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THE STRUCTURE AND CONFIGURATION OF SUCROSE

(α-D-Glucopyranosyl-β-D-Fructofuranoside)

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I. Introduction

Sucrose is by far the most widespread, frequently the most abundant, sugar present in the sap of plants, and has been isolated, often in crude crystalline form, from the richer saps since the dawn of history.[1,2] Sugar cane has been a major commercial source for centuries, but the rise of the sugar beet industry to a comparable status commenced in 1797, when Achard announced that sugar could be extracted from beets on a commercial scale.[3] For many decades cane sugar and, more recently, beet sugar have been the outstanding examples of a very cheap, highly crystalline, organic compound produced in a state of unexcelled purity on a scale of tens of millions of tons a year. When this fact is considered,

(1) A. Chapman and V. W. Chapman, Article on Sugar in "Encyclopedia Britannica," Cambridge, Univ. Press, 11th ed., Vol. 26, p. 32 (1911).

(2) E. O. Von Lippmann, "Die Chemie der Zuckerarten," Vieweg and Sohn, Braunschweig, 2nd ed., pp. 588–596 (1895). (A list of species examined up to 1895, usually by extracting the plant with aqueous alcohol and precipitating any sucrose from the extract as the difficultly soluble strontium saccharate.)

(3) G. Fairrie, "Sugar," Clay and Sons, Bungay, England, 1st ed., p. 24 (1925).

it is interesting to note that the melting point of pure sucrose, given as 180° in 1838 by Peligot,[4] and as 160–161° by Berzelius[5] in 1839, was definitely established only in the last twelve years. Bourne[6] considered that the proper value was 185–186°, and not 160°, as commonly quoted in textbooks. A later opinion that the melting point varied with the rate of heating[7] was invalidated in 1936, when it was found[8] that most previous determinations were lower than the correct value of 188° because minute traces of solvent or impurities brought about partial decomposition during the determination. Such was probably the cause of the difference between the "saccharose A," melting point 171°, and the "saccharose B," melting point 185°, described by Pictet[9] as being prepared by recrystallization from methanol and ethanol, respectively. On the other hand, Pictet's claim that sucrose octaacetate is polymorphous has been supported by other workers,[10–14] who have shown that a more labile form of melting point 69° changes on contact with water, ether and other liquids into the more stable allotrope of melting point 89°, which has a different crystal structure but the same specific rotation in solution.

Hassid, Doudoroff and Barker[15] recently synthesized sucrose, but this outstanding success was obtained by biochemical methods that threw little fresh light upon the constitution of the sugar. A direct proof of structure by an unambiguous chemical synthesis is still lacking. It is the purpose of this review to show that, in spite of this lack, other lines of evidence are concordant enough, and strong enough, to reveal the structure in a convincing way. The scientific literature concerning sucrose is enormous, for the sugar has served for a century as a convenient substrate for very many researches in enzyme, biological and

(4) E. Peligot, *Ann. chim. phys.*, **67**, 113 (1838); *J. prakt. Chem.*, **15**, 65 (1838); a comprehensive review of sugar chemistry.

(5) J. Berzelius, *Poggendorff's Ann.*, **47**, 289 (1839).

(6) B. Bourne, *Chem. News*, **110**, 47 (1914).

(7) K. Sandera and A. Mircev, *Z. Zuckerind. čechoslovak. Rep.*, **59**, 204 (1935); *Chem. Abstracts*, **29**, 2011, 4615 (1935).

(8) S. V. Shah and Y. M. Chakradeo, *Current Sci.*, **4**, 652 (1936); *Chem. Abstracts*, **30**, 4710 (1936).

(9) A. Pictet, *Helv. Chim. Acta*, **13**, 698 (1930).

(10) L. Duparc and R. Galopin, *Helv. Chim. Acta*. **13**, 702 (1930).

(11) M. Frèrejacque, *Compt. rend.* **503**, 731 (1936).

(12) K. Sandera, *Chem. Listy*, **33**, 139 (1939); *Chem. Abstracts*, **33**, 6252 (1939). This article quotes earlier melting points and specific rotations.

