Phytochemistry, Vol 28, No 10, pp 2617-2619, 1989
Printed in Great Britain

0031-9422/89 $3 00 + 0 00

SESQUITERPENOIDS RELATED TO JUVABIONE IN *ABIES PINSAPO*

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(*Received in revised form* 10 *March* 1989)

Key Word Index—*Abies pinsapo*, Pinaceae, sesquiterpenoids, juvabione

Abstract—From the acid fraction of the hexane extract of the wood of *Abies pinsapo*, four new sesquiterpenoids related to juvabione have been isolated: epitodomatuic acid, *cis*-dihydroepitodomatuic acid, 4'-dehydroepitodomatuic acid and 3'-dihydroepitodomatuic acid

INTRODUCTION

Abies pinsapo Boiss. is a tree which grows naturally on the high mountains at the western end of the Betic cordillera (Spain) and on the calcareous chain of Yebala (Northern Morocco). A superficial study of the polar extracts (acetone and diethyl ether) of the heartwood of *A. pinsapo* and its *marocana* subspecies was described in a previous paper [1]. Sitosterol, pinitol and secoisolariciresinol were identified The components of the wood as well as leaves, bark, seeds and resin [2–6] have been studied in other species of *Abies* Bowers *et al.* [7] isolated a substance from *A. balsamea*, that they called juvabione, because it exhibited high insect juvenile hormone activity when tested on the bug *Pyrrhocoris apterus* L. Juvabione and other related substances, which are of phytochemical interest, usually occur in the plant as methyl esters. The assigned stereochemistries are in general wrong [8–10] as shown by the use of the DC and DOR techniques [11].

RESULTS AND DISCUSSION

The acid fraction of the hexane extract from the wood of *A. pinsapo* was treated with diazomethane and four methyl esters (**1–4**) were isolated by column chromatography on silica gel. Upon saponification with methanolic potassium hydroxide these compounds yielded the corresponding natural sesquiterpene acids **5–8**

Saponification of the least polar ester (**1**) gave a substance with the molecular formula $C_{15}H_{24}O_3$ (No $[M]^+$, $[M-H_2O]^+$ at *m/z* 234). Its IR spectrum showed absorption bands due to a ketone carbonyl and an α,β-unsaturated acid (1710 and 1686 cm^{-1}, respectively), as well as a trisubstituted C–C double bond (1646 cm^{-1}). Its ^1H NMR spectrum (Table 1) contained signals of a methyl group bound to a methine (δ0.84, *d*) and two methyls of an isopropyl group (δ0.86, *d*). At δ7.10, a multiplet due to the olefinic β proton of an α,β-unsaturated acid was observed. The acid proton appeared as a broad signal at δ10.00. These data together with the ^{13}C NMR data (Table 2) were consistent with a structure of a carboxylic acid related to juvabione. The positive sign of the optical rotation and the values of the chemical shifts in the ^{13}C NMR spectrum allowed us to establish the stereochemistry of C-4 and C-1'. Thus the δ values for C-3 and C-5 (28 8 and 26.1, respectively) were in agreement with the reported data [12] for an *S* configuration at C-1' of epijuvabione (**1**). Therefore, the acid has structure **5**, namely epitodomatuic acid.

The substance formed on saponification of **2** showed in its mass spectrum a peak at *m/z* 254 ($[M]^+$, $C_{15}H_{26}O_3$), the other spectroscopic properties being very similar to those of **5**. However, in its IR spectrum no C–C double bond absorption was observed, and its ^1H NMR spectrum contained no signals for olefinic protons These data were in agreement with a structure related to dihydrojuvabione. The presence in the ^1H NMR spectrum of a multiplet at δ2.64 showed the equatorial disposition of the geminal proton to the carboxylic group and suggested that the substitution in the cyclohexane ring was *cis*. The ^{13}C NMR spectrum confirmed these deductions, but it did not allow us to assign the configuration at C-1'. Hydrogenation of epijuvabione (**1**) yielded the corresponding *trans* and *cis*-dihydroepijuvabione isomers (**9** and **2**) One of which was identical to ester **2**. This result, together with the fact that all sesquiterpenoids related to juvabione isolated in *A. pinsapo* belonged to the *epi* series, permitted us to propose an *S* configuration at C-1'. Consequently **6** has the structure of *cis*-dihydroepitodomatuic acid.

