PLANT ANTITUMOR AGENTS, 19.[1] NOVEL TRITERPENES FROM *MAPROUNEA AFRICANA*

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ABSTRACT.—Four new pentacyclic triterpenes have been isolated from *Maprounea africana*. These triterpenes are members of the previously unknown urs-12-en-29-oic acid series. The structures of these compounds were deduced from spectral and chemical evidence. The parent compound, maprounic acid, was identified as 3β-hydroxyurs-12-en-29-oic acid. The remaining three triterpenes were identified as maprounic acid 3-*p*-hydroxybenzoate, 7β-hydroxymaprounic acid 3-*p*-hydroxybenzoate, and 2α-hydroxymaprounic acid 2,3-bis-*p*-hydroxybenzoate. Of the four triterpenes, only the 7β-hydroxy derivative exhibited *in vivo* P-388 activity.

A sample of root wood with bark of *Maprounea africana* Muell. Arg. (Euphorbiaceae) was collected in Tanzania. This material was subjected to our standard procedure for preliminary testing of plant extracts for antitumor activity (1). Ethanolic extracts were found to be active in P-388 leukemia (2). This paper describes the isolation, structural elucidation, and structure-activity relationships of four novel triterpenes from this plant. These triterpenes are members of the previously unknown urs-12-en-29-oic acid series.

Fractionation of the alcohol extract of the dried plant, guided by assay in the P-388 leukemia system, revealed that the activity was concentrated successively in the chloroform layer of the chloroform-water partition and the methanol layer of an aqueous methanol-petroleum ether partition. The aqueous methanol residue was then subjected to an 11-tube, large-scale, counter-current distribution (ccd) procedure between aqueous methanol and carbon tetrachloride/chloroform (1). Repeated chromatography (see Experimental section) of the residue from ccd fractions showing the greatest biological activity gave the triterpenes, maprounic acid (3β-hydroxyurs-12-en-29-oic acid, **1**), maprounic acid 3-*p*-hydroxybenzoate (**5**), 7β-hydroxymaprounic acid 3-*p*-hydroxybenzoate (**7**), and 2α-hydroxymaprounic acid 2,3-bis-*p*-hydroxybenzoate (**10**). The isolated yields of **1**, **5**, **7**, and **10** were low, ranging from 0.0010-0.0013%.

The molecular formula of maprounic acid (**1**) was determined to be $C_{30}H_{48}O_3$ by high resolution mass spectrometry (hrms). Important fragment ions were observed at *m/z* 207 and 248, which analyzed for $C_{14}H_{23}O$ (a) and $C_{16}H_{24}O_2$ (b) (figure 1), respectively, by hrms. These fragments are characteristic of Δ^{12}-amyrin derivatives containing a hydroxyl group in ring A or B and a carboxyl group in ring D or E (3). Treating **1** with methyl iodide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (4) gave a monomethyl ester **2**, M^+ at *m/z* 470.3760, $C_{31}H_{50}O_3$. The signal due to the methyl-ester methyl appeared at δ 3.56 in the ^{1}H-nmr spectrum of **2**. Acetylation of **1** gave the monoacetate **3**, M^+ at *m/z* 498.0178. The ^{1}H-nmr signal caused by the acetate methyl in **3** appeared at δ 2.03 and the acetoxy methine proton at δ 4.44. Acetylation of **2** gave the monoacetate monomethyl ester **4**, M^+ at *m/z* 512.3850, $C_{33}H_{52}O_4$. These data suggested that maprounic acid was a pentacyclic triterpene containing a double-bond, carboxyl and hydroxyl functions.

A detailed analysis of the ^{1}H-nmr spectrum of **4** allowed the assignment of map-

[1]For Part 18 in this series, see M.C. Wani, P.E. Ronman, J.T. Lindley, and M.E. Wall, *J. Med. Chem.*, **23**, 554 (1980).

