

Two New Triterpene Acids from *Phellinus pomaceus*

A. G. González

Instituto Universitario de Química Orgánica, Universidad de La Laguna, Tenerife, Spain

J. Bermejo Barrera*

Instituto de Productos Naturales Orgánicos, CSIC, La Laguna, Tenerife, Spain

M. J. Mediavilla Pérez and F. J. Toledo Marante

Escuela Universitaria Politécnica, Las Palmas de Gran Canaria, Spain

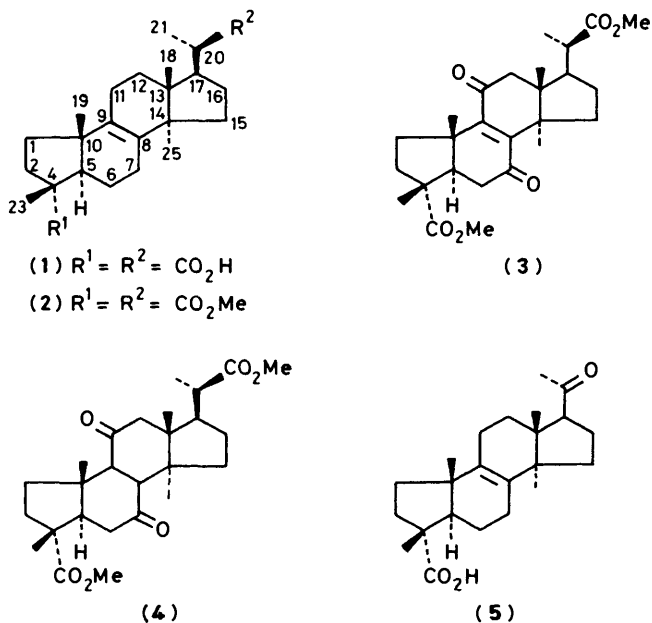
A. Perales

Instituto Rocasolano, CSIC, Departamento de Rayos-X, Serrano 119, 28006 Madrid, Spain

The structures of two new triterpene acids isolated from *Phellinus pomaceus* have been determined by a combination of chemical and physical methods and confirmed by X-ray crystallographic analysis of one of their methylated derivatives. The compounds were identified as 4 β ,14 α -dimethyl-3-nor-5 α -pregn-8-ene-4 α ,20 α -dicarboxylic acid and 4 β ,14 α -dimethyl-20-oxo-3-nor-5 α -pregn-8-ene-4 α -carboxylic acid.

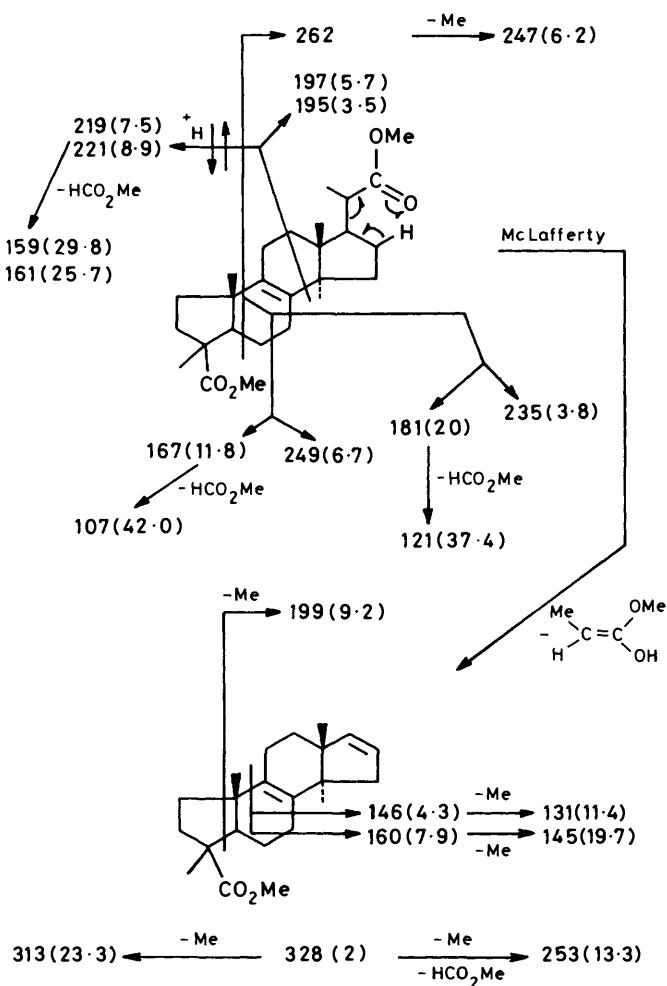
In a previous paper on the constituents of fungi¹ we reported the isolation of two steroids: ergosta-7,22-dien-3-one and ergosta-7,22-dien-3-ol; four pentacyclic triterpenes: friedelin, taraxerol, β -boswellic acid, and ursolic acid; and palmitic and oleic acids.

