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NEW NATURAL DITERPENE ACIDS FROM *JUNIPERUS COMMUNIS**

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Key Word Index—*Juniperus communis*; Cupressaceae; diterpenoids; new labdane and pimarane derivatives.

Abstract—Three new diterpene acids have been isolated from the leaves of *Juniperus communis* and their structures, elucidated by spectroscopic methods, were identified as 7-oxo-13-*epi*-pimara-8,15-dien-18-oic acid, 7α-hydroxysandaracopimaric acid and (14 *S*)-14,15-dihydroxylabda-8(17),13(16)-dien-19-oic acid. Biflavonyls, fatty acids and diterpenoids with known structures were also isolated.

INTRODUCTION

In previous papers of this series we have reported results on the composition of both volatile oil and non-volatile extracts from berries of common juniper [1–3] and other *Juniperus* spp. [4, 5]. In another report we examined the composition of leaves of *Juniperus oxycedrus* [6]. In this paper we report the isolation and structural determination of components of an ethereal extract from *Juniperus communis* leaves.

RESULTS AND DISCUSSION

The ether extract of air-dried leaves was dewaxed and fractionated with aqueous $NaHCO_3$, Na_2CO_3 and NaOH solutions and the acidic fractions were worked up separately. From the NaOH fraction *trans*-communic acid (**1**), isopimaric acid (**2**) and a mixture of 12-hydroxylauric acid and 16-hydroxypalmitic acid were isolated. The Na_2CO_3 contained isopimaric acid (**2**), sandaracopimaric acid (**3**), imbricatalic acid (**4**), isocupressic acid (**5**), and 13,14-epoxyimbricatolic acid (**6**), which had also been isolated from *Juniperus thurifera* [7] and through periodic acid oxidation gave the dinor-compound **7**, another component of *J. thurifera*.

The $NaHCO_3$ fraction contained 7α-hydroxydehydroabietic acid (**8**), two biflavonyls, hinokiflavone and cupressuflavone [6] and the three new diterpenoids, 7-oxo-13-*epi*-pimaric-8,15-dien-18-oic acid, (14 *S*)-14,15-dihydroxylabda-8(17),13(16)-dien-19-oic acid and 7α-hydroxysandaracopimaric acid which were isolated as methyl esters (**9**, **15** and **16**, respectively). The structures of all known compounds were established by physical and spectroscopical data and confirmed by comparison with authentic samples.

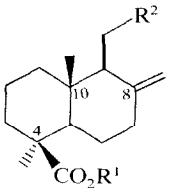
* Preceding paper in this series: Pascual Teresa, J. de *et al.* (1978) *An. Quím.* **74**, 1093.

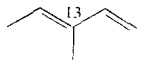
Methyl 7-oxo-13-epi-pimara-8,15-dien-18-oate (**9**)

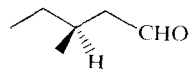
The IR spectrum of **9** showed bands at 1725 and 1240 cm^{-1} which confirmed the presence of an equatorial esterified carboxyl group [8], at 1660 and 1610 cm^{-1} (α,β-unsaturated carbonyl) and at 3070, 990 and 905 cm^{-1} (vinyl substituent). The UV spectrum has an absorption maximum at 244 nm (log ε 4.05) and suggested a trisubstituted conjugated double bond. The ^1H NMR spectrum included signals of three quaternary methyl groups (δ 1.00, 1.12 and 1.27) and those of a typical ABX system of a vinyl group (4.69–5.00, 2H, *m* and 5.50–5.92, 1H *sextet*) but no other resonance of olefinic protons was present. All these data are consistent with a $\Delta^{8,15}$-pimaradiene skeleton with a C_{18}-equatorial carboxyl group and a keto group conjugated with a Δ^8-unsaturation. The carbonyl must be placed at C-7 because the $NaBH_4$ reduction of **9** gave a hydroxy compound, **10**, showing a triplet at δ4.10 for the geminal proton to the OH group (thus excluding the C-14 position), and because the carbonyl or β-hydroxyl groups at C-11 should induce on the C-10 methyl group, larger shifts than those observed (1.12 and 1.06 for **9** and **10**) [9] in both substances.

