CCCXXXI.—*The Preparation and Properties of* dl-*Glyceraldehyde.*

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THE importance of glyceraldehyde is twofold. Not only is it the foundation stone on which the stereochemical configuration of the carbohydrates is constructed, but also it is regarded as an important link in the elusive metabolic chain of chemical reactions by which in the animal body glucose is converted into carbon dioxide and water. Despite this, it has received comparatively little attention owing probably to the great difficulty experienced in preparing it in a pure condition and to its lability and concomitant sensitiveness towards chemical reagents.

Nearly 70 years ago Van Deen (*Nederl. Tydschr. Geneesk.*, 1860, **4**, Aug.) reported that glycerol, when treated with nitric acid or subjected to the passage of an electric current, yielded a fermentable syrup which was optically inactive. Since that time, numerous attempts have been made to prepare glyceraldehyde by the oxidation of glycerol with various agents or by electrolysis of hydroxy-acids, but in every case the yield was most unsatisfactory (for summary see Witzemann, *J. Amer. Chem. Soc.*, 1914, **36**, 1908).

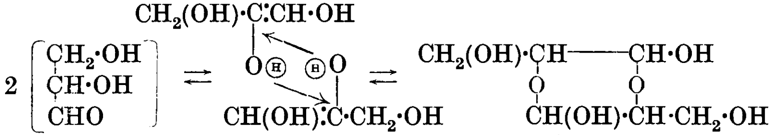
In 1898, Lobry de Bruyn and Adriani (*Rec. trav. chim.*, **17**, 258) made the important discovery that by careful hydrolysis of acraldehyde dibromide, glyceraldehyde could be obtained, and in the same year Wohl (*Ber.*, **31**, 1796, 2394) synthesised the pure aldehyde from acraldehyde through glyceraldehyde diethylacetal. In 1914, Witzemann (*loc. cit.*) proved that glyceraldehyde was formed when glycerol was oxidised with Fenton's reagent (Fenton and Jackson, J., 1899, **75**, 4; *Chem. News*, 1898, **78**, 187); the syrupy product was converted into the diethylacetal by Fischer and Giebe's method (*Ber.*, 1897, **30**, 3055; 1898, **31**, 545), but only 6% of the syrup yielded an acetal, the chief impurity being dihydroxyacetone, which does not form an acetal. Owing to the presence of by-products, the glyceraldehyde, obtained by hydrolysis of the acetal with dilute sulphuric acid, could only be crystallised with great difficulty (compare Meyer and Jacobson, "Lehrbuch der Organische Chemie," I, ii, 157, 171), and the method was too tedious for practical purposes.

Of the many methods which have been used, that of Wohl is the only one which gives glyceraldehyde in a state of purity, entirely free from dihydroxyacetone and in reasonable yield. For this reason the material used for the present observations was prepared directly from acraldehyde (obtained by Moureu's method, *Ann. Chim.*, 1921, **15**, 158; see also Snellgrove, Thesis, London, 1924) in four stages. (1) Acraldehyde was converted into β-chloropropaldehyde diethylacetal by the action of cold absolute alcohol, saturated with dry hydrogen chloride: the yield was greatly improved by the addition of anhydrous calcium chloride. (2) The elements of hydrogen chloride were removed from the chlorinated acetal by distillation with solid potash, with production of acraldehyde diethylacetal. (3) This was oxidised by aqueous potassium permanganate to glyceraldehyde diethylacetal. (4) The last acetal was hydrolysed by means of dilute sulphuric acid, the acid neutralised, and the pure glyceraldehyde caused to crystallise from the concentrated liquor by the addition of methyl alcohol and ether. Each of the four stages proceeds smoothly, but, although the yields have been improved in the present research, they are not good.

Glyceraldehyde, as thus obtained, is a white, non-hygroscopic, slightly sweet powder, which crystallises from aqueous methyl alcohol in colourless, pointed needles. Analysis and the m. p. of its *p*-bromophenylosazone (168°) showed it to be pure (Wohl, *loc. cit.*, gives m. p. 168°).