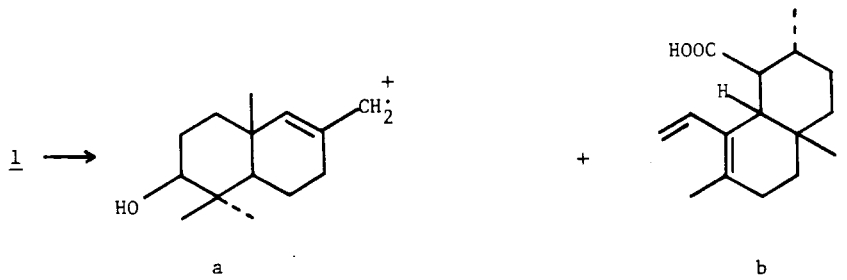


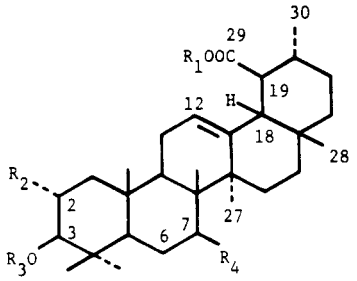
FIGURE 1. Fragment ions observed by hrms.

rounic acid to the urs-12-ene series with the hydroxyl group at C-3 and the carboxyl group at C-29. The high-field region was complex, but indicated the presence of seven methyl groups in agreement with the amyrin skeleton. Shamma *et al.* (5) have studied the relationship between the chemical shift of the highest C-methyl group in the ^{1}H-nmr spectra of pentacyclic triterpenes and the position of the methoxycarbonyl group. It was observed that, in the case of triterpenes, in which the methoxycarbonyl function was at C-28, the highest C-methyl signal was always upfield from δ 0.775. The highest C-methyl signal in the spectrum of **4** appeared at δ 0.79, indicating that the methoxycarbonyl function could not be present at C-28. The ^{1}H-nmr spectrum of **4** indicated a two-proton multiplet at δ 2.3, which was tentatively assigned to the proton at C-18 and the proton on the carbon bearing the methoxycarbonyl function. This assignment was verified by obtaining a spectrum of **4** in CF_3CO_2D in which the C-18 proton appeared as a clean doublet at δ 2.58 (J = 15 Hz). The multiplicity and the coupling constant of this signal suggested that maprounic acid must belong to the urs-12-en-29(or 30)-oic acid series of triterpenes. It remained, therefore, to determine the exact locations of the carboxyl and hydroxyl groups in **1**.

Biogenetic considerations placed the hydroxyl group at C-3. In agreement with this assignment, the C-3 proton in the ^{1}H-nmr spectrum of **2** appeared as a multiplet at δ 3.24, which shifted to δ 4.44 upon acetylation. The shape and chemical shift of this signal were in agreement with a C-3 axial proton (5,6). Therefore, maprounic acid could be either 3β-hydroxyurs-12-en-29-oic acid (**1**) or the corresponding C-30 carboxylic acid. The latter is the known dihydroifflaionic acid isolated from *Adenium obesum* (7). A comparison of the physical properties (mp. ^{1}H-nmr) of maprounic acid with those reported for dihydroifflaionic acid indicated that they were not identical. Therefore, the carboxyl function in maprounic acid must be at C-29.[2] This assignment was further substantiated by the chemical shift of the olefinic proton at C-12. Usually, in members of the urs-12-ene series, this signal appears in the vicinity of δ 5.25. In **4** it appeared downfield at δ 5.50. Molecular models indicate that a carboxylic function at C-29 could deshield the proton at C-12.

The molecular composition of maprounic acid 3-*p*-hydroxybenzoate (**5**) was determined to be $C_{37}H_{52}O_5$ by hrms. Treatment of **5** with methyl iodide and sodium hydride gave the monomethyl ester, monomethyl ether **6**, M^+ at *m/z* 604.4128, $C_{39}H_{56}O_5$. The overall appearance of the ^{1}H-nmr spectrum of **6** was similar to that of **4**. Additionally, the singlet due to the aromatic methyl ether was observed at δ 3.85, and a pair of apparent doublets due to aromatic protons appeared at δ 6.90 and 7.97, each of which integrated for two protons. The methine proton at C-3 was still present at

[2]It is interesting to note that structure **1** was originally assigned to bryonolic acid isolated from the roots of *Bryonia dioica* (Jacq.) (8). However, this acid was later shown to be 3-hydroxymultifluor-8-en-29-oic acid (9).



