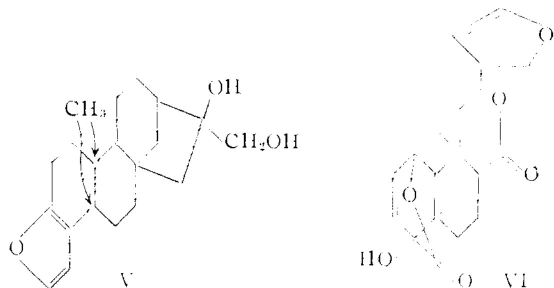
on the carbons of pyrrole are separated into two multiplets with centers at −70 and −54 c.p.s. (in carbon tetrachloride) indicating a distinction between α- and β-hydrogens. The α- and β-protons of thiophene, however, are not spread apart and only one complex multiplet (center at −96 c.p.s. in carbon tetrachloride) appears.



Part B. Application to Certain Natural Products.—Using proton magnetic resonance it took just a few minutes to confirm the nature of furan substitution in cafestol (V)[5b] from the appearance of α- and β-hydrogen resonances at −104 and −63 c.p.s. as doublets of equal intensity and each one-third as intense as the methyl group. The β-monosubstituted furan ring in columbin (VI)[14] was immediately apparent from proton resonances at −113 and −69 c.p.s. (signal ratio of 2:1) for two α- and one β-hydrogen, respectively. The chief bitter principle of citrus, limonin, $C_{26}H_{30}O_8$, whose structure has not yet been described in the literature, is also a β-monosubstituted furan as evidenced by proton resonance at −113 and −74 c.p.s. (signal ratio of 2:1). Such an assignment accounts for the unsaturation in limonin and one of the "ether" oxygens. The −113 and −74 c.p.s. bands are absent in the spectrum of tetrahydrolimonin, the saturated derivative, and those of several other derivatives in which the furan ring has been removed.[15] These data provide the most compelling evidence for the presence of a furan ring in limonin and, in addition, show clearly the type of nuclear substitution.[16]

In conclusion, it should be mentioned that although structural analysis of furans by n-m-r is remarkably straightforward, caution must always be exercised with regard to: (1) the effect of ring substituents, (2) possible interference from other protons attached to sp^2 hybridized carbon, especially in aromatic systems, and (3) applying corrections for solvent and bulk diamagnetic effects on observed shifts.[17]

(14) D. H. R. Barton and D. Elad, *J. Chem. Soc.*, 2085, 2090 (1956).

(15) We hope to publish complete details on these spectra together with an account of extensive structural studies on limonin in the near future.

(16) For a very recent publication on limonin chemistry see A. Malera, K. Schaffner, D. Arigoni and O. Jeger, *Helv. Chim. Acta*, **40**, 1420 (1957).

(17) A. A. Bothner-By and R. E. Glick, *J. Chem. Phys.*, **26**, 1647, 1651 (1957).

URBANA, ILLINOIS

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Steric Inhibition of Periodate Oxidation of Glycosides[1,2]

BY E. F. GARNER,[3] IRWIN J. GOLDSTEIN, R. MONTGOMERY AND F. SMITH

RECEIVED OCTOBER 19, 1957

Large groups have been shown to have a steric effect on the periodate oxidation of glycosides.

Although periodate oxidation is regularly used as a tool in analytical[4] and synthetic organic[5-7] chemistry, there seems to have been little work which demonstrates the primary site of attack of this reagent on an α,β,γ-triol grouping in a hexopyranoside. Reported herein are two instances in which the primary site of oxidation has been determined and which depends upon the directive influence of a bulky group.