In a continuation of our work we now report the structure elucidation of two new triterpene acids, javeroic acid (1) and phellinic acid (5), both of which have a degraded lanosterol skeleton, from *Phellinus pomaceus*.



Javeroic acid (1) was isolated in the free form and purified by alumina chromatography as its dimethyl ester (2), ν_{\max} 1725 cm⁻¹. The ¹H n.m.r. spectrum of the diester showed the presence of two methoxy groups, δ 3.7 (6 H, s), as well as two methyls geminal to a carboxylic ester group, δ 1.25 (6 H, s), and three singlets δ 1.00, 0.97, and 0.78 for 3 methyls.

A study of the mass spectrum of the diester (2) gave the position of the ester groups as being on ring A and at C(20), the latter group showing the characteristic McLafferty rearrangement (Scheme 1).



Scheme 1.

Allylic oxidation of the diester (2) with chromium trioxide-acetic acid gave a yellow diketone (3). This confirms the presence of a double bond at C(8). Its i.r. spectrum shows absorption bands at 1725 and 1668 cm⁻¹ assignable to methyl esters and to the system O=C-C=C-C=O, respectively. The

Table 1. Conformational and configurational characteristics for compound (2) $C_{27}H_{40}O_4$

Torsion angles for ring systems							Cremer's parameters ^a			
Ring	τ_1	τ_2	τ_3	τ_4	τ_5	τ_6	Ring	$\Theta(^{\circ})$	$\Phi(^{\circ})$	$Q(\text{\AA})$
B	3	-2	30	-61	62	-32	B	128.4	61.6	0.52
C	-27	54	-60	37	-7	2	C	50.4	128.5	0.51
A	-43	25	2	-29	43	—	A	—	-3.0	0.45
D	17	13	-38	49	-40	—	D	—	128.6	0.48

Conformational characteristics of the ring substituents with respect to the previous/next ring atom

$$C(13)-C(17)-C(20)-C(22) = 174.0(\text{ap})$$

$$C(16)-C(17)-C(20)-C(22) = 55.8(\text{sc})$$

$$C(5)-C(4)-C(24)-O(4) = -162.3(-\text{ac})$$

$$C(2)-C(4)-C(24)-O(4) = -50.2(-\text{sc})$$

Configurational characteristics for ring substituents

$$[C(14)-C(13)-C(17)-C(20)] = [C(14)-C(13)-C(17)-C(20)] - [C(14)-C(13)-C(17)-C(16)] = -162.3 - (-39.90) = -122.4(\beta)$$

$$[C(10)-C(5)-C(4)-C(24)] = [C(10)-C(5)-C(4)-C(24)] - [C(10)-C(5)-C(4)-C(2)] = 142.1 - 25.2 = 116.9(\alpha)$$

^a D. Cremer and J. A. Pople, *J. Am. Chem. Soc.*, 1975, **97**, 1354.

absorption at $1\ 668\ \text{cm}^{-1}$ is in agreement with that reported for 7,11-dioxo-8-ene diketonic arrangements in other steroidal compounds.² Its mass spectrum showed the molecular ion at $m/z\ 444\ (M^+)$, accompanied by the fragments $m/z\ 429\ (M - \text{Me})^+$, $385\ (M - \text{CO}_2\text{Me})^+$, and $370\ (M - \text{Me} - \text{CO}_2\text{Me})^+$. A more detailed study of the diketone gave fragmentations that are in perfect agreement with the cleavages presented by 7-keto and 11-keto steroids.³

Reduction of this diketone with zinc-acetic acid gave a crystalline product whose mass spectrum indicated absence of the quaternary double bond, the molecular ion appearing at $m/z\ 446\ (M^+)$.

