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RUBITIC ACID, A NEW TRITERPENE ACID FROM *RUBUS FRUTICOSUS*

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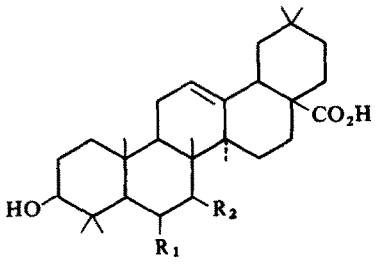
Key Word Index—*Rubus fruticosus*; Rosaceae; triterpene; rubitic acid.

Abstract—A new triterpene acid, rubitic acid was isolated from the alcoholic extract of the whole plant of *Rubus fruticosus*. On the basis of physical methods coupled with chemical investigations, the structure of rubitic acid was shown to be 7α-hydroxy ursolic acid.

INTRODUCTION

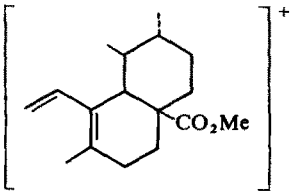
A previous communication [1] reported the isolation and partial structure determination of rubitic acid, an ursane analogue of a 3,6- or 3,7-dihydroxy acid in the oleane series, viz. sumaresinolic (**1**) or rubusic acid (**2**), respectively, which are described in literature [2, 3]. The present paper describes the complete structure elucidation of rubitic acid (**3**).



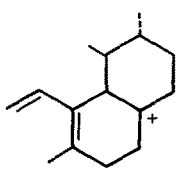
1 $R_1 = OH_2$, $R_2 = H$
2 $R_1 = H_2$, $R_2 = OH$

RESULTS AND DISCUSSION

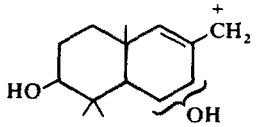
The crude acid obtained from the alcoholic extract of the defatted plant material was esterified with diazomethane. The methyl ester, $C_{31}H_{50}O_4$, by hydrolysis with ethanolic alkali, regenerated pure rubitic acid, $C_{30}H_{48}O_4$. This acid gave a positive Liebermann–Burchard test. Moreover, the IR spectrum of the methyl ester showed the presence of hydroxyl and ester-carbonyl groups. 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 rubitate formed two acetyl derivatives; the monoacetyl derivative was formed easily at room temperature with acetic anhydride and pyridine, whereas the diacetyl derivative was formed only after heating the reaction mixture at 120° for 24 hr. This showed the presence of two hydroxy groups in rubitic acid, of which one was sterically hindered. Since methyl rubitate failed to react with periodic acid it was not a 1,2-dihydroxy compound. The monoacetate, $C_{33}H_{52}O_5$, showed the presence of a hydroxy group and an acetyl carbonyl group, ν_{max}^{Nujol} 3400, 1740 and 1260 cm^{-1}. The diacetate, $C_{35}H_{54}O_6$, showed the absence of a hydroxy group (disappearance of the peak at 3400 cm^{-1}) and the presence of an acetyl carbonyl group (ν_{max}^{Nujol} 1735 and 1280 m^{-1}). Monoacetyl methyl rubitate underwent oxidation with chromic acid in acetic acid and furnished an acetyl monoketonic ester, $C_{33}H_{50}O_5$, which, in its IR spectrum, showed a band at 1720 cm^{-1}, compatible with a six-membered ring ketone. On the other hand, methyl rubitate underwent Sarett oxidation and afforded a diketonic ester, which gave a negative ferric chloride test and a positive Zimmermann colour reaction. Monoacetyl rubitate did not respond to the Zimmermann colour reaction. Diacetyl methyl rubitate was resistant to oxidation with selenium dioxide showing that rubitic acid is a member of the α-amyrin series. Wolff–Kishner reduction by the Barton's modification [5] of the acetyl monoketonic ester gave an acid identical with an authentic sample of ursolic acid which proved that rubitic acid belongs to the ursane family. Diacetyl methyl rubitate underwent partial saponification to a monoacetyl methyl rubitate, different from the one obtained by direct acetylation of methyl rubitate at room temperature. Sarett oxidation of this monoacetyl derivative furnished an acetyl keto