The mechanism of periodate oxidation has been elucidated[8-10] and it appears to proceed through the formation of a five-membered heterocyclic ring involving the iodine atom and the glycol grouping. It is known that a *cis*-glycol group is attacked more readily than the *trans* modification[11-14] and that the rigidity imposed upon a system by bicyclic ring formation renders the *trans*-glycol group inert to attack by periodate.[15-17]

This paper is concerned with two compounds,

(1) Part of this paper is taken from a thesis submitted by E. F. Garner to the Graduate School of the University of Minnesota, in partial fulfillment of the requirements for the degree of Ph.D., 1956.

(2) Paper No. 3816 Scientific Journal Series, Minnesota Agricultural Experiment Station.

(3) Joseph Schlitz Brewing Co., Milwaukee, Wisc.

(4) E. L. Jackson, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 341; J. R. Dryer, "Methods of Biochemical Analysis," Vol. III, ed. by D. Glick, Interscience Pub. Inc., New York, N. Y., 1956, p. 111.

(5) J. C. Sowden, THIS JOURNAL, **72**, 808 (1950).

(6) J. C. Sowden, *ibid.*, **73**, 5496 (1951).

(7) I. J. Goldstein, J. K. Hamilton and F. Smith, *ibid.*, **79**, 1190 (1957).

(8) R. Criegee, *Sitzber. Ges. Beförder. ges. Naturw. Marburg*, **69**, 25 (1934); *C. A.*, **29**, 6820 (1935).

(9) R. Criegee, L. Kraft and B. Rank, *Ann.*, **507**, 159 (1933).

(10) C. C. Price and M. Knell, THIS JOURNAL, **64**, 552 (1942).

(11) C. C. Price and H. Kroll, *ibid.*, **60**, 2726 (1938).

(12) H. Klosterman and F. Smith, *ibid.*, **74**, 5336 (1952).

(13) P. F. Fleury, J. E. Courtois and A. Bieder, *Compt. rend.*, **233**, 1042 (1951).

(14) P. F. Fleury, J. E. Courtois and A. Bieder, *Bull. soc. chim. France*, 118 (1952).

(15) B. H. Alexander, R. J. Dimler and C. L. Mehltretter, THIS JOURNAL, **73**, 4658 (1951).

(16) R. J. Dimler, H. A. Davis and G. E. Hilbert, *ibid.*, **68**, 1377 (1946).

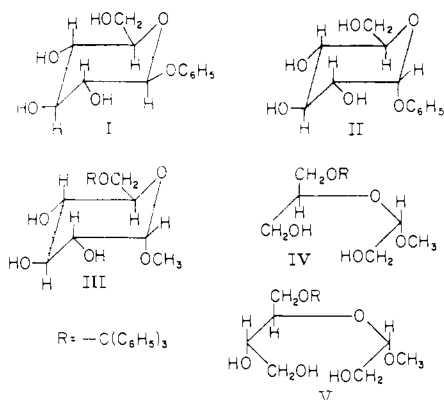
(17) F. Smith, *J. Chem. Soc.*, 633 (1944).

phenyl β-D-glucopyranoside and methyl 6-O-trityl-α-D-glucopyranoside, whose relatively bulky groups direct the primary attack on the sterically unhindered glycol grouping.

While treatment of phenyl α-D-glucopyranoside with 0.08 *N* periodic acid at 5° was complete in 22 hours, treatment of phenyl β-D-glucopyranoside with 0.14 *N* periodic acid at 5° resulted in the rapid consumption of 1 mole of oxidant per mole of glucoside in 2–4 hours but required 12 days before the second mole of periodate was consumed.

To determine the initial point of attack of the periodate upon phenyl β-D-glucopyranoside, the reaction was arrested by neutralizing the periodic acid with barium carbonate after 1 mole of periodic acid had been consumed. The oxidized product was reduced to the corresponding alcohol with sodium borohydride[18] and then hydrolyzed.

