[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

An Evaluation of Hudson's Classical Studies on the Configuration of Sucrose

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The α-configuration of the D-glucopyranose moiety in sucrose has been confirmed by the application of the Hudson's isorotation rules. Evidence is presented that the hydrolysis of sucrose by invertase proceeds without Walden inversion.

The assignment of the α-configuration to the D-glucopyranose component of sucrose was made on the basis of the classical studies of Hudson[1] on the inversion of sucrose with massive amounts of invertase. Under the conditions employed, the rate of inversion was near its maximum and the rate of mutarotation of the liberated D-glucose was near its minimum, while the D-fructose component equilibrated rapidly but measurably. Thus it was demonstrated that prior to mutarotation (downward), the specific rotation of the initially liberated D-glucose, $[\alpha]^0_D+110 \pm 2°$, was identical with the optical activity of the form, now known as α-D-glucopyranose, $[\alpha]_D+109°$. These experiments and the subsequent work of Bailey and Hopkins[2] also indicated that before mutarotation, the process of enzymic hydrolysis is not accompanied by an appreciable change in the optical activity and the initial rotation of the newly produced D-fructose (mutarotating in the levo direction) must be $[\alpha]_D+15$ to $+17°$. On the implicit assumption that enzymic hydrolysis of a glycosidic bond is not accompanied by Walden inversion,[3] the former datum has constituted the main proof for the α configuration of the D-glucose moiety in sucrose. The β-D configuration of the fructofuranose component has been established by the observation that only β-D-fructofuranosides are hydrolyzed by invertase.[4]

In isosucrose the reverse configurations are indicated by the action of a β-D-glucosidase from *Aspergillus niger*[5] and the negative evidence that it is not hydrolyzed by invertase.[5] The above experiments on the configuration of sucrose and isosucrose have been reviewed by Levi and Purves.[6]

Recently it has been noted that the enzymic cleavage of a glycosidic bond may be accompanied by Walden inversion.[7-9] A well known example is provided by the action of β-amylase on amylose with the liberation β-maltose.[10] Thus the experiments of Hudson do not constitute an unequivocal proof for the α configuration of the D-glucose component of sucrose. In this communication evidence will be presented that the action of invertase on sucrose is not accompanied by Walden inversion and the α-configuration of the D-glucopyranose will be confirmed through the application of Hudson's isorotation rules.[11] An application of these rules to sucrose has been made by Klages and Niemann.[12] However, at that time the required D-fructofuranoside derivatives were not characterized and the conclusions attained were based upon optical rotations, in part assumed, which were grossly in error.

According to the isorotation rules, the molecular rotation of the theoretically possible sucrose isomers can be formulated as follows.

$$[M]_{\alpha,\alpha} = G + A + F + B$$
$$[M]_{\alpha,\beta} = G + A + F - B$$
$$[M]_{\beta,\alpha} = G - A + F + B$$
$$[M]_{\beta,\beta} = G - A + F - B$$

In these equations G and F represent the molecular rotation of D-glycopyranose and D-fructofuranose residues and A and B represent the contribution of the corresponding glycosidic centers. The approximate value of these factors can be calculated from the molecular rotations of the methyl D-glucopyranosides and methyl D-fructofuranosides listed in Table I.

In Table II the rotations of sucrose and isosucrose have been compared with the calculated rotations of the four possible isomers. In this table the extreme rotations of the α,α and β,β isomers are in gross disagreement with those of sucrose and isosucrose, its one known isomer. Thus sucrose, having an established β-D-fructofuranosyl component, must

(1) Hudson, *J. Am. Chem. Soc.*, **30**, 1160, 1564 (1908); **31**, 655 (1909); see also Isbell and Pigman, *J. Research Natl. Bur. Standards*, **20**, 773 (1933).

(2) Bailey and Hopkins, *Biochem. J.* (London), **27**, 1957 (1933).

(3) Armstrong, *J. Chem. Soc.*, **83**, 1305 (1903).

(4) Purves and Hudson, *J. Am. Chem. Soc.*, **59**, 49 (1937).

(5) Georg, *Helv. Chim. Acta*, **17**, 1566 (1934); see also Adams, Richtmyer and Hudson, *J. Am. Chem. Soc.*, **65**, 1369 (1943).

(6) Levi and Purves, *Advances in Carbohydrate Chem.*, **4**, 1 (1949).

(7) Koshland, *Biol. Revs. Cambridge Phil. Soc.*, **28**, 416 (1953); *Chem. Abstr.*, **48**, 2134 (1954).

(8) Bunton, Lewis, Llewellyn, Tristram and Vernon, *Nature*, **174**, 560 (1954).

(9) Fitting and Doudoroff, *J. Biol. Chem.*, **199**, 153 (1952).