	R_1	R_2	R_3	R_4
1	H	H	H	H
2	Me	H	H	H
3	H	H	Ac	H
4	Me	H	Ac	H
5	H	H	4-OHC_6H_4CO	H
6	Me	H	4-$OMeC_6H_4CO$	H
7	H	H	4-OHC_6H_4CO	OH
8	Me	H	4-$OMeC_6H_4CO$	OH
9	Me	H	4-$OMeC_6H_4CO$	OAc
10	H	4-OHC_6H_4COO	4-OHC_6H_4CO	H
11	Me	4-$OMeC_6H_4COO$	4-$OMeC_6H_4CO$	H

δ 4.66 even though **6** no longer contained the acetate function of **4**. This evidence, taken together with the similarity in the mass spectra of **1** and **5**, suggested that **5** was the *p*-hydroxybenzoyl ester of maprounic acid. This assignment was confirmed by the formation of **6** from **2** and *p*-anisoyl chloride. The material prepared by the latter route was identical (tlc, hplc, ir, mp) with that prepared by the methylation of **5**.[3]

7β-Hydroxymaprounic acid 3-*p*-hydroxybenzoate (**7**) was obtained as an amorphous powder. The mass spectrum of **7** showed a weak molecular ion at *m/z* 592 and a strong fragment ion at *m/z* 574, corresponding to a loss of a molecule of water from the parent compound. The molecular composition of the fragment ion was determined to be $C_{37}H_{50}O_5$ by hrms, indicating the molecular formula of $C_{37}H_{52}O_6$ for **7**. Treatment of **7** with methyl iodide in the presence of DBU (4) gave the monomethyl ester monomethyl ether **8**, M^+ at *m/z* 620.414, $C_{39}H_{56}O_6$. The singlets due to the methyl-ester methyl and the methyl-ether methyl appeared at δ 3.57 and 3.85, respectively, in the ^{1}H-nmr spectrum of **8**. Acetylation of **8** gave the monomethyl ester, monomethyl ether, monoacetate **9**, M^+ at *m/z* 662.419, $C_{41}H_{58}O_7$. The singlet due to acetate methyl in the ^{1}H-nmr spectrum of **9** appeared at δ 1.98.

The formation of **9** and a cursory examination of its ^{1}H-nmr spectrum indicated that **7** was a hydroxylated derivative of **5**. The problem then reduced to the placement of a secondary hydroxyl group on the maprounic acid portion of **5**. The mass spectrum of **7** showed prominent peaks at *m/z* 574 ($C_{37}H_{50}O_5$, M^+-H_2O), 454 ($C_{30}H_{46}O_3$, M^+-$HOC_6H_4CO_2H$), and 434 ($C_{30}H_{44}O_2$, M^+-H_2O-$HOC_6H_4CO_2H$). The fragment ion at *m/z* 434 involving the elimination of a molecule of water and *p*-hydroxybenzoic acid suggested that the hydroxyl group was not located in ring A. In addition, the presence of a strong retro-Diels-Alder peak a *m/z* 248 ($C_{16}H_{24}O_2$,b) in the mass spectrum of **7** indicated that the hydroxyl group could not be situated in rings C, D, or E. It was, therefore, concluded that the hydroxyl must be present in ring B at C-6 or C-7. The ease of acetylation of **8** to give **9** and the chemical shift (δ 4.78) of the acetoxymethine proton in **9** indicated that the hydroxyl group must be equatorial at C-6 or C-7 (5,6). The acetoxy methine proton of **9** appeared as a doublet of doublets (J = 12, 4 Hz) and, thus, allowed the placement of the hydroxyl group in **7** at C-7.

2α-Hydroxymaprounic acid 2,3-bis-*p*-hydroxybenzoate (**10**) was obtained as an amorphous powder. The molecular composition of **10** was determined to be $C_{41}H_{58}O_7$ by hrms. Treating **10** with methyl iodide and sodium hydride gave the monomethyl ester, dimethyl ether **11**, M^+ at *m/z* 754.4441, $C_{47}H_{62}O_8$. The singlets due to the methyl-ester methyl and the dimethyl-ether methyls appeared at δ 3.57 and 3.78, respectively, in the ^{1}H-nmr spectrum of **11**. Further, examination of the ^{1}H-nmr spectrum of **11** revealed the presence of two *p*-methoxybenzoyl ester moieties. The signals

