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DITERPENOIDS FROM *NEPETA TUBEROSA* SUBSP. *RETICULATA*

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Key Word Index—*Nepeta tuberosa* subsp. *reticulata*; Labiatae; diterpenoids; new isopimarane derivatives; nepetalactones.

Abstract—Three new diterpene compounds have been isolated from *Nepeta tuberosa* subsp. *reticulata* and their structures elucidated by spectroscopic methods. They were identified as diisopimaryl malonate isopimarylmalonic acid and 7-oxo-isopimara-8,15-dien-18-ol. We have also isolated 4aα,7α,7aβ-nepetalactone, 4aα,7α,7aα-nepetalactone, 3α-hydroxy-4α,4aα,7α,7aα-dihydronepetalactone, 4α,4aα,7α,7aα-dihydronepetalactone, isopimaryl acetate, isopimarol, isopimaric acid, 8(14),15-isopimaradien-7α,18-diol, myrceocomunic acid and α-tocopheryl quinone.

INTRODUCTION

In this paper we report the isolation and structural determination of the components of a hexane extract of *Nepeta tuberosa* subsp. *reticulata*. We also report the results on the composition of the volatile oil and partial results concerning the non-volatile extract of the plant.

RESULTS AND DISCUSSION

From the volatile part of the hexane extract of *N. tuberosa* subsp. *reticulata*, four monoterpene cyclopentanoid lactones (**1**–**4**) were isolated and identified according to their spectroscopic properties (Tables 1 and 2) as two nepetalactones **1** and **2** [1], a hydroxylated derivative **3** [2] and a hydrogenated derivative **4** [3]. The ^{13}C NMR values for compound **3** are in agreement with α-stereochemistry for the hydroxyl group, even though there is equilibrium between the α- and β-forms at room temperature [2]. A certain proportion of the β-form must therefore exist. The non-volatile part was dewaxed and fractionated by column chromatography on silica gel or silica gel impregnated with silver nitrate. The acids were purified as their methyl esters (**9**, **10**, **16**).

Compounds **7** and **8** were identified as isopimarol and isopimaric acid, respectively, by spectroscopic methods [4]. According to their ^{1}H NMR spectra, compounds **5**, **6**, **10** and **11** (Table 3) show signals corresponding to the following groups: ABX system: –C–CH=CH$_2$ (5.83, 1H, *dd*; 4.96 1H, *dd*; 4.89, 1H, *dd*), C=CH– (5.36, 1H, *br s*), the characteristic signal of an isopimarane skeleton such as **7** and **8**. Each of them is functionalized with a hydroxyl or acetoxyl or malonyl group. The structure of the natural acetate **5** was confirmed by acetylation of **7**.

In the ^{13}C NMR spectrum of **6** (Table 4) there are 22

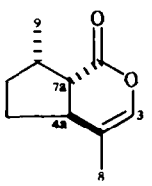
Table 1. ^{13}C NMR chemical shifts for nepetalactones 1–4

Carbon	1*	2	3	4
1	169.98	170.79	177.46	170.72
3	135.83	133.73	99.14	72.86
4	120.43	115.34	39.12	44.80
4a	37.36	40.85	38.95	35.15
5	32.10	(30.99)	32.09	31.85
6	26.09	(33.08)	34.57	34.39
7	30.00	(39.78)	38.71	38.82
7a	49.07	49.50	49.22	49.19
8	14.15	15.45	15.20	15.74
9	17.51	20.28	20.44	20.28

*In compound **1**, assignments are based on DEPT experiments, on H/H (COSY) and on C/H (HCCORR) two-dimensional correlations.

