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TRICYCLIC DITERPENES FROM *HYPTYS DILATATA*

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Key Word Index—*Hyptis dilatata*; Labiatae; diterpenes; abietane; pimarane.

Abstract—Tricyclic diterpenes with the abietane skeleton have been isolated from the aerial parts of *Hyptis dilatata*. In addition to 12 well known compounds, two new compounds have been isolated and identified as 11,12 diacetoxy-7β-methoxy-8,11,13-abietatrien-20,6β-olide (epimethylrosmanol) and 11,12 diacetoxy-7β-ethoxy-8,11,13-abietatrien-20,6β-olide (epiethylrosmanol). Their structures have been determined by spectroscopic methods using 2D correlation experiments (^1H–^{13}C) HMBC and HMQC. Esquirolin B with the pimaran skeleton has been isolated as well.

INTRODUCTION

The genus *Hyptis*, consists of over 400 [1] species that occur in tropical America. Several species from this genus have been studied: they are reputed to possess medicinal properties [2–7], some are used as food flavouring [2, 6–7] and some show insect repellant, antifungal, antibacterial or nematicidal activity [2, 7–9].

The most complete information about this genus comes from Mexico where Miranda *et al.* have studied five of these species: *H. albida* [2], *mutabilis* [10], *oblongifolia* [8], *pectinata* [6], and *verticillata* [11], while Vivar *et al.* have focussed on the species *H. urticoide* [12]. *H. verticillata* and *suavenolens* are the most studied, the former by Heinrich *et al.* [13, 14] and Reese *et al.* [9], and the latter by White [15], Mulherjee *et al.* [16] and Singh *et al.* [17].

Many of these species contain pentacyclic triterpenes [2, 8, 17]; *H. oblongifolia*, *pectinata*, *verticillata* and *urticoide* have α-pyrones [6, 8, 12, 19] with biological activity. Lignans have been obtained from *H. verticillata* [11] and derivatives of pyrrolidin-2-one [9] and a few diterpenes have been isolated (*H. suavenolens* and *fructicosa*) [15, 18].

We have studied the aerial part of *H. dilatata* Jacq. (Panama). Several tricyclic diterpenoids with the abietane structure, where the ring C is aromatic, have been isolated. The structures of two of these compounds is described for the first time and the ^{13}C NMR data is given for the known compounds.

RESULTS AND DISCUSSION

H. dilatata was collected in Veraguas (Panama) where it is used in folk medicine as an insect repellent. From the diethylether-soluble part of the acetone extract of the leaves, several abietanes were isolated, amongst them two new natural compounds **1** and **2**, as their acetyl derivatives. In addition, the well known carnosol (**3**) [20, 21], rosmanol (**4**) [21, 22], methylrosmanol (**5**) [23, 24] have been isolated, and, after acetylation, the acetyl derivatives of: carnosol (**6**) [20, 21], rosmanol (**7**) [21, 24], methylrosmanol (**8**) [24], ethylrosmanol (**9**) [23], isorosmanol (**10**) [28], epirosmanol (**11**) [22], carnosic acid (**12**) [23, 25], its methyl ester (**13**) [26] and pisiferic acid methyl ester (**14**) [25, 27] have also been obtained. Lactone **15** [29] and esquirolin B (**16**) [30], a compound with the pimaran skeleton, have been isolated as well.

Compound **1** showed bands in its IR spectrum indicative of a γ-lactone group (1780 cm^{-1}) and acetyl groups (1740 and 1240 cm^{-1}). In its ^{13}C NMR spectrum signals were observed for 25 carbon atoms. DEPT experiments showed that there were seven methyls (one oxygenated, δ 56.1), three methylenes, five methines (one aromatic and two oxygenated δ 125.5, 74.0 and 77.6) and 10 quaternary carbons (three carbonyls and five aromatics). The mass spectrum showed a $[M]^+$ of *m/z* 444 corresponding to the molecular formula $C_{25}H_{32}O_7$, consistent with a diacetylderivative of a tricylic diterpene diol with an aromatic ring, a γ-lactone group and a methyl ester group.

