Pergamon

PII: S0031-9422(96)00850-3

Phytochemistry, Vol. 45, No. 1, pp. 121–123, 1997

0031-9422/97 $17.00+0.00

NEO-CLERODANE DITERPENOIDS FROM *AJUGA AUSTRALIS* AND *A. ORIENTALIS*

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(*Received* 3 *October* 1996)

Key Word Index—*Ajuga australis*; *A. orientalis*; Labiatae; neo-clerodane diterpenoids; ajugapitin; 14,15-dihydro-15-hydroxyajugapitin; ajugorientin.

Abstract—A new neo-clerodane diterpenoid, ajugorientin, has been isolated from *Ajuga orientalis*. The previously known neo-clerodanes ajugapitin and 14,15-dihydro-15-hydroxyajugapitin have been found in the acetone extract of *A. australis*. The structure of ajugorientin, (11*S*, 13*R*, 16*S*)-6α, 19-diacetoxy-1β-(*E*)-2′-methyl-2′-butenoyloxy-4α.18;11,16;15,16-triepoxy-neo-clerodan-3β-ol, was elucidated by spectroscopic means and by comparison with closely related compounds.

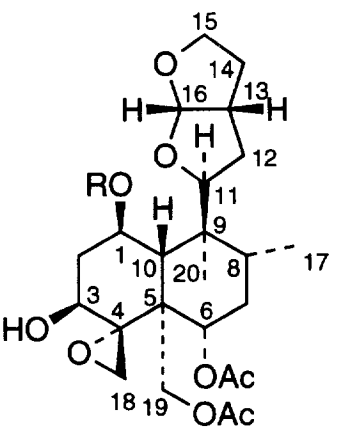
INTRODUCTION

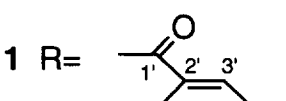
Extensive chemical studies have been carried out on neo-clerodane-type [1] diterpenes of the species belonging to the genus *Ajuga* [2, 3]. Interest in these compounds has been stimulated by their biological activity as insect antifeedants [2, 4]. This report deals with the structural elucidation of a new clerodane diterpene isolated from *A. orientalis* and the identification of two previously known diterpenoids as constituents of *A. australis*.

RESULTS AND DISCUSSION

An acetone extract of the aerial parts of *A. australis*, after column chromatography (see Experimental), provided the neo-clerodane derivatives ajugapitin [5–7] and 14,15-dihydro-15-hydroxyajugapitin [8, 9], the latter as a mixture of C-15 epimers, and both of them previously isolated from *A. chamaepitys*.

An acetone extract of *A. orientalis* (see Experimental) yielded ajugorientin (**1**, $C_{29}H_{42}O_{10}$). Its ^1H and ^{13}C NMR spectra (Table 1) showed essentially the same signals as those present in the spectra of ajugareptansin (**2**), a neo-clerodane diterpene previously found in *A. reptans* [10–12]. The observed differences between the ^1H and ^{13}C NMR spectra of **1** (Table 1) and **2** [10–12] were in agreement with the existence in the former of a tigloyloxy substituent [δ_H 6.82, 1H, *qq*, J = 6.8 and 1.5 Hz (H-3′), 1.80, 3H *dq*, J = 6.8 and 1.0 Hz (Me-4′) and 1.85, 3H, *br s* (Me-5′); δ_C 166.5 *s* (C-1′), 129.2 *s* (C-2′), 137.6 *d* (C-3′), 14.5 *q* (C-4′) and 12.1 *q* (C-5′)] [13] instead of the 2-methylbutyric ester group of the latter [**2**: δ_H 0.90, 3H, *t*, J = 6 Hz (Me-4′) and 1.12, 3H, *d*, J = 6 Hz (Me-5′); δ_C 174.8 *s* (C-1′), 42.0 *d* (C-2′), 26.9 *t* (C-3′), 11.4 *q* (C-4′) and 15.8 *q* (C-5′)] [12].





