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# *NEO*-CLERODANE DITERPENOIDS FROM *AJUGA PSEUDOIVA*

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**Key Word Index**—*Ajuga pseudoiva*; Labiatae; diterpenoids; neo-clerodane derivatives; 2-acetylivain I; dihydro-ajugapitin.

**Abstract**—From the whole plant of *Ajuga pseudoiva* two epimeric *neo*-clerodane diterpenoids, 2-acetylivain I and its C-2 epimer 14,15-dihydro-ajugapitin, have been isolated. The structures were established by spectroscopic and chemical means. 2-Acetylivain I had not been previously reported, while its C-2 epimer had been isolated from *Ajuga chamaepitys*.

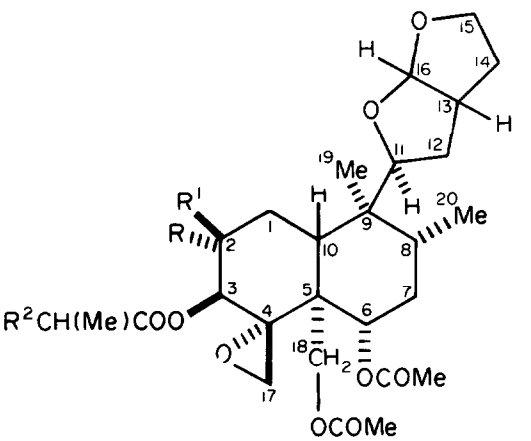
## INTRODUCTION

In our search for new clerodane diterpenoids with potential insect antifeedant activity in *Ajuga* plants [1–3], we have examined the whole plant of *A. pseudoiva* (L.) Schreber. Two clerodane diterpenoids were isolated and their structures (**1** and **3**) established by spectral and chemical correlations with closely related compounds, previously described by us [1–3] and other research groups [4–7].

## RESULTS AND DISCUSSION

The first of these diterpenoids, 2-acetylivain I (**1**), $[\alpha]_D^{20}$ +15.9 ($CHCl_3$; *c* 0.384), had a molecular formula of $C_{30}H_{44}O_{11}$ (580 $[M]^+$), and its IR spectrum revealed the presence of ester groups (1740 and 1240 $cm^{-1}$) and the absence of free hydroxyl groups. The $^1H$ NMR spectrum of compound **1** showed signals attributable to three acetate groups ($\delta$2.12, 2.06 and 1.93) and to a 2-methylpropanoic ester function ($\delta$2.40, 1H, *heptaplet*, $J$ = 6.9 Hz; $\delta$1.09, 6H, *d*, $J$ = 6.9 Hz). The presence of this last function was also confirmed by signals at $\delta$175.1, 35.8, 18.7 and 18.6 in the corresponding $^{13}C$ NMR spectrum. Furthermore, the $^1H$ NMR spectrum of **1** showed absorptions of a tertiary methyl group at $\delta$0.95 (*s*) and of a secondary methyl group at $\delta$0.88 (*d*, $J$ = 6.5 Hz). In addition, the following signals due to ten protons on oxygen-substituted carbon atoms could also be seen: $\delta$5.60 (1H, *d*, $J$ = 4.8 Hz), 5.48 (1H, *d*, $J$ = 3.7 Hz), 5.32 (1H, *br*), 4.82 (1H, *dd*, $J_1$ = 12.2 Hz, $J_2$ = 5.8 Hz), 4.77 and 4.42 (AB system, $J$ = 12.7 Hz), 4.07 (1H, *dd*, $J_1$ = 10.6 Hz, $J_2$ = 6.4 Hz), 3.85 (2H, *dd*, $J_1$ = 8 Hz, $J_2$ = 5.6 Hz) and 2.98 and 2.70 (AB system, $J$ = 4.2 Hz).

