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# SESQUITERPENIC ACIDS FROM *LEUCANTHEMOPSIS PULVERULENTA*

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**Abstract**—From *Leucanthemopsis pulverulenta* (Compositae) ten new sesquiterpenic acids have been isolated. Their structures and absolute stereochemistry were assigned by spectral and chemical evidence; they are the first carboxylic acid members of the cadinane and munrolane series to be found in a natural source.

From the acidic sesquiterpenic fraction of the $CHCl_3$ extract of the aereal parts of *Leucanthemopsis pulverulenta* (Lag.) Heywood,[1] also known as *Pyrethrum hispanicum*, Willk. (Anthemideae Tribe, Compositae), we have isolated several sesquiterpenic acid methyl esters **1–10**. The plant was identified by Prof. B. Casaseca Mena, from the Botany Department of Salamanca University, were a specimen is held (Herbarium No. 8996).

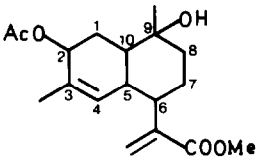
All of them are structurally related, belonging to two stereoisomeric series; six of them are cadinanic acids and the others are muurolanic acids. Spectral and chemical correlations led to their structural and stereochemical assignments.

As far as we know, these are the first reported acids with cadinane and muurolane skeletons, but they are structurally related to the therapeutically interesting amorphane acid isolated by the Xiao-tian Chinese research group[2] from *Artemisia annua*: the (+)-quinghao acid.

The acidic sesquiterpenoid fraction of *Leucanthemopsis pulverulenta* was chromatographed on silica gel after treatment with diazomethane, to afford the individual components as methyl esters **1–10**.

The ester **1**, was a solid of m.p. 104–105° and $[\alpha]_D = -76°$. In its mass spectrum, the molecular ion was not observed, but the fragment at *m/z* 262 ($M^+$–AcOH), was in agreement with the formula $C_{18}H_{26}O_5$. Its IR spectrum showed absorptions ($cm^{-1}$) due to hydroxyl (3400, 1120), acetoxyl (1740, 1250) and conjugated carbomethoxyl (1725) groups and unsaturations C=$CH_2$ and C=CH (3080, 3020, 1630, 880, 825). This functionality was confirmed by the NMR spectrum (see Table 1), which showed signals assignable to four methyl groups (Me–C–OH, Me–C=, Me–COO and COOMe), three olefinic protons ($CH_2$=C–COO and CH=C) and one allylic CH–OAc.

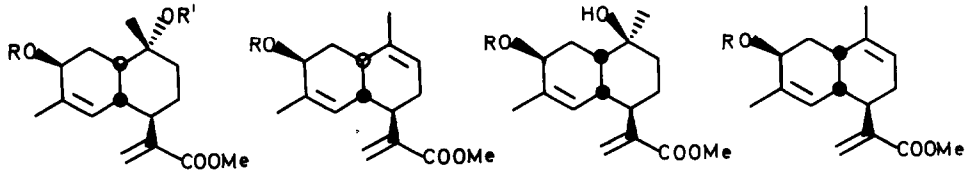
All these data allowed us to propose for the ester **1** the structure:




Remaining to be settled is the stereochemistry (*cis* or *trans* junction between the rings, the relative configuration at the centres $C_2$, $C_6$ and $C_9$ and absolute configuration and the preferred conformation of **1** and related compounds).

Saponification of **1**, followed by treatment with diazomethane, gave a solid of m.p. 181–182° and $[\alpha]_D = -77.5°$, identical with natural compound **2**, named methyl zafronate ($M^+ = 280$) in agreement with the formula $C_{16}H_{24}O_4$, whose $^1H$ NMR spectrum showed the upfield shift of the signal due to the allylic proton at $C_2$, from 5.41 to 3.90 ppm (CH–OAc→CH–OH).

Oxidation of **2** with Jones reagent gave the conjugated ketone **11**, as a viscous oil, $[\alpha]_D = -50.7°$,




**1** R = Ac R' = H
**2** R = H R' = H
**3** R = Ac R' = Et
**4** R = H R' = Et
**5** R = Ac
**6** R = H
**7** R = Ac
**8** R = H
**9** R = Ac
**10** R = H

Table 1. $^1$H NMR data of methyl esters ($CHCl_3$, 200 MHz, TMS)

