cose (10 protons) + 4′-OCH_3 (s, 3.8) at 3.2–4.0; rhamnose -CH_3 at 0.9 (b).

Octaacetyldiosmin was precipitated from ethanol–water: m.p. 129–130°; n.m.r. aromatic protons: H-2′,6′ at 7.42–7.76 (m); H-5′,8 at 6.82–7.2, (m); H-6 at 6.62 (d, J_m = 2.5 c.p.s.); H-3 at 6.45 (s); rhamnoglucosyl and methoxyl protons: glucose H-1,2,3,4 and rhamnose H-1,2,3,4 at 4.65–5.50; 4′-OCH_3 and glucose H-5,6,6 and rhamnose H-5 at 3.50–4.30; and rhamnose -CH_3 at 1.14 (d, J = 6 c.p.s.); acetyl protons at 1.8–2.6.

Apigenin 7-Neohesperidoside (5).—The oxidation procedure described for the preparation of diosmin (4) was followed to convert naringin (2) into 5. The crude apigenin 7-neohesperidoside could be purified by repeated crystallizations from methanol without additional treatment. The pure sample melted at 198–200°: λ_{max}^{MeOH} 340 mμ (log ε 4.45) and 266 mμ (log ε 4.60).

Octa(trimethylsilyl)(apigenin 7-neohesperidoside) had the following n.m.r. spectrum: aromatic protons: H-2′,6′ at 7.70 (d, J_o = 8.5 c.p.s.); H-3′,5′ at 6.80 (d, J_o = 8.5 c.p.s.); H-8 at 6.72 (b); H-3,6 at 6.39 (b); rhamnoglucosyl protons: glucose H-1 at 5.12 (b); rhamnose H-1 at 4.89 (s); rhamnoglucose (10 protons) at 3.20–4.20; rhamnose -CH_3 at 1.20 (d, J = 6 c.p.s.).

Octaacetyl(apigenin 7-neohesperidoside) (m.p. 215–217°) had the following n.m.r. spectrum: aromatic protons: H-2′,6′ at 7.82 (d, J_o = 8.5 c.p.s.); H-3′,5′ at 7.18 (d, J_o = 8.5 c.p.s.); H-8 at 6.98 (d, J_m = 2 c.p.s.); H-6 at 6.66 (d, J_m = 2 c.p.s.); H-3 at 6.5 (s); rhamnoglucosyl protons: glucose H-1,3,4 and rhamnose H-1,2,3,4 at 4.80–5.55; glucose H-2,5,6,6 and rhamnose H-5 at 1.8–2.5; rhamnose -CH_3 at 1.2 (d, J = 6 c.p.s.); acetyl protons at 1.8–2.5.

Compounds 7, 8, and 9 were obtained following the procedure of Pacheco and Grouiller.[7]

Tamarixetin 7-Rutinoside (7).—Hesperidin (2 g.) afforded 1.3 g. of crude tamarixetin 7-rutinoside. After several recrystallizations from methanol, the pure sample melted at 247–249°: λ_{max}^{MeOH} 372 mμ (log ε 3.90), 270 (infl.), and 254 (log ε 4.00). It yielded the aglycone tamarixetin on acid hydrolysis, m.p. 255–257° (lit.[13] m.p. 259–260).

Nona(trimethylsilyl)(tamarixetin 7-rutinoside) had the following n.m.r. spectrum: aromatic protons: H-6′ at 7.70 (m); H-2′ at 7.60 (m); H-5′ at 6.86 (d, J_o = 8.5 c.p.s.); H-8 at 6.60 (d, J_m = 2.5 c.p.s.); H-6 at 6.28 (d, J_m = 2.5 c.p.s.); rhamnoglucosyl and methoxyl protons: glucose H-1 at 5.0 (b); rhamnose H-1 at 4.40 (s); rhamnoglucose (10 protons) at 3.20–4.00; 4′-OCH_3 at 3.88 (s); rhamnose -CH_3 at 0.92 (b).

