208. *Deoxy-sugars. Part XI. Further Observations on the Dische Reaction for 2-Deoxypentoses.*

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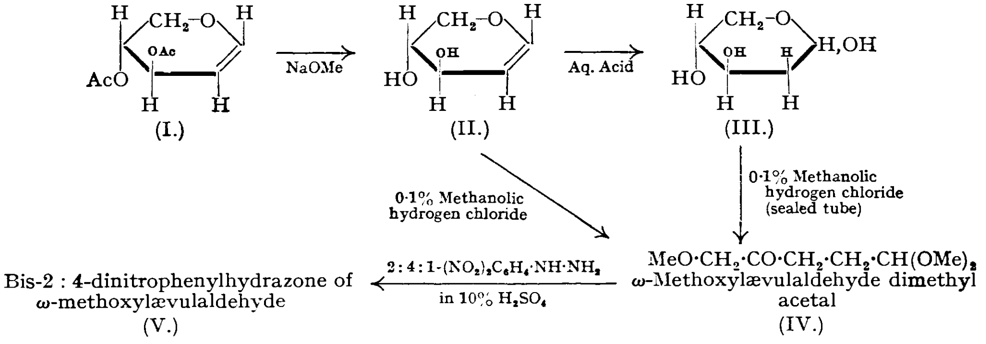
Confirmation is provided from an investigation on 2-deoxyxylose that the reaction between the Dische reagent (diphenylamine in acetic and sulphuric acids) and 2-deoxypentoses, resulting in the development of a characteristic blue colour, depends initially on the conversion of the 2-deoxypentose under acid conditions into ω-hydroxylævulaldehyde.

The specificity of the Dische reaction (*Mikrochemie*, 1930, **8**, 4) is based on the fact that deoxyribonucleic acid contains 2-deoxyribose, for it is this carbohydrate component which is responsible for the reaction with diphenylamine in acetic and sulphuric acids to give the blue colour. However, Pirie (*Brit. J. Exp. Path.*, 1936, **17**, 269) and others have emphasised that great care must be taken in interpreting the results of the test inasmuch as other substances present may interfere with the reaction. For example, reactions with agar and the carbohydrate of carragheen moss may give rise to somewhat similar colours, and yet other complex carbohydrates such as ovomucoid may also give interfering colour reactions.

In view of the important rôle which deoxyribonucleic acid plays in the nucleal material of cells (Mirsky and Pollister, *Proc. Nat. Acad. Sci.*, 1942, **28**, 344; Mirsky, " Advances in Enzymology and Related Subjects," Interscience Publishers, New York, 1943, **3**, 1; Avery, MacLeod, and McCarty, *J. Exp. Med.*, 1944, **79**, 137) and hence of the necessity for its detection and its accurate determination, it seemed desirable to examine this reaction in detail and to discover whether the blue colour was in fact due to the reaction of deoxyribose itself or to some transformation or decomposition product thereof.

Together with other colleagues, one of us (M. S.) (*J.*, 1949, 1222) showed that the reaction depends on the conversion of the 2-deoxypentose under acid condition into ω-methoxylævulaldehyde which then reacts with diphenylamine to give a mixture of solid complexes, some of which under acid conditions give in solution a typical blue colour and have a characteristic absorption band. Recently, we have been investigating the reactions of 2-deoxy-D-xylose (III) and have shown that this, like 2-deoxy-D-ribose, gives a characteristic blue colour when treated with the Dische reagent, and moreover, that the formation of the colour depends on the conversion of the 2-deoxy-D-xylose into ω-hydroxylævulaldehyde.

2-Deoxy-D-xylose (III) was prepared from D-xylose by the modified glycal procedure (Deriaz, Overend, Stacey, Teece, and Wiggins, *J.*, 1949, 1879). It was found that in order to obtain 3 : 4-diacetyl D-xylal (I) from 1-bromo 2 : 3 : 4-triacetyl D-xylose, it was necessary to carry out the reduction with zinc dust and 50% acetic acid at —12°, otherwise the main product was 1 : 2 : 3 : 4-tetra-acetyl D-xylose.

