277. *The Separation of* d-*Fructose from Other Natural Sugars as its* 2 : 3-4 : 5-*Diacetone and* 1 : 2-*Monoacetone Derivatives : Observations on the Behaviour of Acetone Derivatives of Monosaccharides towards Cold Decinormal Sulphuric Acid.*

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A simple procedure is described for the isolation of *d*-fructose from mixtures of monosaccharides as the 2 : 3-4 : 5-diacetone derivative. Glucose, mannose, xylose, and rhamnose do not interfere. The method is not applicable where galactose, arabinose, or fucose are present in quantity; in such instances a procedure leading to the isolation of 1 : 2-monoacetone fructose is adopted.

The diacetone derivatives of glucose, mannose, and xylose and the monoacetone derivatives of rhamnose and ribose (all understood to be derived from the furanose form of the sugars) are hydrolysed to the corresponding monoacetone compounds, or to the free sugar, by several hours' treatment with 0·1N-sulphuric acid at room temperature. On the other hand, 2 : 3-4 : 5-diacetone fructose, and the diacetone derivatives of galactose, fucose, and arabinose (all believed to have pyranose structures) resist this hydrolytic attack. 1 : 2-4 : 5-Diacetone fructose is anomalous : it behaves as if it had a furanose ring although it is generally considered to be derived from fructopyranose. Use being made of this difference in hydrolytic behaviour, a number of sugar separations are facilitated through their readily obtained acetone derivatives.

The identification of *d*-fructose, especially in crude material of biological origin, has always presented difficulty. The only crystalline derivative hitherto employed to this end is the phenylmethylosazone (Neuberg, *Ber.*, 1902, **35**, 960; Neuberg and Mandl, *Arch. Biochem.*, 1946, **11**, 451). This substance is formed in variable yield; when it is isolated from mixtures its physical properties tend to be erratic, and in theory, if not in practice, it may also be formed from *d*-glucose and *d*-mannose. It is also necessary to work in relatively concentrated solutions, so the presence of large amounts of mineral matter, etc., besides other sugars, as often occur in biological extracts, complicates, or may even render impossible, isolation of the phenylmethylosazone. Work in this laboratory demanded an unequivocal means of identifying relatively small amounts (*e.g.*, 100 mg.) of *d*-fructose under conditions such as those described.

It was considered that treatment of the dried material with acetone and a suitable catalyst would provide a convenient means of separating carbohydrates (as monosaccharides) from inert material and at the same time produce derivatives which, directly or indirectly, would

lead to characterisation of the sugars, and this has proved to be the case. Irvine and Garrett (*J.*, 1910, **97**, 1283) observed that 2 : 3-4 : 5-diacetone fructose (the so-called " β "-derivative) displayed remarkable stability towards cold dilute acid, in marked contrast to the behaviour of the 1 : 2-4 : 5-isomer (" α " diacetone fructose). Ohle and Koller (*Ber.*, 1924, **57**, 1566) confirmed this finding and further noted that 1 : 2-5 : 6-diacetone glucose was, like α-diacetone fructose, unstable to cold 0·1N-sulphuric acid, both substances losing at least one *iso*propylidene radical by hydrolysis. Ohle and Koller further demonstrated that, although low concentration of mineral acid catalyst favoured the formation of α-diacetone fructose (cf. E. Fischer, *Ber.*, 1895, **28**, 1164; Irvine and Garrett, *loc. cit.*; Montgomery, *J. Amer. Chem. Soc.*, 1934, **56**, 419), yet increase in concentration of the catalyst led to increasing formation of the β-isomer, resulting in a series of mixed products, until, at a concentration of 4% of sulphuric acid, pure β-diacetone fructose was formed. The use of catalysts such as zinc chloride (H. O. L. Fischer and Taube, *Ber.*, 1927, **60**, 485) favours formation of the α-derivative.

