Pergamon

Phytochemistry, Vol. 49, No. 3, pp. 817–822, 1998
© 1998 Elsevier Science Ltd. All rights reserved
Printed in Great Britain
0031–9422/98/$—see front matter

PII: S0031–9422(98)00168–X

HYDROXYLATED DITERPENOIDS FROM *HALIMIUM VISCOSUM*

JESUS M. L. RODILLA,*† DINA I. M. DE MENDONÇA,† J. G. URONES‡ and R. F. MORO‡

†Departamento de Química, Universidade da Beira Interior, 6200 Covilhã, Portugal; ‡Departamento de Química Orgánica, Universidad de Salamanca, 37008 Salamanca, Spain

(*Received in revised form* 19 *January* 1998)

Key Word Index—*Halimium viscosum*; Cistaceae; chemotype; labdane; *ent*-halimane; valparane; valparolane.

Abstract—Several hydroxylated bi- and tri-cyclic terpenoids have been isolated from the aerial parts of *Halimium viscosum* (Celorico da Beira, Portugal). Together with valparolone, with the same skeleton, two additional compounds have been identified: (4*S*)-2β-acetoxyvalparol-15-en-3-one and (4*S*)-2β-acetoxyvalparol-8(14)-en-3-one. Together with the well-known valpara-3(19),15-dien-2β-ol, valpara-1,13-dien-2α-ol and 2β-acetoxyvalpara-4,15-dien-3α-ol, two new compounds have been isolated, belonging to the valparane class, and identified as valpara-3,15-dien-2β-ol and 3α-hydroxyvalpara-4,15-dien-2-one. There are several bicyclic diterpenoids that should be noted: with labdane skeleton; a diformate and the monoformylderivatives of labd-7-en-3β,15-diol and with halimane skeleton the 15-hydroxy-ent-halim-1(10)-en-18,2β-olide. The total composition of the aerial parts of this plant allows the identification of Celorico da Beira *Halimium viscosum* as a new chemotype. © 1998 Elsevier Science Ltd. All rights reserved

---

INTRODUCTION

Recently, the presence of tricyclic diterpenes of the valparane class in the non-saponificable part of the hexane extract of *Halimium viscosum* (Celorico da Beira) has been discussed. This data together with the one obtained from the study of the acid part of the same extract suggested that this is a new chemotype, different from those previously discussed in the literature. In this work we report the chemical composition of the neutral part in which five valparane diterpenes, three valparolanes, eight labdanes and one lactone derivative with ent-halimane skeleton have been isolated and identified. The comparative study with other chemotypes of *Halimiun viscosum* [1] confirmed the existence of a new chemotype.

RESULTS AND DISCUSSION

The non-saponificable neutral part of the hexane extract from the aerial parts of *Halimium viscosum* (Celorico da Beira, Portugal) was fractionated (see Experimental). The following compounds have been isolated from the most polar fractions either as free alcohols or acetates: valparolone (valparola-2(4),15-dien-3-one) **1** [2], **2**, **3**, **4**, valpara-3(19),15-dien-2β-ol **5** [3], valpara-1, 15-dien-3α-ol **6** [4], **7**, **8**, **9**, 15-hydroxylabd-7-en-3-one **10** [5, 6], **11**, 2β-acetoxyvalpara-4,15-dien-3α-ol **12** [7], 15-hydroxy-ent-halim-1(10)-en-18,2β-olide **13** [8]; labd-7-en-3β,15-diol **14** [8], labda-7,13*E*-diene-3β,15-diol **15** [9] and 3β,7α,15-triacetoxylabd-8(17)-ene **16** [8].