As a basis for the space representation of the sugars, the unimolecular formula (I) for glyceraldehyde is always adopted, but Wohl and Neuberg (*Ber.*, 1899, **32**, 1352; 1900, **33**, 3095) showed by cryoscopic determinations that it was bimolecular (compare glycollaldehyde and dihydroxyacetone) and ascribed to it the formula (II). In aqueous solution, the aldehyde gradually assumes the unimolecular form. In view of the importance of these observations, molecular-weight determinations were repeated with particular care, and the results of Wohl and Neuberg were confirmed.

In preparing the glyceraldehyde for this research, it was noted that the transparent syrup obtained by the hydrolysis of its diethylacetal had most unexpected properties. After standing over-night in a vacuum desiccator, the syrup showed a white opalescence; 24 hours later it had become very viscous and perfectly opaque. These phenomena were noted on only one occasion by Witzemann (*J. Amer. Chem. Soc.*, 1914, **36**, 2223) in his repetition of Wohl's synthesis, and his use of the term " colloidal " in this connexion was unsupported by any evidence. It seemed necessary, therefore, to investigate the molecular complexity of the aldehyde in these various states. The transparent syrup was found to be enolic and, in aqueous solution, unimolecular; the following scheme is therefore suggested as a possible mechanism for the changes described above :



(I. Unimolecular form.) (Enolic form.) (II. Bimolecular form.)

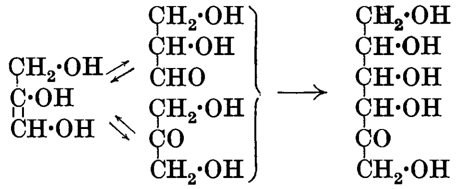
In support of this scheme, the possibility of the formation of a stable six-atom compound from an enol, recently substantiated by Sidgwick (J., 1925, **127**, 907), may be cited. The crystalline bimolecular glyceraldehyde, after repeated crystallisation, was shown by K. H. Meyer's method (*Annalen*, 1911, **380**, 212; Meyer and Kappelmeier, *Ber.*, 1911, **44**, 2718) to contain a trace of the enol.

Fischer and Tafel (*Ber.*, 1887, **20**, 2566) showed that crude glycerose polymerised under the influence of dilute alkali. Wohl and Neuberg (*loc. cit.*) found that pure glyceraldehyde in presence of 1% caustic potash solution readily condensed to β-acrose and also partly split up into dihydroxyacetone. Nef (*Annalen*, 1907, **357**, 214; 1910, **376**, 1; 1914, **403**, 204) showed that the first step in the action of dilute alkali on all carbohydrates was the production of unsaturated enols, which then underwent decomposition by fission at the double bond. Thus the $\gamma\delta$-dienol formed by the action of alkali on glucose breaks down at the ethylene linkage to give glyceraldehyde. The interaction of glyceraldehyde with alkali involves primarily the formation of a sodium salt, the metallic atom being attached to the carbon atom adjacent to the carbonyl group; this salt is next converted, by elimination of sodium hydroxide, into the methylene derivative, and the latter is finally converted into the glycide.

Hence it appeared likely that in dilute alkaline solution the enolic modification of glyceraldehyde could be detected by the coloration with aqueous ferric chloride, and, actually, it was found to give a deeper coloration than glucose under identical conditions.

All sugars in solutions of like concentration have the same refractive index, and tables have been prepared for sugar products by Main (*Int. Sugar J.*, 1907, **9**, 481), Geerligs (*ibid.*, 1908, **10**, 68), and Bryan (*J. Amer. Chem. Soc.*, 1908, **30**, 1443). It was thought that refractometric measurements would provide further proof of the existence of the enol in alkaline solution, and the results fulfilled these expectations both for glucose and for glyceraldehyde.

