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REACTION OF ACETATES OF NEOHESPERIDOSE, RUTINOSE, AND SOME FLAVONOID L-RHAMNOSYL-D-GLUCOSIDES WITH DIHALOMETHYL METHYL ETHERS

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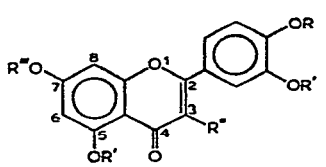
ABSTRACT

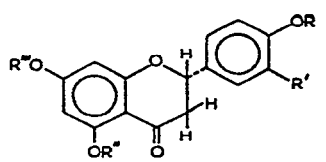
Conversion of the β-hepta-acetate into the *O*-acetylglycosyl halide by means of dihalomethyl methyl ethers occurs more rapidly with rutinose (6-*O*-α-L-rhamnopyranosyl-D-glucose) than with neohesperidose (2-*O*-α-L-rhamnopyranosyl-D-glucose). Further cleavage of the glycosidic link between the L-rhamnose and D-glucose residues occurs readily with dibromomethyl methyl ether, but to a very much lesser extent with the dichloro analogue. Dihalomethyl methyl ethers liberate oligosaccharides less selectively from flavonoid 7-(L-rhamnosyl-D-glucosides) than from the less acid-resistant 3-glycoside, rutin. A method is described for the preparation of β-rutinose hepta-acetate in good yield from rutin deca-acetate.

INTRODUCTION

Recently, the use was reported of dihalomethyl methyl ethers for the selective cleavage of *O*-acetyloligosaccharide glycosides to yield *O*-acetyloligosaccharyl halides[1]. Although various methods have been employed for the selective cleavage of glycosidic linkages, the high yields of acetylated rutinosyl halides obtained from rutin deca-acetate (**1**) by the new procedure suggested that it might be of particular value as a general method for preparing and identifying oligosaccharides from flavonoid compounds. The present investigation was undertaken with a view to extending the reaction to other flavonoid L-rhamnosyl-D-glucosides after establishing the stability of the disaccharide acetates under various conditions.



1 R=R′=R‴=Ac; R″=Hexa-*O*-acetyl-β-rutinosyloxy
2 R=Me; R′=Ac; R″=H; R‴=Hexa-*O*-acetyl-β-rutinosyl
3 R=R′=R‴=Ac; R″=OAc
4 R=R′=R‴=Me; R″=OMe



5 R=Me; R′=OAc; R″=Ac; R‴=Hexa-*O*-acetyl-β-rutinosyl
6 R=R‴=Ac; R′=H; R″=Hexa-*O*-acetyl-β-neohesperidosyl

TABLE I

REACTION OF NEOHESPERIDOSE, RUTINOSE, AND RUTIN ACETATES (0.04 MMOLE) WITH DIBROMOMETHYL METHYL ETHER (0.05 ML) IN CHLOROFORM (0.6 ML) IN THE PRESENCE OF ZINC CHLORIDE (20 MG)

Expt.	*Starting material*	*Bromide formed*	*Time (min)*			
			2	*5*	*30*	*60*
1	β-Neohesperidose hepta-acetate		+++	trace	—	—
		Hexa-*O*-acetyl-α-neohesperidosyl	++	++++	+	—
		Tri-*O*-acetyl-α-L-rhamnopyranosyl	—	trace	+++	++++
2	β-Rutinose hepta-acetate		—	—	—	—
		Hexa-*O*-acetyl-α-rutinosyl	++++	++++	—	—
		Tri-*O*-acetyl-α-L-rhamnopyranosyl	—	trace	++++	++++
3	β-Neohesperidose hepta-acetate		++++	++++	++++	++++
	[in presence of 0.04 mmole of	Hexa-*O*-acetyl-α-neohesperidosyl	—	—	+	+
	penta-*O*-acetylquercetin (3)]	Tri-*O*-acetyl-α-L-rhamnopyranosyl	—	—	—	—
4	β-Neohesperidose hepta-acetate		++++	++++	++	trace
	[in presence of 0.04 mmole of	Hexa-*O*-acetyl-α-neohesperidosyl	—	trace	++	++++
	penta-*O*-methylquercetin (4)]	Tri-*O*-acetyl-α-L-rhamnopyranosyl	—	—	trace	+
Control	Rutin deca-acetate (1)	Hexa-*O*-acetyl-α-rutinosyl	trace	+	++	+++
		Tri-*O*-acetyl-α-L-rhamnopyranosyl	—	—	trace	+

EXPERIMENTAL AND RESULTS

General. — M.ps. are uncorrected, and were determined by the Kofler method. Merck Kieselgel G was employed for thin-layer chromatography (t.l.c.), with benzene–acetone (9:1) as irrigant. Spot intensities were estimated visually after spraying with sulphuric acid, and heating for 15 min at 140°.