From the fraction containing the major content of unsaturated alcohols an unsaturated keto-alcohol was isolated and purified by acetylation. The acetylderivative **2** has an isopropenyl group (Me-C$=$CH$_2$), an acetyl in a Me-CO-CH group (δ 2.64), two methyl singlets (δ 1.02 and 0.69) and a secondary acetoxyl group (AcO-CH), in which the geminal proton (δ 5.17, *dt*, $J_1 = 11.3$, $J_2 = 4.4$) is coupled to the α-H of the carbonyl group (COSY), indicating that a fragment AcO-CH-CH-CO-Me is present in the molecule. The ^{13}C NMR shows in addition to the acetoxyl group signals (δ 169.3 and 21.0), twenty carbon atoms: four methyls, seven methylenes (one olefinic), five methines (one bonded to oxygen) and four quaternary carbons (one carbonyl, one olefinic, δ 207.5; 147.9). The acetoxyl group indicates that it is not a valparane derivative but a valparolane, particularly as valparolane **1** has been isolated from this extract. Thus, the structure of 2-acetoxyvalparol-15-en-3-one is proposed for this compound, in agreement with the 2D data (HMQC and HMBC, Table 1)

The coupling constant between H-2 and H-4 (11.3 Hz) indicated a diaxial relationship with a

---

*Author to whom correspondence should be addressed.

Table 1. NMR data for compounds **2** and **3**, and correlations observed in 2D experiments.

2 (C_6D_6)					**3** ($CDCl_3$)			
Position	C	H		HMBC	C	H		HMBC
1	49.2				55.3			
2	70.6	5.46	1H, *dt*, $J_1 = 11.3$; $J_2 = 5.0$	1, 3, 4,-O_2*C*CH	69.2	4.53	1H, *d*, $J = 9.9$	
3	207.5				143.0			
4	56.7	2.51	1H, *m*	2, 3, 6	124.3	5.56	1H, *m*	
5	27.4				26.0			
6	56.0				56.8			
7	44.6				46.3			
8	55.7				55.0			
9	21.4				21.5			
10	43.4				45.3			
11	35.7				37.1			
12	41.0				41.4			
13	26.4				26.9			
14	46.4	2.51	1H, *m*	7, 8, 13, 15, 16	46.5	2.69	1H, *m*	8, 15, 16, 17
15	147.9				148.4			
16	110.9	4.90; 4.85	1H, *s* each	14, 15, 17	110.3	4.80; 4.78	1H, *s* each	14, 17
17	25.2	1.69	3H, *s*	14, 15, 16	25.0	1.76	3H, *s*	14, 15, 16
18	15.8	0.55	3H, *s*	6, 7, 8, 12	16.0	0.69	3H, *s*	6, 7, 8, 12
19	28.7	1.87	3H, *s*	3, 4	19.7	1.77	3H, *s*	2, 3, 4
20	20.9	0.84	3H, *s*	1, 6, 10, 11	21.2	1.08	3H, *s*	1, 10, 11
CH_3*C*OO-	169.3							
CH_3COO-	21.0	1.64	3H, *s*	-OO*C*CH_3				

dihedral angle of 180° and that the acetoxyl groups are β at C-2 and α at C-4. Thus **2** is (4*S*)-2β-acetoxyvalparol-15-en-3-one.

The unsaturated alcohol **3** (IR 3403 cm^{-1}) showed twenty carbon atoms in the ^{13}C NMR spectrum: four methyls, seven methylenes (one olefinic), five methines (one olefinic and one oxygenated, δ 124.3, 69.2) and four quaternary carbons (two of them olefinic). The mass spectrum had a parent molecular ion at *m/z* 288 according to the formula $C_{20}H_{30}O$ corresponding to a tricyclic diterpenic alcohol with two double bonds. The ^{1}H NMR spectrum showed four methyls, two of them bonded to olefinic carbons. One of them was part of the isopropenyl group (δ 1.76) the other was on the double bond with an allylic secondary hydroxyl group (δ 4.53, 1H, *d*, $J = 9.9$ Hz). HMQC and HMBC (Table 1) allowed the identification of partial structures that are indicated with continuous lines in Fig. 1; no correlations were observed for three methylenes and these are assigned to C-5, C-9 and C-13, in a valparane skeleton. The gemi-proton at C-2 would be a doublet only if the hydroxyl is β and the proton forms a 90° dihedral angle with the hydrogens at C-1. Thus, the proposed structure for **3** is valpara-3,15-dien-2β-ol.