In the ^1H NMR spectrum, in addition to the methyl signals of two acetoxy groups (δ 2.28 and 2.30) on a pentasubstituted aromatic ring (δ 7.39, 1H, *s*), the signals of five other methyls: two on tetrasubstituted carbons (Me-C) (δ 0.96 and 1.00, ea. *s*) a MeO (δ 3.61)

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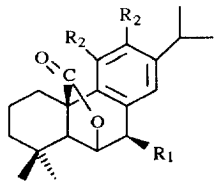
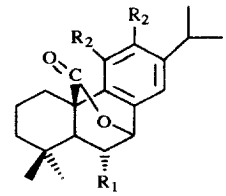
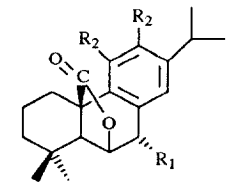


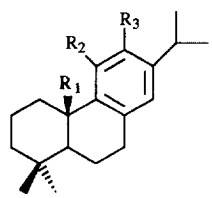
	R_1	R_2
1	OMe	OAc
2	OEt	OAc
11	OAc	OAc



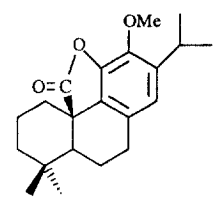
	R_1	R_2
3	H	OH
6	H	OAc
10	OAc	OAc



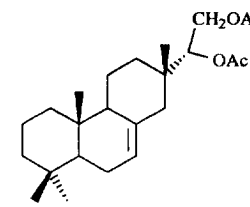
	R_1	R_2
4	OH	OH
5	OMe	OH
7	OAc	OAc
8	OMe	OAc
9	OEt	OAc



	R_1	R_2	R_3
12	HOOC	OAc	OAc
13	MeOOC	OAc	OAc
14	MeOOC	H	OAc



15



16

and two in an isopropyl group (Ar–$CHMe_2$) (δ 2.90, 1H, *hept* and 1.19, 6H, *d*), were observed. The signals of two hydrogens (δ 4.94, 1H, *d*, J = 2.4 and 4.45, 1H, *d*, J = 2.4 Hz) geminal to a lactonic oxygen and to the methoxy, respectively, were also observed, the latter in a benzylic position (based on its chemical shift). These two hydrogens were placed in the partial structure CH(OCO)CH(OMe)Ar(COSY). All these data indicated that **1** was a compound with the abietane skeleton, one of the ring methyls being oxidised and forming part of a γ-lactone ring system. The similarities of the ^1H and ^{13}C NMR spectra of **1** with those of methylrosmanol acetate (**8**) suggested that they may be epimers. In a similar way, the spectroscopic differences between rosmanol triacetate (**7**) and epirosmanol triacetate (**11**) are only shift differences because the coupling constants of H-6 and H-7 are very similar. Taking into account all these data, the structure of diacetoxi-epi-methylrosmanol (11,12-diacetoxy-7β-methoxy-8,11,13-abietatrien-20,6β-olide) was assigned to **1**. H-6 is a doublet because if forms a dihedral angle of 90°, in a similar manner as H-6 of **4**, **5**, **7**, **8**, **9**, **11**.

Compound **2** showed bands in its IR spectrum corresponding to a γ-lactone (1780 cm^{-1}) and acetyl groups (1740 and 1240 cm^{-1}). In its ^{13}C NMR spectrum signals were observed for 26 carbon atoms; seven methyls, four methylenes (one oxygenated, δ 56.1), five methines (one aromatic δ 125.1 and two oxygenated δ 76.8 and 74.7) and 10 quaternary carbons (three carbonyls and five aromatics). In the mass spectrum of **2** the $[M]^+$ was at *m/z* 458 and corresponded to the molecular formula $C_{26}H_{34}O_7$, which is 14 amu higher than that of compound **1**.

The ^1H NMR spectrum showed signals for the same groups as in **1**: one aromatic hydrogen (δ 7.39, 1H, *s*), two tetrasubstituted carbons (Me–C) (δ 0.99 and 0.95, ea *s*), an isopropyl group (Ar–$CHMe_2$) (δ 2.92, 1H, *hept* and 1.18, 1.14, 3H, *d*, each one), two hydrogens next to oxygenated carbons, one of a γ-lactone and the other of an ethyl ether: CH(OCO)CH(OEt)Ar. Comparison of the spectroscopic properties of **2**, **1** and the diacetylderivative of ethylrosmanol (**9**), permited the assignment of the structure of **2** as the diacetate of *epi*-etilrosmanol (11,12-diacetoxi-7β-ethoxy-8,11,13-abietatrien-20,6β-olide).