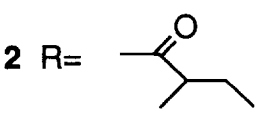


Table 1. ^1H and ^{13}C NMR spectral data for compound **1***

H	1	H	1	J (Hz)	1	C	1	C	1
1α	5.80 *ddd*	15A	3.78 *m*†	1α,2α	4.4	1	69.6 *d*	16	108.5 *d*
2α	~1.80 ‡	15B		1α,2β	5.0	2	37.8 *t*	17	17.9 *q*
2β	2.47 *ddd*	16β	5.56 *d*	1α,10β	8.1	3	64.1 *d*	18	43.8 *t*
3α	4.28 *dd*	Me-17	0.89 *d*	2α,2β	11.6	4	66.7 *s*	19	61.7 *t*
6β	4.82 *dd*	18A	2.95 *d*	2α,3α	6.3	5	44.8 *s*	20	14.4 *q*
7α	1.69 *q*	18B	2.98 *d*	2β,3α	6.8	6	71.4 *d*	OAc	170.2 *s*
7β	~1.90 ‡	19A	4.19 *br d*	6β,7α	11.2	7	32.8 *t*		169.8 *s*
8β	~1.55 ‡	19B	5.04 *d*	6β,7β	4.6	8	33.8 *d*		21.2 *q*
10β	2.32 *d*	Me-20	0.90 *s*	7α,7β	11.2	9	41.8 *s*		21.1 *q*
11α	4.46 *dd*	OAc	2.11 *s*	7α,8β	11.2	10	52.0 *d*	OTig	
12A	§		1.95 *s*	8β,17	6.8	11	84.1 *d*	1′	166.5 *s*
12B	§	OTig ¶		11α,12A	10.6	12	33.6 *t*	2′	129.2 *s*
13β	2.66 *m***	3′	6.82 *qq*	11α,12B	6.5	13	41.9 *d*	3′	137.6 *d*
14A	§	Me-4′	1.80 *dq*	13β,16β	5.1	14	32.8 *t*	4′	14.5 *q*
14B	§	Me-5′	1.85 *br s*	18A,18B	4.2	15	67.8 *t*	5′	12.1 *q*
				19A,19B	12.7				
				19A,6β	<0.3				

* At 500 MHz (^1H) and 125.7 MHz (^{13}C), $CDCl_3$. Chemical shifts (δ values) are relative to residual $CHCl_3$ for ^1H (δ_{CHCl_3} 7.25) and to the solvent for ^{13}C (δ_{CDCl_3} 77.00). Carbons bearing hydrogens were assigned from the HMQC spectrum, which also provided an approximate δ value for some overlapped protons.

† $W_{1/2}$ = 15 Hz.

‡ Partially overlapped signal.

§ These protons resonate between δ 2.05 and 1.40 as overlapped signals.

¶ *J* values (Hz): 3′,4′ = 6.8, 3′,5′ = 1.5, 4′,5′ = 1.0.

** $W_{1/2}$ = 21 Hz.

The location of the tigloyloxy and the two acetoxyl groups (δ_H 2.11 *s* and 1.95 *s*, both 3H; δ_C 170.2 *s*, 169.8 *s*, 21.2 *q* and 21.1 *q*) in **1** was deduced as follows. Comparison of the ^{13}C NMR spectra of **1** (Table 1) and **2** [12] revealed that the C-1, C-3, C-6 and C-19 carbons appeared at the same field in both compounds (**1**, δ 69.6, 64.1, 71.4 and 61.7; **2**, δ 69.5, 63.7, 71.3 and 61.5, respectively), thus establishing that the three ester groups of **1** must be attached to the C-1, C-6 and C-19 positions, as in **2**. The chemical shifts of the H-6β, C-19 methylene and acetoxyl protons of **1** and **2** were identical (**1**, δ 4.82, 4.19, 5.04, 2.11 and 1.95; **2**, δ 4.80, 4.15, 5.00, 2.10 and 1.90, respectively), whereas the H-1α proton of **1** (δ 5.80) was downfield shifted with respect to that of **2** (δ 5.62). These data indicated that the two acetoxyl groups of ajugorientin (**1**) are at the C-6 and C-19 carbons and, consequently, that its tigloyloxy substituent is at the C-1 position, because it is known [14, 15] that the geminal proton of a tigloyloxy group appears paramagnetically shifted with respect to that of a 2-methylbutyrate. Moreover, like ajugareptansin (**2**) and other neo-clerodane derivatives possessing a hexahydrofurofuran side chain at C-9β and a bulky substituent at C-1β, ring A of ajugorientin (**1**) adopts a distorted boat conformation (Table 1, see the coupling values between the H-1α, H-2α, H-2β and H-3α protons) to minimize these steric interactions [10, 11, 15]. This is also in agreement with the presence in **1** of a 1β-tigloyloxy substituent instead of an acetoxyl group.

The absolute stereochemistry of the new diterpenoid was not ascertained. However, on biogenetic grounds, it is reasonable to assume that it possesses a neo-clerodane absolute configuration [1], like other clerodane derivatives isolated from *Ajuga* species [2, 5, 10, 15].

EXPERIMENTAL

General. Plant materials were collected in October 1994 at Alexander Mountain, Central Victoria, Australia (*A. australis*), and in April 1989 near Antalya, Turkey (*A. orientalis*). Voucher specimens of both plants are deposited in the Herbarium of the Dipartimento di Biologia, University of Milan, Italy.

