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MOLLIC ACID 3-β-D-GLUCOSIDE, A NOVEL 1α-HYDROXYCYCLOARTANE SAPONIN FROM *COMBRETUM MOLLE* (COMBRETACEAE).

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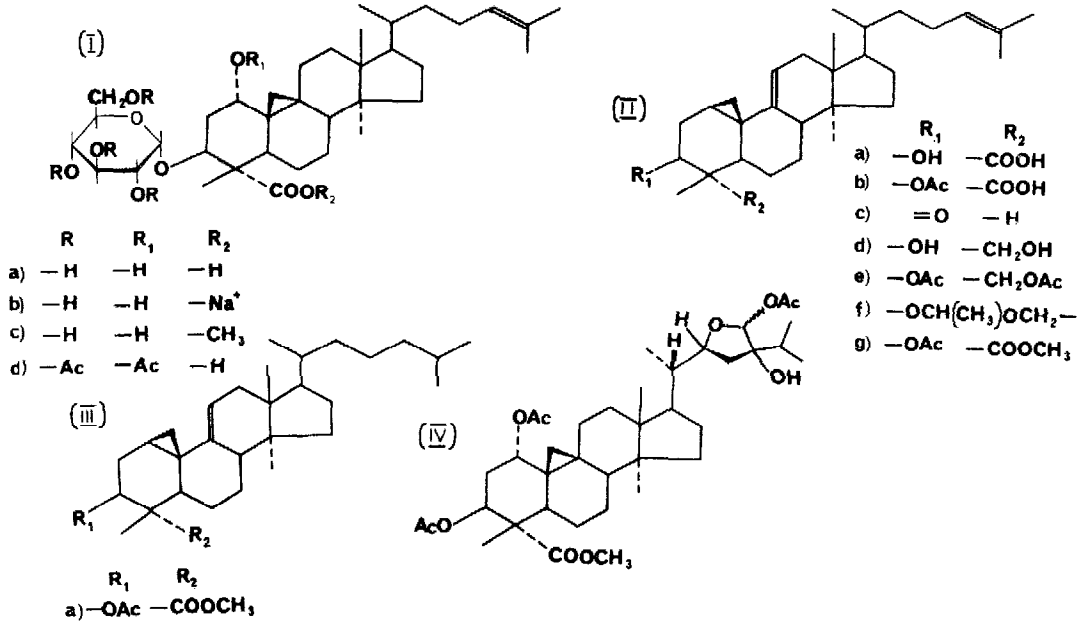
The major constituent of the acetone extract of *Combretum molle* leaves is a colourless, sparingly soluble, crystalline triterpene acid saponin which we have named mollic acid glucoside (Ia), m.p. 248-250° dec., $[\alpha]_D$ + 38° (pyridine), $C_{36}H_{58}O_9$. Titration of this glucoside acid with NaOH shows that it has only one carboxyl function forming a highly water soluble sodium salt (Ib), m.p. 305-307° dec., $C_{36}H_{57}O_9Na$. Mollic acid glucoside readily forms a monomethyl ester (Ic), m.p. 225-226°, $[\alpha]_D$ - 89° (EtOH), $C_{37}H_{60}O_9$, M^+ 648, and a pentaacetate (Id), m.p. 198-200°, $[\alpha]_D$ +22° ($CHCl_3$), $C_{46}H_{68}O_{14}$, M^+ 844, with no hydroxyl bands in the i.r. spectrum.



The ^1H n.m.r. ($CHCl_3$) spectrum of (Id) presents acetoxymethyne signals between δ 5.20 and 4.00, characteristic of the acetylated glucoside moiety. In particular a doublet at δ 4.53 (J8Hz) is assignable to the axially oriented C-1 acetal proton of the glucoside moiety establishing the β-nature of the glucoside link. In addition the mass spectrum of (Id) shows prominent peaks at

m/e 331, 169, 109 and 43 which are characteristic fragments given by glucoside acetates.[1]

