The Characterisation of Mollic Acid 3β-D-Xyloside and its Genuine Aglycone Mollic Acid, two Novel 1α-Hydroxycycloartenoids from *Combretum molle*

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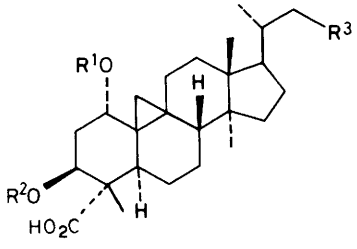
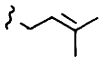
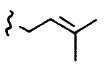
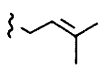
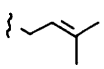
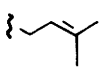
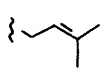
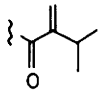
The characterisation of a further novel 1α-hydroxycycloartane glycoside, mollic acid 3-β-D-xyloside, and of its genuine aglycone mollic acid is reported. Three new hydrolysis artifacts of the aglycone have been prepared and ^{13}C .n.m.r. spectral data for these compounds are tabulated.

We previously reported[1] the isolation of mollic acid glucoside (**1**) a 1α-hydroxycycloart-24-en-30-oic acid 3-β-D-glucoside isolated from the acetone extract of *Combretum molle* leaves and showed that the acid hydrolysis of compound (**1**) in tetrahydrofuran (THF) gave the rearranged anhydro aglycone artifact (**8**).

Subsequent investigations have led to the isolation of mollic acid (**3**), the genuine aglycone of (**1**), plus the related compound mollic acid 3β-D-xyloside (**5**). Additional acid-hydrolysis aglycone artifacts of (**1**) have been prepared while soil bacteria were used to hydrolyse (**1**) to mollic acid (**3**). A detailed study of the ^{13}C n.m.r. spectra of the aforesaid compounds has been carried out (Table 1) and the structure of glycoside (**1**) has been confirmed by *X*-ray analysis (Figure 1).[2] The isolation of similar compounds, *e.g.* jessic acid (**7**) from *C. elaeagnoides*,[3] plus work now in progress suggests that this group of cycloartenoids could be common constituents in the leaves of *Combretum* species.



	R^1	R^2	R^3
(**1**)	H	β-D-glucosyl	
(**2**)	Ac	glc(Ac)$_4$	
(**3**)	H	H	
(**4**)	Ac	Ac	
(**5**)	H	β-D-xylosyl	
(**6**)	Ac	xyl(Ac)$_3$	
(**7**)	H	H	

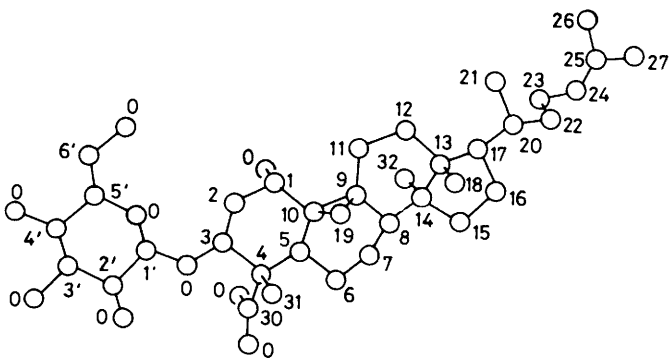


Figure 1. Molecular structure of mollic acid glucoside (**1**) from *X*-ray data[2]

Results and Discussion

Soil bacterial hydrolysis[4] of (**1**) provided mollic acid (**3**) after a variety of hydrolysis attempts using mineral acid had only succeeded in producing aglycone artifacts. Spectral analysis of compound (**3**) and its derivatives showed it to be the genuine aglycone of (**1**). Particularly conclusive was the ^{13}C n.m.r. evidence which indicated that apart from the signals attributed to the sugar carbons and carbons C-2, C-3, and C-4 (which are influenced by glucoside substituent effects) the spectra of compounds (**1**) and (**3**) were identical. A t.l.c. search for unconjugated acid (**3**) in the *C. molle* acetone leaf extract indicated that it was present in low concentration. Isolated by preparative t.l.c. (p.l.c.) as the diacetate, this naturally occurring mollic acid had a m.p. and mass spectrum identical with that of the diacetate of the aglycone (**3**) derived from bacterial hydrolysis.