Table 1. ^{13}C NMR data of compounds **1**–**16**

^{13}C	1*	2	3*	4†	5*†	6	7	8*	9	11	12	13	15*	16
1	26.7	26.5	29.8	28.1	28.2	29.3	26.6	26.6	26.5	26.5	34.5	34.7	42.1	39.8
2	18.9	18.8	19.6	19.9	19.8	18.9	18.9	19.0	18.9	18.8	18.2	18.4	18.3	18.8
3	37.7	37.6	41.8	39.1	38.9	40.8	37.7	37.8	37.7	37.5	41.1	41.1	38.6	42.7
4	31.9	31.8	35.1	31.9	31.9	34.6	31.5	31.4	31.6	31.9	34.0	33.9	32.8	32.8
5	55.2	55.0	46.2	51.0	51.5	44.7	51.1	50.6	50.6	55.4	53.7	53.6	56.4	50.3
6	74.0	74.7	30.5	78.8	74.9	28.0	74.3	74.0	74.5	75.2	19.9	19.7	24.0	19.5
7	77.6	76.8	78.2	69.1	78.5	77.0	69.2	77.4	75.6	70.9	31.9	31.6	32.7	122.6
8	133.7	134.0	133.4	130.4	128.2	138.2	126.9	133.5	133.8	131.8	136.7	136.7	133.0	134.2
9	130.4	130.2	122.9	124.9	125.0	129.3	121.1	130.8	130.8	128.2	132.1	131.9	131.0	52.0
10	47.7	47.6	49.1	47.6	47.7	48.5	46.9	46.8	46.7	47.4	48.0	47.6	49.1	37.0
11	138.1	139.9	144.0	144.7	144.6	139.3	131.4	140.2	140.2	140.2	138.8	138.8	142.7	23.5
12	140.1	140.6	143.4	142.4	142.7	141.1	131.4	141.0	140.6	141.0	140.0	139.7	140.0	33.2
13	142.5	142.3	135.1	136.5	136.3	141.4	142.9	142.3	142.3	142.7	141.5	141.4	139.8	35.4
14	125.5	125.4	112.3	120.2	120.7	118.5	126.9	126.8	126.8	125.0	125.1	125.0	120.2	42.3
15	27.7	27.7	27.5	27.5	27.5	27.7	27.7	27.7	27.6	27.7	27.5	27.4	27.4	78.5
16	22.7	22.7	23.0	22.9	22.8	22.9	22.7	22.8	22.6	22.6	22.6	22.6	22.3	63.2
17	22.7	22.8	23.1	23.1	23.1	22.8	22.6	22.5	22.8	22.9	22.9	22.9	22.8	17.8
18	32.0	31.6	31.9	31.8	32.0	31.6	31.3	31.6	31.4	31.7	32.4	32.4	31.8	33.6
19	22.0	21.9	20.0	22.4	22.6	19.8	21.8	22.1	22.1	21.9	20.1	19.6	23.6	22.3
20	175.8	176.0	176.0	178.3	178.2	173.9	175.7	176.3	176.5	175.8	179.1	175.1	178.0	14.9
OOCMe	168.0	168.0				168.2	170.0	167.8	168.0	168.0	168.1	168.4		171.0
							167.0			171.2				
	168.1	168.0				168.1	167.8	168.0	168.0	168.0	168.0	168.1		170.6
OOCMe	20.3	20.3				20.5	20.7	20.7	20.3	20.3	20.5	20.6		20.9
							20.2			21.0				
	20.7	20.8				20.2	20.7	20.2	20.8	20.8	20.3	20.3		20.8
OMe	56.1				58.3			58.3				51.6	60.6	
OCH2CH3		66.5							66.5					
OCH2CH3		15.8							15.8					

* Assigned by $^{1}H/^{13}C$

† Acetone d_6

EXPERIMENTAL

Spectral analysis

NMR: 200 or 400 MHz for ^{1}H and 50 MHz for ^{13}C. Chemical shifts are given in δ (ppm) and are referenced to the residual $CHCl_3$, 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C, respectively; IR: and film; EIMS: direct inlet system or truth GC/MS-system.

Extraction and isolation.

