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TERPENOIDS FROM *NEPETA TUBEROSA* SUBSP. *RETICULATA* (II)

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Key Word Index—*Nepeta tuberosa*; Labiatae; diterpenoids; new isopimarane derivatives; triterpenoids; steroids.

Abstract—Four new diterpenoid compounds have been isolated from *Nepeta tuberosa* subsp. *reticulata* and their structures elucidated by spectroscopic methods and chemical correlations as abietatrien-3β-ol, isopimara-8,15-dien-7β,18-diol, isopimara-8,15-dien-7α,18-diol and 7α-hydroxy-isopimara-8(14),15-dien-18-yl malonate. We have also isolated the known terpenoids acetyl isocupressic acid, isopimara-8(14),15-dien-7α,18-diol, phytol, ursolic acid, and the steroids sitosterol and sitosterol glucoside.

INTRODUCTION

In a previous paper [1] we reported on the composition of the volatile oil and the non-volatile hexane extract from *Nepeta tuberosa* subsp. *reticulata*. In this paper we report our preliminary results concerning the isolation and structural determination of some new minor compound of the non-volatile extract.

RESULTS AND DISCUSSION

The non-volatile part of the hexane extract of *N. tuberosa* subsp. *reticulata* was dewaxed and fractionated by CC, as in our previous study [1]. A further 10 compounds were isolated and identified in addition to the compounds isolated previously. The acids were purified as methyl esters (**1** and **6**).

The ^{1}H NMR of the methyl ester **1** (IR: 1755, 1740, 1250 cm^{-1}) showed signals of the following groups: Me–C(=)CH–CH_2OAc (*E*), –COOMe, >C=CH_2 and three methyl singlets. On alkaline hydrolysis followed by treatment with diazomethane it gave methyl isocupressate [2, 3].

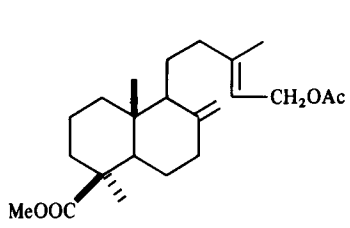
The IR spectrum of compound **2** contained bands corresponding to a hydroxyl group (3400 cm^{-1}) and an aromatic ring (1640, 1610, 1500 cm^{-1}). Its ^{1}H NMR spectrum (Table 1) showed signals of: a 1,2,4-trisubstituted aromatic ring (δ7.17, 1H, *d*, J = 7.8 Hz; 7.01, 1H, *dd*, J = 7.8 and 1.9 Hz; 6.91, 1H, *d*, J = 1.9 Hz); a hydrogen geminal to a secondary hydroxyl group (δ3.30, 1H, *m*) similar to those of 3β-hydroxyderivatives of diterpenes and triterpenes [4, 5]; five methyls, three of them singlets (δ1.20, 1.08 and 0.90); and a doublet of an isopropyl group (δ1.23, 6H, *d*, J = 6.8 Hz). The hydroxyl group was assigned to C-3 due to the fact that Me-18 and Me-19 were shielded with respect to the normal chemical shift owing to a γ-gauche effect induced by the hydroxyl group at C-3 while Me-20 showed a normal chemical shift.

The existence of a 1,2,4-trisubstituted aromatic ring in a compound containing 20 carbon atoms (see Table 2), of an isopropyl group and of a hydroxyl group at 3β, allowed us to assign compound **2** the structure of abietatrien-3β-ol.

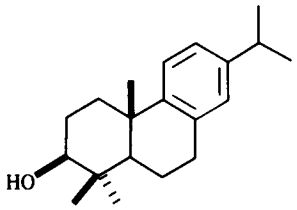
Compounds **3** and **5** showed in their ^{1}H NMR spectrum (Table 1) signals of an ABX system (**3**: δ5.76, *dd*, J = 17.6 and 10.6 Hz, H-15; 4.97, *dd*, J = 17.6 and 1.6 Hz, H-16; 4.92, *dd*, J = 10.7 and 1.6 Hz, H-16; **5**: δ5.74, *dd*, J = 17.4 and 10.9 Hz, H-15; 4.92, *dd*, J = 10.9 and 1.6 Hz, H-16; 4.85, *dd*, J = 17.4 and 1.6 Hz, H-16), characteristic of an isopimarane skeleton [6]. They also showed both signals characteristic of protons geminal to two hydroxyl groups, one of them primary and the other secondary and allylic (see Table 1). Acetylation of **3** gave the diacetate **4** (see Tables 1 and 2). The ^{13}C NMR spectrum of compounds **3** and **5** (see Table 2) confirmed the presence of a double tetrasubstituted bond which could only be situated between positions 9 and 10 an of the skeleton. Oxidation of **3** or **5** with Jones reagent followed by esterification gave **6**, reported in the literature [7]. Accordingly, **3** and **5** were epimers at C-7. The stereochemistry of each was established by comparison of its ^{13}C NMR displacements with those described in the literature of analogous epimers but with a carboxylic group at C-18 [8].