(10) Kuhn, *Ann.*, **443**, 1 (1925).

(11) Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909); **38**, 1566 (1916).

(12) Klages and Niemann, *Ann.*, **529**, 185 (1937).

TABLE I

OPTICAL ROTATIONS OF METHYL D-GLUCOPYRANOSIDES AND METHYL D-FRUCTOFURANOSIDES

Glycoside	Rotation of the free glycoside in H_2O $[\alpha]_D$	Ref.	$[M]_D$	Rotation of the tetraacetate in $CHCl_3$ $[\alpha]_D$	Ref.	$[M]_D$
Methyl α-D-glucopyranoside	+157.9°		+30,600°	+130.6°		+47,300°
Methyl β-D-glucopyranoside	− 32.5		− 6,300	− 18.3		− 6,600
Methyl α-D-fructofuranoside	+ 93	(4, 13, 14)	+18,000	+ 88.1	(4)	+31,900
Methyl β-D-fructofuranoside[a]	− 47	(13–15)	− 9,100	− 26[b]	(15)	− 9,400

[a] Methyl β-D-fructofuranoside and its tetraacetate are not, as yet, known in crystalline form. The others are crystalline. [b] Methanolic solution.

TABLE II

OPTICAL ROTATION OF SUCROSE AND ITS ISOMERS

Sucrose isomers	Rotation of the sugar in H_2O $[\alpha]_D$	Ref.	$[M]_D$	Rotation of the octaacetate in $CHCl_3$ $[\alpha]_D$	Ref.	$[M]_D$
α,α	+142°[a]		+48,600°	+117°[a]		+79,200°
α,β	+ 63[a]		+21,500	+ 56[a]		+37,900
β,α	+ 34[a]		+11,700	+ 37[a]		+25,300
β,β	− 45[a]		−15,400	− 24[a]		−16,000
Sucrose	+ 66.5[b]		+22,700	+ 60[b]		+40,700
Isosucrose	+ 34[b]	(16)	+11,600	+ 20[b]	(16)	+13,600

[a] Calc'd values; (30,600 + 18,000 = 48,600) ÷ 342 = 142. [b] Exptl. values.

be the α,β isomer and conversely, the isosucrose, which is known to have a β-D-glucopyranosyl entity, should be the β,α form. The α,β configuration of sucrose is in agreement with the data obtained from the x-ray analysis of crystalline sucrose sodium bromide dihydrate.[17]

If we accept the glycosidic configurations of sucrose and isosucrose as α,β and β,α, respectively, then it may be calculated that the contribution of the D-fructofuranose residue (F) to the molecular rotation of the corresponding methyl glycosides will be approximately +5000.

$$[M]_{\alpha,\beta} = G + A + F - B = 22{,}700$$
$$[M]_{\beta,\alpha} = G - A + F + B = 11{,}600$$
$$2G \quad + 2F \quad = 34{,}300$$

$$G + A = 30{,}600$$
$$G - A = -6{,}300$$
$$2G \quad = 24{,}300$$

$$2F = 34{,}300 - 24{,}300 = 10{,}000$$
$$F = 5{,}000$$

Then, since $F + B = 18{,}000$

$$B = 18{,}000 - 5{,}000 = 13{,}000$$
$$F - B = 5{,}000 - 13{,}000 = -8{,}000$$

and

$$-8{,}000 \div 194 = -41°.$$

This calculated value, −41°, for the specific rotation (λ 5892.5 Å., 20°, water) of the as yet uncrystallized methyl β-D-fructofuranose, compares favorably with the $[\alpha]_D$ −50°[13,15] and −47°[14] obtained for the sirupy but chromatographically purified[14] substance.

A further implication of this calculation is that the initially liberated D-fructofuranose, $[\alpha]_D$ +15° to +17°, produced by the enzymic inversion of sucrose, is very probably the β-D anomer.

The conclusion that the enzymic cleavage of sucrose by invertase is not accompanied by Walden inversion, is justified by the above considerations and by the experimental fact that the process of hydrolysis before mutarotation of the products is not accompanied by an appreciable change in the optical rotation.

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(13) Purves and Hudson, *J. Am. Chem. Soc.*, **56**, 702, 708 (1934).

(14) Augestad, Berner, and Weigner, *Chemistry & Industry*, 376 (1953); Augestad and Berner, *Acta Chem. Scand.*, **8**, 251 (1954).

(15) Schlubach and Bartels, *Ann.*, **541**, 76 (1939).

(16) Irvine, Oldham, and Skinner, *J. Am. Chem. Soc.*, **51**, 1279 (1929).

(17) Beevers and Cochran, *Proc. Roy. Soc.* (London), **A 190**, 257 (1947).