

IDENTITY OF MAPROUNIC ACID WITH ALEURITOLIC ACID.
REVISION OF THE STRUCTURE OF MAPROUNIC ACID:
X-RAY CRYSTAL STRUCTURE OF *p*-BROMOBENZYL
ACETYLMAPROUNATE¹

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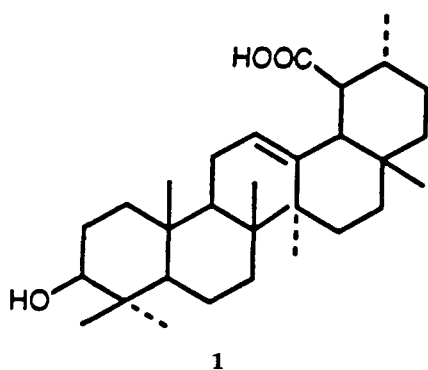
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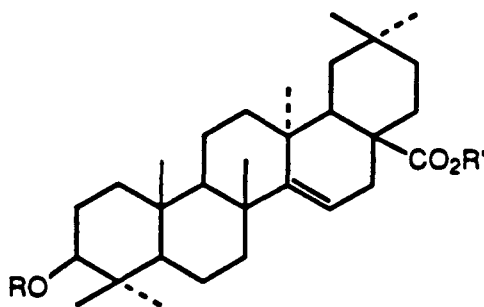
A few years ago, Wani *et al.* (1) reported the isolation and characterization of four pentacyclic triterpenes from *Maprounea africana*. Structures of these compounds were deduced primarily from hrms and ¹H-nmr data. The mass spectrum of one of these, maprounic acid, showed characteristic ions at *m/z* 207 (C₁₄H₂₃O) and 248 (C₁₆H₂₄O₂) arising from retro-Diels-Alder fragmentation around ring C, suggesting that it was a Δ¹²-amyrin derivative containing a hydroxyl group in ring A or B and a carboxyl group in ring D or E (2). Moreover, it has been reported by Shamma *et al.* (3) that in the ¹H-nmr spectrum of triterpenes with an oleanane or ursane skeleton bearing a methoxycarbonyl function at C-28, the highest C-Me signal always lies upfield from δ 0.775. On the basis of this generalization, the appearance at δ 0.79 of the highest C-Me signal in the ¹H-nmr spectrum of methyl acetylmaprounate [4] indicated that the methoxycarbonyl function was not located at C-28. Biogenetic considerations placed the hydroxyl group at C-3, and deshielding of the olefinic proton at C-12 suggested that C-29 was the site of the carboxyl function. Thus, the parent compound, maprounic acid, was identified as 3β-hydroxyurs-12-en-29-oic acid [1]. Soon

after the appearance of the article disclosing these conclusions, Mukherjee *et al.* (4) assigned the same urs-12-ene structure to a pentacyclic triterpene isolated from *Hyptis suaveolens*, also mainly on the basis of mass spectral fragmentation patterns and ¹H-nmr data. Comparison of the melting points of maprounic acid, acetylmaprounic acid [3], and methyl acetylmaprounate [4] with those of the acid from *H. suaveolens* and its corresponding derivatives revealed significant differences. The ¹H-nmr spectrum of acetylmaprounic acid also differed from that of the acetyl derivative of the corresponding acid from *H. suaveolens*. Furthermore, McLean *et al.* (5) very recently reported the isolation of a triterpene acid, acetylaleuritolic acid, from *Maprounea guianenses* with physical and spectral (ms and ¹H-nmr) properties identical to those of 3-acetylmaprounic acid [3]. In order to resolve these ambiguities, we decided to establish unequivocally the structure of maprounic acid by means of a single-crystal X-ray analysis. [It should be noted, however, that the present study to clarify the structural assignment was prompted by the conflicting report of Mukherjee *et al.* (4) and was initiated prior to our knowledge of the report by McLean *et al.* (5).] Because the parent acid [1] did not yield crystals in a form suitable for such a study, the *p*-bromobenzyl derivative 5 was prepared. Recrystallization from MeOH/CHCl₃ yielded clear, colorless needles of 5.