Saponification of **3** gave a colourless oil, the mass spectrum of which showed the molecular ion at *m/z* 250 ($C_{15}H_{22}O_3$). In the IR spectrum, we observed bands at 1643 and 1617 cm^{-1}, assignable to C–C double bonds which were conjugated with carboxyl and ketone carbonyl groups, respectively. The ^1H NMR spectrum showed two doublets at δ1 90 and 2.15 due to methyl groups bound to a double bond and with *trans* and *cis* disposition with respect to a ketone group, as well as two multiplets of olefinic protons at δ6.07 and 7.12. These data were consistent with a structure related to dehydrojuvabione. The ^{13}C NMR spectrum was in accordance with this proposal, the δ values of C-3 (28.7) and C-5 (26.1) being quite remarkable. This and the optical rotation value allowed us to establish the stereochemistry of the molecule, and to assign it the structure of 4'-dehydroepitodomatuic acid (**7**).

Saponification of the most polar ester (**4**) gave a hydroxyacid with the molecular formula $C_{15}H_{26}O_3$ ($[M-H_2O]^+$, *m/z* 236). Its IR spectrum showed the presence of an alcoholic hydroxyl group (3400 cm^{-1}), a carboxyl

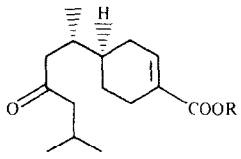
Table 1 ^{1}H NMR spectral data of compounds **5–9***

H	5	6	7	8	9
1	—	2 64*m*	—	—	
2	7 10*m*		7 12*m*	7 10*m*	
3′	—	—	—	3 75*m*	—
4′			6 07*m*		
6′	0 86 *d*	0 88 *d*†	2 15 *d*	0 88 *d*	0 88 *d*†
	(6 4)‡	(6.4)	(1 0)	(6 0)	(6 8)
7′	0 86 *d*	0 89 *d*†	1 90 *d*	0 88 *d*	0 89 *d*†
	(6 4)	(6.4)	(1 0)	(6 0)	(6 8)
8′	0 84 *d*	0 82 *d*	0 90 *d*	0 88 *d*	0 81 *d*
	(6 0)	(6.0)	(6 5)	(6 0)	(6 0)
OMe	—	—	—	—	3 64 *s*

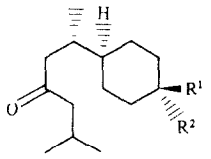
*5 and 9 300 MHz; 6–8 80 MHz ($CDCl_3$, TMS as int standard)

† Interchangeable value

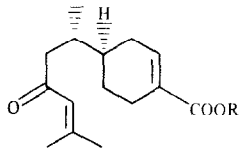
‡ Coupling constants (*J* in Hz) are given in parentheses



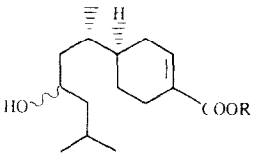
1 R = Me
5 R = H



2 R^1 = COOMe R^2 = H
6 R^1 = COOH R^2 = H
9 R^1 = H R^2 = COOMe



3 R = Me
7 R = H



4 R = Me 3′*S*
8 R = H 3′*S*
10 R = Me 3′*R*

group (1690 cm^{-1}) and a double bond conjugated with the former (1646 cm^{-1}). In the ^{1}H NMR spectrum, overlapping signals of methyl groups bound to methine and of an isopropyl group (δ0.88, *d*, 9H) were present, as were signals of a proton geminal to a hydroxyl group (δ3 75, *m*) and an olefinic proton β to a carboxyl group (δ7.10, *m*). In accordance with these data, the compound had a structure related to 3′-dihydrojuvabione (juvabiol) Again, the ^{13}C NMR data and the optical rotation value permitted us to define the stereochemistry The values of δ28.7 and 26.0 for C-3 and C-5 respectively, were in agreement with those reported by Manville [13] for an *S* configuration at C-1′ in epijuvabiol, whereas the ones corresponding to C-2′ and C-4′ (δ42.4 and 47.9) were consistent with an *S* configuration at C-3′. Therefore, the natural acid was assigned the structure of 3′-dihydroepitodomatuic acid (**8**). In accordance with this proposal, sodium borohydride reduction of epijuvabione (**1**) yielded a mixture of two substances, the ^{13}C NMR of which contained the signals of **4**, and those Manville [13] had assigned to isoepijuvabiol (**10**).