A single proton dd signal in the ^{1}H n.m.r. spectrum of (Id) at δ 4.70 (J_1J_2 3Hz) is assigned to an equatorial acetoxymethyne proton at C-1 of the triterpene moiety. We believe that the shielding of this proton by the 9,19-cyclopropane ring accounts for its relatively high field position. In the same spectrum the five acetoxy methyl group signals appear as sharp singlets between δ 2.09 and 1.96; of the six aliphatic methyl groups two signals at δ 1.70 and 1.63 are typical of the side chain isopropylidene methyl groups, a three proton singlet at δ 1.11 is typical of an axial methyl group α to a carboxyl function, while a broad nine proton singlet at δ 0.93 accounts for the three remaining methyl groups. A pair of one proton doublets at δ 0.73 and 0.50 (J4Hz), the former partly obscured by a methyl group signal, indicates the presence of the isolated cyclopropyl methylene group in (Id). The proximity of the C-1 hydroxyl group explains the uncharacteristic downfield shift of these protons.[2] By using C_6D_6 as solvent these two doublets shift upfield to δ 0.54 and 0.17 (J4Hz) at virtually identical positions to those found in the ^{1}H n.m.r. (C_6D_6) spectrum of triacetyl passifloric acid methyl ester (IV).[3] This similarity and the fact that almost all cyclopropane triterpenes are based on a cycloartane skeleton, suggests that the cyclopropane ring is indeed at the C-9,19 position. The acid induced rearrangement described below confirms this placement.

Acid hydrolysis of (Ia) in THF gives a mixture of aglycones plus glucose, which was identified by co-chromatography and enzymatic indicator paper. Acetylation of the aglycone mixture, followed by chromatographic separation yields as the major product the artifact dehydromollic acid acetate (IIb), m.p. 220-222°, $[\alpha]_D$ + 132°, $C_{32}H_{48}O_4$, M^+ 496. The ^{1}H n.m.r. spectrum of (IIb), shows a three proton multiplet at δ 5.15 due to one acetoxymethyne proton, the single olefinic proton at C-24 of the side chain and a second single olefinic proton introduced as a result of the dehydration reaction. The trisubstituted nature of this introduced double bond was confirmed by ^{13}C n.m.r. spectroscopy. Since the dd signal at δ 4.70 due to the equatorial C-1 proton in the ^{1}H n.m.r. spectrum of (Id) is absent in the spectrum of (IIb), the axial 1α-hydroxy function is, by implication, involved in the acid catylised dehydration reaction as observed and reported for (IV).[3] The remaining acetoxy group in (IIb) and hence the hydroxy group in (IIa) is placed at C-3 by virtue of the cumulative evidence presented here and since the presence of an oxygen function at that position is ubiquitous in all triterpenes. By using C_6D_6 as a solvent for (IIb) the ^{1}H n.m.r. multiplet at δ 5.15 is resolved into a dd at δ 5.71 ($J_1$12, $J_2$6Hz) due to the proton at C-3 and two broad doublets at δ 5.34 (J4Hz), and δ 5.07 (J3Hz) due to the two olefinic protons. The large J_1 value obtained for the C-3 proton indicates that it must be axial and therefore the C-3 oxygen function has to be equatorial. The introduced endocyclic double bond in (IIb) has a marked shielding effect on two of the methyl group ^{1}H n.m.r. signals; these occur at δ 0.93 for (Id) but move to δ 0.83 and 0.57 for (IIb) thereby exposing a three proton d (J5Hz) at δ 0.93. This is characteristic of the C-21 side chain methyl group. The characteristic signals due to the cyclopropane methylene protons also undergo a pronounced environmental change with only a one proton signal visible as a dd ($J_1$8Hz, $J_2$4Hz) at δ 0.08.

The double bond introduction on acid hydrolysis of (Ia) and the resultant upfield shift of the two methyl- and the cyclopropyl proton signals in (IIb) can be rationalised on the basis

that a concerted elimination-rearrangement sequence arises from the protonation and elimination of an axial C-1 hydroxyl group. This elimination is followed by β-migration of the cyclopropane group from C-9,19 to C-1,19 with sequential elimination of the axial 11α-hydrogen. This then explains the high-field shift of the two methyl group signals, since a double bond at 9(11) will shield the axial C-18 and C-28 methyl groups and these, incidentally, are the only methyl group signals that had not yet been assigned. In addition, the splitting of the 1,19-cyclopropane, 19-methylene group signal is due to the 19α-proton coupling with the 19β- and 1β-protons, while the shielding effect of the 9(11)-double bond accounts for the upfield shift of the 19α-proton as has been reported by Bombardelli and co-workers.[3]