The IR spectrum of compound **7** showed bands of an acetate group (1750, 1250 cm^{-1}) (this comes from a previously acetylated fraction) and its ^{1}H and ^{13}C NMR spectra (see Tables 1 and 2) contained signals corresponding to the acetate (δ2.01, 3H, *s*), to a methyl ester (δ3.76, 3H, *s*) and to the methylene of a malonate (^{1}H NMR δ3.39, 2H, *s*; ^{13}C NMR δ41.46) in addition to the signals corresponding to an isopimarane skeleton (the ABX system of a vinyl group at δ5.74, 4.91 and 4.98 and three methyl singlets at δ1.03, 0.87 and 0.83) substituted at C-18.

The isopimarane skeleton had a double trisubstituted bond assignable to position 8-14 of the molecule, the only one in which the olefinic hydrogen was not coupled to α-hydrogens (δ5.67, *d*, J = 1.83 Hz). Moreover, the displacement of the doublet centred at δ5.32 could only be assigned to an allylic hydrogen geminal to an acetoxyl function which had to be at C-7. Hydrolysis of **7** with KOH–MeOH (2M) gave the diol **8**, also isolated as a natural product in this work, whose spectroscopic and physical properties coincide with those described in the



1



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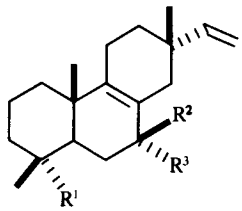
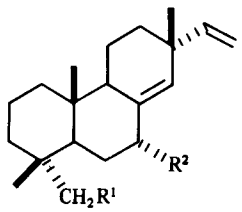


	R¹	R²	R³
3	CH_2OH	H	OH
4	CH_2OAc	H	OAc
5	CH_2OH	OH	H
6	COOMe	$R^2 = R^3 = O$	



	R¹	R²
7	—OCO—CH_2—COOMe	OAc
8	OH	OH

Table 1. ^{1}H NMR data for compounds **2–5** and **7–8**

H	2	3	4	5	7	8 [9]*
3	3.30 *m*					
7	2.77–2.99 *m*	3.82 *br s*	5.08 *br s*	4.15 *dd* (8.1, 1.1)	5.32 *dd* (2.85–2.9)	4.16 *dd* (2.85–2.9)
11	7.17 *d* (7.8)					
12	7.01 *dd* (7.8, 1.9)					
14	6.91 *d* (1.9)				5.67 *d* (1.8)	5.48 *d* (2.1)
15	2.77–2.99 *m* 2.99 *m*	5.76 *dd* (17.6, 10.7)	5.70 *dd* (17.8, 10.4)	5.74 *dd* (17.4, 10.9)	5.74 *dd* (18.1, 10.0)	5.80 *dd* (17.4, 10.7)
16	1.23 *d* (6.8)	4.97 *dd* (17.6, 1.6) 4.92 *dd* (10.7, 1.6)	4.94 *dd* (17.8, 1.6) 4.92 *dd* (10.4, 1.6)	4.92 *dd* (10.9, 1.6) 4.85 *dd* (17.4, 1.6)	4.91 *dd* (10.0, 1.4) 4.89 *dd* (18.1, 1.4)	4.95 *dd* (17.4, 1.4) 4.92 *dd* (10.7, 1.4)
17	1.23 *d* (6.8)	0.95 *s*	0.96 *s*	1.01 *s*	1.03 *s*	1.04 *s*
18	1.08 *s*	3.53 *d* 2.98 *d* (11.3)	3.88 *d* 3.64 *d* (11.0)	3.44 *d* 3.15 *d* (10.9)	3.91 *d* 3.74 *d* (11.2)	3.52 *d* 2.88 *d* (11.5)
19	1.20 *s*	0.74 *s*	0.86 *s*	0.81 *s*	0.83 *s*	0.74 *s*
20	0.90 *s*	0.98 *s*	0.97 *s*	0.07 *s*	0.87 *s*	0.80 *s*
–OAc			2.05 *s* 2.03 *s*		2.01 *s*	
–COOMe					3.76 *s*	
–OCOCH$_2$COOMe					3.39 *s*	

*C_6D_6.