(13) R. P. Linstead, A. Rutenberg, W. G. Dauben and W. L. Evans, *J. Am. Chem. Soc.*, **62**, 3260 (1940).

(14) C. D. West, *J. Am. Chem. Soc.*, **63**, 630 (1941).

(15) W. Z. Hassid, M. Doudoroff and H. A. Barker, *J. Am. Chem. Soc.*, **66**, 1416 (1944); *Science*, **100**, 51 (1944).

physical chemistry. A massive literature concerns the details of its physical properties and its large-scale production. All these subjects are omitted from this review, except in so far as they contribute information concerning the chemical structure and configuration of the sugar.

II. Earlier Observations on Structure

1. Determination of the Sucrose–Invert Sugar Relationship

Precise knowledge of the chemistry of sucrose and glucose began with the establishment of their elementary composition by Lavoisier, Gay-Lussac, Thenard, Dumas, Crum, de Saussure and others. The analyses by Prout[16] were particularly interesting although not exact. Prout showed that combustion of sucrose and glucose in oxygen produced gases equal in volume to the oxygen consumed. Knowing that the combustion of the carbon to carbon dioxide caused no change in the gas volume, he inferred that the two sugars contained oxygen and hydrogen in the ratio characteristic of water. Reviews by Liebig,[17] Peligot[4] and Berzelius[5] summarized the analyses and assigned compositions to sucrose and glucose similar to those accepted today. At that time the equivalent weights of sugars were estimated by a method originated in 1815 by Berzelius, who analyzed the saccharates formed with inorganic hydroxides such as those of lead, the alkaline earths and the alkali metals, or the crystalline double compounds of sodium chloride with sucrose and glucose. The results obtained for sucrose, principally by Peligot[4] and Soubeiran,[18] suggested formulas based on C_{12} units or on even, integral multiples thereof, the exact formula depending on the atomic weights accepted for carbon and oxygen and on the constitution assumed for the saccharates. Such formulas fitted views then current concerning the fermentation of sucrose, which had been studied quantitatively by Gay-Lussac[19] and was known to produce 51.3 and 53.7 parts by weight of carbon dioxide and ethanol, respectively. One mole of sucrose had also been shown to utilize one mole of water during the fermentation.[20] According to Liebig,[17] alcohol ($C_4H_{12}O_2$) was the hydrate of diethyl ether ($C_4H_{10}O$). Sucrose ($C_{12}H_{22}O_{11}$) was a compound of four "atoms" of carbon dioxide, two "atoms" of ether and one "atom" of water of crystallization, the latter being replaceable by bases like lead oxide. The formula of glucose monohydrate was written $C_{12}H_{28}O_{14}$ to show it as a more hydrated form of sucrose.

(16) W. Prout, *Phil. Trans.*, **117**, 355 (1827).
(17) J. Liebig, *Poggendorff's Ann.*, **31**, 321 (1834).
(18) E. Soubeiran, *Ann.*, **43, 223** (1842).
(19) L. J. Gay-Lussac, *Ann. chim. phys.*, [1] **76**, 245 (1810).
(20) J. B. Dumas and P. Boullay, fils, *Ann. chim. phys.*, [2] **37**, 45 (1828).

It was known at an early date that grape sugar, the sugar isolated from diabetic urine[4,21] and the sugar from the acid hydrolysis of starch were identical. The identity, however, was often and more or less tacitly extended to include as "glucose" uncrystallized sirups with the same elementary composition and general properties.[22] This confusing tendency was repeatedly deprecated by Biot,[23] who knew that crystalline glucose rotated plane polarized light to the right, whereas the "uncrystallized glucose" (invert sugar) derived from sucrose by acid hydrolysis was levorotatory.[24] Moreover, Peligot[4] pointed out that crystalline glucose gave no color with concentrated hydrochloric acid, but that sucrose, both before and after acid hydrolysis, did so. The same author was aware that the residue from an incomplete fermentation of sucrose was levorotatory, Persoz being credited with the initial observation both by Berthelot[25] and by Pasteur.[26]