Table 2. ^{1}H NMR chemical shifts for nepetalactones 1–4

	1	2	3	4
3	6.15 *m*	6.15 *m*	5.42 *d* (J = 2.44 Hz)	4.15 1H *dd* (J_1 = 10.74 Hz, J_2 = 3.42 Hz) 3.87 1H *dd* ($J_1 = J_2$ = 10.7 Hz)
8	1.63 *dd* ($J_1 = J_2$ = 1.6 Hz)	1.61 *dd* ($J_1 = J_2$ = 1.1 Hz)	1.05 *d* (J = 6.35 Hz)	0.98 *d* (J = 6.35 Hz)
9	1.03 *d* (J = 6.92 Hz)	1.19 *d* (J = 6.41 Hz)	1.19 *d* (J = 6.35 Hz)	1.20 *d* (J = 6.35 Hz)



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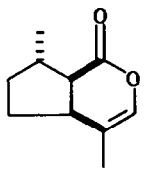

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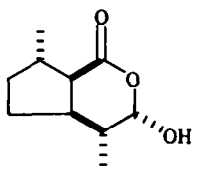

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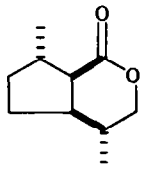

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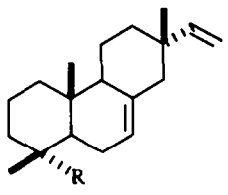
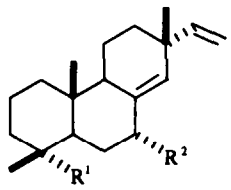
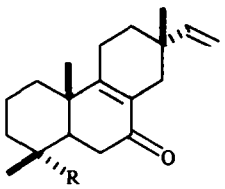


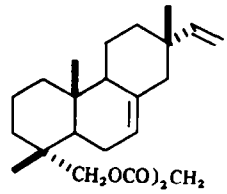
	R
5	CH_2OAc
7	CH_2OH
8	COOH
9	$COOCH_3$
10	$CH_2OCO—CH_2—COOCH_3$



	R^1	R^2
14	CH_2OH	OH
15	CH_2OAc	OAc



	R
11	CH_2OH
12	CH_2OAc
13	$COOCH_3$



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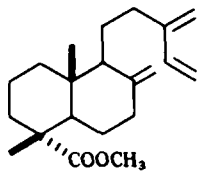

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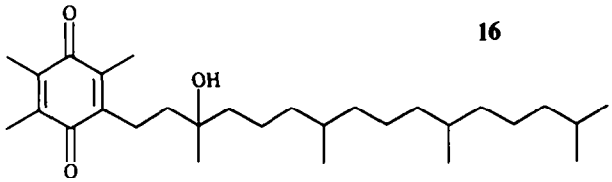

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signals; of these, three are methyl carbons, ten are methylene carbons, four are methines and five correspond to quaternary carbons. In addition to the signals corresponding to the olefinic hydrogens, the ^{1}H NMR spectrum also shows two singlets at δ3.84 (4H) and 3.42 (2H); these suggest the existence of a diisopimaryl malonate. Basic hydrolysis of **6** yielded isopimarol, confirming the proposed structure.

The ^{1}H NMR spectrum of **10**, in addition to the characteristic signals of an isopimaryl (Table 3) and of a methyl ester, presents a singlet (2H) at δ3.39 corresponding to the methylene group of the malonate. The ^{13}C NMR spectrum shows 24 peaks; one of them at δ41.54 is assigned to the malonate methylene carbon. Basic hydrolysis of **10** yielded isopimarol, confirming the proposed structure of isopimaryl-methyl malonate.

Acetylation of one of the fractions obtained from the general chromatography allowed the separation of compound **12**. The IR spectrum of the latter compound showed, in addition to the acetoxyl bands (1750 and 1250 cm^{-1}), other bands corresponding to an α,β-unsaturated carbonyl (1675 cm^{-1}, UV λ_{max} = 249 nm)

