# 265. *Deoxy-sugars. Part I. The Dische Reaction for 2-Deoxypentoses.*

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The nature of the reaction between the Dische reagent (diphenylamine in acetic and sulphuric acids) for deoxyribonucleic acid and 2-deoxy-sugars has been investigated. The reaction is shown to depend upon the conversion of the 2-deoxypentose under acid conditions into ω-hydroxylævulic aldehyde which then reacts with diphenylamine to give a mixture of solid complexes, some of which under acid conditions give a typical blue coloration and a characteristic absorption band. Numerous carbohydrate derivatives studied under the conditions of the reaction give well-defined absorption bands which serve to establish the specificity of the Dische reaction.

Dische (*Mikrochemie*, 1930, **8**, 4) describes a reagent consisting of a mixture of diphenylamine, acetic acid, and concentrated sulphuric acid which on being heated with solutions of deoxyribonucleic acid under carefully controlled conditions gives an intense blue coloration. When suitable concentrations of deoxyribonucleic acid were used (*e.g.*, 0·02—0·2%) the intensity of the blue colour appeared to be proportional to the amount of the deoxyribonucleic acid present. The method was adopted by Sevag, Smollens, and Lackmann (*J. Biol. Chem.*, 1940, **134**, 523) for the determination of deoxyribonucleic acid in cellular material.

The specificity of the Dische reaction 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 to give the blue coloration. However, Pirie (*Brit. J. Exp. Path.*, 1936, **17**, 269) and others have emphasized 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, agar and the carbohydrate of carragheen moss (which may contain *aldehydo*-sugars) give somewhat similar colours and 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, McLeod, and McCarty, *J. Exp. Med.*, 1944, **79**, 137) and hence of the necessity for its detection and its accurate estimation, and more particularly the need for its differentiation from ribonucleic acid (spectrographic analysis failing to distinguish between the two acids), it seemed desirable to examine this reaction in detail and to determine whether the blue coloration was in fact due to the reaction of deoxyribose itself or to some transformation or decomposition product thereof. It was clearly desirable also to determine the origin of similar colorations which might be obtained when using other types of carbohydrate. Accordingly, a large number of carbohydrate and aldehydic substances were submitted to the precise conditions of this test and the results obtained could be classified into several groups according to the coloration produced (see Experimental section, Table I, etc.).

The following were typical: (*a*) Aldohexoses, aldohexose derivatives, and aldopentoses, *e.g.*, glucose, galactose, mannose, glucose-1 phosphate, glucose-6 phosphate, maltose, arabinose, and xylose; these substances gave *no coloration*. (*b*) Aldehydes, *e.g.*, benzaldehyde, salicylaldehyde, piperonal, furfuraldehyde, hydroxymethylfurfuraldehyde; these gave a *green coloration*, whereas acetaldehyde and cinnamaldehyde gave *brown colours*. (In these tests with liquid aldehydes the concentration of the aldehyde was probably greatly in excess of the prescribed amount of 10 mg. because 1 drop was used). (*c*) Ketoses, *e.g.*, fructose, sucrose, and sorbose, gave *green colorations*. (*d*) Hexals, *e.g.*, lactal, triacetyl glucal, and galactal, gave a *pink-violet coloration*. (*e*) Thymonucleic acid, 2-deoxy-D- and -L-ribose, D- and L-arabinal, and furfuryl alcohol gave the intense *characteristic blue coloration*. (*f*) Lævulic acid (as I) which is the final common acid-hydrolytic product of 2-deoxyribose, arabinal, and furfuryl alcohol, gave *no coloration*.

These preliminary tests showed that, in addition to thymonucleic acid, 2-deoxy-L-ribose (IV), L-arabinal (V), and furfuryl alcohol (III) also give the typical blue coloration. It was clearly desirable to discover the underlying reason for the fact that such structurally dissimilar compounds could exhibit a similarity of behaviour with this reagent. It seemed probable that the substance actually responsible for the coloration was some common acid degradation product which could be produced from all four of these substances, and such a common product is lævulic acid. However, the negative test given by this acid showed that it could not be the

colour-producing intermediate, so it was decided that an aldehyde precursor might be responsible and the isolation of such a substance was therefore attempted.

When we observed that furfuryl alcohol gave the characteristic blue colour in the Dische test, we noted that for some time it has been known that furfuryl alcohol can be converted by treatment with methyl-alcoholic hydrogen chloride into the dimethyl acetal of ω-methoxylævulic aldehyde (II), and that this moreover on further hydrolysis with methanolic hydrogen chloride can be transformed into methyl lævulate (I) (see Pummerer and Gump, *Ber.*, 1923, **56**, 999; Pummerer, Guyot, and Birkofer, *Ber.*, 1935, **68**, 480). It was therefore decided to prepare this aldehyde and to investigate its behaviour under the conditions of the Dische reaction.

Accordingly (III) was boiled for 3 hours with methanolic hydrogen chloride (0·1%) (Pummerer and Gump, *loc. cit.*) and thereby was converted into the acetal (II) (a colourless liquid) which was characterised by formation of the crystalline *bis*-2 : 4-*dinitrophenylhydrazone* of the aldehyde. ω-Hydroxylævulic aldehyde or its derivatives, under the conditions of the Dische reaction, did indeed give the characteristic blue coloration, and quantitative measurements on the Spekker photoelectric absorptiometer (described below) showed that the intensity of the colour was slightly greater than that given by equivalent weights of (III), (IV), (V), or deoxyribonucleic acid.

Attempts were next made to convert both 2-deoxyribose and arabinal into (II). L-Arabinal with 0·1% methanolic hydrogen chloride was readily converted into (II), which was identified as before by formation of its bis-2 : 4-dinitrophenylhydrazone. On the other hand, 2-deoxyribose with 0·1% methanolic hydrogen chloride gave, not (II), but a new crystalline methylglycoside of 2-deoxyribose, which will be described in detail in a later communication. From its behaviour on acid hydrolysis it appeared to be β-methyl-2-deoxyribopyranoside and it also gave the characteristic blue colour on being treated with the Dische reagent.