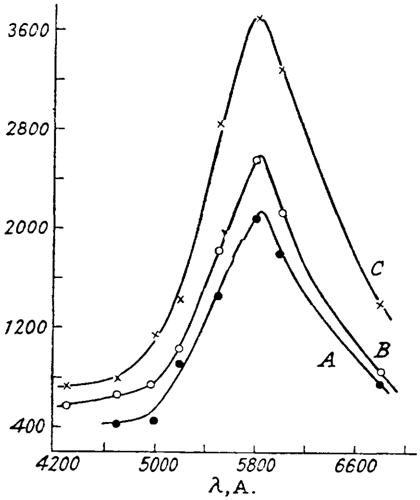


Diacetyl D-xylal (I) was deacetylated smoothly by sodium methoxide in dry methanol and afforded D-xylal (II) in good yield. Reacetylation afforded (I). With 5% sulphuric acid at 0° D-xylal gave 2-deoxy-D-xylose (III) (cf. Levene and Mori, *J. Biol. Chem.*, 1929, **83**, 803), which gave a very strong Dische test. Treatment with aniline in ethanol yielded a crystalline *anilide* which served to characterise further this deoxy-sugar.

When D-xylal (II) was heated under reflux with 0·1% methanolic hydrogen chloride it was readily converted into ω-methoxylævulaldehyde dimethyl acetal (IV) (a colourless liquid), characterised by formation of the crystalline bis-2 : 4-dinitrophenylhydrazone of the aldehyde

(V) (cf. Deriaz, Stacey, Teece, and Wiggins, *loc. cit.*). With 2-deoxy-D-xylose (III) more drastic conditions were required to effect the same conversion; *e.g.*, it was necessary to heat the sugar with 0·1% methanolic hydrogen chloride in a sealed tube at 145—150° for one hour to obtain any significant amounts of (IV).

Quantitative Dische tests were carried out on (II), (III), and (IV) in the manner described by Deriaz *et al.* (*loc. cit.*) and the absorption was measured in a Spekker absorptiometer. The figure shows the absorption curves obtained by plotting ε against λ, where ε is the molecular extinction coefficient, *i.e.*, *I* (Spekker reading) × *M*/concn. (in g./l.), and λ is the wave-length of the light transmitted. It is seen that ε is greatest for the ω-methoxylævulaldehyde dimethyl acetal (IV) and least for 2-deoxy-D-xylose (III). This is as expected, since D-xylal (II) is more easily converted into the keto-acetal (IV) than is the deoxy-pentose.



A. 2-*Deoxy*-D-*xylose.*
B. D-*Xylal.*
C. *ω-Methoxylævulaldehyde dimethyl acetal.*

These results indicate that both of the possible 2-deoxypentose isomers are able to be converted into (IV), the compound which reacts under acid conditions with diphenylamine to give the blue colour characteristic of the Dische test. Consequently, the reaction can be used only as a test for 2-deoxypentoses and not specifically for 2-deoxyribose. The presence of the latter sugar in deoxypentosenucleic acids needs supplementary confirmation by alternative methods (see Chargaff, Vischer, Doniger, Green, and Misani, *J. Biol. Chem.*, 1949, **177**, 405) to that of the Dische test before it can be accepted unequivocally as 2-deoxy-D-ribose.

EXPERIMENTAL.

D-*Xylal.*—3 : 4-Diacetyl xylal (17·6 g.) was dissolved in dry methanol (120 c.c.) containing sodium (150 mg.). The solution was kept for 2 days and then carbon dioxide was bubbled through it. The solvent was removed by evaporation under diminished pressure, and the syrupy residue extracted with ethyl acetate. The extract was evaporated to dryness, and the residue distilled. A colourless viscous syrup (7·6 g., 75%) was obtained, b. p. 100—105° (bath temp.)/0·05 mm., which crystallised. The solid was crystallised from ethanol–ether as prisms, m. p. 51°, $[\alpha]_D$ $-259° \pm 3°$ (*c*, 1·0 in water) (Levene and Mori, *J. Biol. Chem.*, 1929, **83**, 803, record m. p. 49—50°, b. p. 109—112°/1·2 mm., $[\alpha]_D^{26}$ −254·5° in water, −238·5° in ethanol).

