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TERPENOIDS AND OTHER CONSTITUENTS OF *EUCALYPTUS GLOBULUS*

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Key Word Index—*Eucalyptus globulus*; Myrtaceae; wood; bark; triterpenes; ursanes; oleanes; lupanes; vomifoliol; phenols; daucosterol.

Abstract—The wood of *Eucalyptus globulus* contains several pentacyclic diterpenes with oleane, ursane and lupane skeleta. In addition to the well known compounds β-amyrin, erythrodiol, uvaol, acetyloleanolic, acetylbetulinic, acetylursolic, betulinic, ursolic, 23-hydroxyursolic and *trans-p*-methoxycinnamoyloxy-ursolic acids, three new triterpenoids have been isolated: methyl *cis-p*-methoxycinnamoyloxyoleanolate, methyl *cis-p*-methoxycinnamoyloxyursolate and methyl 11α-methoxy-3-acetoxyursolate.

INTRODUCTION

The forestry industry makes an important contribution to the Portuguese economy (12% of total export trade in 1991) [1]; 37% of the industry is dedicated to *Eucalyptus* with *E. globulus* Labill being the most abundant species (95%). Since 1875, *Eucalyptus* plantations have been cultivated on a large scale and since 1957 have been dedicated almost exclusively to paper pulp production. As part of our studies on the chemical components of *E. globulus*, we have examined the bark and wood, separately, for useful secondary metabolites. We have isolated several pentacyclic triterpenes, some of them described here for the first time.

RESULTS AND DISCUSSION

The benzene-soluble fraction of an acetone extract of the wood of *E. globulus* afforded eight main fractions (I–VIII).

The main component of fraction I was β-amyrin (**1**) [2]. A mixture of methyl esters separated from fraction II was shown by GC-mass spectrometry to contain acetylursolic acid, (**2**), the major component of the benzene extract (13%), acetyloleanolic acid, (**3**) and acetylbetulinic acid, (**4**) [3].

Erythrodiol (**5**) and uvaol (**6**) were isolated from fraction III and identified by comparison of their physical properties with those reported in the literature [2–5]. Compounds **7** and **8** (betulinic acid methyl ester) [2, 3] were isolated by column chromatography from fraction IV.

Compound **7** showed in the ^{1}H NMR spectrum the following groups: Me—COO—CH (δ 2.03, *s* and 4.49, *dd*, $J_1 = 7.2$ Hz and $J_2 = 8.9$ Hz), MeO—CH—CH=C (δ 3.23, *s*; 3.76, *dd*, $J_1 = 3.5$ Hz and $J_2 = 8.6$ Hz; and 5.45, *d*, $J = 3.4$ Hz), COOMe (δ 3.60, *s*) and seven methyl groups, five singlets (δ 1.13, 1.02, 0.84 (6H) and 0.73) and two doublets (δ 0.93, $J = 7.8$ Hz, 6H).

Comparison of the ^{1}H and ^{13}C NMR data (Table 1) with those of **2** allowed the assignment of **7** as an ursane derivative with an allylic methoxyl group at C-11. The multiplicity of the *gem*-hydrogen indicated an α-stereochemistry for the methoxyl group; therefore, **7** can be assigned as 11α-methoxyacetylursolic acid methyl ester.

Two methyl esters, **9** and **10**, were isolated from fraction V. According to the NMR data compound **9** was an ursane derivative and compound **10** an oleane derivative. Both of them were esterified by *cis-p*-methoxy-cinnamic acid at C-3. Thus, **9** is *cis-p*-methoxy-cinnamoyloxyursolic acid methyl ester and compound **10** is *cis-p*-methoxy-cinnamoyl-oxyoleanolic acid methyl ester.

Fraction VI afforded *trans-p*-methoxycinnamoyl-oxyursolic acid methyl ester (**11**) and ursolic acid methyl ester (**12**) [3, 6].

