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NEW MONOTERPENE γ-LACTONES WITH AN UNUSUAL CARBON SKELETON FROM *NEPETA TUBEROSA*

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Key Word Index—*Nepeta tuberosa* ssp *tuberosa*, Labiatae, Monoterpenic lactones, 1,5-dioxo-2-oxahexahydroindane derivatives

Abstract—From the aerial parts of *Nepeta tuberosa* ssp. *tuberosa* four new monoterpenic γ-lactones with an unusual carbon skeleton were isolated. Their structures were established by means of two dimensional NMR spectroscopy and are in agreement with a 1,5-dioxo-2-oxa-hexahydroindane skeleton.

INTRODUCTION

In the course of our systematic research on the Genus *Nepeta* we have studied, among others, *Nepeta tuberosa ssp. tuberosa*, a species native to the south-western part of the Iberian Peninsula. As part of this work, we report here, for the first time, the isolation and structural determination of some constituents of the non-volatile part of a hexane extract. Their structures were established by 2D NMR spectroscopy (H–H; H–C, normal and long range couplings) The couplings observed, especially in the H–C spectra, correspond to a highly substituted 1,5-dioxo-2-oxa-hexahydroindane skeleton.

RESULTS AND DISCUSSION

Lactones **1a**, **1b**, **2a** and **2b** were isolated from the non-volatile part of the *n*-hexane extract of *Nepeta tuberosa* ssp *tuberosa*. In a first approach to the identification of these compounds it was unclear that these substances belonged to the unusual monoterpenoid skeleton because all of them showed a great similarity in the proton pattern upfield, as well as a broad singlet downfield, with nepetalactones In particular, the latter peak (δ6.3 or 6 8, $CDCl_3$ solutions) resembled the olefinic proton signal characteristic of these cyclopentane monoterpenes usually isolated from the esential oil of these species [1, 2].

From inspection of the ^{1}H NMR spectra, in addition to the broad singlet observed downfield, the presence of four methyl groups (two of them characteristic of acetyl groups, another a tertiary methyl group and the last a secondary methyl group) was clear, especially for compounds **1a** and **2a** For compounds **1b** and **2b** it was necessary to record the ^{1}H NMR spectra in benzene solutions to suppress the great overlap observed between the two upfield methyl group signals

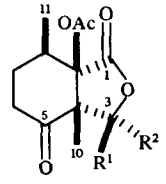
In addition to the methyl groups, the ^{13}C NMR spectra showed two methylenes, two methynes (one of them bonded to an oxygen function) two quaternary carbon atoms (one of them bearing one of the acetyl groups) as well as for quaternary carboxylic carbon atoms, where the one resonating at lower field corresponded to the carbonyl of a ketone (δ200)

Two-dimensional NMR experiments (H–H, H–C) were undertaken using the major compound **1b** for unambiguous proof of the basic structure proposed. In addition to assigning each proton to its corresponding carbon atom, we were able to demonstrate the atoms connectivity in agreement with the skeleton proposed by using long-range couplings (atoms separated by two or three bonds) 2D spectra were recorded in $CDCl_3$ and C_6D_6 solutions.

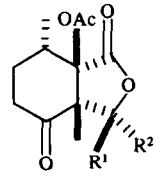
The results allowed the assignment of almost the whole structure, but no couplings were observed for the carbonyl group of the acetyl substituent at C-9. However, the couplings observed between other atoms and the quaternary carbon supporting the acetyl group make an unambiguous assignment of this position possible

The presence of four asymmetric centers (C-3, C-4, C-8 and C-9) required the use of other techniques such as circular dichroism and solvent effect experiments such as ASIS (aromatic solvent induced shifts) to determine the relative and absolute configuration for each compound.

The configuration at C-3 was easily determined by comparison of the chemical shifts observed with those given in the literature for carbohydrates [3, 4], especially those of the α-carbon to the ring oxygen-function when it is functionalized with a hydroxyl or acetoxyl group. In fact, α- and β-configurations are clearly distinguished by ^{13}C NMR as well as ^{1}H NMR. When the *gem*-hydrogen is in the α-configuration, the anomeric carbon atom



	R^1	R^2
1a	OAc	H
1b	H	OAc



	R^1	R^2
2a	OAc	H
2b	H	OAc

appears downfield (4 or 5 ppm) with respect to the chemical shift observed for the β-configuration, while chemical shifts for *gem*-hydrogens show the inverse relationship, that is, the α-H appears 0.5 ppm upfield compared with its α-counterpart

