Phytochemistry, Vol. 21, No. 12, pp. 2909–2911, 1982.
Printed in Great Britain.

0031-9422/82/122909-03$03.00/0

DITERPENOIDS FROM *AJUGA CHAMAEPITYS*: TWO *NEO*-CLERODANE DERIVATIVES

AMPARO HERNÁNDEZ, CONRAD PASCUAL, JESÚS SANZ and BENJAMÍN RODRÍGUEZ

Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, Madrid-6, Spain

(*Received* 22 *April* 1982)

Key Word Index—*Ajuga chamaepitys*; Labiatae; diterpenoids; *neo*-clerodane derivatives; ajugapitin; dihydro-ajugapitin; Horeau's method.

Abstract—From the whole plant of *Ajuga chamaepitys* two new *neo*-clerodane diterpenoids, ajugapitin and its dihydro derivative, have been isolated. Their structures were established mainly by spectroscopic means.

INTRODUCTION

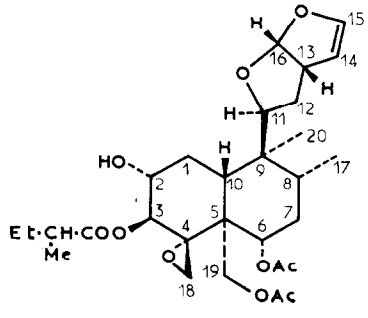
In our search for new natural diterpenoids in the Labiatae plants[1–4], we have examined the whole plant of *Ajuga chamaepitys*, a species which grows throughout Europe. From this plant two new diterpenoids have been isolated. Their structures and absolute configurations (**1** and **2**) were established on the basis of spectroscopic evidence, chemical correlation, application of Horeau's method and by comparison with closely related compounds.

RESULTS AND DISCUSSION

The first of the new diterpenoids, ajugapitin (**1**), had a $C_{29}H_{42}O_{10}$ molecular formula and its IR spectrum was consistent with the presence of a hydroxyl group (3500 cm^{-1}), three ester groups (1740, 1735, 1725 and 1250 cm^{-1}), a vinyl ether function (3030, 1620 and 730 cm^{-1}) and an oxirane ring (3080 cm^{-1}).

The ^1H NMR spectrum of ajugapitin (**1**) showed signals for two acetate groups (δ 2.13 and 1.94) and for a 2-methylbutyric ester function (δ 2.37, 1H, *sextet*, $J = 6.9$ Hz; 1.12, 3H, *d*, $J = 6.9$ Hz; 0.90, 3H, *t*, $J = 7$ Hz). The presence of this last function in the molecule of ajugapitin was also supported by the appearance of a fragment ion at *m/z* 448 in its mass spectrum[5]. In addition, the ^1H NMR spectrum of ajugapitin (**1**) (Table 1) showed signals of a tertiary methyl group at δ 0.98 (*s*), of a secondary methyl group at 0.85 (*d*, $J = 7$ Hz) and of an olefinic proton at 4.81 (*dd*, $J_1 = 2.9$ Hz, $J_2 = 2.8$ Hz). The following signals due to 10 protons on carbon atoms bearing oxygen atoms could also be seen: 6.47 (1H, *dd*, $J_1 = 2.8$ Hz, $J_2 = 2$ Hz, vinyl ether proton), 6.05 (1H, *d*, $J = 6.1$ Hz), 5.24 (1H, *d*, $J = 9.9$ Hz), 4.79 and 4.41 (AB system, $J = 12.4$ Hz), 4.70 (1H, *dd*, $J_1 = 11.3$ Hz, $J_2 = 4.6$ Hz), 4.03 (1H, *dd*, $J_1 = 11.3$ Hz, $J_2 = 4.6$ Hz), 3.65 (1H, *septuplet*, $J_1 \cong J_2 \cong 10$ Hz, $J_3 \cong J_4 \cong 5$ Hz) and 2.80 and 2.57 (AB system of an α, α-disubstituted oxirane ring, $J = 4.3$ Hz). The signals at δ 5.24 and 2.80 are reciprocally long-range coupled ($J < 0.5$ Hz), which was revealed by double resonance experiments. In addition, the ^1H NMR spectrum of ajugapitin (**1**) showed a one-proton multiplet at δ 3.56 ($W_{1/2} = 15$ Hz), a one-proton doublet of double doublets at 2.59 ($J_1 = 12$ Hz, $J_2 = 5$ Hz, $J_3 = 2.6$ Hz) and a one-proton doublet at 2.11 ($J = 5$ Hz).