a
m/e 262



b
m/e 203



c
m/e 223

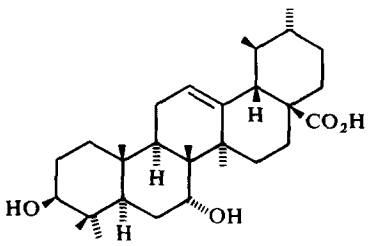
methyl ester, $C_{33}H_{50}O_5$, which gave a positive Zimmermann colour reaction for a 3-keto group. Thus, of the two hydroxyl groups, which are secondary, the one which was easily acylable was placed at the C-3 position in the pentacyclic triterpene skeleton. Rubitic acid was thus a hydroxy ursolic acid, and as it could not be a 1,3-diol, the second hydroxyl group must be situated in a ring other than ring A. In the MS of methyl rubitate (M^+ *m/e* 486, $C_{31}H_{50}O_4$), the most abundant ions at *m/e* 262 and 203 corresponded with the fragmentation observed with Δ^{12}-triterpenoids [6] (fragments **a** and **b**). An ion at *m/e* 223 corresponded to the fragment **c**, which proved that the second hydroxyl group was situated in ring B.

The NMR spectral study of the 3-acetyl keto-methyl rubitate showed no one proton singlet for a methine proton at C-5 but instead, a two proton multiplet at δ 2.48, corresponding to methylene protons at C-6. The second hydroxyl group was, therefore, situated at C-7 and not at C-6, α- to a ketone at C-7. The appearance of singlets at δ 5.27 and at δ 3.62 corresponded to the olefinic proton and methyl ester group, respectively. The structure of rubitic acid can therefore be represented as **3**.

Stereochemistry of rubitic acid

Rubitic acid was easily converted to its 3-acetyl derivative. Therefore, the hydroxyl group at C-3 in rubitic acid was assigned the same configuration as in α-amyrin. This was further supported by the greater ease of hydrolysis of 3-acetyl methyl rubitate. This was finally confirmed by the conversion of 3-acetyl-7 keto methyl rubitate to ursolic acid which contains a 3β-equatorial-OH group. The carboxyl group attached to C-17 in ursolic acid has been proved to be β- and since rubitic acid was correlated with ursolic acid, the carboxyl group attached to C-17 in rubitic acid must also be β.

The C-7 hydroxyl group was readily oxidized without difficulty to the C-7 keto compound which was highly hindered. This hindred keto group could not be reduced with sodium borohydride nor did it undergo Wolff–Kishner reduction under normal conditions. The hydroxyl group at C-7 in rubitic acid was not acetylated under the usual conditions and when acetylated under drastic conditions, this acetoxy group resisted saponification under normal conditions. These facts suggest that the hydroxyl group at C-7 was α-axial. Therefore, the stereochemistry of rubitic acid can be assigned as 3-β-7-α-dihydroxyursa-12-en-28-oic acid (**3**).



3

EXPERIMENTAL

All mps are uncorr. NMR spectra were recorded at 60 MHz with TMS as internal standard.

Isolation of rubitic acid as the methyl ester. Air-dried finely powdered whole plant of *Rubus fruticosus* was defatted with petrol (60–80°) in a Soxhlet for 36 hr. The defatted plant material was dried and further extracted with $CHCl_3$ for 40 hr. 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 till free from 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 CH_2N_2 was removed and the soln concd and filtered. The filtrate was concd and taken up with $CHCl_3$ and chromatographed over Al_2O_3. The ester of rubitic acid was obtained from a C_6H_6 fraction as colourless needles after washing the column with petrol. It was crystallized from C_6H_6–petrol (1:2), mp 182–183° $[\alpha]_D$ +58° ($CHCl_3$). (Found: C, 76.54; H, 10.32. Calc. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.35%).