Closely associated with mollic acid glucoside (**1**) in the acid fraction of the crude acetone extract is a compound which was identified by spectral, chemical, and t.l.c. analysis as mollic acid 3β-D-xylopyranoside (**5**). A slight difference in solubility between these two compounds in ethanol enabled glucoside (**1**), the less soluble, to be obtained pure by repeated recrystallisation whereas preparative high-pressure liquid chromatography

Table 1. ^{13}C N.m.r. spectra data (δ_C/p.p.m.; 20 MHz) for mollic acid glucoside (**1**), mollic acid xyloside (**5**), mollic acid (**3**), mollic acid glucoside penta-acetate (**2**), mollic acid xyloside tetra-acetate (**6**), mollic acid diacetate (**4**), and the hydrolysis products (**9**) and (**10**)

Carbon atom	(1)*	(5)*	(3)*	(2)*	(2)†	(6)†	(4)†	(9)†	(10)†
1	72.6	72.6	72.8	76.3	75.5	75.5	75.1	18.9	18.8
2	37.4	37.5	38.5	33.9	32.9	33.0	30.7	27.8	28.9
3	81.7	81.4	70.8	82.2	80.8	80.3	73.3	74.9	72.2
4	54.8	54.9	55.7	55.9	53.8	53.8	52.7	50.3	51.8
5	38.0	38.0	37.7	39.4	38.9	38.9	38.5	44.2	44.3
6	23.2	23.0	23.4	22.7	22.2	22.2	22.1	19.9	19.7
7	28.5	28.7	28.4	28.5	28.0	28.2	27.9	28.2	28.1
8	48.2	48.4	48.2	46.7	46.6	46.4	46.2	45.0	44.9
9	21.3	21.2	21.1	21.7	21.4	21.4	21.5	143.4	143.8
10	30.6	30.4	30.5	28.5	27.3	27.4	27.9	29.0	28.9
11	25.9	26.1	25.9	25.4	24.6	24.6	24.6	114.6	114.1
12	37.0	37.0	36.9	37.0	35.4	35.5	35.5	36.5	36.1
13	45.9	45.8	45.8	45.8	45.1	45.2	45.2	45.0	44.9
14	49.5	49.5	49.3	49.5	48.8	48.9	48.9	47.0	47.0
15	33.6	33.5	33.5	33.4	32.7	32.7	32.9	33.7	33.6
16	26.6	26.4	26.5	26.7	26.1	26.1	26.4	27.4	28.1
17	52.9	52.9	52.8	52.8	52.4	52.3	52.4	51.0	50.9
18	18.8[a]	18.8[a]	18.7[a]	18.8[a]	17.8[a]	17.7[a]	17.6[a]	12.4[a]	12.7[a]
19	29.8	30.0	29.9	28.5	28.0	28.0	27.9	25.7	25.1
20	36.2	36.4	36.3	35.9	36.1	36.1	36.0	36.2	36.1
21	18.4	18.8	18.3	18.1	18.3	19.0	18.4	18.5	18.5
22	36.4	36.5	36.3	36.4	36.5	36.4	36.5	36.5	36.9
23	25.6	25.5	25.5	25.6	25.0	25.0	25.1	25.1	25.1
24	126.1	125.2	125.9	126.0	125.2	125.2	125.4	125.4	40.4
25	131.0	131.0	130.8	131.0	130.8	130.9	130.8	130.8	74.8
26	25.9	25.7	25.9	25.9	25.7	25.7	25.6	25.7	27.8
27	17.9	18.0	17.8	17.9	17.5	17.6	17.6	17.7	20.6
30	180.0	180.2	179.9	180.0	181.3	181.1	181.2	176.4	178.0
31	10.4	10.5	9.6	9.9	8.9	8.9	9.4	11.2	10.1
32	19.7[a]	19.7[a]	19.6[a]	19.4[a]	19.1[a]	19.0[a]	19.0[a]	14.6[a]	14.5[a]
1′	105.6	106.5		102.8	101.8	101.8			
2′	75.8	75.5		72.2	71.8	71.5			
3′	78.3	78.1		73.7	72.5	71.0			
4′	72.0	71.2		70.0	68.8	69.1			
5′	77.9	67.1		72.5	71.3	62.1			
6′	63.2			63.1	62.3				
25-$COCH_3$									49.0

* In [$^2H_5$]pyridine. † In $CDCl_3$.

[a] Assignments interchangeable.

(h.p.l.c.) separation of the resultant mother liquors yielded xyloside (**5**). The isolation and identification of the remaining constituents in this mother liquor are still in progress.

