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J. Nat. Prod., **1992**, 55 (7), 845-850 • DOI:
10.1021/np50085a002 • Publication Date (Web): 01 July 2004

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DC 20036

A NEW CLERODANE-TYPE DITERPENOID FROM *EPERUA LEUCANTHA*

DINORAH AVILA,¹ JOSÉ D. MEDINA,*

Centro de Química, Instituto Venezolano de Investigaciones Científicas,
Apartado 21827, Caracas 1020-A, Venezuela

and ANTHONY J. DEEMING

Department of Chemistry, University College London, UK

ABSTRACT.—The structure of a new diterpene isolated from the seed pods of *Eperua leucantha* has been determined by spectroscopic evidence as (–)-7 β -hydroxycyclo-8(17),13E-dien-15-oic acid, isolated as its methyl ester, **1**. Norhardwikiic acid, 16-oxo-15,16H-hardwikiic acid, and 2-oxokolavenic acid have also been identified as their methyl ester derivatives **2**, **3**, and **4**, respectively.

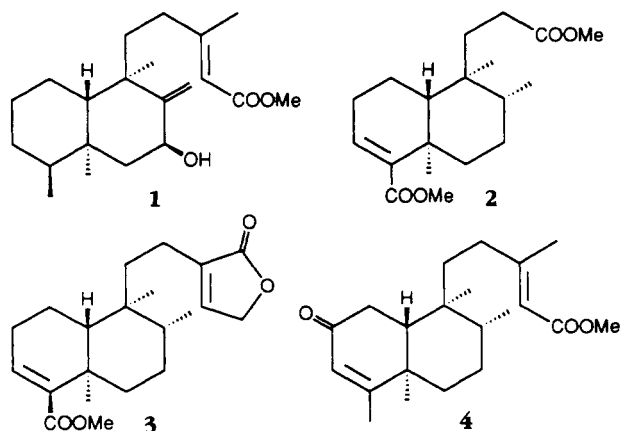
In the course of our continuing investigation of plants of the genus *Eperua* (1–4), we studied the seed pods of *Eperua leucantha* Benth. (Leguminosae).

Chromatography of the acidic fraction from the C₆H₆ extract afforded a mixture of acids that was treated with CH₂N₂ to obtain the methyl ester derivatives. This mixture was separated by preparative layer chromatography to give a new clerodane-type diterpenoid **1**, as well as the methyl esters **2**, **3**, and **4** of nor-hardwikiic acid, 16-oxo-15,16H-hardwikiic acid, and 2-oxokolavenic acid, respectively.

The ¹H-nmr spectrum of **1** showed a singlet at δ 3.68 for the methoxyl group, which demonstrates the existence of only one carboxylic moiety in the molecule, and the ir spectrum exhibits absorptions at 1720, 1644, and 1150 cm⁻¹ ($\nu_{C=O}$, $\nu_{C=C}$, ν_{C-O-C} , respectively), indicating an α,β -unsaturated methyl ester.

There are also signals for two tertiary and one secondary methyl group (δ 0.74 s, 1.04 s, 0.81 d, $J = 6.3$ Hz). A vinylic methyl group is evidenced by a signal at δ 2.15, which is coupled ($J = 1.2$ Hz) to a broad singlet for a vinylic proton at δ 5.64, both characteristic of an unsaturated side chain of a bicyclic diterpene with an *E* configuration (5).

The molecular formula of **1** was determined to be C₂₁H₃₄O₃, on the basis of elemental analysis and ¹³C-nmr pulse experiments. This formula requires five degrees of



¹On leave from Universidad del Zulia, Maracaibo, Venezuela, for graduate work.

unsaturation. In addition, the existence of four methyl groups (δ 21.2, 19.1, 18.0, 15.7), five quaternary (δ 167.2, 162.2, 161.2, 40.2, 39.3), four methine (δ 114.9, 69.5, 48.4, 36.6), and seven methylene (δ 99.4, 37.5, 37.4, 36.3, 34.4, 27.2, 20.4) carbons was shown by a DEPT experiment.