The presence of two carbonyl chromophores (ketone and lactone) allows the determination of the spatial orientation of the ring substituents especially those linked to the interannular carbon atoms [5, 6]. Both chromophores show a positive Cotton effect for the four compounds From these experimental results, theoretical approaches using molecular models indicate that there are two possible ring-functions that give rise to a positive CD curve. a *trans*-function where both substituents in the interannular positions are axial and a *cis*-function where the methyl group attached to C-4 is in an equatorial position, while the acetoxyl group at C-9 remains in an axial position. In most five and six membered lactones the ring itself is chiral Thus, the position of one or two key atoms with reference to the chromophore determines, or has the greatest influence on, the sign of the lactone Cotton effect, in our case the location above or below the place of the β-carbon atom in the lactone ring However, even though the structure with a *trans*-ring function shows a positive Cotton effect for both chromophores, the results obtained by ASIS experiments do not agree with an axial position for the methyl group linked to C-4, but instead support an axial configuration for the methyl group at C-8 (Table 1, compounds **2a** and **2b**) Furthermore, the *cis* ring-function is not only in agreement with the equatorial configuration for the methyl group attached to C-4 but also supports the equatorial configuration for the methyl group linked to C-8 for compounds **1a** and **1b** and the axial configuration for the same substituent in the case of compounds **2a** and **2b** In fact the ASIS experiments leads to a $\Delta\delta_{CDCl_3-C_6D_6}$ characteristic for equatorial methyl groups (0 06–0 15 ppm) [5, 7] in the case of lactones **1a** and **1b** and also the characteristic $\Delta\delta$(0 2 to 0 4 ppm) of an axial methyl group in lactones **2a** and **2b**

^{1}H and ^{13}C NMR data are summarized in Tables 1 and 2, respectively

EXPERIMENTAL

^{1}H NMR, Bruker 200 MHz $CDCl_3$, C_6D_6, TMS as int standard, ^{13}C NMR, 50 3 MHz

Extraction and isolation The air-dried aerial parts (3 5 kg) of *N tuberosa* ssp *tuberosa* collected in Cáceres (Spain) were extracted with *n*-hexane at room temp over 4 weeks, affording after evapn 76 77 g (2 1%) of crude extract After exhaustive steam distillation, 4 99 g of essential oil and 71 64 g of the non-volatile part were obtained The non-volatile part was dewaxed with MeOH, after evapn, the soluble fraction was subjected to flash chromatography (silica gel 60, 230–400 mesh) using *n*-hexane–Et_2O (1 1), Et_2O and MeOH as eluents The last fraction collected with *n*-hexane–Et_2O (1 1) after acetylation with Ac_2O and pyridine and repeated flash chromatography afforded compounds **1a** and **1b** The first fraction eluted with Et_2O afforded compounds **2a** and **2b**, respectively after acetylation and further purification by prep TLC and repeated CC

1,5-Dioxo-2-oxa-3(R),9(R)-diacetyl-4(R),8(R)-dimethylhexahydroindane (**1a**) Colourless oil (80 mg) $C_{14}H_{18}O_7$ $[\alpha]_D^{25} = +113\,84^\circ$ ($CHCl_3$, *c* 4 87) IR ν_{max}^{film} cm^{-1} 3500 (w), 1820, 1790, 1760, 1730, 1390, 1220 1190, 1000 CD (MeOH)

Table 2 ^{13}C NMR spectral data (50 3 MHz, $CDCl_3$, δ)

C	1a	1b	2a	2b
1	167 59	168 70	167 52	168 59
3	97 47	93 07	97 57	93 03
4	56 09	57 61	56 17	57 62
5	204 81	205 85	204 73	205 59
6	26 24	26 90	26 22	27 04
7	36 51	34 89	36 47	34 94
8	35 23	35 89	35 25	35 93
9	84 13	82 99	84 21	83 16
10	14 10	14 05	14 14	14 00
11	17 51	14 05	17 52	14 09
12	170 14	169 32	170 13	169 23
13	20 69	20 54	20 75	20 55
14	170 58	170 08	170 59	169 95
15	20 35	20 23	20 40	20 26

Table 1 ^{1}H NMR spectral data (200 MHz, $CDCl_3$ or C_6D_6 δ pm)

	1a		1b		2a		2b	
H	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6
3	6 25	6 48	6 80	6 98	6 31	6 49	6 82	6 98
	*$\Delta\delta = -0\,23$		$\Delta\delta = -0\,18$		$\Delta\delta = -0\,18$		$\Delta\delta = -0\,16$	
10	1 27	1 17	1 14	1 08	1 32	1 16	1 16	1 10
	$\Delta\delta = 0\,10$		$\Delta\delta = 0\,06$		$\Delta\delta = 0\,16$		$\Delta\delta = 0\,06$	
11	1 08	0 94	1 14	0 97	1 13	0 92	1 15	0 92
	$J = 6\,83$ $\Delta\delta = 0\,14$		$J = 6\,46$ $\Delta\delta = 0\,17$		$J = 6\,86$ $\Delta\delta = 0\,21$		$J = 6\,60$ $\Delta\delta = 0\,23$	
13	2 04	1 42	2 07	1 59	2 09	1 37	2 08	1 52
	$\Delta\delta = 0\,62$		$\Delta\delta = 0\,48$		$\Delta\delta = 0\,72$		$\Delta\delta = 0\,46$	
15	2 11	1 51	2 12	1 62	2 16	1 48	2 13	1 54
	$\Delta\delta = 0\,60$		$\Delta\delta = 0\,50$		$\Delta\delta = 0\,68$		$\Delta\delta = 0\,59$	