EXPERIMENTAL

The wood was collected in June in Sierra Bermeja (Málaga, Spain) and was identified by Professor F Valle (Department of Botany, University of Granada) The air-dried material (5 2 kg) was crushed and extracted with hexane in a Soxhlet apparatus for 12 hr The extract (29 8 g) was defatted and, after dissolving in Et_2O, was extracted with NaOH solns (5, 2 5, and 10%) giving 10 85 g of a neutral fraction and 8 16 g of an acid one The latter was treated with CH_2N_2 to yield the methyl esters which are easier to isolate and purify

Table 2 ^{13}C NMR chemical shifts of compounds **1–10** ($CDCl_3$, TMS as int standard)*

C	1	2	3	4	5	6	7	8	9	10
1	130 2	39.5	130 2	130 1	130 2	39 5	129 9	129 9	43 3	130 1
2	139 2	27 1	139 5	139 7	141 6	27 0	141 9	142 3	27 1†	139 6
3	28 4	26 6†	28 5	28 3	28 8	26 9†	28 7	28 7	29 3‡	27 3
4	37 7	41 8	37 8	38 4	37 7	41 8	37 7	38 5	41 8	36 9
5	26 0	25 6†	26 2	25 9	26 1	25 6†	26 1	26 0	27 9‡	26 4
6	24 9	27 1	25 0	25 0	24 7	27 0	24 7	24 8	29 0†	25 0
7	167 7	175 6	168 0	167 9	172 8	181 3	172 8	172 5	176 3	167 8
8	51 4	51 4	51 5	51 9	—	—	—	-	51 4	51 3
1′	32 8	33 1	33 6	33 0	32 9	33 2	33 6	33.1	33 5	33 6
2′	47 9	48 0	49 0	42 3	48 0	48 1	49 0	42 4	48 0	42 7
3′	210 3	210 9	201 0	67 5	210 7	211.5	200 9	67 7	210 7	68 1
4′	52 3	52 3	124 1	47 8	52 5	52 4	124 1	47 9	52 4	46 9
5′	24 5	24 5	155 3	24 6	24 6	24 6	155 4	24 8	24 5	24 4
6′	22 5	22 5	20 7	22 2†	22 6†	22 7‡	20 8	23 4	22 5	21 8†
7′	22 5	22 5	27.7	23 2†	22 7†	22 6‡	27 7	22 3	22 5	23 5†
8′	16 3	16 8	16.4	15 5	16 5	16 9	16 4	15 6	16 5	16 0

*1–4, 7 20 MHz, 5, 6, 8–10 75 MHz

†, ‡ Interchangeable values

The reaction mixture was chromatographed under pressure on a silica gel column eluted with hexane–Et_2O mixtures Further chromatography on silica gel columns yielded the pure esters: **1** (170 mg), **2** (330 mg), **3** (300 mg) and **4** (84 mg).

Epijuvabione (**1**) Eluted with hexane–Et_2O (30·1), $[\alpha]_D^{25}$ +60.0° ($CHCl_3$; *c* 1.18)

cis-*Dihydroepijuvabione* (**2**). Eluted with hexane–Et_2O (49 1), $[\alpha]_D^{25}$ 0.0° ($CHCl_3$, *c* 1 02).

4′-*Dehydroepijuvabione* (**3**). Eluted with hexane–Et_2O (97 3). $AgNO_3$–silica gel (1 4) gave pure **3**, $[\alpha]_D^{25}$ +84 0° ($CHCl_3$, *c* 0.51).

