



AJUGAPYRIN A, A *NEO*-CLERODANE DITERPENE FROM *AJUGA PYRAMIDALIS*

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Abstract—A new neo-clerodane, ajugapyrin A, as well as the iridoid glycosides 8-acetylharpagide and harpagide have been isolated from the aerial parts of *Ajuga pyramidalis*. The structure of ajugapyrin A was established by chemical and spectroscopic means and comparison with those of closely related compounds. © 1997 Elsevier Science Ltd

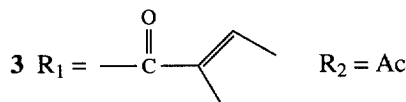
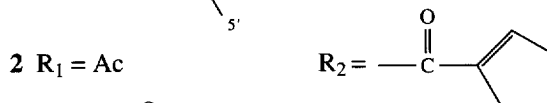
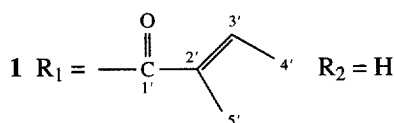
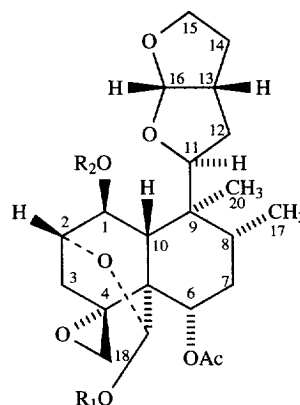
INTRODUCTION

In the course of our systematic studies of neo-clerodane diterpenoids within the genus *Ajuga* [1–4], we have investigated the aerial parts of *A. pyramidalis*.

We report here on the isolation and structure determination of a new neo-clerodane diterpene, which was named ajugapyrin A (**1**).

RESULTS AND DISCUSSION

Ajugapyrin A (**1**) was assigned the molecular formula $C_{27}H_{38}O_9$ (EIMS) and its IR spectrum was consistent with the presence of a free hydroxyl group (3403 cm^{-1}) and ester groups (1723 , 1698 , 1654 cm^{-1}), one of which was probably a tigloyloxy moiety (1654 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) revealed the presence of an acetoxyl group and a tigloyloxy substituent [δ 7.06 (1H, *qq*, $J_1 = 7.1\text{ Hz}$; $J_2 = 1.2\text{ Hz}$); δ 1.81 (3H, *m*), δ 1.87 (3H, *m*)] [2], together with characteristic signals of a neo-clerodane structure (Me-17 at δ 0.89, *d*, $J = 6.2\text{ Hz}$ and Me-20 at δ 1.19, *s*) possessing a $4\alpha,18$ -oxyrane ring (δ 2.88, *d*, and 3.08, *d*, $J = 4.5\text{ Hz}$) and a hexahydrofurofuran moiety (see the H-11, H-13, H₂-15 and H-16 proton resonances in Table 1). The presence of the hexahydrofurofuran moiety was confirmed by the presence of significant peaks at m/z 113, 91, 83 and 69 in the mass spectrum of **1** [5]. The ^1H NMR spectrum of **1** was almost identical with that of 14,15-dihydrojodrelin T (**2**) [6]. In fact the observed differences were consistent with the presence in the former of an tigloyloxy group at



C-19 β instead of the acetoxyl and a β -hydroxyl group at C-1 instead of a tigloyloxy group. Acetic-anhydride-pyridine treatment (see Experimental) of **1** yielded derivative **3**, the IR spectrum of which was devoid of hydroxyl absorption and whose ^1H NMR spectrum (Table 1) showed a paramagnetically shifted signal of H-1 α (δ 5.40 *dd*, $J_1 = 2.0\text{ Hz}$, $J_2 = 4.7\text{ Hz}$). The relative configuration of **1** was deduced from a NOESY experiment. The ^1H -equatorial showed NOEs with H-

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Table 1. ^1H NMR spectral data of compounds **1** and **3** (250 MHz, CDCl_3 , TMS as int. standard)*

H	1	3	J, Hz	1	3
1 α	3.97 <i>br t</i>	5.40 <i>dd</i>	1 α , 2 β	3.2	4.7
2 β	4.40 <i>m</i> †	4.23 <i>m</i>	1 α , 10 β	2.4	2.6
3 α (eq.)	2.27 <i>dt</i>	2.58 <i>dt</i>	2 β , 3 α	2.7	2.4
3 β (ax.)	1.67 <i>m</i>	1.62 <i>m</i>	2 β , 3 β	2.7	2.0
6 β	4.64 <i>dd</i>	4.64 <i>dd</i>	6 β , 7 α	11.5	11.2
10 β	1.97 <i>d</i>	2.06 <i>d</i>	6 β , 7 β	4.8	5.0
11 α	4.08 <i>dd</i>	4.07 <i>dd</i>	8 β , 17	6.2	6.2
13 β	2.83 <i>m</i>	2.86 <i>m</i>	11 α , 12 α	5.7	5.6
15 (2H)	3.86 <i>m</i>	3.88 <i>m</i>	11 α , 12 β	10.9	11.0
16 β	5.63 <i>d</i>	5.66 <i>d</i>	13 β , 16 β	5.1	5.1
Me-17	0.89 <i>d</i>	0.90 <i>d</i>	18A, 18B	4.5	4.5
18A‡	2.88 <i>d</i>	2.91 <i>q</i>	3', 4'	7.1	7.1
18B§	3.09 <i>d</i>	—	3', 5'	1.2	1.2
19 α	6.77 <i>s</i>	6.79 <i>s</i>			
Me-20	1.19 <i>s</i>	1.25 <i>s</i>			
3'	7.06 <i>qq</i>	7.09 <i>qq</i>			
Me-4'	1.87 <i>m</i>	1.89 <i>m</i>			
Me-5'	1.81 <i>m</i>	1.82 <i>m</i>			
OAc	1.79 <i>s</i>	1.80 <i>s</i>			
OAc	—	2.06 <i>s</i>			
OH	1.63 <i>br s</i>	—			

