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NEO-CLERODANE DITERPENOIDS FROM *AJUGA CHAMAEPITYS*

FRANCISCO CAMPS, JOSE COLL and ORIOL DARGALLO

Instituto de Química Bio-Orgánica, C S I C, Jorge Girona Salgado, 16-22, Barcelona-34, Spain

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Key Word Index—*Ajuga chamaepitys*, Labiatae, diterpenoids, *neo*-clerodane derivatives, 15-ethoxy-14-hydroajugapitin, 14-hydro-15-hydroxyajugapitin

Abstract—From the whole plant of *Ajuga chamaepitys*, 15-ethoxy-14-hydroajugapitin and a C-15 epimeric mixture of 14-hydro-15-hydroxyajugapitin have been isolated

INTRODUCTION

Recently, Hernández *et al* [1] isolated two *neo*-clerodane diterpenoids, ajugapitin (**1**) and 14,15-dihydroajugapitin (**2**), from *Ajuga chamaepitys* L In the context of our ongoing interest in potential insect antifeedants, we have continued the study of this plant since compound **2** is the C-2 epimer of ivain IV (**6**), a clerodane diterpenoid, previously characterized in our laboratory from *A iva* [2], with high antifeedant activity against larvae of Egyptian cotton leafworm *Spodoptera littoralis* [X Bellés, F Camps, J Coll, A. Cortel, O Dargallo and M D Piulachs, unpublished work] We have recently reported the simultaneous occurrence of both types of C-2 epimers in *A pseudoiva* [3]

In the present communication, we report on the isolation and identification from *A chamaepitys* of two new clerodane structures, 15-ethoxy-14-hydroajugapitin (**3**) and a C-15 epimeric mixture of 14-hydro-15-hydroxy-ajugapitin (**4**), which are closely related to **1** and **2**

RESULTS AND DISCUSSION

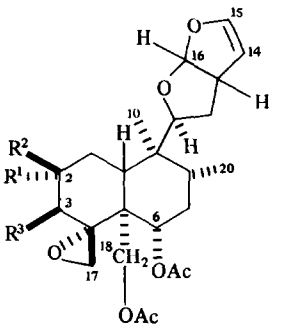
The first of these diterpenoids, 15-ethoxy-14-hydro-ajugapitin (**3**), had a molecular formula of $C_{31}H_{48}O_{11}$ (549 60168 $[M-EtOH]^+$), and its IR spectrum was consistent with the presence of a free hydroxyl group (3450 cm^{-1}) and ester groups (1730 and 1250 cm^{-1})

The ^1H NMR spectrum of **3** showed signals for two acetate groups (δ2 12 and 1 94) and for a 2-methylbutyric ester function (δ0 94, 3H, *t*, $J = 4$ 5 Hz, 1 17, 3H, *d*, $J = 7$ Hz, 2 45, 1H, *sextet*, $J = 6$ 0 Hz) as present in dihydroajugapitin (**2**) (Table 1) It also exhibited the following

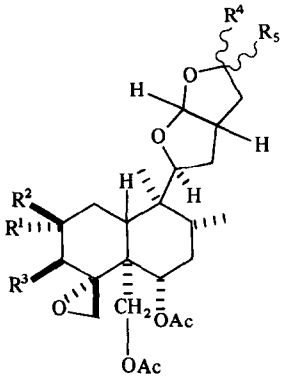
Table 1 ^1H NMR spectral data of compounds **2** and **3** (80 MHz, $CDCl_3$, TMS as int standard) and compounds **5** and **7** (200 MHz, $CDCl_3$, TMS as int standard)

H	2	3	5	7
2	3 63 *m*	3 65 *br*		4 18 *br*
3	5 23 *d* (9 9)*	5 22 *d* (9 9)	5 95 *s*	5 44 *d* (2)
6	4 69 *dd* (11 3, 5)	4 80 *dd* (11, 5)	4 80 *dd* (11, 5)	4 80 *dd* (10, 5)
11	4 11 *dd* (11 3, 5)	4 48 *dd* (11, 5)	4 12 *dd* (11 5, 5)	4 48 *dd* (11, 6)
15	3 87 *m*	5 09 *d* (5)		5 12 *d* (6)
16	5 68 *d* (5 5)	5 81 *d* (5 3)	6 09 *d* (5 6)	5 86 *d* (5)
17	2 56 and	2 58 and	2 74 and	2 70 and
	2 80 AB (4)	2 81 AB (4)	2 93 AB (4)	3 00 AB (3 5)
18	4 40 and	4 40 and	4 63 and	4 48 and
	4 77 AB (12 3)	4 82 AB (12)	4 92 AB (12 6)	4 78 AB (12)
19	0 98 *s*	0 98 *s*	1 06 *s*	0 98 *s*
20	0 85 *d* (7)	0 85 *d* (7 5)	0 89 *d* (4 4)	0 92 *d* (6 5)
$C\underline{H}_3CH_2CH(CH_3)CO$	0 90 *t* (6 9)	0 94 *t* (4 5)	0 94 *t* (4 5)	
$CH_3CH_2CH(C\underline{H}_3)CO$	1 12 *d* (7)	1 17 *d* (7)	1 17 *d* (6 8)	
$CH_3CH_2C\underline{H}(CH_3)CO$	2.45 *sextet* (6)	2 45 *sextet* (6)	2 45 *sextet* (6)	
$(C\underline{H}_3)_2CHCO$				1 16 *d* (6 5)
$(CH_3)_2C\underline{H}CO$				2 57 *heptuplet* (6)
$C\underline{H}_3CH_2O$		1 20 *t* (6 3)		1 20 *t* (6)
$CH_3C\underline{H}_2O$		3 40 and 3 60		3 44 and 3 80
		ABX_3 (8, 6)		ABX_3 (9, 6)

