Properties of Periodate-oxidised Polysaccharides. Part V. Micro-determination of Molecular Weights of Sugar Osazones from Measurements of Ultra-violet-light Absorption.*

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Light-absorption data for ten sugar osazones, ranging in molecular weight from that of glycerosazone to that of a disaccharide osazone, have been determined. The extinction curves are characterised by the existence of three maxima and by a constant value of ε at the absorption maximum of longest wave-length. This enables molecular weights to be determined with an error of less than ±2%. The light absorption spectra of sugar osazones and some related compounds are discussed.

THE degradation of various periodate-oxidised polysaccharides by phenylhydrazine leads to the production of mixtures of osazones of different molecular weights which can be separated by adsorption chromatography (Part IV *). Identification of these osazones by melting-point determinations was frequently unreliable, and conversion into osotriazoles was generally impracticable with the small quantities of osazones available. We were therefore prompted to examine the possibility of their identification by spectrophotometric methods.

Engel (*J. Amer. Chem. Soc.*, 1935, **57**, 2419) reported light-absorptions for some osazones of various sugars and sugar derivatives, but did not give values for the molecular extinction coefficients. We have, therefore, repeated some of his determinations and extended the range of osazones examined. The extinction curves of sugar osazones are characterised by the existence of three regions of maximum absorption (I, 256 mμ; II, 308—314 mμ; III, 395—399 mμ) illustrated for glycerosazone in Fig. 1, and further by little variation in value of ε, especially in region III where the differences come within the limits of experimental error (Table 1, Nos. 1—10). We have thus the conditions required for spectrophotometric determination of molecular weights (cf. Strain, *J. Biol. Chem.*, 1938, **123**, 425; Cunningham, Dawson, and Spring, *J.*, 1951, 2305). The accuracy of the

TABLE 1.

		I		II		III	
No.		$\lambda_{max.}$ (mμ)	$\varepsilon_{max.}$	$\lambda_{max.}$ (mμ)	$\varepsilon_{max.}$	$\lambda_{max.}$ (mμ)	$\varepsilon_{max.}$
Osazones.							
1	Glycerose	256	19,280	308—310	9,970	395—397	20,430
2	Erythrose	256	19,620	309	10,300	397	20,420
3	Arabinose	256	19,100	309—311	10,450	393—397	20,270
4	Xylose	256	19,610	308—312	10,440	397	20,360
5	Rhamnose	256	19,470	309—312	10,480	396—397	20,240
6	Galactose	256	20,230	309—311	10,840	395—397	20,700
7	Glucose	256	19,950	308—312	10,560	395—398	20,310
8	Maltose	256—257	21,450	309—314	10,640	399	20,430
9	Lactose	256—257	20,670	309—313	11,170	396—399	20,330
10	Melibiose	256	18,510	310—312	10,660	393—395	20,100
Bisphenylhydrazones.							
11	Glyoxal	—	—	302—304	10,110	378	46,410
12	Methylglyoxal	—	—	302—304	11,950	364	46,250
13	Diacetyl	—	—	305	14,190	352	46,400
Osotriazoles.							
14	Diacetyl	274—275	19,310				
15	Glycerose	266—277	18,970				
16	Glucose	267—278	18,670				

* Part IV, Barry and Mitchell, *J.*, 1954, 4020.

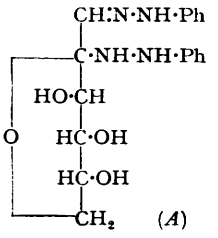
method is illustrated in Table 2 where the molecular weights of ten sugar osazones are calculated, by means of the average value, $\varepsilon_{max.\ III} = 20{,}360$.

Included in Table 1 (Nos. 11—13) are light-absorption data for the " osazones " of glyoxal, methylglyoxal, and diacetyl which, as was pointed out by Engel (*loc. cit.*), differ markedly (see also Fig. 1) from those of the sugar osazones. (For this reason we think it preferable to name these compounds as bisphenylhydrazones.) Engel attributed this

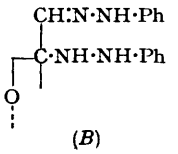
TABLE 2.

Osazone of :	*c* (mg./100 ml.)	$E_{max.}$	*M*, found	*M*, calc.	Error (%)
Glycerose	0·492	0·375	267	268	−0·4
Erythrose	0·486	0·333	297	298	−0·3
Arabinose	0·584	0·361	329	328	+0·3
Xylose	0·572	0·355	328	328	0
Rhamnose	0·620	0·367	344	342	+0·6
Galactose	0·614	0·355	352	358	−1·7
Glucose	0·610	0·346	359	358	+0·3
Maltose	0·606	0·238	518	520	−0·4
Lactose	0·596	0·233	521	520	+0·2
Melibiose	0·590	0·228	527	520	+1·3