Mass spectral, ^1H n.m.r., and ^{13}C n.m.r. evidence showed clearly that the sugar moiety was the point of difference between glycosides (**1**) and (**5**). The mass spectral fragmentation pattern attributed to the aglycone in compound (**6**), the peracetylated derivative of (**5**), is identical with that in the spectrum of mollic acid glucoside penta-acetate (**2**), whereas the fragments attributed to the sugar moieties in each spectrum are quite different. Peaks at *m/z* 331, 169, 109, and 43 due to the glucopyranoside tetra-acetate moiety in (**2**) have been replaced by prominent fragments at *m/z* 259, 216, 199, 157, and 97, typical of a pentopyranoside triacetate moiety,[5] in compound (**6**). ^1H N.m.r. signals of tetra-acetate (**6**) support the presence of a xyloside moiety since the two 6′-H proton signals prominent in the spectrum of compound (**2**) are absent. In addition the spectrum of compound (**6**) shows four acetate methyl signals whereas the spectrum of compound (**2**) has five. The similarity of the chemical shifts and the magnitudes of the anomeric proton coupling in the two spectra indicated that the nature of the glycoside linkage is identical in each compound. This was confirmed by ^{13}C n.m.r. evidence which showed that the anomeric carbon resonates at δ_C 101.8 p.p.m. in the peracetylated glycosides (**2**) and (**6**) and at δ_C 105.6 p.p.m. and 106.5 p.p.m. in the spectra of the free glycosides (**1**) and (**5**) respectively, thus indicating a β-D-glycopyranoside link for both.[6] In addition the spectra of glucosides of (**1**) and (**2**) show six carbohydrate carbons whereas the spectra of xylosides (**5**) and (**6**) show only five (Table 1).

This evidence confirms that compound (**5**) contains a pentopyranoside moiety linked by means of a β-D-glycoside bond to the aglycone. The anomeric and D configurations of the xyloside were further indicated by molecular rotation calculations using Klyne's rule (Table 2).[7]

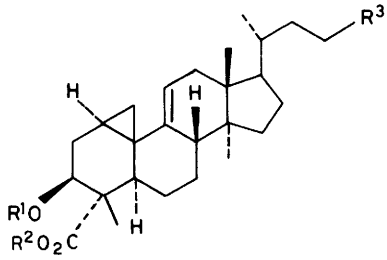
Finally the sugar residue in the water-soluble fraction from the mineral-acid hydrolysis of compound (**5**) co-chromatographed with xylose on both paper chromatography and t.l.c., which confirmed the carbohydrate moiety as xylose. Thus compound (**5**) is mollic acid β-D-xylopyranoside.

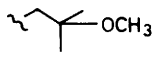
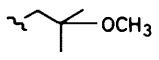
Hydrolysis Products.—Owing to the labile nature of the C-1 hydroxy group adjacent to the cyclopropyl ring, attempts to prepare mollic acid (**3**) from its glucoside (**1**) by mineral-acid hydrolysis led to several interesting aglycone artifacts the formation of which is dependent on the solvent system used.

Table 2. The molecular rotations of mollic acid β-D-xyloside (**5**) and mollic acid (**3**) (in pyridine) compared with the molecular rotations of β- and α-D-xylopyranosides

$[M]_D$ mollic acid xyloside (**5**)		$[M]_D$ mollic acid (**3**)		Δc		$[M]_D$[a] methyl β-D-xyloside	$[M]_D$[a] methyl α-D-xyloside	Assigned Configuration at C-1′ of the D-xylose moiety
+238.6°	−	+292.1°	=	−53.5°	:	−107°	+249°	β

[a] W. S. Woo, S. S. Kang, H. Wagner, O. Seligmann, and V. M. Chari, *Planta Med.*, 1978, **34**, 87.



	R^1	R^2	R^3
(**8**)	H	H	
(**9**)	Ac	H	
(**10**)	H	CH_3	
(**11**)	H	H	
(**12**)	H	CH_3	

Artifacts (**10**)—(**12**) were obtained by methanolic hydrolysis while the use of THF led to the previously reported artifact (**8**).[1] In both solvents a concerted elimination–rearrangement sequence arises from the protonation and elimination of the axial C-1 hydroxy function with concomitant in-phase sigmatropic suprafacial migration of C-19 from C-9 to C-1 with configurational inversion leaving C-9 as a carbocation (Figure 2). This is followed by elimination of the axial 11α-hydrogen. In methanol this reaction is accompanied by esterification of the carboxy function and the addition of either methanol or water across the 24(25)-double bond to give the compounds (**10**)—(**12**). The modification of the side-chain and esterification of the carboxy function in artifacts (**10**)—(**12**) was confirmed mainly by spectroscopic methods.