The presence of the enolic modification of glyceraldehyde in alkaline solution elucidates the mechanism of acrose formation as outlined by Schmitz (*Ber.*, 1913, **46**, 2327). The reaction is not, however, the auto-condensation of glyceraldehyde, but its condensation with dihydroxyacetone, which may be assumed to result from the enolic form of the aldehyde :



$$\begin{array}{l} \\ \\ CH_2\cdot OH \\ C\cdot OH \\ \| \\ CH\cdot OH \end{array} \begin{array}{c} \\ \\ \rightleftharpoons \\ \\ \\ \rightleftharpoons \end{array} \left.\begin{array}{l} CH_2\cdot OH \\ CH\cdot OH \\ CHO \\ CH_2\cdot OH \\ CO \\ CH_2\cdot OH \end{array}\right\} \longrightarrow \begin{array}{l} CH_2\cdot OH \\ CH\cdot OH \\ CH\cdot OH \\ CH\cdot OH \\ CO \\ CH_2\cdot OH \end{array}$$

That an enolic form of glyceraldehyde was present in alkaline solution had been foreshadowed, since Fischer (*Ber.*, 1895, **28**, 1149) suggested that " sodium glucosate," formed by the action of sodium hydroxide on glucose, had the structure

$$OH\cdot CH_2\cdot[CH\cdot OH]_3\cdot C(OH){:}CH\cdot ONa,$$

and Wohl and Neuberg (*loc. cit.*) postulated an unsaturated alcohol of the type $X\cdot C(OH){:}CH\cdot OH$ as an intermediate phase in the conversion of glyceraldehyde into β-acrose under the influence of dilute alkali. The existence of this unsaturated labile form is now definitely proven.

The close resemblance between glyceraldehyde and glucose in chemical properties suggests the possibility of either a 1 : 2- or a 1 : 3-oxide structure rather than the generally accepted straight-chain aldehydic form.

Owing to its extreme lability, glyceraldehyde has hitherto resisted attempts to methylate it completely, but the work is being continued, and it is hoped to publish the results shortly. From the preliminary work, it would appear that glyceraldehyde falls into line with other sugars in its resistance to propylene-oxide formation.

EXPERIMENTAL.

Preparation of Glyceraldehyde.—β-*Chloropropaldehyde diethylacetal.* To absolute alcohol (2 mols.), cooled in an ice-bath, powdered anhydrous calcium chloride ($\frac{1}{6}$ mol.; excess should be avoided) was added, and acraldehyde (1 mol.) admitted in small portions at a time with continuous shaking. Dry hydrogen chloride (1 mol.) was now bubbled through the mixture, the temperature of the bath being kept below 5°; the mixture, which became yellow, was kept for 24 hours, during which it separated into two layers. The lower oily layer was drawn off, neutralised with finely powdered sodium bicarbonate, and dried over solid potassium hydroxide without removing the alcohol. Small quantities of the oil were recovered from the supernatant liquor by passing further quantities of hydrogen chloride. The yield was 90% of the theoretical. The pale yellow oil so obtained had a characteristic but not unpleasant odour.

Acraldehyde diethylacetal. (In this preparation exact adherence to the specified conditions is essential.) To 200 g. of the above crude oil in a 1-litre copper flask, 400 g. of pure, dry, finely powdered potassium hydroxide were added slowly in small quantities at a time with vigorous shaking and cooling. The mixture was then distilled very slowly through a three-bulb Young fractionating column; the main bulk passed over between 120° and 130°. This was dried with potassium carbonate and redistilled, the fraction distilling between 120° and 128° being collected (yield, 70%). The acraldehyde acetal was a colourless, mobile liquid with an agreeable ester-like odour, slightly soluble in water, but miscible in all proportions with alcohol and ether. It is readily hydrolysed in the cold by dilute hydrochloric acid.

Glyceraldehyde diethylacetal. The acraldehyde acetal (100 g.) was emulsified by vigorous shaking with distilled water (1200 c.c.), and the whole cooled to 0°. To the cold emulsion was added, drop by drop with continuous and vigorous shaking, the calculated quantity of a 4% aqueous potassium permanganate solution. The mixture, after being kept for 12 hours at 0°, was heated for ½ hour on the water-bath and filtered from the precipitated manganese dioxide, which was well pressed and then twice washed with 50 c.c. of warm water. The warm filtrate and washings were treated with potassium carbonate solution (500 g. per litre) and immediately cooled; the glyceraldehyde diethylacetal, which separated as a yellow oil on the surface of the liquor, was removed, and the lower aqueous layer extracted four times with ether. The oil and the extracts were combined, and the ether was removed. The residual acetal was redistilled under diminished pressure, b. p. 130°/20 mm. A small amount of unchanged acraldehyde acetal can be recovered. The yield of glyceraldehyde diethylacetal is variable; in no case was it greater than 60%, and in one experiment it fell to 30% for no accountable reason (Wohl and Neuberg reported 40%; Witzemann, 54%). The loss here cannot be explained, since the oxidation itself proceeds quite smoothly.