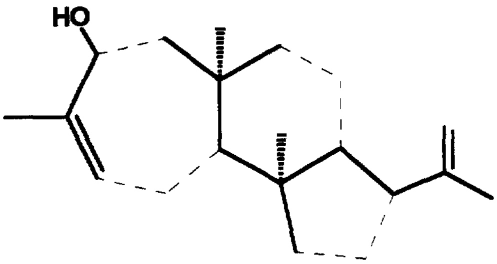


Fig. 1.

The unsaturated hydroxy-ketone **4** (IR 3448, 1718 cm^{-1}) showed in its ^{13}C NMR spectrum twenty carbon atoms: four methyls, six methylenes (one olefinic), five methines (two of them olefinic) and five quaternary carbons (one carbonyl, one olefinic and one oxygenated). The following partial structures are identified by ^{1}H and COSY Me-C(=CH_2), Me-C-OH, C-CH=CH-CH and -C-CO-CH_2-C-.

The parent molecular ion (M^+ at *m/z* 302) corresponded to a $C_{20}H_{30}O_2$ formula, in agreement with a monohydroxylated tricyclic diterpene, with two double bonds and a ketone with a valparane skeleton. In this skeleton the hydroxyl group has to be at C-3, the disubstituted double bond at C-4 and the ketone at C-2, all in agreement with the partial structures already identified.

HMQC and HMBC data are summarised in Table 2. The continuous lines on Fig. 2 indicated the partial structures confirmed by the data. The methylenes which do not show correlations could be assigned to C-9 and C-13 by comparison with other known

Table 2. NMR data for compounds **4** and **11**, and correlations observed in 2D experiments for the compound 4.

4 (CDCl$_3$)					11 (CDCl$_3$)		
Position	C	H		HMBC	C	H	
1	51.4	2.64; 2.17	1H, *d*, J = 11.0 each	2, 3, 11	48.3		
2	213.6				76.3	4.90	1H, *ddd*, $J_1 = 12.5$; $J_2 = 7.8$; $J_3 = 4.9$
3	76.7				209.0		
4	132.2	5.76	1H, *dd*, $J_1 = 10.4$, $J_2 = 1.2$	2, 3	38.8	3.10	1H, *m*
5	131.9	5.87	1H, *dd*, $J_1 = 10.4$, $J_2 = 6.7$	6	35.4		
6	54.3	1.82	1H, *dt*, $J_1 = 6.7$, $J_2 = 1.2$		58.8		
7	43.8				48.2		
8	54.4				141.7		
9	21.7				23.1		
10	40.8				44.7		
11	43.1				41.0		
12	41.0				44.4		
13	26.9				28.1		
14	45.4	2.65	1H, *m*		138.4		
15	147.8				26.7		
16	110.4	4.82; 4.79	1H, *s* each		21.6	1.03	3H, *d*, $J = 6.8$
17	25.1	1.75	3H, s	14, 15, 16	23.3	0.87	3H, *d*, $J = 6.8$
18	16.4	0.87	3H, *s*	6, 7, 8, 12	21.3	1.00	3H, *s*
19	27.5	1.35	3H, *s*	2, 3, 4	30.0	2.14	3H, *s*
20	23.9	0.97	3H, *s*	1, 6, 10, 11	27.0	1.06	3H, *s*
$CH_3\mathit{C}OO$-					171.4		
$\mathit{C}H_3COO$-					21.4	2.00	3H, *s*

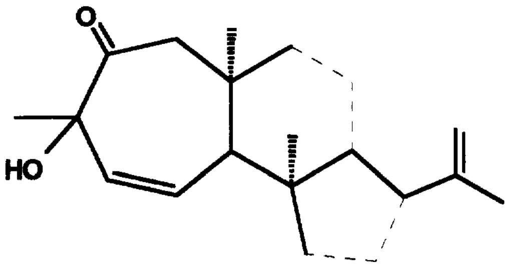


Fig. 2.

compounds. The proposed structure for **4** is 3α-hydroxyvalpara-4,15-dien-3-one. The α-stereochemistry for the hydroxyl group was proposed by comparison with other products [2, 10].

Compounds **7**, **8**, and **9** were a diformyl derivative, and two monoformylderivatives, respectively. Their structures were determined by mild alkaline hydrolysis of each to give labd-7-en-3β,15-diol [9].