From fraction VII, after esterification and acety-

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Table 1. ^{13}C NMR data for compounds **2**, **7**, **9** and **10** (62.9 MHz; $CDCl_3$; TMS as int. standard)

C	2	2 (C_6D_6)	7	9	10
1	38.2	38.2	39.1	38.3	38.2
2	23.5	23.9	23.8	23.5	27.7
3	80.9	80.5	80.7	80.8	80.8
4	37.6	37.8	37.9	37.7	36.7
5	55.3	55.6	55.2	55.3	55.3
6	18.2	18.5	18.2	18.2	18.2
7	32.9	33.2	33.3	32.9	32.6
8	39.5	39.8	38.0	39.5	39.3
9	47.4	47.7	52.3	47.4	47.5
10	36.8	36.9	42.0	36.9	36.9
11	23.3	23.5	76.1	23.3	23.3
12	125.4	125.9	124.9	125.5	122.3
13	138.1	138.6	142.5	138.2	143.2
14	41.9	42.3	42.4	41.9	41.6
15	28.0	28.4	29.7	28.0	27.6
16	24.2	24.6	24.1	24.2	24.2
17	48.1	48.3	47.6	48.1	48.0
18	52.8	53.3	52.3	52.8	52.8
19	39.0	39.4	38.8	38.8	45.8
20	38.8	39.2	38.6	39.0	31.9
21	30.6	30.9	30.5	30.6	30.6
22	36.6	37.0	36.5	36.6	36.6
23	28.0	28.2	28.1	28.1	28.1
24	16.7	17.0	16.7	16.7	15.4
25	15.5	15.6	16.9	15.5	16.7
26	16.9	17.2	18.4	17.1	16.9
27	23.5	23.9	22.9	23.5	25.9
28	178.1	177.2	177.9	178.1	178.1
29	17.0	17.3	17.0	16.9	33.1
30	21.3	21.3	21.3	21.2	23.6
$-O\underline{C}H_3$	51.4	51.2	51.5	51.4	51.5
$\underline{C}H_3-CO_2O$	21.2	20.9	21.1	—	—
$CH_3-\underline{C}O_2O$	171.0	169.9	170.9	—	—
$-O\underline{C}H_3$	—	—	54.4	55.2	55.4
1′	—	—	—	166.3	166.3
2′	—	—	—	117.8	117.8
3′	—	—	—	143.0	143.0
4′	—	—	—	127.5	127.5
5′–9′	—	—	—	132.1	132.1
6′–8′	—	—	—	113.4	113.4
7′	—	—	—	160.3	160.3

Assignments based on DEPT experiments and, particularly in the case of **2**, on 2D NMR experiments, $^1H-^{13}C$ XHCORR and COLOC.

lation, was isolated methyl 3β,23-diacetoxy-12-ursen-28-oate (**13**) [3] and from fraction VIII tetraacetyldaucosterol (**14**) [3, 6].

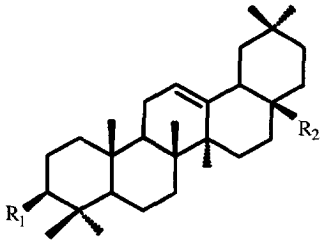
The bark of *E. globulus* was extracted with boiling hexane and then with acetone at room temperature. From the benzene-soluble part of the acetone extract were isolated: vomifoliol (**15**) [6], 2,6-dimethoxy-p-benzoquinone (**16**) and the acetates of 3,4,5-trimethoxyphenol (**17**) and 2,4,6-trimethoxyphenol (**18**).

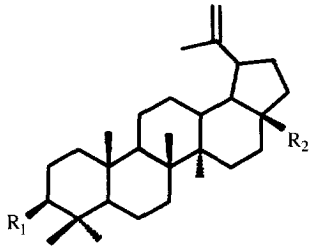
EXPERIMENTAL

Spectral analysis. Mps uncorr; NMR: 250 MHz for ^1H and 62.9 MHz for ^{13}C. Chemical shifts are given in δ (ppm) and are referenced to the residual $CHCl_3$, 7.26 ppm for ^1H and 77.0 ppm for ^{13}C, respectively; or C_6D_6, 7.15 ppm for ^1H and 128.0 ppm for ^{13}C. EIMS: VG Trio 1000, 70 eV.