[3]The UV spectrum of the sample prepared from **5** showed a shoulder at 310 nm. All attempts to remove the long wavelength contaminant from **5** and **6** by chromatographic methods were unsuccessful.

for the methine protons alpha to these ester moieties appeared as a doublet at δ 5.12 (J = 10 Hz) and a multiplet at δ 5.40. The doublet could be assigned to the methine proton at C-3, which was now coupled to only one proton at C-2. The value of the coupling constant indicated a diaxial relationship of the protons at C-2 and C-3. The multiplet at δ 5.40 was then assigned to the proton at C-2, thus confirming the assigned structure **10**.

BIOLOGICAL ACTIVITY AND STRUCTURE-ACTIVITY RELATIONSHIPS.—Initial assessment of the cytotoxic activity of various *Maprounea* extracts or crude chromatographic fractions was conducted in parallel, using the inhibition of growth of cells in both the 9KB and 9PS assays (2). The same fractions were also tested *in vivo*, utilizing the P-388 leukemia assay. The results on the relatively crude fractions clearly indicated that the 9KB assay was not a useful procedure for demonstrating any concentration of activity in the *Maprounea* fractions. However, excellent correlation was noted between the 9PS and P-388 assays, and concentration of activity in chromatographic fractions was facilely demonstrated by 9PS assays. Fractions from which compounds **1, 5,** and **10** were isolated were inactive both in 9PS and P-388. On the other hand, the crude chromatographic fraction from which **7** was isolated was highly active in both 9PS and P-388. Table 1 presents the data for the pure compounds **1, 5, 7,** and **10.** It will be noted that the 7β-hydroxyl analog **7** was highly active in 9PS, whereas the remaining compounds were at least four orders of magnitude less active. Compound **7** also showed good activity in P-388, but was relatively inactive in 9KB. Further testing in P-388 of all of the *Maprounea* triterpenes is in progress. However, as noted previously, the crude fractions from which **1, 5,** and **10** were isolated were inactive, and it is unlikely that the pure compounds will show activity.

TABLE 1. Biological activity of compounds isolated from *Maprounea africana*.

Compound	Test System		
	In vitro		*In vivo*
	9PS	9KB	P-388
1	5×10^{1}	—	—
5	3×10^{1}	—	—
7	2×10^{-4}	$>1 \times 10^{1}$	163% at 0.4 mg/kg[a]
10	1×10^{0}	—	—

[a]A good dose response was observed, 136% at 0.2 mg/kg, 127% at 0.1 mg/kg, and 111% at 0.05 mg/kg.

The structure-activity relationship in the *Maprounea* compounds is most interesting. The 7β-hydroxyl function in compound **7** seems to be an absolute requirement for the high 9PS cytotoxic activity, as compound **5,** identical to **7** except for replacement of the 7β-hydroxyl moiety by hydrogen, is devoid of *in vitro* activity. Both the specific cause of the observed cytotoxicity and the reason for the requirement of a 7β-hydroxyl function for activity in this series remain to be elucidated.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Infrared spectra were measured as KBr discs or CH_2Cl_2 or $CHCl_3$ solutions with a Perkin-Elmer 267 spectrophotometer. Ultraviolet spectra were measured in MeOH using a Cary-14 spectrophotometer, and 250 MHz proton nmr spectra were obtained in $CDCl_3$ using TMS as an internal standard with a Brüker WM250 spectrometer. Mass spectra were obtained with an Associated Electrical Industries MS-902 instrument. Rotations were recorded on a Perkin-Elmer 141 polarimeter.

PLANT MATERIAL.—The plant material was supplied through the auspices of the Drug Research and Development Branch, National Cancer Institute, by the Medicinal Plant Resources Laboratory, Plant Genetics and Germplasm Institute, Agricultural Research Service, USDA, Beltsville, MD. A herbarium specimen documenting this collection is deposited in the Herbarium of the National Arboretum, Agricultural Research Service, USDA, Washington, DC. The plant material (roots) was collected in Tanzania in April 1975.

