REACTION BETWEEN 2-AMINO-2-DEOXY-D-GLUCOSE AND 2,4-PENTANEDIONE

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INTRODUCTION

The reaction between 2-amino-2-deoxy-D-glucose and 2,4-pentanedione¹⁻⁷ has attracted attention because of its relation to the Elson-Morgan procedure^{8,9} for the estimation of aldosamines. Although it is agreed that when the reaction is carried out under neutral conditions the product is 3-acetyl-2-methyl-5-(D-*arabino*-tetrahydroxybutyl)pyrrole (I), there are discrepancies in the physical properties (melting point) ascribed to this substance^{1-4, 7, 10}, and in no case has a rigorous proof of the structure been given. It is possible that more than one substance is produced



and, indeed, when the reaction is performed in a slightly alkaline medium, at least four compounds giving positive pyrrole tests to the Ehrlich reagent (*p*-dimethylaminobenzaldehyde-hydrochloric acid) have been detected⁵; two of these were identified⁶ as 2-methylpyrrole and 3-acetyl-2-methylpyrrole. We report now the results of a recent investigation of this reaction; this study is an extension of previous work^{3,4}.

RESULTS AND DISCUSSION

Preparation of 3-acetyl-2-methyl-5-(D-*arabino*-tetrahydroxybutyl)pyrrole (I) is best carried out from 2-amino-2-deoxy-D-glucose hydrochloride, with the equivalent amount of sodium carbonate, and 2,4-pentanedione in aqueous acetone, at room temperature and neutral pH. The almost pure product (I) crystallizes from the reaction medium, as a monohydrate (m.p. $106-107^{\circ}$), in yields of over 80%; crystallization of the monohydrate from ethanol gives the anhydrous form (m.p. $142-142.5^{\circ}$). Other products (detected chromatographically) of the reaction under these conditions are 3-acetyl-2-methylpyrrole and 2-deoxy-2-[2-(4-oxo-2-pentenyl)amino]-D-glucose (II) (see below). When the reaction is performed at a higher temperature and substituting ethanol for acetone, substantial amounts of 3-acetyl-5-[2-(3,4-dihydroxytetrahydrofuryl)]-2-methylpyrrole (III) are also produced. Compound (III) can be detected in aged reaction mixtures of 2-amino-2-deoxy-D-glucose and 2,4-



pentanedione, and often in the mother liquor of crystallization of compound (I). If the reaction is carried out using 2-amino-2-deoxy-D-glucose hydrochloride and the equivalent amount of sodium methoxide in ethanol, as indicated by Boyer and Fürth², yields of the pyrrole (I) are lower, and the whole procedure is more involved. In this case, the anhydrous form (m.p. $142-142.5^{\circ}$) is directly obtained.

Evidence for the structure of compound (I) is as follows. When treated with Ehrlich reagent, compound (I) gives an unstable red colour having maxima at 530 m μ (strong) and *ca*. 500 m μ (weaker) (Fig. 1). The absorption at 530 m μ decreases with time, and that at 500 m μ increases. Both the hydrated and the anhydrous form of the compound have the ultraviolet absorption pattern (maxima at 210, 246, and 290 m μ) typical of a methyl pyrrol-3-yl ketone¹¹. The infrared spectrum shows the NH and C=O bands at the frequencies observed in other methyl pyrrol-3-yl ketones¹². Acetylation of either form gives the same tetra-acetyl derivative. Oxidation with sodium metaperiodate, or with minium (Pb₃O₄) in acetic acid, furnishes 4-acetyl-5-methyl-2-pyrrolecarboxaldehyde (VI); the preparation using minium can be accomplished with an over-all yield of 50% from 2-amino-2-deoxy-D-glucose hydrochloride. Oxidation of compound (VI) with silver oxide-sodium hydroxide gives 4-acetyl-5-methyl-2-pyrrolecarboxylic acid (VII) which can be decarboxylated to known⁶ 3-acetyl-2-methylpyrrole*.