Under more drastic conditions such as obtain when 2-deoxyribose was heated under pressure with 0·1% methanolic hydrogen chloride at 180°, a mixture of methyl lævulate and (II) was obtained. Separation of the two liquids was effected by treatment of the mixture with 10% methanolic potassium hydroxide, by means of which the methyl ester was hydrolysed and the unaffected (II) was then extracted by ether. It was identified as previously described.

It is thus established that 2-deoxyribose, arabinal, and furfuryl alcohol are all convertible into one and the same degradation product, namely ω-methoxylævulic aldehyde, isolated in the form of its dimethyl acetal (II). Attempts to obtain the same compound directly from thymus deoxyribonucleic acid were, however, not successful, although after treatment with methanolic hydrogen chloride and distillation of the product a liquid was obtained which did give a positive Dische reaction and probably contained (II).

With another colleague, we have obtained from sperm deoxyribonucleic acid, a deoxyribose derivative of thymine which does indeed yield ω-hydroxylævulic aldehyde on acid treatment.

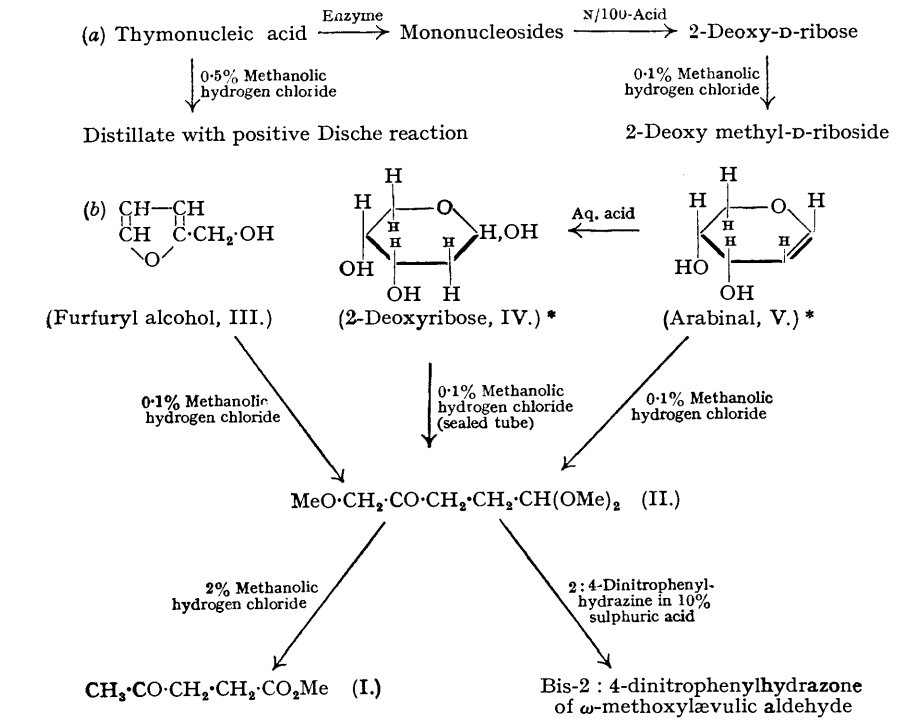
The main reactions are summarized on p. 1224.

In view of the importance of deoxyribonucleic acid in natural products and of the need for its accurate detection and determination, the specificity of the Dische reaction has been further examined quantitatively by a study of the absorption curves of the coloured reaction products obtained by heating a wide variety of carbohydrate derivatives with the reagent.

In each case highly purified specimens of the various substances were used and the following experimental procedure was adopted so that exactly comparable results were obtained. The Dische reagent (6 c.c.) was mixed with water (3 c.c.) containing a known weight of the substance to be tested (final concentration of substance, 0·02—1·0 mg./c.c.), and the mixture heated in a standard test-tube in a briskly-boiling water-bath (3·25 min.). After exactly 15 minutes' cooling in ice-water, the absorption was measured immediately in a Spekker absorptiometer, and the absorption curve obtained by plotting $\varepsilon$ against $\lambda$, where $\varepsilon$ is the molecular extinction coefficient [*i.e.*, $I$ (Spekker reading) $\times$ $M$/conc. (in g./l.)], and $\lambda$ is the wave-length of the light transmitted. It was found that the substances tested could be divided sharply into four classes (Figs. 1—4), as follows.

(1) *Substances giving the characteristic blue coloration.* Deoxyribose derivatives and related compounds gave absorption curves showing a characteristic band with the maximum at $\lambda$ 5800 (see Fig. 1) identical with that given by (II). These results are in accordance with the view given previously that the compound responsible for the characteristic blue coloration is ω-hydroxylævulic aldehyde and that only those substances which can be transformed into this compound will give a positive specific reaction. From Fig. 1 it is seen that of all the substances examined $\varepsilon$ is greatest for the keto-acetal (II) and that D- and L-arabinal and furfuryl alcohol, which are more

easily converted into the keto-acetal than is 2-deoxyribose, show a greater intensity of colour than the latter, whereas on the other hand stable glycosidic derivatives of deoxyribose show a




diminished value for ε. It is of interest that dimethyl β-methyl-2-deoxy-L-ribopyranoside shows a much lower value of ε than do the other derivatives—a result which can now be expected in