Table 2. ^{13}C NMR data for compounds **1–8**

C	1†	2	3	4	5	6*	7	8 [9]*
1	38.39‡	37.11	35.90	35.43	35.99	36.34	38.61	38.76
2	20.07	28.19	18.38	18.09	18.19	17.94	18.10	18.38
3	3931‡	78.90	35.90	35.58	34.87	37.06	35.83	35.21
4	44.45	39.09	37.23	36.32	37.35	46.79	36.57	37.79
5	55.56	50.04	38.83	41.23	43.27	44.82	42.38	39.56
6	26.36	19.00	28.11	26.07	29.67	34.86	27.92	28.74
7	38.39‡	30.76	69.39	71.68	72.94	198.90	75.24	73.34
8	148.09	134.75	126.36	122.96	127.13	129.28	137.06	139.40
9	56.56	146.92	142.43	144.60	141.46	164.53	46.48	46.32
10	40.33	37.44	38.36	38.08	38.35	39.25	38.13	38.30
11	21.98	124.34	21.16	21.49	21.58	22.72	18.41	18.38
12	38.85‡	123.97	34.70	34.44	34.66	33.90	34.31	34.35
13	142.78	145.78	34.90	34.97	34.87	34.39	37.61	37.59
14	118.35	126.81	39.56	38.50	37.65	33.76	134.23	134.14
15	61.41	33.50	146.18	145.95	145.69	145.45	147.72	148.38
16	16.49	23.96	111.26	111.14	111.13	111.65	111.26	110.67
17	106.39	23.96	27.63	27.70	28.57	27.29	25.95	25.78
18	20.98	28.19	70.90	72.63	71.89	177.75	73.61	70.98
19	177.67	15.34	17.84	17.39	17.55	16.44	17.53	18.07
20	12.69	24.86	18.47	18.41	20.19	18.31	15.06	14.86
–COOMe	51.01					52.07		
–OCOMe	170.97			170.99			170.12	
–OCOMe	28.86			20.94			21.61	
–OCO–CH$_2$–COOMe							41.46	
–OCO–CH$_2$–COOMe							52.46	
–OCO–CH$_2$–COOMe							166.86	
–OCO–CH$_2$–COOMe							166.30	

*C_6D_6

†These data are not included in the bibliography

‡These signals may be interchangeable

literature [9]. Accordingly, **7** was identified as methyl malonate of 7α-acetyl-isopimara-8(14),15-dien-18-yl.

Also isolated were phytol (**9**), sitosterol (**10**), ursolic acid (in the form of methyl ester) (**11**) and sitosterol glucoside (**12**) whose structures were established by comparison with authentic samples and with the data in the literature.

EXPERIMENTAL

Mps uncorr.; ^{1}H NMR: 200 MHz., $CDCl_3$, TMS as int. standard; ^{13}C NMR: 50.3 MHz.

Extraction and isolation. The plant was collected in Ciudad Rodrigo (Salamanca, Spain) in July 1983. The dried plant (5.7 kg) was extracted with *n*-hexane at room temp. for 4 weeks. After evapn of the solvent, the extract (148.1 g) was subjected to vapour-current distillation, yielding 8.10 g volatile fraction and 140.0 g non-volatile fraction. The composition of volatile fraction and partial composition of the non-volatile fraction was reported in previous papers. Other compounds of the dewaxed non-volatile fraction were isolated by CC and prep. TLC.

Abietatrien-3β-ol (**2**). Colourless crystals (mp 109–111°, *n*-hexane), $C_{20}H_{30}O$. $[\alpha]_D$ + 37.26° ($CHCl_3$; *c* 0.95); IR ν_{max}^{film} cm^{-1}: 3400, 1640, 1610, 1500, 1460, 1380, 1050, 1030, 940, 880, 820, 750; ^{1}H NMR: see Table 2.

Isopimara-8,15-dien-7α,18-diol (**3**). Colourless oil, $C_{20}H_{32}O_2$. $[\alpha]_D$ + 54.5° ($CHCl_3$; *c* 2.3); IR ν_{max}^{film} cm^{-1} 3400, 3090, 1650, 1470, 1390, 1030, 920, 750; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

Isopimara-8,15-dien-7α,18-diol diacetate (**4**). Colourless oil, $C_{24}H_{36}O_4$. $[\alpha]_D$ + 55.0° ($CHCl_3$; *c* 1.73); IR ν_{max}^{film} cm^{-1}: 3090, 1750, 1650, 1480, 1390, 1250, 910; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

Isopimara-8,15-dien-7β,18-diol (**5**). Colourless crystals (mp 95–97°; Et_2O) $C_{20}H_{32}O_2$. $[\alpha]_D$ + 29.5° ($CHCl_3$; *c* 0.9); IR ν_{max}^{film} cm^{-1}: 3400, 3090, 1650, 1470, 1390, 1070, 920; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

7α-Acetyl-isopimara-8(14),15-dien-18-yl and methyl malonate (**7**). Colourless oil, $C_{26}H_{38}O_6$. $[\alpha]_D$ + 9.7° ($CHCl_3$; *c* 1.15); IR ν_{max}^{film} cm^{-1}: 3400, 3090, 1770, 1750, 1650, 1470, 1390, 1250, 920; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2.

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