Tetrahydroxybutyl-furans and -pyrroles similar to compound (I) lose a molecule of water in the tetrahydroxybutyl chain giving 1,4-anhydro-derivatives¹³. 3-Acetyl-2-methyl-5-(D-*arabino*-tetrahydroxybutyl)pyrrole (I), upon heating in the dry state *in vacuo*, or in neutral or slightly acid solutions, readily gives compound (III) which affords a diacetate. The results of its periodate oxidation, also in agreement with

^{*}This reaction sequence was described in the paper^{4a} of 1950, but the sample of 3-acetyl-2methylpyrrole (m.p. 75°) then obtained was not completely pure, cf. the product (m.p. 94–95°) synthesized⁶ afterwards from aminoacetaldehyde and 2,4-pentanedione.

structure (III), have been reported previously^{4b}. Similarly, the *N*-alkyl derivatives of the pyrrole (I), substances (VIII) and (IX), give the corresponding anhydroderivatives (IV) and (V).



The reaction between 2-amino-2-deoxy-D-glucose and 2,4-pentanedione in dilute aqueous solution at pH 9.5-10 gives 3-acetyl-2-methyl-5-(D-arabino-tetrahydroxybutyl)pyrrole (I) in 30% yield. This substance is therefore a product of the reaction under conditions similar to those prescribed in the analytical method of Elson and Morgan. Other pyrrole compounds previously identified in the reaction mixture obtained under these conditions are 2-methylpyrrole (yield 10%) and 3-acetyl-2-methylpyrrole⁶. As compound (I), when heated with p-dimethylaminobenzaldehyde in hydrochloric acid, gives (see above) a red colour absorbing maximally at 530 mu (i.e., the wavelength used in the analytical method), it can be considered to be a chromogen in the Elson-Morgan reaction. It should be pointed out in this connection that in several modifications¹⁴⁻¹⁸ of the Elson-Morgan method colours having absorption maxima at 530 m μ are obtained. This fact seems to be in conflict with the current view^{6,9} that 2-methylpyrrole, which gives with the p-dimethylaminobenzaldehyde-hydrochloric acid reagent a colour with maximum at 548 m μ^6 , is the main chromogen. To ascertain whether steam-volatile 2-methylpyrrole derives from the more complex pyrrole (I) under the conditions prevailing in the analytical method, this compound was heated in dilute aqueous solution at pH 10. No pyrrole compound could be detected in a steam distillate of the reaction mixture. Also, the absorption curve given by compound (I) (preheated with sodium carbonate at pH 10) and the Ehrlich reagent is practically the same as that obtained with a non-pretreated solution of (I) (Fig. 1). If any conversion of compound (I) into 2-methylpyrrole had occurred, it would have produced a shift of the absorption to 548 mu.

The formation of pyrrole (I) from 2-amino-2-deoxy-D-glucose and 2,4pentanedione in a mixture of triethylamine and methanol has been reported⁷ recently. We have repeated this work and established the identity of the product so obtained with the one described above. Under these conditions, the enamine (II) can be isolated. Cessi and Serafini-Cessi⁷, who first obtained this compound, proposed structure (II) on the basis of its ultraviolet absorption, and quantitative hydrolysis to 2-amino-2-deoxy-D-glucose and 2,4-pentanedione. Additional evidence is now

presented with the infrared spectrum of compound (II), which shows a band at 1613 cm⁻¹ (C=O group in an intramolecularly bonded β -amino- α,β -unsaturated ketone^{19–21}), and a broad, strong band centred at ca. 1543 cm⁻¹. Compound (II) gives a tetra-O-acetyl derivative, the infrared spectrum (carbon tetrachloride solution) of which has bands at 1616, 1577, and 1495 cm⁻¹, typical of chelated β -amino- α , β -unsaturated ketones¹⁹⁻²¹. As in other compounds of this class¹⁹. no NH stretching absorption was observed either in solution or in the solid state. Both compound (II) and its tetra-O-acetyl derivative have high, positive, optical rotations, suggestive of α -D anomeric configurations. Accordingly, these compounds are better represented by formulae (X) and (XI). 2-Deoxy-2-[2-(4-oxo-2-pentenyl)amino]- α -D-glucose (II or X), when stored in aqueous solution at room temperature, cyclized to a mixture of 3-acetyl-2-methylpyrrole and 3-acetyl-2-methyl-5-(D-arabinotetrahydroxybutyl)pyrrole (I). D-Erythrose could be detected chromatographically in the reaction mixture. When compound (II) was heated at pH 10, the product was 3-acetyl-2-methylpyrrole. 2-Methylpyrrole was detected in only trace amounts (see also ref. 7).

