two other protons (also coupled to each other), appearing as a double doublet at δ 4.35 and a triplet at δ 4.88, respectively. This AMX grouping is characteristic of the methylene dihydrofuran system present in the tanshinone-type quinones isolated from *S. miltiorrhiza* [15]. The further presence in the same spectrum of an aromatic AB system as two doublets of one

proton each centred at δ 7.48 and 7.63 also agreed with a structure such as that of cryptotanshinone which was confirmed by comparison of the spectral data for this substance in the literature [15] with those of our product.

The HRMS of product **2** gave the molecular structure, $C_{19}H_{20}O_4$, and its IR spectrum showed bands for a hydroxy group (3473 cm^{-1}) and an intense band at 1613 cm^{-1} assignable to an o-benzoquinone group. Its ^1H NMR spectrum showed an aromatic AB system as doublets at δ 7.53 and 7.67 analogous to that shown by the 6-H and 7-H protons of cryptotanshinone and only two angular methyls. Nonetheless, the AMX system of the 16-H and 15-H protons typical of the methyldihydrofuran grouping of cryptotanshinone was still to be seen but the 15-H proton appeared as a quintet, all of which situated the hydroxy group on C-17 which was confirmed by the presence of two multiplets centred at δ 4.50 and 4.97 superimposed on the AMX system and assignable to the $-CH_2OH$. These data are completely consistent with the structure of 17α-hydroxytanshinone for **2** which has just now, simultaneously with the preparation of this paper, been described elsewhere [16].

Compound **4** was isolated as a colourless oil with an HRMS molecular formula of $C_{19}H_{26}O_4$. Its IR spectrum possessed the typical bands of a phenol group (3558 cm^{-1}) and a tertiary alcohol group (3486 cm^{-1}). The presence in the ^1H NMR spectrum (run in $CDCl_3$) of a singlet proton at δ 10.0 was evidence of the aldehyde nature of this carbonyl group while the aromatic ring was shown to be present by an aromatic proton as a singlet at δ 7.32 and the characteristic signals for an isopropyl group on an aromatic. When the ^1H NMR spectrum was taken in benzene, the signals of the isopropyl methyls and those of the angular methyls could be separated perfectly making it clear that the three angular methyls were all present. Two singlet signals (in the $CDCl_3$ spectrum) interchangeable with deuterium oxide at δ 2.81 and 5.48 could be assigned to the alcohol and phenol hydroxy groups, respectively. The low chemical shift of one of the angular methyls (δ 1.67) together with its shift to δ 2.01 when the spectrum was taken in C_6H_5N clearly revealed that it was opposite to a hydroxy group.

Scheme 4



With the molecular formula $C_{19}H_{26}O_4$, an aromatic ring, a phenol group, an alcohol group and an aldehyde, the molecule must be tricyclic and the fourth oxygen must be an ether bridge. A signal for a singlet carbon at δ 115 in the ^{13}C NMR spectrum showed the existence of a hemiacetal grouping and thus the ether bridge must be closed on the carbon bearing the tertiary hydroxyl. All these date agree with the structure **4** for this substance which we have called salvicanaraldehyde. The biosyntetic origin of this substance is consistent with the biological oxidation of 6,7-didehydroferruginol via the intermediate **8** with participation of singlet-state oxygen as indicated in Scheme **3** and **4**.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were recorded on Bruker AMX400 and WP200SY spectrometers. IR spectra were taken on a Perkin-Elmer 1600 (FTIR) spectrophotometer and UV spectra on a Perkin. Elmer 550SE instrument. High resolution mass spectra were run on a VG-Micromass ZAB-F at 70 eV.

Isolation of Products. S. munzii Eplig is a Mexican species endemic to Baja California which grows on stony desert scrubland in the Rosario district near to the Sierra de Juárez and San Diego and is subjected to intense and protacted solar irradiation.

The ground roots were extracted with distilled acetone at room temperature and the resulting extract (2.789 g) was chromatographed on a dry column of silica gel with mixtures of n-hexane-EtOAc, n-hexane-$CHCl_3$ and Cl_2CH_2-acetone as solvents, the following compounds were obtained.