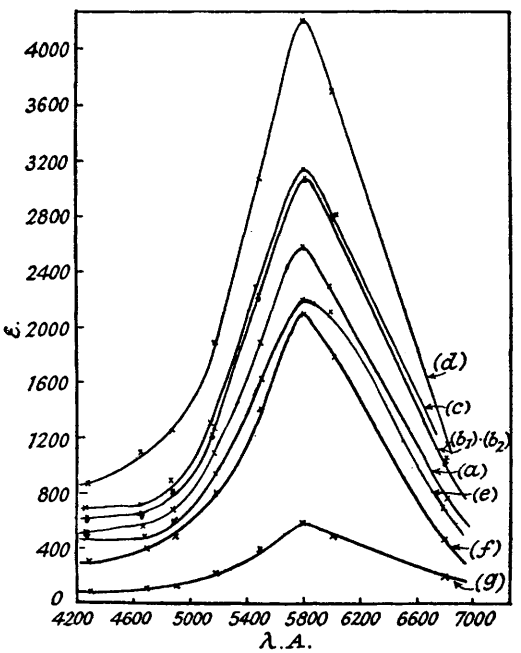


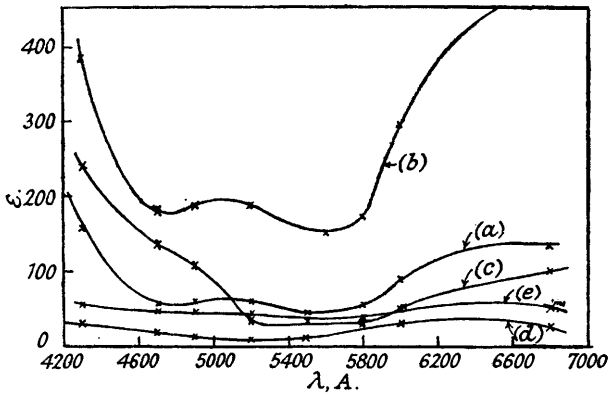

FIG. 1.

(*a*) *2-Deoxy-D-ribose.*
($b_1$) D-*Arabinal.*
($b_2$) L-*Arabinal.*
(*c*) *Furfuryl alcohol.*
(*d*) *ω-Methoxylævulic aldehyde dimethyl acetal.*
(*e*) *Sodium thymonucleate.*
(*f*) *β-Methyl-2-deoxy-L-ribopyranoside.*
(*g*) *Dimethyl derivative of* (*f*).

* For convenience, formulae (IV) and (V) have been written as the D-isomers although the reactions described were carried out with the L-isomers.

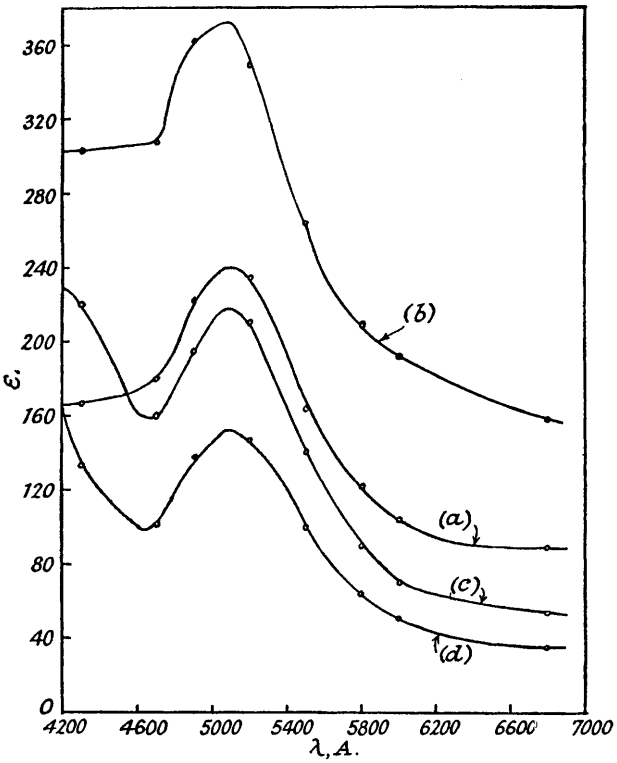
that the transformation of this compound into ω-hydroxylævulic aldehyde would be much more difficult inasmuch as it involves the cleavage of an ether methyl group. Other derivatives

FIG. 2.




(*a*) *Hydroxymethylfurfuraldehyde.*
(*b*) *Bis-(2-furfurylmethyl) ether.*
(*c*) *Tetra-acetyl oxyglucal.*
(*d*) *Aldol.*
(*e*) *Glyceraldehyde.*

FIG. 3.




(*a*) *Lactal.*
(*b*) *Triacetyl glucal.*
(*c*) *Hexa-acetyl lactal.*
(*d*) *Hexa-acetyl maltal.*

which gave a positive result in this reaction were α-methyl-2-deoxy-L-ribopyranoside, αβ-methyl-2-deoxy-D- and -L-ribofuranosides, dimethyl αβ-methyl-2-deoxy-L-ribofuranoside, 2-deoxy-D-ribose anilide, and β-2-deoxy-D-ribose penta-acetate.

(2) *Substances giving no interfering coloration.* It was established that the following

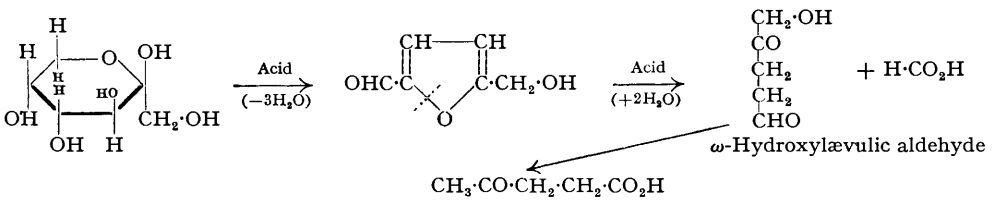
substances gave no colour in the given concentrations (mg./c.c.) and that the colours given with concentrations 10 times these quoted do not interfere in this reaction: Glucose (0·16), arabinose (0·13), maltose (0·26), lactose (0·3), fructose (0·23), sucrose (0·37), sorbose (0·3), ascorbic acid (0·18), acetaldehyde (0·2), formaldehyde (0·2), benzaldehyde (0·2), acetone (0·2), lævulic acid (0·3).

(3) *Substances giving a green coloration.* It has been mentioned by Pirie (*loc. cit.*) that certain substances such as agar and the polysaccharide of carragheen moss which may contain *aldehydo*-sugars produce interfering green colorations in this reaction. However, in experiments carried out with 3 : 6-anhydroglucose and with 3 : 6-anhydro-α-methylmannoside, which, as the free sugars, may possess aldehydic properties under some conditions, only very pale green colorations were obtained. On the other hand, examination of certain aldehydes such as aldol, glyceraldehyde, and hydroxymethylfurfuraldehyde revealed that these substances give a green coloration of higher intensity, but in all cases the value of ε is much less than that shown by deoxypentose derivatives. The absorption curves in these cases (Fig. 2) show no striking band.

