

**751.** *Toluene-*p*-sulphonylhydrazones of the Pentose Sugars, with Particular Reference to the Characterisation and Determination of Ribose.*

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Toluene-*p*-sulphonylhydrazones of L-arabinose, D-ribose, D-xylose, D-lyxose, and L-fucose have been prepared in good yield and form useful derivatives for characterisation of these sugars. Under standard conditions, the formation of crystalline D-ribose toluene-*p*-sulphonylhydrazone is quantitative and may be used for the determination of D-ribose. The optical rotatory powers of solutions of these derivatives in dry pyridine, and in pyridine containing small amounts of water and acid, have been studied.

AMONGST the pentose sugars, D-ribose occupies a unique position in that it is a component of the nucleic acids, found in all animal and plant cells, and of certain coenzymes and vitamins. The characterisation and determination of ribose are, therefore, of considerable importance. Crystalline derivatives of ribose have been described; for example, ribosebenzimidazole (Dimler and Link, *J. Biol. Chem.*, 1943, **150**, 345), ribose "diphenylmethane-dimethyl-dihydrazone" (von Braun, *Ber.*, 1913, **46**, 3949), and ribose diethyl thioacetal (Kenner, Rhodda, and Todd, *J.*, 1949, 1613). Ribose has been determined hitherto either by conversion into furfuraldehyde, which may be determined as the phloroglucide (Schorger, *Ind. Eng. Chem.*, 1928, **15**, 748; Dorée, "Methods of Cellulose Chemistry," Chapman and Hall, London, 1947, p. 381), by colorimetric methods (cf. Dunstan and Gillam, *J.*, 1949, S 140; Barker, *J.*, 1950, 1636), or by separation on the paper partition chromatogram and subsequent estimation by sodium meta-periodate (Hirst and Jones, *J.*, 1949, 1659) or colorimetrically by use of aniline phthalate (Blass, Macheboeuf, and Nunez, *Bull. Soc. Chim. biol.*, 1950, **32**, 130).

We have observed that ribose readily yields a highly crystalline toluene-*p*-sulphonylhydrazone, which is sparingly soluble in methanol, ethanol, or water, the normal solvents for sugars. The reagent, toluene-*p*-sulphonylhydrazide is a crystalline solid and possesses the advantages

of stability and ease of preparation. Calibration with varying quantities of D-ribose showed that, under standard conditions, the formation of the toluene-*p*-sulphonylhydrazone is quantitative and thus affords a gravimetric procedure for the determination of ribose (see Table, p. 3418). The reaction is not, however, specific, since arabinose, xylose, lyxose, and fucose readily yield crystalline toluene-*p*-sulphonylhydrazones in good yield under these conditions and will, therefore, interfere with the determination of ribose. This is not a serious drawback, however, since ribose normally does not occur in Nature in admixture with other sugars. Even so, the difficulty may be circumvented by chromatographic separation before the formation of the derivatives. The above toluene-*p*-sulphonylhydrazones form useful derivatives for the characterisation of pentoses, since their preparation is easy and the products may be identified by physical constants and also by fission with dilute acid or by heating them with benzaldehyde, whereafter the liberated pentose may be identified in the usual way on the paper chromatogram.

The use of toluene-*p*-sulphonylhydrazide for the characterisation of sugars was first described by Freudenberg and Rümmler (*Annalen*, 1924, 440, 45), who isolated derivatives of glucose and hamamelose; and, later, Freudenberg and Raschig (*Ber.*, 1929, 62, 373) formed fucose toluene-*p*-sulphonylhydrazone. This reagent has been used with advantage in conjunction with partition chromatography for the characterisation of arabinose, xylose, and ribose in the complex mixture arising from the condensation of glyceraldehyde with glycollic aldehyde (Hough and Jones, *J.*, 1951, 1122) and for the characterisation of L-fucose in the fission products of linseed mucilage (*Linum usitatissimum*) (Easterby and Jones, unpublished results).