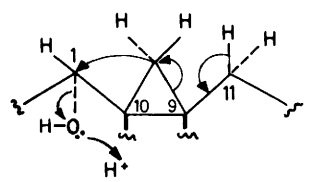


Figure 2.

Mass spectrometry showed fragments corresponding to the presence of methoxy ($M^+ - 32$, lost as CH_3OH) and methoxycarbonyl functions ($M^+ - 32 - 59$) in (**10**); a methoxy ($M^+ - 32$) and carboxy function ($M^+ - 18 - 45$) in (**11**), and a methoxycarbonyl and an extra hydroxy function ($M^+ - 18 - 59 - 18$) in (**12**). Otherwise the fragmentation patterns for these compounds were essentially the same as that of (**8**).[1]

The ^1H n.m.r. spectrum shows that the two methyl signals at δ_H 1.63 and 1.70, which belong to the side-chain isopropylidene group in glycoside (**1**), have shifted upfield to give a two-methyl singlet at δ_H 1.16 in compound (**10**). In addition the spectrum of compound (**10**) showed two three-proton singlets at δ_H 3.18 and 3.64, corresponding to a methoxy methyl and a methoxycarbonyl methyl respectively. In conjunction with the mass spectral evidence this suggested that methanol had added across the side-chain double bond in mollic acid (**3**) to form compound (**10**) and that the carboxy function had been esterified. It follows that in artifact (**12**) water rather than methanol has added across the side-chain double bond. Similar isopropylidene addition products, not reported here, were obtained by subjecting lanosterol to identical reaction conditions.

Experimental

M.p.s were determined with a Kofler micro hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141M polarimeter at 20—25 °C and i.r. spectra were recorded from KBr discs with a Perkin-Elmer 221 spectrophotometer. U.v. spectra were recorded on a Unicam SP 1800 spectrophotometer for solutions in ethanol and mass spectra were obtained with an A.E.I. MS 902 spectrometer. ^1H N.m.r. spectra were recorded with Varian T-60 or XL-100 spectrometers and a Varian CFT-20 spectrometer was used for ^{13}C n.m.r. spectra. Because the compounds characterised gave proton signals in the region δ 0.08—0.50, $CHCl_3$ instead of $SiMe_4$ was used as internal standard. P.l.c. and column chromatography were carried out on Merck silica gel (7731) and (7729) respectively. A Waters Prep LC/System 500A fitted with a Waters 2.5 cm semipreparative column packed with Merck silica gel (7731) was used for the h.p.l.c. separation. T.l.c. was carried out on Merck silica gel GF_{254}. Light petroleum refers to that fraction with b.p. 60—65 °C.

Isolation of Triterpenoid Acids.—Air-dried leaves (4.34 kg) were milled and defatted with boiling light petroleum for 48 h before they were extracted (Soxhlet) with acetone for 3 days. A sparingly soluble, white, crystalline solid (33 g) consisting of a mixture of acidic compounds separated from the acetone solution as it cooled.

Repeated recrystallisations of this mixture from ethanol yielded crystals of mollic acid glucoside (**1**) (15 g), m.p. 248—250 °C (decomp.); $[\alpha]_D^{23}$ +38° (*c* 1.278 in C_5H_5N)

(Found: C, 68.0; H, 9.45. $C_{36}H_{58}O_9$ requires C, 68.10; H, 9.21%); $\lambda_{max.}$ 208 nm (ε 3 800); $\nu_{max.}$ 3 595—3 280 (4 × OH), 3 040 (cyclopropyl CH), 2 640 (CO_2H dimer), 1 705, 1 450, 1 360, 1 265, 1 100—1 020, 990, 915, 890, and 810 cm^{-1}; δ_H(Polysol) 0.39 and 0.63 (each 1 H, d, *J* 4 Hz, 19-H), 0.92 (6 H, br s, 21- and 32-H_3), 1.03 (3 H, s, 31-H_3), 1.56 (3 H, s, Me), 1.63 (3 H, s, Me), 2.90—4.90 (hydroxymethyne protons), and 5.06 (1 H, t, 24-H); δ_C([$^2H_5$]pyridine) see Table 1.