* Spectral parameters were obtained by first order approximation. All assignments were confirmed by double resonance experiments.

† $W_{1,2} = 9.2$ Hz, collapsed into *t* after addition of D_2O .

‡ Exo hydrogen with respect to ring B.

§ Endo hydrogen with respect to ring B.

11 α , whereas H-11 α showed cross-peaks with Me-17, Me-20 and H-7. On the other hand H-19 α showed cross-peaks with Me-17, Me-20 and H-7 α . Moreover, the H-6 β -axial proton showed NOEs with H-10 β , H-7 β and H β -18. These results showed unambiguously that **1** possessed the same stereochemistry as **2**. This neo-clerodane diterpene is the first to be isolated from an *Ajuga* species with a C-19, 2- α -hemiacetal function like those of some neo-clerodanes isolated from *Scutellaria* species [6–9].

The absolute configuration of ajugapyrin A (**1**) was not ascertained. However, on biogenetic grounds, it may be supposed that **1** belong to the neo-clerodane series like the other diterpenoids isolated from *Ajuga* species [1–5, 10].

EXPERIMENTAL

Plant material. Collected in August 1995 near Smoljan, Bulgaria and voucher specimens (no. 16819) are deposited in the Herbarium of the Department of Botany at the Higher Institute of Agriculture in Plovdiv, Bulgaria.

Extraction and isolation of ajugapyrin A (1). Dried and finely powdered aerial parts (30 g) of *Ajuga pyramidalis* were extracted with Me_2CO (0.5 l) at room temp. for 5 days. After removal of the solvent, the residue (2.8 g) was dissolved in 50 ml 60% aq. Me_2CO ,

cooled for 24 hr at 2–5° and filtered (this process was repeated $\times 3$). The combined filtrates were extracted with CHCl_3 . The extract was dried over Na_2SO_4 and evapd *in vacuo* to give 0.5 g gum (bitter fr.).

The bitter fr. was chromatographed on a silica gel column (10 g, Merck, no. 7734, deactivated with 15% H_2O). Elution with CHCl_3 –MeOH (99:1) yielded crude ajugapyrin A (**1**). Further elution with CHCl_3 –MeOH (47:3) gave 8-acetylharpagide (30 mg) and harpagide (25 mg).

Ajugapyrin A (1). Mp 198–200° (Et_2O –petrol); $[\alpha]_{\text{D}}^{20} + 23.1^\circ$ (CHCl_3 ; *c* 0.157); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3403, 3076, 2966, 2940, 2900, 2873, 1723, 1698, 1654, 1378, 1294, 1261, 1157, 1092, 1082, 1066, 1052, 1019, 966, 942, 918, 899, 876, 732; EIMS (70 eV, direct inlet), *m/z* (rel. int.): 506 $[\text{M}]^+$ (0.12), 488 (0.9), 446 (0.7), 423 (7), 407 (2), 387 (1), 348 (2), 175 (8), 113 (100), 91 (11), 83 (38), 69 (61), 43 (28). Found: C, 62.19; H, 7.41, $\text{C}_{17}\text{H}_{38}\text{O}_4$; requires: C, 64.01, H 7.56%. ^1H NMR; Table 1.

Acetylation of 1. Compound **1** (12 mg) was treated with a mixt. of Ac_2O (0.6 ml) and pyridine (1.2 ml) at room temp. for 24 hr. Work up in the usual manner gave **3** (10 mg). Amorphous solid, mp. 98–102°. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3074, 2962, 2931, 2875, 1734, 1724, 1651, 1373, 1240, 1141, 1083, 1024, 968, 920, 900, 756, 667; EIMS (70 eV, direct inlet), *m/z* (rel. int.): $[\text{M}]^+$ absent, 448 $[\text{M}-\text{C}_5\text{H}_7\text{O}_8]^+$ (0.8), 389 (0.9), 329 (1), 315 (2), 276

(4), 216 (8), 171 (20), 113 (100), 111 (4), 91 (37), 83 (47), 69 (31), 43 (70). ¹H NMR: Table 1.

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