Extraction and isolation of the diterpenoids. Dried and powdered aerial parts of *A. australis* R. Br. Prod. (1370 g) were extracted with Me_2CO (10 l) at room temp. for 7 days. After removal of the solvent, the residue (79 g) was subjected to CC (silica gel Merck No. 7734, deactivated with 15% H_2O, w/v, 400 g) eluting with a petrol–EtOAc gradient. Elution with petrol–EtOAc (4:1) gave ajugapitin (18 mg) [5–7] and elution with EtOAc–petrol (3:2) yielded 36 mg of 14,15-dihydro-15-hydroxyajugapitin [8, 9]. These previously known compounds were identified by conventional methods, by comparison with data already described in the literature and, in the case of ajugapitin, by direct comparison (mmp, TLC) with an authentic sample [5].

Dried and powdered aerial parts of *A. orientalis* L. (430 g) were extracted as above yielding an extract (11

g) which was chromatographed as above. Elution with EtOAc-petrol (1:1) gave impure **1** (30 mg). Rechromatography (silica gel and Et_2O-petrol 2:1) yielded ajugorientin (**1**, 10 mg) as a white amorphous powder.

Ajugorientin (**1**). $[\alpha]_D^{24}$ −23.7° ($CHCl_3$; *c* 0.392). IR ν_{max}^{KBr} cm^{-1}: 3470 (OH), 3080, 1710, 1650 (tigloyloxy), 1740, 1250 (OAc), 2930, 2880, 1460, 1370, 1140, 1070, 1030, 920, 900; ^1H NMR: Table 1; ^{13}C NMR: Table 1; EIMS (70 ev, direct inlet) *m/z* (rel. int.): 550 $[M]^+$ (0.02), 521 (0.08), 451 $[M\text{-}OTig]^+$ (0.1), 450 $[M\text{-}HOTig]^+$ (0.1), 421 (0.7), 380 (0.3), 243 (0.7), 201 (2), 159 (3), 113 [C-9 side chain]$^+$ (91), 83 $[Tig]^+$ (43), 69 (78), 55 (58), 43 (100). (Found: C, 63.38; H, 7.56. $C_{29}H_{42}O_{10}$ requires: C, 63.25; H, 7.69%.)

Acknowledgements—We thank Dr Claude C. Culvenor (Chemistry Department, La Trobe University, Bundoora, Australia) for help in obtaining the plant material of *A. australis*. This work was supported by research grants from MURST-CNR (Italy), DGICYT (Spain, PB93-0154) and the bilateral project CNR-CSIC between the ICTPN (Italy) and the Instituto de Química Orgánica (Spain).

REFERENCES

1. Rogers, D., Unal, G. G., Williams, D. J., Ley, S. V., Sim, G. A., Joshi, B. S. and Ravindranath, K. R., *Journal of the Chemical Society, Chemical Communications*, 1979, 97.
2. Merritt, A. T. and Ley, S. V., *Natural Products Reports*, 1992, **9**, 243.
3. Rodríguez-Hahn, L., Esquivel, B. and Cárdenas, J., *Progress in Chemistry of Organic Natural Products*, 1994, **63**, 107.
4. Camps, F. and Coll, J., *Phytochemistry*, 1993, **32**, 1361.
5. Hernández, A., Pascual, C., Sanz, J. and Rodríguez, B., *Phytochemistry*, 1982, **21**, 2909.
6. Boneva, I. M., Mikhova, B. P., Malakov, P. Y., Papanov, G. Y., Duddeck, H. and Spassov, S. L., *Phytochemistry*, 1990, **29**, 2931.
7. Camps, F., Coll, J. and Dargallo, O., *Phytochemistry*, 1984, **23**, 2577.
8. Camps, F., Coll, J. and Dargallo, O., *Phytochemistry*, 1984, **23**, 3213.
9. Camps, F., Coll, J., Dargallo, O., Rius, J. and Miravitlles, C., *Phytochemistry*, 1987, **26**, 1475.
10. Camps, F., Coll, J., Cortel, A. and Messeguer, A., *Tetrahedron Letters*, 1979, 1709.
11. Camps, F., Coll, J. and Cortel, A., *Chemistry Letters*, 1981, 1093.
12. Camps, F., Coll, J. and Cortel, A. *Chemistry Letters*, 1982, 1053.
13. Joseph-Nathan, P., Wesener, J. R. and Günther, H., *Organic Magnetic Resonance*, 1984, **22**, 190.
14. Malakov, P. Y., Papanov, G. Y., de la Torre, M. C. and Rodríguez, B., *Phytochemistry*, 1991, **30**, 4083.
15. Malakov, P. Y., Papanov, G. Y., Perales, A., de la Torre, M. C. and Rodríguez, B., *Phytochemistry*, 1992, **31**, 3151.