(1-*Acetoxy*-4α-*carboxy*-4β,14α-*dimethyl*-9β,19-*cyclo*-5α-*cholest*-24-*en*-3β-*yl* 2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranoside* (*Mollic Acid Glucoside Penta-acetate*) (**2**).—Acetylation of mollic acid glucoside (**1**) (5 g) with acetic anhydride–pyridine yielded the penta-acetate (**2**) (6 g), m.p. 198—200 °C (from light petroleum); $[\alpha]_D^{23}$ +22.2° (*c* 1.868 in $CHCl_3$) (Found: C, 65.25; H, 8.0% *M*$^+$, 844. $C_{46}H_{68}O_{14}$ requires C, 65.38; H, 8.11%; *M*, 844); $\lambda_{max.}$ 208 nm (ε 3 900); $\nu_{max.}$ 1 750 (acetoxy C=O), 1 707 (carboxy C=O), 1 630, 1 435, 1 365, 1 250—1 200 (acetate C–O–C), 1 035, 990, and 905 cm^{-1}; δ_H($CHCl_3$) 0.50 (1 H, d, *J* 4 Hz, 19-H), 0.78 (1 H, d, partially obscured, 19-H), 0.93 (9 H, br s, 18-, 21-, and 32-H_3), 1.11 (3 H, s, 31-H_3), 1.63 (3 H, s, CH_3), 1.70 (3 H, s, CH_3), 1.99, 2.02, 2.06, 2.10, and 2.12 (each 3 H, s, acetoxy CH_3), 3.66 (1 H, br m, 5′-H), 4.13 (2 H, br m, 6′-H_2), 4.58 (1 H, d, *J* 8 Hz, 1′-H), 4.70 (1 H, t, $J_1 = J_2 = 3$ Hz, 1-H_β), 4.95 (2 H, m, 2′- and 4′-H), and 5.16 (3 H, br m, 3′-, 3-, and 24-H); δ_H(C_6H_6) 0.19 and 0.56 (each 1 H, d, *J* 4 Hz, 19-H); δ_C($CDCl_3$) see Table 1; *m*/*z* (70 eV) 844 (*M*$^+$, 3%), 784 (*M* − CH_3CO_2H, 5), 769 (*M* − CH_3CO_2H − CH_3, 4), 740 (*M* − CH_3CO_2H − CO_2, 4), 513 (*M* − sugar, <1), 453 (14), 463 (24), 421 (7), 392 (14), 391 (13), 377 (7), 367 (7), 331 (sugar, 41), 271 (7), 259 (6), 231 (8), 205 (13), 169 (sugar, 98), 109 (sugar, 38), 69 (34), and 43 (CH_3CO, 100).

Isolation of 4α-*Carboxy*-4β,14α-*dimethyl*-1α-*hydroxy*-9β,19-*cyclo*-5α-*cholest*-24-*en*-3β-*yl* β-D-*xylopyranoside* (*Mollic Acid Xyloside*) (**5**).—The mother liquor from the recrystallisation purification of mollic acid glucoside (**1**) yielded a mixture (2 g) enriched in mollic acid xyloside (**5**). H.p.l.c. separation of this mixture on a Waters Associates Prep LC/System 500(A) fitted with a 2.5 cm Semipreparative Column (P/N 84980) packed with silica gel and using a solvent gradient consisting of ethyl acetate–ethanol (0—10%) yielded crystals of *xyloside* (**5**) (0.19 g), m.p. 235—237 °C (decomp.); $[\alpha]_D^{24}$ +39.5° (*c* 0.76 in C_5H_5N) (Found: C, 70.1; H, 9.1. $C_{35}H_{56}O_8$ requires C, 69.53; H, 9.27%); $\lambda_{max.}$ 210 nm (ε 2 409); $\nu_{max.}$ 3 400br (OH), 3 040 (cyclopropyl CH), 2 625 (CO_2H dimer), 1 700 (carboxy C=O), 1 450, 1 375, 1 260, 1 160, 1 110—1 020, 990, 920, 890, and 820 cm^{-1}; δ_C([$^2H_5$]pyridine) see Table 1.