In order to establish the relative arrangement of the protons, a series of proton-decoupling experiments was carried out. As a result of irradiation at δ 6.47, the olefinic proton double doublet at 4.81 collapsed into a doublet ($J = 2.9$ Hz). Irradiation at 4.81 transformed the double doublet at 6.47 into a doublet ($J = 2$ Hz) and at the same time the multiplet at 3.56 was clearly modified. Irradiation at 6.05 also modified the multiplet at 3.56, and on irradiation at this last signal, the double doublet at 6.47 collapsed into a doublet ($J = 2.8$ Hz), the doublet at 6.05 appeared as a singlet and the olefinic proton double doublet at 4.81 collapsed into a doublet ($J = 2.8$ Hz). Furthermore, by irradiating at 3.65, the doublets at 5.24 and 2.11 collapsed into singlets and the doublet of double doublets signal at 2.59 was transformed into a double doublet ($J_1 = 12$ Hz, $J_2 = 2.6$ Hz). Finally, irradiation at 2.37 transformed the doublet signal at 1.12 into a singlet, thus confirming the presence of a 2-methylbutanoate moiety in the molecule of ajugapitin (**1**).



1

2 14,15-DIHYDRO

Table 1. ^{1}H NMR spectral data of compounds **1** and **2** (270 or 90 MHz, $CDCl_3$, chemical shifts in δ-values from TMS)

	1	2
H-1β	2.59 *ddd*	*
H-2β	3.65 *ddd*	3.63 *m*
H-3α	5.24 *d*	5.23 *d*
H-6β	4.70 *dd*	4.69 *dd*
H-11α	4.03 *dd*	4.11 *dd*
H-13β	3.56 *m*	*
H-14	4.81 *dd*	*
H-15	6.47 *dd*	3.87 *m*†
H-16	6.05 *d*	5.67 *d*
3H-17	0.85 *d*	0.88 *d*
H-18	2.80 *br d*	2.80 *br d*
H-18′	2.57 *d*	2.56 *d*
H-19	4.79 *d*	4.77 *d*
H-19′	4.41 *br d*	4.40 *br d*
3H-20	0.98 *s*	0.98 *s*
MeCOO–	2.13 *s*	2.13 *s*
	1.94 *s*	1.95 *s*
CH_2–Me | Me–CH–COO–	—	—
Me-4′	0.90 *t*	0.90 *t*
Me-2″	1.12 *d*	1.12 *d*
CH-2′	2.37 *sextet*	*

J (Hz): 1β, 1α = 12; 1β, 2β = 5; 1β, 10β = 2.6; 2β, 1α = 10; 2β, 3α = 9.9; 6β, 7α = 11.3; 6β, 7β = 4.6; 8, 17 = 7; 11α, 12 = 11.3; 11α, 12′ = 4.6; 13β, 14 = 2.9; 13β, 15 = 2; 13β, 16β = 6.1; 14, 15 = 2.8; 18, 18′ = 4.3; 18, 3α < 0.5: 19, 19′ = 12.4; 19′, 6β < 0.5. 2-Methylbutyric ester: 2′, 2″ = 6.9; 4′, 3′ = 7.

*Signal not measured.

†A 2H signal.