¹Part 29 in the series Plant Antitumor Agents. For part 28 in this series see: M.C. Wani, A.W. Nicholas, and M.E. Wall, *J. Med. Chem.*, **30**, 2317 (1987).



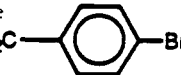
sion angles³ indicate that cyclohexane rings A and B are in chair forms whereas the C and E rings approximate to twist-boat forms. Analysis of corresponding ring D angles in terms of deviations from symmetry-related values for boat, twist-boat, and 1,3-diplanar forms indicates that this ring has a conformation which departs significantly from all three and is intermediate in character.



2 R=R'=H

3 R=Ac, R'=H

4 R=Ac, R'=Me

5 R=Ac, R'=H₂C-

The crystal structure of **5** was solved by the heavy-atom method. Full-matrix least-squares refinement of atomic parameters converged at $R = 0.047$ ($R_w = 0.071$)² over 2474 reflections. Fractional coordinates of the nonhydrogen atoms are listed in Table 1. A view of the solid-state conformation is provided in Figure 1. The results of this study show that the earlier formulation of maprounic acid as 3 β -hydroxyurs-12-en-29-oic acid [**1**] was in error and that this compound is, in fact, identical with the taraxer-14-ene derivative, aleuritolic acid [**2**] (**5**). Bond lengths in **5** lie close to expected values (6). Endocyclic tor-

The fragments observed at m/z 207 ($C_{14}H_{23}O$) and 248 ($C_{16}H_{24}O$) in the mass spectrum of maprounic acid may alternatively be accommodated by the fragmentation scheme depicted in Figure 2. Thus, it is obvious that structural assignments based on fragmentation data can be quite misleading. It also ap-

² $R = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|}$; $R_w = \frac{[\sum w(|F_o| - |F_c|)^2]}{\sum w|F_o|^2}^{1/2}$.

³Endocyclic torsion angles (ω_{ij} , $\sigma \pm 0.5-0.8^\circ$) about the bonds between atoms i and j follow: $\omega_{1,2} -58.3$, $\omega_{2,3} 63.6$, $\omega_{3,4} -58.7$, $\omega_{4,5} 52.1$, $\omega_{5,10} -49.7$, $\omega_{1,10} 51.2$ in ring A; $\omega_{5,6} -63.0$, $\omega_{6,7} 57.4$, $\omega_{7,8} -48.8$, $\omega_{8,9} 50.9$, $\omega_{9,10} -56.8$, $\omega_{5,10} 61.0$ in ring B; $\omega_{8,9} -59.2$, $\omega_{9,11} 27.7$, $\omega_{11,12} 30.3$, $\omega_{12,13} -53.8$, $\omega_{13,14} 19.5$, $\omega_{8,14} 35.4$ in ring C; $\omega_{13,14} -42.4$, $\omega_{14,15} 8.5$, $\omega_{15,16} 42.9$, $\omega_{16,17} -58.6$, $\omega_{17,18} 26.1$, $\omega_{13,18} 22.9$ in ring D; $\omega_{17,18} 20.8$, $\omega_{18,19} -63.2$, $\omega_{19,20} 37.7$, $\omega_{20,21} 24.9$, $\omega_{21,22} -67.3$, $\omega_{17,22} 41.2$ in ring E.

TABLE 1. Fractional Coordinates and Equivalent Isotropic Thermal Parameters for the Non-hydrogen Atoms of Compound **5**, with Estimated Standard Deviations in Parentheses.