In the present work the above observations have been confirmed and extended. The presence of 5% (v/v) of sulphuric acid in the acetone condensation (optimum duration, 6 hours) produces over 70% of the theoretical yield of β-diacetone fructose while diacetone glucose, under similar conditions, is formed to the extent of 50—55%. If a mixture of glucose and fructose, or sucrose itself, is treated in the above manner, the products are mainly β-diacetone fructose and diacetone glucose along with minor quantities of monoacetone derivatives. Partition of this mixture between chloroform and water leaves the " mono "-constituents in the aqueous phase. Six hours' treatment of the material obtained from the chloroform phase with 0·1N-sulphuric acid effects hydrolysis of the diacetone glucose and the α-diacetone fructose to the monoacetone derivatives; pure β-diacetone fructose is obtained by partition into chloroform. If all the " mono "-residues are brought into the dry state, recondensed with acetone, treated with dilute acid, and partitioned as before, the total yield of β-diacetone fructose may approach 80% of the theoretical.

It having been established that fructose could satisfactorily be separated from glucose, it seemed desirable to determine whether differences in hydrolytic behaviour existed among the acetone derivatives of other natural monosaccharides, and to what extent their presence might interfere with the isolation of β-diacetone fructose. The required derivatives were prepared under the conditions described above (which are not necessarily optimum for sugars other than fructose). Those compounds which are considered to be derived from the pyranose form of the parent sugar, *viz.*, the diacetone derivatives of *d*-galactose, *l*-arabinose, and *l*-fucose, all proved completely resistant to the action, for 6 hours, of 0·1N-sulphuric acid. In contrast, the furanose compounds, diacetone *d*-mannose, diacetone *d*-xylose, and monoacetone *l*-rhamnose, behaved similarly to diacetone glucose and were converted into substances which could not be extracted from water by chloroform. Crude " monoacetone *d*-ribose " (see Experimental) is hydrolysed comparatively slowly, but, although it is partitioned into chloroform, the restricted occurrence of the parent sugar is unlikely to cause difficulties. It was found practicable to isolate β-diacetone fructose from a mixture composed of 200 mg. each of *d*-fructose and six other sugars, despite the presence of both diacetone *d*-galactose and diacetone *l*-arabinose in the final product of the chloroform–water partition.

A possible explanation of the marked difference in stability towards acid exhibited by the two types of diacetone sugar may lie in the fact that in each example possessing a furanose sugar ring the labile *iso*propylidene radical is united through a primary alcoholic group. However, in the instance of the labile radical of α-diacetone fructose, if we assume that the hitherto accepted pyranose structure is correct, two secondary *cis*-alcoholic groups (4 and 5) are engaged. Such configurations are stable when the sugar ring is pyranose, and labile when it is furanose. The 1 : 2-monoacetone fructose derived from α-diacetone fructose is well known to possess a pyranose structure. It may be possible that α-diacetone fructose is really a derivative of fructofuranose having its *iso*propylidene radicals attached through positions 1 : 2 and 4 : 6, and that hydrolysis to the monoacetone derivative is accompanied by enlargement of the ring. Our present knowledge of the chemistry of the ketoses is too scanty definitely to forbid such a transformation.

If it is desired to separate fructose from sugars which form pyranose derivatives, with acetone, condensation catalysed by 0·3% (v/v) of sulphuric acid will lead to the production of α-diacetone fructose. The action of dilute acid will hydrolyse the latter to 1 : 2-monoacetone fructose which can be partitioned from chloroform into water, leaving the pyranose diacetone compounds in the organic solvent. The yield of monoacetone fructose thus obtained is good.

Experimental.

Evaporation of solvents was conducted below 50°, under diminished pressure. M. p's. are uncorrected. Measurements of optical activity were made in a 2-dm. tube. Acetone was kept, for at least 2 weeks, over calcium chloride before use.

α-Diacetone d-*Fructose.*—(a) *Catalyst, zinc chloride.* The method of H. O. L. Fischer and Taube (*loc. cit.*) was followed. From 2 g. of fructose, the yield (not improved by using varying amounts of catalyst) was 3·8 g., after recrystallisation from ligroin (b. p. 60—80°); m. p., 119°, $[\alpha]_D^{16°}$ (water) — 161·0°; (chloroform) — 147·3°.

