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THE STRUCTURE OF LEOCARDIN, TWO EPIMERS OF A DITERPENOID FROM *LEONURUS CARDIACA*

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Key Word Index—*Leonurus cardiaca*; Labiatae; diterpenoid; labdane derivative; leocardin.

Abstract—From the aerial part of *Leonurus cardiaca* a new labdane diterpenoid, leocardin, has been isolated and its structure was established by spectroscopic and chemical means. It has been shown to be 8β-acetoxy-9α,13α,15,16-bisepoxy-15-hydroxy-7-oxo-labdan-6β,19-olide present as a C-15 epimeric mixture.

INTRODUCTION

A number of years ago Brieskorn and Broshek isolated from *Leonurus cardiaca* seven compounds the structures of which were not be established [1]. We have examined the aerial parts of *Leonurus cardiaca* from which we have isolated an C-15 epimeric (15α or 15β-hydroxy) mixture of diterpenoids. The structure is established as 8β-acetoxy-9α,13α,15,16-bisepoxy-15-hydroxy-7-oxo-labdan-6β-19-olide (**1a** or **1b**). This epimeric mixture was not isolated previously [1].

RESULTS AND DISCUSSION

We now present evidence in support of structure **1a/b** for leocardin. The epimeric mixture showed IR absorption characteristic of a hydroxyl group (3520 cm^{-1}), a cyclohexanone (1722 cm^{-1}) a γ-lactone (1780 cm^{-1}) and an ester group (1760 cm^{-1}). The ^1H NMR spectrum (Table 1) contained signals attributable to three pairs of tertiary methyl groups at δ0.70, 0.72, 1.28, 1.29, 1.79 and 1.84 (C-20, C-18 and C-17).

The AB quartets at 3.81, 4.05 and 4.13, 4.32 (J = 10 Hz)

Table 1. ^1H NMR spectral data of compounds **1a/b**, **2**, **3** and **4** (400 MHz in $CDCl_3$ solution, TMS as internal standard)

	2	**1a**	**1b**	**4**	**3**
H-1β	1.28 *m*				
H-1α	1.40 *ddd*				
H-2β	1.50 *dddd*				
H-2α	1.58 *m*				
H-3β	2.26 *br dd*			2.13 *br d*	
H-3α	1.33 *m*				
H-5	2.74 *d*	2.79 *d*	2.72 *d*	3.28 *br d*	2.97 *d*
H-6	4.99 *d*	5.00 *d*	4.98 *d*	4.54 *dd*	5.52 *d*
H-7	—	—	—	5.20 *d*	
H-11β	2.42 *br dd*				
H-11α	1.85 *dd*		2.35 *m*	2.7–2.85 *m*	
H-12β	2.03 *ddd*				2.68 *m*
H-12α	2.08 *br dd*				2.56 *m*
H-14α	2.79 *br dd*	2.32 *d*	2.42 *br d*	6.27 *br d*	6.27 *br s*
H-14β	2.50 *d*	1.90 *d*	1.97 *dd*		
H-15	—	5.67 *br d*	5.49 *br d*	7.34 *dd*	7.37 *dd*
H-16α	4.50 *br d*	4.32	4.13 *d*	7.25 *br s*	7.24 *br s*
H-16β	4.30 *d*	3.81	4.05 *d*		
H_3-17	1.79 *s*	1.84	1.79 *s*	2.27 *s*	1.92 *s*
H_3-18	1.28 *s*	1.29	1.28 *s*	1.61 *s*	1.30 *s*
H-20	0.73 *s*	0.70	0.72 *s*	0.51 *s*	0.75 *s*
OAc	2.15 *s*	2.15 *s*		2.23 *s*	2.16 *s*

J (Hz), **2**: 1α, 1β = 2α, 2β = 11α, 11β = 12β, 12α = 13; 1α, 2β = 2β, 3α = 11β, 12α = 12; 1α, 2α = 3.5; 2β, 3β = 4; 3α, 3β = 15; 5, 6 = 6; 11β, 12β = 7; 11α, 12α = 5.5; 14α, 14β = 17.5; 14β, 16β = W 0.5; 16α, 16β = 10.

J (Hz), **1a/b**, **3**: 5, 6 = 6; 14′, 14 = 13, 14 = 5.5; 14, 15 = 1; 15, 16′ = 10.

were due to the H-16 protons. The signals at 1.90 (*br d*), 1.97 (*dd*) and 2.32 (*dd*), 2.42 (*br d*) were assigned to the H-14 protons (J = 13 Hz), while the doublets at 2.72 and 2.79 (J = 6 Hz) were due to H-5. The doublets at 4.98 and 5.00 were assigned to H-6 (J = 6.0 Hz), while the broad doublets at 5.49 and 5.67 were due to two methine protons at H-15 (J = 5.0 Hz).