Atom	x	y	z	B(Å ²)
C-1	0.4854(3)	-0.0671(2)	-0.2860(8)	4.2(1)
C-2	0.4230(4)	-0.1040(2)	-0.2811(9)	4.7(1)
C-3	0.3942(3)	-0.1070(2)	-0.0813(8)	3.7(1)
C-4	0.3586(3)	-0.0615(2)	-0.0089(7)	3.4(1)
C-5	0.4210(3)	-0.0227(2)	-0.0237(7)	3.1(1)
C-6	0.3999(3)	0.0256(2)	0.0524(8)	3.9(1)
C-7	0.4710(3)	0.0556(2)	0.0884(8)	3.9(1)
C-8	0.5200(3)	0.0619(2)	-0.0892(7)	3.0(1)
C-9	0.5315(3)	0.0122(2)	-0.1817(7)	3.3(1)
C-10	0.4606(3)	-0.0176(2)	-0.2193(7)	3.3(1)
C-11	0.5840(3)	0.0148(2)	-0.3567(8)	4.0(1)
C-12	0.6410(3)	0.0553(2)	-0.3513(8)	3.6(1)
C-13	0.6655(3)	0.0693(2)	-0.1489(7)	3.0(1)
C-14	0.5970(2)	0.0803(2)	-0.0283(7)	3.0(1)
C-15	0.6063(3)	0.1061(2)	0.1313(8)	3.8(1)
C-16	0.6806(3)	0.1292(2)	0.1740(8)	4.1(1)
C-17	0.7097(3)	0.1510(2)	-0.0102(8)	3.4(1)
C-18	0.7187(3)	0.1138(2)	-0.1700(7)	3.2(1)
C-19	0.8010(3)	0.1002(2)	-0.1944(8)	3.9(1)
C-20	0.8495(3)	0.1418(2)	-0.2572(8)	3.8(1)
C-21	0.8246(3)	0.1880(2)	-0.1603(9)	4.2(1)
C-22	0.7851(3)	0.1768(2)	0.0253(9)	4.2(1)
C-23	0.2859(3)	-0.0496(2)	-0.1100(10)	5.0(1)
C-24	0.3393(3)	-0.0690(2)	0.2032(9)	5.0(1)
C-25	0.4092(3)	0.0043(2)	-0.3753(9)	4.7(1)
C-26	0.4854(3)	0.0993(2)	-0.2184(9)	3.9(1)
C-27	0.7057(3)	0.0274(2)	-0.0549(9)	4.3(1)
C-28	0.6542(3)	0.1888(2)	-0.0751(8)	3.7(1)
C-29	0.8442(4)	0.1480(3)	-0.4752(10)	5.9(1)
O-30	0.9304(3)	0.1318(2)	-0.2021(10)	5.3(1)
C-31	0.3376(2)	-0.1449(1)	-0.0686(6)	4.5(1)
O-32	0.3601(3)	-0.1875(2)	-0.0142(9)	4.6(1)
C-33	0.4242(3)	-0.1969(2)	0.0147(9)	7.3(1)
O-34	0.2980(4)	-0.2214(2)	0.0021(11)	6.3(2)
O-35	0.6281(2)	0.1924(1)	-0.2313(6)	4.9(1)
C-36	0.6374(2)	0.2192(1)	0.0641(6)	5.2(1)
C-37	0.5825(3)	0.2551(2)	0.0165(13)	5.9(1)
C-38	0.5051(3)	0.2335(2)	0.0154(10)	4.9(1)
C-39	0.4639(4)	0.2349(2)	-0.1451(11)	5.4(1)
C-40	0.3931(3)	0.2141(2)	-0.1493(11)	5.5(1)
C-41	0.3657(3)	0.1921(2)	0.0092(11)	5.4(1)
C-42	0.4077(4)	0.1903(2)	0.1747(11)	5.8(2)
C-43	0.4771(4)	0.2116(2)	0.1783(10)	5.7(2)
Br	0.27202(5)	0.16292(3)	0.0067(2)	8.87(2)

pears that the generalization of Shamma *et al.* (3) concerning the location of a methoxycarbonyl function at C-28 and the appearance of the highest C-Me signal upfield from δ 0.775 does not apply to the taraxerane skeleton.

In summary, the results of the present study confirm the structure of aleuritolic

acid derived by 2D-nmr spectroscopy by McLean *et al.* (5). In view of these findings, it would be of interest to establish firmly the structure of the isomeric acid obtained from *H. suaveolens* (4) either by detailed 2D-nmr studies or by X-ray crystallography. This is especially important because the same structure **1**