On the basis of the above mentioned data we assigned the structure dimethyl 4 β ,14 α -dimethyl-3-nor-5 α -pregn-8-ene-4 α ,20 α -dicarboxylate (dimethyl javeroate) to compound (2).

The detailed structure of compound (2) was established by X-ray crystallography. The conformational characteristics for the molecule of the compound $C_{27}H_{40}O_4$ (Figure) are given in

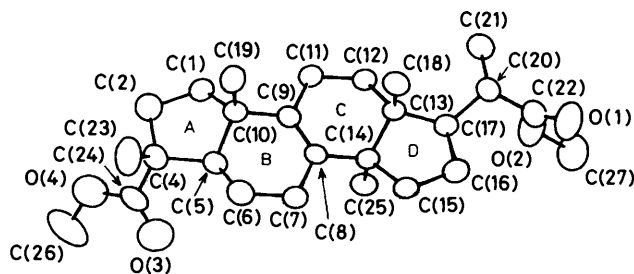
**Figure.** X-Ray molecular structure of dimethyl javeroate (2)

Table 1. Table 2 gives the final atomic co-ordinates for non-H atoms. The torsional angles ($^{\circ}$) are given in a clockwise direction, starting at C(9) ($\tau_1 = 3$), C(11) ($\tau_1 = -27$), C(10) ($\tau_1 = -43$), and C(17) ($\tau_1 = 17$), for rings B, C, A, and D, respectively. Rings B and C have an envelope conformation due to the influence of the unsaturation at the C(8)-C(9) bond, and this is indicated by the torsion angles (Table 1). The conformation of ring A is an envelope with the flap at C(10). Ring D has a conformation between a half-chair and an envelope. The asymmetric parameters⁴ for the ring system show that the dominant symmetry for rings B, C, and A is mirror symmetry; in ring D the asymmetric parameters show that both symmetries, rotational and mirror, are present: ($D_s^{13}\ 0.099$, D_2^{16}

Table 2. Final atomic co-ordinates ($\times 10^4$) for non-H atoms of compound (2) with e.s.d.s in parentheses

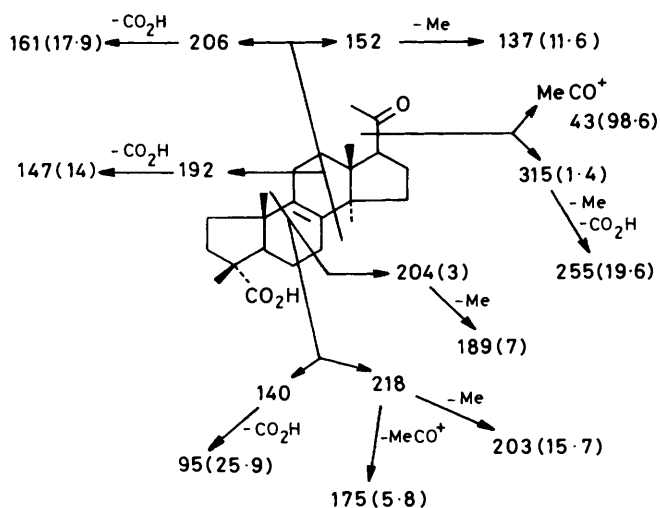
Atom	x	y	z
O(1)	4 394(7)	1 266(2)	1 975(8)
O(2)	4 660(7)	908(2)	5 250(7)
O(3)	41(8)	-2 598(2)	-226(14)
O(4)	1 912(7)	-3 184(2)	-60(10)
C(1)	6 075(6)	-2 139(2)	1 542(9)
C(2)	5 233(7)	-2 598(2)	307(10)
C(4)	3 002(7)	-2 503(2)	-1 342(9)
C(5)	2 756(6)	-1 984(2)	-1 013(8)
C(6)	1 257(6)	-1 713(2)	-2 951(8)
C(7)	1 166(6)	-1 233(2)	-2 030(8)
C(8)	3 072(6)	-1 062(2)	-196(7)
C(9)	4 759(6)	-1 309(2)	678(7)
C(10)	4 877(6)	-1 781(2)	-255(7)
C(11)	6 673(6)	-1 124(2)	2 504(8)
C(12)	6 597(6)	-642(2)	3 407(8)
C(13)	5 024(6)	-346(2)	1 596(7)
C(14)	2 959(6)	-601(2)	856(7)
C(15)	1 525(6)	-238(2)	-679(8)
C(16)	2 330(7)	215(2)	566(9)
C(17)	4 470(6)	116(2)	2 416(7)
C(18)	5 694(7)	-278(2)	-533(8)
C(19)	5 805(2)	-1 737(2)	-2 195(9)
C(20)	5 897(7)	524(2)	2 623(8)
C(21)	7 997(8)	457(2)	4 468(11)
C(22)	4 918(8)	943(2)	3 197(10)
C(23)	2 668(9)	-2 654(2)	-3 859(11)
C(24)	1 497(8)	-2 765(2)	-458(12)
C(25)	2 248(7)	-693(2)	2 966(8)
C(26)	535(11)	-3 452(3)	647(14)
C(27)	3 687(11)	1 292(3)	5 942(11)