| Nº H | 1 | 2 | 5 | 6 | 7 | 8 | 9* | 1o | 11** | 18** |
|---|---|---|---|---|---|---|---|---|---|---|
| $H_{1a}$ | 1.34 dd J=8,1o Hz | 1.4o dd J=8,1o Hz | 1.45 dd J=8,1o Hz | 1.36 dd J=8,1o Hz | 1.38 dd J=2,6 Hz | 1.35 dd J=2,5 Hz | 1.4o dd J=2,5 Hz | 1.28 dd J=2,5 Hz | 2.8o m | 2.9o m |
| $H_{1e}$ | 2.48 dd J=2,6 Hz | 2.36 dd J=2,6 Hz | 2.35 dd J=2,5 Hz | 2.38 dd J=2,5 Hz | 2.65 dd J=1.5,2 Hz | 2.38 dd 1.5,2 Hz | 2.34 dd J=1.5,2 Hz | 2.3o dd 1.5,2 Hz | | |
| $H_2$ | 5.41 dd J=6,8 Hz | 3.9o dd J=6,8 Hz | 5.45 dd J=5,8 Hz | 3.78 dd J=5,8 Hz | 5.2o dd J=1.5,2 Hz | 3.93 dd J=1.5,2 Hz | 5.2o dd J=1.5,2 Hz | 3.95 dd J=1.5,2 Hz | - | - |
| $H_4$ | 5.34 br s | 5.1o br s | 5.32 br s | 5.1o br s | 5.68 dd J=1.5,5 Hz | 5.55 dd J=1.5,5 Hz | 5.5o dd J=1.5,5 Hz | 5.48 dd J=1.5,5 Hz | 6.4o br s | 6.67 d J=5 Hz |
| $H_5$ | 2.5o ddd J=1.5,9,1o Hz | 2.6o ddd J=1.5,9,1o Hz | 2.76 ddd J=2,6,9 Hz | 2.8o ddd J=2,6,9 Hz | 2.45 ddd J=5,1o,12 Hz | 2.54 ddd J=5,1o,12 | 2.38 ddd J=5,8,1o Hz | 2.35 ddd J=5,8,1o Hz | 2.45 dd J=9,1o Hz | 2.56 ddd J=5,1o,12 |
| $H_6$ | 2.3o m | 2.3o m | 2.5o m | 2.6o m | 2.7o ddd J=4,1o,12 Hz | 2.76 ddd J=4,1o,12 Hz | 2.43 ddd J=5,5,8 Hz | 2.47 ddd J=5,5,8 Hz | 2.3o m | 2.3o m |
| $H_7$ | 1.34 m | 1.34 m | 2.6o m | 2.6o m | 1.5o m | 1.6o m | 2.2o m | 2.2o m | 1.4o m | 1.5o m |
| $H_8$ | | | 5.32 br s | 5.1o br s | | | 5.6o td J=1.5,8 Hz | 5.6o m | | |
| $H_{1o}$ | 1.8o ddd J=ℓ,1o,1o Hz | 2.1o ddd J=2,1o,1o Hz | 2.95 ddd J=2,9,1o Hz | 3.1o ddd J=2,9,1o Hz | 1.85 ddd J=2,6,1o Hz | 2.15 ddd J=2,6,1o Hz | 2.68 ddd J=2,5,1o Hz | 2.7o ddd J=2,5,1o Hz | 2.1o m | 2.1o m |
| $H_{13}$ | 5.57 d J=2 Hz | 5.6o d J=2 Hz | 5.55 d J=2 Hz | 5.45 d J=2H | 5.72 d J=2 Hz | 5.68 d J=2 Hz | 5.6o d J=2 Hz | 5.63 d J=2 Hz | 5.6o s | 5.7o s |
| $H_{13'}$ | 6.3o d J=2 Hz | 6.18 d J=2 Hz | 6.25 d J=2 Hz | 6.15 d J=2 Hz | 6.28 d J=2 Hz | 6.25 d J=2 Hz | 6.25 d J=2 Hz | 6.28 d J=2 Hz | 6.3o s | 6.27 s |
| $H_{14}$ | 1.2o s | 1.13 s | 1.65 br s | 1.65 br s | 1.2o s | 1.3o s | 1.8o d J=1.5 Hz | 1.8o d J=1.5 Hz | 1.2o s | 1.22 s |
| $H_{15}$ | 1.58 br s | 1.63 br s | | | 1.68 d J=1.5 Hz | 1.8o d J=1.5 Hz | 1.75 d J=1.5 Hz | 1.75 d J=1.5 Hz | 1.68 br s | 1.7o br s |
| MeCO | 2.o7 s | - | 2.o2 s | - | 2.o8 s | - | 2.1o s | - | - | - |
| COOMe | 3.76 s | 3.65 s | 3.7o s | 3.68 s | 3.75 s | 3.78 s | 3.76 s | 3.75 s | 3.72 s | 3.7o s |

* $C_3D_6O$ ; ** 6o MHz

$M^+ = 278$ ($C_{16}H_{22}O_4$) and UV with $\lambda_{max}$ at 239 nm ($\epsilon = 14{,}760$) (CH=C–C=O).[3]

The IR spectrum of **11** showed absorptions ($cm^{-1}$) due to hydroxyl (3300) and conjugated carbomethoxyl (1725, 1270) groups, one conjugated ketone (1675) and unsaturations (3080, 1640, 890, 825) and its $^1H$ NMR spectrum (see Table 1), was in agreement with the structure. The signal due to the olefinic proton H-4. appeared as a singlet, suggesting the *trans*-junction between the rings,[4] with a $H_4$–$H_5$ dihedral angle of about 90°.

This stereochemistry was confirmed by the CD curve of **11**, recorded in MeOH, showing dichroic absorptions at 208 nm ($\Delta\epsilon = +7.78$), 239 nm ($\Delta\epsilon = -11.25$) and 333 nm ($\Delta\epsilon = +1.13$).

The application of the Snatzke rule for transoid enones[5] and the helicity rules[6,7] to the observed signs of the CE, allowed us to assign the 5(S) and 10(R)[8] configurations to **11**.