1α-*Acetoxy*-4α-*carboxy*-4β,14α-*dimethyl*-9β,19-*cyclo*-5α-*cholest*-24-*en*-3β-*yl* 2,3,4-*Tri*-O-*acetyl*-β-D-*xylopyranoside* (*Mollic Acid Xyloside Tetra-acetate*) (**6**).—Mollic acid xyloside (**5**) (50 mg) was treated with acetic anhydride and pyridine at room temperature (15 h) to give the *tetra-acetate* (**6**) (48 mg), m.p. 253—256 °C (from ethanol–light petroleum); $[\alpha]_D^{24}$ +13.5° (*c* 0.424 in $CHCl_3$) (Found: C, 66.7; H, 8.6%; *M*$^+$, 772. $C_{43}H_{64}O_{12}$ requires C, 66.84; H, 8.29%; *M*, 772); $\lambda_{max.}$ 210 nm (ε 4 429); $\nu_{max.}$ 3 045 (cyclopropyl CH), 1 735 (acetoxy C=O), 1 700 (carboxy C=O), 1 435, 1 365, 1 270—1 210 (acetate C–O–C), 1 170, 1 100, 1 090, 1 060, 1 035, 995, 990, 935, 900, 890, 870, and 800 cm^{-1}; δ_H($CHCl_3$) 0.50 (1 H, d, *J* 4 Hz, 19-H), 0.93 (9 H, br s, 18-, 21-, and 32-H_3), 1.10 (3 H, s, 31-H_3), 1.63 (3 H, s, Me), 1.69 (3 H, s, Me), 2.05 (9 H, br s, 3 × acetoxy Me), 2.10 (3 H, s, acetoxy Me), 3.36 (2 H, br m, 5′-H_2), 4.57 (1 H, d, *J* 8 Hz, 1′-H), 4.73 (1 H, t, $J_{2\alpha} = J_{2\beta} = 3$ Hz, 1-H_β), and signals at 4.90—5.07 (5 H, br m, 2′- 3′-, and 4′-H, 3-H_α, and 24-H); δ_C($CDCl_3$) see Table 1; *m*/*z* (70 eV) 772 (*M*$^+$, 1%), 712 (5), 697 (3), 669 (2), 665 (>1), 652 (1), 643 (2), 630 (2), 601 (2), 599 (3), 453 (11), 436 (47), 421 (9), 408 (2), 391 (17), 377 (3), 367 (3), 336 (3), 325 (6), 284 (5), 259 (42), 231 (13), 216 (7), 205 (12), 199 (48), 185 (11), 175 (6), 173 (9), 171 (12), 159 (10), 157 (100), 139 (70), 131 (14), 121 (14), 119 (21), 109 (23), 107 (20), 105 (26), and 97 (90).

Identification of the Sugar Moiety from Glycoside (**5**).—The glycoside (**5**) (60 mg) was refluxed in THF (50 cm^3) containing conc. hydrochloric acid (2.5 cm^3) until t.l.c. analysis indicated that hydrolysis was complete (3—4 h). The solution was diluted with water (30 cm^3) and the solvent volume was reduced by half under reduced pressure. The resultant precipitate was filtered off and the hydrolysate, after neutralisation on a small Amberlite [IR-45 (OH)] column (*ca.* 6 cm × 1 cm diam.), was taken to dryness under reduced pressure. Water (0.5 cm^3) was added to the solid residue and the solution was co-chromatographed with glucose, mannose, fructose, arabinose, xylose, and ribose using a method described previously.[8] Xylose was the only sugar present in the hydrolysate.

Hydrolysis Products.—Preparation of mollic acid (**3**) *by soil bacterial hydrolysis.* Soil (500 g) collected from five different localities was slurried with water (1 dm^3), and the mixture was kept overnight and then filtered. The resultant filtrate (500 cm^3) was added to a buffered solution (500 cm^3) (prepared as described by Yosioka *et al.*[4]) containing the sodium salt of mollic acid glucoside (**1**) (3 g). After a 10 day incubation period at 30 °C the culture broth was acidified with conc. hydrochloric acid and the resultant precipitate was chromatographed on a short column (ethyl acetate as eluant) to yield needles of 4α-*carboxy*-4β,14α-*dimethyl*-9β,19-*cyclo*-5α-*cholest*-24-*ene*-1α,3β-*diol* (*mollic acid*) (**3**) (1.7 g), m.p. 210—212 °C; $[\alpha]_D^{21}$ +61.9° (*c* 0.21 in C_5H_5N) (Found: *M*$^+$, 472.3546. $C_{30}H_{48}O_4$ requires *M*, 472.3552); $\lambda_{max.}$ 210 nm (ε 3 956); $\nu_{max.}$ 3 485, 3 350, 3 045 (cyclopropyl CH), 2 625 (CO_2H dimer), 1 700 (carboxy C=O), 1 660, 1 440, 1 370, 1 270, 1 185, 1 085, 1 045, 1 000, 920, and 870 cm^{-1}; δ_C([$^2H_5$]pyridine) see Table 1; *m*/*z* (70 eV), 472 (30%), 457 (6), 454 (24), 439 (11), 436 (40), 429 (6), 421 (6), 409 (8), 392 (30), 388 (9), 377 (10), 361 (6), 359 (10), 341 (8), 325 (9), 281 (29), 259 (36), 231 (10), 207 (100), 205 (23), 199 (28), 191 (11), 189 (9), 187 (15), 185 (15), 183 (10), 175 (11), 157 (45), 139 (40), 121 (32), 119 (40), and 109 (46).