Confirmation of the position and orientation of the carboxyl function was obtained. The β-hydroxyacid (IIa), m.p. 250-253°, $C_{30}H_{46}O_3$, M^+ 454, prepared by base hydrolysis of (IIb) is oxidised by Jones' reagent to the corresponding β-keto acid, which readily decarboxylates to the nor-ketone (IIc), m.p. 196-203°, $C_{29}H_{44}O$, M^+ 408. Since the hydroxyl-, and keto functions in (IIa) and (IIc) respectively must be at C-3, the eliminated carboxylic acid group has to be at C-4. The ^1H n.m.r. spectrum of the nor-ketone (IIc) no longer has a methyl group signal at δ 1.11. The configuration of the carboxylic acid function was established by reducing the methyl ester of (IIb) with LAH to the diol (IId), m.p. 160-165°, $C_{30}H_{48}O_2$ and acetylating it to the diacetate (IIe), m.p. 167-170°, $C_{34}H_{52}O_4$. The ^1H n.m.r. spectrum of (IIa) shows a two proton singlet at δ 3.83 which is characteristic of a primary equatorial acetoxymethyl group.[4] In addition the diol (IId) forms an ethylidene derivative (IIf), m.p. 160-165°, $C_{32}H_{50}O_2$, which further confirms the 1,3 relationship of the oxygen functions at C-3 and C-4.

The side chain is typically that of cycloartane according to the ^1H n.m.r. evidence outlined so far. This is supported by mass spectral data which shows that all the mollic acid derivatives have fragments at (M^+ - 111) and (M^+ - 111 - 42) due to the loss of the unsaturated C_8H_{15} side chain and side chain plus ring D respectively.[5] On hydrogenation of the side chain double bond, these fragments appear at (M^+ - 113) and (M^+ - 113 - 42). This also provides evidence that no oxygen functions exist on the side chain or on the D ring. Finally, ^{13}C n.m.r. ($CDCl_3$) spectroscopy confirmed the similarity of the mollic acid side chain to that of lanosterol and cycloartenol as presented in the TABLE. A detailed report on the structure determination of mollic acid and related compounds will be presented elsewhere.

TABLE. Carbon-13 Chemical Shifts.[a]

	C-17	C-20	C-21	C-22	C-23	C-24	C-25	C-26	C-27
Cycloartanol[6]	52.5	36.0	18.3	36.4	24.0	39.4	28.0	22.5	22.7
Lanost-8-ene-3β-ol[7]	50.7	36.5	18.8	36.5	24.2	39.6	28.1	22.6	22.8
(IIIa)	52.1	36.6	18.8	36.5	24.1	39.6	28.1	22.6	22.8
Cycloartenol[8]	52.4	36.0	18.0	36.4	25.0	125.3	130.8	17.6	25.7
Lanost-8,24-diene-3β-ol[7]	50.7	36.6	18.8	36.3	25.0	125.3	130.8	17.6	25.7
(IIc)	52.1	36.7	18.8	36.5	25.0	125.3	130.8	17.6	25.7

[a] In parts per million relative to internal TMS.

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References.

1. I.A. Pearl and S.F. Darling, Phytochemistry, 1968, 7, 831-837.

2. R.H. Takata and P.J. Scheuer, Tetrahedron, 1976, 32, 1077-1080.

3. E. Bombardelli, A. Bonati, B. Gabetta, E.J. Martinelli, G. Mustich and B. Danieli, Phytochemistry, 1975, 14, 2661-65.

4. A. Gaudamer, J. Polonsky and F. Wenkert, Bull. Soc. Chim., 1964, 407.

5. H. Budzikiewicz, C. Djerassi and D.H. Williams, Structural Elucidation of Natural Products by Mass Spectrometry, Volume II, Holden-Day, Inc., San Francisco, California, 1964, 94.

6. F. Khuong-Huu, M. Sangare, V.M. Chari, A. Bekaert, M. Devys, M. Barbier and G. Lukacs, Tetrahedron Letters, 1975, 22 + 23, 1787-90.

7. S.A. Knight, Organic Magnetic Resonance, 1974, 6, 603-611.

8. L. Radics, M. Kajtar-Peredy, S. Corsano and L. Standoli, Tetrahedron Letters, 1975, 48, 4287-4290.