(4) *Substances giving a pink-violet coloration.* Examination of several hexal derivatives showed that all these substances gave a pink-violet colour in this reaction, the absorption curves (Fig. 3) having a band with maximum absorption at λ 5100, but the values of ε were much lower than for the corresponding pental series. This reaction might prove useful for the detection of derivatives of the hexal type.

From these experiments it seems clear that the presence of carbohydrate derivatives and decomposition products, or of aldehydes, while perhaps giving apparently interfering coloration during superficial examination in the test-tube, is unlikely to affect the comparative results of deoxyribose estimations if the experimental procedure is rigidly applied with suitable concentrations of material and with careful determination of light-absorption data.

In the course of the previous work it was noticed, when using a modified Dische reagent which contained a higher concentration than usual of sulphuric acid, that certain keto-sugars gave a blue colour apparently similar to that given by deoxypentoses in the standard test. It is known, however, that fructose is easily transformed by acid into hydroxymethylfurfuraldehyde and that on further acid hydrolysis this in turn gives lævulic acid. It was thought therefore that the blue coloration might again result from ω-hydroxylævulic aldehyde which might be formed as an intermediate product in this series of reactions as follows:




Various keto-carbohydrate derivatives were therefore treated by the following modified procedure; the Dische reagent (5 parts) was mixed with concentrated sulphuric acid (1 part) and water (3 parts) containing a known weight of the substance to be tested (final concentration of substance, 0·02—1·0 mg./c.c.), and the mixture heated on a briskly-boiling water-bath for 5 minutes and then kept at room temperature for 15 hours before measurement of the colour intensity on a Spekker absorptiometer.

2-Deoxyribose was also submitted to this modified procedure. The following results were obtained.

(1) *Substances giving a blue coloration.* (*a*) Deoxyribose and related substances gave a characteristic blue coloration, and the absorption curves were similar to those shown in Fig. 1 but ε was less than in the case of the standard test owing most probably to rapid hydrolysis of some deoxypentose to lævulic acid under these conditions.

(*b*) The following substances (see Fig. 4) also gave an intense blue coloration but the absorption curves are quite distinct from those obtained from the deoxypentose derivatives and show two bands with maximum absorption at λ 5200 and λ 6300 approx.

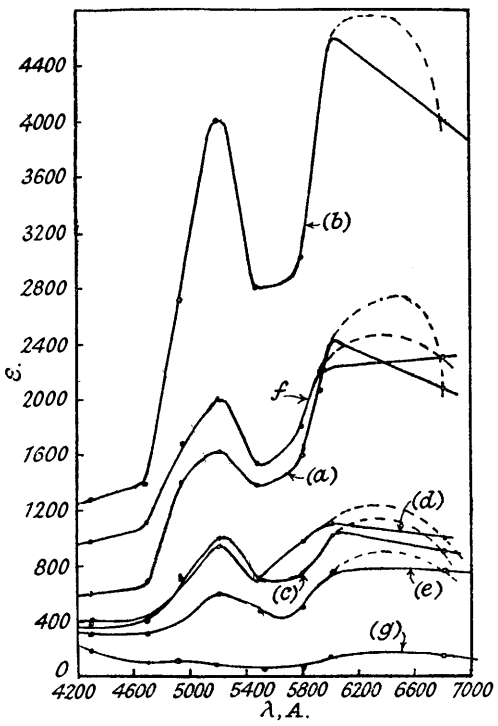
It is seen from Fig. 4 that hydroxymethylfurfuraldehyde and the related bis-(2-furfurylmethyl) ether give absorption curves of the same shape as that obtained with ketohexoses, although with the first two substances ε has a greater value. It is therefore probable that in this reaction ketohexoses decompose to give hydroxymethylfurfuraldehyde and that this then reacts with the diphenylamine reagent. There is no clear indication of the formation of the keto-acetal (II) in this reaction. It is possible that this reaction could provide a useful method

for the detection of ketoses, and a comparison of ε at λ 5200 might be developed as a method of determination for keto-sugars.

(2) *Substances giving a green coloration.* Certain aldehydes such as aldol and furfuraldehyde give green colorations in this reaction but in all cases the value of ε is extremely low (see Fig. 4, Curve *g*, for furfuraldehyde).

(3) *Substances giving no coloration.* The following substances gave no coloration in this reaction : glucose (0·3 mg./c.c.), arabinose (0·14), lactose (0·3), ascorbic acid (0·18), acetaldehyde (0·2), benzaldehyde (0·2), formaldehyde (0·2), acetone (0·2).

FIG. 4.




(*a*) *Hydroxymethylfurfuraldehyde.*
(*b*) *Bis-(2-furfurylmethyl) ether.*
(*c*) *Fructose.*
(*d*) *Sucrose.*
(*e*) *Sorbose.*
(*f*) *Tetra-acetyl oxyglucal.*
(*g*) *Furfuraldehyde.*

A comparison of diphenylamine oxidation products with the products of the Dische reaction was now made, for it is well known that diphenylamine gives a blue-purple coloration when treated with certain oxidising agents such as potassium dichromate in acid solution. It has been shown (see Knop, *J. Amer. Chem. Soc.*, 1924, **46**, 263; Kolthoff and Sarver, *ibid.*, 1930, **52**, 4179) that the reaction proceeds according to the following scheme :