The acetal is a colourless, viscous liquid with a caustic taste. It is miscible with water, alcohol, and ether, and is readily hydrolysed by dilute mineral acids. Its aqueous solution reduces Fehling's solution in the cold, and in acetic acid it gives a phenylosazone.

dl-*Glyceraldehyde.* 55 G. of the acetal were dissolved in *N*/10-sulphuric acid (550 g.) and kept at room temperature for 48 hours, then at 38° for 24 hours, and allowed to cool. The acid was exactly neutralised with the calculated weight of barium hydroxide, and the whole kept for 2 hours. The precipitated barium sulphate was filtered off and washed with a minimum volume of water. The filtrate was concentrated at 40° in a vacuum over calcium chloride. The further small amount of barium sulphate which had separated when concentration was half completed was filtered off, and concentration continued. Success in crystallisation depends on the *complete* removal of water from the syrup. After one month, a pale yellow, transparent syrup was obtained, which on standing over-night in the vacuum desiccator showed a white opalescence, and after a further 24 hours became creamy and opaque. Trituration with small amounts of pure ice-cold methyl alcohol containing 1% of ether caused immediate separation of the crystalline glyceraldehyde; yield, 60% of the theoretical. (Excess of ether must be avoided, otherwise the product is sticky.)

Properties.—The glyceraldehyde was obtained as a white, non-hygroscopic, slightly sweet powder, m. p. 133°; after being washed with methyl alcohol, dried on porous tile, recrystallised from 40% aqueous methyl alcohol, and dried over phosphorus pentoxide in a vacuum for one week, it had m. p. 138·5° (Wohl gives 138°, and Witzemann 142°), unaltered by further recrystallisation and desiccation (Found : C, 39·8; H, 6·7. Calc. : C, 40·0; H, 6·7%). It crystallises from aqueous methyl alcohol in short, colourless needles, which are sparingly soluble in water, ethyl alcohol, and ether, and decompose above their m. p. to a sticky, brown, caramel-like mass. Its *p*-bromophenylosazone melted at 168°.

To the properties already described (Abderhalden, " Biochemisches Handlexikon," II, 1911) may be added the following : It is insoluble in benzene, petroleum, and pentane. Its solubility in water at 18° is 3 g. per 100 c.c., and it has $d_{18^\circ}^{18^\circ}$ 1·455.

Molecular-weight Determinations.—(a) *The crystalline solid.* A freshly prepared solution containing 0·1480 g. in 25 c.c. of water gave $\Delta = 0·061°$, hence $M = 180·6$. After the solution had stood for one week, Δ was found to be 0·122°, hence $M = 90·3$. Thus dissociation into the unimolecular form occurs in aqueous solution.

(b) *The transparent syrup*, from which the crystals separated, is apparently unimolecular : 0·1204 g. in 25 c.c. of water gave $\Delta = 0·097°$; $M = 92·4$.

The Action of Alkalis and the Existence of an Enolic Modification.—Cold dilute sodium hydroxide (both $N/10$ and N) readily dissolves glyceraldehyde, which passes through all the various changes described by Nef, finally giving β-acrose, polysaccharides, and lactic acid. The alkaline solution resinifies gradually on being warmed. The aldehyde is less soluble in 50% sodium hydroxide and rapidly resinifies in the hot solution. Its behaviour with alkali is thus similar to that of other aliphatic aldehydes.

