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METABOLITES OF THE POLYPORACEAE II. CARBOXYACETYLQUERCINIC ACID - A NOVEL TRITERPENE CONJUGATE FROM DAEDALEA QUERCINA

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Until recently[1], the only reported naturally occurring fungal triterpene conjugates were acetates.[2] We here report the structure of a new ketonic triterpene malonate Ia, the major metabolite of Daedalea quercina

This compound, carboxyacetylquercinic acid, Ia, $C_{34}H_{52}O_7$ has been obtained by silicic acid chromatography of the acetone extracts of the sporophores and had, after repeated crystallisation, m.p. 211° $[\alpha]_D$ - 31° (pyridine). It was more easily isolated in the form of its dimethyl ester Ib, $C_{36}H_{56}O_7$, m.p. 101 - 103°, $[\alpha]_D$ - 2.8° ($CHCl_3$) by silicic acid chromatography of esterified extracts.

Titration showed that Ia was a dibasic acid and treatment of Ia or Ib with aqueous methanolic base gave malonic acid and the hydroxy acid Ic, $C_{31}H_{50}O_4$, m.p. 203 - 205° $[\alpha]_D$+ 22° (pyridine). The latter with diazomethane afforded the hydroxyester Id, $C_{32}H_{52}O_4$, m.p. 141 - 142°, $[\alpha]_D$ + 50.8° ($CHCl_3$).

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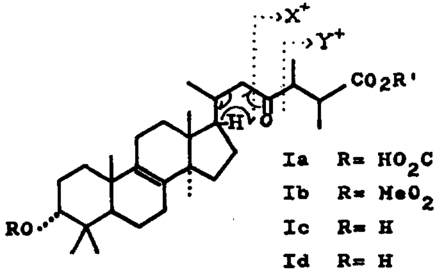
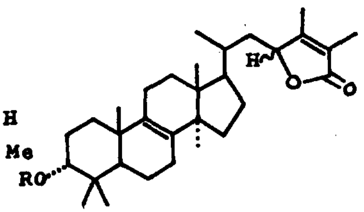


Ia R= $HO_2C.CH_2.CO$ R'= H
Ib R= $MeO_2C.CH_2.CO$ R'= Me
Ic R= H R'= H
Id R= H R'= Me
Ie R= H R'= Et



IIa R= H
IIb R= $MeO_2C.CH_2.CO$

The multiple carbonyl absorption shown by Ib in the IR was attributed to the twin carbonyl peaks typical[3] of a malonate (1758, 1736 $cm.^{-1}$) superimposed upon peaks due to an ester (1736 $cm.^{-1}$) and a ketone (1716 $cm.^{-1}$). The last function was evident from the C.D. of Ia ($\Delta\epsilon_{284} = -0.8$). The malonate grouping gave rise in the NMR of Ib to singlets at 6.30 τ (3 H, OMe) and 6.70 τ (2 H, $-O.CO.CH_2.CO.O-$) and to a multiplet at 5.30 τ (1H, $\overset{|}{\underset{|}{\mathrm{HC}}}$.O.CO.R), while Ia gave only the last two of these signals (at 6.57 and 5.27 τ respectively).

In the side chain of Ib the grouping $-CO.CHMe.CHMe.CO_2Me$ gave rise to 3 proton doublets (J = 6 c/s) in the NMR at 8.88 and 8.96 τ (Me at C_{24} and C_{25}) which collapsed to singlets upon irradiation in the vicinity of a 2 proton multiplet at 7.3 τ (-$\overset{|}{\mathrm{C}}$H.CO- at C_{24} and C_{25}). A peak at 6.33 τ (3 H) due to the CO_2Me at C_{25} showed apparent fine splitting (3 c/s) which disappeared when the spectrum was determined at 130° in tetrachloroethane and was thus attributed to the presence of rotamers in which the methoxyl groups have slightly different magnetic environments.[4]

Three of the principal fragments in the mass spectrum of Ib, namely those at m/e 585, 467 and 309, *represent successive losses of* 15 (CH_3), 118 ($MeO_2C.CH_2CO_2H$) *and* 158 mass units from the parent ion ($M^+ = 600$).[5] The last of these transitions corresponds to the elimination of $CH_2=C(OH).CHMe.CHMe.CO_2Me$ by McLafferty rearrangement e.g. as shown in Ib. Abundant ions at m/e 143 and m/e 115 correspond to fragments of the side chain resulting from cleavages α to the ketonic carbonyl group (ions X^+ and Y^+ in I). This interpretation of the side chain fragmentation was confirmed by comparison of the mass spectra of the methyl ester Id (M^+ = 500; cracking pattern below 485 mass units identical to that of Ib) and of the ethyl ester Ie, $C_{33}H_{54}O_4$, m.p. 102 - 104°. The appropriate peaks in the latter were shifted up by 14 mass units.