Extraction and isolation. The wood (3.7 kg) of *E. globulus* was cut into very small pieces and extracted with Me_2CO at room temp., affording, after evapn of the solvent, 28.8 g (0.8% w/w) crude extract, which was partitioned with boiling C_6H_6 (14 g, 48%), boiling EtOAc (7.8 g, 27%), *n*-BuOH (0.56 g, 2.1%) and H_2O (6.3 g, 21.7%). A 4.5-g portion of the benzene-soluble part was subjected to CC on silica gel and sepd in 8 main frs by elution with hexane–EtOAc mixts of increasing polarity.

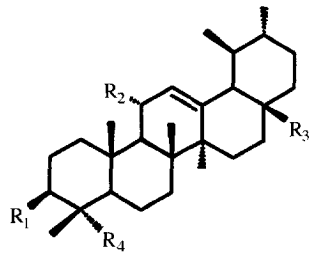
β-Amyrin (**1**) (34.7 mg, 1.0%) was isolated from fr. I (*n*-hexane–EtOAc, 9:1). A mixt. sepd from fr. II



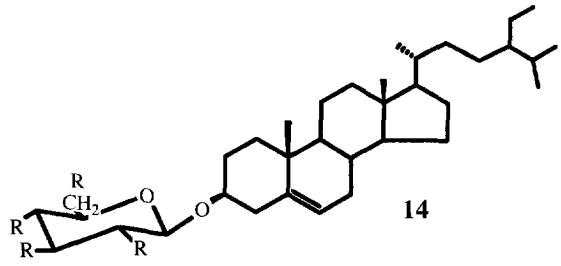


	R_1	R_2
1	OH	Me
3	OAc	COOMe
5	OH	CH_2OH
10	*p*-MeO-C_6H_4-CH$\overset{Z}{=}$CH-COO	COOMe

	R_1	R_2
4	OAc	COOMe
8	OH	COOMe



	R_1	R_2	R_3	R_4
2	OAc	H	COOMe	Me
6	OH	H	CH_2OH	Me
7	OAc	MeO	COOMe	Me
9	*p*-MeO-C_6H_4-CH$\overset{Z}{=}$CH-COO	H	COOMe	Me
11	*p*-MeO-C_6H_4-CH$\overset{E}{=}$CH-COO	H	COOMe	Me
12	OH	H	COOMe	Me
13	OAc	H	COOMe	CH_2OAc



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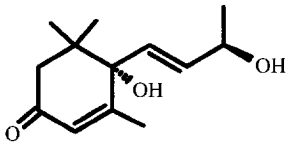
R = OAc

(685.2 mg, 15.5%, *n*-hexane–EtOAc, 9:1) was esterified with CH_2N_2 and sepd by CC (*n*-hexane–EtOAc, 9:1) giving a mixt. of acetylated triterpenes (24.0 mg). GC-MS allowed identification of the methyl ester of **2** and the acids **3** and **4**. The same column afforded **2** (180.7 mg) as the major compound and 185.2 mg (*n*-hexane–EtOAc, 1:1) of *β*-sitosterol.

Fr. III (70.1 mg, *n*-hexane–EtOAc, 4:1) afforded **5**



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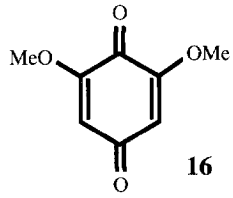

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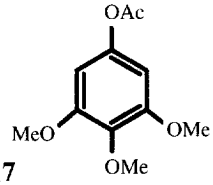

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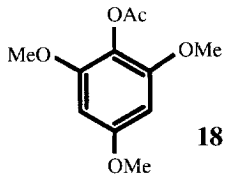

18

(15 mg) and (**6**) (10 mg) and a mixt. (45 mg) of **5** and **6**.