The presence of a hexahydrofurofuran system in **1** was inferred from the existence in its mass spectrum of the base peak at *m/z* 113, as well as fragment ions with high intensity at *m/z* 83, 81 and 69. The presence of metastable ion *m** 42.1 enabled the fragment ions at *m/z* 113 and 69 to be related as has been described for other clerodane diterpenoids [8]. The occurrence of the hexahydrofurofuran moiety was confirmed by the appearance in the $^1H$ NMR spectrum of **1** of an acetalic proton at $\delta$5.60 and of signals at $\delta$4.07 and 3.85 attributable, respectively, to C-11 and C-15 protons, and of signals at $\delta$106.3, 84.9 and 68.3 attributable, respectively, to C-16, C-11 and C-15 in the corresponding $^{13}C$ NMR spectrum. An identical absorption pattern for this hexahydrofurofuran ring has been previously found in several *neo*-clerodane diterpenoids, whose structures have been firmly established by X-ray analysis [4–6, 9–12].




| | R | R¹ | R² |
|---|---|---|---|
| 1 | H | Me COO | Me |
| 2 | H | HO | Me |
| 3 | HO | H | Et |
| 4 | H | HO | Et |

The above data suggested a *neo*-clerodane diterpenoid structure for compound **1**, related with those of ivains, previously isolated in this laboratory from *A. iva* [1, 2]. This is in agreement with what could be expected from taxonomic considerations. In fact, as shown in Table 1, the $^1H$ NMR spectral data of **1** revealed a close similarity with those of ivain I (**2**), except for the presence of a third acetyl group which should be located at C-2 to account for the

deshielding effect observed on the αH-2 absorption. Likewise, the presence of this group also explained the 2.9 ppm downfield and 1.5 and 2.0 ppm upfield shifts of the C-2, C-1 and C-3 absorptions, respectively, in the $^{13}C$ NMR spectrum of **1** when compared with the corresponding absorptions in the spectrum of **2** (cf. Table 2).

The structure of 2-acetylivain I for compound **1** was further confirmed by direct acetylation of **2** with acetic anhydride in pyridine and the complete identity of the spectral and chromatographic features of the resulting synthetic compound with those of the natural product.

14,15-Dihydroajugapitin (**3**) was also present in *A. pseudoiva*. The $^1H$ NMR, IR and mass spectral data, $R_f$ value on TLC and $[\alpha]_D^{20}$ −41.5° ($CHCl_3$; *c* 0.2) were in agreement with those of an authentic sample [7]. In addition, we recorded the previously unreported $^{13}C$ NMR spectrum of **3** which was compared with that of ivain IV (**4**) [1, 2], a natural C-2 epimer of **3**. As shown in Table 2, the $^{13}C$ NMR spectrum of **3** was in agreement with the structural assignment, exhibiting the expected effects due to the orientation change of the C-2 substituent [13]. Comparison of the chemical shifts differences, as depicted in Table 3, shows an excellent correlation for all positions except the γ-one. This could be rationalized by the conformational changes in ring A due to the difference in substitution pattern when compared with the model cyclohexanols.

The isolation of compounds **1** and **3** from *A. pseudoiva* is of interest because it reveals the co-existence in the same plant of two *neo*-clerodane diterpenoids epimeric at C-2. Thus 2-acetylivain I (**1**) has β-acetoxy group at C-2 whereas 14,15-dihydroajugapitin (**3**) has an α-hydroxyl group.

Table 1. $^1H$ NMR spectral data of compound **1** (80 MHz, $CDCl_3$, TMS as internal standard)

| H | 1 | 2 |
|---|---|---|
| 2α | 5.32 *br* | 4.18 *br* |
| 3α | 5.48 *d* | 5.48 *d* |
| 6β | 4.82 *dd* | 4.82 *dd* |
| 11α | 4.07 *dd* | 4.14 *dd* |
| 15(2H) | 3.85 *m* | 3.90 *m* |
| 16 | 5.60 *d* | 5.68 *d* |
| 17 | 2.98 *d* | 2.98 *d* |
| 17′ | 2.70 *d* | 2.74 *d* |
| 18 | 4.77 *d* | 4.76 *d* |
| 18′ | 4.42 *d* | 4.48 *d* |
| 19(3H) | 0.95 *s* | 0.98 *s* |
| 20(3H) | 0.88 *d* | 0.88 *d* |
| $C\underline{H}_3COO$– | 2.12 *s* | 2.14 *s* |
| | 2.06 *s* | |
| | 1.93 *s* | 1.94 *s* |
| $C\underline{H}_3CCOO$– | 1.09 *d* | 1.14 *d* |
| $C\underline{H}COO$– | 2.40 *h* | 2.54 *h* |