EXTRACTION AND FRACTIONATION.—Milled roots of *M. africana* (17.24 kg) were exhaustively extracted at 50° with 95% ethanol. The extract was evaporated to dryness and partitioned between chloroform and methanol-water (1:9). The chloroform solution was evaporated to dryness and partitioned between petroleum ether-methanol-water (10:9:1). The aqueous methanol fraction was concentrated under vacuum and lyophilized to a powder (155 g). The lyophilized powder was then subjected to a ccd in 4-liter separatory funnels using CCl_4-$CHCl_3$-MeOH-H_2O (35:15:40:10). Fractions showing the greatest biological activity were combined and concentrated (48 g).

The residue from the ccd tubes was then chromatographed on silica gel (Mallinckrodt, SilicAR CC-7, 2.27 kg) and eluted with chloroform and increasing amounts of methanol. A total of 500 fractions of 80 ml each was collected; like fractions were combined after tlc evaluation. Further processing of these fractions is detailed in the individual isolation sections that follow.

ISOLATION OF MAPROUNIC ACID (**1**).—Fractions 148-171 eluted from the column with chloroform-methanol (98:2) afforded a residue (9.06 g) that was further chromatographed on silica gel (Mallinckrodt, SilicAR CC-7, 900 g) using chloroform-methanol (99:1) as the eluant and collecting a total of 300 fractions of 20 ml each. Similar fractions were combined after tlc evaluation. Fractions 174-210 gave a residue (1.94 g) that was chromatographed on silica gel (E. M Reagents, 170 g) eluting with ethyl acetate-hexane (1:5) and collecting 20-ml fractions to give a total of 250 fractions. Concentration of fractions 95-155 afforded crystalline maprounic acid (**1**) (220 mg): mp, 305-307°; $[\alpha]^{20}D$ +12.8° (C = 0.40, pyridine); ir ν max (KBr) 3400 (OH), 1686 (acid C=O) cm^{-1}; ms, *m/z* 456 (M^+, 31.4%) 441 (28.6), 438 (12.9), 248 (97), 234 (100), 189 (97); mass measurement, obsd 456.3604, calcd for $C_{30}H_{48}O_3$ 456.3603; obsd 248.1777, calcd for $C_{16}H_{24}O_2$ 248.1775.

METHYLATION OF **1**.—Maprounic acid (**1**) (100 mg) was suspended in CH_3CN (10 ml). To this, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.5 ml) was added, and the mixture was stirred for 1 h, at which time CH_3I (1 ml) was added. The reaction was allowed to continue for 12 h. The mixture was then diluted with water (20 ml) and extracted with Et_2O (3 × 25 ml). The combined ether extracts were dried (Na_2SO_4) and evaporated to dryness. Crystallization of the residue from MeOH afforded methyl maprounate (**2**) (81 mg): mp, 202-204°; ir ν max (KBr) 3450 (OH), 1728 (ester C=O) cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 0.79 (3H, s), 0.92 (15H, m) 0.96 (3H, s), 2.34 (2H, m, C-18, C-19), 3.24 (1H, m, C-3), 3.56 (3H, s, CO_2Me), 5.48 (1H, dd, J = 8, 4 Hz, C-12); ms, *m/z* 470 (M^+, 21.2%), 262 (60), 248 (60), 189 (100); mass measurement, obsd 470.3761, calcd for $C_{31}H_{50}O_3$ 470.3760; obsd 262.1936, calcd for $C_{17}H_{26}O_2$ 262.1932.

ACETYLATION OF **1**.—A solution of **1** (7 mg) in pyridine (2 ml) and acetic anhydride (1 ml) was stirred overnight. Ice water (20 ml) was then added, and the solution was stirred for 2 h. The mixture was then extracted with CH_2Cl_2 (3 x 20 ml). The combined CH_2Cl_2 extracts were washed with dilute HCl and water, dried (Na_2SO_4), and evaporated to dryness. Crystallization of the residue from MeOH gave maprounic acid acetate **3** (4 mg): mp, 304-306°; ν max (CH_2Cl_2) 1726 (ester C = O), 1698 (acid C = O), 1245 (ester C-O) cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 0.85 (3H, s), 0.89 (3H, s). 0.93 (15H, m), 2.03 (3H, s, OAc), 2.30 (2H,m, C-18, C-19), 4.5 (1H, m, C-3), 5.50 (1H, dd, J = 8, 4 Hz, C-12); ms, *m/z* 498 (M^+, 3%), 248 (29.3), 234 (100), 189 (89.7); mass measurement, obsd 498.3705, calcd for $C_{32}H_{50}O_4$ 498.3708.