(b) *Catalyst, 0·3% sulphuric acid.* 5 G. of fructose were shaken with 50 ml. of dry acetone containing 0·15 ml. of concentrated sulphuric acid; after 24 hours the sugar was completely dissolved. The reaction mixture was kept for a further 24 hours, stirred with anhydrous sodium carbonate to remove acid, filtered, and evaporated. The residue was dissolved in water, the pH adjusted to 8·5—9 by addition of dilute sodium hydroxide, and the solution distilled under reduced pressure to remove mesityl oxide. The solution was then shaken with a little charcoal, filtered, and three times extracted with its own volume of chloroform. On evaporation of the dried (Na_2SO_4) extract, 5·29 g. of crystalline material were left. Recrystallised from ligroin (b. p. 60—80°), 4·79 g. (66%) of pure α-diacetone fructose were obtained, having m. p. 119°, $[\alpha]_D^{16°}$ (chloroform) — 147·3°.

β-Diacetone Fructose.—(a) *Catalyst, 4% sulphuric acid* (cf. Ohle and Koller, *loc. cit.*). 5 G. of fructose were shaken with 50 ml. of dry acetone containing 2 ml. of sulphuric acid. The sugar dissolved rapidly and, after being kept for 3 hours, the solution was worked up in the manner described for the α-isomer. The crude product (3·7 g.) was crystalline; it proved, however, to consist of mixed crystals of the α- and the β-isomer which could not be separated by fractional crystallisation; m. p. *ca.* 80°, $[\alpha]_D^{16°}$ (water) — 62·8°. (Ohle and Koller observed similar inseparable mixtures when using lower concentrations of catalyst.) This mixture corresponds to one of 25% "α" with 75% "β"; 3·2 g. were kept for 3 hours in 0·1N-sulphuric acid solution. After neutralisation of the acid with dilute sodium hydroxide, the solution was three times extracted with its own volume of chloroform. On evaporation of the solvent, 2·4 g. (75% of the mixed crystals taken) of needles, m. p. 93—94°, remained. After recrystallisation from ligroin (b. p. 60—80°), the m. p. rose to 95—96° and $[\alpha]_D^{16°}$ (water) was — 33·1° (*c*, 7); these constants agree with those previously recorded.

(b) *Catalyst, 5% sulphuric acid.* 2·5 G. of fructose were shaken for 4 hours with 25 ml. of acetone containing 1·25 ml. of sulphuric acid, and the mixture treated as before. The chloroform phase yielded 2·7 g. (74%) of crystals, m. p. 90°, $[\alpha]_D^{16°}$ (water) —41·9°, corresponding to a mixture of 93% "α" with 7% "β".

The aqueous phase from the chloroform extraction was evaporated to dryness, and 0·5 g. of a colourless solid obtained; this was treated with 10 ml. of acetone (5% H_2SO_4) in the manner described above for 4 hours. After the customary separation by partition, 0·4 g. of crystalline diacetone fructoses was obtained, bringing the total yield to 3·1 g. 2·5 G. of this material were subjected to the action of 0·1N-sulphuric acid for 2 hours; the yield of pure β-diacetone fructose (m. p. 95—96°) was 2·3 g. (*ca.* 79%).

Ohle and Koller stated that the specific rotation (in water) of β-diacetone fructose varies with the concentration of the solute. That this is not the case is shown by the following observations:

Concn., g./100 ml.	7·0	3·5	1·0
$[\alpha]_D^{16°}$	—33·1°	—33·1°	—32·95°

The specific rotation in chloroform was also found to be — 24·8° over a range of concentrations.

The Stability of β-Diacetone Fructose towards 0·1N-*Sulphuric Acid.*—352 Mg. of pure β-diacetone fructose were dissolved in the acid, and the solution kept at room temperature for 6 hours. After the pH of the solution had been brought to about 9 by addition of sodium hydroxide, it was three times extracted with chloroform. Evaporation of the chloroform phase yielded 350 mg. (99·5%) of crystals, m. p. 95°, $[\alpha]_D^{16°}$ (water) — 32·9° (*c*, 2·5).