2-Amino-2-deoxy-D-glucose and ethyl acetoacetate react in aqueous solution at ρ H 9.5–10 to give ethyl 2-methyl-3-pyr clearboxylate and ethyl 2-methyl-5-(D-arabino-tetrahydroxybutyl)-3-pyrrolecarboxylate (XII), in the approximate ratio



2:3, and the over-all yield is 50%. No 2-methylpyrrole was found. In the reaction in the presence of triethylamine, the tetrahydroxybutyl ester (XII) was the only product to be detected chromatographically and isolated.

The formation of pyrrole compounds from amino sugars and β -dicarbonyl compounds is probably a stepwise process, the mechanism of which is still uncertain. The isolation of compound (II), and its conversion into 3-acetyl-2-methyl-5-(D-arabino-tetrahydroxybutyl)pyrrole (I) and 3-acetyl-2-methylpyrrole, suggests that enamines of the type (XIII) may be the first intermediates in these reactions. A similar point of view has been expressed previously by Cornforth and his co-workers²², and by Gottschalk²³. However, the low yield of 2-methylpyrrole obtained from the enamine (II), when heated at pH 9–10, in comparison with that obtained from 2-amino-2-deoxy-D-glucose and 2,4-pentanedione under similar conditions, seems to indicate that some other mechanism(s) may be operative. The mechanism of the reaction between 2-amino-2-deoxy-D-glucose and 2,4-pentanedione will be discussed elsewhere²⁴, under the more general context of the reaction of monosaccharides and β -dicarbonyl compounds.

Carbohydrate Res., 1 (1965) 261-273

EXPERIMENTAL

Melting points are uncorrected. Concentrations and evaporations of solvents were carried out *in vacuo* at temperatures not over 50° . Paper chromatography was carried out on Whatman No. I paper by the horizontal technique at room temperature using butan-I-ol-ethanol-water (I0:1:2), unless otherwise stated. Detection reagents used were silver nitrate-sodium hydroxide for monosaccharides and polyhydroxylic compounds, and chromatographic Ehrlich reagent (prepared by dissolving I.o g of *p*-dimethylaminobenzaldehyde in 30 ml of ethanol, adding 30 ml of concentrated hydrochloric acid, and diluting with butan-I-ol to I00 ml) for pyrrole derivatives. The analytical Ehrlich reagent was prepared by dissolving 0.5 g of *p*-dimethylaminobenzaldehyde in 6 ml of ethanol and 6 ml of conc. hydrochloric acid⁶. The Ehrlich reagent for other purposes was prepared as indicated in the literature²⁵. The u.v. spectra were obtained with a Beckman DU spectrophotometer. The i.r. spectra were obtained with a Beckman IR-5A instrument, and those marked (*) with a Perkin-Elmer 237 instrument.

3-Acetyl-2-methyl-5-(D-arabino-tetrahydroxybutyl)pyrrole (I)

2-Amino-2-deoxy-D-glucose hydrochloride (21.6 g, 0.1 mole) and 5.3 g (0.05 mole) of sodium carbonate were dissolved in 75 ml of water, and 10.0 g (0.1 mole) of 2,4-pentanedione and 12 ml of acetone added. The resulting solution was left at room temperature. Paper chromatography after 20 min showed the presence of the following substances (R_F): (I) (0.53), 2-deoxy-2-[2-(4-0x0-2pentenyl)amino]-D-glucose (X) (0.62), and 3-acetyl-2-methylpyrrole (0.89). When sprayed with the Ehrlich reagent, compound (I) produces a red colour which turns green, and compound (X) an orange colour that turns yellow. Crystallization of compound (I), as a monohydrate, began after 10–12 h. After three days, the crystalline mass was filtered off and dried in the air; yield, 21.3 g (85%), m.p. 103–105°. Two recrystallizations from water gave the analytical sample, m.p. 106–107°, $[\alpha]_{\rm D}^{18}$ -34° (c 0.9, water); $\lambda_{\rm max}$ (ethanol) 211, 247, 290 m μ (ε 16785, 7699, 5820); $v_{\rm max}$ (Nujol) 3279, 1626 cm⁻¹.

Anal. Calc. for C₁₁H₁₇NO₅. H₂O: C, 50.56; H, 7.33; N, 5.36. Found: C, 50.79,, H, 7.58; N, 5.23.