0.111°). The junctions of rings A/B and C/D are *trans*. The configurations of the substituents at C(17) and C(4) (methoxycarbonyl) (Table 1) are β and α , respectively, with an *S* configuration at C(20).

Phellinic acid (5), $C_{23}H_{34}O_3$, showed i.r. absorptions at ν_{max} $3\ 500\text{--}2\ 400\ \text{cm}^{-1}$ and $1\ 700\text{--}1\ 680\ \text{cm}^{-1}$ for an acid group and a ketone. The ^1H n.m.r. spectrum is similar to that of the previous products, showing the presence of a methyl ketone, $\delta\ 2.15\ (3\ \text{H}, \text{s})$ as well as a methyl geminal to a carboxylic group [$\delta\ 1.25\ (3\ \text{H}, \text{s})$] and two singlets, $\delta\ 1.00$ and 0.66 , for three methyls.

The fragmentations observed in its mass spectrum allowed us to assign the methyl ketone to be situated at C(20) and the carboxy group on ring A (Scheme 2).

Consequently, the structure of phellinic acid (5) is proposed



Scheme 2.

to be 4 β ,14 α -dimethyl-20-oxo-3-nor-5 α -pregn-8-ene-4 α -carboxylic acid.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus, i.r. spectra on a Perkin-Elmer 257 spectrophotometer for CHCl₃ solutions, u.v. spectra on a Perkin-Elmer 402 spectrophotometer, n.m.r. spectra on a Perkin-Elmer R-12B (60 MHz) spectrometer for solutions in CDCl₃ with tetramethylsilane as internal standard, mass spectra on a VG Micromass ZAB-1F spectrometer, and optical rotations on a Perkin-Elmer 141 polarimeter. Unless otherwise stated all t.l.c. and column chromatography was done on Merck silica gel. Extracts were dried over Na₂SO₄.

Extraction and Isolation.—*Phellinus pomaceus* is a perennial fungus of woody consistency, belonging to the family, Polyporaceae, class Basidiomycete, and is found parasitizing the trunks of laurisilva trees.

The fungus (15 kg), collected at El Bailadero (Tenerife, Canary Islands), was pulverized and extracted with ethanol for about 1 month. The extract was concentrated from water-chloroform, and the organic layer was dried (Na₂SO₄) and percolated through alumina (activity III). Evaporation of the filtrate gave a residue (150 g), chromatography of which on silica gel (5 kg) gave, on elution with n-hexane and a mixture of n-hexane-ethyl acetate, friedelin (200 mg), taraxerol (86 mg), ergosta-7,22-dien-3-one (120 mg), a mixture of palmitic and oleic acids (70 mg), β -boswellic (32 mg), ursolic (120 mg) acids, and javeroic and phellinic acids.

