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Note

Chemistry of rutin. Mass-spectral characterization of rutinose heptaacetate*

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That rutinose and quercetin are hydrolysis products of rutin, either through enzymic[1] or weakly acidic[2] catalysis, has long been known. The synthesis of rutinose heptaacetate (**1**) from 2,3,4-tri-*O*-acetyl-L-rhamnopyranosyl bromide and 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose, and subsequent deacetylation of **1**, led to an assignment[3] of the structure 6-*O*-(β-L-rhamnopyranosyl)-D-glucopyranose to rutinose. On the basis of periodate-oxidation studies[4], the structure of rutinose was revised to 6-*O*-(α-L-rhamnopyranosyl)-D-glucopyranose. Quite recently, a novel procedure for preparation of rutinose heptaacetate from rutin decaacetate has been described[5].

In the first report[2] of hydrolysis of rutin in dilute acetic acid, rutinose heptaacetate (**1**) was obtained in rather low yield by acetylation of the crude sugar fraction. By extending the period of hydrolysis and making other minor procedural modifications, **1** can be obtained in nearly twice the yield previously cited. In addition to customary analytical characterization, we have measured the mass spectrum of **1** (*m/e* values in Experimental).

The spectrum does not show a molecular-ion peak at 620. However, peaks are observed at *m/e* 561 and 560, as would be expected by loss of CH_3CO_2 and CH_3CO_2H fragments, respectively, from an ion of mass 620. The peaks listed (Experimental) are generally of low relative intensity, but can be plausibly accounted for in terms of stepwise losses of CH_3CO_2, CH_3CO_2H, and CH_2CO fragments. Such decomposition is known to be characteristic of acetic esters[6] and, in particular, of sugar polyacetates[7].

By far the strongest peak in the spectrum is that at *m/e* 43, attributed[7] to CH_3CO^+. Other prominent peaks can be accounted for by characteristic ether cleavages[8] as shown by the broken lines in Fig. 1. Moreover, the two strongest peaks in the high-mass region, those at *m/e* 317 and 273, are the two strongest peaks in the entire spectrum when measurement is made at a lower ionizing potential. Smaller peaks occur at *m/e* 331, 330 (from hydrogen migration, also characteristic of ethers)[8c],

*Dedicated to the memory of Professor M. L. Wolfrom.

and 289. Relatively strong peaks at *m/e* 257, 215, and 213 can be shown by metastable peaks to arise by further breakdown of major primary products.

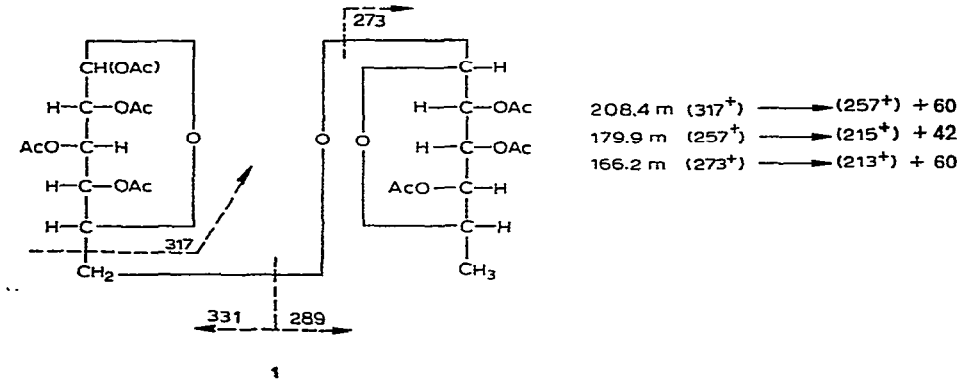

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Fig. 1. Principal pathways for fragmentation of rutinose heptaacetate (1) in the mass spectrometer. Transitions corresponding to metastable peaks (m) are shown.

In addition to peaks associated with rutinose heptaacetate, a series of peaks occur at *m/e* 534, 474, 445, 432, 415, 414, and 372. These may originate from an acetylated proanthocyanidin hydrate, a specific example of which would be a diarylglycerol derivative [$C_{15}H_{11}O_8(COCH_3)_5$]. The proanthocyanidin area has been reviewed by Freudenberg[9]. A proanthocyanidin hydrate could be a contaminant of rutin, despite purification of the rutin by recrystallization from water. In terms of the presently accepted structure[3], a proanthocyanidin hydrate can not arise by hydrolysis of rutin under non-reductive conditions.