Nonaacetyl(tamarixetin 7-rutinoside) had the following n.m.r. spectrum: aromatic protons: H-2′,6′ at 7.50–7.85 (m); H-5′,8 at 6.90–7.20 (m); H-6 at 6.70 (d, J_m = 2.5 c.p.s.); rhamnoglucosyl and methoxyl protons: glucose H-1,2,3,4 and rhamnose H-1,2,3,4 at 4.65–5.50; glucose H-5,6,6 and rhamnose H-5,4′-OCH_3 at 3.50–4.20; rhamnose -CH_3 at 1.15 (d, J = 6 c.p.s.); acetyl protons at 1.80–2.50.

Tamarixetin 7-Neohesperidoside (8).—Neohesperidin (0.2 g.) yielded 0.12 g. of 8: m.p. 266–269°; λ_{max}^{MeOH} 372 mμ (log ε 2.90), 270 (infl.), and 255 (4.02).

Non(trimethylsilyl)(tamarixetin 7-neohesperidoside) had the following n.m.r. spectrum: aromatic protons: H-2′ at 7.76 (m); H-6′ at 7.74 (m); H-5′ at 6.84 (d, J_o = 8.5 c.p.s.); H-8 at 6.74 (d, J_m = 2.5 c.p.s.); H-6 at 6.36 (d, J_m = 2.5 c.p.s.); rhamnoglucosyl and methoxyl protons: glucose H-1 at 5.18 (b); rhamnose H-1 at 4.92 (s); rhamnoglucose (10 protons) at 3.30–4.10; 4′-OCH_3 at 3.82 (s); rhamnose -CH_3 at 1.24 (d, J = 6 c.p.s.).

Nonaacetyl(tamarixetin 7-neohesperidoside) had the following n.m.r. spectrum: aromatic protons: H-2′,6′ at 7.50–7.82 (m); H-5′,8 at 6.90–7.18 (m); H-6 at 6.74 (d, J_m = 2.5 c.p.s.); rhamnoglucosyl and methoxyl protons: glucose H-1,3,4 and rhamnose H-1,2,3,4 at 4.80–5.60; glucose H-2,5,6,6 and rhamnose H-5,4′-OCH_3 at 3.70–4.40; rhamnose -CH_3 at 1.22 (d, J = 6 c.p.s.); acetyl protons at 1.85–2.50.

Kaempferol 7-Neohesperidoside (9).—Naringin (2 g.) yielded 1.4 g. of crude 9. After recrystallization from methanol, crystals were obtained which sintered at 205° and melted at 252–255°: λ_{max}^{MeOH} 368 mμ (log ε 4.18), 328 (infl.), 266 (4.17), and 252 (infl.).

Nona(trimethylsilyl)(kaempferol 7-neohesperidoside) had the following n.m.r. spectrum: aromatic protons: H-2′,6′ at 8.4 (d, J_o = 8.5 c.p.s.); H-3′,5′, 8 at 6.65–7.02 (m); H-6 at 6.4 (d, J_m = 2.0 c.p.s.); rhamnoglucosyl protons: glucose H-1 at 5.12 (b); rhamnose H-1 at 4.92 (s); rhamnoglucose (10 protons) at 3.25–4.15; rhamnose -CH_3 at 1.24 (d, J = 6 c.p.s.).

Nonaacetyl(kaempferol 7-neohesperidoside) (m.p. 144–145°) had the following n.m.r. spectrum: aromatic protons: H-2′,6′ at 7.84 (d, J_o = 8.5 c.p.s.); H-3′,5′ at 7.26 (d, J_o = 8.5 c.p.s.); H-8 at 7.06 (d, J_m = 2.5 c.p.s.); H-6 at 6.76 (d, J_m = 2.5 c.p.s.); rhamnoglucosyl protons: glucose H-1,3,4 and rhamnose H-1,2,3,4 at 4.80–5.60; glucose H-2,5,6,6 and rhamnose H-5 at 3.70–4.35; rhamnose -CH_3 at 1.22 (d, J = 6 c.p.s.); acetyl protons at 1.85–2.5.

Acknowledgment.—This investigation was supported by Grant F-130 from the Robert A. Welch Foundation and Grant GM-11111-03 from the National Institutes of Health. M. F. C. was an National Institutes of Health predoctoral trainee (Grant 5TI GM-789), 1962–1965.