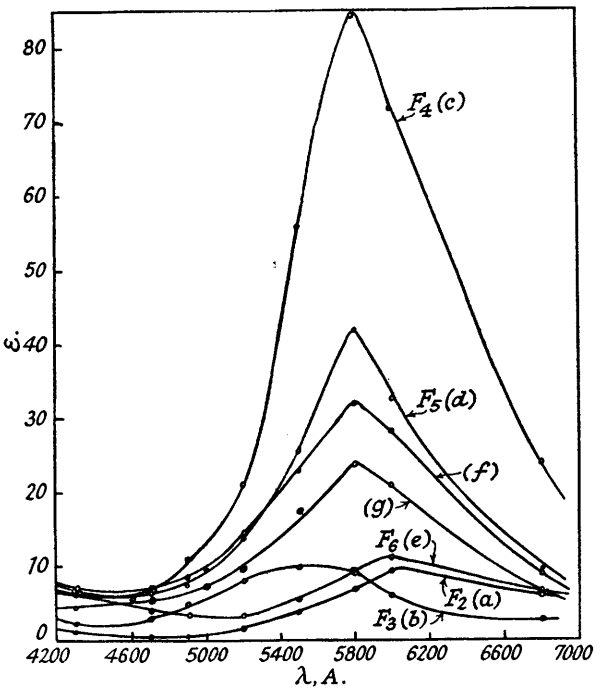
$$2NHPh_2 \xrightarrow{-H_2} \underset{\text{Diphenylbenzidine.}}{NHPh\cdot C_6H_4\cdot C_6H_4\cdot NHPh} \rightleftharpoons \underset{\text{Diphenylbenzidine-violet.}}{NPh{:}C_6H_4{:}C_6H_4{:}NPh} + 2H^+ + 2e$$

It might have been objected that the blue colour developed in the Dische reaction was related to diphenylbenzidine-violet, so this substance was prepared (see p. 1231) and two series of absorption measurements were taken with different solutions. It was found that the general character of the curves was similar to that shown by the blue of the Dische reaction; diphenylbenzidine-violet, however, had the maximum at λ 5500 instead of λ 5800. When diphenylbenzidine was put into acid of the same concentration as that of the Dische reagent it gave a curve with maximum at λ 5800. However, this blue colour was unstable to heat and disappeared on standing, whereas the colour given with the Dische reagent was quite stable and only changed in intensity over long periods of time.

The ultra-violet absorption of diphenylbenzidine-violet was compared with that given by

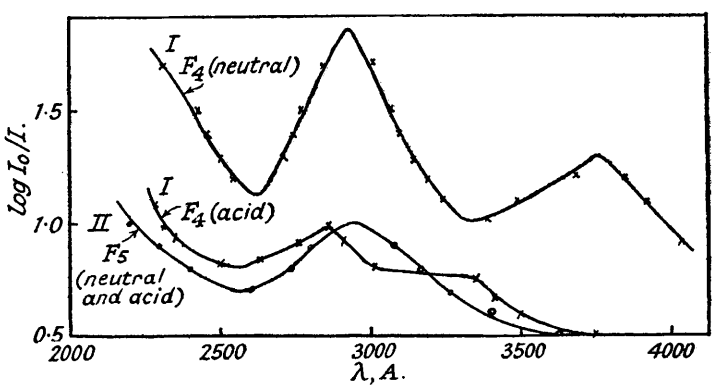
fraction $F_4$, the true "Dische" blue fraction actually isolated as described below from the products of the Dische reaction (Fig. 6): $F_4$ shows two bands, λ 2960 (log $I_0/I$ = 1·9) and λ 3750 (log $I_0/I$ = 1·3), in neutral solution, and one band, λ 2850 (log $I_0/I$ = 1·0), in acid

FIG. 5.




(*a*) $F_2$, *aq. alcohol acidified to* 5N.
(*b*) $F_3$, ,, ,, ,,
(*c*) $F_4$, ,, ,, ,,
(*d*) $F_5$, *neutral alcohol.*
(*e*) $F_6$, *acidified alcohol.*
(*f*) *Furfuryl alcohol (heated with Dische reagent).*
(*g*) *ω-Methoxylævulic aldehyde dimethyl acetal (heated with Dische reagent).*

FIG. 6.




*Ultra-violet absorption spectra of $F_4$ and $F_5$.*

solution. On the other hand, diphenylbenzidine-violet shows no band between λ 2300 and λ 4500.

For the following reasons we consider that the blue coloration of the Dische reaction is not identical with diphenylbenzidine-violet: (*a*) In the Dische reaction there is a very great excess

of diphenylamine necessary for production of the blue colour, so it would not be possible for the oxidation reaction to take place, for insufficient oxygen would be present to produce diphenylbenzidine-violet. (*b*) The Dische colour appears to be stable on being kept or on being heated, whereas diphenylbenzidine-violet is unstable to heat and disappears on being kept. (*c*) The ultra-violet spectrum differs from that of $F_4$ obtained from the Dische reaction as described below.

Finally, we have attempted the isolation of the actual substance responsible for the blue colour in the Dische reaction. It has been suggested by Cohen (*J. Biol. Chem.*, 1944, **138**, 691) that condensation of secondary amines with aldohexoses, aldopentoses, or aldehydes of the type CHO·CH(OH)·R″ does not result in the formation of coloured products because these substances can participate in the Amadori reaction (*Atti R. Accad. Lincei*, 1925, **2**, 337; 1931, **13**, 72, 195), the course of the reaction then proceeding to the ketone (*C*), in which the length of the conjugated system is not increased. With deoxypentoses and substances of the type CHO·$CH_2$R″, however, the reaction cannot proceed beyond stage (*B*), thus increasing the number of conjugated double

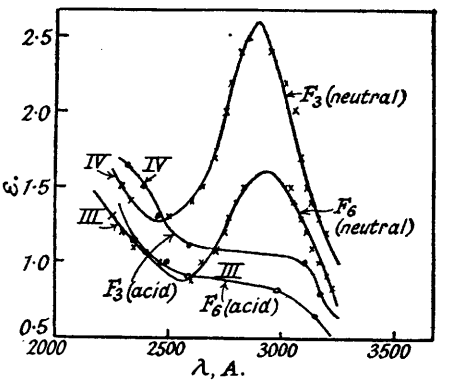
$$\mathrm{NHRR' + OCH{\cdot}CHR''{\cdot}OH \longrightarrow \overset{+}{N}RR'{:}CH{\cdot}CHR''{\cdot}O\overset{-H+}{H} \longrightarrow}$$
$$\underset{(A.)}{} \quad \underset{(B.)}{\mathrm{NRR'{\cdot}CH{:}CHR''{\cdot}OH}} \longrightarrow \underset{(C.)}{\mathrm{NRR'{\cdot}CH_2{\cdot}COR'}}$$

bonds, particularly if R″ also contains a conjugated system. The specificity of a special tryptophan reaction for deoxypentoses is ascribed to the formation of compounds of this type (Cohen, *loc. cit.*). It was also suggested that the reddish-purple colour obtained by treatment of deoxyribose with the Dische reagent at room temperature is due to a similar condensation, whereas the blue coloration obtained on heating was thought to involve a more complex series of reactions.