The full significance of these observations was brought out in the period 1846 to 1856 by Dubrunfaut,[27] who established or confirmed the following facts by direct experiment. Pure sucrose, when carefully hydrolyzed by weak acids, yielded 105 parts of invert sugar, which was weighed after drying at 100° *in vacuo*. Contrary to the current opinion, invert sugar was not transformed to glucose by crystallization; although a certain proportion of well-characterized glucose might separate, the mother sirup became more levorotatory and, therefore, had to contain a different sugar. The same inference was drawn from the fact that, when invert sugar was fermented by yeast, and particularly in a lactic acid fermentation, the glucose was consumed preferentially, and strongly levorotatory sirups were obtained by interrupting the fermentations. When milk of lime was added to a 10% aqueous solution of invert sugar and the resulting, sparingly soluble, crystalline basic compound was recovered and decomposed with oxalic acid, the new constituent was isolated as a practically pure sirup. This sirup, carefully dried, had the same elementary composition as invert sugar or glucose and yielded the same amounts of alcohol and carbon dioxide when fermented by yeast. The specific rotatory power, about four times more levorotatory than that of invert sugar, confirmed the identity of the new sugar (which he simply

(21) L. Pasteur, *Ann. chim. phys.*, [3] **31**, 67 (1851).

(22) For example, L. J. Thenard, L. J. Gay-Lussac, J. B. Biot and J. B. Dumas Report to the French Academy of Sciences, *Compt. rend.*, **7**, 106 (1838).

(23) For example, J. B. Biot, *Compt. rend.*, **15**, 619, 693 (1842).

(24) J. B. Biot, *Compt. rend.* **17**, 755 (1843).

(25) M. Berthelot, *Compt. rend.*, **50**, 980 (1860).

(26) L. Pasteur, *Ann. chim. phys.*, [3] **58**, 323 (1860); an extensive literature review is included.

(27) A.-P. Dubrunfaut, *Compt. rend.*, **25**, 307 (1847); **29**, 51 (1849); **42**, 901 (1856).

termed "sucre liquide") with that obtained by hydrolyzing the polysaccharide inulin.   Moreover, the magnitude of the levorotation decreased markedly with increase of temperature and at twice the rate displayed by the rotation of invert sugar.   The composition, optical rotation and rotation change with temperature peculiar to the latter were duplicated by a synthetic mixture of equal parts of crystalline glucose and the new levorotatory amorphous sugar, his "sucre liquide."   This sugar, the discovery of which is one of the great services that Dubrunfaut rendered to science, later received the name "levulose" from Berthelot and eventually the present scientific name "D-fructose" from Fischer.[28] Dubrunfaut summarized his results in the equation:

$$2\,C_{12}H_{11}O_{11} + 2\,HO \rightarrow \quad C_{12}H_{12}O_{12} \quad + \quad C_{12}H_{12}O_{12}$$

Sucre de canne	Glucose	Sucre liquide
$[\alpha]_j = +73.8°$	$[\alpha]_j = +53.2°$	$[\alpha]_j = -106°$
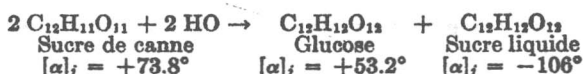
in which the atomic weights of carbon and oxygen were taken as 6 and 8, respectively.

Modern atomic weights gained acceptance in the 1860's.[29]   About twenty years later Raoult[30] determined the molecular weight of sucrose as 342, and that of invert sugar as 180, by noting the freezing points of their dilute aqueous solutions.   Kiliani,[31] employing the cyanohydrin synthesis, then showed conclusively that D-glucose was a six-carbon aldose, a result that was quickly supported by the new physical method.[32,33] Levulose, or D-fructose, known for years as the "uncrystallizable sugar," was finally crystallized by Jungfleisch and Lefranc,[34] whose success made it easy to confirm the elementary composition of the sugar,[27,35-37] determine its molecular weight[32] and recognize it as a six-carbon 2-ketose.[38] By 1890, Dubrunfaut's fundamental equation for the cleavage of sucrose became generally accepted in the familiar form:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose	D-Glucose	D-Fructose
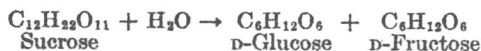
(28) E. Fischer, *Ber.*, **23**, 930 (1890).