Compound **11**, isolated as the acetate of a secondary alcohol, showed in addition to the acetoxyl group (AcOC*H*, δ 4.90) an acetyl group (*Me*-COO, δ 2.14), an isopropyl group (CH*Me*$_2$) with restricted rotation (δ 1.03 and 0.87, $J = 6.8$ Hz) and two methyl singlets (δ 1.06, 1.00) (Table 2). The geminal hydrogen to the acetoxyl group (δ 4.90) had a multiplicity of *ddd* and it was coupled to the α′-H to the carbonyl (COSY). The ^{13}C NMR spectrum, in addition to the acetoxyl group, showed twenty carbon atoms: five methyls, six methylenes, four methines and five quaternary carbons (two of them olefinic, δ 141.7 and 138.4).

The acetoxyl group indicated, as in compound **2**, a valparolane skeleton and they differ only in the double bond position, being a tetrasubstituded double bond, in this compound ($\Delta^{8,14}$). Thus, the proposed structure is (4*S*)-2β-acetoxyvalparol-8(14)-en-3-one.

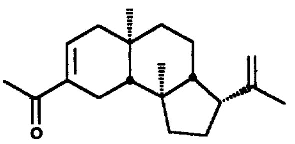
EXPERIMENTAL

Spectral analysis

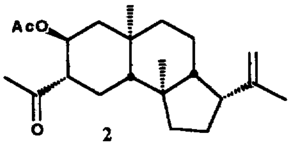
NMR: 400 or 250 MHz for ^{1}H and 100.1 or 62.9 MHz for ^{13}C. Chemical shifts are given in δ (ppm) and are referenced to the residual CHCl$_3$, 7.26 ppm for the ^{1}H and 77.0 ppm for ^{13}C.

Extraction and isolation

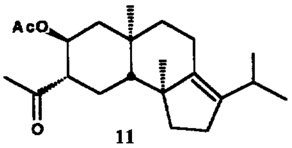
Aerial parts of *Halimium viscosum* (1.3 kg) collected in Celorico da Beira, Souto do Bispo (Guarda, Portugal) in 08/11/1993 by M. Alves, J. Forte and A. Matos. A specimen was deposited at the Herbarium



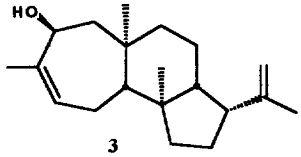
1



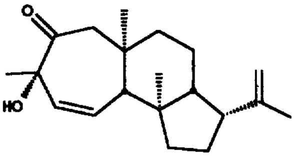
2



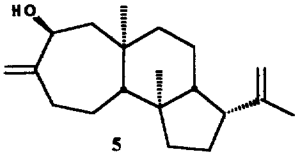
11



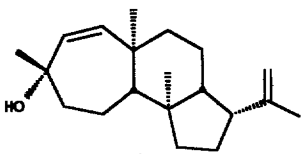
3



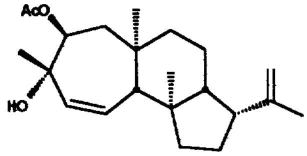
4



5



6



12

of the University of Coimbra, without number (COI); were dried and extracted with *n*-hexane in a Soxhlet apparatus for 24 hr. The extract (124.8 g) was dewaxed with MeOH (9.5 g) and then extracted with 4% NaOH (91.4 g). The neutral fraction weighed 23.8 g. A part of the neutral fraction (18.0 g) was saponified with 10% KOH/MeOH and the neutral part (12.1 g) was crystallised giving as major component the 3β,15-dihydroxy-7-labdene (5.9 g). The remaining part (7.2 g) was chromatographed on a silica gel column with *n*-hexane-EtOAc mixtures giving seven fractions (I-VII). CC of faction IV on silica gel/10% $AgNO_3$ gave **1** (30 mg), **11** (36 mg). CC of fraction V afforded **3** (73 mg), **4** (31 mg), **5** (45 mg), **6** (22 mg) and fraction VI gave **7** (47 mg), **8** (52 mg), **9** (93 mg) and **10** (79 mg). Fraction VII gave **2** (17 mg), **12** (41 mg), **13** (49 mg), **14** (65 mg), **15** (56 mg), **16** (385 mg).