Alkaline hydrolysis of methyl rubitate. Me rubitate (200 mg) was dissolved in 15 ml 20% ethanolic KOH and refluxed for 8 hr. The solvent was then removed from the reaction mixture and H_2O added and filtered. The filtrate was acidified with HCl in the cold and extracted with Et_2O. The ethereal extract was washed with H_2O till free from acid and dried over dry Na_2SO_4. The Et_2O was distilled off to leave a white amorphous solid of rubitic acid, crystallized from EtOH, mp 252–254°, $[\alpha]_D$ +65° (MeOH) (Found: C, 76.21; H, 10.29. Calc. for $C_{30}H_{48}O_4$: C, 76.23; H, 10.24%).

Monoacetate of methyl rubitate. Me rubitate was acetylated at room temp. and the product was chromatographed over Si gel. Monoacetyl Me rubitate was obtained from a C_6H_6 fraction as colourless crystalline solid, crystallized from C_6H_6, mp 202–203°, $[\alpha]_D$ +68° ($CHCl_3$) (Found: C, 74.95; H, 9.97. Calc. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.91%).

Diacetate of methyl rubitate. Me rubitate (100 mg) was treated with Ac_2O and Py and the reaction mixture refluxed at 120° for 24 hr. It was then processed in the case of the monoacetate and the solid obtained was chromatographed over Si gel. Diacetyl Me rubitate was obtained as a colourless solid from the petrol–C_6H_6 (1:1) fraction after washing the column with petrol and crystallized from petrol–C_6H_6 (1:1), mp 230°, $[\alpha]_D$ +72° ($CHCl_3$). (Found: C, 73.60; H, 9.51. Calc. for $C_{35}H_{54}O_6$: C, 73.65; H, 9.54%).

3-Acetyl monoketonic ester of rubitic acid. To a soln of 3-acetyl keto Me rubitate (75 mg) in HOAc (20 ml) was added a soln of chromic acid (30 mg) in HOAc (5 ml) with constant stirring and the soln was kept overnight. The excess chromic acid was destroyed by MeOH and the soln was poured into crushed ice. The ppt. obtained was filtered, washed till free of acid, dried and crystallized from C_6H_6, mp 238°. (Found: C, 75.23; H, 9.51. Calc. for $C_{33}H_{50}O_5$: C, 75.25; H, 9.57%).

Sarett oxidation of methyl rubitate. Me rubitate was subjected to Sarett oxidation and the product was purified by chromatography over Al_2O_3. Diketo Me rubitate, crystallized from C_6H_6, mp 240°, was obtained from the C_6H_6 fraction after eluting the column with petrol ether. (Found: C, 77.19; H, 9.65. Calc. for $C_{31}H_{46}O_4$: C, 77.14; H, 9.61%).

Selenium dioxide oxidation of diacetyl methyl rubitate. Diacetyl Me rubitate (25 mg) was dissolved in HOAc (2 ml) and refluxed with pure SeO_2 (30 mg) for 5 hr. The excess reagent and the red metallic Se deposited during the reaction were filtered and the filtrate was poured into crushed ice. The solid residue was filtered, washed till free from acid and dried. It was crystallized from petrol–C_6H_6 (1:1) mp 230°. It did not depress the mp with diacetyl Me rubitate.

Wolff–Kishner reduction of 3-acetyl monoketonic ester of rubitic acid. 3-Acetyl mono ketonic ester of rubitic 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 282–284°, identical with authentic ursolic acid (mp, mmp and IR).

Saponification of diacetyl methyl rubitate. Diacetyl Me rubitate

(50 mg) was dissolved in 3% methanolic KOH (10 ml) and refluxed for 2 hr. It was cooled, transferred to a porcelain basin and MeOH allowed to evaporate. $.H_2O$ was added and the ppt. filtered, washed free from alkali and dried. The dried mass was crystallized from C_6H_6–MeOH, mp 210° (Found: C, 74.99; H, 9.87. Calc. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.91%).

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