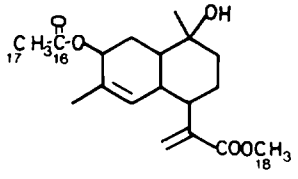
These configurations agreed also with those deduced by comparison of the CD curve of **11** with that reported for the ketone **12**, isolated from *Jasonia tuberosa*,[9] which suggested also the configurations 6(R) and 9(R), and the preferred ss-s conformation depicted.[10]

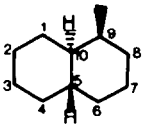
The 9(R) configuration in **1** and **2**, was also suggested by the comparison of the NMR signals due to the geminal–OH methyl group (1.18 ppm) with those exhibited by some related compounds derivated from α-cadinol,[11] and for the $^{13}C$ NMR signal at 18.88 ppm, which is a proof of axial disposition of the methyl group at $C_9$[12] (see Table 2), and was confirmed by the regioselective dehydratation of **1** with $POCl_3$/pyridine, giving only product **13**, of $[\alpha]_D = +20.9°$, whose $^1H$ NMR spectrum showed the loss of the signal due to the geminal–OH methyl group, and two additional signals due to the new $CH_2$=C group. This was evidence for the equatorial

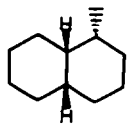
Table 2. $^{13}C$ NMR spectra of 1 and 7 compared with *trans*- and *cis*-9-methyldecalin shift ($\delta$) in ppm relative to TMS

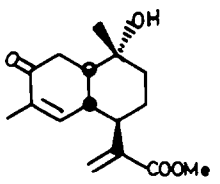
| Carbon atom | 1 | trans-9-methyldecalin | 7 | cis-9-methyldecalin |
|---|---|---|---|---|
| 1 | 31.1o ($\Delta\delta$ = -8.8)* | 42.44 | 28.44 ($\Delta\delta$ = -8.3)* | 36.39 |
| 2 | 73.84 ($\Delta\delta$ = +49.1)** | 22.25 | 7o.58 ($\Delta\delta$ = +49.1)** | 22.81 |
| 3 | 133.89 | 27.42 | 131.21 | 24.48 |
| 4 | 128.63 | 29.36 | 131.21 | 28.1o |
| 5 | 45.46 | 29.36 | 39.62 | 28.1o |
| 6 | 41.o9 | 27.42 | 36.48 | 24.48 |
| 7 | 29.16 | 22.25 | 27.59 | 22.81 |
| 8 | 42.16 | 42.44 | 34.49 | 36.39 |
| 9 | 71.22 ($\Delta\delta$ =+36.1)* | 34.18 | 71.51 ($\Delta\delta$ =+4o.1)* | 32.93 |
| 1o | 49.49 ($\Delta\delta$=+2.3)* | 46.17 | 4o.55 ($\Delta\delta$ =-o.6)* | 41.76 |
| 11 | 143.26 | - | 144.12 | - |
| 12 | 125.3o | - | 124.16 | - |
| 13 | 167.25 | - | 167.9o | - |
| 14 | 18.88 | 19.74 | 29.24 | 28.22 |
| 15 | 21.o5 | - | 21.19 | - |
| 16 | 17o.73 | - | 17o.71 | - |
| 17 | 2o.72 | - | 2o.66 | - |
| 18 | 51.77 | - | 51.77 | - |

(*) $\delta_C^{ROH} - \delta_C^{RH}$ ; (**) $\delta_C^{ROAc} - \delta_C^{RH}$ [21]

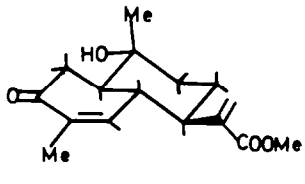


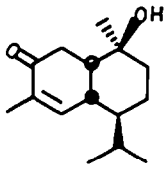






11

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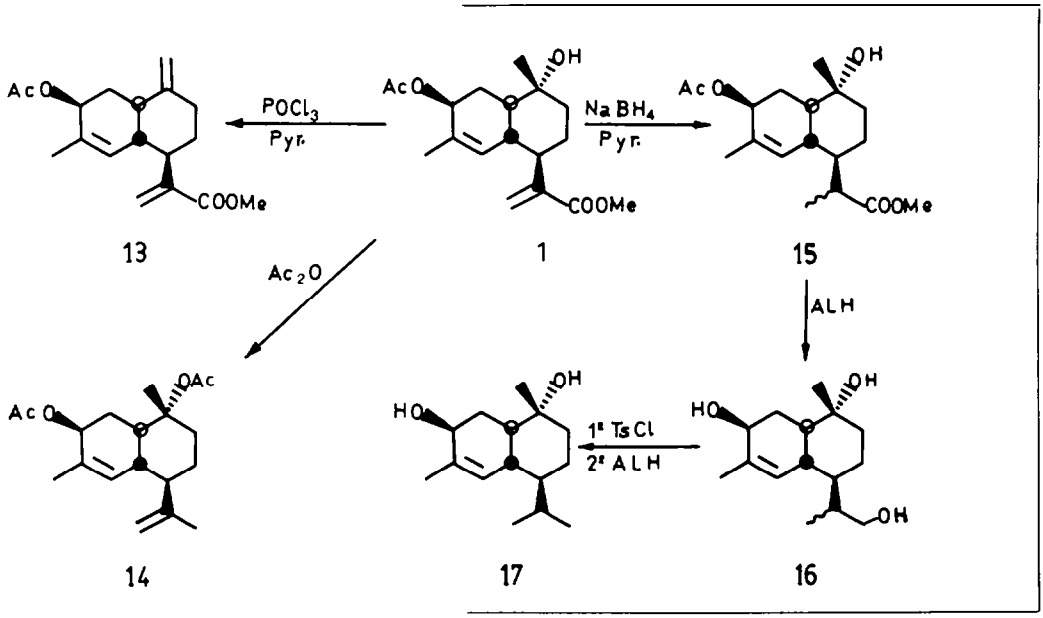
disposition of hydroxyl group at $C_9$,[13] and so on, for its *cis*-stereochemistry to the angular H-10. These results agreed also with the fact that when **1** was treated with $Ac_2O$/pyridin at reflux temperature, gave an oily diacetate of $[\alpha]_D = -70°$, whose $^1H$ NMR spectrum showed the downfield shift of the signal due to the methyl group at $C_9$, and one additional signal at 1.98 ppm, due to the new acetoxyl group.