On the other hand, when the ^{1}H NMR spectrum of ajugapitin (**1**) was obtained under D_2O exchange, the disappearance of the signal at δ 2.11 and a modification of the multiplicity of the signal at 3.65 (now a *sextet*, $J_1 \cong J_2 \cong 10$ Hz, $J_3 = 5$ Hz) were observed. Thus, the signal at 3.65 must be assigned to the geminal proton of a secondary hydroxyl group, which is responsible for the absorption at 3500 cm^{-1} in the IR spectrum of ajugapitin (**1**) (see above).

On the basis of these results, and in agreement with structure **1** for ajugapitin, the following assignment for the protons could be made. The signals at δ 6.47, 6.05, 4.81, 4.03 and 3.56 were assigned to the protons at C-15, C-16, C-14, C-11 and C-13, respectively. An identical behaviour for this tetrahydrofuro-furan ring has been previously found in several *neo*-clerodane diterpenoids [6–11] whose structures have been firmly established by X-ray analysis. The methyl signals at 0.98 and 0.85 were assigned to the C-20 and C-17 protons, respectively. The AB system at 4.79 and 4.41 was assigned to the protons of the C-19 esterified hydroxymethylene group, and the signals at 5.24 and 4.70 were attributed to the 3α-axial and 6β-axial protons, respectively, which are attached to carbon atoms bearing esterified secondary hydroxyl groups. The AB system at 2.80 and 2.57 was assigned to the C-18 oxirane protons, one of which (signal at 2.80) is long-range coupled with the 3α-axial proton (see above). This behaviour has been previously observed in some *neo*-clerodane diterpenoids isolated from *Ajuga* species, as ajugarin-I [12] and ajugamarin [13]. The signal of the geminal proton of the free hydroxyl group (δ 3.65) was assigned to the 2β-axial proton, because it is *trans*-diaxially coupled with the 3α-H ($J_{2\beta,3\alpha} = 9.9$ Hz). Finally, the signal at 2.59 was assigned to the 1β-equatorial proton ($J_{1\beta,1\alpha} = 12$ Hz, $J_{1\beta,2\beta} = 5$ Hz, $J_{1\beta,10\beta} = 2.6$ Hz) in a 2α-hydroxy-*neo*-clerodane skeleton [14, 15].

The location of the two acetyl groups at the C-6 and C-19 positions is supported by the ^{1}H NMR data of ajugapitin (**1**) (see above and Table 1), which showed for their C-6, C-17, C-19 and C-20 protons and for the two acetates identical values to the ones reported for several diterpenoids [5, 6, 8, 12, 13, 16–19] having a ring B identical to ajugapitin (**1**). On the other hand, the chemical shift of the 3α-axial proton of compound **1** (δ 5.24) agrees with the reported values for 3β-(2-methyl)-butanoic [20] or 3β-(2-acetoxy-2-methyl)-butanoic [6, 8] ester groups in 2-hydroxy-4α,18-epoxy-clerodane derivatives. The attachment of the acetyl groups at the C-6 and C-19 positions and the 4α-configuration of the oxirane ring in the molecule of ajugapitin (**1**) are also supported on the basis of biogenetic considerations, because all the diterpenoids isolated until now from *Ajuga* species possess these structural features [5, 10–13, 17, 20].

Finally, the *neo*-clerodane absolute configuration [10] of ajugapitin (**1**) was established by application of the Horeau method [21], which defined as *R* the absolute stereochemistry of its C-2 equatorial alcohol (see Experimental).

The 14, 15-dihydro derivative (**2**) of ajugapitin (**1**) was also present in *Ajuga chamaepitys*. The ^{1}H NMR data of compound **2** (Table 1) supported this structure. Moreover, hydrogenation of ajugapitin (**1**) [16] yielded a product identical in all respects to compound **2**. The molecular rotation difference between compounds **2** and **1** ($\Delta[\phi]_D + 165.9$) also confirmed the *neo*-clerodane absolute configuration of these diterpenoids [9]. In fact, compound **2** is the C-2 epimer of ivain-4, a *neo*-clerodane diterpenoid isolated from *Ajuga iva* [20].