(4*S*)-2β-*Acetoxyvalparol*-15-*en*-2-*one* (**2**)

Colourless oil. IR ν_{max}^{film}cm^{-1}: 3441, 1706, 1668, 1445, 1382, 1223, 752. ^{1}H and ^{13}C NMR in C_6D_6 see Table 1. ^{1}H NMR in $CDCl_3$ δ(ppm): 5.17 (1H, *dt*, $J_1 = 11.3$ Hz, $J_2 = 4.4$ Hz, H-2); 4.80 and 4.78 (1H, *s*, H-16) each; 2.68 (1H, *m*, H-14); 2.64 (1H, *dt*, $J_1 = 11.3$ Hz, $J_2 = 5.0$ Hz, H-4); 2.17(3H, *s*, Me-19); 1.95 (3H, *s*, CH_3COO-); 1.74 (3H, *s*, Me-17); 1.02 (3H, *s*, Me-20); 0.69 (3H, *s*, Me-18). GC-MS, 70 eV, *m/z* (rel. int.): 304 $[M-43]^+$ (18), 286 (52), 271(25), 244 (32), 243 (58), 230 (21), 217 (20), 203 (35), 187 (25), 175 (29), 161 (31), 159 (39), 147 (36), 133 (56), 119 (70), 105 (57), 91 (84), 79 (84), 67 (100), 55 (79).

Valpara-3,15-*dien*-2β-*ol* (**3**)

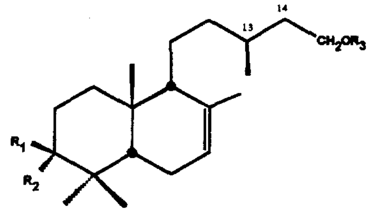
Colourless oil, IR ν_{max}^{film}cm^{-1}: 3403, 3085, 1712, 1629, 886, 746. ^{1}H NMR and ^{13}C NMR see Table 1. GC-MS, 70 eV, *m/z* (rel. int.): 228 $[M]^+$ (34), 225 (15), 189 (26), 188 (16), 187 (19), 177 (23), 173 (15), 161 (20), 160 (15), 159 (44), 147 (42), 146 (37), 145 (40), 137 (18), 135 (17), 134 (21), 133 (38), 131 (27), 121 (32), 119 (42), 117 (15), 109 (21), 107 (32), 106 (17), 105 (49), 95 (27), 94 (27), 93 (48), 91 (54), 84 (25), 83 (20), 81 (40), 80 (22), 79 (69), 77 (23), 69 (42), 68 (39), 67 (85), 56 (18), 55 (100).

3α-*Hydroxyvalpara*-4,15-*dien*-2-*one* (**4**)

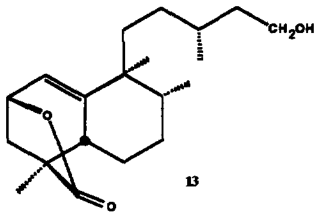
Colourless oil, IR ν_{max}^{film}cm^{-1}: 3448, 1718, 1458, 1369, 1247, 753, 663. ^{1}H and ^{13}C NMR see Table 1.

3β,15-*Diformyloxylabd*-7-*ene* (**7**)

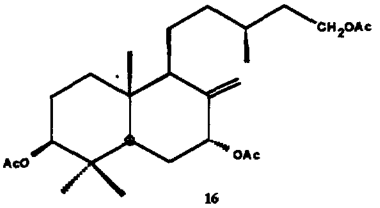
Colourless oil, IR ν_{max}^{film}cm^{-1}: 1725, 1458, 1375, 1166, 974. ^{1}H and ^{13}C NMR see Tables 3 and 4. GC-MS, 70 eV, *m/z* (rel. int.): 364 $[M]^+$ (1), 196 (12), 190 (10), 189 (33), 175 (11), 150 (11), 147 (14), 135 (38), 134 (11), 133 (20), 123 (29), 122 (31), 121 (100), 120 (11),



	R_1	R_2	R_3	
7	OCHO	H	CHO	
8	OH	H	CHO	
9	OCHO	H	H	
10	=O		H	
14	OH	H	H	
15	OH	H	H	Δ^{13E}



13



16

Table 3. ^1H NMR data for compounds **7-9**.