Preparation of reagents and samples. — The procedure of Gross and Karsch[2] was employed for the preparation of dibromomethyl methyl ether, and dichloromethyl methyl ether was prepared by the method of Rieche, Gross, and Höft[3].

Hesperidin, naringin, quercetin, and rutin (Koch–Light Laboratories) were acetylated with acetic anhydride–pyridine, and recovered from water in the usual way. Diosmin octa-acetate (**2**) was prepared from hesperidin octa-acetate (**5**) by dehydrogenation with selenium dioxide in acetic anhydride[4]. Penta-*O*-methylquercetin (**4**) was prepared by treatment of quercetin with methyl sulphate and potassium carbonate in acetone[5]. β-Neohesperidose hepta-acetate was synthesized as described by Koeppen[6], and β-rutinose hepta-acetate was prepared from compound **1** as described below. All samples were dried to constant weight at 110° under diminished pressure before use.

Reactions with dibromomethyl methyl ether. — A solution of the compound (0.04 mmole) and zinc chloride (20 mg) in chloroform (0.6 ml) was boiled, and dibromomethyl methyl ether (0.05 ml) was added. The reaction was monitored by t.l.c. A control with compound **1** was run in each case, and the compounds were studied both individually and simultaneously. Tables I and II contain the results of several experiments in which no differences in the reaction rates of the rutin acetate controls could be detected by t.l.c.

Reaction with dichloromethyl methyl ether. — Solutions of neohesperidose and rutinose acetates (0.04 mmole) in chloroform (0.6 ml), with various quantities of zinc chloride, were boiled, and examined periodically by t.l.c. after addition of various amounts of dichloromethyl methyl ether (Table III). Flavonoid L-rhamnosyl-D-glucoside acetates were examined as follows. A solution of the compound (0.04 mmole) and zinc chloride (40 mg) in chloroform (0.6 ml) was boiled, and treated with dichloromethyl methyl ether (0.1 ml). A control with compound **1** was run in each case, and the reaction mixtures were examined by t.l.c. after 1, 3, and 6 h (Table IV).

*Preparation of 1,2,3,4-tetra-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-6-deoxy-α-*L*-manno-pyranosyl)-β-*D*-glucopyranose (β-rutinose hepta-acetate, cf. ref. 1).* — A mixture of rutin deca-acetate (**1**; 1.0 g), dry, finely powdered zinc chloride (1.0 g, analytical reagent grade), and anhydrous chloroform (15 ml) was boiled, and dichloromethyl methyl ether (2.5 ml) was added. After 3 h, the solution was decanted, and the residue was washed with chloroform (2 × 10 ml). The combined chloroform solutions were washed with iced water, saturated, aqueous sodium hydrogen carbonate, and water until the washings were neutral to litmus, filtered through anhydrous sodium sulphate, and evaporated to dryness under diminished pressure at 30°. The dry residue (0.78 g)

TABLE II

REACTION OF FLAVONOID L-RHAMNOSYL-D-GLUCOSIDE ACETATES (0.04 MMOLE) WITH DIBROMOMETHYL METHYL ETHER (0.05 ML) IN CHLOROFORM (0.6 ML) IN THE PRESENCE OF ZINC CHLORIDE (20 MG)

Expt.	*Starting material*	*Bromide formed*	*Time (min)*		
			15	*60*	*120*
1	Diosmin octa-acetate (2)	Hexa-*O*-acetyl-α-rutinosyl	—	—	—
		Tri-*O*-acetyl-α-L-rhamnopyranosyl	+	+++	++++
2	Hesperidin octa-acetate (5)	Hexa-*O*-acetyl-α-rutinosyl	+	+	++
		Tri-*O*-acetyl-α-L-rhamnopyranosyl	trace	++	+++
3	Naringin octa-acetate (6)	Hexa-*O*-acetyl-α-neohesperidosyl	—	trace	++
		Tri-*O*-acetyl-α-L-rhamnopyranosyl	trace	+	++
Control	Rutin deca-acetate (1)	Hexa-*O*-acetyl-α-rutinosyl	++	+++	++++
		Tri-*O*-acetyl-α-L-rhamnopyranosyl	trace	+	+