Chromatographic analysis revealed the presence of glycerol in addition to small amounts of glucose; no erythritol was detected. Also present in the hydrolyzate were D-glyceraldehyde and glycolic aldehyde. The glycerol, which was isolated and characterized as its crystalline tri-*p*-nitrobenzoate, is derived from carbon atoms 4, 5 and 6 of the glucoside. The D-glyceraldehyde, identified as its crystalline dimedone and 2,4-dinitrophenylhydrazine compound, is derived from carbon atoms 1, 2 and 3 of the cleaved glycoside, while the glycolic aldehyde, recognized as the bis-2,4-dinitrophenylhydrazone of glyoxal, arose from carbon atoms 1 and 2. There is thus little doubt that the oxidation proceeded by a primary attack between the hydroxyl groups at C_3 and C_4, for only in this manner can the presence of glycerol and the absence of erythritol be explained. That a small portion of the glycoside was unattacked while another small amount underwent complete oxidation is shown by the chromatographic detection of glucose and the isolation of glycolic aldehyde, respectively.



An interpretation of the course of this reaction may be deduced from conformational analysis. If the possible strainless ring conformations of phenyl α- and β-D-glucopyranosides are considered, excluding the six boat forms of Sachse[19] and Mohr[20] since they have been precluded on a physico-chemical basis,[21,22] it is seen that two possibilities for both the α- and β-anomers exist. Reeves has shown,[23] by extending the work of Hassel and Ottar[24] to the interaction of glycosides with cuprammonium solution, that both anomers of the phenyl D-glucosides will exist in the same form, the C_1 conformation. An inspection of the formulas of these two compounds shows that the only apparent difference in the two molecules resides at C_1. The hydroxyl and phenyl groups of the β-anomer I are all equatorial and the same is true for the α-anomer II with the exception that the phenyl group is axial. Because of this difference it is concluded that the phenyl group in the β-anomer is sufficiently close to the hydroxyl on C_2 to hinder the attack of periodate between C_2 and C_3, and consequently the molecule is attacked by periodate between C_3 and C_4.

In the second instance of this phenomenon of steric inhibition of periodate oxidation, the reaction of methyl 6-O-trityl-α-D-glucopyranoside with sodium periodate at room temperature in aqueous acetone did not proceed to completion. Thus even after 6 days, only 1.7 moles of periodate per mole of glucoside was consumed.

Reduction of the isolated product yielded a mixture of the two alcohols IV and V, which upon acid hydrolysis gave glycerol, identified as its crystalline tritosylate, and erythritol, characterized as its tetratosylate. Methylation studies conducted upon the mixture of the two alcohols (IV and V) gave rise to L-α-O-methylglyceritol and L-3,4-di-O-methylerythritol which were separated by column chromatography and characterized as their *p*-nitrobenzoates.

Consideration of the C_1 conformation[23] of methyl 6-O-trityl-α-D-glucopyranoside (III) shows that the hydroxyl group at C_4 is hindered by the bulky triphenylmethyl grouping at C_6 and hence the initial attack is confined largely to the bond between C_2 and C_3.

Experimental

Unless stated otherwise all evaporations were conducted *in vacuo* at 40–50°.

Oxidation of Phenyl β-D-Glucopyranoside with Periodic Acid.—To a solution of phenyl β-D-glucopyranoside dihydrate (0.7375 g., m.p. 174–175°, $[\alpha]^{21}D$ −72° in water (c 1))[25] in water (25 ml.) cooled to 5°, was added 0.4 *N* periodic acid (35 ml.) and the volume adjusted to 100 ml. with water at 5°. The oxidation reaction was conducted at 5°. Consumption of periodate per mole of glycoside determined by the standard procedure[26] was as follows: 1.04 moles (after 2 hr.), 1.13 (3 hr.), 1.46 (48 hr.), 1.65 (96 hr.), 1.79 (124 hr.), 1.86 (180 hr.), 1.94 (252 hr.), 2.01 (288 hr.).

After 288 hr. the reaction was arrested by adding a slight excess of dilute barium hydroxide and then solid carbon dioxide to remove the excess of the barium hydroxide. The dialdehyde in the filtered solution showed $[\alpha]^{26}D$ −161° based upon the theoretical yield.