(29) C. Graebe, "Geschichte der organischen Chemie," J. Springer, Berlin, pp. 223–33 (1920).

(30) F. M. Raoult, *Compt. rend.*, **94**, 1517 (1882).

(31) H. Kiliani, *Ber.*, **19**, 1128 (1886).

(32) H. T. Brown and G. H. Morris, *Chem. News*, **57**, 196 (1888).

(33) B. Tollens and F. Mayer, *Ber.*, **21**, 1566 (1888).

(34) E. Jungfleisch and E. Lefranc, *Compt. rend.*, **93**, 547 (1881).

(35) A. Herzfeld, *Ann.*, **244**, 274 (1888); a review of the literature is included.

(36) H. Winter, *Ann.*, **244**, 295 (1888).

(37) M. Hönig and S. Schubert, *Monatsh.*, **8**, 529 (1887).

(38) H. Kiliani, *Ber.*, **19**, 221 (1886).

2. *Suggested Constitutional Formulas*

The expansion of these molecular formulas into satisfactory constitutional formulas was intimately linked with the growth of knowledge concerning the optical mutarotation of crystalline D-glucose freshly dissolved in water. Dubrunfaut[39] was apparently the first to record that the initial specific rotation of D-glucose monohydrate in water was nearly twice the final value, which was reached only after the solution had stood for some hours or had been heated. Dubrunfaut made the observation while developing a polarimetric method for estimating sucrose and invert sugar. A few years later, Pasteur[40] observed in detail the change with time shown by the rotation of a 10% solution of the crystalline D-glucose-sodium chloride compound. According to reviews by Brown and Pickering[41] and by Von Lippmann,[42] such mutarotations were attributed to the establishment of equilibria in solution between a colloidal crystal aggregate and a molecularly dispersed state, or between a crystalline and an amorphous form, a hydrated and a solvated form, or to the formation and hydrolysis of an ethylene oxide ring. The speculation which eventually proved correct was made by Tollens,[43] who considered that the mutarotation of D-glucose and D-fructose, as well as some abnormal properties of their carbonyl groups, was to be explained by their existence as unstable cyclic hemiacetals. By analogy, Tollens assigned the mixed cyclic acetal structure I to the nonreducing sucrose molecule, although the exact positions of the acetal rings were left undecided. Such a formula agreed with the existence of a presumed

I
Sucrose (Tollens, 1883)

II
Sucrose (Fischer, 1893)
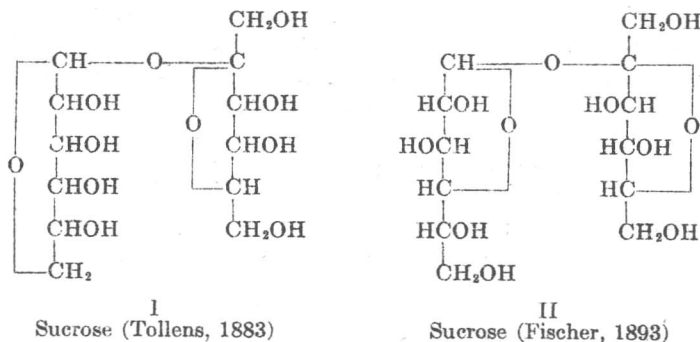
(39) A.-P. Dubrunfaut, *Compt. rend.*, **23**, 38 (1846); *Ann. chim. phys.*, [3] **18**, 99 (1846).

(40) Ref. 21, p. 95. Although Pasteur did not realize it at that early time, all of his observations except the first fit a first-order equation quite well.

(41) H. T. Brown and S. U. Pickering, *J. Chem. Soc.*, **71**, 756 (1897).