Studies of sugar amine derivatives, stimulated by the occurrence of this type of linkage in the nucleic acids and certain coenzymes, have revealed that they can assume a number of different forms, possibly existing in the  $\alpha$ - and  $\beta$ -orientations of both the furanoside and pyranoside modifications, and that, in solution, there is a ready interchange of these various forms, presumably through the acyclic Schiff's base (Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 855; Honeyman and Tatchell, *J.*, 1950, 967; Butler, Laland, Overend, and Stacey, *J.*, 1950, 1433; Ellis and Honeyman, *Nature*, 1951, 167, 239). Although Honeyman and Tatchell (*loc. cit.*) and Ellis and Honeyman (*loc. cit.*) have pointed out that the existence of the aniline derivative of  $\alpha$ -D-ribofuranoside has not yet been firmly established, it has been suggested that this substance is partly converted in moist pyridine into the  $\beta$ -isomer, whereas in pyridine containing acidic catalysts it gives an equilibrium mixture of the  $\alpha\beta$ -pyranosides; in dry pyridine, mutarotation is arrested and therefore mutarotation occurs only in pyridine containing water or acid (Howard, Kenner, Lythgoe, and Todd, *loc. cit.*). In contrast, D-ribose toluene-*p*-sulphonylhydrazone mutarotates in dry redistilled pyridine ( $[\alpha]_D + 23^\circ \longrightarrow +14^\circ$ ), and in moist pyridine, the initial specific rotation is enhanced ( $[\alpha]_D + 42.5 \longrightarrow +16^\circ$ ). On the other hand, xylose and arabinose toluene-*p*-sulphonylhydrazones do not show mutarotation and give approximately the same specific rotation ( $[\alpha]_D + 3^\circ$  and  $-36^\circ$  respectively) in dry redistilled pyridine and in moist pyridine. However, the addition of a little hydrochloric acid to pyridine solutions of the above derivatives caused a marked change in their optical rotatory power. Thus, the arabinose derivative mutarotated from  $[\alpha]_D - 7^\circ$  to  $-13.5^\circ$  and the xylose derivative gave a constant specific rotation of  $-14^\circ$ . Thus, it may well be that the pentose toluene-*p*-sulphonylhydrazones are capable of existing in more than one modification, although there is no evidence available as yet to show whether the molecule is of amino-sugar or of Schiff's base type. In view of the marked effect that a trace of impurity in pyridine has on the initial and final specific rotations of certain of these derivatives, caution should be observed when optical rotatory power is applied to these or analogous derivatives as a criterion of identity or purity.

#### EXPERIMENTAL.

Unless otherwise stated, dry redistilled pyridine, prepared by distillation from potassium hydroxide pellets, was employed. M. p.s are uncorrected.

**General Procedure.**—The aldose is dissolved in methanol (10 ml.), to which is then added a solution (10 ml.) of toluene-*p*-sulphonylhydrazide in methanol (7 g. in 100 ml.). The solution is heated under reflux on the boiling water-bath for 30 minutes and then stored in the refrigerator. After approx. 20 hours, the crystalline residue is isolated on a tared filter, washed with ice-cold methanol (30 ml.), dried under reduced pressure over silica gel at 80° for 30 minutes, cooled in a desiccator, and weighed.

D-Ribose thus readily gave a crystalline *toluene-p-sulphonylhydrazone*, m. p. 164°,  $\{[\alpha]_D + 23^\circ$  (c, 0.7 in pyridine)  $\longrightarrow +14^\circ$  (16 h.);  $[\alpha]_D + 42^\circ$  (c, 1.4 in wet pyridine)  $\longrightarrow +14^\circ$  (17 h.);  $[\alpha]_D + 13^\circ$  (c, 1.4 in pyridine containing a little concentrated hydrochloric acid)  $\longrightarrow +9^\circ$  (24 h.);  $[\alpha]_D + 55^\circ$  (c, 0.86 in commercial pyridine)  $\longrightarrow +20^\circ$  (24 h.)\} (Found: C, 45.5; H, 5.5; N, 8.9.  $C_{12}H_{18}O_6N_2S$  requires C, 45.3; H, 5.7; N, 8.8%), which was only sparingly soluble in boiling methanol, ethanol, or water.