*$\Delta\delta = \delta_{CDCl_3} - \delta_{C_6D_6}$

nm $\Delta\varepsilon_{290} = +0.19$, $\Delta\varepsilon_{230} = +0.50$ ^{1}H NMR and ^{13}C NMR see Tables 1 and 2

1,5-*Dioxo-2-oxa*-3(S),9(R)-*diacetyl*-4(R)-8(R)-*dimethylhexahydroindane* (**1b**) Colourless oil (200 mg) $C_{14}H_{18}O_7$. $[\alpha]_D^{25} = +21.35°$ ($CHCl_3$, *c*2.13) IR ν_{max}^{film} cm^{-1}: 3500 (w), 1810, 1790, 1750, 1720, 1375, 1220, 1180, 1150, 1000. CD (MeOH) nm $\Delta\varepsilon_{290} = +0.27$, $\Delta\varepsilon_{230} = +0.45$. ^{1}H NMR and ^{13}C NMR: see Tables 1 and 2

1,5-*Dioxo-2-oxa*-3(R),9(R)-*diacetyl*-4(R),8(S)-*dimethylhexahydroindane* (**2a**) Colourless oil. (24 mg) $C_{14}H_{18}O_7$ $[\alpha]_D^{25} = +34.8°$ ($CHCl_3$; *c*2.4) IR ν_{max}^{film} cm^{-1}. 3450 (w), 1820, 1790, 1760, 1730, 1470, 1390, 1250, 1200, 1130, 1000 CD (MeOH) nm· $\Delta\varepsilon_{290} = +0.64$, $\Delta\varepsilon_{230} = +1.39$. ^{1}H NMR and ^{13}C NMR see Tables 1 and 2

1,5-*Dioxo-2-oxa*-3(S),9(R)-*diacetyl*-4(R),8(S)-*dimethylhexahydroindane* (**2b**) Colourless oil (40 mg) $C_{14}H_{18}O_7$. $[\alpha]_D^{25} = +17.47°$ ($CHCl_3$, *c*1.75). IR ν_{max}^{film} cm^{-1}: 3500 (w), 1800, 1770, 1760, 1720, 1480, 1390, 1220, 1180, 990, CD (MeOH) nm $\Delta\varepsilon_{290}$ +0.15, $\Delta\varepsilon_{230}$ +0.51 ^{1}H NMR and ^{13}C NMR see Tables 1 and 2

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A SESQUITERPENE-COUMARIN ETHER AND AN ACETYLENIC COMPOUND FROM *TANACETUM HETEROTOMUM*

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Key Word Index—*Tanacetum heterotomum*, Compositae, sesquiterpene-coumarin ethers, acetylenic compounds, spiroketal-enoletherpolyines

Abstract—The aerial parts of *Tanacetum heterotomum* afforded in addition to known compounds, a new spiroketalenoletherpolyine and a new sesquiterpene-coumarin ether. The structures were elucidated by spectral methods.

INTRODUCTION

Tanecetum species have been investigated for their sesquiterpene lactones and other compounds. Since *Tanacetum heterotomum* Bornm. is an endemic plant in Turkey, it was investigated in order to find its compounds.

RESULTS AND DISCUSSION

The aerial parts of *T. heterotomum* contain known compounds, taraxasterol, lupeyl acetate, epifriedenol, isofraxidin [1], 6,7,8-trimethoxycoumarin, 6′,7′-dimethoxyfeselol (**1**) [2], a C_{14} acetylenic compound (**2**) [3], and two new compounds, a spiroketalenoletherpolyine (**3**) and a sesquiterpene-coumarin ether (**4**). The structures of the compounds were established by spectral methods.

The IR spectrum of **3** showed an acetylene band at 2160 cm^{-1}, an ester band at 1740 and 1260 cm^{-1} and unsaturation at 1680 cm^{-1}. The high resolution mass spectrum gave a molecular peak at *m/z* 300.136 ($C_{18}H_{20}O_4$). Although the ^{1}H NMR spectrum of **3** was very close to that of spiroketalenoletherpolyine (**5**) previously found in *Tanacetum parthenium* [4], they were not identical (Table 1) To understand the differences,