(42) Ref. 2, pp. 125–30.

(43) B. Tollens, *Ber.*, **16**, 921 (1883).

octaacetate[44] and a presumed octanitrate[45-48] of sucrose, although these fully substituted derivatives were not crystallized and reliably analyzed until 1887[49] and 1919,[50] respectively. A recent review by Hudson[51] commemorates Emil Fischer's classic work that led to the assignment of stereochemical configurations to D-glucose and D-fructose. These configurations are included in the Fischer formula II for sucrose, together with his suggestion that the cyclic structure was five membered in both halves of the molecule. This suggestion was based in part upon Baeyer's strain theory,[52] which stated that five-membered rings formed most readily, and in part upon a previous assumption that the sugar lactones contained rings of that size.[53]

Consideration of a structure such as II shows that both the D-glucoside and D-fructoside portions may have an alpha or a beta configuration about the glycosidic carbon atoms and that four isomers are therefore possible. The line of thought that eventually identified sucrose with the proper isomer commenced about 1830, when Dubrunfaut, as stated by Pasteur,[26] observed that the first action of aqueous yeast suspensions was to invert the sugar. Sucrose was fermented more slowly than D-glucose.[54] Berthelot[25] later succeeded in separating the inverting from the fermenting action by extracting the macerated yeast with water. Alcohol when mixed with the extract precipitated a yellow nitrogenous mass that retained the capacity to invert sucrose and that resembled protein in being coagulated by heat or nitric acid. O'Sullivan and Tompson[55] reviewed subsequent improvements up to 1890 in the preparation of the active agent, "invertin,"[56] or invertase,[55] and have also to be credited with an admirable preliminary study of its mode of action. They noted the freezing point of a dilute, invertase-containing sucrose solution at various times and followed the decrease in molecular weight from that of sucrose (342) to that of invert sugar (180) as inversion proceeded. Knowing by this means the fraction of sucrose inverted at any time, comparison with corresponding values calculated from the polarimetric

(44) A. Herzfeld, *Ber.*, **13**, 265 (1880).
(45) C. F. Schoenbein, *Poggendorff's Ann.*, **70**, 100 (1847).
(46) A. Sobrero, *Compt. rend.*, **24**, 247 (1847).
(47) A. H. Elliott, *J. Am. Chem. Soc.*, **4**, 147 (1882).
(48) W. Will and F. Lenze, *Ber.*, **31**, 68 (1898).
(49) A. Herzfeld, *Z. Ver. deut. Zucker-Ind.*, **37**, 422 (1887), quoted from ref. 14.
(50) E. J. Hoffman and V. P. Hawse, *J. Am. Chem. Soc.*, **41**, 235 (1919).
(51) C. S. Hudson, *J. Chem. Education*, **18**, 353 (1941).
(52) A. Baeyer, *Ber.*, **18**, 2267 (1885).
(53) E. Fischer, *Ber.*, **26**, 2400 (1893).
(54) H. Rose, *J. prakt. Chem.*, **23**, 393 (1841).
(55) C. O'Sullivan and F. W. Tompson, *J. Chem. Soc.*, **57**, 834 (1890).
(56) E. Donat, *Ber.*, **8**, 795 (1875).

changes showed that the latter gave a misleading underestimate of its extent. When the inversion was arrested, and the invertase inactivated, instantaneously by adding the minimum amount of alkali, the rotation swiftly decreased to a final constant value which was characteristic of the particular degree of inversion, and which was concordant with the molecular weight determinations. The results showed that the cleavage of sucrose by invertase followed a monomolecular course but that the cleavage products were liberated in an unstable, more dextrorotatory, state. These products promptly assumed their final rotations when their "bi-rotation" was catalyzed by the added alkali. Since the spontaneous mutarotation of ordinary D-fructose was known to be small and swift, it was surmised that the "bi-rotation" was that of freshly liberated α-D-glucose and that sucrose was an α-D-rather than a β-D-glucoside. The above information made it possible to study the influence of temperature and hydrogen ion concentration on invertase in a quantitative way. The fact that the inversion obeyed the first-order rate equation for simple organic reactions also tended to invalidate the controversial supposition[26] that yeasts and enzymes could not be expected to observe such physical laws because their reactions were governed by vital forces. With the exception of Armstrong,[57] later workers overlooked the systematic error in the polarimetric method of following the inversion by invertase until Hudson[58] redirected attention to the correct observations of O'Sullivan and Tompson. It is interesting to note that Barth's[59] data showed as early as 1878 that inversion by invertase obeyed the equation for a first-order reaction over the range from 3% to 70 or 75% of completion. Although unrecognized at the time, Barth's success arose from the fact that he followed the inversion by the increase in the alkaline-copper-reducing power of the solution. This method of estimation is obviously insensitive to mutarotation effects.