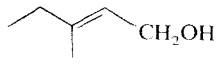
As shown by ^{13}C NMR spectroscopy [10], the conformation of ring C in Δ^8-pimarenes must be **11a** or **12a**, regardless of the configuration at C-13 and the free energy calculations based on data for isolated cyclohexanes. Furthermore, compounds **9** and **10** do show in the ^1H NMR spectrum a sextet for the X part of the vinyl ABX system. This unusual multiplicity has been observed when the vinyl group is axial in a cyclohexene but not when it is equatorial [11, 12]. Hence, the vinyl group must be axial and the relative configuration as shown in **11a**.

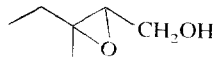
The CD of **9** ($\Delta\varepsilon_{339}+0.84$, $\Delta\varepsilon_{249}-0.77$, $\Delta\varepsilon_{213}+3.52$) showed maxima opposed in sign to those of **13** ($\Delta\varepsilon_{339}-0.5$, $\Delta\varepsilon_{246}+1.97$, $\Delta\varepsilon_{210}-2.42$), a compound recently isolated by Bohlmann *et al.* [13]. These data and the established rules for the CD of enones [14, 15] led us to conclude that the ketoester **9** belongs to the

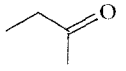


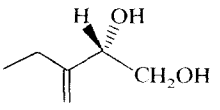
1 $R^1 = H$ $R^2 =$ 

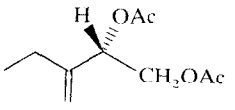
4 $R^1 = H$ $R^2 =$ 

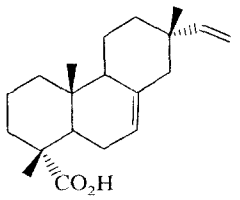
5 $R^1 = H$ $R^2 =$ 

6 $R^1 = H$ $R^2 =$ 

7 $R^1 = H$ $R^2 =$ 

15 $R^1 = Me$ $R^2 =$ 

16 $R^1 = Me$ $R^2 =$ 



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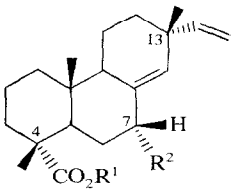
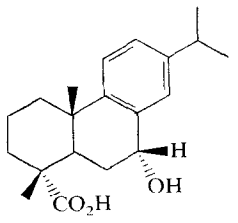


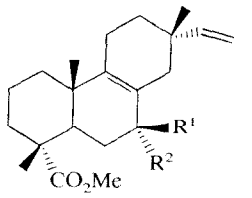
3 $R^1 = R^2 = H$
17 $R^1 = Me$ $R^2 = OH$



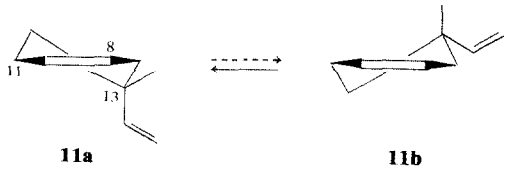
8

normal 5α-pimarane series, like all the other pimaranes isolated from *J. communis*, and so the absolute configuration at C-13 must be *S*.

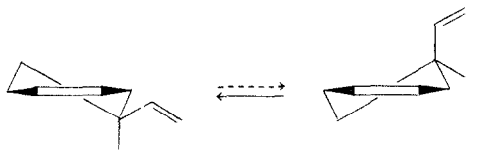
The CD curve of **9** was also in agreement with the axial conformation of the vinyl group. The smaller dichroic absorption of **9** relative to **13** at 246 nm could



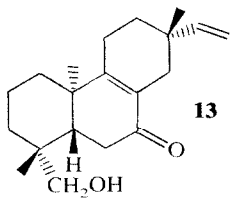
9 $R^1, R^2 = O$
10 $R^1 = OH$ $R^2 = H$



11a **11b**



12a **12b**



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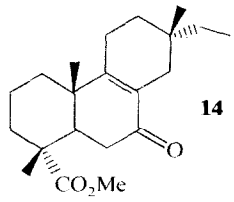

14

be rationalized if some coupling between the axial vinyl and the enone π-electrons is assumed [16].