Conversion of α- into β-Diacetone Fructose.—1·300 G. of α-diacetone fructose were dissolved in 20 ml. of acetone containing 1 ml. of sulphuric acid. After being kept for 6 hours at room temperature, the product was isolated by the usual procedure. The yield of crystals was 1·202 g. (92·5%); $[\alpha]_D^{16°}$ was — 32·7° (chloroform), corresponding to a mixture of 90% "β" with 10% "α". The material was therefore treated with 0·1N-sulphuric acid for 6 hours, and the β-diacetone fructose isolated by partition into chloroform. 1·010 G. of needles were obtained, corresponding to 93·5% of the β-diacetone compound estimated to be present in the product of the acetone treatment. The material had $[\alpha]_D^{16°}$ (chloroform) — 24·8° (*c*, 5·78), and after recrystallisation from ligroin (b. p. 60—80°) melted at 95—96°.

Action of Acetone containing 5% of Sulphuric Acid on Some Monosaccharides.—The finely powdered sugar was shaken for 6 hours (unless stated to the contrary) with 20 parts (v/w) of acetone containing 5% (v/v) of the catalyst. The reaction mixture was treated as in the preparation of α-diacetone fructose, and the product isolated by triple chloroform extraction from water.

(*a*) *d*-Glucose (5 g.). Crude yield (crystalline) 4·59 g. (63%); recrystallised from ligroin (b. p. 60—80°), 3·84 g. (53%), m. p. 110°; $[\alpha]_D^{16°}$ (chloroform) — 12·3° (*c*, 5·6).

(*b*) *d*-Galactose (5 g.). After 24 hours, 0·9 g. of sugar remained undissolved; yield (syrup) 3·55 g. (60%); $[\alpha]_D^{16°}$ (chloroform) — 55·0° (*c*, 2·14), (water) — 42·8° (*c*, 2·77).

(*c*) *d*-Mannose (2 g.). 1·802 G. of crystals, m. p. 123°, were obtained. This material was pure, the m. p. not being raised by crystallisation from ethanol; $[\alpha]_D^{16°}$ (methanol–water, 2 : 3) showed downward mutarotation, from + 7·8° to + 3·9° (const. after 24 hours) (*c*, 1·29). In chloroform solution (*c*, 5·9) similar behaviour was observed, $[\alpha]_D$ falling from + 8·7° to a constant value (24 hours) of + 5·7°.

(*d*) *d*-Fructose (5 g.). The crude product was treated with cold 0·1N-sulphuric acid for 6 hours. The yield of pure material (from ligroin, b. p. 60—80°) was 5·40 g. (75·8%), m. p. 95°, $[\alpha]_D^{16°}$ (chloroform) — 24·6° (*c*, 3·9).

(*e*) *l*-Fucose. 535 Mg. yielded 670 mg. (84%) of syrup which crystallised (needles), m. p. 35—37°.

Distillation in a high vacuum failed to raise the m. p. Recrystallisation was readily effected from 50% aqueous methanol, yielding needles, m. p. 35—36°, $[\alpha]_D^{16°}$ (chloroform) + 53·6° (*c*, 2·35).

(*f*) *l*-Arabinose (5 g.). Crude yield, crystalline, 6·59 g. (86%). Recrystallised from water, the substance, 4·80 g. (66·5%) had m. p. 41—42° and $[\alpha]_D^{16°}$ (water) + 5·4° (*c*, 2·88), (chloroform) — 13·2° (*c*, 4·2).

(*g*) *d*-Xylose (2 g.). Crude yield (syrup) 1·82 g. (59·5%), $[\alpha]_D^{16°}$ (water) + 13·0° (*c*, 1·32), (chloroform) + 5·0° (*c*, 2·68).

(*h*) *d*-Ribose. 270 Mg. yielded 129 mg. of a syrupy " monoacetone " condensation product.

Although a reasonable yield is in each instance obtainable by the above procedure, it is emphasised that, the preparation of β-diacetone fructose excepted, methods already described in the literature may for individual examples prove superior.