For this reason we examined quantitatively the reddish-purple colour produced by deoxyribose and the Dische reagent in the cold. The curves obtained (*a*) after 24 hours and (*b*) after 48 hours showed that maximum absorption again took place at λ 5800, and it was therefore clear that the reaction follows the same course in the cold as on heating.

In order to gain further information on this question an attempt was made to isolate the substance responsible for the blue coloration and to investigate its properties. The method used was to treat either 2-deoxyribose or arabinal with the Dische reagent in the usual way. The sulphuric acid was neutralised with barium acetate, and the coloured products separated from the filtered concentrated liquid by extraction with ether. Separation of the coloured products from unreacted diphenylamine was achieved by means of an alumina chromatogram. A small portion of one fraction gave an intense blue coloration on treatment with acid, and the product in the fraction was obtained in the form of an orange-coloured powder.

FIG. 7.




*Ultra-violet absorption spectra of $F_3$ and $F_6$.*

This material, with several others—fractions $F_1$—$F_6$—was made on a larger scale by reaction of furfuryl alcohol with diphenylamine. It was clear that the one fraction ($F_4$), although obtained in only about 10% yield of the total, was the actual material responsible for the typical blue colour, since it had the absorption band at λ 5800 characteristic of ω-hydroxylævulic aldehyde in the test. Another fraction $F_5$ actually gave the blue coloration in neutral solvents and possessed a similar absorption curve (see Figs. 6 and 7). However, it is clear that these two fractions are not the main product of the reaction, and the blue coloration obviously cannot be identified with the presence of a single condensation product of the secondary amine.

These results would indicate that the Dische reaction is unlikely to be of high reliability in the absolute quantitative sense even when rigorously applied with the use of the Spekker absorptiometer.

On the other hand, with 2-deoxyribose or the dimethyl acetal as a standard, it is of great value for comparative estimations particularly of 2-deoxyribonucleic acid derivatives.

## EXPERIMENTAL.

*Preparation of ω-Methoxylævulic Aldehyde Dimethyl Acetal.*—(i) *From furfuryl alcohol.* Dry furfuryl alcohol (100 g.) was heated under reflux for 3 hours with 0·1% anhydrous methanolic hydrogen chloride

(1 l.). After cooling and neutralisation with silver carbonate followed by evaporation of the methanol under diminished pressure, a yellowish-brown liquid (70 g.), b. p. (bath temp.) 65—140°/6 mm., was obtained. Purification by formation of the bisulphite compound, followed by extraction with ether to remove furfuryl methyl ether and decomposition of the bisulphite compound, gave a colourless liquid (43 g.) which distilled over the range 74—120°/6 mm. This was carefully fractionated to give the following colourless liquids: (*a*) 11·2 g., b. p. 80°/6 mm.; (*b*) 4·0 g., b. p. 80—84°/6 mm.; (*c*) 26·2 g., b. p. 84—90°/6 mm. The distillate (*c*) was refractionated 3 times more and finally a colourless liquid (10·8 g.), b. p. 89—90°/6 mm., $n_D^{18°}$ 1·4236 (Found: OMe, 51·6. Calc. for $C_8H_{16}O_4$: OMe, 52·9%), was obtained. This ω-methoxylævulic aldehyde dimethyl acetal in minute amounts gave a positive Dische reaction.

The dimethyl acetal was characterised by its conversion into the *bis*-2 : 4-*dinitrophenylhydrazone* of the aldehyde by treating it with a 1% solution of 2 : 4-dinitrophenylhydrazine in 10% (vol.) aqueous sulphuric acid at 37° for 24 hours. A yellow solid separated, which after four recrystallisations from hot ethyl acetate gave yellow needles, m. p. 221° (Found: C, 44·3; H, 3·43; N, 23·3; OMe, 6·28. $C_{18}H_{18}O_9N_8$ requires C, 44·1; H, 3·67; N, 22·9; OMe, 6·33%).

Treatment of the dimethyl acetal with boiling 2% hydrogen chloride transformed it into methyl lævulate, identified as the 2 : 4-dinitrophenylhydrazone, m. p. 141°.

(ii) *From* L-*arabinal.* After various trial experiments L-arabinal (1 g.) (prepared by the method of Meisenheimer and Jung, *Ber.*, 1927, **60**, 1462) was heated under reflux with 0·1% methanolic hydrogen chloride (10 c.c.) for 3 hours. After neutralisation with silver carbonate followed by evaporation of the methyl alcohol under reduced pressure a colourless liquid, b. p. 96—106° (bath temp.)/6 mm., $n_D^{18°}$ 1·4317, was obtained (0·4 g.) (Found: OMe, 41·2%). This distillate gave a strong Dische reaction. Conversion into the 2 : 4-dinitrophenylhydrazone by the method used in (i) yielded a yellow solid which, after several recrystallisations from ethyl acetate, gave yellow needles, m. p. and mixed m. p. with preparation in (i) 221° (Found: C, 44·5; H, 3·92%).

The isolation of this dimethyl acetal was then attempted from 2-deoxy-L-ribose as starting material. It is known that L-arabinal is easily convertible into 2-deoxy-L-ribose by treatment with N-sulphuric acid, but the conversion of the deoxypentose into the dimethyl acetal was found to be much more difficult than is the case in (i) or (ii) (above), showing, in our opinion, that it is unlikely that these last two substances pass through the deoxy-sugar stage in their conversion into the keto-acetal.