Position	**7** H		**8** H		**9** H	
3	4.63	1H, *dd*, $J_1=9.0$; $J_2=6.7$	3.22	1H, *dd*, $J_1=10.1$; $J_2=5.7$	4.62	1H, *dd*, $J_1=9.4$; $J_2=6.4$
7	5.39	1H, *bs*	5.37	1H, *bs*	5.37	1H, *m*
15	4.25	2H, *m*	4.19	2H, *m*	3.68	2H, *m*
16	0.86	3H, *d*, J =6.3	0.92	3H, *d*, J=5.5	0.87	3H, *d*, J=6.4
17	1.68	3H, *s*	1.60	3H, *s*	1.66	3H, *s*
18	0.95	3H, *s*	0.95	3H, *s*	0.94	3H, *s*
19	0.88	3H, *s*	0.83	3H, *s*	0.86	3H, *s*
20	0.79	3H, *s*	0.74	3H, *s*	0.78	3H, *s*
*H*COOC-3	8.13	1H, *s*			8.12	1H, *s*
*H*COOC-15	8.06	1H, *s*	8.04	1H, *s*		

119 (46), 109 (20), 108 (21), 107 (56), 105 (24), 96 (11), 95 (50), 94 (27), 93 (36), 91 (18), 83 (13), 82 (23), 81 (63), 80 (11), 79 (27), 69 (37), 68 (30), 67 (26), 57 (11), 55 (50).

15-*Formyloxy-3β-hydroxylabd-7-ene* (**8**)

Colourless oil, IR ν_{max}^{film}cm^{-1}: 3428, 1725, 1664 1463, 1382, 1180, 1052. ^1H NMR and ^{13}C NMR see Tables 3 and 4. GC-MS, 70 eV, *m/z* (rel. int.): 318 $[M-H_2O]^+$ (1), 124 (35), 109 (100), 105 (10), 95 (11), 91 (11), 81 (16), 79 (11), 69 (16), 55 (20).

3*β-Formyloxy-15-hydroxylabd-7-ene* (**9**)

Colourless oil, IR ν_{max}^{film}cm^{-1}: 3416, 1718, 1458, 1375, 1184, 1051. ^1H NMR and ^{13}C NMR see Tables 3 and 4. GC-MS, 70 eV, *m/z* (rel. int.): 336 $[M]^+$ (3), 275

Table 4. NMR data for compounds **7**-**9** and **12** ($CDCl_3$).

Position	**7**	**8**	**9**	**12**
1	36.8	37.1	37.2	50.4
2	24.0	27.3	24.0	75.4
3	81.1	79.1	81.2	74.9
4	37.4	38.6	37.4	136.7
5	49.7	49.5	49.7	130.6
6	23.2	23.4	22.2	56.2
7	121.7	122.0	121.6	45.1
8	135.2	135.1	135.4	53.9
9	55.0	55.1	55.1	21.1
10	36.5	36.6	36.5	44.2
11	24.4	24.3	24.4	37.3
12	39.5	39.5	39.5	41.2
13	30.6	30.5	30.4	26.9
14	35.0	35.0	39.8	45.8
15	62.4	62.4	61.2	147.8
16	19.5	19.5	19.7	110.6
17	21.9	21.9	21.9	25.0
18	27.7	27.8	27.7	16.1
19	16.2	15.0	16.2	21.1
20	13.6	13.5	13.6	20.0
3-HCOO-	161.2		161.2	
15-HCOO-	161.2	161.2		
CH_3COO-				170.8
CH_3COO-				21.4

(15), 235 (23), 207 (11), 190 (23), 189 (71), 187 (15), 175 (18), 161 (12), 159 (10), 148 (10), 147 (24), 145 (12), 136 (13), 135 (44), 134 (18), 133 (33), 131 (11), 124 (17), 123 (58), 122 (36), 121 (97), 120 (20), 119 (78), 112 (13), 111 (20), 109 (38), 108 (28), 107 (89), 106 (13), 105 (42), 99 (30), 98 (20), 97 (16), 96 (22), 95 (87), 94 (36), 93 (55), 91 (32), 85 (18), 84 (17), 83 (24), 82 (33), 81 (100), 80 (17), 79 (43), 77 (13), 71 (15), 70 (16), 69 (61), 67 (40), 57 (21), 56 (11), 55 (77).