Recovery of Acetone Sugars after Treatment with Cold 0·1N-*Sulphuric Acid.*—Weighed amounts of the sugars were condensed with acetone (5% sulphuric acid) for 6 hours, and the products, isolated by the procedure described above, subjected to the action of 0·1N-sulphuric acid at room temperature for the times stated. Any material extractable by chloroform was then recovered and weighed. The findings are tabulated below :

Sugar.	Wt. used, mg.	Chloroform-extractable material : Wt., mg. Hrs. in acid : 2.	6.	Yield (%) of sugar taken. 2.	6.	Diacetone cpd. resistant to 0·1N-H_2SO_4, %.
d-Glucose	500	82	0	11·3	0	0
d-Mannose	500	80	0	11·0	0	0
d-Xylose	500	49	0	6·4	0	0
l-Rhamnose	500	55 *	0	8·9 *	0	0
d-Ribose.....	270	—	50 *†	—	14·6 *	—
d-Galactose	390	345	340	68	65	95·6
l-Arabinose	500	663	663	86·4	86·4	100
l-Fucose	535	670	633	84·2	83·3	94·4

* Monoacetone derivatives.

† This material is probably a mixture of 2 : 3-monoacetone ribofuranose with the anhydro-derivative described by Levene and Stiller (*J. Biol. Chem.*, 1933, **102**, 187); sufficient material was not available for accurate investigation.

Monoacetone *d*-ribose is considered by Levene and Stiller (*loc. cit.*) to be substituted in positions 2 and 3 and to have a furanose ring; the behaviour of the crude product described above is intermediate between those compounds which have furanose rings and those resistant substances where the sugar ring is pyranose. It is possible that the acid-resistant portion is the anhydro-fraction of the crude material.

Fission of Sucrose : Separation of d-*Fructose from* d-*Glucose.*—1·00 G. of finely powdered sucrose was shaken for 6 hours with 20 ml. of dry acetone containing 1·25 ml. of sulphuric acid. The crude product, isolated by the customary procedure, was a crystalline mixture of diacetone glucose with the two diacetone fructoses. Treatment of this with 0·1N-sulphuric acid for 6 hours, followed by partition between chloroform and water, yielded 0·66 g. (86·8%) of crystalline material which, after recrystallisation from ligroin (b. p. 60—80°), weighed 0·593 g. (78%) and had m. p. 95° and $[\alpha]_D^{16°}$ (chloroform) — 24·7° (*c*, 5·2).

Separation of β-Diacetone Fructose from a Mixture of Seven Monosaccharides.—A mixture of 200 mg. of each of the following sugars was treated in the manner described for the foregoing experiment : *d*-glucose, *d*-galactose, *d*-mannose, *l*-arabinose, *d*-xylose, *l*-rhamnose, and *d*-fructose. After the acid treatment the crude product became partly crystalline. As it weighed 450 mg. it obviously contained diacetone galactose and diacetone arabinose as well as β-diacetone fructose. The crystals were drained on porous tile, after trituration with ice-cold ligroin (b. p. below 40°); recrystallisation in the usual way yielded 90 mg. of β-diacetone fructose, m. p. 95° alone or mixed with authentic substance.

Separation of Fructose, as the 1 : 2-*Monoacetone Derivative, from a Sugar yielding an Acid-stable Diacetone Derivative.*—If the mixture of sugars is condensed with acetone containing 0·3% of sulphuric acid as catalyst, as noted above, the fructose yields the acid-unstable α-diacetone derivative. (Galactose and arabinose yield 11% and 90% of their respective diacetone derivatives.) A mixture of 1·008 g. of α-diacetone fructose with 1·046 g. of diacetone galactose was kept in 0·1N-sulphuric acid solution for 4 hours at 16°. The unchanged diacetone galactose was extracted with chloroform, as in preceding experiments. The aqueous phase was made alkaline (phenolphthalein) with potassium hydrogen carbonate and heated at 100° for 30 minutes to destroy traces of reducing sugar. The solution was then evaporated. and the solid residue extracted three times with 50 ml. portions of boiling, dry ethyl acetate. On evaporation of this extract, 0·76 g. (89%) of nearly pure, crystalline 1 : 2-monoacetone *d*-fructose was obtained; m. p. 119°, $[\alpha]_D^{17°}$ (water) — 158·8°. After recrystallisation from dry ethyl acetate, the m. p. rose to 121—122° and $[\alpha]_D^{17°}$ (water) was — 159·4° (*c*, 3·4). From the chloroform phase of the partition 0·981 g. of diacetone *d*-galactose was recovered after evaporation of the solvent.

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