The above monohydrate (2 g) was dissolved in 20 ml of boiling ethanol. On cooling, 1.7 g of the anhydrous form of compound (I) crystallized, m.p. 141–142°. Two additional recrystallizations from the same solvent gave a product having m.p. 142–142.5°, $[\alpha]_D^{18}$ –52° (c 0.7, water); λ_{max} 210, 246, 290 m μ (ε 19990, 8278, 6081).

Anal. Calc. for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.04; N, 5.75. Found: C, 54.41; H, 7.01; N, 5.82.

Recrystallization of the anhydrous form, m.p. 142–142.5°, from water regenerated the monohydrate, m.p. 104–105°.

Anal. Found: C, 50.66; H, 7.09; N, 5.43.

The mother liquor of the crystallization of compound (I) (monohydrate) was concentrated and extracted with ether. The ethereal extracts were dried (MgSO₄) and concentrated. Thin-layer chromatography [silica gel; light petroleum-ether (I:I)] showed the presence of a substance having the same mobility as 3-acetyl-2-methylpyrrole. Evaporation of the solvent left a dark oily residue that was not further examined.

Preparation of compound (I) according to Boyer and Fürth² gave a 60% yield of product, m.p. and mixed m.p. with the sample described above $142-142.5^{\circ}$. Boyer and Fürth give m.p. 137° .

The reaction in the presence of triethylamine⁷ was carried out as follows: 2.0 g (9 mmole) of 2-amino-2-deoxy-D-glucose hydrochloride, 25.0 g (0.25 mole) of 2,4-pentanedione, 25 ml of triethylamine, and 1 ml of pyridine in 50 ml of methanol were heated at 55° for 15 h. Evaporation of solvents left a syrup that crystallized on treatment with a little hot water. A portion (0.5 g) of this material (1.3 g) was recrystallized twice from water yielding compound (I) monohydrate, m.p. and mixed m.p. with the sample described above 106-107°. Another sample (0.5 g) was recrystallized from ethanol, as indicated before, yielding compound (I), m.p. and mixed m.p. 142-142.5°.

3-Acetyl-2-methyl-5-(D-arabino-tetra-acetoxybutyl)pyrrole

A solution of 1.0 g of compound (I) in 6 ml of pyridine was treated with 6 ml of acetic anhydride at 0°. After storage for 48 h in the refrigerator, the product was precipitated by pouring the reaction mixture onto ice. After recrystallization from ethanol-water (1:1), the product (71%) had m.p. $132-133^{\circ}$, ν_{max} (chloroform) 3460, 3300, 1730, 1650, 1585, and 1524 cm⁻¹.

Anal. Calc. for C₁₅H₂₄NO₅: C, 55.47; H, 6.13; N, 3.41. Found: C, 55.26; H, 6.38; N, 3.70.

4-Acetyl-5-methyl-2-pyrrolecarboxaldehyde (VI)

(a) A solution of compound (I) (1.1 g, 4.5 mmole) in water was treated with a slight excess of a saturated solution of sodium metaperiodate. After 30 min, the crystalline solid was collected, washed with cold water, and recrystallized from acetone-water (1:1) to yield 0.33 g (49%) of compound (VI), m.p. 144-145°, λ_{max} (ethanol) 228, 298 (ε 14960, 17840), ν_{max} (chloroform) 3436, 3279, 3012, 2817, 1650, 1567, and 1504 cm⁻¹.

(b) A suspension of the monohydrate of compound (I) (0.65 g, 2.5 mole) in 80 ml of acetic acid-water (1:3) was treated under vigorous stirring with 20.5 g (0.03 mole) of minium (Pb₃O₄), added in small portions over a period of 9 h, and stirring was continued for a further 14 h. The reaction mixture was extracted with ether (7×20 ml), and the combined extract washed with water (3×20 ml) and dried (Na₂SO₄). Evaporation of the solvent left crude compound (VI) (0.3 g, 59%), m.p. 139-142°. Recrystallization from acetone-water gave the analytical sample, m.p. 144-144.5°.

Anal. Calc. for C₈H₉NO₂: C, 63.56; H, 6.00; N, 9.26. Found: C, 63.38; H, 5.98; N, 9.62.