**1** *J* (Hz): 2α, 3α = 3.7; 6β, 7α = 12.2; 6β, 7β = 5.8; 11α, 12 = 10.6; 11α, 12′ = 6.4; 13β, 16β = 4.8; 17, 17′ = 4.2; 18, 18′ = 12.7; 8, 20 = 6.5; 2-methylpropionic ester: 2′, 3′ = 6.9 Hz. **2** *J* (Hz): 2α, 3α = 2.5; 6β, 7α = 11.5; 11α, 12 = 11.5; 13β, 16β = 4; 17, 17′ = 4; 18, 18′ = 12; 8, 20 = 6.5; 2-methylpropionic ester: 2′, 3′ = 7.

Table 2. $^{13}C$ NMR spectral data of compounds **1**–**4** (20 MHz, $CDCl_3$ TMS as internal standard)

| C | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 26.9 | 28.4 | 30.4 | 28.4 |
| 2 | 70.9 | 68.0 | 72.4 | 68.6 |
| 3 | 66.7 | 68.7 | 71.4 | 68.8 |
| 4 | 60.5 | 61.2 | 62.9 | 61.3 |
| 5 | 45.9 | 45.8 | 45.7 | 46.1 |
| 6 | 71.8 | 71.4 | 71.8 | 71.7 |
| 7 | 32.6 | 32.8 | 32.8 | 32.9 |
| 8 | 33.9 | 33.6 | 35.6 | 36.0 |
| 9 | 40.2 | 39.7 | 42.5 | 39.9 |
| 10 | 42.1 | 41.8 | 42.1 | 42.0 |
| 11 | 84.9 | 85.0 | 84.6 | 85.2 |
| 12 | 32.4 | 32.2 | 32.6 | 32.4 |
| 13 | 41.3 | 40.0 | 40.6 | 40.1 |
| 14 | 33.2 | 31.5 | 33.4 | 31.7 |
| 15 | 68.3 | 68.0 | 40.6 | 40.1 |
| 16 | 106.3 | 107.4 | 107.8 | 107.6 |
| 17 | 44.3 | 43.8 | 43.5 | 43.9 |
| 18 | 62.0 | 61.4 | 61.5 | 61.6 |
| 19 | 16.3 | 15.9 | 16.3 | 16.0 |
| 20 | 13.7 | 13.5 | 13.8 | 13.7 |
| $\underline{C}H_3COO$– | 21.0 | 20.9 | 21.0 | 21.0 |
| | 21.0 | 20.6 | 20.9 | 20.9 |
| | 20.6 | | | |
| $CH_3\underline{C}OO$– | 170.6 | 170.7 | 171.2 | 170.7 |
| | 170.0 | 169.8 | 170.2 | 169.8 |
| | 169.5 | | | |
| $CH\underline{C}OO$ | 175.1 | 174.8 | 175.7 | 175.3 |
| $\underline{C}HCOO$ | 35.8 | 35.7 | 41.2 | 40.9 |
| $\underline{C}H_3CHCO$ | 18.7 | 18.6 | 16.3 | 16.0 |
| | 18.6 | 18.5 | | |
| $\underline{C}H_2CHCO$ | — | — | 26.7 | 26.6 |
| $\underline{C}H_3CH_2CH$ | — | — | 11.2 | 11.1 |

Table 3. Chemical shift difference comparison for axial or equatorial substitution in C-2

| Δδ | α(2) | β(1) | β(3) | γ(4) | γ(10) | δ(5) | δ(9) | δ(17) |
|---|---|---|---|---|---|---|---|---|
| Calc. | −5.4 | −2.4 | −2.4 | −4.7 | −4.7 | 0.9 | 0.9 | 0.9 |
| Found | −3.8 | −2.0 | −2.6 | −1.6 | −0.1 | 0.4 | −2.6 | 0.4 |

## EXPERIMENTAL

EIMS (DI method), 70 eV; CC: silica gel 60 (70–230 mesh; Merck), silica gel $HF_{254}$ (Merck) and $Al_2O_3$ 90 [70–230 mesh; Merck; activity I (Brockmann Scale)].