Methylation of javeroic acid (1), 4 β ,14 α -dimethyl-3-nor-5 α -pregn-8-ene-4 α ,20 α -dicarboxylic acid. A solution of the dicarboxylic acid (200 mg) in ether (20 ml) was treated with diazomethane, prepared from methyl(nitroso)urea, at room temperature for 24 h. The mixture was evaporated to dryness under reduced pressure. Alumina chromatography of the residue and recrystallization from methanol afforded needles of the diester (2) (180 mg), m.p. 125–126 °C; [α]_D +101° (c 3.6 in CHCl₃); ν_{\max} (CHCl₃) 1725 cm⁻¹ (CO₂Me); δ_{H} (60 MHz; CDCl₃) 3.70 (6 H, s), 2.80 (1 H, m), 2.50 (1 H, m), 1.25 (6 H, s), 1.00 (3 H, s), 0.97 (3 H, s), and 0.78 (3 H, s) (Found: M^+ , 416.2977. C₂₆H₃₈O₄ requires M , 416.2925). Other significant peaks in the low-resolution m.s. were at m/z 401 (M - Me,

67.5%), 357 (M - CO₂Me, 12.1), 341 (M - HCO₂Me, 100), 313 (M - Me - MeCH=C(OH)OMe, 23.3), and 2.81 (M - 2HCO₂Me - Me, 10.3).

Oxidation of Diester (2).—To a solution of compound (2) (120 mg) in benzene (10 ml) was added a saturated solution of chromium(VI) oxide in acetic acid-water (90:10), and the mixture was stirred at room temperature for 24 h. It was neutralized with saturated aqueous NaHCO₃ and extracted with benzene to give a yellow oil (100 mg) which, after chromatography on alumina, behaved like a single product by t.l.c. [benzene-EtOAc (9:1)]; ν_{\max} (CHCl₃) 1725 (ester) and 1668 cm⁻¹ (diketone); δ_{H} (60 MHz; CDCl₃) 3.68 (6 H, s), 2.50 (1 H, m), and 1.25, 1.21 and 1.18 (15 H, s) (Found: M^+ , 444.2476. C₂₆H₃₆O₆ requires M , 444.2510). Other significant peaks in the low-resolution m.s. were at m/z 429 (M - Me, 3.0%), 385 (M - CO₂Me, 2.9), and 370 (M - Me - CO₂Me, 2.5). This product was identified as dimethyl 4 β ,14 α -dimethyl-7,11-dioxo-3-nor-5 α -pregn-8-ene-4 α ,20 α -dicarboxylate (3).

Hydrogenation of Compound (3).—Compound (3) (110 mg) was treated with acetic acid (10 ml) and powdered Zn (700 mg) for 1 h. The acetic acid was removed under reduced pressure and the residue was chromatographed through silica gel [hexane-EtOAc (8:2) as eluant], a single product being obtained which was identified as dimethyl 4 β ,14 α -dimethyl-7,11-dioxo-3-nor-5 α -pregnane-4 α ,20 α -dicarboxylate (4), δ_{H} (60 MHz; CDCl₃) 3.68 (6 H, s), and 1.25 and 1.20 (15 H, s) (Found: M^+ , 446.2594. C₂₆H₃₈O₆ requires M , 446.2665). Other significant peaks in the low-resolution m.s. were at m/z 431 (M - Me, 9.0%), 415 (M - OMe, 19.2), 386 (M - HCO₂Me, 25.3), and 358 (M - MeCH=C(OH)OMe, 21).