The mass spectrum contained an ion at m/z 109 which is a characteristic fragment seen with many diterpenoids [2, 3]. The ^{13}C NMR spectrum showed that the carbon atoms were disposed as four methyl groups, seven methylene, three methine carbon atoms and eight fully substituted carbon atoms including three carbonyl groups (199.9, 179.1, 168.6). Careful examination of the ^{1}H NMR spectrum allowed the resonances to be accommodated in the structure **1a/b** (Scheme 1). In agreement with this hypothesis Jones reagent oxidation of the epimeric mixture **1a/b** for 15 min yielded a bis lactone (**2**). The presence of an acetyl group in **2** at the C-8 position was firmly supported by its ^{1}H NMR spectrum. The C-17 methyl group resonance was a singlet, while the position of the carbonyl group followed from the downfield shift of H-6. The IR, ^{1}H NMR, ^{13}C NMR and mass spectral data (Tables 1 and 2) firmly established the structure **2**. Moreover, the traces of acid in $CDCl_3$ (or $CHCl_3$) transformed **1a/b** first to **3**, which was only detected by ^{1}H NMR. After work up compound **4** and a mixture of the epimers of **5** were obtained, but they could not be obtained pure (Table 1, Scheme 1). A similar retro-aldol reaction has been observed at galeopsin [4].

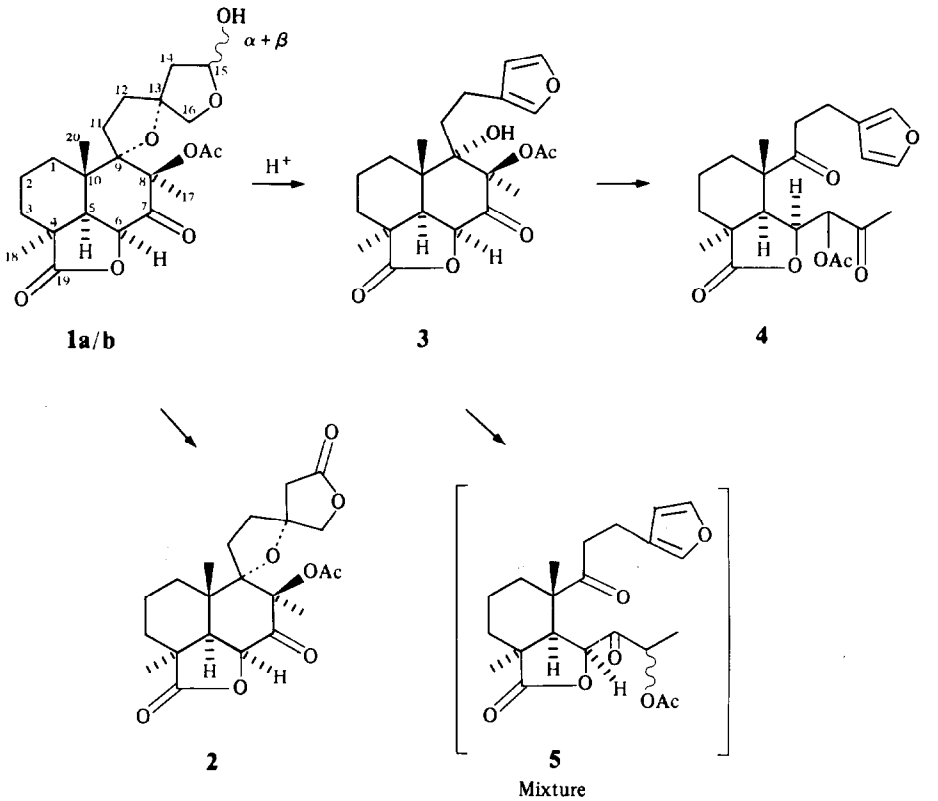
The stereochemistry at the chiral centres and the *trans*-junction or rings A and B were deduced from spin decoupling and an NOE experiment (Table 3). Inspection of molecular models suggests that ring B must be present in a Twist-conformation.

EXPERIMENTAL

Mps were determined on a Kofler apparatus and are uncorr. Plant materials were collected in July 1983 near Plovdiv, Bulgaria.