The results in the table show that solutions (1—4%) of ribose in methanol may be determined with an accuracy of  $\pm 1\%$ .

Ribose used (g.)	Yield of derivative (g.)	Ribose found (g.)	Error (g.)	Ribose used (g.)	Yield of derivative (g.)	Ribose found (g.)	Error (g.)
0.038	0.029	0.014	-0.024	0.187	0.389	0.187	0.000
0.083	0.161	0.077	-0.006	0.287	0.586	0.282	-0.005
0.100	0.227	0.109	+0.009	0.377	0.778	0.383	+0.006
0.109	0.226	0.109	0.000				

The effect of adding other sugars, which also give the derivative (see below), was examined. (i) A mixture of D-ribose (0.2808 g.) and L-arabinose (0.0376 g.) in methanol (10 ml.) was treated as above. The crystalline product was removed and, from its weight (0.6273 g.), it is clear that the arabinose has interfered with the estimation of ribose. (ii) D-Ribose (0.1902 g.) and D-xylose (0.0174 g.) were converted into the derivatives as described above and yielded only D-ribose toluene-*p*-sulphonylhydrazone (0.3854 g., 98% yield); hence the xylose has not interfered with the determination. (iii) D-Ribose (0.4148 g.) and D-xylose (0.1586 g.), treated as above, gave only D-ribose toluene-*p*-sulphonylhydrazone (0.6842 g., 75% yield), m. p. and mixed m. p. 164°. Hence, the xylose derivative, although not crystallising with the ribose derivative, has interfered with the determination.

L-Fucose (0.0914 g.) was treated with the reagent as above. The crystalline product (0.1584 g., 70%) was removed, washed with a little ice-cold methanol, and dried, to give fine white needles of L-fucose toluene-*p*-sulphonylhydrazone, m. p. 169°,  $[\alpha]_D -19^\circ$  (10 min.; *c*, 2.2 in pyridine)  $\rightarrow -9^\circ$  (23 h.) (Found: C, 46.9; H, 5.8; N, 8.6. Calc. for  $C_{18}H_{20}O_6N_2S$ : C, 47.0; H, 6.02; N, 8.4%).

L-Arabinose (0.1280 g.) gave a crystalline toluene-*p*-sulphonylhydrazone (0.2104 g., 78%), m. p. 153—154°,  $[\alpha]_D +3^\circ$  (*c*, 2.8 in pyridine, const.),  $[\alpha]_D +2^\circ$  (*c*, 2.1 in moist pyridine, const.),  $[\alpha]_D -7^\circ$  (*c*, 0.89 in pyridine containing a little concentrated hydrochloric acid)  $\rightarrow -13.5^\circ$  (24 h.) (Found: C, 45.5; H, 5.6; N, 8.9.  $C_{12}H_{18}O_6N_2S$  requires C, 45.3; H, 5.7; N, 8.8%).

D-Xylose (0.2338 g.) gave a toluene-*p*-sulphonylhydrazone (0.2450 g.) which crystallised as fine, white needles, m. p. 149°,  $[\alpha]_D -36^\circ$  (*c*, 1.8 in pyridine, const.),  $[\alpha]_D -36^\circ$  (*c*, 1.3 in moist pyridine, const.),  $[\alpha]_D -14^\circ$  (*c*, 1.8 in pyridine containing a little concentrated hydrochloric acid, const.),  $[\alpha]_D -14^\circ$  (*c*, 1.9 in commercial pyridine)  $\rightarrow -1^\circ$  (24 h.) (Found: C, 45.1; H, 5.7; N, 9.0.  $C_{12}H_{18}O_6N_2S$  requires C, 45.3; H, 5.6; N, 8.8%).

D-Lyxose gave a crystalline derivative, m. p. 141° (Found: N, 9.3.  $C_{12}H_{18}O_6N_2S$  requires N, 8.8%).

The derivatives of xylose, arabinose, lyxose, and fucose may be recrystallised from methanol. Derivatives of galactose, rhamnose, and fructose could not be obtained by the above procedure.

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