4-Acetyl-5-methyl-2-pyrrolecarboxylic acid (VII)

A suspension of silver oxide in water was prepared by dissolving 7.4 g (44 mmole) of silver nitrate in 15 ml of water, and adding 75 ml of N sodium hydroxide. Compound (VI) (3.0 g, 20 mmole) was added, and the mixture was heated under reflux for 40 min. The cooled reaction mixture was filtered and the solid residue washed several times with water. The filtrate and the washings were mixed and acidified (Congo Red) with dilute nitric acid. The acid (VII), precipitated as a dark solid (2.5 g), was filtered off and washed with water. The mother liquor was extracted with ether (3×10 ml), and the combined extracts were dried (MgSO₄). Evaporation of the solvent left an additional 0.2 g of compound (VII). Crude compound (VII) (2.7 g) was dissolved in 10 ml ethanol, applied to a column $(25 \times 3 \text{ cm})$ of alumina, and eluted with a mixture of conc. ammonia (sp. gr. 0.973) and ethanol (1:9). The eluates giving a positive reaction to the Ehrlich reagent were collected and concentrated. Acidification with hydrochloric acid gave 1.2 g (36%) of compound (VII), m.p. 255-256°. Recrystallization from ethanol-water (2:1) gave the analytical sample, m.p. 255–256° (dec.), λ_{max} (ethanol) 252, 284 m μ (ϵ 9533, 9400); ν_{max} (Nujol) 3279, 2632 (broad), 1653, 1575, 1508 cm⁻¹.

Anal. Calc. for $C_8H_9NO_3$: C, 57.47; H, 5.43; H, 8.38; neutralization equivalent, 167. Found: C, 57.48; H, 5.52; N, 8.40; neutralization equivalent, 170.

3-Acetyl-2-methylpyrrole

4-Acetyl-5-methyl-2-pyrrolecarboxylic acid (VII) (100 mg) was heated at 250–260° (bath temperature) in a tube fitted with a "cold finger". The sublimate (51 mg), m.p. 86–89°, was recrystallized from light petroleum (40–70°) to give pure 3-acetyl-2-methylpyrrole, m.p. 95–97° (lit.⁶, 94–95°); λ_{max} (ethanol) 243, 282 m μ (ϵ 7822, 5822).

Anal. Calc. for C₇H₉NO: C, 68.26; H, 7.44; N, 11.38. Found: C, 68.20; H, 7.12; N, 11.30.

3-Acetyl-5-[2-(3,4-dihydroxytetrahydrofuryl)]-2-methylpyrrole (III)

(a) Compound (I) monohydrate (5.0 g) was heated to constant weight over phosphorus pentoxide at $70^{\circ}/20$ mm [wt. loss: 0.7 g (calc. for two mol. of water, 0.8 g)]. The residual material had m.p. 159–163° and, after recrystallization from ethanol, m.p. 165–166°, [α]_D – 104° (c 1, water).

(b) Compound (I) monohydrate (2 g) in water (10 ml) was heated at 100° for 3 h. Evaporation of the solvent left a crystalline residue which, after recrystallization from ethanol, gave compound (III) (1.1 g, 64%), m.p. 163–164°.

(c) Compound I monohydrate (5.0 g) dissolved in 25 ml of water containing 0.25 ml of acetic acid was heated at 100° for 3 h. Working up as before gave 2.6 g (60%) of compound (III), m.p. 165–166°.

Anal. Calc. for C₁₁H₁₅NO₄; C, 58.66; H, 6.71; N, 6.21. Found: C, 58.67; H, 6.64; N, 6.45.

3-Acetyl-5-[2-(3,4-diacetoxytetrahydrofuryl)]-2-methylpyrrole

Acetylation of compound (III), as indicated before for compound (I), gave the corresponding diacetyl derivative (84%) which, after recrystallization from ethanol, had m.p. 133.5-134.5°.

Anal. Calc. for C₁₅H₁₉NO₆: C, 58.28; H, 6.19; N, 4.52. Found: C, 58.53; H, 6.13; N, 4.82.

3-Acetyl-5-[2-(3,4-dihydroxytetrahydrofuryl)]-1-ethyl-2-methylpyrrole (IV)

A solution of compound (VIII) (0.2 g) in 2.5 ml of water was heated at 50° for 7 h. The crystalline material which separated on cooling was collected and recrystallized from ethanol, yielding 0.12 g (64%) of compound (IV), m.p. 189–191°, $[\alpha]_{D}^{17}$ –142° (c 3.5, pyridine); λ_{max} (ethanol) 219, 252 m μ (ϵ 20982, 7827, 6034). Anal. Calc. for C₁₃H₁₉NO₄: C, 61.66; H, 7.50; N, 5.53. Found: C, 61.83;

H, 7.53; N, 5.80.