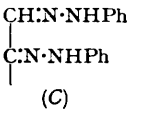
difference to the presence in the sugar osazones of an oxygen atom attached to $C_{(3)}$, whereas Percival and his co-workers have put forward evidence for the existence in glucosazone of a 2 : 6-oxide ring as in (*A*) (Summary in *Adv. Carbohydrate Chem.*, 1948, **3**, 29). While one should not be too rigid in the correlation of structure with ultra-violet-light absorption, one is tempted nevertheless to make a few observations regarding the relation between the structure of osazones and bisphenylhydrazones and their ultra-violet spectral absorption. Now the light-absorption properties of all the sugar osazones examined are closely similar, so, if one accepts an oxide ring structure for glucosazone, the same chromophoric grouping (*B*) must be present in each. Glycerosazone will then have a 2 : 3-oxide ring, and the tetrosazones and pentosazones presumably 2 : 4- and 2 : 5-oxide rings, respectively. Furthermore, in melibiosazone where $C_{(6)}$ of the reducing glucose residue is already involved in a glycosidic link, the oxide ring must again be different from that of glucosazone. The necessity for these varied assumptions, and Engel's positive evidence (*loc. cit.*) that 3 : 4 : 6-tri-*O*-methylglucosazone shows ultra-violet-light absorption closely similar to that of glucosazone, make it difficult to accept Percival's cyclic structure for glucosazone. Percival's case is based on his failure to prepare a methylated derivative of glucosazone containing a 6-methoxyl group, and he puts forward as additional evidence the findings of Diels, Cluss, Stephen, and König (*Ber.*, 1938, **71**, 1189) that triphenylmethyl chloride, a reagent which normally reacts readily with primary alcohol groups, does not react with glucosazone. We have no alternative explanation to offer of the lack of reactivity of the $CH_2{\cdot}OH$ group.



(*A*)



(*B*)



(*C*)

Percival suggests (*loc. cit.*) that in aqueous ethanolic solution an equilibrium is set up between the cyclic and acyclic modifications of glucosazone, such as is believed to obtain in solutions of the sugars themselves. The ultra-violet spectral absorption of glucosazone must then be due to the presence of the two chromophoric groupings, (*B*) and (*C*). Any variation in the proportions of the cyclic and acyclic modifications would be expected to show itself in altered ultra-violet absorption characteristics. As a result of the close

similarity of light absorption of all the sugar osazones, one would be thus compelled to postulate that the same equilibrium is reached in all cases. This would hardly be expected in view of the different structures involved, and it is evident that at the high dilutions used in the determination of ultra-violet absorption, the osazones are present only in the acyclic form.

The osotriazoles obtained from sugar osazones and diacetyl bisphenylhydrazone (Table 1, Nos. 14—16) show very similar light absorption (Fig. 2), indicating the absence in sugar osotriazoles of interaction between hydroxyl groups and the chromophore, such as obtains with osazones.

A satisfactory explanation of the apparently conflicting viewpoints referred to above must await more detailed investigation such as an extension of Percival's methylation

FIG. 1. FIG. 2.

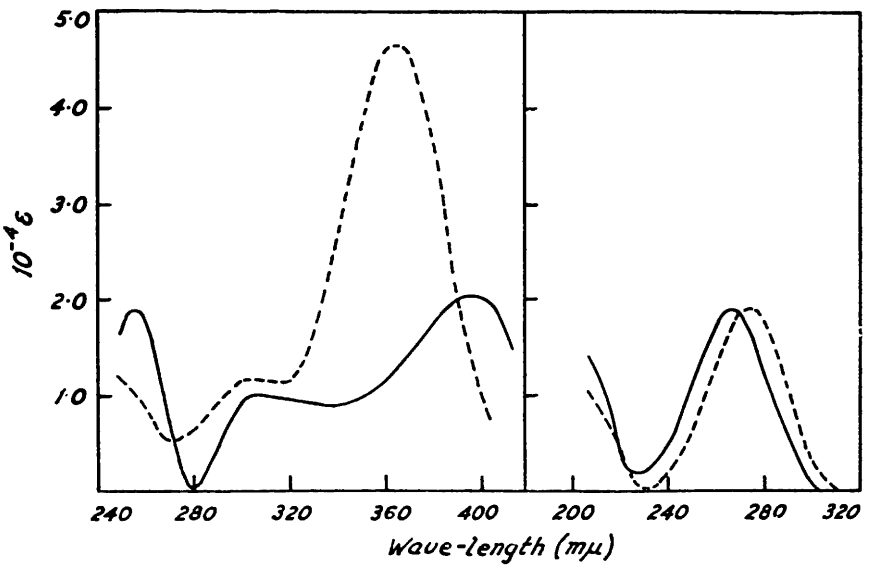


FIG. 1. *Extinction curves of glycerosazone* (——) *and methylglyoxal bisphenylhydrazone* (– – – –).
FIG. 2. *Extinction curves of the osotriazoles of glycerose* (——) *and diacetyl* (– – – –).

experiments to osazones of different molecular size. This might show whether in all these osazones a hydroxyl group is in fact involved in ring formation.

Experimental.—Light-absorption measurements were made with a Beckman Model DU spectrophotometer, a 1-cm. quartz cell, and 95% aqueous ethanol as solvent.

The osazones were prepared in the usual way and crystallised twice from aqueous ethanol. Glycerosazone and erythrosazone were obtained from the degradation of oxyinulin and oxystarch, respectively, by phenylhydrazine (Part IV, *loc. cit.*).

Glycerosotriazole was prepared by reduction with lithium aluminium hydride of 4-formyl-2-phenyl-2 : 1 : 3-triazole which is readily obtained by periodate oxidation of glucosotriazole. The product which was purified by adsorption on alumina and elution with benzene had m. p. 63—64°.

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