Table 3. ^{1}H NMR data for compounds **5**, **6**, **10**–**15**

Compound	H-7	H-14	H-15	H-16	H-17	H-18	H-19	H-20	–OCO–CH_2–COO–	–OCO–CH_2–$COOCH_3$	–CH_2–OCO–CH_3	–$COOCH_3$
5*	5.36 *s br*		5.38 *dd* (*J* = 17.52; 10.68)	a 4.96 *dd* (*J* = 17.58; 1.15) b 4.89 *dd* (*J* = 10.68; 1.15)	0.89 *s*	a 3.82 *d* b 3.72 *d* (*J* = 10.80)	0.97 *s*	0.93 *s*			2.07 *s*	
6	5.36 *s br*		5.83 *dd* (*J* = 17.52; 10.68)	a 4.96 *dd* (*J* = 17.52; 1.4) b 4.89 *dd* (*J* = 10.68; 1.4)	0.89 *s*	3.84 *s*	0.98 *s*	0.93 *s*	3.42 *s*			
10	5.33 *s br*		5.80 *dd* (*J* = 17.51; 10.69)	a 4.92 *dd* (*J* = 17.51; 1.4) b 4.86 *dd* (*J* = 10.69; 1.4)	0.86 *s*	a 3.87 *d* b 3.76 *d* (*J* = 10.80)	0.95 *s*	0.90 *s*	3.39 *s*	3.74 *s*		
11			5.69 *dd* (*J* = 17.49; 10.74)	a 4.92 *dd* (*J* = 10.79; 1.32) b 4.84 *dd* (*J* = 17.49; 1.32)	1.01 *s*	a 3.41 *d* b 3.10 *d* (*J* = 10.95)	0.87 *s*	1.10 *s*				
12			5.69 *dd* (*J* = 17.49; 10.74)	a 4.92 *dd* (*J* = 10.79; 1.32) b 4.84 *dd* (*J* = 17.49; 1.32)	0.99 *s*	a 3.77 *d* b 3.68 *d* (*J* 11.1)	0.94 *s*	1.11 *s*			2.04 *s*	
13			5.70 *dd* (*J* = 17.58; 10.74)	a 4.95 *dd* (*J* = 10.74; 1.09) b 4.87 *dd* (*J* = 17.58; 1.09)	1.00 *s*		1.26 *s*	1.11 *s*				3.66 *s*
14	4.18 *dd* (*J* = 2.3; 3.1)	5.48 *s*	5.75 *dd* (*J* = 17.36; 10.69)	a 4.94 *dd* (*J* = 17.36; 1.50) b 4.92 *dd* (*J* = 10.69; 1.50)	1.05 *s*	a 3.52 *d* b 2.88 *d* (*J* = 11.47)	0.74 *s*	0.80 *s*				
15	5.31 *dd* (*J* = 2.83)	5.66 *s*	5.75 *dd* (*J* = 17.14; 11.40)	a 4.88 *dd* (*J* = 17.14; 1.44) b 4.89 *dd* (*J* = 11.40; 1.44)	1.03 *s*	a 3.86 *d* b 3.59 *d* (*J* = 11.0)	0.84 *s*	0.86 *s*			2.06 *s* 1.99 *s*	

*According to C/H (HCCORR) and H/H (COSY) experimental results, we have modified the methyl group assignments for compounds **5** and **6**.
Coupling constants (*J*) in Hz.