Wilhelmy[60] was one of several who studied the polarimetric changes occurring when sucrose was hydrolyzed by acids. His articles, which are now classics in physical chemistry thanks largely to Ostwald's[61] early recognition of their importance, developed the mathematical expressions to describe the progress of a unimolecular reaction. They showed that the inversion of sucrose by several dilute acids obeyed the equation, although the extent of inversion was calculated directly from the observed polarimetric readings. Other investigators cast doubt upon the latter claim until Hudson[62] pointed out in a review that the mutarotations of

(57) E. F. Armstrong, J. Chem. Soc., 83, 1305 (1903).
(58) C. S. Hudson, J. Am. Chem. Soc., 30, 1160 (1908).
(59) M. Barth, Ber., 11, 474 (1878).
(60) L. Wilhelmy, Poggendorff's Ann., 81, 413, 499 (1850).
(61) W. Ostwald, J. prakt. Chem., 29, 385 (1884).
(62) C. S. Hudson, J. Am. Chem. Soc., 32, 885 (1910).

D-glucose and D-fructose were practically instantaneous in the acid solutions that Wilhelmy used and could produce no appreciable time lag in his measurements. Although this circumstance has, until now, made inversion by acids of little value as a tool for determining the glycosidic configurations in sucrose, the precision and ease with which the reaction can be followed have made it invaluable for the study of hydrogen ion concentration, common ion and strong electrolyte effects, activation energy, analytical methods, and other matters of interest to physical chemists. Articles including reviews of this field are available.[63-68] The inversion, with or without invertase or acid, has also been useful in studying the chemical effect of the deuterium ion,[69-72] of high pressure,[73] of sonic and supersonic energy[74,75] of ultraviolet light[76-78] of high-frequency electric energy[79] and of variation in the dielectric constant of the solvent.[80]

III. DETERMINATION OF THE CYCLIC STRUCTURES IN SUCROSE[81]

1. *The D-Glucopyranosyl Ring*

The discovery, by Purdie and his collaborators,[82] that the reaction between a lower alkyl iodide and the silver salt of a simple hydroxy acid yielded a proportion of the corresponding alkoxy ester, led to the silver

(63) E. O. Von Lippmann, Ref. 2, pp. 725–736.

(64) L. J. Heidt and C. B. Purves, *J. Am. Chem. Soc.*, 60, 1206 (1938); 62, 1006 (1940).

(65) A. Lamble and W. C. M. Lewis, *J. Chem. Soc.*, 107, 233 (1915).

(66) G. Scatchard, *J. Am. Chem. Soc.*, 48, 2259 (1926).

(67) D. I. Hitchcock and Ruth B. Dougan, *J. Phys. Chem.*, 39, 1177 (1935).

(68) J. M. Sturtevant, *J. Am. Chem. Soc.*, 59, 1528 (1937).

(69) E. W. R. Steacie, *Z. physik. Chem.*, B27, 6 (1934).

(70) P. Gross, H. Suess and H. Steiner, *Naturwissenschaften*, 22, 662 (1934).

(71) E. A. Moelwyn–Hughes, *Z. physik. Chem.*, B26, 272 (1934).

(72) E. A. Moelwyn–Hughes and K. F. Bonhoeffer, *Naturwissenschaften*, 22, 174 (1934).