The $\beta$-equatorial disposition of acetoxyl (or hydroxyl) group at $C_2$ was assigned by the NMR coupling constant $J_{2,3}$ ($W_{1/2} = 17$ Hz) in agreement with the 2(S) configuration.

The structure and stereochemistry assigned to **1–2** and related compound **3–4**, was confirmed by the following transformations.




Selective reduction[14] of **1** with $NaBH_4$/pyridine, gave **15**, whose $^1H$ NMR spectrum showed the absence of the $CH_2$=C group signal, so as one additional doublet at 1.15 ppm (J = 7 Hz, MeCHCOOMe). Treatment of **15** with LAH/ether, gave **16**, which by treatment with TsCl/pyridine,[15] followed by reduction with LAH, gave 2$\beta$-hydroxy-$\alpha$-cadinol, **17**, of m.p. 171–172° and $[\alpha]_D = -60.3°$, whose spectral data were identical with those reported for the diol synthetized by Lin *et al.* from $\alpha$-cadinol.[11]

Thus the stereochemistry of natural compounds **1–4** were confirmed as (2S,5S,6R,9R,10R).

The ester **5** was an oil of $[\alpha]_D = -11.7°$; its mass spectrum did not show the molecular ion, but the fragment at *m/z* 244 ($M^+$–AcOH) agreed with the formula $C_{18}H_{24}O_4$. Its IR spectrum showed typical absorptions of acetoxyl and carbomethoxyl groups and double bonds (see Experimental) and its $^1H$ NMR spectrum (Table 1) showed signals due to four methyl groups (two Me–C=, Me–COO and COOMe), four olefinic protons ($C=CH_2$ and two C=CH) and one allylic proton CH–OAc.

The ester **6** was an oil of $[\alpha]_D = +7.9°$and $M^+ = 262$, in agreement with the formula $C_{16}H_{22}O_3$. Its IR spectrum showed bands assignable to hydroxyl (3400) and conjugated carbomethoxyl (1725, 1260) groups and double bonds $C=CH_2$ and C=CH (3080, 1665, 900, 825). The $^1H$ NMR spectrum showed signals due to three methyl groups (two Me–C= and COOMe), four olefinic protons and one allylic CH–OH. Acetylation of **6** gave a monoacetate identical to **5**.

All these data and comparison of the multiplicity of the $^1H$ NMR signal due to $H_2$ and $H_4$, with those of the $^1H$ NMR spectrum of **1**, allowed us to propose for **5** the structure and absolute configuration methyl (2S,5S,6R,10R) 2-acetoxycadin-3,8,11(13)-trien-12-oate and for **6** methyl (2S,5S,6R,10R) 2-hydroxycadin-3,8,11(13)-trien-12-oate or methyl ledesmate.

The ester **7** was a solid of m.p. 99–101° and $[\alpha]_D = -113°$. Saponification of **7**, followed by treatment with diazomethane, gave a solid of m.p. 175–178° and $[\alpha]_D = -180°$, which was identical with the natural compound **8**.

The mass spectra of **7–8** did not show the molecu-

lar ions but fragments at $m/z$ 280 ($M^+-CH_2=C=O$) and 262 ($M^+$-AcOH) in the case of **7**, and $m/z$ ($M^+-H_2O$) and 244 ($M^+$ – two $H_2O$) in the case of **8**, agreed respectively with formulae $C_{18}H_{26}O_5$ and $C_{16}H_{24}O_3$.

The mass spectral fragmentations, and the IR and NMR spectra of **7–8** were very similar to those exhibited by **1–2**, and showed respectively, the presence of the same functional groups.

Oxidation of **8** with Jones reagent, gave the conjugated ketone **18**, as a viscous oil of $[\alpha]_D = -121°$ and $M^+ = 278(C_{16}H_{22}O_4)$, which spectral properties were very similar to those of **11** (see Table 1). The most significant difference was that NMR signal due to the olefinic proton $H_4$, conjugated with the carbonyl group, appeared now, as a broad doublet at 6.67 ppm ($J = 7$ Hz), and it became a broad singlet by irradiation at 2.80 ppm. Application of the Karplus rule,[16] allowed us to conclude that the CH-4/H-5 dihedral angle was about 40°.