*J in Hz



1 Ajugapitin R^1 = OH, R^2=H R^3=OCO(Me)CHEt



		R^1	R^2	R^3	R^4	R^5
2	14, 15 – Dihydroajugapitin	HO	H	EtCH(Me)COO	H	H
3	15–Ethoxy –14 – hydroajugapitin	HO	H	EtCH(Me)COO	H	OEt
4	14 – Hydro –15 – hydroxyajugapitin	HO	H	EtCH(Me)COO	H	OH
5		–O–		EtCH(Me)COO	–O–	
6	Ivain – IV	H	HO	EtCH(Me)COO	H	H
7	Ivain – III	H	HO	Me_2CHCOO	H	OEt

signals due to protons on carbon atoms bearing oxygen atoms δ5 22 (1H, *d*, *J* = 9 9 Hz, H-3), 4 80 (1H, *dd*, *J* = 11 and 5 Hz, secondary acetate), 4 82 and 4 40 (AB system, *J* = 12 Hz, primary acetate), 3.65 (1H, *br*, H-2) and 2 81 and 2.58 (AB system, *J* = 4 Hz, α,α-disubstituted oxirane ring) In addition, characteristic signals of a tertiary methyl group (δ0 98, *s*) and of a secondary methyl group (δ0 85, *d*, *J* = 7 5 Hz) were also observed Likewise, the presence of a hexahydrofuranofuran system was inferred from an acetal proton signal at δ5 81 (*d*, *J* = 5 3 Hz) and of signals at δ4 48 (*dd*, *J* = 11 and 5 Hz) and 5 09 (1H, *d*, *J* = 5 Hz), attributable, respectively, to H-16, H-11 and H-15 This last absorption and the appearance of a methyl group signal at δ1 20 (*t*, *J* = 6 3 Hz) and methylene protons signals at δ3 40 and 3 60 (*dd*, *J* = 8 and 6 Hz) suggested the substitution of an H-15 proton for an ethoxy group, as found previously for ivain III (7) [2] (Table 1) Furthermore, the occurrence of this C-15 substituted hexahydrofuranofuran moiety was confirmed by the presence in its mass spectrum of significant peaks at *m/z* 157, 111, 81 and 69, as well as of metastable ions at *m* 78 5 and 42 9 [2]

The second compound, 14-hydro-15-hydroxyajugapitin (**4**) had a molecular formula of $C_{29}H_{44}O_{11}$ (550 [M $-H_2O]^+$) and its IR spectrum revealed the presence of free hydroxyl (3450 cm^{-1}) and ester (1730 and 1250 cm^{-1}) groups Its ^1H NMR spectrum showed essentially the same signals as those present in the spectrum of **3**, except for the H-15 signal which appeared as a double doublet at δ5 56 (1H, *dd*, *J* = 10 5 and 5 1 Hz) and for the H-16 signal which appeared as two doublets at δ5 76 and 5 80 (1H, *d*, *J* = 4 5 Hz) suggesting an epimeric mixture at this site This suggestion was further confirmed by the presence of practically a double number of signals in the ^{13}C NMR spectrum of **4** (Table 2).

Oxidation of **4** with chromium trioxide in pyridine yielded the 2,15-dioxo derivative **5** with a molecular formula of $C_{29}H_{40}O_{11}$ (479 46877 [M $-C_5H_9O]^+$) Its IR spectrum revealed the presence of a γ-lactone moiety (1780 cm^{-1}) and broad carbonyl absorption of ketone

Table 2 ^1H and ^{13}C NMR spectral data of the epimeric mixture 4 (80 and 20 MHz respectively, $CDCl_3$, TMS as int standard)