Extraction of Leonurus cardiaca. Dried and finely powdered *Leonurus cardiaca* aerial parts (3.2 kg) were extracted with Me_2CO (22 l.) for 5 days. After evaporation, the residue was taken up in CH_2Cl_2 washed with H_2O dried and the solvent evapd. The residual gum (16 g) was passed through a silica gel column (800 g) (Merck No 7734 deactivated with 15% H_2O). Elution with CH_2Cl_2–petrol (2:8) gave leocardin (**1a/b** 80 mg), which crystallized from Me_2CO–petrol; mp 180–186°; IR ν_{max}^{KBr} cm^{-1}: 3520, 2950, 2885, 1780, 1760, 1722, 1460, 1380, 1250, 1220, 1130, 1140, 800 and 750. MS (75 eV, direct inlet) m/z (rel. int.): 422.194 $[M]^+$ (12) ($C_{22}H_{30}O_8$), 380 (60) [M − ketene]$^+$, 363 (88) [M − OAc]$^+$, 362 (52) [M − HOAc]$^+$, 334 (22), 319 (36), 273 (35), 221 (28), 209 (56), 193 (78), 183 (55), 109 (100), 95 (58), 81 (68).

Oxidation of **1a/b** *to* **2**. To a soln of **1a/b** (30 mg) in Me_2CO was added a few drops of Jones reagent at room temp. during 15 min. Work up in the usual manner yielded a crystalline product (24 mg) which was recrystallized from CH_2Cl_2–petrol to yield pure substance, mp 125–128°. IR ν_{max}^{KBr} cm^{-1}: 3040, 2980, 2800,



Scheme 1.

Table 2. ^{13}C NMR chemical shifts (in values from TMS) of compounds **1a/b** and **2** in $CDCl_3$ at 100.6 MHz

	1a/b	2
C-1	29.8/29.7	29.4 *t*
C-2	17.8	17.6 *t*
C-3	31.2	31.1 *t*
C-4	41.6	41.5 *s*
C-5	47.6/47.4	47.2 *d*
C-6	75.8/75.9	75.7 *d*
C-7	199.9/199.6	199.4 *s*
C-8	89.8/89.6	89.0 *s*
C-9	97.4/96.6	97.5 *s*
C-10	41.3/41.1	40.9 *s*
C-11	29.4/29.3	29.1 *t*
C-12	35.6/36.7	35.8 *t*
C-13	90.7/90.5	86.4 *s*
C-14	46.7/45.6	41.2 *t*
C-15	99.4/99.1	174.2 *s*
C-16	75.1/75.3	76.0 *t*
C-17	26.8/26.7	26.7 *q*
C-18	23.4/23.5	23.5 *q*
C-19	179.1	178.9 *s*
C-20	17.1	17.5 *q*
OAc	21.9	22.0 (Me)
>C=O	168.6	168

1790, 1770, 1730, 1620, 1490, 1440, 1420, 1390, 1230, 1180, 1130, 1040, 1020, 950, 900, 770 and 750. MS (75 eV, direct inlet) *m/z* (rel. int.): $[M]^+$ (absent), 378, 168 $[M - \text{ketene}]^+$ (100) ($C_{20}H_{26}O_7$), 362 (53) $[M - OAc]^+$, 359 $[378 - Ac]^+$ (39), 289 (25), 261 (23), 207 (44), 194 (20), 181 (28), 109 (58).

Table 3. NOE effects with compound **2** (% in parentheses)

Irradiation at	NOE
H-5	H-6 (10); H-17 (8); H-18 (5); H-1α (6)
H-6	H-5 (10); H-17 (6); H-18 (6)
H-16α	H-16β (10); H-17 (4)
H-16β	H-16α (10); H-12β (3)
H-17	H-5 (4); H-6 (12); H-16α (10)
H-18	H-5 (12); H-6 (14)
H-20	H-11α (8); H-11β (6)

Preparation of **4** *from* **1a/b**. A trace of HCl in $CDCl_3$ (0.5 ml) after 12 hr transformed **1a/b** partially into **3** (^1H NMR) and prep. TLC (Et_2O, 100%), gave **3**, **4** (3 mg) and starting material. Compound **4**, IR ν_{max}^{KBr} cm^{-1}: 3140, 3118, 2980, 1760, 1730, 1698, 1503, 1242, 1030, 880, 789. MS (75 eV, direct inlet) *m/z* (rel. int.): 404.184 $[M]^+$ (82), $C_{22}H_{28}O_7$, 344 $[M - HOAc]^+$ (4), 282 $[M - CHOCH_2CH_2-$ $]^+$ (40), 239 $[282 - COMe]^+$ (12), 222 $[282 - HOAc]^+$ (18), 193 $[239 - HCOH]^+$ (25), 179 $[239 - HOAc]^+$ (33), 151 (31), 137 (30), 123 [$]^+$ (55), 109 (61), 95 $[123 - CO]^+$ (68), 81 [$]^+$ (100).

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