Determination of the Primary Site of Oxidative Cleavage of Phenyl β-D-Glucopyranoside Dihydrate using Periodic Acid.—To an aqueous solution of phenyl β-D-glucopyranoside dihydrate (0.9220 g.) cooled to 5° was added 0.2 *N*

(18) M. Abdel-Akher, J. K. Hamilton and F. Smith, THIS JOURNAL, **73**, 4691 (1951).
(19) H. Sachse, *Z. physik. Chem.*, **10**, 203 (1892).
(20) E. Mohr, *J. prakt. Chem.*, [2] **98**, 315 (1918).
(21) A. Scattergood and E. Pacsu, THIS JOURNAL, **62**, 903 (1940).
(22) F. Goren, D. R. Kauzmann and W. R. Walter, *J. Chem. Phys.*, **7**, 327 (1939).
(23) R. E. Reeves, THIS JOURNAL, **72**, 1499 (1950).
(24) P. F. Hassel and F. Ottar, *Acta Chem. Scand.*, **1**, 929 (1947).
(25) B. Helferich and E. Schmitz-Hillebrecht, *Ber.*, **66**, 378 (1933).
(26) P. F. Fleury and J. Lange, *J. pharm. chim.*, **17**, 107, 196 (1933).

periodic acid (150 ml.) and the volume adjusted to 250 ml. with water at 5°. After keeping for 4 hr. in the dark, the periodate consumption had reached 0.98 mole per mole of glucoside. The reaction mixture was neutralized ($BaCO_3$) and filtered giving a solution (300 ml.) which showed $[\alpha]^{22}D$ −0.50° (2-dm. tube). Reduction was accomplished by adding sodium borohydride (0.15 g.) to the solution and allowing the reaction mixture to stand overnight when the rotation had become constant, $[\alpha]^{21}D$ −0.24° (2-dm. tube). The solution was neutralized with glacial acetic acid (tested with litmus) and evaporated to dryness. To the residue dry pyridine (150 ml.) and acetic anhydride (100 ml.) were added. After standing overnight at room temperature the mixture was poured into ice-water and the 2,3,4,6-tetra-*O*-acetyl derivative of the unchanged phenyl β-D-glucopyranoside (0.2 g., m.p. and mixed m.p. 125° after recrystallization from ethanol) was removed.

The aqueous filtrate was extracted with chloroform (3 × 100 ml.); the combined extracts were dried ($CaCl_2$) and evaporated to dryness. The resulting amber-colored liquid was deacetylated[27] by dissolving in absolute methanol and adding a catalytic amount of sodium. After standing overnight, evaporation of the methanol gave a light brown sirup (0.1458 g.) which showed $[\alpha]^{23}D$ −103° in water (*c* 0.4).

To a portion (0.37 g.) of the sirup in water (10 ml.) was added concentrated sulfuric acid (0.28 ml.) and the solution was refluxed for 1 hr. Neutralization ($BaCO_3$), filtration and evaporation gave an amber-colored solution. Paper chromatographic analysis using butan-1-ol:ethanol:water (5:1:4) as the irrigating solvent[28] and Tollens solution as the spray reagent revealed the presence of glucose and glycerol but no erythritol.

Glycolic aldehyde was identified as the bis-2,4-dinitrophenylhydrazone of glyoxal, m.p. and mixed m.p. 323–326°, by boiling the remaining 30 ml. of the aqueous solution (see above) in 1 *N* sulfuric acid for 1.5 hr., neutralizing ($BaCO_3$), distilling about one-half of the solution at atmospheric pressure and adding 2,4-dinitrophenylhydrazine to the distillate.