Mollic Acid Diacetate (**4**).—Mollic acid (**3**) (0.2 g) was acetylated with acetic anhydride–pyridine and the product was eluted through a short column of silica gel with light petroleum to give needles of the *diacetate* (**4**) (0.19 g), m.p. 115—117 °C (from light petroleum); $[\alpha]_D^{23}$ +45.1° (*c* 1.07 in $CHCl_3$) (Found: *M*$^+$, 556.3778. $C_{34}H_{52}O_6$ requires *M*, 556.3763); $\nu_{max.}$ 3 045 (cyclopropyl CH), 2 620 (CO_2H dimer), 1 700—1 730 (carboxy and acetoxy C=O), 1 430, 1 370, 1 225, 1 115, 1 085, 1 030, and 925 cm^{-1}; δ_H($CHCl_3$) 0.51 (1 H, d, *J* 4 Hz, 19-H), 0.91 (9 H, br s, 3 × CH_3), 1.18 (3 H, s, 31-H_3), 1.60 and 1.66 (each 3 H, s, CH_3), 1.96 and 2.10 (each 3 H, s, acetoxy CH_3), 4.70 (1 H, t, $J_{2\alpha} = J_{2\beta} = 3$ Hz, 1-H_β), 5.13 (1 H, t, $J_{23\alpha} = J_{23\beta} = 6$ Hz, 24-H), and 5.51 (1 H, dd, $J_{2\beta}$ 12-, $J_{2\alpha}$ 6 Hz, 3-H_α); δ_H(C_6H_6) 0.13 and 0.53 (each 1 H, d, *J* 4 Hz, 19-H); *m*/*z* (70 eV) 556 (*M*$^+$, 11%), 496 (8), 487 (2), 481 (3), 472 (4), 454 (3), 450 (4), 445 (2), 436 (100), 421 (16), 414 (4), 408 (2), 393 (39), 391 (42), 377 (8), 367 (6), 352 (4), 325 (18), 309 (4), 297 (4), 284 (8), 279 (10), 269 (5), 257 (7), 248 (10), 231 (25), 217 (13), 205 (28), 191 (14), 185 (30), 171 (29), 169 (20), 145 (53), 131 (65), 119 (75), 109 (90), 105 (99), 95 (90), 81 (86), and 69 (89); δ_C($CDCl_3$) see Table 1.

Isolation and Identification of Naturally Occurring Mollic Acid (**3**) [*as the Diacetate* (**4**)] *in the Crude* Combretum molle *Acetone Extract.*—T.l.c. analysis indicated the presence of mollic acid (**3**) in the crude acetone extract (1 g), which was treated with acetic anhydride–pyridine (12 h). Mollic acid

diacetate (**4**) (*ca.* 10 mg), m.p. 112—116 °C, was isolated by p.l.c. of the product. The authenticity of this compound was established by co-chromatography and by comparison of its mass spectrum with that of authentic diacetate (**4**). The mass spectral data for this compound were as follows; *m/z* (70 eV) 556 (M^+, 10%), 496 (17), 487 (1), 481 (4), 472 (3), 454 (5), 452 (7), 450 (4), 445 (2), 436 (100), 421 (17), 408 (12), 393 (73), 391 (37), 377 (20), 367 (7), 352 (5), 325 (13), 309 (6), 297 (3), 281 (10), 279 (13), 269 (4), 259 (7), 248 (20), 231 (13), 217 (8), 205 (18), 203 (17), 191 (12), 183 (9), 171 (18), 169 (10), 145 (22), 131 (27), 119 (37), 109 (48), 105 (45), 95 (61), 81 (50), and 69 (75).