ACETYLATION OF **2**.—A solution of **2** (25 mg) in pyridine (4 ml) and acetic anhydride (2 ml) was stirred overnight. The solution was worked up as in the preparation of **3** to give a residue which, upon crystallization from MeOH, gave methyl maprounate acetate **4** (16 mg): mp, 234.5-237°; ir ν max (KBr) 1726 (ester C = O), 1245 (C-O) cm^{-1}; ^{1}H-nmr ($CDCl_3$ δ 0.84 (3H, s), 0.86 (3H, s), 0.92 (15H, brs), 2.04 (3H, s, OAc), 2.34 (2H,m, C-18, C-19), 3.56 (3H, s, CO_2Me), 4.44 (1H, m, C-3), 5.48 (1H, dd, J = 8, 4 Hz, C-12); ms, *m/z* 512 (M^+, 31.2%), 452 (31.3), 262 (37.5), 248 (68.8), 189 (100), mass measurement, obsd 512.3869, calcd for $C_{33}H_{52}O_4$ 512.3864, obsd 262.1936, calcd for $C_{17}H_{26}O_2$ 262.1932.

ISOLATION OF MAPROUNIC ACID *P*-HYDROXYBENZOATE (**5**).—Fractions 172-200, eluted from the column with chloroform-methanol (98:2) gave a residue (1.86 g) that was further chromatographed on silica gel (E.M. Reagents, 400 g), eluting with increasing amounts of methanol in chloroform. A total of 220 fractions of 15 ml each was collected, and like fractions were combined after tlc evaluation. Fractions

160-220 gave a residue (900 mg) that was rechromatographed on silica gel (E.M. Reagents, 200 g), eluting with increasing amounts of ethyl acetate in hexane. A total of 270 fractions of 15 ml each was collected and like fractions were combined after tlc evaluation. Fractions 145-180, eluted with ethyl acetate-hexane (7:3) upon concentration, yielded maprounic acid *p*-hydroxybenzoate (**5**) (175 mg): mp, 308-311°; $[\alpha]^{20}D$ +32.5° (c = 0.79, pyridine); uv, λ max (MeOH) 256 (log ε 4.17) nm; ir ν max (KBr) 3350 (OH), 1682 (ester C = O), 1608 (aromatic); ms, *m/z* 576 (M^+, 1.1%), 438 (12.3), 423 (13.8), 248 (37.2), 234 (66.2), 189 (75.4), 121 (100); mass measurement, obsd 576.3820, calcd for $C_{37}H_{52}O_5$ 576.3814.

METHYLATION OF **5**.—Maprounic acid *p*-hydroxybenzoate (**5**) (100 mg) was dissolved in *N,N*-dimethylformamide (DMF) (5 ml) and added to NaH (13.1 mg) suspended in DMF (10 ml). The mixture was stirred for 1 h, at which time CH_3I (1 ml) was added and the stirring continued overnight. The mixture was diluted with water (20 ml) and extracted with ether (3 x 25 ml); the combined ether extracts were dried (Na_2SO_4) and evaporated to dryness. Crystallization of the residue from MeOH gave methyl maprounate *p*-methoxybenzoate (**6**) (48 mg): mp, 228-231°; uv λ max (MeOH) 256 (log ε 4.24) nm; ir, ν max (KBr) 1702 (C = O), 1608 (aromatic) cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 0.91 (3H, s), 0.93 (3H, s), 0.94 (9H, brs), 0.97 (3H, s), 1.0 (3H, s), 2.36 (2H, m, C-18, C-19), 3.57 (3H, s, CO_2Me), 3.85 (3H, s, OMe), 4.66 (1H, m, C-3), 5.54 (1H, dd, *J* = 8, 4 Hz, C-12), 6.90 (2H, d, *J* = 9 Hz, ArH), 7.97 (2H, d, *J* = 9 Hz, ArH); ms, *m/z* 604 (M^+, 2.5%), 452 (26.5), 262 (23.5), 248 (49.0), 189 (79.4), 203 (23.5); 135 (100), 161 (17.6); mass measurement, obsd 604.4119, calcd for $C_{39}H_{56}O_5$ 604.4126.