In the mass spectrum, the molecular ion is not observed, and the largest fragment is at 316 amu ($[M - H_2O]^+$; m/z 302 $[M - MeOH]^+$ at 20 eV). A fragment at m/z 207, attributed to the loss of the side chain ($C_7H_{11}O_2$), must have an oxygenated function, which is inferred from the ir spectrum (ν_{OH} 3350 cm^{-1}) and confirmed in the 1H -nmr spectrum by a signal centered at δ 4.30 (dd, $J = 12$ and 6 Hz) for a proton geminal to the hydroxyl group. The multiplicity of the signal indicates that CH-OH must be adjacent to a methylene group, and the coupling constants suggest an axial configuration for the proton under consideration. The mass spectrum also shows an intense peak at m/z 95, typical of clerodane-type diterpenes (6).

In addition, the 1H -nmr spectrum exhibits two signals at δ 4.9 and 4.7 assignable to an exocyclic methylene group. Both signals show long range coupling ($J = 1$ Hz) with the proton geminal to the hydroxyl group, indicating an allylic position for the CH-OH moiety, which justifies the abnormal downfield shift observed for the oxymethine proton. It also shows absence of peaks in the δ 1.7–2.1, indicating that there are no allylic protons in the molecule.

These facts indicate that the hydroxyl group and the exocyclic methylene must be located either at ring A or B of a clerodane skeleton, in the case of ring A as a 3β -OH and a $\Delta^{4(18)}$ unsaturation, or in ring B as a 7β -OH and a $\Delta^{8(17)}$ unsaturation.

Singh and Singh (7) report a clerodane with a 3β -OH and a $\Delta^{4(18)}$ unsaturation but, unfortunately, do not give ^{13}C data. However, all examples in the literature (8–11) of unstrained systems with similar arrangement give values between 145 and 155 ppm for

TABLE 1. ^{13}C -nmr Spectral Data of Compounds 1–4.

Carbon	Compound			
	1	2	3	4
C-1	27.2	18.0	18.0	35.6
C-2	34.4	27.0 ^a	27.2 ^a	199.8
C-3	20.4	136.8	136.7	125.5
C-4	36.6 ^a	142.3	143.4	167.0
C-5	39.3	37.5	37.6	39.8
C-6	37.5 ^b	32.9	35.8	34.9 ^a
C-7	69.5	28.0	27.1 ^a	26.8
C-8	161.2	36.3	36.0	36.6
C-9	40.2	38.4	38.7	38.7
C-10	48.4	46.6	46.6	45.6
C-11	36.2 ^a	35.8	36.3	34.0 ^a
C-12	37.4 ^b	27.1 ^a	17.4	35.6
C-13	162.2	167.7	134.9	160.2
C-14	114.9		142.3	115.2
C-15	167.2		70.0	172.2
C-16	19.1		174.1	18.9
C-17	99.4	15.8	15.8	15.8
C-18	15.7	174.6	167.6	18.3
C-19	21.2	20.6	20.6	19.1
C-20	17.9	17.3	19.0	17.6
OMe	50.8	51.0	50.1	50.7

^{a,b}Interchangeable values within a column.

C-4 and between 104 and 115 ppm for the equivalent of C-18. In our case, carbons involved in the exocyclic methylene absorb at 161.2 and 99.4 ppm (Table 1). The only possible explanation for this fact has to be hydrogen bonding between a 7β -OH group and C-15 carbonyl, which somehow affects chemical shifts of the above-mentioned carbons.

The stereochemistry at C-18 and C-19 is inferred from the ^{13}C -nmr spectrum. The signal at higher field (δ 15.7) indicates that C-18 is β -oriented, since if it were α -oriented, it would show resonance around δ 24.0, as has been observed for similar compounds (12). On the other hand, the signal at δ 21.2 for C-19 implies that this methyl group has a *trans* relation with H-10, since otherwise it would absorb around δ 30.0 (13-15).

All the information above, and the negative optical rotation $[\alpha]^{25}_{\text{D}} - 10^\circ$, allow us to propose the structure of $(-)$ - 7β -hydroxycleroda-8(17),13*E*-dien-15-oic acid methyl ester for compound **1**.

The structures of compounds **2** and **3** were determined through their spectral data, which fully agree with those for the same compounds isolated from *Grangea maderaspatana* (16), identified as the methyl esters of norhardwikiic and 16-oxo-15,16*H*-hardwikiic acids, respectively. The spectral data obtained for **4** coincide with the corresponding data for methyl 2-oxokolavenoate (17). Since there is no report in the literature about crystal data for clerodane-type compounds, we felt it important to have such a report, and the structure of **4** was confirmed by means of X-ray diffraction methods (Figure 1, Table 2 and Experimental section). Table 1 shows the ^{13}C -nmr data for compounds **1-4**.