(73) F. V. Sander, Jr., *J. Biol. Chem.*, 148, 311 (1943).

(74) E. W. Flosdorf and L. A. Chambers, *J. Am. Chem. Soc.*, 55, 3051 (1933).

(75) S. Sokolov, *Tech. Phys. U. S. S. R.*, 3, 176 (1936); *Chem. Abstracts*, 31, 4182 (1937).

(76) N. Taketomi and K. Miura, *J. Soc. Chem. Ind. Japan*, 33, 99 (1930); *Chem. Abstracts*, 24, 2942 (1930).

(77) A. Guillaume and G. Tanret, *Bull. soc. chim. biol.*, 18, 556 (1936); *Chem. Abstracts*, 30, 5503 (1936).

(78) L. J. Heidt, *J. Am. Chem. Soc.*, 61, 3223 (1939).

(79) S. P. Voskresenskii and E. N. Malashenko, *J. Gen. Chem.* (*U. S. S. R.*), 9, 1118 (1939); *Chem. Abstracts*, 33, 8119 (1939).

(80) C. J. Plank and H. Hunt, *J. Phys. Chem.*, 45, 1403 (1941).

(81) See also W. N. Haworth, "The Constitution of Sugars," Arnold and Co., London, pp. 17–33, 68–71 (1929).

(82) T. Purdie and W. Pitkeathly, *J. Chem. Soc.*, 75, 153 (1899).

oxide–methyl iodide method of methylation. Purdie and Irvine[83] were the first to use the new procedure in the carbohydrate field and their well-known articles include the first description of octamethylsucrose. The preparation of this compound was greatly facilitated by Haworth's[84] observation that the methylation of sucrose with dimethyl sulfate and strong aqueous caustic soda yielded an uncrystallized "heptamethyl-sucrose" in one operation. Remethylation of the latter by the earlier method then gave octamethylsucrose as a slightly viscid, colorless liquid, boiling point 176° at 0.05 mm., with a dextrorotation of 69.3° in methanol.[85]

Later improvements in technique rendered such substances very readily accessible.[86,87] The existence of a homogeneous octamethyl-sucrose, together with that of an octapropionate,[88] confirmed Tollens' view[43] that only eight hydroxyl groups were present in the sucrose molecule. Moreover, since no molecular rearrangement has ever been found to occur during the methylation of nonreducing carbohydrates, the hydroxyl positions in sucrose were those occupied by methyl ether groups in octamethylsucrose. Since these groups are very stable, they retained their positions during the cleavage of the methylated sucrose to a tetra-methyl-D-glucose and a tetramethyl-D-fructose. It followed that these two products would not have been methylated in positions originally shielded from methylation by the ring structures of sucrose.

Contrary to experience with the unsubstituted sugar, no optical inversion took place when the octamethyl derivative was completely hydrolyzed with hot, dilute mineral acid,[83] the overall decrease in specific rotation being[84] from $+66.7°$ to $+57.0°$. After isolation, the portion of the product that crystallized was found to be identical with a tetra-methyl-D-glucose, melting point 96° and $[\alpha]^{20}_D + 81.3°$ in water (final),[86] which could also be prepared by the complete methylation and subsequent hydrolysis of ordinary methyl D-glucoside.[83,85] The positions of the methyl ether groups in this tetramethyl-D-glucose were established by several researches, of which the first, by Irvine and Hirst,[89] showed that a certain trimethyl-D-glucose (III) could be oxidized to a *dimethyl*saccharic acid (IV). The loss of one substituent without the simultaneous cleavage of the hexose chain was clear evidence that the missing unit had occupied the sixth or terminal position in the trimethyl-D-glucose. The same

