which allowed strips of paper to be run along the photocell entrance (Leloir & Paladini, 1951).

Sugars and their esters were revealed with aniline phthalate reagent (Partridge, 1949).

Liberation of phosphate with phenylhydrazine. This procedure was described by Deuticke & Hollmann (1939) for the estimation of fructosediphosphate. The analytical procedure has been modified by Dr Cardini as follows:

Reagents: (*a*) 6% (w/v) phenylhydrazine hydrochloride in water (decolorized with charcoal if necessary); (*b*) saturated solution of sodium acetate; (*c*) saturated Na_2SO_3.

The samples and phosphate standards in 0·5 ml. of water plus 0·1 ml. of (*a*), 0·05 ml. of (*b*) and 0·1 ml. of (*c*) were heated 30 min. in a boiling-water bath. After cooling 0·75 ml. of 5N-H_2SO_4, 0·75 ml. of 2·5% ammonium molybdate and water to a total vol. 7·5 ml. were added. After 10 min. the optical density was measured at 660 mμ. Controls heated without phenylhydrazine were run at the same time.

SUMMARY

1. Purified preparations of uridine-diphosphate-glucose (UDPG) were studied by paper chromatography and found to be contaminated with uridylic acid and a substance UDPX.

2. The uridylic acid obtained by degradation of UDPG has been identified as uridine-5′-phosphate.

3. The alkaline degradation products of UDPG are uridine-5′-phosphate and a cyclic phosphate ester of glucose, probably esterified at positions 1 and 2 of the glucose. This ester decomposes with acid or alkali giving glucose-1-phosphate (25%) and glucose-2-phosphate (75%).

4. The contaminating substance UDPX appears to have the same structure as UDPG except that it contains an unidentified component instead of glucose.

The studies with synthetic uridine-5′-phosphate and with many samples of rare sugars were possible owing to the kindness of Prof. A. R. Todd, F.R.S., and the identification of glucose-2-phosphate by the generosity of Mrs K. R. Farrar. We wish to express our thanks to them as well as to Prof. E. E. Galloni for the X-ray diffraction studies, to Dr C. E. Cardini for his co-operation with the phenylhydrazine method and to Dr J. T. Park for a sample of the *Staphylococcus aureus* compound.

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The Structure of Urorosein

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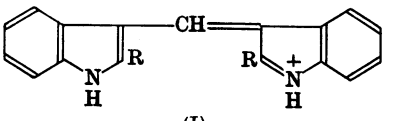
Urorosein is a red pigment obtained by the action of mineral acids on the tryptophan oxidation product indole-3-aldehyde (Ellinger & Flamand, 1909). Fearon & Boggust (1950) have reviewed earlier work on the pigment and assigned to it the structure dehydro-indolo-3′:2′:2:3-carbazole. This is the correct systematic name for the structure as shown by Fearon & Boggust and described by them as indolo-3′:2′:2:3-carbazole. It is, however, difficult to see how such a structure containing only a *p*-quinone-diimine chromophore could be so intensely coloured and, moreover, the absorption spectrum as quoted shows a close correspondence to those of the di-3-indolylmethenes described by König (1925). It

seemed desirable, therefore, to re-investigate the structure of this pigment. Fearon & Boggust prepared urorosein sulphate to which they assigned the formula $(C_{18}H_{10}N_2SO_4)_3H_2SO_4$ and further stated that no evidence could be obtained of the splitting off of an aldehyde side chain in the course of formation of the pigment from two molecules of indole-3-aldehyde.

We have repeated the preparation of the sulphate and have made, in addition, the chloride, bromide and perchlorate. Analyses of these salts showed that they are in fact derived from a base of formula $C_{17}H_{12}N$, and it is thus clear that one carbon atom is



(I)

lost in the course of the condensation. We have further shown that this carbon atom is in fact lost as formic acid, which was identified in a distillate of the mother liquors from the preparation of the pigment. Ellinger & Flamand (1909) had earlier shown that 2-methylindole-3-aldehyde was converted by acids into 2:2′-dimethyl-di-3-indolylmethene salts (I; R = Me) with the elimination of formic acid, so that it appeared from our results that indole-3-aldehyde reacted in an exactly similar manner to give di-3-indolylmethene salts (I; R = H). A synthesis of such salts by the reaction of indole with ethyl orthoformate followed by treatment with acids was described by König (1911). We repeated this work and prepared di-3-indolylmethene chloride, bromide, sulphate and perchlorate. These salts were identical in every way with the urorosein salts obtained from indole-3-aldehyde. Melting points were found to be unsatisfactory criteria of identity, since salts all decompose rather indefinitely at temperatures varying with the rate of heating. Identity was therefore established by X-ray powder photographs and absorption spectra. The statement of König (1911), that the sulphate obtained by the orthoformate method and Ellinger & Flamand's urorosein sulphate are spectroscopically identical, is thus confirmed. As Fearon & Boggust observe, the salts tend to dissociate in dilute solution and the absorption spectra were therefore determined in the presence of free acid. The intense colour of the salt is of course due to resonance, the positive charge being shared between the two nitrogen atoms in two equivalent mesomeric structures.