Fr. IV (300 mg, *n*-hexane–EtOAc, 4:1) was treated with CH_2N_2 and after CC afforded **7** (18.5 mg, *n*-hexane–EtOAc, 9:1), identified as 3β-acetyl-11α-methoxyursolic acid methyl ester, **8** (52.5 mg, *n*-hexane–EtOAc, 9:1) and **6** (15.2 mg).

Fr. V (*n*-hexane–EtOAc, 4:1, 435.5 mg, 10.0%) was treated with CH_2N_2 and purified by CC, affording **9** (12.4 mg, *n*-hexane–EtOAc, 49:1) identified as methyl *cis*-p-methoxycinnamoyloxyursolate and **10** (10.2 mg, *n*-hexane–EtOAc, 49:1) identified as methyl *cis*-*p*-methoxy-cinnamoyloxyoleanolate.

Fr. VI (98.9 mg, 2.5%, *n*-hexane–EtOAc, 7:3) was esterified with CH_2N_2 and subjected to CC to afford **11** (15.6 mg) and **12** (30.1 mg).

Fr. VII (135.2 mg, 3.2%, *n*-hexane–EtOAc, 1:1) was esterified with CH_2N_2 and acetylated. CC afforded **13** (36.7 mg, *n*-hexane–EtOAc, 9:1) identified as methyl 3β,23-diacetoxy-12-ursen-28-oate.

Fr. VIII (400 mg, 10%, EtOAc) was acetylated and subjected to CC to give 22.6 mg **14**.

The dried bark of *E. globulus*, fine cut, was extracted with hot hexane and then with Me_2CO at room temp. affording 57.7 g crude extract (6.0%). Fractionation, as before, was carried out with hot C_6H_6 (0.98 g, 1.6%), EtOAc (2.6 g, 4.5%); *n*-BuOH (6.5 g, 11.2%) and H_2O (25.8 g, 44.6%). The benzene-soluble fr. on CC eluting with hexane-EtOAc (3:2) gave three frs.

Fr. I (414.2 mg) was acetylated and chromatographed to afford **17** and **18**.

Fr. II (hexane–EtOAc, 3:2) afforded **16** (50.0 mg) identified as 2,6-dimethoxy-*p*-benzoquinone.

Fr. III (EtOAc) afforded 48.9 mg of a crystalline product identified as **15**.

Methyl 3β-acetoxy-11α-methoxy-12-ursen-28-oate (**7**). Mp 140.5–141.0°; $[\alpha]_D = -82°$ ($CHCl_3$, 0.25%). IR ν_{max}^{KBr} cm^{-1}: 2945, 2857, 1728, 1603, 1460, 1368, 1246, 1082, 1030, 806; ^1H NMR ($CDCl_3$) δ(ppm): 5.45 (1H, *d*, $J = 3.4$ Hz, H-12), 4.49 (1H, *dd*, $J_1 = 7.2$ Hz, $J_2 = 8.9$ Hz, H-3), 3.76 (1H, *dd*, $J_1 = 3.5$ Hz, $J_2 = 8.6$ Hz, H-11), 3.60 (3H, *s*, $CO_2C\underline{H}_3$), 3.23 (3H, *s*, $OC\underline{H}_3$), 2.26 (1H, *d*, $J = 11.7$ Hz, H-18), 2.03 (3H, *s*, $C\underline{H}_3CO_2$), 1.13 (3H, *s*, Me-27), 1.02 (3H, *s*, Me-25), 0.93 (2 × 3H, *d*, $J = 7.8$ Hz, Me-29, Me-30), 0.84 (2 × 3H, *s*, Me-23, Me-24), 0.73 (3H, *s*, Me-26), ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 542 $[M]^+$ (1.6), 512 (1.4), 484 (6.8), 483 (17.9), 452 (3.9), 400 (6.3), 299 (4.0), 292 (7.4), 233 (5.1), 205 (6.0), 203 (10.4), 199 (6.8), 167 (17.0), 149 (16.6), 125 (21.1), 111 (21.0), 97 (37.6), 83 (42.6), 69 (62.1), 57 (100), 55 (82.8).