(83) T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903); **87**, 1022 (1905).
(84) W. N. Haworth, *J. Chem. Soc.*, (a) **107**, 8 (1915); (b) **117**, 199 (1920).
(85) W. N. Haworth and J. Law, *J. Chem. Soc.*, **109**, 1314 (1916).
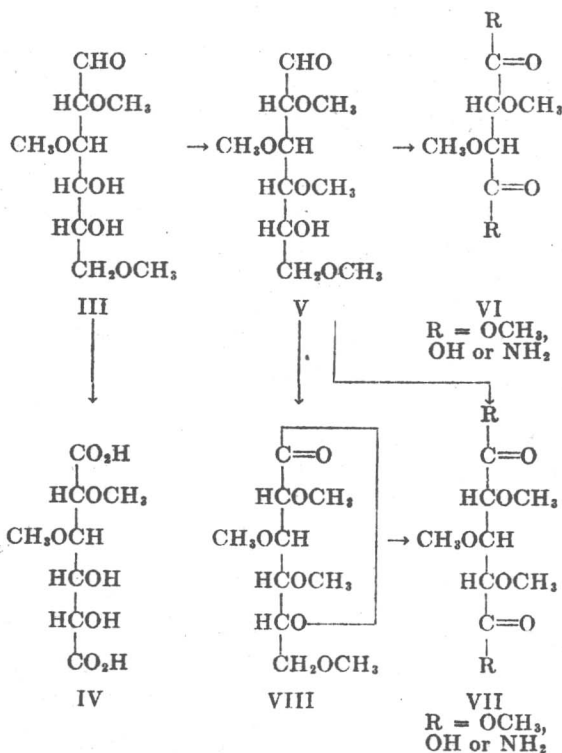(86) E. S. West and R. F. Holden, *J. Am. Chem. Soc.*, **56**, 930 (1934).
(87) E. Pacsu and S. M. Trister, *J. Am. Chem. Soc.*, **61**, 2442 (1939).
(88) C. D. Hurd and K. M. Gordon, *J. Am. Chem. Soc.*, **63**, 2657 (1941).
(89) J. C. Irvine and E. L. Hirst, *J. Chem. Soc.*, **121**, 1213 (1922).

conclusion was true of the tetramethyl-D-glucose (V), because it was readily obtained by the further methylation of the trimethyl derivative. The oxidation of the tetramethyl-D-glucose itself was then undertaken by Hirst,[90] who employed nitric acid of density 1.42, first at room temperature and finally at 90°. After forming the methyl ester of the acidic product, distillation yielded a main fraction corresponding in properties to a 60:40 mixture of a dimethyl dimethoxysuccinate (VI, R = OCH_3) and a dimethyl trimethoxyglutarate (VII, R = OCH_3). The constituents of the mixture were separated in pure form as the crystalline, sparingly soluble diamides. One of those, identified as the diamide of internally compensated, optically inactive *xylo*-trimethoxyglutaric acid, (VII, R = OH), accounted for 15 to 20% of the tetramethyl-D-glucose, and the other, the dextrorotatory (L-*threo*)-dimethoxysuccinamide, (VI, R = NH_2), for 25 to 40%. Although it was true that none of VII was isolated unless the oxidizing conditions were favorable, the fact that it arose at all was proof that three adjacent positions in the tetramethyl-D-glucose were methylated. These positions were the second, third and

```
      CHO              CHO                    R
                                              |
                                              C=O
      HCOCH₃           HCOCH₃              HCOCH₃
 CH₃OCH      → CH₃OCH           →  CH₃OCH
      HCOH             HCOCH₃               C=O
                                              |
      HCOH             HCOH                   R
  CH₂OCH₃          CH₂OCH₃
      III              V                     VI
                                          R = OCH₃,
                                          OH or NH₂
                                              |
                                              R
                                              |
                                              C=O
      CO₂H             C=O                  HCOCH₃
      HCOCH₃           HCOCH₃
 CH₃OCH         CH₃OCH           →  CH₃OCH
      HCOH             HCOCH₃               HCOCH₃
      HCOH             HCO                    C=O
                                              |
      CO₂H          CH₂OCH₃                  R
      IV               VIII                  VII
                                          R = OCH₃,
                                          OH or NH₂
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(90) E. L. Hirst, *J. Chem. Soc.*, **350** (1926).