By reaction of ethyl orthoformate for a longer period with excess of indole, a colourless product was obtained; this was tri-3-indolylmethane, which on heating with acids was converted into di-3-indolylmethene salts with elimination of a molecule of indole. Tri-3-(2-methylindolyl)methane has been shown to behave similarly.

In agreement with Fearon & Boggust, we have found that indole-3-acetic acid yields a red pigment, apparently urorosein, on warming with acids in the presence of oxidizing agents, but we have been unable to confirm their statement that a similar pigment is obtained from 2-methylindole.

EXPERIMENTAL

Preparation of urorosein salts

Preparation from indole-3-aldehyde. Finely powdered indole-3-aldehyde (0·5 g.) was heated at 70° for 1 hr. with 35% (w/v) $HClO_4$ (400 ml.) with stirring. After standing overnight the product, which had crystallized partially during the heating, was collected and recrystallized from acetic acid. *Urorosein perchlorate* formed deep-red needles with an intense green reflex and containing a molecule of acetic acid of crystallization. (Found: C, 56·3; H, 4·0; N, 7·0. $C_{17}H_{13}N_2ClO_4.C_2H_4O_2$ requires C, 56·8; H, 4·2; N, 6·95%).

Other salts. Urorosein chloride. This was obtained in the form of deep-red needles from ethanol-acetone. (Found: C, 72·3; H, 4·9; Cl, 12·4. $C_{17}H_{13}N_2Cl$ requires C, 72·7; H, 4·6; Cl, 12·6%.)

The *bromide* crystallized in the form of deep-red prisms from ethanol. (Found: C, 62·4; H, 4·3; Br, 24·6. $C_{17}H_{13}N_2Br$ requires C, 62·8; H, 4·0; Br, 24·5%.) *Urorosein sulphate* formed deep-red plates from acetic acid. (Found: C, 60·1; H, 4·0; N, 8·0. $C_{17}H_{13}N_2.HSO_4$ requires C, 59·8; H, 4·1; N, 8·2%.) These salts were prepared exactly similarly to the perchlorate using 20% (w/v) HCl, 20% (w/v) HBr and 30% (w/v) H_2SO_4.

Preparation from indole and ethyl orthoformate. Indole (1·2 g.) and ethyl orthoformate (0·8 g.) were dissolved in ethanol (5 ml.), 1 drop of dilute ethanolic HCl added as catalyst and the mixture refluxed for 1 hr. After cooling, the deep-yellow solution was diluted with ethanol (50 ml.) and then 20% (w/v) $HClO_4$ (50 ml.) added with stirring. Urorosein perchlorate crystallized out and was collected and recrystallized as above. The sulphate, chloride and bromide were prepared similarly using the appropriate acids in place of $HClO_4$.

The ultraviolet absorption spectra, determined with a Beckmann spectrophotometer, of the bromides and perchlorates prepared by the two methods above in 95% ethanol containing 5% HBr and $HClO_4$ respectively were compared and found to be identical, having maxima at 258, 286, 487 and 530 mμ. X-ray powder photographs of the bromides prepared by the two methods were identical.

The action of NH_3 on urorosein salts gave the yellow free base in crystalline form: the material was, however, unstable and could not be obtained analytically pure.

Tri-3-indolylmethane

Indole (2 g.) and ethyl orthoformate (0·8 g.) were dissolved in ethanol (7 ml.) one drop of ethanolic HCl added and the mixture refluxed for 6 hr. Towards the end of the heating some pale-yellow crystalline material began to separate. After cooling the solid was filtered off and recrystallized from ethanol. *Tri-3-indolylmethane* formed

colourless prisms, m.p. 244–246°. (Found on a sample dried at 100°/10 mm.: C, 83·2; H, 5·0; N, 11·6. $C_{25}H_{19}N_3$ requires C, 83·0; H, 5·25; N, 11·6%.)

Detection of formic acid

Indole-3-aldehyde (0·2 g.) was treated with 10% (w/v) HCl (100 ml.) at 60° for 20 min. and the urorosein chloride filtered off. The mother liquor was distilled at atmospheric pressure and formic acid was detected in the first portion of the distillate by reduction to formaldehyde with Mg and reaction with chromotropic acid (Feigl, 1946).

SUMMARY

Urorosein salts are shown to be derived from di-3-indolylmethene and can be prepared either from indole-3-aldehyde or from indole and ethyl orthoformate.

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