Table 4. ^{13}C NMR data for compounds 5, 6, 10 and 12–15

Carbon	5*	6	10	12	13	14	15
1	39.39	39.27	39.32	35.35†	36.34†	38.76	38.65
2	18.12	17.97	17.98	17.84	17.94	18.38	18.17
3	36.28	36.17	36.23	35.15†	37.06†	35.21	36.01
4	36.24	36.39	36.45	36.50	46.79	37.49	36.39
5	44.88	44.87	44.75	44.78	44.82	39.56	42.18
6	23.63	23.60	23.57	33.51†	34.86†	28.74	27.84
7	121.21	121.07	121.12	198.93		73.34	75.39
8	135.61	135.59	135.58	129.07	129.28	139.40	136.95
9	51.91	51.84	51.91	165.33	164.53	46.32	46.80
10	35.37	35.32	35.34	39.56	39.25	38.30	38.14
11	20.23	20.18	20.21	22.97	22.72	18.38	18.41
12	36.33	36.17	36.13	33.73†	33.70†	34.35	34.35
13	36.87	36.83	36.83	34.42	34.39	37.59	37.60
14	46.19	46.18	46.18	33.73†	33.76†	134.14	134.34
15	150.44	150.36	150.37	145.49	145.45	148.38	147.74
16	109.26	109.25	109.25	111.50	111.65	110.67	111.23
17	21.59	21.53	21.56	27.68	27.69	25.78	25.98
18	75.35	74.28	74.20	72.15	177.75	70.98	72.48
19	18.05	17.97	17.98	17.28	16.44	18.07	17.67
20	15.49	15.63	15.43	18.84	18.31	14.86	15.06
–OCO–$\underline{C}H_3$	21.03			20.92			21.61, 20.92
–O$\underline{C}$O–CH_3	171.26			170.92			169.96, 170.83
–O$\underline{C}$O)$_2CH_2$		166.59					
–OCO)$_2\underline{C}H_2$		41.84					
–COO$\underline{C}H_3$					52.07		
–$\underline{C}$OOCH$_3$					177.75		
–CH_2OCO–$\underline{C}H_2$–COOMe			41.54				
–CH_2OCO–CH_2–COO$\underline{C}H_3$			52.34				
–CH_2O$\underline{C}$O–CH_2–COOMe			166.45				
–CH_2OCO–CH_2–$\underline{C}$OOMe			166.91				

*In compound 5, assignments are based on DEPT experiments and on C/H (HCCORR) two-dimensional correlations.

†These signals may be interchanged.

and a vinyl (3090, 1630, 920 cm^{-1}) group, respectively.

The ^{1}H NMR spectrum of **12** presents signals corresponding to the following groups: ABX system C–CH =CH_2 (5.83, 1H, *dd*; 4.96, 1H, *dd*; 4.89, 1H, *dd*), –CH_2–O–CO–CH_3 (AB system: 3.77 and 3.68 J = 11.1 Hz; 2.03, 3H, *s*) and three singlet methyl groups (1.11, 0.99, 0.94). These spectroscopic data show that the carbonyl group is conjugated with a tetrasubstituted double bond, which must be in the position 8–9 in a pimarane skeleton, and that the carbonyl group is at C-7, because the Me-20 signal appears at δ 1.11. If the carbonyl group were at C-11, then the Me-20 signal would appear downfield [5].

Basic hydrolysis of compound **12** yielded **11**, whose oxidation with Jones' reagent and subsequent esterification yielded **13**. The spectroscopic and physical properties are identical to those described in the literature [5].

The physical and spectroscopic properties of compound **14** (^{1}H NMR and ^{13}C NMR in C_6D_6) revealed an isopimaradienic skeleton [6] identical to that described for 8(14),15-isopimara-dien-18-diol [8]. Acetylation of compound **14** yielded the acetate **15**. Compound **16** was identified as methylmyrceocomunate by comparison with the data appearing in the literature [8, 9].

The IR spectrum of compound **17** shows bands corresponding to a hydroxyl group (3550 cm^{-1}) and of an α,β-unsaturated ketone (1660 cm^{-1}). The UV absorption spectrum shows λ_{max} 262 nm (ε = 17 000), which is typical for a quinonic function. The ^{1}H NMR spectrum shows three singlet methyls on a quinonic ring (δ 2.05, 3H, *s*; 1.98, 6H, *s*), à methyl geminal to a hydroxyl group (1.22, 3H, *s*) and four doublet methyls (0.88, 9H, *d*, J = 6.35 Hz; 0.86, 3H, *d*, J = 5.86 Hz). The ^{13}C NMR spectrum clearly shows a quinonic ring totally substituted together with an aliphatic chain of the phytol type with a hydroxyl group at C-3'. The physical and spectroscopic properties agree with those described in the literature for α-tocopherylquinone [10, 11].

EXPERIMENTAL

Mps (Kofler hot apparatus) are uncorr.; ^{1}H NMR: 200 MHz, $CDCl_3$, TMS as internal standard; ^{13}C NMR: 50.3 MHz.