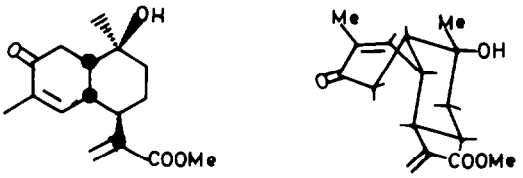
Comparison of this dihedral angle with those reported for the stereoisomer sesquiterpenes with cadalene skeleton,[4] suggested that **18** was a conjugated ketone with muurolane skeleton, with *cis*-junction between the rings and $H_5$ in *trans* with $H_6$.

The *cis*-relative stereochemistry of the hydroxyl at $C_9$ was suggested by the regioselective dehydratation of **7**, to give only one product with a new unsaturation at C-8,[13] which was identical with the natural product **9**, $[\alpha]_D = -70.5°$, whose spectral data were comparable with those of **5** (see Table 1).

The NMR signals for the allylic proton at $C_2$ in **7–10** appeared as a dd ($J = 1.5$ and 2 Hz); this was evidence for the "pseudoequatorial" disposition of $H_2$ and for the *cis* relative stereochemistry of the acetoxyl (or hydroxyl) group at $C_2$, with respect to the angular proton $H_5$ and $H_{10}$.[17]

The CD curve of the ketone **18** recorded in MeOH, showed dichroic absorptions at 357 nm ($\Delta\epsilon = -0.06$), 308 nm ($\Delta\epsilon = +0.32$), 240 nm ($\Delta\epsilon = -7.79$) and <195 nm ($\Delta\epsilon$ negative).

All these data, the application of the helycity rules[6,7] and data collected,[18–20] allowed us to assign to ketone **18** and the related compounds **7–8**, the preferred conformation *ss–s*[10] and the absolute configurations (5S,6R,9S,10S) for **18**, (2S,5S,6R,9S,10S) for **7–8** and (2S,5S,6R,10S) for **9–10**:

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The $^{13}C$ NMR data for **1** and **7** agreed also with the assigned structures to **1–10** (see Table 2). The $^{13}C$ NMR signals have been assigned using the known rules of the chemical shift of $^{13}C$,[12,21] by $^1H$ single frequency off-resonance (SFORD) and selective decoupling techniques.

The chemical shift for the different carbon atoms have been calculated in base of the reported data for *trans*-9-methyldecalin and *cis*-9-methyldecalin,[22] considering the interactions due to the hydroxyl group as a substituent.[21]

## EXPERIMENTAL

M.ps are uncorrected and were determined on a Kofler hot stage apparatus. UV spectra were recorded in EtOH on a Beckmann DK-2 spectrometer. $^1H$ NMR spectra were recorded on a Hitachi Perkin–Elmer R-24 (60 MHz) spectrometer using TMS as an internal standard, and $^{13}C$ NMR spectra were recorded on a Bruker WP2SY spectrometer. MS were obtained on a Hewlett–Packard 5930 A. CD curves were measured on a Jobin–Yvon Dichrograph III.

*Collection of plants. L. pulverulenta* was collected at the end of May 1980, at the municipal district of Zafrón, near Ledesma (Salamanca, Western Spain).

*Extraction and isolation.* The aereal parts (2300 g) were extracted with hot $CHCl_3$, and the crude extract was fractionated as previously reported,[23] to give, after treatment of the acidic fraction, with diazomethane, 4.410 g of methyl esters (35.80% of the sesquiterpenic fraction and 5.89% of the row $CHCl_3$ extract).

Methyl esters were chromatographed on a 250 g of Si gel column and eluted with hex–ether, 1:1 to give:

| Fr. | mg | % | Composition | |
|---|---|---|---|---|
| 1 | 190 | 4.35 | **5** | **9** |
| 2 | 270 | 6.20 | **3** | |
| 3 | 472 | 11.00 | **6** | **10** |
| 4 | 200 | 4.52 | **4** | |
| 5 | 245 | 5.68 | **7** | |
| 6 | 510 | 11.83 | **1** | |
| 7 | 2400 | 54.90 | **2** | **8** |

and different components were purificated by prep. CC, TLC or recrystallization.

*Methyl 2 - acetoxy - 9 - hydroxycadin - 3,11(13) - dien - 12 - oate or methyl 2-acetylzafronate*, **1**. Recrystallization of fraction 6 in hexane, gave pure **1**, m.p. 104–105° and $[\alpha]_D = -76°$ (c, 1.65, $CHCl_3$) IR (melted, $\nu$, $cm^{-1}$): 3400, 1740, 1725, 1630, 1445, 1380, 1250, 1120, 1040, 1030, 975, 940, 915, 880, 850, 825. MS, $m/z$ (%): 262 ($M^+$–AcOH, 18), 244(15), 230(14), 212(31), 185(37), 172(67), 144(70), 132(100), 110(33), 99(74), 93(96), 91(98), 72(44), 43(55).

*Acetylation of* **1**. A soln of 97 mg of **1** in 3 ml of pyridine, 3 ml of $Ac_2O$ was refluxed for 12 h. After the usual treatment, 107 mg of methyl 2,9-diacetoxycadin-3,11(13)-dien-12-oate, **14**, of $[\alpha]_D = -70.3°$ (c, 3.56, $CHCl_3$) were obtained. IR (film, $\nu$, $cm^{-1}$): 1745, 1720, 1630, 1445, 1375, 1240, 1105, 1030, 980, 930, 890, 875, 830. $^1H$ NMR ($CDCl_3$, $\delta$, ppm): 1.50 (3H, s), 1.60 (3H, br s), 1.98 (3H, s), 2.07 (3H, s), 3.70 (3H, s), 5.30 (1H, br s), 5.35 (1H, m), 6.27 (1H, s). MS, $m/z$ (%): 262(8), 244(8), 212(15), 202(8), 197(8), 185(20), 132(100), 93(40), 43(95).