In an analytical oxidation, there was a consumption of 1.05 mol. of periodic acid (calc., 1.0 mol.).

3-Acetyl-1-butyl-5-[2-(3,4-dihydroxytetrahydrofuryl)]-2-methylpyrrole (V)

A saturated solution of 0.2 g of compound (IX) in water was heated at 50°



Fig. 1. Colour reaction given by compound (I) and *p*-dimethylaminobenzaldehyde: ——— after 3 h; $\cdot - \cdot - \cdot$ after 7 h; $- - - \cdot$ compound (I) previously heated at pH 9.5.

Carbohydrate Res., 1 (1965) 261-273

for 24 h; compound (V) crystallized on cooling. Recrystallization from ethanol yielded 0.1 g (53%) of the analytically pure substance, m.p. 167–168°, $[\alpha]_D^{17}$ –116° (c 0.8, pyridine); λ_{max} (ethanol) 220, 251, 283 m μ (ε 18243, 7268, 5463).

Anal. Calc. for C₁₅H₂₃NO₄: C, 64.05; H, 8.19; N, 4.98. Found: C, 63.96; H, 8.34; N, 4.87.

The compound consumed 1.0 mol. of periodic acid (calc., 1.0 mol.).

Colour reaction of compound (1) with the Ehrlich reagent

A solution of compound (I) (1.0 mg) in water (1 ml) was diluted with ethanol (5 ml) and the analytical Ehrlich reagent (0.5 ml) was added. The mixture was heated under reflux in a boiling-water bath for 15 min, cooled in an ice-water bath, and the red colour measured against a solution similar to the reaction mixture but for omission of the *p*-dimethylaminobenzaldehyde. The absorption curves at different intervals of time are shown in Fig. 1.

A solution of compound (I) (50.0 mg) in 25 ml of a sodium carbonate-sodium hydrogen carbonate buffer (pH 9.5) was heated at 100° for 20 min. After being cooled, a portion (0.5 ml) of this solution was diluted with water (0.5 ml) and ethanol (5 ml), and treated with the analytical Ehrlich reagent, as indicated before. The absorption curve of the colour developed appears in Fig. 1.

Treatment of 3-acetyl-2-methyl-5-(D-arabino-tetrahydroxybutyl)pyrrole (I) with sodium carbonate at pH 9–10

A solution of compound (I) (60.0 mg) in 10 ml of a buffer of sodium carbonatesodium hydrogen carbonate (pH 9.55) was heated at 100° for 30 min. The reaction mixture was distilled at 20 mm pressure until it was reduced to a volume of 2 ml, the distillate being collected in a flask immersed in an ice-salt bath. A portion of this distillate did not develop any colour when treated with the analytical Ehrlich reagent as indicated before.

Formation of compound (I) from 2-amino-2-deoxy-D-glucose and 2,4-pentanedione at pH 9-10

2-Amino-2-deoxy-D-glucose hydrochloride (5.4 g, 25 mmole) and 2,4pentanedione (7.5 g, 75 mmole) were dissolved in 175 ml of water containing sodium carbonate (6 g) and sodium hydrogen carbonate (0.5 g), and the pH was adjusted to 9.7 by addition of 5N sodium hydroxide. The solution was diluted to 200 ml, heated at 100° for 30 min, and cooled, and the pH was brought to 7.0 by addition of Zeokarb 225 (H⁺ form). The resin was filtered off and the filtrate extracted repeatedly with ether until the extracts no longer gave a reaction with the Ehrlich reagent. The aqueous fraction was concentrated until crystallization of compound (I) started, and then refrigerated. Compound (I) monohydrate, m.p. $101-103^{\circ}$, was collected and air-dried. Concentration of the mother liquor, and treatment of the syrup with a little hot water afforded a second crop, m.p. $103-105^{\circ}$. Total yield, 2.0 g (30%). After recrystallization from water, it had m.p. and mixed m.p. 106-107°.

Anal. Found: C, 50.77; H, 7.48; N, 5.45.