Colorimetric Measurements with Ferric Chloride Solution.—5 C.c. of 1% aqueous solutions of glucose and glyceraldehyde were each kept at 38° for 10 minutes; 5 c.c. of 0·1% caustic soda solution were then mixed with each, and the mixtures were kept at 38° for a further 10 minutes. On the addition of 1 c.c. of 10% aqueous ferric chloride, the glyceraldehyde solution showed a deeper red coloration than did the glucose solution, and therefore apparently contained a higher percentage of the enolic form. A quantitative estimation was then made with the colorimeter : 1% Aqueous solutions of glucose and glyceraldehyde were used; 2 c.c. of each were kept for 10 minutes at 38° with 7 c.c. of $N/10$-sodium hydroxide. To each was added 1 c.c. of 0·1% aqueous ferric chloride, and the solutions were cooled to room temperature.

The readings on the colorimetric scale were 1·46 and 1·15 for glucose and glyceraldehyde, respectively. Hence, the ratio of enol in glyceraldehyde to that in glucose = 1·27 : 1. Similar results were obtained with several different preparations.

Refractometric Measurements.—Readings were taken with a Pulfrich refractometer at 20° with sodium light. The following solutions were prepared :

(*a*) *Neutral solution.* 2 C.c. of 1% glucose solution were warmed at 38° for 5 minutes with a mixture of 2 c.c. of *N*/10-hydrochloric acid and 2 c.c. of *N*/10-sodium hydroxide, and cooled to room temperature.

(*b*) *Acid treatment.* As in (*a*), but the alkali was not added until after the cooling.

(*c*) *Alkali treatment.* As in (*a*), but the acid was omitted until after the cooling.

(*d*) A 1% aqueous solution of glucose, as a standard.

Solutions similar to (*a*), (*b*), (*c*), and (*d*) were prepared with glyceraldehyde. The readings obtained show that there is a decrease of 2′ in the angle of refraction of both glucose and glyceraldehyde in alkaline solution, indicating the production of an unsaturated form. These results were confirmed on several occasions with different preparations.

Nature of solution.	Glucose.	Glyceraldehyde.
(*d*) Aqueous (1%)	67° 2′	67° 2′
(*a*) Neutral (⅓%)	67° 9′	67° 8′
(*b*) Acid (⅓%)	67° 9′	67° 8′
(*c*) Alkaline (⅓%)	67° 7′	67° 6′

There is no change in the refractive index of the glyceraldehyde solution after standing for one month.

From (*d*) the refractive index for a 1% solution = 1·33412, whereas that for water is 1·33311 under the same conditions. Hence $[R_L]_D$ = 19·2 (calc. for $C_3H_6O_3$: 19·14; for enolic form : 20·08), showing that in aqueous solution no appreciable amount of the enolic form exists, a fact which is substantiated in that ferric chloride gives no coloration.

Bromine Titration for Estimation of the Enol.—A suspension of glyceraldehyde in chloroform slowly decolorises a cold solution of bromine in the same solvent. An alcoholic solution of glyceraldehyde rapidly decolorises alcoholic bromine at —7°.

The bromine titration was carried out as follows : A solution of 0·1 g. of glyceraldehyde in 250 c.c. of absolute alcohol was cooled in ice and salt for ½ hour. Ice-cold *N*/10-alcoholic bromine solution was run in slowly from a burette until the yellow colour of the bromine just persisted (10—12 c.c. usually); 2—3 c.c. of a 10% alcoholic solution of β-naphthol, freshly prepared, were added to remove the excess of bromine, followed by 15 c.c. of 10% potassium iodide solution, and the mixture was boiled on the water-bath for 10 minutes. The resulting pale yellow solution was cooled and titrated against $N/100$-sodium thiosulphate (usually 1—2 c.c.). The average of four experiments gave enol = 0·54%.

An alcoholic solution of the syrup from which the solid glyceraldehyde was obtained readily decolorised alcoholic bromine solution. Bromine titrations on this syrup (0·1 g. in 50 c.c. of absolute alcohol) gave 5·7% of enol (mean of four experiments).

These results show that, although the crystalline form of glyceraldehyde is contaminated with a little enol, the syrup from which the crystals are obtained contains ten times as much.

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