*Dehydration of* **1**. To a stirred soln of 100 mg of **1** in 4 ml of dry pyridine at 0°, 1.6 ml of $POCl_3$ were added dropwise and the soln was kept at room temp for 6 h. The soln was poured on ice water and extracted with ether. The ethereal layer was washed with 2N HCl, aq sodium bicarbonate and water, dried over $Na_2SO_4$ and evaporated to give 91 mg of methyl 2-acetoxycadin-3,11-(13)-dien-12-oate, **13**, of $[\alpha]_D = +20.9°$ (c, 2.0, $CHCl_3$). IR (film, $\nu$, $cm^{-1}$): 3080, 1740, 1725, 1660, 1630, 1440, 1370, 1240, 1080, 1020, 980, 955, 890, 855, 820. $^1H$ NMR ($CDCl_3$, $\delta$, ppm): 1.62 (3H, s), 2.10 (3H, s), 3.77 (3H, s), 4.58 and 4.72 (2H, 2 s), 5.38 (1H, br s), 5.43 (1H, m), 5.52 (1H, s), 6.28 (1H, s). MS, $m/z$ (%): 262(2), 244(7), 185(12), 184(12), 143(11), 132(60), 117(17), 105(17), 91(34), 61(60), 43(100).

*Deacetylation of* **1**. To a soln of 85 mg of **1** in 2 ml of MeOH, 3 ml of a methanolic 5% NaOH soln were added and it was kept at room temp overnight. Evaporation of the solvent and extraction with AcOEt, after 2H HCl

acidification, gave 58 mg of an acid which by treatment with diazomethane, gave 60 mg of **2**.

*Methyl* 2,9 - *dihydroxycadin* - 3,11(13) - *dien* - 12 - *oate*; *methyl zafronate*, **2**. M.p. 181–182° (acetone) and $[\alpha]_D = -77.5°$ (c, 1.6, EtOH). IR (KBr, $\nu$, $cm^{-1}$): 3330, 1725, 1630, 1450, 1140, 1120, 1040, 1020, 960, 925, 890, 825. MS, *m/z* (%): 280 ($M^+$, 7), 262 ($M^+-H_2O$, 14), 244 (262–$H_2O$, 12), 230(32), 212(29), 185(40), 149(92), 139(68), 132(77), 99(93), 71(75), 43(100).

*Oxidation of* **2** *with Jones reagent*. To a soln of 60 mg of **2** in 10 ml of acetone, 1 ml of Jones reagent was added and the reaction mixture was kept at room temp for 15 min. After destroying the excess reagent with MeOH, the extraction with ether gave 54 mg of methyl 9-hydroxy-2-oxocadin-3,11(13)-dien-12-oate, **11**, of $[\alpha]_D = -50.3°$ (c, 1.3, $CHCl_3$). IR (film, $\nu$, $cm^{-1}$): 3300, 3080, 1725, 1675, 1640, 1450, 1380, 1270, 1200, 1115, 1090, 965, 935, 890, 850, 825. MS, *m/z* (%): 278 ($M^+$, 1), 260(2), 220(4), 200(2), 119(34), 117(39), 95(68), 93(100), 61(81), 43(8). UV: $\lambda_{max}^{EtOH}$ at 239 nm ($\epsilon = 14{,}760$). CD(MeOH): $\Delta\epsilon_{209} = +8.02$, $\Delta\epsilon_{239} = -11.2$, $\Delta\epsilon_{332} = +1.13$.

*Reduction of* **1** *with* $NaBH_4$. To a stirred soln of 400 mg of **1** in 10 ml of pyridine, 400 mg of $NaBH_4$ were slowly added, keeping the stirring for 72 h at 60°. 10 ml of aq 10% $KIO_3$ and 10 ml of water were added and the reaction mixture was kept for 15 min, then extracted with ether. The ethereal extract was washed with 2N HCl and water, dried with $Na_2SO_4$ and evaporated to give 320 mg of a product, which after being chromatographed on 20 g of Si gel, gave by elution with $CHCl_3$–$Et_2O$, 9:1, 190 mg of methyl 2 - acetoxy - 9 - hydroxycadin - 3 - en - 12 - oate, **15**, of $[\alpha]_D = -79.1°$ (c, 2.3, $CHCl_3$). IR (film, $\nu$, $cm^{-1}$): 3330, 1735, 1710, 1450, 1430, 1370, 1240, 1205, 1165, 1120, 1040, 1025, 975, 940, 910, 875, 840, 820. NMR ($CDCl_3$, $\delta$, ppm): 1.07 (3H, s), 1.15 (3H, d, J = 7 Hz), 1.63 (3H, br s), 2.07 (3H, s), 2.85 (1H, br t, J = 7 Hz), 3.74 (3H, s), 5.36–5.62 (2H, m). MS, *m/z* (%): 264 ($M^+$–AcOH, 3), 246(264-$H_2O$, 4), 204(9), 177(18), 159(33), 119(33), 105(20), 93(24), 91(29), 43(100).