Methyl 3β-(cis-p-methoxycinnamoyloxy)-12-ursen-28-oate (**9**). Mp 219–220°; $[\alpha]_D = -564°$ ($CHCl_3$, 0.19%). IR ν_{max}^{KBr} cm^{-1}: 2924, 1726, 1630, 1605, 1512, 1462, 1377, 1159, 1034, 985, 849; ^1H NMR ($CDCl_3$) δ(ppm): 7.68 (2H, *d*, $J = 8.7$ Hz, H-5′/H-9′), 6.86 (2H, *d*, $J = 8.7$ Hz, H-6′/H-8′), 6.82 (1H, *d*, $J = 12.7$ Hz, H-3′), 5.82 (1H, *d*, $J = 12.7$ Hz, H-2′), 5.23 (1H, *t*, $J = 3.5$ Hz, H-12), 4.55 (1H, *dd*, $J_1 = 7.2$ Hz, $J_2 = 8.3$ Hz, H-3), 3.81 (3H, *s*, $OC\underline{H}_3$), 3.59 (3H, *s*, $CO_2C\underline{H}_3$), 2.22 (1H, *d*, $J = 11.6$ Hz, H-18), 1.06 (3H, *s*, Me-27), 0.92 (3H, *s*, Me-25), 0.91 (3H, *d*, $J = 6.5$ Hz, Me-30), 0.88 (3H, *d*, $J = 6.0$ Hz, Me-29), 0.86 (3H, *s*, Me-24), 0.80 (3H, *s*, Me-23), 0.73 (3H, *s*, Me-26), ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 630 $[M]^+$, 453 (5.9), 369 (2.6), 264 (9.3), 262 (50.7), 249 (4.9), 204 (11.1), 203 (55.5), 190 (45.7), 161 (100), 133 (45.5), 95 (19.1), 81 (26.2), 69 (39.5), 57 (40.0), 55 (45.5).

Methyl 3β-(cis-p-methoxycinnamoyloxy)-12-olean-28-oate (**10**) Mp 208–209°; IR ν_{max}^{KBr} cm^{-1}: 2924, 1725, 1629, 1605, 1511, 1462, 1377, 1156, 1033, 984, 849; ^1H NMR ($CDCl_3$) δ(ppm): 7.68 (2H, *d*, $J = 8.7$ Hz, H-5′/H-9′), 6.86 (2H, *d*, $J = 8.7$ Hz, H-6′/H-8′), 6.82 (1H, *d*, $J = 12,8$ Hz, H-3′), 5,80 (1H, *d*, $J = 12.8$ Hz, H-2′), 5.25 (1H, *t*, $J = 3.5$ Hz, H-12), 4.55 (1H, *dd*, $J_1 = 4.9$ Hz, $J_2 = 9.8$ Hz, H-3), 3.81 (3H, *s*, $OC\underline{H}_3$), 3.61 (3H, *s*, $CO_2C\underline{H}_3$), 2.85 (1H, *m*, H-18), 1.12 (3H, *s*, Me-27), 0.94 (3H, *s*, Me-25), 0.91 (3H, *s*, Me-30), 0.87 (2 × 3H, *s*, Me-29, Me-24), 0.84 (3H, *s*, Me-23), .071 (3H, *s*, Me-26), ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 630 $[M]^+$, 453 (5.0), 369 (2.5), 264 (9.0), 262 (50.0), 249 (5.0), 204 (11.0), 203 (55.6), 190 (45.5), 161 (100), 133 (45.6), 95 (19.0), 81 (26.2), 69 (39.4), 57 (39.0), 55 (45.5).

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