Epijuvabiol (**4**) Eluted with hexane–Et_2O (17·3). $AgNO_3$–silica gel (1 4) gave **4**, $[\alpha]_D^{25}$ +50.2° ($CHCl_3$; *c* 0 77). Saponification [MeOH–KOH, room temp, 8 (**3**) i.r 21 hr] of the above esters yielded the corresponding natural acids A shorter reaction period was used for **3** because longer periods led to retroaldolization. The reaction mixture was processed in the usual way

Epitodomatuic acid (**5**). Colourless oil. $[\alpha]_D^{25}$ +71 2° ($CHCl_3$, *c* 1 07), IR ν_{max}^{film} cm^{-1}: 3300–2500, 1710, 1686, 1646, 1462, 1423, 1366, 1273, 1085, 1030, 945, 739, MS (probe) 70 eV, *m/z* (rel int) 234 $[M-H_2O]^+$ (15), 177 (12), 153 (20), 152 (57), 134 (100), 127 (36), 107 (44), 85 (41), 79 (40), 57 (56); UV λ_{max}^{MeOH} nm (log ε) 221 (3.4)

cis-*Dihydroepitodomatuic acid* (**6**). Syrup. $[\alpha]_D^{25}$ 0.0° ($CHCl_3$, *c* 1 19); IRν_{max}^{film} cm^{-1} 3500–2500, 1706, 1452, 1371, 1238, 1181, 1136, 958, 842, MS (probe) 70 eV, *m/z* (rel int)· 254 $[M]^+$(1), 154 (66), 136 (76), 127 (84), 109 (78), 108 (74), 85 (85), 57 (100)

4′-*Dehydroepitodomatuic acid* (**7**) Solid Mp (uncorr) 56–58°, $[\alpha]_D^{25}$ +94 0° ($CHCl_3$, *c* 0.71), IR ν_{max}^{film} cm^{-1} 3700–2400, 1681, 1643, 1617, 1427, 1378, 1272, 1211, 1126, 1075, 1032, MS (probe) 70 eV, *m/z* (rel. int.) 251 $[M+1]^+$ (1), 223 (1), 152 (2), 149 (8), 120 (3), 87 (10), 85 (61), 83 (100), 47 (17), 44 (13); UV λ_{max}^{MeOH}nm (log ε) 246 (4.2)

3′-*Dihydroepitodomatuic acid* (**8**). Syrup. $[\alpha]_D^{25}$ +51.6° ($CHCl_3$, *c* 0.97), IRν_{max}^{film} cm^{-1}: 3700–2200, 3400, 1690, 1646, 1462, 1423, 1264, 1141, 949, 760, 701, MS (probe) 70 eV, *m/z* (rel. int) : 236 $[M-H_2O]^+$ (8), 179 (20), 152 (19), 151 (15), 124 (100), 123 (42), 109 (11), 107 (19), 83 (10), 79 (11), 69 (19), 57 (8),, UV λ_{max}^{MeOH}nm (log ε) 219 (3 5)

Hydrogenation of epijuvabione (**1**) 297 mg of **1** was dissolved in MeOH (20 ml) containing Pd/C catalyst (10% Pd, 300 mg). Hydrogenation was performed at room temp at 1 6 atm for 4 hr. After removing the catalyst and solvent the crude product (250 mg) chromatographed on a silica gel column eluted with hexane–Et_2O (24.1). Two products, (**2** and **9**) in roughly equal amounts, were isolated

Reduction of epijuvabione (**1**) *with* $NaBH_4$ Compound **1** (1 2 mmol) in MeOH (5 ml) was treated with $NaBH_4$ (1 2 mmol) at room temp for 24 hr The soln was neutralized with 2 M HCl, the MeOH evapd and the residue extracted with Et_2O (3 × 50 ml) The extracts were washed (3 × 50 ml), dried over Na_2SO_4 and then evapd to give a mixture (256 mg) which was chromatographed on a silica gel column eluted with hexane–Et_2O mixtures (4 1 and 3 1) 190 mg of a mixture of epijuvabiol (**4**) and isoepijuvabiol (**10**) was obtained ^{13}C NMR, Table 2.

Acknowledgements—We wish to thank Junta de Andalucía for financial support, Professor F Valle (Department of Botany, Faculty of Sciences, University of Granada) for identification of the plant material, and Isabel J Macdonald for her assistance in the translation of the text.

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