Presence of the rutinose moiety in rutin is clearly demonstrated by hydrolytic experiments[1,2], and more recently by syntheses of rutin in two different laboratories[10,11]. Methylation of rutin has been investigated in the present work in an effort to achieve permethylation of this flavonol glycoside. The procedure employed involves partial methylation by interaction of rutin with methyl sulfate and aqueous alkali, and then repeated further methylation steps with methyl sulfate and dry sodium hydroxide in tetrahydrofuran. The initial methylation process is carried out in such a way that it is violently exothermic, and partial oxidative degradation may well occur. Furthermore, completely consistent results have not been realized. In several instances, especially when an additional *p*-phenylazobenzoylation (azoylation) step was introduced to remove partial methylation products, the procedure has given a colorless solid for which combustion and methoxyl analyses are in agreement with theory for a decamethyl ether of rutin. In other experiments, however, an unstable, crystalline, yellow product or yellow oils were obtained.

Acidic hydrolysis of the colorless methylation product of rutin yields the known quercetin 3′,4′,5,7-tetramethyl ether and a methylated sugar fraction. Azoylation and subsequent chromatographic purification of the azoylated sugars by the general procedure of Reich[12] and Coleman[13] gave a crystalline azoyl derivative

whose analysis and m.p. indicate that it is 1,6-di-*O*-azoyl-2,3,4-tri-*O*-methyl-D-glucopyranose. Thus, methylation and hydrolysis indicate that the D-glucose portion of the rutinose moiety in rutin, as well as in the isolated sugar rutinose, has the pyranoid ring structure. All attempts to detect 2,3,4-tri-*O*-methyl-L-rhamnopyranose were unsuccessful.

EXPERIMENTAL

General. — Melting points were measured in capillary tubes and are uncorrected. Mass spectra were measured with 70-volt and low-voltage (9.5 volts, nominal) electrons on a Consolidated Model 21-103 mass spectrometer, with the source and inlet at 250°. Microanalyses were determined by Alfred Bernhardt, Geller Laboratories, and Micro-Tech Laboratories.

Rutinose heptaacetate (**1**). — Rutin trihydrate (N.F. X, 10 g, recrystallized from water) was heated with 300 ml of 10% aqueous acetic acid for 12 h and the sugar fraction was isolated by the general procedure of Zemplén[2]. The crude sugar was acetylated with 20 ml of acetic anhydride and 4 g of anhydrous sodium acetate for 9 h at steam-bath temperature. The clear reaction mixture was poured into a large excess of water and kept overnight. Evaporation of the resulting mixture to dryness *in vacuo* gave a residue which was washed several times with hot water. The residue then was extracted with chloroform; the chloroform extract was washed with water, dried, and evaporated to dryness. The resulting residue was treated with ether and nucleated with **1**, whereupon a colorless, crystalline product, m.p. 168–169°, was obtained. The oily residue from the ether treatment was dissolved in methanol and refrigerated to give **1** as a colorless, crystalline solid; yield, 1.5 g, m.p. 168–169°. Analytically pure **1** was obtained by repeated recrystallization from ether and drying *in vacuo* for 24 h; m.p. 169–170°, lit.[3] m.p. 168.5–169°.

Anal. Calc. for $C_{12}H_{15}O_{10}(COCH_3)_7$: C, 50.36; H, 5.85; O, 43.87; CH_3CO, 48.55; C (CH_3), 19.38. Found: C, 50.86; H, 5.83; O, 43.28; CH_3CO, 47.04; C (CH_3), 18.23.

The mass spectrum showed peaks at *m/e* 561, 560, 518, 501, 500, 459, 458, 441, 399, 398, 381, 339, 338, 331, 330, 317, 297, 296, 289, 273, 257, 215, 213, and 43, attributed to rutinose heptaacetate, and at *m/e* 534, 474, 445, 432, 415, 414, and 372, seemingly unrelated to the main series and attributed to an impurity. Peaks of *m/e* less than 213, except for the intense peak at 43, are not listed here.