Lastly, the structure of **9** was confirmed by partial hydrogenation to yield **14**, a substance with identical IR and comparable (different solvent) ^1H NMR spectra to those of the compound previously synthetized by Herz and Hall [17].

Methyl-(14 S)-14, 15-*dihydroxylabda*-(17), 13(16)-*dien*-19-*oate*(**15**)

The molecular formula of **15**, $C_{21}H_{34}O_4$, was deduced from the MS data of its diacetate. The IR spectrum showed intense bands of hydroxyl (3400 cm^{-1}) vinylidenic (3070, 1640, 890 cm^{-1}) and axial carboxylic ester groups (1725, 1230, 1190, 1160 cm^{-1}) [8]. The ^1H NMR spectrum showed signals of three methyl singlets (δ0.51, 1.20 and 3.61), a hindered multiplet of two methylene protons of a primary alcohol (3.65), another multiplet (4.14) of a proton geminal with a secondary allylic hydroxyl group, two broad singlets (4.50 and 4.86) of an exocyclic methylene and two vinylidenic protons at 4.95 and 5.12. The ^1H NMR spectrum of the diacetate **16** showed an ABX system (3.72–4.33 AB part and 5.14–5.36 X part) of the geminal protons to both acetoxyl groups which, consequently, should be vicinal. These data suggested that **15** had a labdane structure. As a definitive proof of the structure, a substance with identical spectral properties was obtained from methyl isocupressate by oxidation with singlet oxygen. The CD of diol **15** measured in CCl_4 containing $Pr(fod)_3$ showed a negative dichroic absorption ($\Delta\varepsilon_{317} - 1.2$) which led us to assign the configuration *S* for C-14 [18].

The same sign of the CE is shown by a halimic acid derivative with the same side-chain ($\Delta^{13(16)}$, (14*S*)-hydroxydihydrohalimic acid [19]), whose stereochemistry was also established by Horeau's method.

Methyl 7α-hydroxysandaracopimarate (**17**)

The MS of **17** showed the M^+ at *m/e* 332, in agreement with the molecular formula $C_{21}H_{32}O_3$. The IR spectrum showed bands of a hydroxyl group, vinyl unsaturation and an equatorial carboxyl group. The ^{1}H NMR spectrum had four methyl singlets at δ0.84, 1.08, 1.22 and 3.70 and the absorption pattern of vinylic protons was identical with that of sandaracopimaric acid (**3**). The multiplet at 4.16 ($W_{1/2}$ = 9 Hz) of the geminal proton to the secondary axial OH group and the observed deshielding of 0.25 ppm for the proton at C-14 suggested the localization of the hydroxy group at C-7. These properties are identical to those of a photoxidation product of methyl isopimarate (**2** methyl ester) described by Fourrey *et al.* [20]. Another substance related to **17**, 7α-hydroxy-(−)-pimara-8(14),15-dien-19-oic acid, was isolated by Yanagisawa *et al.* [21] form *Aralia cordata.*

EXPERIMENTAL

Optical rotations were measured in $CHCl_3$ soln, IR spectra in film, ^{1}H NMR (60 MHz) generally in $CDCl_3$ with TMS as int. standard; chemical shifts are in ppm. MS at 70 eV. Si gel G and Si gel 60 (Merck) were used in PLC and CC separations.