PREPARATION OF **6** FROM **2**.—Methyl maprounate (**2**) (30 mg) was dissolved in pyridine (4 ml) and an excess of *p*-anisoyl chloride (1 ml) added. The mixture was stirred for 72 h. Usual work-up gave **6** (16 mg) identical with the sample prepared from **5**.[3]

ISOLATION OF 7β-HYDROXYMAPROUNIC ACID 3-*P*-HYDROXYBENZOATE (**7**).—Fractions 279-297, eluted from the column with chloroform-methanol (96:4) gave a residue (2.1 g) that was further chromatographed on silica gel (E.M. Reagents, silica gel H, 200 g), eluting with chloroform and increasing amounts of methanol. A total of 370 fractions of 10 ml each was collected. Fractions 261-310 eluted with chloroform-methanol (92:8), upon concentration gave a residue (435 mg). A portion (369 mg) was rechromatographed on a prepacked silica gel column (E.M. Reagents, Lobar, size B) eluting with chloroform-methanol (98:2) and collecting a total of 70 fractions of 18 ml each. Fractions 9-32 gave 7β-hydroxymaprounic acid 3-*p*-hydroxybenzoate (**7**) (189 mg): $[\alpha]^{20}D$ +8.0° (c = 0.65, pyridine); uv, λ max (MeOH) 256 (log ε 4.16) nm; ir, ν max (KBr) 3400 (OH), 1692 (C = O), 1608 (aromatic). 1274, 1166 cm^{-1}; ms, *m/z* 592 (M^+, 1.2%), 577 (2.7), 574 (3.1), 548 (3.1), 546 (4.3), 454 (2.7), 436 (9.4), 358 (11.8), 248 (18.8), 234 (37.5), 203 (37.5), 189 (53.1), 121 (100); mass measurement, obsd 574.3649, calcd for $C_{37}H_{50}O_5$ 574.3658; obsd 436.3339, calcd for $C_{30}H_{44}O_2$ 436.3340.

METHYLATION OF **7**.—A quantity of **7** (50 mg) was suspended in CH_3CN (10 ml) and DBU (1 ml) added. To this CH_3I (1 ml) was added and the mixture was stirred overnight. The mixture was diluted with water, extracted with ether (3 x 25 ml), dried (Na_2SO_4) and evaporated to give crude methyl 7β-hydroxymaprounate 3-*p*-methoxybenzoate (**8**) (49 mg). This was further purified by silica gel chromatography and crystallization from ether-hexane to give pure **8** (17 mg): mp, 260-264°; uv, λ max (MeOH) 256 (log ε 4.32) nm; ir, ν max ($CHCl_3$) 1706 (C = O), 1606 (aromatic), 1290, 1258, 1168 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 0.90 (3H, s), 0.95 (9H, m), 1.00 (3H, s), 1.04 (3H, s), 2.35 (2H, m, C-18, C-19), 3.57 (3H, s, CO_2Me), 3.85 (3H, s, OMe), 4.67 (1H, dd, *J* = 12, 4 Hz, C-3), 5.51 (1H, dd, *J* = 8, 4 Hz, C-12), 6.90 (2H, d, *J* = 9 Hz, ArH), 7.96 (2H, d, *J* = 9 Hz, ArH); ms, *m/z* 620 (M^+, 9.5%), 602 (8.6), 560 (22.9), 468 (19.1), 450 (31.8), 262 (27.7), 248 (57.9), 203 (31.6), 189 (63.2), 135 (100); mass measurement, obsd 620.4140, calcd for $C_{39}H_{56}O_6$ 620.4074.