The configuration at the C-2 carbon atom of the 2-methylbutyric ester moiety of these diterpenoids (compounds **1** and **2**) was not ascertained. However, for biogenic reasons, we suppose that it is *S*, as has been established for some *Ajuga* diterpenoids by X-ray analysis [5, 17, 20].

Since the *neo*-clerodane diterpenoids isolated from *Ajuga* species are insect antifeedants or have antitumour, antimicrobial, or antifungal properties, interest in them has grown rapidly of late.

EXPERIMENTAL

Mps were determined on a Kofler apparatus and are uncorr. Elemental analyses were carried out in our Institute with the help of an automatic analyser. Plant materials were collected in August 1981, in Villamoñico (Valderredible, Santander, Spain) and voucher specimens were deposited in the Herbarium of the Faculty of Biology (Oviedo University, Spain).

Extraction and isolation of the diterpenoids. Dried and finely powdered *S. chamaepitys* (L.) Schreber whole plants (2.1 kg) were extracted with EtOH (10 l.) at room temp. for 1 week. After filtration the solvent was evaporated yielding a gum (101 g) which was subjected to dry-CC over Si gel (900 g, Merck No. 7734, deactivated with 15% H_2O). Elution with *n*-hexane–$CHCl_3$ (1:1) yielded ajugapitin (**1**, 76 mg) and elution with $CHCl_3$ gave dihydro-ajugapitin (**2**, 32 mg).

Ajugapitin (**1**). Mp 196–198° (from Et_2O–*n*-hexane); $[\alpha]_D^{20}$ −70.3° ($CHCl_3$; *c* 0.256); IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 3080 (oxirane), 1740, 1735, 1725, 1250 (ester groups), 3030, 1620, 730 (vinyl ether), 2990, 2950, 2920, 2890, 1465, 1390, 1370, 1205, 1145, 1095, 1000, 950. ^1H NMR (270 MHz, $CDCl_3$): see Results and Discussion and Table 1. EIMS (75 eV, direct inlet) *m/z* (rel. int.): 550 $[M]^+$ (1), 521 (0.2), 448 (0.5), 440 (0.4), 435 (0.3), 388 (0.8), 381 (0.5), 375 (0.8), 339 (3.6), 331 (2.3), 325 (5), 187 (11), 169 (10), 111 (34, tetrahydrofuro-furan fragment ion), 85 (25), 83 (12), 81 (13), 69 (11), 57 (75), 55 (14), 43 (100), 41 (18). (Found: C, 63.10; H, 7.52. $C_{29}H_{42}O_{10}$ requires: C, 63.25; H, 7.69%.)

Dihydo-ajugapitin (**2**). Mp 212–214° (from EtOAc–Et_2O); $[\alpha]_D^{20}$ −40.0° ($CHCl_3$; *c* 0.254); IR ν_{max}^{KBr} cm^{-1}: 3495 (OH), 3080 (oxirane), 1740, 1725 *br*, 1275, 1250 (ester groups), 2990, 2950, 2890, 1460, 1370, 1180, 1150, 1030, 975, 890, 830. ^1H NMR (90 MHz, $CDCl_3$): see Table 1. EIMS (75 eV, direct inlet) *m/z* (rel. int.): 552 $[M]^+$ (0.05), 493 (0.05), 451 (1), 450 (0.05), 437 (0.1), 423 (0.1), 421 (0.1), 339 (1), 325 (1.5), 218 (3), 200 (3), 187 (4), 113 (100, hexahydrofuro-furan fragment ion), 85 (7), 83 (5), 55 (10), 69 (25), 57 (30), 43 (25). (Found: C, 63.19; H, 7.96. $C_{29}H_{44}O_{10}$ requires: C, 63.02; H, 8.03%.)