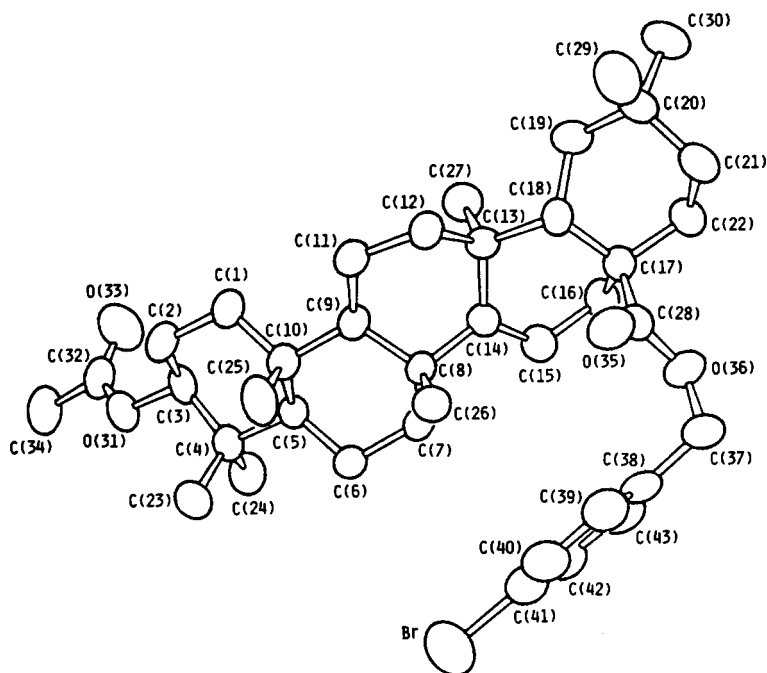


FIGURE 1. Structure and solid-state conformation of **5**; hydrogen atoms have been omitted for clarity.

was also originally assigned to bryonolic acid isolated from *Bryonia dioica* (7); however, this compound was later shown to be 3-hydroxymultiflor-8-en-29-oic acid (8).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—

Melting points were recorded by a Kofler hot-stage apparatus and are uncorrected. The ir and nmr data were obtained on a Perkin-Elmer 467 and an EM-390 instrument, respectively; mass determinations were made on an MS-902 spectrometer, and combustion analysis was performed at Atlantic Microlab, Atlanta, Georgia.

p-BROMOBENZYL 3-ACETYLMAPROUNATE [**5**].—The *p*-bromobenzyl ester of 3-acetylma-

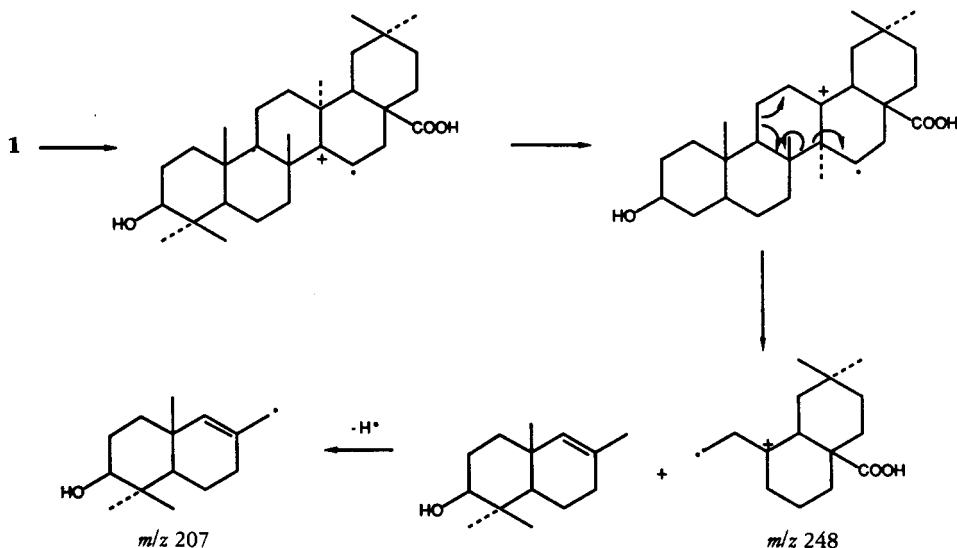


FIGURE 2. Fragment ions observed by hrms.