Extraction and isolation. The plant was collected in Ciudad Rodrigo (Salamanca, Spain) in July 1983 (a specimen has been deposited in the Botany Department of Salamanca University). The dried plant (5.7 kg) was extracted with *n*-hexane at room temp. for 4 weeks. After evaporation of the solvent, 148.10 g of

extract was obtained. The extract was subjected to vapour-current distillation, yielding 8.10 g (volatile fraction) and 140.0 g (non-volatile fraction). CC of the volatile fraction gave compounds **1**, **2**, **3** and **4**. The non-volatile fraction was dewaxed with MeOH, yielding 76.3 g of the dewaxed extract which yielded the other compounds by CC and preparative TLC.

Diisopimaryl malonate (**6**). Colourless oil. $C_{43}H_{64}O_4$. $[\alpha]_D$ +25.16° (*c* 0.62; $CHCl_3$); IR ν_{max}^{Film} cm^{-1}: 3090, 1750, 1650, 1470, 1390, 920; ^1H NMR and ^{13}C NMR: see Tables 3, 4.

Isopimaryl-methyl malonate (**10**). Colourless oil. $C_{24}H_{36}O_4$. $[\alpha]_D$ −4.7° (*c*, 1.70; $CHCl_3$); IR ν_{max}^{Film} cm^{-1}: 3090, 1770, 1750, 1650, 1460, 1390, 1160, 1040, 920; ^1H NMR: see Table 3; ^{13}C NMR: see Table 4.

7-Oxo-iso-pimara-8,15-dien-18-ol (**11**). Colourless oil $C_{20}H_{30}O_2$. $[\alpha]_D$ +85.4° (*c* 1; $CHCl_3$); IR ν_{max}^{Film} cm^{-1}: 3450, 3100, 1660, 1630, 1470, 1390, 1230, 1080, 920, 670; ^1H NMR: see Table 3.

7-Oxo-isopimara-8,15-dien-18-ol acetate (**12**). Colourless oil. $C_{22}H_{32}O_3$. $[\alpha]_D$ +73.4° (*c* 1.2; $CHCl_3$); IR ν_{max}^{Film} cm^{-1}: 3090, 1750, 1675, 1630, 1480, 1390, 1250, 1050, 920. ^1H NMR Table 3; ^{13}C NMR: see Table 4. UV λ_{max} nm: 249 (ε = 7500).

Tocopherylquinone (vitamin E) quinone (**17**). Yellow oil. $C_{29}H_{50}O_3$. $[\alpha]_D$ +2.86° (*c* 1.4; $CHCl_3$); IR ν_{max}^{Film} cm^{-1}: 3550, 1660, 1480, 1390, 1320, 730; ^1H NMR: δ0.86 (3H, *d*, *J* = 5.86 Hz, Me-15'), 0.88 (9H, *d*, *J* = 6.35 Hz, Me-7',11',15'), 1.2 (3H, *s*, Me-3'), 1.98 (6H, *s*, Me-2,3) 2.03 (3H, *s*, Me-5), 2.55 (2H, *m*, C-1'); ^{13}C NMR: δ187.68 (CO), 187.24 (CO), 144.55 (C-6), 140.58 (C-5)*, 140.49 (C-2)*, 140.23 (C-3)*, 72.69 (C-3'), 42.39 (C-4'), 40.35 (C-2'), 39.44 (C-14'), 37.69 (C-6'), 37.50 (C-10'), 37.35 (C-12'), 32.84 (C-11'), 28.01 (C-15'), 26.64 (Me-3'), 24.83 (C-13'), 24.54 (C-9'), 22.73 (Me-15'), 22.64 (Me-15'), 21.46 (C-1'), 21.39 (C-5'), 19.79 (Me-11')*, 19.74 (Me-7')*, 12.35 (Me-2)*, 12.27 (Me-3)*, 11.95 (Me-5).

*These signals may be interchangeable.

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