Extraction and isolation. Juniperus communis (female gametophyte) was collected in Soria (Spain), September 1974. Dried leaves (1.5 kg) were extracted with refluxing Et_2O for 48 hr. Dewaxing with MeOH gave waxes (44.8 g) and the remaining product in Et_2O soln was extracted with aq. satd $NaHCO_3$ and 4% NaOH solns to yield, respectively, 17.8, 21.5 and 4.4 g, leaving 24.3 g of neutral fraction. The NaOH fraction after CC on Si gel (150 g) yielded *trans*-communic acid (**1**) (0.62 g eluted with C_6H_6), isopimaric acid (**2**) (0.95 g, C_6H_6–Et_2O, 9 : 1), fatty acids (1.3 g, C_6H_6–Et_2O, 9 : 1 and 8 : 2), 12-hydroxylauric acid and 16-hydroxypalmitic acid (0.8 g, C_6H_6–Et_2O, 1 : 1). All compounds had properties as described previously [2]. The Na_2CO_3 fraction by CC on Si gel (500 g) yielded **1**+**2** (5.7 g C_6H_6), **2**+sandaracopimaric acid (**3**) (5.15 g, C_6H_6–Et_2O, 9 : 1), imbricatalic acid (**4**) (2.24 g, C_6H_6–Et_2O, 9 : 1), isocupressic acid (**5**) (5.9 g, C_6H_6–Et_2O, 7 : 3) and, after esterification and CC, methyl 13,14-epoxyinbricatolate (**6**) (21 mg C_6H_6–Et_2O, 9 : 1). Properties as described previously [3, 7]. The $NaHCO_3$ fraction by CC on Si gel (550 g) yielded **2**(2.8 g), fatty acids (2.6 g), **5**(1.61 g), methyl 7-oxo-13-*epi*-pimara-8,15-dien-18-oate (**9**) (123 mg after esterifn and new CC, C_6H_6–Et_2O, 9 : 1), methyl-(14*S*)-14,15-dihydroxylabda-8(17),13(16)-dien-19-oate (**15**) (125 mg after esterifn and CC, C_6H_6–Et_2O. 8 : 2), hinokiflavone (23 mg by PLC on Si gel, MeC_6H_5–HOAc–Py, 4 : 1 : 1), cupressuflavone (152 mg, C_6H_6–EtOAc, 3 : 7), methyl-7α-hydroxydehydroabietate (8 Me ester) (40 mg after esterification and PLC purification C_6H_6–Et_2O, 9 : 1) and methyl-7α-hydroxysandaracopimarate (**17**) (77 mg, purified as **8**).

Methyl 7-oxo-13-epi-pimara-8,15-dien-18-oate (**9**). Colourless oil. $C_{21}H_{30}O_3$; $[\alpha]_D$ +77.6°(*c* 0.76); IR ν_{max} cm^{-1}: 3070, 1725, 1660, 1610, 1240, 990, 905; UV λ_{max} nm (log ε): 244 (4.05); ^{1}H NMR: δ 1.00 (3H, *s*, Me-13), 1.12 (3H, *s*, Me-10), 1.27 (3H, *s*, Me-4), 3.61 (3H, *s*, OMe), 4.69–5.00 (2H, *m*, =CH_2), 5.50–5.92 (1H, *m*, —CH=). CD (*n*-hexane): $\Delta\varepsilon_{339}$ +0.84, $\Delta\varepsilon_{249}$ −0.77 and $\Delta\varepsilon_{213}$ +3.52.

Methyl-7α-hydroxy-13-epi-pimara-8,15-dien-18-oate (**10**). To a MeOH soln of **9** (46 mg), $NaBH_4$ (10 mg) was added and refluxed for 10 min. Recovery of the product as usual gave **10** (41 mg). $C_{21}H_{32}O_3$, oil, $[\alpha]_D$ +42.9° (*c* 0.67); IR ν_{max} cm^{-1}: 3400, 3070, 1725, 1640, 1240, 990, 905; ^{1}H NMR: δ1.00 (3H, *s*, Me-13), 1.06 (3H, *s*, Me-10), 1.22 (3H, *s*, Me-4), 3.62 (3H, *s*, OMe), 4.10 (1H, *t*, *J* = 8 Hz, H-7), 4.60–4.98 (2H, *m*, =CH_2) and 5.46–5.92 (1H, *m*, —CH=).