(13) S. R. Gupta and T. R. Seshadri, *J. Chem. Soc.*, 3063 (1954).

Relative Rates of Hydrolysis of the Anomeric 1-O-Acetyltetra-O-methyl-D-glucopyranoses

MARTIN VAN DYKE,[1] S. G. SUNDERWIRTH, AND GESTUR JOHNSON[2]

Department of Chemistry, Colorado State University, Fort Collins, Colorado

Received May 14, 1965

Of the eight conformations (six boat and two chair) of the pyranose ring, the boat forms are generally rejected as being unlikely or energetically unstable.[3,4] The two chair conformations of the anomeric glucopyranoses are shown in Figure 1. The work of Reeves[5] has shown that for both anomers the favored conformation is that shown as C1. Using proton magnetic resonance Lemieux and co-workers[6] have shown that the anomeric pentaacetates of glucose, galactose, and mannose exist in the C1 conformation. The favored conformation (C1) of the β anomer has the hydroxyl group on carbon 1 in the equatorial position while the favored conformation (C1) of the α anomer has the hydroxyl on carbon 1 in the axial position. Since equatorial groups are sterically less hindered than axial groups, the more stable anomer would be the β anomer. This agrees with the well-known fact that an aqueous solution of glucose is composed of about 63% of the β anomer and about 37% of the α anomer.

On the same basis, the favored conformations of the anomeric 1-O-acetyltetra-O-methyl-D-glucopyranoses would be predicted to be the C1 conformation. The C1 conformation of the β anomer has the acetyl group in the equatorial position while the α anomer has the acetyl in the axial position in the C1 conformation. Examination of molecular models shows that the carbonyl of the equatorial acetyl group is more exposed to attack than the carbonyl in the axial position. On this basis it would be predicted that the β anomer would undergo alkaline hydrolysis faster than the α anomer. A study of the mechanism of ester hydrolysis

(1) Based upon a dissertation submitted in partial fulfillment of the requirements for a Ph.D. degree at Colorado State University.

(2) To whom all requests for reprints should be made.

(3) E. Gorin, W. Kauzmann, and J. Walter, *J. Chem. Phys.*, **7**, 327 (1939).

(4) O. Hassel and B. Ottar, *Acta Chem. Scand.*, **1**, 929 (1947).

(5) R. E. Reeves, *J. Am. Chem. Soc.*, **72**, 1499 (1950).

(6) R. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *ibid.*, **79**, 1005 (1957); **80**, 6098 (1958).

TABLE I
KINETIC DATA

Sample	$\beta:\alpha$	Time, sec.	Concn. of β, moles/l.	β/β_0	Concn. of α, moles/l.	α/α_0	r
A	31:69	0	0.000147		0.000331		
		300	0.000109	0.741	0.000297	0.897	2.77
		600	0.000082	0.557	0.000273	0.825	3.04
		900	0.000065	0.443	0.000252	0.761	2.98
		1200	0.000050	0.340	0.000213	0.644	2.45
		1800	0.000022	0.150	0.000140	0.423	2.21
B	78:22	0	0.000364		0.000109		
		300	0.000289	0.794	0.000099	0.912	2.50
		600	0.000231	0.635	0.000080	0.737	1.49
		1200	0.000151	0.415	0.000058	0.534	1.46
		3600	0.000037	0.103	0.000036	0.334	2.07
						Av.	2.39