ACETYLATION OF **8**.—A quantity of **8** (19 mg) in pyridine (0.2 ml) and acetic anhydride (0.2 ml) was stirred overnight. Usual work-up followed by crystallization from chloroform-methanol afforded methyl 7β-acetoxymaprounate 3-*p*-methoxybenzoate (**9**) (11 mg): mp, 292-295°, uv λ max (MeOH) 356 (log ε 4.28) nm; ir, ν max 1732 (C=O), 1608 (aromatic), 1254, 1168 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 0.91 (12H, m), 0.96 (3H, s), 1.02 (3H, s), 1.14 (3H, s), 1.98 (3H, s, OAc), 2.35 (2H, m, C-18, C-19), 3.57 (3H, s, CO_2Me), 3.85 (3H, s, OMe), 4.72 (1H, dd, *J* = 11, 4 Hz, C-3), 4.78 (1H, dd, *J* = 12, 4 Hz, C-7), 5.52 (1H, dd, *J* = 8, 4 Hz, C-12), 6.90 (2H, d, *J* = 9 hz, ArH), 7.96 (2H, d, *J* = 9 Hz, ArH); ms, *m/z* 662 (M^+, 28.6%), 602 (32.1), 456 (75.0), 248 (50.0), 189 (60.7), 135 (100); mass measurement, obsd 662.4188, calcd for $C_{41}H_{58}O_7$ 662.4183.

ISOLATION OF 2α-HYDROXYMAPROUNIC ACID 2,3-BIS-*P*-HYDROXYBENZOATE (**10**).—Fractions 298-340, eluted from the column with chloroform-methanol (90:10) afforded a residue (4.39 g) that was chromatographed on silica gel (E.M. Reagents, Silica Gel H, 400 g), eluting with increasing amounts of methanol in chloroform. A total of 800 fractions of 18 ml each was collected, and like fractions were combined after tlc evaluation. Fractions 621-680 eluted with chloroform-methanol (97:3) gave a residue (554

mg), which was further chromatographed on silica gel (E.M. Reagents, Silica Gel H, 60 g) eluting with chloroform and increasing amounts of methanol. A total of 210 fractions of 15 ml each was collected. Fractions 139-210 eluted with chloroform-methanol (96:4) gave, upon concentration, 2α-hydroxymaprounic acid 2,3-bis-*p*-hydroxybenzoate (**10**) (210 mg): uv, λ max (MeOH) 256 (log ε 4.46) nm; ir, ν max (KBr) 3400 (OH) 1685 (C=O), 1608 (aromatic), 1278, 1165 cm^{-1}; ms, *m/z* 712 (M^+, 0.5%), 668 (1.4), 666 (2.1), 574 (1.0), 421 (13.3), 341 (12.1), 248 (11.4), 234 (24.2), 203 (47.0), 189 (54.5), 121 (100); mass measurement, obsd 712.3979, calcd for $C_{44}H_{56}O_8$ 712.3973.

METHYLATION OF **10.**—A quantity of **10** (50 mg) in DMF (5 ml) was added to a suspension of NaH (20 mg) in DMF (10 ml) and the mixture was stirred for 1 h at which time CH_3I (1 ml) was added and the mixture stirred overnight. Usual work-up afforded, after crystallization from MeOH, methyl 2α-hydroxymaprounate 2,3-bis-*p*-methoxybenzoate (**11**) (31 mg): mp, 271-273°; $[\alpha]^{20}D$ −41.9° (c = 0.80, pyridine); uv, λ max (MeOH) 256 (log ε 4.51) nm; ir, ν max (CH_2Cl_2) 1714 (C = O), 1608 (aromatic), 1172 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 0.93 (15H, m) 1.08 (3H, s), 1.17 (3H, s), 2.35 (2H, m, C-18, C-19), 3.57 (3H, s, CO_2Me), 3.78 (3H, s, OMe), 3.79 (3H, s, OMe), 5.12 (1H, d, *J* = 10 Hz, C-3), 5.40 (1H, m, C-2), 5.51 (1H, dd, *J* = 8, 4 Hz), 6.78 (2H, d, *J* = 9 Hz, ArH), 6.81 (2H, d, *J* = 9 Hz, ArH), 7.82 (2H, d, *J* = 9 Hz, ArH), 7.89 (2H, d, *J* = 9 Hz, ArH); ms, *m/z* 754 (M^+, 6.0%), 694 (13.4), 507 (14.9), 450 (24.6), 435 (25.4), 262 (10.4), 248 (64.2), 203 (55.2), 189 (89.6), 135 (100); mass measurement, obsd 754.4441, calcd for $C_{47}H_{62}O_8$ 754.4443.

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