The γ relationship of the keto and ester functions in the side chain of Ib results in the formation of butenolides under various conditions. Thus methanolic sodium methoxide afforded two isomeric hydroxybutenolides (IIa), $C_{31}H_{48}O_3$: A, m.p. 270 - 274° and B, m.p. 228 - 229° with almost identical IR, UV, NMR and mass spectral properties [$\nu_{max.}^{CHCl_3}$ 1742, 1683 cm.$^{-1}$; $\lambda_{max.}^{MeOH}$ 217 (ε 14, 600); NMR signals at 8.04 and 8.18 τ (each 3H, $\text{Me}\overset{|}{\text{C}} = \text{C}<$),[6] multiplet at 5.20 τ (1H, $\text{H}\underset{|}{\overset{|}{\text{C}}}$.O.CO.R of butenolide)]. Reaction of Ib with p-toluenesulphonic acid in refluxing benzene gave an intimate mixture of the butenolide malonates IIb, $C_{35}H_{52}O_6$, m.p. 100 - 102°.

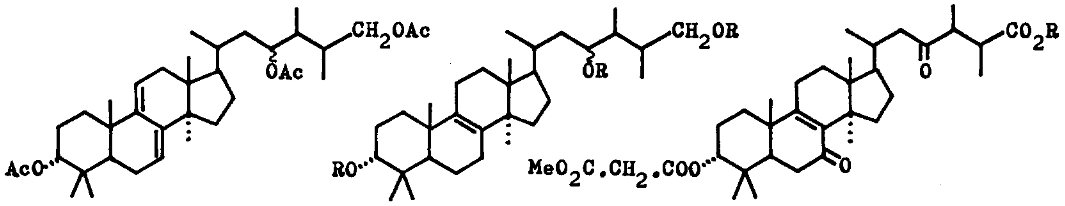
TABLE 1.

τ values of H at C_3

3β - alcohols		3α - alcohols	
Methyl eburicoate	6.78	methyl polyporenate A	6.63
Methyl dehydrotumulosate	6.75	methyl dihydropolyporenate A	6.65
24-dihydrolanosterol	6.78	methyl 3-epidehydrotumulosate	6.60
γ-lanosterol	6.75	methyl isopolyporenate A	6.63

The configuration for the 3 substituent in Ia is suggested by the small half-width (ca 5 cps) of the multiplet due to H at C_3 and by its position in the spectra of Id (6.54 τ) and IIa (6.58 τ) compared to that found for various 3α-and 3β-hydroxylated lanostane derivatives (Table I).

The triterpene skeleton of Ib suggested by similarities with methyl polyporenate A and by the co-occurrence of polyporenic acid C and dehydrotumulosic acid[2] (isolated as their methyl esters) was confirmed by conversion of Ib into the mixture of epimeric dienes III,



III

IVa R= H
IVb R= Ac

V

$C_{37}H_{58}O_6$, m.p. 143 - 144°; (2 H multiplet at 4.5 τ due to olefinic H), the latter showing the chromophore ($\lambda_{max.}$ 236, 243, 252 mμ) characteristic of a 7, 9 (11) lanostadiene.[2] This transformation involved LAH reduction of Ib to give a mixture of the epimeric triols IVa, $C_{31}H_{54}O_3$, m.p. 199.5 - 201° and selenium dioxide oxidation of the corresponding triacetates IVb, $C_{37}H_{60}O_6$, m.p. 159 - 160°. Additional evidence for the presence of the Δ^8 double bond is provided by the oxidation of Ib by trifluoroperacetic acid which yields a conjugated ketone, $C_{36}H_{54}O_8$, m.p. 137 - 139°, the UV absorption (λ_{max}. 253 mμ; ε 8, 600) and CD ($\Delta\epsilon_{340}$ = + 1.1) of which are consistent with structure V[7].

The new structural features shown by Ia (malonate ester, 23-keto function together with carboxyl group at C_{25}) are also present in several other metabolites of D. quercina. The structures of these will be reported in a future communication.

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4. This effect was not shown by methyl 24 (28) - dihydropolyporenate A (cf ref. 2) which has a similar side chain but lacks a 23-keto function.

5. The composition of these ions was confirmed by mass measurement, each transition being accompanied by the appropriate metastable ion.

6. Each signal was finely split, probably due to long range coupling with the proton at C_{23}. Cf. the C_{30} triterpene butenolide, abieslactone (S. Matsunaga, J. Okada and S. Vyeo, Chem. Comm., 525 (1965).

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