2-Deoxy-2-[2-(4-oxo-2-pentenyl)amino]-D-glucose (X)

The method of Cessi and Serafini-Cessi⁷ is incorporated in the following procedure. A mixture of 10.8 g (0.05 mole) of 2-amino-2-deoxy-D-glucose hydrochloride, 15.0 g (0.15 mole) of 2,4-pentanedione, 15 ml of triethylamine, and 220 ml of methanol was heated until dissolution occurred, and then under reflux for 5 min. The mixture was concentrated to half its volume and refrigerated. The crystal mass of compound (X) (9.2 g, 70%), m.p. 109–111°, was filtered off and washed with ethanol. After recrystallization from ethanol, it had m.p. 111–112°, $[\alpha]_D^{24} + 215^\circ$ (c 2, water); λ_{max} (ethanol) 312 m μ (ε 18700); ν_{max} (Nujol) 3185, 1613, 1543 cm⁻¹. Cessi and Serafini-Cessi⁷ give m.p. 107° (for a monohydrate), $[\alpha]_D^{20} + 181^\circ$ (water).

Anal. Calc. for C₁₁H₁₉NO₆: C, 50.56; H, 7.33; N, 5.36. Found: C, 50.41; H,7.52; N, 5.24.

Paper chromatography of an analytical sample of compound (X) showed, with the silver nitrate spray, a spot of R_F 0.58, and traces of compound (I) (R_F 0.51) and 2-amino-2-deoxy-D-glucose. The presence of the last two substances is considered to be due to partial cyclization [to give (I)] and hydrolysis of compound (X). The Ehrlich reagent spray showed only the spot of R_F 0.58.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-[2-(4-oxo-2-pentenyl)amino]-α-D-glucopyranose (XI) A solution of compound (X) (1.0 g) in 4 ml of pyridine was treated with 4 ml of acetic anhydride at 0°, and stored at room temperature for two days. The reaction mixture was poured onto ice, and the solid precipitate filtered and washed

with water. Recrystallization from ethanol gave 1.0 g (62%) of compound (XI), m.p. 174–175°, $[\alpha]_D^{18}$ 194° (c I, pyridine); v_{max} (KBr) 1754, 1739, 1610, 1569, 1513 cm⁻¹ (*); v_{max} (carbon tetrachloride) 1754, 1616, 1577, 1495 cm⁻¹ (*).

Anal. Calc. for C₁₉H₂₇NO₁₀: C, 53.14; H, 6.34; N, 3.49. Found: C, 52.89; H, 6.40; N, 3.48.

Transformation of compound (X) into compound (I) and 3-acetyl-2-methylpyrrole A solution of 15.0 g of compound (X) in 230 ml of water was left at room temperature. After three days, it was extracted with ether (5 × 15 ml), and the combined extracts were dried (Na₂SO₄) and evaporated. The residue (2.1 g), m.p. 88-94°, was recrystallized from ethanol-water (4:1) to yield 3-acetyl-2-methylpyrrole, m.p. and mixed m.p. with the sample described above 95-97°.

Anal. Found: C, 68.42; H, 7.01; N, 11.64.

The aqueous fraction was concentrated to yield 4.6 g (32%) of compound (I) (monohydrate) which, after recrystallization from water, had m.p. and mixed m.p. 106-107°. Paper chromatography (descending, butan-1-ol-acetic acid-water

4:1:5 upper layer) of the mother liquor showed the presence of a compound $(R_F 0.42)$ having the same mobility as D-erythrose.

Treatment of compound (X) at pH 9–10

A solution of compound (X) (60.0 mg) in 10 ml of a sodium carbonate-sodium hydrogen carbonate buffer (pH 9.5), was heated at 100° for 30 min. The cooled reaction mixture was brought to μ .I 7.0 by addition of Zeokarb 225 (H⁺ form), the resin filtered off, and the filtrate concentrated *in vacuo* (50-60° [bath]/20 mm) to a volume of 2 ml. The distillate was collected in a cooled (ice-salt) receiver. A portion (0.5 ml) of the distillate was diluted with water (0.5 ml) and ethanol (5 ml), and treated with 0.5 ml of the analytical Ehrlich reagent, as indicated above. No colour reaction developed in the cold, and only a very weak colour upon heating. Another portion (0.5 ml) of the distillate, treated with water (0.5 ml) and the Ehrlich reagent (0.5 ml) in the same way, developed in the cold a weak pink colour that had maximal absorption at 549 m μ . Thin-layer chromatography [silica gel; light petroleum-ether (1:1)] of the distillation residue showed the presence of 3-acetyl-2-methylpyrrole.