TABLE III

REACTION OF NEOHESPERIDOSE AND RUTINOSE ACETATES (0.04 MMOLE) IN CHLOROFORM (0.6 ML) WITH DICHLOROMETHYL METHYL ETHER UNDER VARIOUS CONDITIONS

Dichloromethyl methyl ether (ml)	*Zinc chloride (mg)*	*β-Hepta-acetate*	*Time required for complete conversion into α-chloride (h)*	*Tri-O-acetyl-α-L-rhamnopyranosyl chloride detected after 6 h*
0.05	20	Neohesperidose	5	trace
0.05	20	Rutinose	1	+
0.10	20	Neohesperidose	4.25	trace
0.05	0	Neohesperidose	No reaction after 6 h	0
0.05	40	Neohesperidose	5	trace
0.10	40	Neohesperidose	2	+
0.10	40	Rutinose	0.25	++

was treated with a solution of mercuric acetate (0.8 g) in glacial acetic acid (16 ml) for 2 h at room temperature, the mixture was diluted with chloroform (50 ml), washed with water (5 × 20 ml), and evaporated to dryness, and the residue was dissolved in boiling ethanol (25 ml). A yellow precipitate (0.08 g) which formed on cooling was removed by filtration and discarded. On further standing, the filtrate deposited colourless prisms of β-rutinose hepta-acetate (0.261 g, 43.3%), m.p. 165–167°. The mother liquor was purified by t.l.c., the major component being located on the chromatoplates by spraying with water. After elution with acetone, and crystallization from ethanol, a further amount (0.098 g, 16.0%) of β-rutinose hepta-acetate, m.p. 168–169°, was obtained; total yield, 0.359 g (59.3%). After one recrystallization from ethanol, the pure compound had m.p. 169°, $[\alpha]_D^{19}$ −30.5° (*c* 2.56, chloroform); lit.[7], m.p. 168–169°, $[\alpha]_D^{24}$ −29.7° (chloroform).

β-Rutinose hepta-acetate was similarly prepared from compound **1** (1.0 g) in chloroform (15 ml) by treatment with zinc chloride (0.5 g) and dibromomethyl methyl ether (1.2 ml); a reaction period of 2 h generally gave the best results. However, as the highest yield of pure compound was only 32% by this procedure, the method employing dichloromethyl methyl ether is to be preferred.

DISCUSSION

The results presented in Table I reveal a significant difference in the rate of reaction of dibromomethyl methyl ether with the β-hepta-acetates of rutinose and neohesperidose. Whereas the former was completely converted into the α-bromide within 2 min, less than 50% of the latter had undergone conversion during this period, and a trace of unreacted acetate was still apparent after 5 min. Under similar conditions, but with the less-reactive dichloromethyl methyl ether, this difference in conversion rates was very much greater (Table III). Thus, conversion of β-rutinose hepta-acetate into the α-chloride was complete after 1 h, whereas conversion of β-neohesperidose hepta-acetate required a reaction period of 5 h.

Despite the high yields of *O*-acetylrutinosyl halides obtained from compound **1** by previous authors[1], the present results show that attack of the dihalomethyl methyl ethers at the link between the aglycone group and the disaccharide is by no means specific, nor is it in some cases even preferential (Table II). Both rutinose and neohesperidose acetates, after conversion into the α-bromides, undergo further attack at the glycosidic bond between the L-rhamnose and D-glucose residues, resulting in complete cleavage within 30 and 60 min respectively (Table I). The apparent, greater stability of this linkage in hexa-*O*-acetyl-α-rutinosyl bromide, formed from rutin acetate (**1**), indicated a protective effect due to the quercetin moiety. This was clearly demonstrated with β-neohesperidose hepta-acetate when the reaction was studied in the presence of equivalent amounts of penta-*O*-acetyl- (**3**) or penta-*O*-methyl-quercetin (**4**) (Table I). T.l.c. revealed that dibromomethyl methyl ether reacted preferentially with acetate **3**, converting it rapidly and quantitatively into a more

TABLE IV

REACTION OF FLAVONOID L-RHAMNOSYL-D-GLUCOSIDE ACETATES (0.04 MMOLE) WITH DICHLOROMETHYL METHYL ETHER (0.1 ML) IN CHLOROFORM (0.6 ML) IN THE PRESENCE OF ZINC CHLORIDE (40 MG)