The remaining undistilled aqueous solution was treated with an alcoholic solution of dimedone and allowed to stand for 48 hr. at 5°. The dimedone derivative of D-glyceraldehyde showed $[\alpha]^{22}D$ +205° in ethanol (*c* 0.2), m.p. and mixed m.p. 198–204°, after recrystallization from 50% aqueous ethanol.

Authentic D-glyceraldehyde, prepared by the method of Fischer and Baer,[29] gave the dimedone derivative,[29] m.p. 199–204° and $[\alpha]^{24}D$ +208° in ethanol (*c* 0.2).

Identification of the Glycerol Moiety.—After separating the dimedone derivative of D-glyceraldehyde, the aqueous solution was evaporated to dryness and the residue extracted with cold ethanol. Ethanol was removed and the residual liquid was dissolved in anhydrous pyridine (5 ml.) and treated with *p*-nitrobenzoyl chloride (0.25 g.). The reaction mixture was heated at 60° for 30 min., cooled, and poured into saturated sodium bicarbonate solution to give glycerol tri-*p*-nitrobenzoate (0.12 g.), m.p. and mixed m.p. 196.5–198°.

Periodate Oxidation of Phenyl α-D-Glucopyranoside with Periodic Acid.—Phenyl α-D-glucopyranoside monohydrate (0.0473 g.) was treated with 0.08 *N* periodic acid (250 ml.) in the dark at 5° in the manner described above. The molar periodate consumption per mole of glucoside was as follows: 0.61 (after 2 hr.), 1.21 (4 hr.), 1.51 (9 hr.), 2.01 (15 hr.), 2.02 (22 hr.) (constant value). After 22 hr. the reaction mixture was neutralized ($BaCO_3$) and filtered to give a solution (300 ml.) which showed $[\alpha]^{21}D$ +158°, based upon the theoretical yield of the dialdehyde.

Periodate Oxidation of Methyl 6-*O*-Trityl-α-D-glucopyranoside.—The oxidation of methyl 6-*O*-trityl-α-D-glucopyranoside with sodium periodate and reduction of the oxidation product with sodium borohydride were performed as previously described[7] to give a mixture of the two dihydric alcohols IV and V.

Identification and Characterization of Glycerol and Erythritol Obtained by Detritylation and Hydrolysis of the Reduced Dialdehydes.—Detritylation and hydrolysis of the mixture of the dihydric alcohols IV and V (550 mg.) was accomplished by dissolving the latter in 0.57 *N* ethereal hydrogen chloride (5 ml.). After standing in a refrigerator for 1.5 hr., the reaction mixture was allowed to come to room temperature and then poured into water (25 ml.). The ethereal layer was separated after agitation and re-extracted with water (40 ml.). The combined aqueous acidic extracts were boiled for 5 hr. Neutralization ($PbCO_3$), filtration and concentration gave a sirup which was freed from inorganic salts by extraction with ethanol. Paper chromatographic analysis of the colorless sirup (152 mg.) revealed the presence of two components, glycerol and erythritol. These were separated by means of sheet paper chromatography (Whatman No. 1) using butan-1-ol:ethanol:water (5:1:4)[28] as the irrigating solvent.

Glycerol (61.3 mg.) was identified as its crystalline tritosyl derivative, m.p. and mixed m.p. 102°, while the erythritol (23.1 mg.) was obtained crystalline, m.p. 118°, and characterized as its tetratosylate, m.p. and mixed m.p. 165°.

Methylation studies on the reduced dialdehyde from III together with the isolation and characterization of L-3,4-di-*O*-methylerythritol are described elsewhere.[7]

Acknowledgment.—The authors thank the Office of Ordnance Research, U. S. Army, for financial support (Contract No. DA-11-022-ORD-999) which helped to defray the expenses of this work.

ST. PAUL, MINNESOTA

(27) G. Zemplén, *Ber.*, **59**, 1254 (1926).

(28) S. M. Partridge and R. G. Westall, *Biochem. J.*, **42**, 238 (1948).

(29) H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, **17**, 622 (1934).