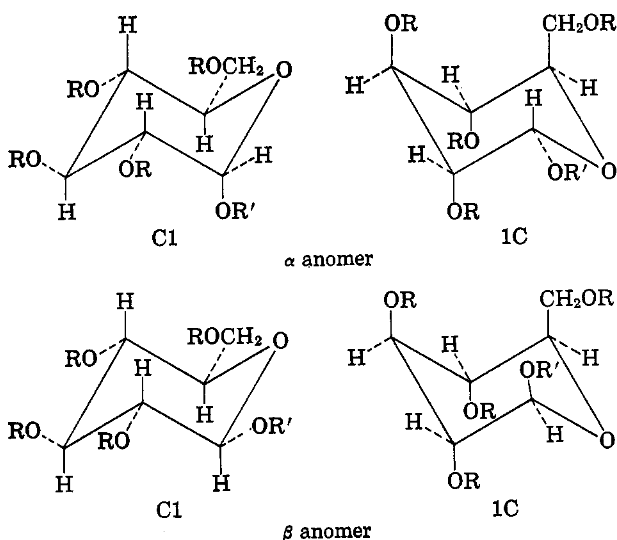

Figure 1.—Chair conformations of α- and β-D-glucopyranose and their derivatives: OR = OH, CH_3O; OR′ = OH, CH_3O, CH_3COO.

lends further evidence to support this prediction. The formation of the anion intermediate, which is the rate-determining step, involves a change from sp^2 to sp^3 hybridization. This change would have a lower free energy of activation in the case of the equatorial acetyl group than in the case of the axial acetyl group.

There is some experimental evidence to support the contention that equatorial acetyl groups hydrolyze faster than axial acetyl groups.[7] It is also known that in the saponification of esters the equatorial ester groups are removed more readily than the axial ester groups.[8]

Methyl-α-D-glucopyranoside was completely methylated using dimethyl sulfate and concentrated sodium hydroxide.[9] Subsequent hydrolysis of this methylated product yielded tetra-O-methyl-α-D-glucopyranose. The tetra-O-methyl-α-D-glucopyranose was then acetylated using acetic anhydride with either pyridine or sodium acetate as catalysts.

The acetylation of tetra-O-methyl-D-glucopyranose was first reported by Levene and Cortese[10] and then later by Wolfrom and Husted.[11] Neither paper reports which anomer was the major product. It is generally known that the product obtained by acetylating D-glucose depends upon the temperature and the nature of the catalyst as well as upon the anomeric form of the starting material.[12] In the work reported here the use of pyridine in the cold or sodium acetate with heat yielded a mixture of the two anomers. This was detected by g.l.p.c. using a DEGS column at 200°, which clearly resolved the acetylation product into two components. Using acetic anhydride and pyridine in the cold (5°), the α anomer was found to be the major product. When sodium acetate was used as a catalyst and the mixture was heated, the major product of the acetylation was the β anomer.

It was not possible to separate the anomers completely, although some enrichment was observed by fractional distillation using a Todd distillation apparatus. The hydrolysis studies were carried out on mixtures of the two anomers. Two samples, one rich in the α anomer and the other rich in the β anomer, were analyzed for carbon, hydrogen, methoxyl, and acetyl. The results of the analyses agreed closely to the values calculated from the formulas of the 1-O-acetyltetra-O-methyl-D-glucopyranoses.

Data obtained by optical rotation measurements on mixtures of the anomers permitted assignment of the two peaks obtained by g.l.p.c. to the α and β anomers, respectively. It was found that a typical mixture rich in the anomer eluted first from g.l.p.c. was always more dextrorotatory than a mixture which contained a lesser amount of the first anomer. Therefore, according to Hudson's rule,[13] the anomer eluted first from the g.l.p.c. is the α anomer.

The hydrolysis was carried out on the two mixtures mentioned above. The extent of hydrolysis was followed by analysis of the reaction mixture with g.l.p.c. The hydrolysis was carried out in base, aliquots were removed periodically, and the reaction was quenched with a slight excess of hydrochloric acid. These samples were extracted with chloroform and analyzed with the g.l.p.c. It was possible by this means to determine the amount of the β and α anomers present at any time (t).

(7) (a) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953); (b) A. Furst and P. A. Plattner, *Helv. Chim. Acta*, **32**, 275 (1949).
(8) M. S. Newman, "Steric Effects in Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1956, pp. 222, 223.
(9) E. S. West and R. F. Holden, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, pp. 800–803.
(10) P. A. Levene and F. Cortese, *J. Biol. Chem.*, **98**, 17 (1932).
(11) N. L. Wolfrom and D. R. Husted, *J. Am. Chem. Soc.*, **59**, 2559 (1937).
(12) T. Purdie and J. C. Irvine, *J. Chem. Soc*, **83**, 1021 (1903).
(13) C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).

