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NEO-CLERODANE DITERPENOIDS FROM ROOTS OF *LINARIA SAXATILIS* VAR. *SAXATILIS*

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Key Word Index—*Linaria saxatilis* var. *saxatilis*; Scrophulariaceae; diterpenoids; *neo*-clerodanes.

Abstract—Two new and four known *neo*-clerodane diterpenoids were isolated from an *n*-hexane extract of the roots of *Linaria saxatilis* var. *saxatilis*. The structures of the new compounds were established from the spectral data as 15,16-diacetoxy-15,16-epoxy-*neo*-cleroda-3,12*Z*-diene, and 15,16-diacetoxy-12,13-15,16-diepoxy-14-hydroxy-*neo*-clerod-3-ene.

INTRODUCTION

The genus *Linaria* belongs to the family Scrophulariaceae (Subfam. Scrophularioidea, tribe Antirrhinae) and several species have been used in traditional medicine for the treatment of vascular disorders (*L. vulgaris*) [1], and for their tonic and antiscorbutic (*L. cymbalaria*) [2], laxative (*L. japonica*) [3] and diuretic (*L. cymbalaria* and *L. japonica*) [2, 3] effects.

For several years, our group has been studying the chemical composition of varieties of *Linaria saxatilis*. Continuing on the same line, we have addressed the chemical composition of the roots of *L. saxatilis* var. *saxatilis*, a plant native to the north and centre of the Iberian Peninsula, on which we have already published studies concerning the chemical composition of the aerial parts [4, 5], which mainly contain unsaturated *neo*-clerodanes at position 4(18). The chemical composition of the var. *glutinosa* [6–8] has also been studied and its roots contain six new clerodanes unsaturated at position C-3. In the present work, we report the results of study of the chemical composition of an *n*-hexane extract of the roots of the var. *saxatilis*.

RESULTS AND DISCUSSION

From an acetone-dewaxed *n*-hexane extract of the roots of *L. saxatilis*, six compounds of *neo*-clerodane structure and the triterpene friedelin were isolated and identified. The *neo*-clerodane nature of the isolated diterpenes and the presence of an unsaturation at C-3 were established from comparison of their spectroscopic data with those for clerodanes previously isolated from the roots of the var. *glutinosa*. Compounds **1**, **2**, **4** and **5** are common to the roots of both varieties, while **3** and **6** were only isolated from the var. *saxatilis* and are described as new natural products.

The structure of **3** was established by comparison of the ^{13}C and ^{1}H NMR data with those for 15,16-diacetoxy-15,16-epoxy-*neo*-cleroda-4(18),12*Z*-diene (**7**) [4]. The signals assignable to the side chain almost reproduce those of the compound, whose 12*Z*-stereochemistry was established by NOE experiments. For the signals assignable to the bicyclic moiety, the main differences are the absence of an olefinic methylene and the presence of a methine both in ^{1}H and ^{13}C NMR (δ5.10 and 120.5) indicating the existence of a double bond between C-3/C-4 instead of the Δ4(18)-double bond of **7**. The structure proposed for **3** is that of 15,16-diacetoxy-15,16-epoxy-*neo*-cleroda-3,12*Z*-diene, which is isolated with a small proportion of its epimer at C-15, similar to the case for **7**.

The structure of compound **6** was assigned by comparison with that of 15,16-diacetoxy-15,16-epoxy-*neo*-clerod-3-ene (**5**), which was also obtained from the var. *glutinosa*, and its structure was unequivocally established by H–C heteronuclear correlations and some NOE difference experiments [6]. The only observable difference between **5** and **6** lies in the presence in **6** of an additional secondary alcohol function (IR: 3510 cm^{-1}; ^{1}H NMR: δ4.25 and ^{13}C NMR: δ71.4); this was confirmed by acetylation which yielded the acetate **6a**. The additional hydroxyl function was located at C-14 because the geminal proton to the acetate at C-15 was a doublet of 2 Hz, which also indicated the *trans*-configuration between both functions. The absolute configuration of the side chain could correspond to that shown in the figure or to its enantiomers.

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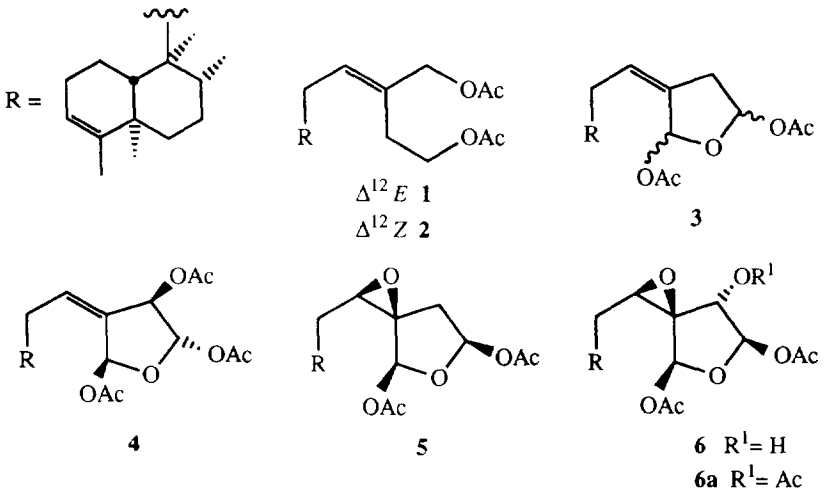

EXPERIMENTAL

General. Optical rotations: $CHCl_3$. IR: NaCl, ν_{max} values are expressed in cm^{-1}. ^1H (200.13 MHz) and ^{13}C (50.3 MHz) NMR: $CDCl_3$ with TMS as int. standard. Chemical shifts are reported in δ (ppm) and coupling constants are in Hz. EIMS: 70 eV. Flash chromatography: silica gel (Merck No. 9385).