2β-*Acetoxyvalparol*-8(14)-*en*-3-*one* (**11**)

Colourless oil. IR ν_{max}^{film}cm^{-1}: 1731, 1705, 1623, 1464, 1375, 1241, 1044. ^{1}H NMR and ^{13}C NMR see Table 1.

2β-*Acetoxyvalpara*-4,15-*dien*-3β-*ol* (**12**)

Colourless oil. IR ν_{max}^{film}cm^{-1}: 3408, 3060, 1740, 1632, 1238, 1040, 890. ^{1}H NMR: δ 5.73 (1H, *dd*, $J_1 = 12.8$, $J_2 = 2.1$ Hz, H-1), 5.49 (1H, *dd*, $J_1 = 12.8$, $J_2 = 4.0$ Hz, H-2), 4.90 (1H, *dd*, $J_1 = 10.3$, $J_2 = 4.4$ Hz, H-4), 4.82 and 4.80 (1H, *s*, H-16) ea, 2.69 (1H, *m*, H-14), 2.07 (3H, *s*, -$OCC\mathit{H}_3$), 1.76 (3H, *s*, Me-19), 1.32 (3H, *s*, Me-19), 1.02 (3H, *s*, Me-20), 0.81 (3H, *s*, Me-18). ^{13}C NMR see Table 4. GC-MS, 70 eV, *m*/*z* (rel. int.): 248 (8), 162 (8), 124 (25), 109 (52), 91 (14), 81 (13), 69 (30), 55 (100).

Acknowledgements—To Anna M. Lithgow, Servicio General de Resonancia Magnética Nuclear, Facultad de Ciencias Químicas, Universidad de Salamanca. This work is partially supported by the Program INTERREG II, no 05/REG II/6/96 and I&D "Materiais Texteis e do Papel".

REFERENCES

1. de Mendonça, D. I. M. D., Rodilla, J. M. L., Lithgow, A. M. and Marcos, I. S., *Phytochemistry*, 1997, **40**, 1301.
2. Urones, J. G., Marcos, I. S., Basabe, P., Alonso, C. A., Diez Martín, D., Garrido, N. M. and Oliva, I. M., *Tetrahedron Letters*, 1990, **31**, 5665.
3. Urones, J. G., Basabe, P., Marcos, I. S., Alonso, C., Oliva, I. M., Garrido, N. M., Diez Martín, D. and Lithgow, A. M., *Tetrahedron Letters*, 1992, **33**, 5269.
4. Urones, J. G., Marcos, I. S., Basabe, P., Alonso, C. A., Oliva, I. M., Garrido, N. M. and Lithgow, A. M., *Phytochemistry*, 1993, **34**, 747.
5. Zdero, C., Bohlmann, F., King, R. M. and Robison, H., *Phytochemistry*, 1988, **27**, 2835.
6. Urones, J. G., Marcos, I. S., Oliva, I. M., Garrido, N. M., Haggett, J. and Humphreys, V. M., *Phytochemistry*, 1995, **38**, 663.
7. PhD thesis, University of Salamanca, Spain, 1990.
8. Pascual Teresa, J., Urones, J. G., Basabe, P., Carrillo, H. and Muñoz, M. A. G., *Phytochemistry*, 1985, **24**, 791.
9. De Pascual Teresa, J., Urones, J. G., Basabe, P. and Llanos, A., *Anales de Quimica*, 1972, **73**, 1029.
10. Urones, J. G., Marcos, I. S., Alonso, C. A., Moro, R. F. and Rodilla, J. M. L., *Phytochemistry*, 1993, **34**, 569.