Since the hydrolysis, assumed to be second order, is of the homocompetitive type,[14] the following equations may be employed to calculate the relative rate ratios.

$$-\frac{d[\beta]}{dt} = k_\beta[\beta][OH]$$

and

$$-\frac{d[\alpha]}{dt} = k_\alpha[\alpha][OH]$$

then

$$\frac{k_\beta}{k_\alpha} = \frac{\log \frac{[\beta]}{[\beta_0]}}{\log \frac{[\alpha]}{[\alpha_0]}} = r$$

r equals the ratio of the specific reaction rate constants. Table I shows the data obtained in typical hydrolyses of two mixtures of the anomers. Knowing the initial concentrations of the α and β anomers and knowing the concentration of these anomers at various times as the reaction proceeds makes it possible to calculate r. This value was found to be approximately 2.4. Therefore, the β anomer has a specific reaction rate constant approximately 2.4 times as great as that of the α anomer.

Experimental Section

Materials.—An Aerograph Model A600B Hy Fi gas chromatograph with hydrogen flame-ionization detector (Wilkens Instrument and Research, Inc., Walnut Creek, Calif.) was used for the analyses. A Leeds and Northrup Model H recorder with a Model 207 Disc Integrator was attached. Separation of the anomers was accomplished with a 20% diethylene glycol succinate column on 60–80-mesh Chromosorb W (10 ft. × 1/8 in. i.d.). The instrument was operated at a column temperature of 200°, injector temperature of 225°, nitrogen carrier gas flow rate of 25 cc./min., and a hydrogen gas flow rate of 20 cc./min.

Tetra-O-methyl-α-D-glucopyranose.—Methyl-α-D-glucopyranose (27 g., 0.139 mole, Eastman Practical P658) was methylated using dimethyl sulfate (Matheson Coleman and Bell, DX 1950) and concentrated sodium hydroxide (40%) following the procedure of West and Holden.[9] Two additional methylations using the same quantities of the reagents were carried out to ensure complete methylations. The product obtained was purified by distillation at 110–112° and 0.5 mm., yielding 21.7 g. of clear colorless syrup which gave only one peak when analyzed by g.l.p.c. on a DEGS column, indicating complete methylation. The methyl tetra-O-methyl-α-D-glucopyranoside was hydrolyzed with 2 N HCl for 9 hr. A steam distillation apparatus was used for the hydrolysis. Steam was passed into the distilling flask containing the methylated sugar and 400 ml. of the acid. The volume of the aqueous layer was kept constant by heating the flask externally as required. After 9 hr. the hydrolysis was complete as determined by the absence of all peaks in a g.l.p.c. analysis using the DEGS column. Norit was added to the hot hydrolysis solution. When cool, the solution was filtered, saturated with Na_2SO_4, and extracted with ten 100-ml. portions of $CHCl_3$. The combined chloroform extracts were dried over anhydrous Na_2SO_4, decolorized with Norit, and filtered. The $CHCl_3$ was removed under reduced pressure using a hot water bath. The product solidified on cooling. The yield of crude tetra-O-methyl-α-D-glucopyranose was 18.3 g. Repeated recrystallizations from petroleum ether (b.p. 30–60°) gave a melting point of 91–92° (lit.[9] m.p. 90–93°).

Anal. Calcd.: C, 50.8; H, 8.47; OCH_3, 52.5. Found: C, 51.42; H, 8.63; OCH_3, 49.31.

(14) (a) T. S. Lee and I. M. Kolthoff, *Ann. N. Y. Acad. Sci.*, **53**, 1093 (1951); (b) T. S. Lee in "Technique of Organic Chemistry," Vol. VIII, Interscience Publishers, Inc., New York, N. Y., 1953, pp. 100–108.