5,6-Didehydro-7-hydroxy-taxodone (**1**) (30 mg) isolated as a colourless crystalline solid: $[M]^+$ at *m/z* 330.1320 (calc.for $C_{20}H_{26}O_4$, 330.1340); UV λ_{max} (EtOH) nm: 328, 287, 256; IR υ_{max} cm^{-1} ($CHCl_3$): 3580, 2590, 1595, 1445, 1320, 1270, 1190; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.27, 1.33 (3H, d, J=6.45Hz, Me-16, Me-17), 1.47 (6H, s, Me-18, Me-19), 1.69 (3H, s, Me-20), 2.95 (1H, br d, J=7.60Hz, H-1α), 3.05 (1H, hept, J=6.45Hz, H-15), 5.72 (1H, br s, OH), 5.86 (1H, s, OH), 7.14 (1H, s, OH), 7.73 (1H, s, H-14); ^1H NMR (200 MHz, Py-d_5) δ: 1.31 (3H, d, J=6.80Hz, Me-16, Me-17), 1.65, 1.69 (each 3H, s, Me-18, Me-19), 1.96 (3H, s, Me-20), 3.64 (1H, m, H-1α), 3.68 (1H, hept, J=6.80Hz, H-15), 8.25 (1H, s, H-14); ^{13}C NMR (200 MHz, Py-d_5) δ: 18.32 (t, C-2), 22.86 (q, C-16), 23.14 (q, C-17), 27.62 (q, C-18), 27.5 (d, C-15), 28.33 (q, C-19), 28.40 (q, C-20), 30.62 (t, C-1), 36.70 (s, C-4), 36.90 (t, C-3), 41.40 (s, C-10), 116.4o (d, C-14), 121.58 (s, C-8), 123.80 (s, C-13), 140.30 (s, C-11), 142.50 (s, C-9), 144.20 (s, C-5), 144.60 (s, C-6), 149.90 (s, C-7), 180.50 (s, C-12); EIMS (rel. int.) *m/z*: 330 $[M]^+$ (37), 287 (12), 260 (100).

Acetylation of 1: Compound **1** (16.8 mg) was acetylated with Ac_2O in Py at r. t. for 60 h. affording the four products: **1a** (17.5 mg), **1b** (2.0 mg), **1c** (1.0 mg) and **1d** (1.5 mg) after chromatographic purification.

Diacetate 1a: UV λ_{max} (EtOH) nm: 244; IR υ_{max} cm^{-1} ($CHCl_3$): 2585, 1760, 1650, 1600, 1455, 1420, 1360, 1325, 1250, 1180, 1170, 1100, 1050, 1010; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.21, 1.24 (each 3H, d, J=7.0Hz, Me-16, Me-17), 1.22 (3H, s, Me-18, Me-19, Me-20), 2.32 (3H, s, -OCOMe), 2.36 (6H, s, -

OCOMe), 2.95 (1H, hept, J=7.0Hz, H-15), 8.11 (1H, s, H-14); EIMS (rel. int.) *m/z*: 456 $[M]^+$ (30), 414 (54), 372 (56), 330 (45), 303 (92), 260 (100), 231 (17).

Diacetate 1b: UV λ_{max} (EtOH) nm: 255; IR υ_{max} cm^{-1} ($CHCl_3$): 2580, 1760, 1670, 1595, 1360, 1300; ^1H NMR (200 MHz, $CDCl_3$) δ: 0.96 (6H, d, J=6.10Hz, Me-16, Me-17), 1.22 (3H, s, Me-20), 1.57 (6H, s, Me-18, Me-19), 2.32 (6H, s -OCOMe), 2.68 (1H, m, H-1α), 2.91 (1H, hept, J=6.10Hz, H-15), 8.03 (1H,s,H-14); EIMS (rel. int.) *m/z*: 414 $[M]^+$ (2), 354 (7), 326 (15), 308 (79), 280 (79), 251 (36).

Diacetate 1c: UV λ_{max} (EtOH) nm: 262, 274; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.24, 1.27 (each 3H, d, J=6.10Hz, Me-16, Me-17), 1.46, 1.48 (each 3H, s, Me-18, Me-19), 1.57 (3H, s, Me-20), 2.34, 2.38 (each 3H, s, -OCOMe), 2.97 (1H, hept, J=6.10Hz, H-15), 7.05 (1H, s, OH), 8.14 (1H, s, H-14).