Expt.	*Starting material*	*Chloride formed*	*Time (h)*		
			1	*3*	*6*
1	Diosmin octa-acetate (2)	Hexa-*O*-acetyl-α-rutinosyl	—	+	trace
		Tri-*O*-acetyl-α-L-rhamnopyranosyl	trace	++	++
2	Hesperidin octa-acetate (5)	Hexa-*O*-acetyl-α-rutinosyl	++	++	++
		Tri-*O*-acetyl-α-L-rhamnopyranosly	—	trace	trace
3	Naringin octa-acetate (6)	Hexa-*O*-acetyl-α-neohesperidosyl	trace	+	+
		Tri-*O*-acetyl-α-L-rhamnopyranosyl	—	trace	trace
Control	Rutin deca-acetate (1)	Hexa-*O*-acetyl-α-rutinosyl	+++	++++	++++
		Tri-*O*-acetyl-α-L-rhamnopyranosyl	—	trace	+

mobile product. Consequently, the neohesperidose acetate remained largely unchanged, even after 60 min. With ether **4**, conditions were established which more closely paralleled the rate of formation of *O*-acetylrutinosyl bromide from **1**; conversion of β-neohesperidose hepta-acetate into the α-bromide required an extension of reaction time from 5 to 60 min, and, as with **1**, only a small proportion of *O*-acetyl-L-rhamnosyl bromide was formed.

Substitution of dichloromethyl methyl ether for the dibromo analogue resulted in a marked, general decrease in reactivity, and no significant cleavage of disaccharide to monosaccharide was observed, even after 6 h. Increasing the concentration of the ether or zinc chloride alone had no significant effect on the rate of conversion of β-neohesperidose hepta-acetate into the α-chloride, although the catalytic effect of zinc chloride is clearly illustrated (Table III). However, doubling the concentration of both the ether and zinc chloride decreased the required reaction time by about 75% without causing any significant increase in the formation of *O*-acetyl-L-rhamnosyl chloride, except after 6 h. These modified conditions were subsequently adopted in experiments with flavonoid L-rhamnosyl-D-glucoside acetates (Table IV), as the rate of formation of *O*-acetylrutinosyl halide from **1** was similar to that observed under the experimental conditions with dibromomethyl methyl ether (Table II).

From Tables II and IV, it would appear that the use of dihalomethyl methyl ethers for the selective cleavage of oligosaccharides from flavonoid compounds is of limited applicability. This is not altogether unexpected if resistance to acid hydrolysis is taken as an index of the general stability of glycosidic bonds. Flavonol 3-glycosides, for instance, are cleaved under fairly mild conditions of acid hydrolysis, and the use of boiling, 10%, aqueous acetic acid has been described[8] as a preparative procedure for obtaining rutinose from rutin, indicating that the glycosidic link between L-rhamnose and D-glucose is more stable than that between D-glucose and quercetin in this compound. Many 7-glycosyloxyflavonoids, on the other hand, are hydrolyzed only under quite drastic conditions, and at 100°, hydrolysis of diosmin occurs only slowly, even in concentrated sulphuric acid[9]. Similar findings were obtained in the present study of the action of dihalomethyl methyl ethers on flavonoid 7-(L-rhamnosyl-D-glucosides). With both the dichloro- and dibromo-methyl methyl ethers, the tri-*O*-acetyl-α-L-rhamnopyranosyl halide was the main cleavage product from diosmin acetate (**2**), and insufficient disaccharide was liberated from hesperidin and naringin acetates (**5**, **6**) for adaptation on a preparative scale to be of much value.

With **1**, however, the findings of Bognár *et al.*[1] were confirmed. Cleavage of the oligosaccharide is best accomplished with dichloromethyl methyl ether, although, even with the dibromo analogue, β-rutinose hepta-acetate can be prepared in higher yield (32%) than by the acetic acid method[8] of Zemplén and Gerecs (net yield, 22%). In the present modified procedure, this compound was prepared directly from the intermediate *O*-acetyl-α-rutinosyl halide (not isolated) by reaction with mercuric acetate in acetic acid. It is of interest that mercuric acetate in benzene catalyzes the condensation of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide with 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl chloride to yield hexa-*O*-acetyl-α-rutinosyl chloride[7],

whereas, in acetic acid, it converts this product quantitatively at room temperature into the β-hepta-acetate.

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