Acid Hydrolysis of Mollic Acid Glucoside (**1**) *in Methanol.*—The glucoside (**1**) (5 g) was dissolved in methanol (1 dm^3) containing conc. hydrochloric acid (50 cm^3), and the solution was refluxed for 36 h. After the addition of water (250 cm^3) the solution was concentrated under reduced pressure (to 500 cm^3) and the resultant precipitate was collected by filtration. Column chromatographic separation of this mixture (3 g) (silica gel, 50 g) using a solvent gradient of light petroleum, ethyl acetate, and ethanol (100—0, 0—100, and 0—10% respectively) yielded two crystalline products. The less polar of these, and also the major aglycone, was *25-methoxy-4α-methoxycarbonyl-4β,14α-dimethyl-1β,19-cyclo-5α-cholest-9(11)-en-3β-ol* (**10**) (900 mg), m.p. 180—185 °C; $[\alpha]_D^{23}$ +113.4° (*c* 1.102 in $CHCl_3$) (Found: C, 77.0; H, 10.5%; M^+, 500. $C_{32}H_{52}O_4$ requires C, 76.80; H, 10.40%; *M*, 500); $\lambda_{max.}$ 209 nm (ε 6 290); $\nu_{max.}$ 3 430 (O–H), 3 060 (cyclopropyl C–H), 1 715 (ester C=O), 1 632 (C=C), 1 460, 1 430, 1 365 (Me), 1 250, 1 225, 1 190, 1 055 (OH), 990, and 820 cm^{-1}; $\delta_H(CHCl_3)$ 0.08 (1 H, dd, $J_{1\alpha}$ 8-, $J_{19\beta}$ 4 Hz, 19-H_α), 0.54 and 0.79 (each 3 H, s, CH_3), 0.86 (3 H, d, *J* 5 Hz, 21-H_3), 1.00 (3 H, s, 31-H_3), 1.12 (6 H, s, 26- and 27-H_3), 3.18 (3 H, s, 25-OCH_3), 3.70 (3 H, s, CO_2CH_3), 4.05 (1 H, dd, $J_{2\beta}$ 12-, $J_{2\alpha}$ 6 Hz, 3-H_α), and 5.05 (1 H, m, 11-H); *m/z* (70 eV) 500 (M^+, 42%), 485 (17), 482 (40), 468 (30), 453 (28), 450 (20), 435 (17), 423 (47), 409 (13), 391 (16), 370 (11), 357 (17), 355 (14), 339 (28), 337 (16), 315 (10), 297 (19), 245 (24), 211 (17), 204 (16), 197 (15), 185 (33), 171 (19), 145 (19), 131 (34), 119 (19), 109 (24), 95 (40), 81 (30), and 73 (100).

The second crystalline product, and also the more polar in the mixture, was *4α-carboxy-25-methoxy-4α,14β-dimethyl-1β,19-cyclo-5α-cholest-9(11)-en-3β-ol* (**11**) (56 mg), m.p. 198—202 °C (Found: M^+, 486.3705. $C_{31}H_{50}O_4$ requires *M*, 486.3708); $\nu_{max.}$ 3 410 (OH), 3 060 (cyclopropyl C–H), 2 620 (carboxylic acid dimer), 1 690 (carboxy C=O), 1 460, 1 370 (Me), 1 270, 1 200, 1 080, 1 050 (OH), and 975 cm^{-1}; *m/z* (70 eV) 486 (M^+, 18%), 471 (13), 468 (18), 454 (41), 439 (29), 436 (19), 423 (18), 421 (18), 409 (12), 392 (12), 391 (12), 383 (13), 370 (12), 341 (29), 325 (17), 283 (14), 231 (18), 205 (18), 145 (18), 131 (24), 119 (23), 109 (24), 95 (36), 81 (29), 73 (100), 69 (41), and 55 (36).

P.l.c. was used to isolate a third minor product, *4α-methoxycarbonyl-4β,14α-dimethyl-1β,19-cyclo-5α-cholest-9(11)-ene-3β,25-diol* (**12**) (15 mg), m.p. 171—172 °C; $\nu_{max.}$ 3 430 (OH), 3 075 (cyclopropyl CH), 1 715 (ester C=O), 1 650 (C=C), 1 460, 1 430, 1 365 (Me), 1 255 (ester), 1 230, 1 095, 1 050 (OH), and 975 cm^{-1}; *m/z* (70 eV) 486 (M^+, 25%), 468 (52), 453 (33), 435 (21), 426 (18), 409 (79), 391 (11), 382 (15), 369 (16), 356 (23), 339 (15), 337 (19), 297 (16), 285 (23), 245 (38), 211 (24), 197 (28), 185 (57), 171 (34), 131 (68), 109 (43), 95 (72), 91 (34), 81 (56), and 69 (100).

Compounds (**10**) and (**11**) were further characterised by their acetates, m.p. 238—242 °C and 163—166 °C respectively; and the acid (**11**) was converted into the methyl ester (**10**) by diazomethane esterification.[9]

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