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RUBINIC ACID, A TRITERPENE ACID FROM *RUBUS FRUTICOSUS*

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Key Word Index—*Rubus fruticosus*, Rosaceae, rubinic acid, triterpene

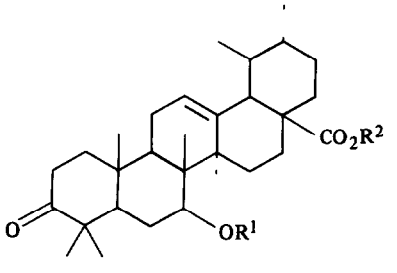
Abstract—A new triterpene acid, rubinic acid, was isolated from the chloroform extract of the leaves of *Rubus fruticosus* On the basis of physical methods and chemical degradation experiments, its structure was determined as 7α-hydroxyursonic acid

INTRODUCTION

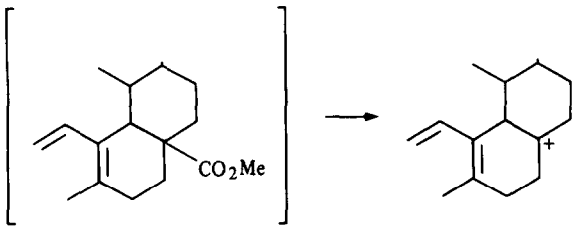
Previous communications [1, 2] reported the isolation and structure elucidation of a new pentacyclic triterpene, rubitic acid, an ursane analogue of the 3,7-dihydroxy acid of the oleanane series, rubusic acid [3] The present paper deals with the isolation and structure elucidation of another new pentacyclic triterpene acid, rubinic acid (**1**), from the leaves of *Rubus fruticosus*

RESULTS AND DISCUSSION

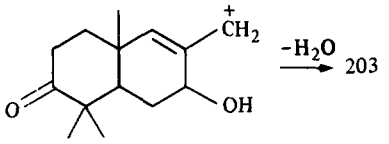
The crude acid obtained from the chloroform extract of the defatted plant material was esterified with diazomethane The methyl ester obtained after chromatography, named methyl rubinate (**2**), $C_{31}H_{48}O_4$, crystallized from benzene–petrol, mp 171–172°, $[\alpha]_D^{25}$ +49° ($CHCl_3$) and was found to be homogeneous on TLC Methyl rubinate by hydrolysis with ethanolic alkali regenerated pure rubinic acid (**1**), $C_{30}H_{46}O_4$, crystallized from methanol–chloroform, mp 259–261°, $[\alpha]_D^{25}$ +72° (MeOH) This acid gave a positive Liebermann–Burchard test for triterpene The IR spectrum of methyl rubinate showed peaks at ν_{max}^{Nujol} 3460, 1710 and 1725 cm^{-1} for a hydroxyl group, 6-membered ketone and a carbomethoxy group, respectively The appearance of three peaks in the region 1400–1350 cm^{-1} and two peaks in the region 1330–1240 cm^{-1} indicated that it was probably an ursane derivative [4] Methyl rubinate showed a positive Zimmermann colour reaction confirming the presence of a keto function at the C-3 position in the triterpene skeleton Acetylation of methyl rubinate with pyridine and acetic anhydride at room temperature did not form any acetate but it did form an acetate (**3**) after heating the reaction mixture at 120° for 24 hr The acetate **3**, $C_{33}H_{50}O_5$, was crystallized from benzene mp 215–216°, $[\alpha]_D^{25}$ +60° ($CHCl_3$) The IR spectrum of acetyl methyl rubinate showed strong peaks at ν_{max}^{Nujol} 1750 cm^{-1} for an acetate and at 1710 cm^{-1} for a $>C=O$ group, no peak was observed in the hydroxyl region Methyl rubinate on Sarett oxidation gave the corresponding diketone $C_{31}H_{46}O_4$, crystallized from benzene, mp 240° It did not exhibit any UV-absorption for an α- or β-diketone Thus the hydroxyl group of rubinic acid must be situated in a ring other than ring A In the mass spectrum of methyl rubinate (M^+ at m/z 484, $C_{31}H_{48}O_4$), the most abundant ions at m/z 262 and 203 corresponded with the fragmentation observed with Δ^{12}-triterpenoids [5] (fragments **a** and **b** in Scheme 1) An ion at m/z 221 corresponded with fragment **c** and proved that the hydroxyl group was situated in ring B Thus the hydroxyl group of methyl rubinate must be either at C-6 or C-7 in ring B On Barton's modification of the Wolff–Kishner reduction [6], 3-acetyl monoketonic ester (prepared by reduction, acetylation and oxidation of methyl rubinate) gave ursolic acid identical with the authentic sample This fact proved that rubinic acid belongs to the ursane family The ^1H NMR spectrum of methyl rubinate showed sharp singlets at δ0 76 (3H), 0 82 (3H), 0 89 (3H), 0 94 (3H), 0 97 (3H), 1 04 (3H) and 1 13 (3H) for seven tertiary methyl groups There was a triplet centred at δ5 29 (J = 3 4 Hz) for the C-12 olefinic proton The signal at 2 95 (1H, *m*) was attributed to one carbinyl proton at C-3 The broad singlet at 1 82 (1H) which disappeared on D_2O treatment was attributed to the proton of the hydroxyl group The presence of a C-28 carbomethoxy group was confirmed by the appearance of a sharp singlet at 3 36 [7] Therefore, rubinic acid is a hydroxy derivative of ursonic acid and the position of the hydroxyl group is either at C-6 or C-7 Finally the Sarett oxidation product of methyl rubinate was found to be identical in all respect (mp, mmp and superimposable IR) with diketo methyl rubitate which is 7-keto methyl ursonate On the basis of the above findings, rubinic acid can be represented as **1**