Phellinic Acid (5), 4 β ,14 α -Dimethyl-20-oxo-3-nor-5 α -pregn-8-ene-4 α -carboxylic acid.—This compound (30 mg) had m.p. 218–220 °C (from MeOH) (Found: C, 76.8; H, 9.85. C₂₃H₃₄O₃ requires C, 77.05; H, 9.56%). λ_{\max} (EtOH) 236, 243, and 296 nm (log ϵ 3.25, 3.26, and 3.01); ν_{\max} (CHCl₃) 3500–2400 and 1680–1700 cm⁻¹ (acid and ketone); δ_{H} (60 MHz; CDCl₃) 2.90 (1 H, t, J 9 Hz), 2.15 (3 H, s), 1.25 (3 H, s), 1.00 (6 H, s), and 0.66 (3 H, s). Peaks in the high-resolution m.s. were at m/z 358.2501 (M^+ , 19.1%), 343.2299 (M - Me, 16), 298.2256 (M - Me - CO₂H, 22.4), and 297.2218 (M - Me - HCO₂H, 95.9).

X-Ray Crystallographic Analysis of the Dimethyl Ester of Javeroic Acid.—A crystal of approximate dimensions of 0.4 × 0.3 × 0.2 mm was selected to measure the 4052 reflexions on a four-circle diffractometer with graphite-monochromated Cu-K α radiation (λ = 1.5418 Å) to θ = 65° by the $\theta/2\theta$ mode. No intensity decay was observed during the experiment. The crystal was monoclinic, a = 7.0935(2), b = 29.403(1), c = 6.1170(2) Å, β = 110.45(1)°, space group $P2_1$, Z = 2, V = 1195.37(1) Å³, D_c = 1.1574 g cm⁻³; no absorption correction was applied (μ = 5.665 cm⁻¹). The crystal structure was solved by MULTAN⁵ with 250 E_s > 1.50. After refinement with the 2741 [I > 2 $\sigma(I)$] observed intensities and a value of R = 8.4%, a difference map showed the major portion of the H-atoms. The rest were located in successive Fourier difference maps. A weighting scheme⁶ designed to have no dependence on $\langle w\Delta^2 F \rangle$ vs. $\langle F_o \rangle$ and vs. $\langle \sin\theta/\lambda \rangle$ was selected. A weighted full-matrix least-squares anisotropic refinement,⁷ using the 2741 observed reflections, converged to the R = 4.4% and R_w = 5.1%, respectively. During this refinement the parameters for the H-atoms were not refined, but were included in the structure-factor calculations. To each H-atom was assigned an isotropic

thermal parameter of the atom to which the H-atom was bonded.*

The absolute configuration was confirmed by comparison of the 50 more relevant Bijvoet differences:

$$R_1 = \Sigma\{|F_o(+h) - F_o(-h)\} - \{F_c(+h) - F_c(-h)\}|/N = 0.086 \text{ (0.194 for the reversed enantiomorph).}$$

Average Bijvoet ratio:

$$R_2 = \Sigma|R_o - R_c|/N = 1.006 \text{ (1.012 for the reversed enantiomorph), and:}$$

$$R_3 = \Sigma|\Delta I_o = I_c|/\Sigma|\Delta I_o| = 0.656 \text{ (1.560 for the reversed enantiomorph), with } N = 50, R_o = \Delta I_o/\langle F_o^2 \rangle,$$

$$R_c = \Delta I_c/\langle F_c^2 \rangle, \Delta I_o = F_o^2(+h) - F_o^2(-h), \text{ and}$$

$$\Delta I_c = F_c^2(-h)$$

Acknowledgements

We thank Professor García-Blanco for his support and the Centro de Proceso de Datos, Ministerio de Educación y Ciencia

* Tables of thermal parameters and atomic co-ordinates for non-H atoms, atomic co-ordinates and isotropic parameters for H-atoms, bond distances and angles, torsion angles, and some interatomic distances between non-bonded atoms are deposited as supplementary material SUP 56431 (13 pp.). For details of the Supplementary Publications Scheme see Instructions for Authors (1986), *J. Chem. Soc., Perkin Trans. I*, 1986, issue 1. Structure factors are available from the editorial office on request.

(Madrid) for the use of a Univac computer. One of us (J. B. B.) thanks the Comisión Asesora de Investigación Científica y Técnica (Madrid) for financial support. Thanks are also due to the AIETI (Madrid) for further financial support.

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Received 22nd April 1985; Paper 5/661