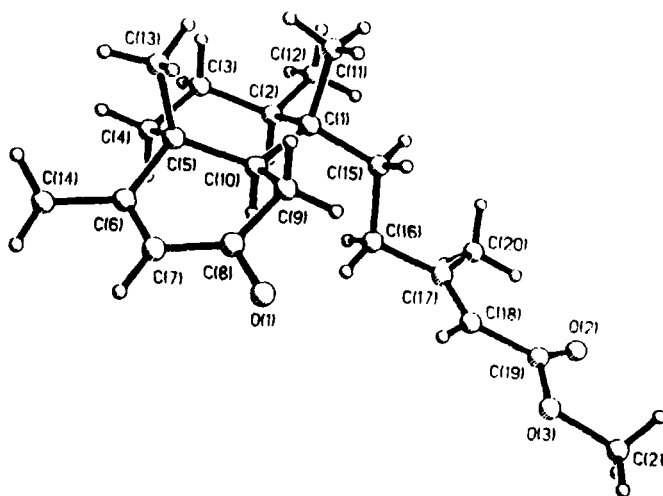


FIGURE 1. The molecular structure of methyl 2-oxokolavenoate [4].

EXPERIMENTAL

PLANT MATERIAL.—Sample identified by R. Liesner, Missouri Botanical Garden, 1979. Present sample identified by E. Medina, Centro de Ecología, IVIC. The seed pods of *E. leucantha* were collected in March 1987 from San Carlos de Río Negro (Venezuelan Amazonas). A voucher specimen is deposited under No. M-243 at the Botanical Garden, Caracas, Venezuela.

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected. Elemental analysis was performed in a Leco CHN-800 analyzer. Optical rotations were measured in a Carl Zeiss polarimeter in CHCl_3 solutions; uv was measured in a Perkin-Elmer 559 UV-VIS in EtOH solutions. The ir spectra were recorded in a Nicolet 5DX FTIR spectrophotometer. ^1H - and ^{13}C -nmr spectra were recorded in a Bruker

TABLE 2. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameter ($\text{\AA}^2 \times 10^3$) for Compound 4.

	x	y	z	U(eq) ^a
C-1	2278(11)	41192	7889(9)	60(2)
C-2	1029(13)	3901(11)	6648(11)	80(3)
C-3	405(12)	2740(12)	6712(10)	84(3)
C-4	1709(12)	1898(11)	6816(10)	77(3)
C-5	2911(11)	2079(11)	8083(10)	63(3)
C-6	4384(12)	1299(10)	8029(10)	73(3)
C-7	5866(15)	1635(12)	8530(11)	96(3)
C-8	6280(16)	2630(13)	9207(11)	93(3)
C-9	4893(13)	3415(12)	9229(11)	87(3)
C-10	3518(11)	3286(10)	8052(9)	63(3)
C-11	1360(13)	4357(13)	9179(11)	97(4)
C-12	-362(15)	4720(14)	6410(13)	121(5)
C-13	2117(13)	1722(12)	9379(10)	89(3)
C-14	4071(16)	205(13)	7471(13)	119(5)
C-15	3114(12)	5273(11)	7659(10)	77(3)
C-16	4246(12)	5343(11)	6528(10)	83(3)
C-17	4943(12)	6447(11)	6356(10)	72(3)
C-18	6418(12)	6678(12)	6846(9)	72(3)
C-19	7336(12)	7694(13)	6850(10)	71(3)
C-20	3845(16)	7290(13)	5642(13)	125(5)
C-21	9879(15)	8510(13)	7457(14)	116(4)
O-1	7603(9)	2868(14)	9685(10)	163(5)
O-2	6853(9)	8539(10)	6359(9)	111(4)
O-3	8863(8)	7556(10)	7390(7)	99(3)

^aEquivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor.

AM300 instrument working at 300 and 75.5 MHz, respectively; in all cases TMS was used as internal standard. Eims were obtained from a Kratos MS25RFA spectrometer operating at 70 eV; X-ray structure determination was made with a Nicolet R3v/m diffractometer.