1 $R^1 = H$, $R^2 = H$
2 $R^1 = H$, $R^2 = Me$
3 $R^1 = Ac$, $R^2 = Me$

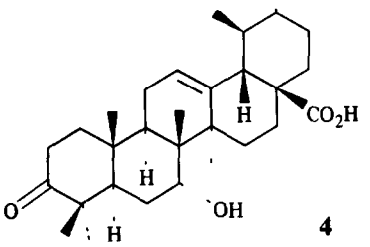


a, m/z 262 b, m/z 203



c, m/z 221

Scheme 1



4

Stereochemistry of rubinic acid

The hydroxyl group at C-7 in rubinic acid could not be acetylated under usual conditions and when acetylated under drastic conditions the acetoxy group resisted saponification under normal conditions These facts suggest that the hydroxyl group of rubinic acid at C-7 is α-axial

The carboxyl group attached to C-17 in ursolic acid has been proved to be β and since rubinic acid was correlated with ursolic acid, the carboxyl group attached to C-17 in rubinic acid must also be β Therefore the stereochemistry of rubinic acid can be assigned and the structure is 3-keto-7α-hydroxy-ursa-12-en-28-oic acid (**4**)

EXPERIMENTAL

All mps are uncorr ^{1}H NMR spectra were recorded at 90 MHz with TMS as internal standard

Isolation of rubinic acid as methyl ester Air dried, finely powdered leaves of *Rubus fruticosus* were defatted with petrol (60–80°) in a Soxhlet for 36 hr The defatted plant material was dried and further extracted with $CHCl_3$ The $CHCl_3$ extract on concn yielded a dark brown tarry mass It was taken up with Et_2O and the soln was extracted with aq alkali (3%) The alkali extract on acidification with HCl in the cold yielded a light green ppt which was filtered, washed with H_2O until free from mineral acid and dried This dried residue was dissolved in MeOH and the soln was treated with excess of ethereal CH_2N_2 at 0° and was kept overnight The excess of CH_2N_2 was removed and the soln concd and filtered The filtrate was evaporated and the residue was taken up with $CHCl_3$ and chromatographed over alumina The ester of rubinic acid was obtained from the petrol–C_6H_6 (1 1) fraction as colourless crystals after first eluting the column with petrol Methyl rubinate was crystallized from C_6H_6–petrol (1 1), mp 171–172° $[\alpha]_D^{25}$ +49° ($CHCl_3$)

Alkaline hydrolysis of methyl rubinate Methyl rubinate (100 mg) was dissolved in 10 ml of 20% ethanolic KOH and refluxed for 8 hr The hydrolysed product was crystallized from MeOH–$CHCl_3$ mixture, mp 259–261°, $[\alpha]_D^{25}$ +72° (MeOH)

Acetate of methyl rubinate Methyl rubinate (100 mg) was treated with Ac_2O and pyridine and the reaction mixture was refluxed at 120° for 24 hr Acetyl methyl rubinate, $C_{33}H_{50}O_5$, thus obtained was crystallized from C_6H_6, mp 215–216°, $[\alpha]_D^{25}$ +60° ($CHCl_3$)

Sarett oxidation of methyl rubinate Methyl rubinate was subjected to Sarett oxidation and the product was purified by chromatography over Al_2O_3 Diketone, $C_{31}H_{46}O_4$, crystallized from C_6H_6, mp 240° was obtained from the C_6H_6 fraction after eluting the column with petrol

3-*Acetyl ketonic ester* The Sarett oxidation product of methyl rubinate on reduction with KBH_4 gave a hydroxy monoketonic ester, $C_{31}H_{48}O_4$, mp 224°, which did not show the Zimmermann colour reaction The borohydride reduction product on treatment with Ac_2O and pyridine at room temperature yielded 3-acetyl 7-keto methyl rubinate, $C_{33}H_{50}O_5$, mp 237–238°

Wolff–Kishner reduction of the 3-acetyl ketonic ester of rubinic acid The 3-acetyl ketonic ester of rubinic acid was subjected to Wolff–Kishner reduction under drastic conditions The reaction mixture was poured into crushed ice, acidified with cold dil HCl (1 1) and separated into neutral and acid parts in the usual way The acid part was treated with charcoal in EtOH The product was crystallized from EtOH as needles, mp 283–284°, identical with authentic ursolic acid (mp, mmp and IR)

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REFERENCES

1 Ganguly, S N (1970) *Chem Ind* 869
2 Sarkar Alok and Ganguly, S N (1978) *Phytochemistry* **17**, 1983
3 Bhattacharya, A K and Dutta, H K (1969) *J Indian Chem Soc* **46**, 381
4 Snatzke, G, Lampert, F and Tschesche, R (1962) *Tetrahedron* **18**, 1417
5 Budzikiewicz, G, Wilson, J M and Djerassi, C (1963) *J Am Chem Soc* **85**, 3688
6 Barton, D H R, Ives, D A J and Thomas, B R (1955) *J Chem Soc* 2056
7 Shamma, M, Glick, E R and Mamma, R O (1962) *J Org Chem* **27**, 4512