Methylation of rutin. — Rutin (3 g, purified by the Couch procedure[14]) was suspended in 20 ml of methyl sulfate. Aqueous alkali (9 g of sodium hydroxide 97% in 18 ml of water) was added all at once. A violent reaction occurred, which subsided after *ca.* one h. The reaction mixture then was treated with 70 ml of tetrahydrofuran. The organic phase was separated and treated twice with 7 ml of methyl sulfate and excess powdered sodium hydroxide. The mixture was heated for 2 h on a steam bath, filtered, and to the filtrate was added 7 ml of methyl sulfate and powdered sodium hydroxide. The resulting mixture was kept overnight at room temperature. Iterative

treatment (2 to 3 times), with 7 ml of methyl sulfate and excess powdered sodium hydroxide, with the final mixture being kept for 3 days at room temperature and then being refluxed for one h, gave a tetrahydrofuran solution (dried over anhydrous magnesium sulfate) which was chromatographed on acidic alumina (Woelm). The column was developed with tetrahydrofuran or benzene and the total effluent was collected. Removal of the solvent gave a red oil (positive sulfur and Mg–HCl tests, negative $FeCl_3$), which was treated with 30% aqueous sodium hydroxide and heated for 30 min on a steam bath. The aqueous mixture was extracted with tetrahydrofuran, and the dried extract was chromatographed on acidic alumina. Removal of the solvent from the total effluent gave a solid residue (negative $FeCl_3$ and sulfur tests, positive Mg–HCl), m.p. *ca.* 55–66°.

To remove partially methylated material, the solid product in 30 ml of dry pyridine was treated with excess *p*-phenylazobenzoyl (azoyl) chloride at room temperature overnight, with protection from atmospheric moisture. Water (10 ml) and excess sodium hydrogen carbonate were added, followed by acetone. The inorganic material thus precipitated was filtered off, the solvent was removed *in vacuo* from the filtrate, and the resulting residue was dissolved in chloroform. Excess azoyl chloride, azoic acid, and azoic anhydride were removed by chromatography of the solution on acidic alumina. The red chloroform effluent was evaporated and the residue was dissolved in ethanol. Addition of water caused precipitation of reddish, colloidal material. After the addition of charcoal, the mixture was filtered through a funnel at 60° (to avoid oxidation). The filtrate was extracted three times with chloroform and the combined extracts were dried and evaporated. A solution of the residue in tetrahydrofuran was chromatographed on acidic alumina with retention of the total effluent. Removal of the solvent *in vacuo* gave the decamethyl ether of rutin as a solid, colorless, water-soluble residue (negative $FeCl_3$, positive Mg–HCl tests), melting unsharply near 103°, with marked softening at 73°.

Anal. Calc. for $C_{27}H_{20}O_6(OCH_3)_{10}$: C, 59.19; H, 6.71; OCH_3, 41.33; C (CH_3), 2.00. Calc. for $C_{27}H_{19}O_7(OCH_3)_9$: C, 58.69; H, 6.57; OCH_3, 37.9; C (CH_3), 2.04. Found: C, 58.91; H, 6.73; OCH_3, 40.74; C (CH_3), 1.82.

Hydrolysis of methylated rutin. — Methylated rutin (2 g, prepared as in the foregoing experiment in 30 ml of 95% ethanol and 50 ml of 6% hydrochloric acid was refluxed for 8 h. Water (50 ml) was added, and the resulting mixture was refrigerated overnight. The precipitated quercetin 3′,4′,5,7-tetramethyl ether, m.p. 194°, lit.[15] m.p. 193-196°, was filtered off. The filtrate was neutralized with barium carbonate, excess barium carbonate was removed, and the solvent was removed *in vacuo* from the filtrate. The solid residue was extracted with tetrahydrofuran, and the extract was evaporated to give an oil. The latter was dried in a vacuum desiccator and azoylated by the procedure of Coleman[13]. The azoyl derivatives were subjected to two chromatographic steps. First, a chloroform solution was chromatographed on acidic alumina to remove azoyl chloride, azoic acid, and azoic anhydride. The effluent was then concentrated to 10 ml, and chromatographed on silicic acid ($H_2SiO_3 \cdot H_2O$). Development with 3:1 (v/v) petroleum ether–benzene gave a single, brightly colored

zone, which was removed mechanically. Elution with 3:1 (v/v) chloroform–methanol gave the 1,6-bis(azoate) of 2,3,4-tri-*O*-methyl-D-glucopyranose, m.p., after crystallization from 95% ethanol, 138.5–139° (lit.[16] m.p. 133°).

Anal. Calc. for $C_{25}H_{34}N_4O_8$: C, 65.82; H, 5.37; N, 8.77. Found: C, 65.81; H, 5.28; N, 8.86.

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