prounic acid [3] was prepared from the acid 3 and *p*-bromobenzyl bromide using DBU in MeCN in the same fashion as the methyl ester 4 which was reported earlier (1). Thus, 3-acetylmaprounic acid [3] (228 mg, 0.458 mmol) was suspended in MeCN (2.5 ml), and DBU (76 mg, 75 μ l, 1.0 mmol) was added to give a clear yellow solution. The addition of *p*-bromobenzyl bromide (150 mg, 0.600 mmol) resulted in the rapid formation of a thick, pale green slurry. After 1 h, H₂O (10 ml) was added and the mixture was extracted with EtOAc. The organic phase was back-extracted with aqueous NaHCO₃, washed with H₂O, dried (Na₂SO₄) and evaporated to afford 5 as a beige-yellow solid (298 mg, 97%). Recrystallization from MeOH/CHCl₃ gave the derivative as clear, colorless needles, mp 178.5–180.5°; ν max (CHCl₃) 2920 (s), 2863, 1722 (s), 1490 (w), 1469, 1461, 1391 (w), 1380, 1368, 1257, 1160, 1010 cm⁻¹; ¹H nmr (90 MHz, CDCl₃) δ 4.94 (ABq, 2, *J* = 12 Hz, 21 Hz, ArCH₂O), 7.20 (d, 2, *J* = 8 Hz, aromatic), 7.47 (d, 2, *J* = 8 Hz, aromatic), with the remainder of the spectrum closely matching that for the parent 3-acetylmaprounic acid: *m/z* (%) (2.9) 666, [M]⁺ 668, 606 (3.8), 608, 498 (5.8), 437 (38.5), 416 (4.8), 418, 247 (46.2), 233 (40.4), 203 (34.6), 189 (100). Calcd for C₃₉H₅₅BrO₄, C 70.15, H 8.30, Br 11.97; found C 70.06, H 8.32, Br 12.05.

X-RAY CRYSTAL STRUCTURE ANALYSIS OF *p*-BROMOBENZYL 3-ACETYLMAPROUNATE [5].⁴—Crystal data: C₃₉H₅₅BrO₄, MW = 667.78, orthorhombic, space group P2₁2₁2₁, *a* = 18.011(4), *b* = 28.217(9), *c* = 7.031(1) Å (from 25 orientation reflections, 37° < θ < 46°), *V* = 3573.3 Å³, *Z* = 4, *D*_c = 1.241 g/cm³, μ (CuK α radiation, λ = 1.5418 Å) = 18.4 cm⁻¹. Crystal dimensions: 0.12 × 0.18 × 0.40 mm. Intensity data (+*b*, +*k*, +*l*; 3633 reflections; θ max = 67°) recorded on an Enraf-Nonius CAD-4 diffractometer [CuK α radiation, incident-beam graphite monochromator; ω -2 θ scans, scanwidth (1.15 + 0.14 tan θ)°], yielded 2474 reflections [*I* > 3.0 σ (*I*)] which were retained for the analysis. Lorentz, polarization, and empirical absorption (T max:Tmin = 1.00:0.89) corrections were applied to the data.

The crystal structure was solved by the heavy-atom approach. Initial bromine atom coordinates were derived from a Patterson map, and the remaining non-hydrogen atoms were located in a series of weighted and difference Fourier syntheses. Following the refinement of non-hydrogen

⁴Atomic 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.

atom positional and anisotropic temperature factor parameters by several rounds of full-matrix least-squares calculations, a difference Fourier synthesis yielded hydrogen atom positions. Continuation of the least-squares iterations, with hydrogen atoms included at their calculated positions, led to *R* = 0.050. Introduction of the imaginary contributions to the anomalous dispersion corrections into the structure-factor calculations verified that the absolute configuration of 5, and hence of 2, was as expected, correctly represented as shown. Thus, for coordinates corresponding to the absolute stereochemistry represented by 5, *R* was 0.049 whereas a significantly higher value (*R* = 0.054) was obtained when coordinates for the enantiomer were used. Several further rounds of least-squares refinement of parameters for the correct enantiomer led to convergence at *R* = 0.047 (*R*_w = 0.071).

Crystallographic calculations were performed on PDP11/44 and MicroVAX II computers by use of the Enraf-Nonius Structure Determination Package. For the structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were from the literature (9). In the least-squares calculations, $\sum w\Delta^2$ [$w = 1/\sigma^2(|F_o|)$, $\Delta = (|F_o| - |F_c|)$] was minimized.

ACKNOWLEDGMENTS

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