Plant material. Linaria saxatilis var. *saxatilis* was collected in July 1991 at Campo de Ledesma (Salamanca, Spain) and identified by Prof. M. Ladero. A voucher specimen is deposited at the Botany Dept. of Pharmacy, Salamanca (Register No. SALAF 21386).

Extraction and isolation. Once triturated, air-dried roots (750 g) were extracted by the Soxhlet procedure with *n*-hexane for 15 hr, and resulting extract was cooled overnight at $-20°$. The soluble fr. was defatted with Me_2CO, yielding 6.4 g of a viscous material from which, after repeated CC and/or prep. TLC and/or crystallization, the following compounds were isolated: **1** and **2** (22 mg), **3** (81 mg), **4** (208 mg), **5** (380 mg), friedelin (17 mg) and **6** (23 mg).

15,16-*Diacetoxy*-15,16-*epoxy*-neo-*cleroda*-3,12*Z*-*diene* (**3**). Eluted with *n*-hexane–EtOAc (8:2); $[\alpha]_D$: −41.9° (*c* 1.25). IR ν_{max} cm^{-1}: 1750, 1670, 1200, 1050, 880. ^1H NMR: Table 1, and ^{13}C NMR: Table 2.

15,16-*Diacetoxy*-12,13-15,16-*diepoxy*-14-*hydroxy*-neo-*clerod*-3-*ene* (**6**). Eluted with *n*-hexane–EtOAc (8:2); $[\alpha]_D$: −0.4° (*c* 1.4). IR: 3500, 1750, 1640, 1220, 1080, 1030, 1000, 900, 850. ^1H NMR: Table 1, and ^{13}C NMR: Table 2.

14,15,16-*Triacetoxy*-12,13-15,16-*diepoxy*-neo-*clerod*-3-*ene* (**6a**). By acetylation of 10 mg **6** with Ac_2O–pyridine,

Table 1. ^1H NMR data for compounds **3** and **6** (200 MHz, $CDCl_3$, TMS as int. standard; *J* (Hz) in parentheses)

H	3	6
3	5.10 *m*	5.20 *m*
12	5.70 *m*	3.40 *t* (6.0)
14	2.60–3.00 *m*	4.25 *m*
15	6.30 *d* (5.4)	6.30 *d* (2.0)
16	6.70 *s*	6.05 *s*
Me-17	0.85 *d* (6.1)	0.88 *d* (6.1)
Me-18	1.50 *br s*	1.57 *br s*
Me-19	1.00 *s*	1.01 *s*
Me-20	0.70 *s*	0.77 *s*
$MeCO_2$	2.00 *s*	2.06 *s*
	2.05 *s*	2.11 *s*

Table 2. ^{13}C NMR data for compounds **3** and **6** (50.3 MHz, $CDCl_3$, TMS as int. standard)

C	3	6
1	26.7	26.8
2	19.0	21.0
3	120.5	120.8
4	144.3	143.8
5	39.9	39.8
6	37.6	36.7
7	27.5	27.3
8	36.6	37.6
9	38.3	37.8
10	47.7	48.2
11	36.6	32.3
12	125.6	57.2
13	133.1	68.9
14	36.8	71.4
15	97.0	103.6
16	93.9	99.6
17	16.1	16.2
18	17.9	17.9
19	19.8	19.1
20	17.7	17.5
$MeCO_2$	169.7	169.1
		169.3
$MeCO_2$	20.6	21.0
		19.9

9 mg **6a** was obtained. Mp 117 (*n*-hexane). IR ν_{max} cm^{-1}: 1760, 1640, 100, 890. EIMS *m/z* (rel. int.): 418 ([M]$^+$ − AcOH, 6), 300 (4), 282 (6), 246 (7), 215 (8), 189 (48), 175 (77), 133 (42), 119 (71), 107 (86), 95 (100). ^{1}H NMR: δ0.78 (3H, *s*, H-20), 0.89 (3H, *d*, J = 6.0 Hz, H-17), 1.03 (3H, *s*, H-19), 1.58 (3H, *br s*, H-17), 2.04 (3H, *s*, OAc), 2.08 (3H, *s*, OAc), 2.10 (3H, *s*, OAc), 3.40 (1H, *t*, J = 6 Hz, H-12), 5.12 (1H, *m*, H-3), 5.21 (1H, *br s*, H-14), 6.17 (1H, *s*, H-16), 6.25 (1H, *s*, H-15).

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