*Reduction of* **15** *with* LAH. Treatment of 190 mg of **15** with 150 mg of LAH in dry ether, followed by prep. CC on 10 g silica gel, gave, by elution with AcOEt, 150 mg of methyl 2,9,12-trihydroxycadin-3-en-12-oate, **16**, of $[\alpha]_D = -70.4°$ (c, 1.25, EtOH) IR (melted, $\nu$, $cm^{-1}$): 3300, 1450, 1375, 1250, 1210, 1120, 1060, 1035, 1020, 920, 870, 830. NMR ($C_3D_6O$, $\delta$, ppm): 0.95 (3H, d, J = 7 Hz), 1.07 (3H, s), 1.70 (3H, br s), 3.25–3.80 (3H, m), 5.60 (1H, br s). ME, *m/z* (%): 254 ($M^+$, 3), 246(2), 221(2), 218(3), 200(3), 177(11), 159(42), 119(19), 105(18), 93(21), 91(22), 69(25), 43(100).

*2β-Hydroxy-α-cadinol*, **17**. To a stirred soln of 136 mg of **15** in 2 ml dry pyridine at 0°, 89 mg of TsCl were added, keeping the stirring for 30 min. The reaction mixture was left for six more hours at 0°, monitoring the course of reaction by TLC. When the reaction was over, the mixture was poured on ice water and extracted with ether. The organic extract was washed with 2N HCl, aq sodium bicarbonate and water, dried with $Na_2SO_4$ and evaporated, to give 90 mg of tosylate, which was treated with 90 mg of LAH in 4 ml of dry ether and kept at room temp for 12 h, monitoring the reaction by TLC. The usual treatment of the ethereal extract followed by percolation on 6 g of silica gel (elution with hexane–$Et_2O$ 1:1) gave 30 mg of 2β-hydroxy-α-cadinol, **17**, m.p. 171–172° and $[\alpha]_D = -60.3°$ (c, 0.3, $CHCl_3$). IR (KBr, $\nu$, $cm^{-1}$): 3300, 1450, 1430, 1390, 1370, 1320, 1295, 1250, 1210, 1125, 1080, 1060, 1035, 1020, 1010, 915, 885, 870, 825. $^1H$ NMR ($CDCl_3$, $\delta$, ppm): 0.78, (3H, d, J = 7 Hz), 0.92 (3H, d, J = 7 Hz), 1.12 (3H, s), 1.75 (3H, br s), 4.20 (1H, d, J = 6 Hz), 5.57 (1H, m, $W_{1/2} = 4$ Hz). MS, *m/z* (%): 238 ($M^+$, 36), 223(3), 220(2), 205(14), 202(18), 187(17), 177(24), 159(39), 149(36), 135(36), 121(37), 95(49), 91(49), 79(47), 69(45), 43(100).

*Methyl* 2 - *acetoxy* - 9 - *ethoxycadin* - 3,11(13) - *dien* - 12-*oate*, **3**. It was eluted in CC with hexane–ether 9:1 as an oil of $[\alpha]_D = -71°$ (c, 1.97°, $CHCl_3$). IR (film, $\nu$, $cm^{-1}$): 1740, 1725, 1630, 1445, 1380, 1240, 1200, 1150, 1070, 1030, 950, 820. $^1H$ NMR ($CDCl_3$, $\delta$, ppm): 1.11 (3H, t, J = 7 Hz), 1.13 (3H, s), 1.55 (3H, br s), 2.05 (3H, s), 3.40 (1H, q, J = 7 Hz), 3.75 (3H, s), 5.35 (1H, m), 5.40 (1H, s), 6.25 (1H, s).MS, *m/z* (%): 262(8), 244(7), 230(7), 212(13), 185(18), 132(52), 99(100), 91(48), 86(52), 71(60), 43(59).

*Methyl* 2 - *hydroxy* - 9 -*ethoxycadin* - 3,11(13) - *dien* - 12-*oate*, **4**. The oily pure compound was eluted in CC with hexane–ether 1:1, $[\alpha]_D = -68.5°$ (c, 4.5, $CHCl_3$). IR (film, $\nu$, $cm^{-1}$): 3300, 1725, 1640, 1450, 1390, 1250, 1200, 1160, 1140, 1110, 1080, 1050, 950, 920, 885, 820. $^1H$ NMR ($CDCl_3$, $\delta$, ppm): 1.18 (3H, s), 1.23 (3H, t, J = 7 Hz), 1.70 (3H, br s), 3.37 (2H, q, J = 7 Hz), 3.70 (3H, s), 4.00–4.20 (1H, m), 5.18 (1H, br s), 5.53 (1H, s), 6.25 (1H, s). MS, *m/z* (%): 308 ($M^+$, 2), 262(4), 244(4), 230(5), 212(8), 185(15), 132(45), 99(100), 91(52), 86(51), 71(81), 43(52).

*Methyl* 2 - *acetoxycadin* - 3,8,11(13) - *trien* - 12 - *oate or* 2-*acetylledesmate*, **5**. It was eluted in CC on Silica gel with benzene, as colourless oil of $[\alpha]_D = -11.7°$ (c, 5.6, $CHCl_3$). IR (film, $\nu$, $cm^{-1}$): 3080, 1740, 1725, 1630, 1440, 1375, 1245, 1150, 1100, 1025, 1000, 970, 825. MS, *m/z* (%): 244 ($M^+$–AcOH, 8), 243(13), 229(6), 211(18), 183(20), 169(21), 156(21), 143(23), 132(100), 115(38), 91(50), 43(38).