*Treatment of* 2-*Deoxy*-L-*ribose with* 0·1% *Methanolic Hydrogen Chloride.*—In a preliminary experiment, 2-deoxy-L-ribose was heated with 0·1% methanolic hydrogen chloride under reflux for 3 hours, and after neutralisation of the acid and removal of the methanol by evaporation under reduced pressure, extraction with ether gave a syrup which failed to yield any distillate of b. p. below 110°/6 mm. (the dimethyl acetal has b. p. 89—90°/6 mm.), but which crystallised on being kept. This substance was identified as a methylglycoside of 2-deoxy-L-ribose, and it also gave the characteristic blue colour in the Dische test.

Since 0·1% methanolic hydrogen chloride failed to disintegrate the sugar ring, the experiment was repeated with 0·6% methanolic hydrogen chloride but the same glycosidic product was still obtained. It was therefore evident that more drastic methods of hydrolysis were needed, but it was also essential that these should not be too drastic since the dimethyl acetal if formed would be directly converted into methyl lævulate. Consequently, some efforts were made to determine the stability of the acetal.

*Decomposition of* ω-*Methoxylævulic Aldehyde Dimethyl Acetal with Methanolic Hydrogen Chloride.*—A 10% solution of the dimethyl acetal, heated under reflux with methanolic hydrogen chloride (3%) for 1½ hours, was completely transformed into methyl lævulate, having a negative Dische reaction and identified as the 2 : 4-dinitrophenylhydrazone, m. p. 141°. This experiment showed that it was unlikely that the dimethyl acetal could be isolated by acid hydrolysis at ordinary temperatures and pressures so that sealed tube experiments were then investigated.

In a typical example the dimethyl acetal was heated with 0·1% methanolic hydrogen chloride in a sealed tube at 100°; 65% was destroyed after being heated for 45 minutes. A further rise in temperature seemed to have little effect on the rate of decomposition.

*Treatment of* 2-*Deoxy*-L-*ribose with* 0·1% *Methanolic Hydrogen Chloride in a Sealed Tube.*—A series of experiments showed that the methylglycoside formed by heating under reflux was still the chief product when the reaction was carried out in a sealed tube at 128—130° for 1½ hours, and that decomposition of the glycoside did not take place until the temperature was raised to 180°. It was therefore evident that a high yield of the dimethyl acetal could not be expected but that the most favourable conditions for the isolation of this substance from L-deoxyribose would be a brief treatment at 180°.

2-Deoxy-L-ribose (3 g.) was therefore heated in a sealed tube with 0·1% methanolic hydrogen chloride (30 c.c.) at 180° for 30 minutes. After neutralisation, removal of the methanol, and extraction with ether, a distillate (0·86 g.), b. p. 85—110°/6 mm., was obtained which gave a positive Dische reaction, but quantitative measurements of the blue coloration and methoxyl determinations revealed that it consisted of methyl lævulate (90%) with only 10% of the dimethyl acetal. Separation of these two substances by means of dilute aqueous sodium hydroxide was impracticable, but a separation was effected by keeping the mixture overnight with methanolic potassium hydroxide (10%) and subsequently refluxing it for 20 minutes. Extraction with ether gave a syrup which gave the bis-2 : 4-dinitrophenylhydrazone, m. p. 221°, shown conclusively by means of an *X*-ray powder photograph to be identical with that prepared from an authentic specimen of ω-methoxylævulic aldehyde dimethyl acetal.

*Treatment of Deoxyribonucleic Acid from Thymus Gland with Methanolic Hydrogen Chloride.*—So far it has not been possible to obtain deoxyribose from deoxyribonucleic acid by direct chemical methods (*i.e.*, without use of enzymes), the hydrolytic methods necessary to break down the acid into nucleotides resulting in the transformation of the deoxy-sugar into lævulic acid.

Treatment of thymus deoxyribonucleic acid with 0·5% methanolic hydrogen chloride under reflux for long periods showed no dimethyl acetal or glycoside formation, but preliminary experiments using 0·5% methanolic hydrogen chloride in a sealed tube indicated that some decomposition occurred. Finely ground thymus deoxyribonucleic acid (1·3 g.) was therefore heated in a sealed tube with methanolic

hydrogen chloride (8 c.c., 0·5%) for 1 hour at 135°. After neutralisation, removal of the methanol, and extraction with ether, a small amount of distillate, b. p. 60—90°/6 mm., was collected, and this had a positive Dische reaction. By suitable adjustment of experimental conditions it is therefore probable that by this means it will be possible to isolate a methyldeoxyribose or the dimethyl acetal from the nucleic acid.

*Preparation of Diphenylbenzidine-violet.*—Diphenylamine (0·113 g.) was dissolved in 1 l. of N-sulphuric acid, the solution kept at 50°, and an aqueous solution of potassium dichromate (calculated quantity for the first stage of the reaction on p. 1227) slowly added with brisk stirring. When the addition was complete the solution was boiled and diphenylbenzidine separated as a fine white powder, m. p. 244°. Another equivalent of potassium dichromate was then added and the diphenylbenzidine was converted into diphenylbenzidine-violet. Its ultra-violet absorption was measured on two solutions: (*a*) 0·2 g./l., (*b*) 0·05 g./l. :

| (*a*) λ, A. | Spekker reading. | (*a*) λ, A. | Spekker reading. | (*b*) λ, A. | Spekker reading. | (*b*) λ, A. | Spekker reading. |
|---|---|---|---|---|---|---|---|
| 4300 | 0·372 | 5500 | 0·758 | 4300 | 0·231 | 5500 | 0·492 |
| 4700 | 0·428 | 5800 | 0·712 | 4700 | 0·270 | 5800 | 0·468 |
| 4900 | 0·536 | 6000 | 0·579 | 4900 | 0·332 | 6000 | 0·377 |
| 5200 | 0·646 | 6800 | 0·272 | 5200 | 0·413 | 6800 | 0·192 |

*Condensation of Deoxyribose with the Dische Reagent.*—Deoxyribose (*ca.* 2 mg.) was dissolved in water (6 c.c.) and mixed with the Dische reagent (12 c.c.) and the mixture was heated in a boiling water-bath for 3 minutes. After cooling in ice-water the sulphuric acid of the Dische reagent was neutralised by addition of barium acetate solution (2·5 g. in 5 c.c. water). After centrifuging, the solution was extracted several times with ether. By evaporation of the solvent and testing the residue, it was found that the compound responsible for the blue coloration was completely extracted with ether.