H	δ	C	δ	C	δ
2	3 60 *br*	1	30 3	14	33 3
3	5 21 *d* (9 9)*	2	72 1	14′	33 2
6	4 80 *dd* (11, 5)	3	71 5	15	98 6
11	4 05 *dd* (11, 5)	4	62 7	15′	98 3
15	5 56 *dd* (10 5, 5 1)	5	45 6	16	109 1
		5′	45 5	16′	107 2
16	5 80 *d* (4 5)	6	32 8	17	43 5
16′	5 76 *d* (4 5)	7	32 9	18	61 4
17	2 50 and	8	35 7	19	16 3
	2 75 AB (4)	8′	35 4	19′	16 1
18	4 38 and	9	40 9	20	13 7
	4 79 AB (12)	10	42 3	20′	13 6
19	0 98 *s*	11	83 3	$\underline{C}H_3COO$	20 9
20	0 86 *d* (7)	11′	82 8	$\underline{C}H_3COO$	20 8
$C\underline{H}_3CH_2CH(CH_3)CO$	0 94 *t* (4 5)	12	32 1	$CH_3\underline{C}OO$	171 1
$CH_3CH_2CH(C\underline{H}_3)CO$	1 16 *d* (7)	12′	32 0	$CH\underline{C}OO$	175 7
$CH_3CH_2C\underline{H}(CH_3)CO$	2 45 *sextet* (6)	13	40 1	$CH\underline{C}OO'$	175 6
				$\underline{C}H_3CHCOO$	16 6
				$\underline{C}H_2CHCOO$	26 5
				$\underline{C}H_3CH_2COO$	11 0

*J in Hz

and ester groups (1740 cm^{-1}), as well as the absence of hydroxyl groups The ^1H NMR spectrum of **5** exhibited essentially the same signals as those of **3** and **4**, except for H-3 which now appeared as a singlet at δ 5 95 and the corresponding disappearance of H-2 and H-15 absorptions

EXPERIMENTAL

EIMS (DI method), 70 eV, CC silica gel 60 (70–230 mesh, Merck), silica gel $HF_{254+366}$ (Merck) and Al_2O_3 90 [70–230 mesh, Merck, activity I (Brockmann Scale)]

Plant materials were collected in May 1983 in Villanna (Gerona, Spain) and voucher specimens were deposited in the Instituto de Quimica Bio-Orgánica C S I C (Barcelona, Spain) and identified by Mr Joan Fisse

Extraction and isolation of the diterpenoids Dried and finely powdered whole plants (400 g) were extracted with Et_2O (4 l) at 35° for 1 week The solvent was evaporated to yield a gum (11 6 g) which was treated with Me_2CO to precipitate the accompanying waxes After filtration, the solvent was evaporated to yield a residue (9 25 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 **3** was eluted with the hexane–EtOAc (1 2) system, and a fraction enriched in diterpenoid **4** was eluted with EtOAc Both fractions were purified separately by successive CC over silica gel HF and Al_2O_3 gel eluted with hexane–EtOAc (1 1, 1 2 and 0 1) yielding finally 15-ethoxy-14-hydroajugapitin (**3**, 6 mg) and 14-hydro-15-hydroxy-ajugapitin (**4**, 107 mg)

15-Ethoxy-14-hydroajugapitin (**3**) $[\alpha]_D^{20}$ −3 53° ($CHCl_3$, *c* 3 4), IR ν_{max}^{KBr} cm^{-1} 3450 (OH), 1730, 1250 (ester groups), 1150, 1020, ^1H NMR (80 MHz) see Table 1, MS *m/z* (rel int) 550 $[M-EtOH]^+$ (7), 375 (2), 339 (10), 325 (17), 281 (4), 265 (5), 248 (5), 230 (2), 218 (11), 217 (7), 200 (10), 187 (19), 169 (9), 157 (29, ethoxyhexahydrofuranofuran fragment ion), 149 (10), 111 (81), 85 (37), 83 (18), 81 (20), 69 (19), 57 (100), 55 (24), 43 (92)

2,15-Dioxo derivative **5** A soln of **4** (55 mg) in C_5H_5N (3 ml) was added to a mixture of CrO_3 (70 mg) in 3 ml C_5H_5N at 0° and the mixture was stirred for 22 hr Then, Et_2O (6 ml) and EtOAc (1 ml) were added and after 10 min the suspension was filtered over celite The solvent was evaporated at 40° yielding a crude oil of **4** and **5** which was purified by CC over silica gel HF and eluted with hexane–EtOAc (1 2) to yield **5** (15 mg) $[\alpha]_D^{20}$ +1 41° ($CHCl_3$, *c* 6 4), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1780 (γ-lactone), 1740 (ketone group), 1740, 1230 (ester groups), ^1H NMR (200 MHz). see Table 1, MS *m/z* (rel int) 479 $[M-C_5H_9O]^+$ (3), 463 (5), 437 (3), 421 (4), 420 (13), 378 (2), 330 (2), 301 (2), 234 (5), 217 (4), 200 (2), 187 (7), 181 (6), 175 (7), 127 (19, 15-oxohexahydrofuranofuran fragment ion), 111 (3), 85 (46), 83 (10), 81 (11), 69 (9), 57 (100), 55 (15), 43 (54) (Found C, 61 46, H, 6 90 $C_{29}H_{40}O_{11}$ requires C, 61 76, H, 7 15)

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