*Methyl* 2 - *hydroxycadin* - 3,8,11(13) - *trien* - 12 - *oate or methyl ledesmate*, **6**. Colourless oil of $[\alpha]_D = +7.4°$ (c, 0.80, $CHCl_3$)·IR (film, $\nu$, $cm^{-1}$): 3400, 1725, 1665, 1635, 1445, 1380, 1280, 1260, 1200, 1150, 1050, 1040, 900, 870, 825. MS, *m/z* (%): 262 ($M^+$, 8), 244(8), 230(8), 212(16), 202(16), 185(29), 184(29), 169(19), 143(48), 132(100), 117(34), 91(45), 77(23), 43(37).

*Methyl* 2 - *acetoxy* - 9(S) - *hydroxymuurol* - 3,11(13) - *dien* - 12 - *oate or methyl* (9S)2-*acetylepizafronate*, **7**. It was isolated as a solid of m.p. 99–101° and $[\alpha]_D = -113°$ (c, 2.1, $CHCl_3$), by crystallization of frac. 5 in hexane. IR (melted, $\nu$, $cm^{-1}$): 3400, 1740, 1725, 1640, 1450, 1380, 1250, 1200, 1150, 1070, 1030, 990, 960, 950, 890, 860. EM, *m/z* (%): 280 ($M^+-CH_2C=O$, 3), 262(11), 244(4), 230(8), 212(11), 185(16), 132(85), 113(33), 110(32), 93(67), 91(100), 77(56), 43(78).

*Deacetylation of* **7**. Saponification of 85 mg of **7**, followed by treatment with diazomethane, gave 60 mg of methyl 2,9-dihydroxymuurol - 3,11(13) - dien - 12 - oate identical in all respects with natural compound **8**.

*Methyl* 2,9(S) - *dihydroxymuurol* - 3,11(13) - *dien* - 12 - *oate or* (9S) *methyl epizafronate*, **8**. M.p. 175–178° and $[\alpha]_D = -180°$ (c, 2.0, EtOH). IR (nujol, $\nu$, $cm^{-1}$): 3360, 1730, 1640, 1450, 1380, 1300, 1260, 1200, 1150, 1050, 990, 950, 890, 870, 820. MS, *m/z* (%): 262 ($M^+-H_2O$, 6), 244(2), 212(4), 202(8), 132(27), 95(24), 91(21), 59(26), 43(100).

*Dehydratation of* **7**. Treatment of 40 mg of **7** with $POCl_3$/pyridine for 6 h at room temp, as was previously described for **1**, gave 35 mg of a colourless oil identical in all respect with natural compound **9**.

*Methyl* 2 - *acetoxymuurol* - 3,8,11(13) - *trien* - 12 - *oate or methyl epi* - 2 - *acetylledesmate*, **9**. It was an oil of $[\alpha]_D = -70.5°$ (c, 1.9, $CHCl_3$). IR (film, $\nu$, $cm^{-1}$): 1740, 1725, 1630, 1440, 1375, 1245, 1210, 1150, 1030, 970, 900, 855, 820. MS, *m/z* (%): 262 ($M^+-CH_2C=O$, 2), 244 ($M^+$–AcOH, 3), 239(4), 212(8), 184(18), 156(21), 143(23), 132(100), 117(32), 91(62), 43(48).

*Methyl* 2 - *hydroxymuurol* - 3,8,11(13) - *trien* - 12 - *oate or methyl epiledesmate*, **10**. It was an oil of $[\alpha]_D = -84.9°$ (c, 0.83, $CHCl_3$). IR (film, $\nu$, $cm^{-1}$): 3400, 1720, 1630, 1440, 1380, 1280, 1250, 1200, 1140, 1055, 1030, 950, 890, 870, 850, 820. MS, *m/z* (%): 262 ($M^+$, 6), 244 ($M^+-H_2O$, 4), 230(5), 229(5), 212(14), 197(10), 184(31), 163(48), 143(48), 132(100), 117(32), 91(43).

*Oxidation of* **8**. Treatment of 50 mg of **8** with Jones reagent, as previously described for **2**, gave 24 mg of methyl 2 - oxo - 9(S) - hydroxymuurol - 3,11(13) - dien - 12 - oate, **18**, as a colourless oil of $[\alpha]_D = -121°$ (c, 1.8·$CHCl_3$). IR (film, $\nu$, $cm^{-1}$): 3300, 1720, 1670, 1640, 1450, 1375, 1285, 1270, 1250, 1190, 1140, 1060, 900, 890, 870, 850, 825. MS, *m/z* (%): 278 ($M^+$, 3), 260 ($M^+-H_2O$, 15), 245(7), 228(15), 200(25), 185(42), 148(72), 138(34), 110(53), 109(100), 91(38), 79(32), 71(29), 43(92). CD (MeOH): $\Delta\epsilon_{195} = (-)$, $\Delta\epsilon_{240} = -7.69$, $\Delta\epsilon_{308} = +0.32$, $\Delta\epsilon_{357} = -0.06$.

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