Plant materials were collected in August 1982 in Totana (Murcia, Spain) and voucher specimens were deposited at the Herbarium of the Facultad de Farmacia (Universidad Complutense, Madrid, Spain) and identified by Dr. J. Borja.

*Extraction and isolation of the diterpenoids.* Dried and finely powdered whole plants (570 g) were extracted with $Et_2O$ (4 l.) at 35° for 1 week. The solvent was evapd yielding a gum (14 g) which was treated with $Me_2CO$ to precipitate the accompanying waxes. After filtration, the solvent was evapd yielding a residue (9.60 g) which was fractionated by dry CC over silica gel (180 g) eluted with 600 ml hexane–EtOAc (5:1), 400 ml hexane–EtOAc (5:2, 5:3, 5:4, 1:1, 1:2), 800 ml EtOAc, and 800 ml MeOH. A fraction enriched in diterpenoid **1** was eluted with the hexane–EtOAc (1:1) and (1:2) systems, and a fraction enriched in diterpenoid **3** was eluted with EtOAc. Both fractions were purified separately by successive CC over silica gel H and $Al_2O_3$ gel eluted with hexane–EtOAc (2:1, 1:1, 1:2 and 0:1) yielding finally 2-acetylivain I (**1**) (5 mg) and 14,15-dihydroajugapitin (**3**) (20 mg).

*2-Acetylivain I* (**1**). $[\alpha]_D^{20}$ +15.9° ($CHCl_3$; *c* 0.384); IR $\nu_{max}^{CCl_4}$ $cm^{-1}$: 3080 (oxirane), 1740, 1240 (ester groups), 1150, 1020; $^1H$ NMR (80 MHz): see Table 1; $^{13}C$ NMR (20 MHz): see Table 2; MS *m/z* (rel. int.): 580 $[M]^+$ (0.01), 520 (0.05), 451 (0.1), 437 (0.1), 432 (0.04), 421 (0.1), 408 (0.3), 378 (0.3), 335 (0.2), 312 (0.13), 293 (0.4), 257 (0.8), 248 (0.5), 230 (1), 218 (0.9), 217 (1, 1), 200 (1.6), 187 (6), 169 (3.4), 114 (11), 113 (100, hexahydrofurofuran fragment ion), 85 (0.7), 83 (9), 69 (43), 57 (4), 55 (7), 43 (21).

*14,15-Dihydroajugapitin* (**3**). $[\alpha]_D^{20}$ −41.5° ($CHCl_3$; *c* 0.2); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3495 (OH), 3080 (oxirane), 1740, 1240 (ester groups), 1150, 1020; $^{13}C$ NMR (20 MHz): see Table 2; MS *m/z* (rel. int.): 552 $[M]^+$ (0.3), 493 (0.3), 451 (0.8), 450 (0.05), 437 (0.1), 423 (0.1), 421 (0.8), 339 (1.5), 325 (1), 218 (8), 200 (8.8), 187 (17), 113 (100, hexahydrofurofuran fragment ion), 85 (32), 83 (31), 69 (32), 57 (20), 55 (21), 43 (33).

*Acetylation of* **2**. Compound **2** (26 mg) was dissolved in 0.3 ml dry $C_5H_5N$ adding immediately 0.3 ml $Ac_2O$ and the mixture was stirred at room temp. for 6 hr. MeOH (1 ml) was added and after 10 min the solvent was evapd yielding a mixture of 2-acetylivain I and ivain I. The acetate was purified by CC over silica gel H and eluted with hexane–EtOAc (1:1) yielding finally 15 mg 2-acetylivain I.

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