The aerial part of *Hyptis dilatata* collected in December 1995 in Santiago de Veraguas, Panamá, was dried (270 g) and extracted for 48 h with Me_2CO at room temp. (25°C). After percolation, the solvent was removed under vacuum at 35°, giving an extract (20.7 g) which was extracted with Et_2O. The Et_2O-soluble extract (13.17 g) was fractionated with hexane and hexane-soluble portion (4.7 g) chromatographed on silica gel with hexane-EtOAc to give methylcarnosic acid diacetate (**13**) (6 mg). The hexane-insoluble residue (8.5 g) was chromatographed on a silica gel (cc) with a mixture of hexane-EtOAc; 5 fractions (I–V) were collected. From Fraction I was isolated carnosol (**3**) (800 mg), rosmanol (**4**) (200 mg) and methylrosmanol (**5**) (500 mg). Fractions II to V were acetylated (Ac_2O and pyr.) and after repetitive chromatography with a mixtures of hexane-EtOAc, hexane-Et_2O and hexane-$CHCl_3$, the following diterpene acetates were isolated: from Fraction II ethylrosmanol (**9**) (10 mg), epiethylrosmanol (**2**) (10 mg) and pisiferic acid methyl ether (**14**) (8 mg) after esterification of the more polar part with CH_2N_2; from Fraction III esquirolin B (**16**) (13 mg), isorosmanol (**10**) (7 mg), epirosmanol (**11**) (8 mg), epimethylrosmanol (**1**) (20 mg) and the γ-lactone (**15**) (40 mg); from Fraction IV, carnosic acid (**12**) (200 mg) and from fraction V the acetyl derivatives of carnosol (**6**), methylrosmanol (**8**) and rosmanol (**7**).

Diacetylepimethylrosmanol (**1**). $[\alpha]_D^{25}$ +47.9 (*c* 0.5; $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 1780, 1371, 1204, 1175, 1101, 1026, 733; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.39 (1H, *s*, H-14), 4.94 (1H, *d*, J = 2.4 Hz, H-7), 4.45 (1H, *d*, J = 2.4 Hz, H-6), 3.61 (3H, *s*, OMe), 2.90 (1H, *hep*, 6.8 Hz, H-15), 2.30 (3H, *s*, MeCOO), 2.28 (3H, *s*, MeCOO), 2.08 (1H, *s*, H-5), 1.19 (6H, *d*, J = 6.8 Hz,

H-16 and H-17), 1.00 (3H, *s*, H-19), 0.96 (3H, *s*, H-18); EIMS 70 eV *m/z* (rel. int.): 444 $[M]^+$ (6), 416 (3), 402 (3), 374 (2), 361 (23), 360 (100), 316 (16), 314 (27), 284 (8), 258 (12), 215 (14).

Diacetylepiethylrosmanol (**2**). $[\alpha]_D^{25}+49.7$ (*c* 0.5; $CHCl_3$);IR ν_{max}^{film} cm^{-1}: 2965, 1780, 1371, 1204, 1175, 1101, 1026, 758; ^1H NMR (400 MHz, $CDCl_3$): δ 7.39 (1H, *s*, H-14), 4.90 (1H, *d*, $J = 2.7$ Hz, H-7), 4.55 (1H, *d*, $J = 2.7$ Hz, H-6), 3.87 (1H, *dq*, $J = 9.0$ and 7.0 Hz OCH_2CH_3), 3.73 (1H, *dq*, $J = 9.0$ and 7.0 Hz OCH_2CH_3), 2.92 (1H, *hep*, $J = 6.9$ Hz, H-15), 2.30 (3H, *s*, MeCOO), 2.28 (3H, *s*, MeCOO), 2.08 (1H, *s*, H-5), 1.3 (OCH_2CH_3, *t*, $J = 7.0$ Hz), 1.18 and 1.14 (each 3H, *d*, $J = 6.9$ Hz, H-16 and H-17), 0.99 (3H, *s*, H-19), 0.95 (3H, *s*, H-18); EIMS 70 eV *m/z* (rel. int.): 458 $[M]^+$ (8), 430 (4), 388 (19), 374 (100), 344 (11), 330 (33), 328 (9), 284 (28), 272 (12), 259 (18), 231 (11), 215 (28).

Esquirolin B (**16**). $[\alpha]_D^{25}+24.0$ (*c* 0.2; $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 2926, 2870, 1746, 1462, 1443, 1370, 1242, 1040, 976; ^1H NMR (400 MHz, $CDCl_3$): δ 5.3 (1H, *m*, H-7), 4.91 (1H, *dd*, $J = 2.5$ and 9.1 Hz, H-15), 4.41 (1H, *dd*, $J = 2.5$ and 11.8 Hz, H_A-16), 4.01 (1H, *dd*, $J = 9.1$ and 11.8 Hz, H_B-16), 2.09 and 2.02 (3H, *s*, each, MeCOO), 0.91 (3H, *s*), 0.86 (3H, *s*), 0.84 (6H, *s*); EIMS 70 eV *m/z* (rel. int.): $[M^+-60]^+$ 330 (7), 270 (20), 255 (70); 243 (13), 229 (17), 160 (22), 131 (37), 109 (70), 105 (65), 91 (75), 79 (58), 69 (63), 55 (100).

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