Methyl 7-oxo-13-epi-pimar-8-en-18-oate (**14**). **9** (65 mg) was hydrogenated in C_6H_6 soln at atm. pres. in the presence of PtO_2 (28 mg) for 5 hr. After PLC on Si gel, **14** (35 mg) was obtained. $[\alpha]_D$ +17.4° (*c* 1.09); IR ν_{max} cm^{-1}: 1720, 1660, 1610, 1240; ^{1}H NMR (CCl_4): δ 0.82 (3H, *s*, Me-13), 0.83 (3H, *t*, *J* = 7 Hz, H-16), 1.11 (3H, *s*, Me-10), 1.22 (3H, *s*, Me-4), 3.64 (3H, *s*, OMe). MS *m/e* (rel. int.): 332 (M^+, 7), 303 (8), 289 (6), 273 (4), 91 (100). CD (*n*-hexane) $\Delta\varepsilon_{339}$ + 0.51, $\Delta\varepsilon_{240}$ − 1.96, $\Delta\varepsilon_{212}$ + 2.57.

Methyl (14S)-14,15-dihydroxylabda-8(17),13(16)-dien-19-oate (**15**). Colourless oil. $C_{21}H_{34}O_4$; $[\alpha]_D$ +42.9° (*c* 1.56); IR ν_{max} cm^{-1}: 3400, 3070, 1725, 1640, 1230, 1190, 1160, 990, 890; ^{1}H NMR (CCl_4): δ0.51 (3H, *s*, Me-10), 1.20 (3H, *s*, Me-4). 3.61 (3H, *s*, OMe), 3.65 (2H, *m*, —CH_2—OH), 4.14 (1H, *m*, —CHOH—), 4.50 (1H, *br.s* H-17), 4.86 (1H, *br.s*, H-17), 4.95 (1H, *br.s*, H-16), 5.12 (1H, *br.s*, H-16). CD (CCl_4, concn: 1.5 mM **16** and 0.2 mM Pr(fod)$_3$): $\Delta\varepsilon_{317}$ − 1.2. *Diacetate:* oil, $[\alpha]_D$ +39.3° (*c* 1.21); IR ν_{max} cm^{-1}: 3060, 1740, 1720, 1640, 1230, 1150, 1035, 885; ^{1}H NMR (CCl_4): δ0.49 (3H, *s*, Me-10), 1.15 (3H, *s*, Me-4), 1.97 (3H, *s*, OAc), 2.02 (3H, *s*, OAc), 3.55 (3H, *s*, OMe), 4.07 (2H, *m*, AB part of an ABX system H-15), 4.48 (1H, *br.s* H-17), 4.84 (1H, *br.s* H-17). 4.89 (1H, *br.s* H-16), 5.02 (1H, *br.s* H-16), 5.24 (1H, *q*, X part of the ABX system H-14). MS *m/e* (rel. int.): 374 (M^+ − 60, 1.5), 314 (13), 299 (5), 255 (16), 254 (14), 239 (13), 121 (100).

Photoxidation of methyl isocupressate. To **5** Me ester (220 mg) in *iso*-PrOH (31 ml) 6 mg of Rose Bengal were added and the stirred mixture was exposed to sunlight for 6 hr. The *iso*-PrOH was evapd, MeOH and $NaBH_4$ (307 mg) were added, the mixture stirred at room temp. for 30 min, worked up as usual and after PLC on Si gel (C_6H_6–Et_2O, 6 : 4) gave 46 mg of methyl-14,15-dihydroxylabda-8(17),13(16)-dien-19-oate, $[\alpha]_D$ +52.5° (*c* 1.6) with identical R_f, IR and NMR to **15**.

Methyl-7α-hydroxysandaracopimarate (**17**). Viscous oil. $C_{21}H_{32}O_3$; $[\alpha]_D$ −41.0° (*c* 0.84); IR ν_{max} cm^{-1}: 3400, 3070, 1720, 1640, 1245, 995, 905; ^{1}H NMR: δ0.84 (3H, *s*, Me-10), 1.08 (3H, *s*, Me-13), 1.22 (3H, *s*, Me-4), 3.70 (3H, *s*, OMe), 4.16 (1H, *m*, $W_{1/2}$ = 9Hz), 4.80–5.10 (2H, AB part of an ABX system, H-16), 5.55 (1H, *br.s*, H-14) and 5.60–6.05 (1H, X part of the ABX system, H-15); MS *m/e* (rel. int.): 332 (M^+, 2), 330 (3), 315 (3), 271 (4), 255 (7), 203 (11), 177 (22), 121 (67), 55 (100).

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