2-*Deoxy*-D-*xylose.*—D-Xylal (6 g.) was treated with ice-cold 5% sulphuric acid (60 c.c.). The mixture was kept at 0° for 5 hours and then neutralised first with barium hydroxide and then with barium carbonate. The barium sulphate was removed by centrifuging and the solution was decolorised by filtering through charcoal. The solution was concentrated and the syrupy residue was dissolved in dry methanol (25 c.c.) and passed through a short column of activated alumina. The solution was evaporated to dryness and afforded colourless syrupy material (1·3 g.) which readily reduced Fehling's solution and gave a very strong Dische test. Trituration of this with methanol gave crystals which, recrystallised from methanol–ether, had m. p. 82—86°, and $[\alpha]_D^{19}$ −25·5° (initial) changing to −1·9° (equilibrium in 25 minutes; *c*, 0·5 in water) [Levene and Mori, *loc. cit.*, give m. p. 92—96° and $[\alpha]_D^{22}$ −2·0° (equilibrium in 20 minutes in water)].

2-*Deoxy*-D-*xylose Anilide.*—2-Deoxy-D-xylose (0·882 g.) was dissolved in dry methanol (10 c.c.) containing freshly distilled aniline (0·9 g.) and the mixture was heated under reflux for 3·5 hours. The solvent was removed by evaporation under diminished pressure and trituration of the syrupy residue with ethanol induced it to crystallise. It was recrystallised from ethanol, and 2-*deoxy*-D-*xylose anilide* (0·12 g.) was obtained as colourless needles, m. p. 137°, $[\alpha]_D^{19}$ −37·4° (6 minutes) changing to −20° (equilibrium after 24 minutes; *c*, 0·35 in water) (Found : C, 63·2; H, 7·0. $C_{11}H_{15}O_3N$ requires C, 63·1; H, 7·2%).

Preparation of ω-Methoxylævulaldehyde Dimethyl Acetal.—(i) *From* D-*xylal.* D-Xylal (1·3 g.) was heated under reflux with 0·1% methanolic hydrogen chloride (10 c.c.) for 4 hours. After neutralisation with silver carbonate, followed by evaporation of the methanol under diminished pressure, a colourless liquid, b. p. 80—90° (bath temp.)/*ca.* 8 mm., $n_D^{17.5}$ 1·4310, was obtained (0·38 g.). It gave a positive Dische reaction in minute amounts. When this keto-acetal (0·355 g.) was treated with a clear solution of 2 : 4-dinitrophenylhydrazine (0·9 g.) in 10% sulphuric acid at 38° for 24 hours a yellow precipitate separated and was collected. The precipitate was taken up in ethyl acetate (25 c.c.), and the solution filtered from insoluble material. Evaporation of the solvent gave a solid, which was recrystallised from chloroform. Yellow needles of the bis-2 : 4-dinitrophenylhydrazone were obtained, having m. p. 220—221° alone or in admixture with an authentic specimen [Deriaz *et al.*, *loc. cit.*, report b. p. 96—106° (bath temp.)/6 mm., n_D^{18} 1·4317, for the keto-acetal, and m. p. 221° for its bis-2 : 4-dinitrophenylhydrazone].

(ii) *From* 2-*deoxy*-D-*xylose.* 2-Deoxy-D-xylose (0·8 g.) was dissolved in 0·1% hydrogen chloride

(10 c.c.), and the solution heated in a sealed tube at 145—150° for one hour. The dark reaction mixture, was neutralised with silver carbonate and filtered through charcoal, and the solvent removed by evaporation under diminished pressure. The residue was fractionally distilled, and a liquid was obtained (0·1 g.), b. p. 89—90°/8 mm., n_D^{19} 1·4307, which when treated with a 1% solution of 2 : 4-dinitrophenylhydrazine in 10% (vol.) aqueous sulphuric acid at 37° for 24 hours gave the bis-2 : 4-dinitrophenylhydrazone (m. p. 221°) identical with that described above.

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