1-O-Acetyltetra-O-methyl-D-glucopyranose. Acetylation of Tetra-O-methyl-α-D-glucopyranose Using Acetic Anhydride and Pyridine.—Redistilled acetic anhydride (10 g., 0.098 mole, Eastman 99–100%) and 13.5 ml. of redistilled pyridine were cooled in a flask to 5°. To this solution 2 g. (0.008 mole) of tetramethylglucose was added with stirring until it completely dissolved. The flask was stoppered, put in a tightly closed jar, and kept at 5° for 2 days. The pyridine and the acetic anhydride were then removed under reduced pressure using a warm water bath. Toluene (10 ml.) was added and then removed under reduced pressure with a warm water bath. Toluene (10 ml.) was added a second time and again removed. The syrup obtained was dissolved in ethyl ether and transferred to a distilling flask. The ether was removed under reduced pressure and the remaining syrup was vacuum distilled. The fraction collected distilled at 128–130° at 3 mm. The yield was 1.87 g. of 1-O-acetyltetra-O-methyl-D-glucopyranose of which 65% was the α anomer and 35% the β anomer as determined with a DEGS column by g.l.p.c.

Acetylation of Tetra-O-methyl-α-D-glucopyranose Using Acetic Anhydride and Sodium Acetate.—Redistilled acetic anhydride (15 g., 0.147 mole) and 0.75 g. of fused sodium acetate were placed in a small flask and the mixture was brought to a boil. The heating was stopped and 2 g. (0.008 mole) of tetramethylglucose was added with stirring. The stirring was continued for a few minutes and the flask was then cooled under tap water to room temperature. To remove the volatile liquids, 15 ml. of toluene and 10 ml. of ethyl ether were added and the liquids were removed under reduced pressure. Ethyl ether (25 ml.) was added to the crude syrup remaining and the mixture was filtered to remove the sodium acetate. After removal of the ether under reduced pressure, the remaining syrup obtained was vacuum distilled; the fraction collected distilled at 130° and 3 mm. The yield of 1.8 g. was found to be approximately 40% α and 60% β 1-O-acetyltetra-O-methyl-D-glucopyranose. By using a Todd fractionation apparatus with a takeoff temperature of 135° and 3.5 mm. some enrichment in the α anomer was observed in the first fraction collected and some enrichment in the β anomer in the last fraction. Two samples of the following composition were prepared with the elemental analyses as indicated. *Anal.* Calcd.: C, 51.80; H, 7.90; methoxyl, 44.60; acetyl, 15.46. Found for sample A: C, 51.75; H, 8.01; methoxyl 44.74; acetyl, 15.48; $\alpha:\beta$ ratio, 69:31. Found for sample B: C, 51.52; H, 7.77; methoxyl, 44.84; acetyl, 15.39; $\alpha:\beta$ ratio, 22:78. The optical rotation for the α anomer was $[\alpha]^{25}D$ +165.4° (c 1.6, chloroform), for the β anomer $[\alpha]^{25}D$ −17.4° (c 1.6, chloroform).

Hydrolysis Procedures.—In the hydrolysis the two samples (A and B above) of different ratios of α to β were used. Two 0.132-g. samples of the same mixtures of anomers were each placed in a 500-ml. three-necked, round-bottom flask. Each flask was equipped with a mechanical stirrer in one opening and a thermometer in a second opening, the third opening being reserved for the removal of aliquots at periodic time intervals. The flasks were placed in an ice–water bath and cooled to 0°. An alcohol–base solution was prepared consisting of 13.7 ml. of 0.037 N NaOH and enough ethanol (95%) to bring the volume to the 100-ml. mark at 0°. A blank of 13.7 ml. of water and enough alcohol to bring the volume to the 100-ml. mark was also prepared. The alcohol–base solution was added to one of the 1-O-acetyl samples and the blank was added to the other. At timed intervals 10-ml. samples were taken from each with a pipet and quickly drained into erlenmeyer flasks each containing 10 ml. of water and 2 ml. of 0.047 N HCl. As rapidly as possible the quenched samples were extracted with four 10-ml. portions of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate, concentrated to a syrup, and stored at −20° until analyzed with a g.l.p.c. The syrups were dissolved in 100 μl. of 95% ethanol and 2 μl. of each solution was injected into the instrument.

Acknowledgments.—Part of this research was supported by a Research Grant to S. G. S. from the Faculty Improvement Committee of Colorado State University. M. V. D. gratefully acknowledges the financial support received from a National Science Foundation Fellowship.