Reaction between 2-amino-2-deoxy-D-glucose and ethyl acetoacctate

(a) At pH 9-10. 2-Amino-2-deoxy-D-glucose hydrochloride (10.8 g, 0.05 mole) and 13.0 g (0.1 mole) of ethyl acetoacetate were dissolved in 350 ml of a buffer prepared by dissolving 10.6 g of sodium carbonate and 1.5 g of sodium hydrogen carbonate in 500 ml of water. The pH was adjusted to 9.7 by addition of 5N sodium hydroxide, and the resulting solution diluted with water to 400 ml, heated to 100° for 30 min, and cooled. The pH was brought to 7.2 by addition of Amberlite IR-120 (H⁺ form), the resin was removed, and the filtrate extracted with ether (4×20 ml, and afterwards in a continuous extractor for 9 h). The combined extracts were dried (Na₂SO₄) and evaporated to leave a crystalline residue of ethyl 2-methyl-3-pyrrolecarboxylate (1.4 g, 18%), m.p. 75-78°. After recrystallization from ethanolwater (2:1), it had m.p. 79.5-81.5°; λ_{max} (ethanol) 225, 256 mµ (ε 7555, 6442); ν_{max} (chloroform) 3484, 3436, 3154, 3012, 1689, 1582, 1502 cm⁻¹. Lit.²⁶, m.p. 78-79°. Anal. Calc. for C₈H₁₁NO₂: C, 62.72; H, 7.23; N, 9.1. Found: C, 62.44;

H, 7.38; N, 9.11.

A sample (197 mg) of the above ester was refluxed with 13 ml of 3.25N sodium hydroxide for 2 h. The resulting solution was filtered, ice-cooled, and acidified (to Congo Red) with conc. hydrochloric acid. On storage in the refrigerator, crystallization of 2-methyl-3-pyrrolecarboxylic acid took place. Recrystallization from water yielded 67 mg (42%) of product, m.p. 171–173° (dec.) (lit.^{26, 27}, 169°].

The aqueous solution, after the extraction with ether, was concentrated to half its volume and refrigerated. Ethyl 2-methyl-5-(D-arabino-tetrahydroxybutyl)-3-pyrrolecarboxylate (XII)¹³ (4.0 g), m.p. 139–140°, was filtered off and dried. Concentration of the mother liquor afforded additional product (0.2 g), m.p. 138–140°

(total yield 39%). After recrystallization from water, the product had m.p. and mixed m.p. $141-142^{\circ}$.

(b) In the presence of triethylamine. A mixture of 10.8 g (0.05 mole) of 2-amino-2-deoxy-D-glucose hydrochloride, 13.0 g (0.1 mole) of ethyl acetoacetate, 13 ml of triethylamine, and 200 ml of methanol was heated gently until complete dissolution occurred, and then under reflux for 5 min. Paper chromatography [descending, butan-1-ol-ethanol-water-ammonia (40:10:49:1)] showed the presence of Dglucosamine and a substance having the same mobility (R_F 0.73) as compound (XII). Evaporation of the solvents left a residue that crystallized upon treatment with ether. Recrystallization from ethanol afforded pure compound (XII), m.p. and mixed m.p. 141-142°.

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SUMMARY

2-Amino-2-deoxy-D-glucose and 2,4-pentanedione react in neutral solution giving 3-acetyl-2-methyl-5-(D-arabino-tetrahydroxybutyl)pyrrole (I), and minor amounts of 3-acetyl-2-methylpyrrole and 2-deoxy-2-[2-(4-oxo-2-pentenyl)amino]-D-glucose (II). The structure of compound (I) is demonstrated and its properties described. The pyrrole (I) is also a product of the reaction at pH 9-10, and its possible role as a chromogen in the Elson-Morgan method for the estimation of 2-amino-2-deoxy-D-glucose is discussed. Directions for the preparation of compound (II) are given, and its structure and properties studied. Reaction between 2-amino-2-deoxy-D-glucose and ethyl acetoacetate at pH 9-10 yields ethyl 2-methyl-5-(D-arabino-tetrahydroxybutyl)-3-pyrrolecarboxylate (XII) and ethyl 2-methyl-3-pyrrolecarboxylate.

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