Diacetate 1d: UV λ_{max} (EtOH) nm: 245, 301, 344; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.27, 1.30 (each 3H, d, J=6.20Hz, Me-16, Me-17), 1.60 (9H, s, Me-18, Me-19, Me-20), 2.36 (3H, s, -OCOMe), 3.03 (1H, hept, J=6.20Hz, H-15), 7.69 (1H, s, H-14); EIMS (rel. int.) *m/z*: 372 $[M]^+$ (18), 330 (30), 287 (12), 260 (100).

17-Hydroxycryptotanshinone (2) (2 mg) isolated as a reddish solid: $[M]^+$ at *m/z* 312.1020 (calc. for $C_{19}H_{20}O_4$, 312.1136); UV λ_{max} (EtOH) nm: 292, 241; IR υ_{max} cm^{-1} ($CHCl_3$): 3473, 3017, 2981, 1613, 1422, 1072; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.30, 1.32 (each 3H, s, Me-18, Me-19), 3.22 (2H, t, $-CH_2$-1), 3.79 (1H, m, H-15), 4.50 (2H, m, H-6β, H-17), 4.97 (2H, m, H-16α, H-17), 7.53, 7.67 (each 1H, d, J=8.0Hz, H-6, H-7); EIMS (rel. int.) *m/z*: 312 $[M]^+$ (46), 281 (31), 253 (100), 185 (11), 165 (19), 115 (16).

Salvicanaraldehyde (4) (5 mg) isolated as a colourless oil: $[M]^+$ at *m/z:* 318.1126 (calc. for $C_{19}H_{26}O_4$, 318.1140); UV λ_{max} (EtOH) nm: 281, 231; IR υ_{max} cm^{-1} ($CHCl_3$): 3557, 2997, 2963, 1686, 1616, 1432, 1219, 1097; ^1H NMR (200 MHz, $CDCl_3$) δ: 1.27 (12H, m, Me-16, Me-17, Me-18, Me-19), 1.67 (3H, s, Me-20), 2.22 (1H, m, H-1β), 2.81 (1H, s, OH-5), 3.28 (1H, hept, H-15), 5.48 (1H, s, OH-12), 7.32 (1H, s, H-14), 10.04 (1H, s, -CHO); ^1H NMR (200 MHz, C_6D_6) d: 1.09, 1.15 (each 3H, s, Me-18, Me-19), 1.20, 1.29 (each 3H, d, J=7.0Hz, Me-16, Me-17), 1.70 (3H, s, Me-20), 2.45 (1H, s, OH-5), 3.39 (1H, hept, J=7.0Hz, H-15), 5.53 (1H, s, OH-12), 7.39 (1H, s, H-14), 10.00 (1H, s, -CHO); ^1H NMR (200 MHz, Py-d_5) δ: 1.17, 1.33 (each 3H, s, Me-18, Me-19), 1.27, 1.32 (each 3H, d, J=7.0Hz, Me-16, Me-17), 2.01 (3H, s, Me-20), 2.35 (1H, m, H-1α), 3.61 (1H, hept, J=7.0Hz, H-15), 7.60 (1H, s, H-14), 8.30 (1H, s, OH-12), 10.35 (1H, s, -CHO); ^{13}C NMR (200 MHz, C_6D_6) δ: 17.72 (q, C-20), 19.39 (q, C-16), 22.44 (q, C-17), 24.50 (q, C-18), 26.44 (q, C-19), 27.60 (t, C-2), 30.15 (d, C-15), 37.55 (t, C-3), 37.85 (s, C-4), 39.45 (t, C-1), 51.35 (s, C-10); 115.01 (s, C-5), 125.08 (d, C-14), 126.76 (s, C-9), 134.64 (s, C-8), 137, 65 (s, C-11), 142.00 (s, C-13), 144.21 (s, C-12), 189.70 (s, C-7); EIMS (rel. int.) *m/z*: 318 $[M]^+$ (23), 303 (1), 290, (28), 275 (7), 247 (89), 219 (100).

ACKNOWLEDGEMENTS: This work has been partly subsidized by Grant PB 910763 (CICYT) and Ramón Areces Foundation. We should also like to thank the botanist Prof. J. Delgadillo of the University from Baja California (Mexico) for collecting the plant material.

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