EXTRACTION AND FRACTIONATION.—The dried and ground seed pods (2.5 kg) were extracted in a Soxhlet using petroleum ether (40–60°) and C_6H_6 as solvents. The C_6H_6 extract (33 g) was partitioned with 10% aqueous KOH; the organic layer was dried over $MgSO_4$, and evaporation of solvent left the neutral fraction (6.6 g). The aqueous phase was adjusted to pH 3 with HCl and extracted (3X) with Et_2O . Evaporation of the solvent yielded the acidic fraction (22 g). A portion (12 g) of this fraction was chromatographed on Si gel (Merck®, 70–230 mesh, 500 g) using Et_2O /petroleum ether mixtures of increasing polarity as eluent. Fractions of 200 ml were collected. Fractions 12–16 (20% Et_2O /petroleum ether) afforded a mixture of acids (800 mg) which was methylated with CH_2N_2 and purified by preparative tlc (Si gel, 2 mm, 50% Et_2O /petroleum ether), developed successively three times.

ISOLATION OF (-)-7 β -HYDROXYCLERODA-8(17), 13E-DIEN-15-OIC ACID METHYL ESTER [1].—The most polar compound of the preparative layer gave a crystalline solid, needles (17 mg), mp 84–87°. *Anal.* found C 75.5, H 10.3%; $C_{21}H_{34}O_3$ requires C 75.4, H 10.4%. $[\alpha]_D^{25} - 10^\circ$ ($c = 0.15$, $CHCl_3$); ir (KBr) ν max 3350, 3000, 2920, 2870, 1720, 1644, 1225, 1150 cm^{-1} ; 1H nmr ($CDCl_3$) δ 0.74 (3H, s, H-19), 0.81 (3H, d, $J = 6.3$ Hz, H-18), 1.04 (3H, s, H-20), 2.15 (3H, d, $J = 1.2$ Hz, H-16), 3.68 (3H, s, OMe), 4.27–4.30 (1H, dd, $J_{ax,ax} = 12$ Hz, $J_{ax,eq} = 6$ Hz, H-7 α), 4.73 (1H, bs, H₂-17), 4.92 (1H, bs, H₂-17), 5.64 (1H, bs, H-14); ^{13}C nmr ($CDCl_3$, 75.5 MHz) see Table 1; eims m/z (%) $[M]^+$ ($C_{21}H_{34}O_3$) 334; $[M - 18]^+$ 316 (0.8), $[M - MeOH]^+$ 302 (3), $[M - MeOH - 15]^+$ 287 (1), 242 (7), 189 (37), 159 (27), 133 (30), 119 (45), 105 (48), 95 (60), 55 (79), 41 (100).

ISOLATION OF NORHARDWIKIIC ACID METHYL ESTER [2].—The least polar compound of the above cited tlc was a colorless oil: $[\alpha]_D^{25} - 70^\circ$ ($c = 0.3$, $CHCl_3$); ir (film) ν max 2957, 2929, 1731, 1662, 1437, 1239 cm^{-1} ; 1H nmr ($CDCl_3$) 300 MHz δ 0.77 (3H, s, H-20), 0.81 (3H, d, $J = 6$ Hz, H-17), 1.25 (3H, s, H-19), 3.66 (3H, s, OMe), 3.68 (3H, s, OMe), 6.6 (1H, t, $J = 3$ Hz, H-3); ^{13}C nmr ($CDCl_3$, 75.5

(MHz) see Table 1; eims m/z (%) $[M]^+$ ($C_{19}H_{30}O_4$) 322 (3), $[M - H_2O]^+$ 304 (3), $[M - MeOH]^+$ 290 (100), 275 (1), $[M - C_4H_7O_2]^+$ 235 (19), $[M - C_4H_7O_2 - MeOH]^+$ 203 (35), 175 (45), 139 (64).

ISOLATION OF 16-OXO-15, 16H-HARDWIKIIC ACID METHYL ESTER [3].—The same preparative layer afforded another colorless oil: $[\alpha]_D^{25} - 70^\circ$ ($c = 0.3$, $CHCl_3$); $uv \lambda_{max}$ (EtOH) 213 nm ($\log \epsilon 3.8$); ir (film) ν_{max} 3020, 2957, 2930, 1753, 1708, 1435, 1233 cm^{-1} ; 1H nmr ($CDCl_3$, 300 MHz) δ 0.77 (3H, s, H-20), 0.82 (3H, d, $J = 7$ Hz, H-17), 1.26 (3H, s, H-19), 3.68 (3H, s, OMe), 4.77 (2H, bs, H-15), 6.62 (1H, t, $J = 3$ Hz, H-3), 7.12 (1H, s, H-16); ^{13}C nmr ($CDCl_3$, 75.5 MHz) see Table 1; eims m/z (%) $[M]^+$ ($C_{21}H_{30}O_4$) 346; $[M - MeOH]^+$ 314 (100), $[M - MeOH - 15]^+$ 299 (7), 271 (16), 235 (10), 175 (66), 139 (75), 107 (65).