*Condensation of* D-*Arabinal with Diphenylamine.*—A slightly larger-scale experiment was then carried out with D-arabinal as the starting material. D-Arabinal (80 mg.) was dissolved in water (240 c.c.), the solution mixed with the Dische reagent (480 c.c.), and the whole heated in 10-c.c. portions on a boiling water-bath for 3 minutes. After cooling, the solution was freed from sulphate ions, centrifuged, and extracted with ether as before, and the extracts were dried ($Na_2SO_4$) and evaporated to dryness. Finally, the residue was dissolved in dry benzene, and the solution poured on an alumina column (30 cm. × 1 cm.). The chromatogram was developed by elution with benzene–alcohol (100 : 2, parts by vol.) and the coloured products were retained by the column, the diphenylamine passing through. The fractions of the eluted solution were collected, evaporated to dryness, and tested with acid (5N). One fraction had an intense blue coloration. This gave an orange solution in alcohol and on evaporation of the solvent gave a yellow amorphous solid, soluble in ether and most organic solvents except light petroleum. This yellow solid gave an intense blue coloration with acid of the same composition as used in the Dische reagent.

*Condensation of Furfuryl Alcohol with Diphenylamine.*—Furfuryl alcohol (0·75 g.) was dissolved in water (500 c.c.) and mixed with a solution of diphenylamine (10 g.) in acetic acid (1 l.) and concentrated sulphuric acid (27·5 c.c.) was added. Portions (100 c.c.) of this solution were then heated separately in a boiling water-bath for exactly 3 minutes and then cooled in ice water and finally combined. Barium acetate (calculated quantity for neutralisation of the sulphuric acid) was then added, the colour of the solution changing from blue to green, and the mixture was extracted three times with ether. The ethereal extracts, which contained excess of diphenylamine, acetic acid, and coloured products, were evaporated under reduced pressure until the water and acetic acid were completely removed. The dark green residue (consisting chiefly of diphenylamine )was dissolved in benzene–light petroleum (b. p. 60—80°) (7 : 3) (50 c.c.), and the solution passed through an alumina column (30 cm. × 1 cm.). The coloured products were absorbed and the diphenylamine was separated by eluting the column successively with benzene–petrol (8 : 2) and benzene–petrol (9 : 1). The coloured products were then removed from the column by elution with absolute alcohol, and the solution evaporated to dryness giving a dark green residue (0·5 g.). This was dissolved in benzene–petroleum (7 : 3), and the column eluted successively with benzene–petroleum (4 : 1), benzene–petroleum (9 : 1), benzene, and benzene–alcohol (200 : 1), and after many refractionations seven principal fractions were obtained and their colour reactions with 5N-sulphuric acid were investigated. The results were as follows :

| Fraction. | Weight, mg. | Appearance. | Coloration with 5N-$H_2SO_4$. |
|---|---|---|---|
| $F_1$ | 31·5 | Colourless syrup | No coloration |
| $F_2$ | 28·7 | Pale yellow syrup | Pale blue-green |
| $F_3$ | 27·5 | Yellow syrup | Purple |
| $F_4$ | 29 | Yellow amorphous solid | Intense blue |
| $F_5$ | 12·5 | Blue amorphous solid | Blue : no change |
| $F_6$ | 88 | Yellow-brown solid | Green |
| $F_7$ | 91 | Brown solid | Green |

*Absorption data of fractions $F_2$—$F_6$.* Each fraction (weighed quantity) was dissolved in a known volume of alcohol, and sufficient acid added to make the solution 5N. After 30 minutes' standing, the absorption was measured on a Spekker absorptiometer and in each case ε (0·1%; 1 cm.) was calculated. The curves thus obtained may well compare with those for furfuryl alcohol and ω-methoxylævulic aldehyde dimethyl acetal on heating with the Dische reagent. The results are shown in Fig. 5, and it is clear that the fraction mainly responsible for the blue coloration is $F_4$. This substance is a yellow, amorphous, optically inactive solid, very insoluble in water but soluble in most organic solvents except light petroleum; it gives a yellow solution in benzene and a deep orange-yellow solution in alcohol. It behaves as an indicator, giving a yellow coloration in alkali and green in acetic acid, changing to blue about pH 1·5.

$F_5$, which gives a similar absorption curve, gives a blue solution in neutral solvents and a yellow coloration in alkali, and thus may be closely related structurally to $F_4$.

*Ultra-violet absorption spectra of fractions $F_3$—$F_6$.* These ultra-violet absorptions have been examined both in neutral and in 5N-acid solution, the concentrations being 3·3 mg. %, and the results are summarised in the following table (see Figs. 6 and 7).

| | | Absorption band. | | | | Absorption band. | |
|---|---|---|---|---|---|---|---|
| Fraction. | Solvent.* | λ. | log $I_0/I$. | Fraction. | Solvent.* | λ. | log $I_0/I$. |
| $F_3$ | N.A. | 2900 | 2·6 | $F_5$ | N.A. | 2910 | 1·0 |
| $F_3$ | A.A. | (No band) | — | $F_5$ | A.A. | 2910 | 1·0 |
| $F_4$ | N.A. | 2900 | 1·9 | $F_6$ | N.A. | 2940 | 1·6 |
| | | 3750 | 1·3 | $F_6$ | A.A. | (No band) | — |
| $F_4$ | A.A. | 2850 | 1·0 | | | | |

* N.A. = Neutral alcohol; A.A. = aqueous alcohol acidified to 5N.

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