Application of the Horeau method [21] *to compound* **1**. A mixture of (±)-α-phenylbutyric anhydride (0.232 mmol) and ajugapitin (**1**, 0.082 mmol) in pyridine soln (2 ml) was kept at room temp. for 18 hr; $\alpha_1 = -2.580$; $\alpha_2 = -2.456$; $\alpha_1 - 1.1\alpha_2 = +0.121$, configuration 2*R*.

Dihydro-ajugapitin (**2**) *from ajugapitin* (**1**). Ajugapitin (**1**, 16 mg) in EtOAc (10 ml) was hydrogenated over 5% Pd/C (5 mg) as previously described for clerodin [16]. Work-up in the usual manner yielded a compound identical in all respects (mp, mmp, IR, MS and $[\alpha]_D$) with natural dihydro-ajugapitin (**2**).

Acknowledgements—We thank Dr. J. Borja, Department of Botany, Faculty of Pharmacy, Madrid, for botanical classification of the plant material. This work was supported in part by the 'Comisión Asesora de Investigación Científica y Técnica', Madrid (Grant No. 11/81).

REFERENCES

1. García-Alvarez, M. C., Marco, J. L., Rodríguez, B., Savona, G. and Piozzi, F. (1982) *Phytochemistry* **21**, 2559.
2. Marco, J. L., Rodríguez, B., Savona, G. and Piozzi, F. (1982) *Phytochemistry* **21**, 2567.
3. Eguren, L., Perales, A., Fayos, J., Rodríguez, B., Savona, G. and Piozzi, F. *J. Org. Chem.* (in press).
4. Savona, G., Bruno, M., Paternostro, M., Marco, J. L. and Rodríguez, B. (1982) *Phytochemistry* **21**, 2563.
5. Camps, F., Coll, J. and Cortel, A. (1981) *Chem. Letters* 1093.
6. Kato, N., Shibayama, S., Munakata, K. and Katayama, C. (1971) *J. Chem. Soc. Chem. Commun.* 1632.
7. Kato, N., Munakata, K. and Katayama, C. (1973) *J. Chem. Soc. Perkin Trans.* 2, 69.
8. Kato, N., Shibayama, M. and Munakata, K. (1973) *J. Chem. Soc. Perkin Trans.* 1, 712.
9. Harada, N. and Uda, H. (1978) *J. Am. Chem. Soc.* **100**, 8022.
10. Rogers, D., Unal, G. G., Williams, D. J., Ley, S. V., Sim, G. A., Joshi, B. S. and Ravindranath, K. R. (1979) *J. Chem. Soc Chem. Commun.* 97.
11. Kubo, I., Kido, M. and Fukuyama, Y. (1980) *J. Chem. Soc. Chem. Commun.* 897.
12. Kubo, I., Lee, Y-W., Balogh-Nair, V., Nakanishi, K. and Chapya, A. (1976) *J. Chem. Soc. Chem. Commun.* 949.
13. Shimomura, H., Sashida, Y., Ogawa, K. and Iitaka, Y. (1981) *Tetrahedron Letters* **22**, 1367.
14. Stapel, G., Menssen, H. G. and Snatzke, G. (1980) *Planta Med.* **38**, 366.
15. Bruno, M., Savona, G., Pascual, C. and Rodríguez, B. (1981) *Phytochemistry* **20**, 2259.
16. Barton, Sir D. H. R., Cheung, H. T., Cross, A. D., Jackman, L. M. and Martin-Smith, M. (1961) *J. Chem. Soc.* 5061.
17. Camps, F., Coll, J., Cortel, A. and Messeguer, A. (1979) *Tetrahedron Letters* 1709.
18. Hosozawa, S., Kato, N. and Munakata, K. (1973) *Phytochemistry* **12**, 1833.
19. Hosozawa, S., Kato, N. and Munakata, K. (1974) *Phytochemistry* **13**, 308 and 1019.
20. Camps, F., Coll, J. and Cortel, A. (1981) *Rev. Latinoam. Quim.* **12**, 81.
21. Horeau, A. and Nouaille, A. (1971) *Tetrahedron Letters* 1939.