ISOLATION OF 2-OXOKOLAVENIC ACID METHYL ESTER [4].—Fraction 20 (30% Et_2O /petroleum ether) from the chromatographic column afforded a yellow oil, which was methylated with CH_2N_2 and purified by preparative tlc (Si gel, 2 mm, 50% Et_2O /petroleum ether), developed successively three times. The compound was obtained as colorless prisms from Et_2O /EtOH: mp 92–94°; $[\alpha]_D^{25} - 17^\circ$ ($c = 0.3$, $CHCl_3$); $uv \lambda_{max}$ (EtOH) 223 ($\log \epsilon 4.3$); ir (KBr) ν_{max} 3020, 2960, 2900, 1711, 1665, 1645, 1230 cm^{-1} ; 1H nmr ($CDCl_3$, 300 MHz) δ 0.82 (3H, s, H-20), 0.84 (3H, d, $J = 6$ Hz, H-17), 1.12 (3H, s, H-19), 2.34 (1H, ABX, $J_{gem} = 17.7$ Hz, $J_{eq,ax} = 4.4$ Hz, H_{eq-1}), 2.40 (1H, ABX, $J_{gem} = 17.7$ Hz, $J_{ax,ax} = 13.3$ Hz, H_{ax-1}), 3.68 (3H, s, OMe), 5.65 (1H, bs, H-14), 5.73 (1H, bs, H-3); ^{13}C nmr ($CDCl_3$, 75.5 MHz) see Table 1; eims m/z (%) $[M]^+$ ($C_{21}H_{32}O_3$) 332 (6), $[M - OMe]^+$ 301 (8), $[M - MeOH]^+$ 300 (10), 285 (16), $[M - C_7H_{11}O_2]^+$ 205 (39), 135 (44), 121 (48), 109 (71), 95 (100). X-ray:¹ the single crystal X-ray structure of compound 4 was determined using a Nicolet R3v/m diffractometer operating with Mo-K α radiation ($\lambda 0.71073 \text{ \AA}$) at 20°. Crystal data: $C_{21}H_{32}O_3$, $M = 332.53 \text{ g}\cdot\text{mol}^{-1}$; monoclinic, $P2_1$, $a = 8.265$ (2), $b = 12.220$ (3), $c = 9.866$ (2) \AA ; $\beta = 95.60$ (2)°; $U = 991.7$ (4) \AA^3 ; $Z = 2$; $D_c = 1.11 \text{ g}\cdot\text{cm}^{-3}$; $\mu(\text{Mo-K}\alpha) = 0.68 \text{ cm}^{-1}$; $F(000) = 364$; $R = 0.0838$; $R_w = 0.0870$, where $R_w = [\sum_w(|F_o| - |F_c|)^2 / \sum_w |F_o|^2]^{1/2}$, after refinement of 953 intensity data with $I_o > 3\sigma(I_o)$ with $9 \leq 2\theta \leq 23^\circ$. Oxygen atoms refined anisotropically. All carbons were refined isotropically and H atoms were included in the model in idealized position and allowed to ride on the corresponding C atoms with C-H distance fixed at 0.96 \AA and thermal parameters at 0.08 \AA^2 . Atomic coordinates are given in Table 2. Reflection intensities were corrected for Lorentz and polarization effects, and crystal decay, even though it was minimal, was checked by measuring the intensities of three standard reflections periodically throughout the data collection. All calculations were carried out using a Micro Vax II computer running SHELXTL-Plus (18).

ACKNOWLEDGMENTS

This paper is a contribution of the Amazonas Project of IVIC. Partial support of CONICIT (Venezuela), OAS and UNEP-UNESCO is hereby acknowledged. D. Avila is thankful to the Universidad del Zulia, (Venezuela) for a fellowship. We acknowledge E. Medina (Centro de Ecología, IVIC) for identification of present sample of plant material.

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¹Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK.

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Received 19 April 1991