Isolation of some Precursors of 2-Methylpyrrole in the Elson–Morgan Reaction

By FRANCA SERAFINI-CESSI AND C. CESSI*

Istituto di Patologia Generale, Università di Bologna, 40126 Bologna, and Istituto di Patologia Generale, Università di Cagliari, 09100 Cagliari, Italy

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1-*C*-(1-Acetylacetyl)-2-deoxy-2-(1-methyl-3-oxobut-1-enyl)amino-D-galactitol is obtained from the condensation of 2-amino-2-deoxy-D-galactose with pentane-2,4-dione in anhydrous solvent. On treatment with hot alkali it gives 2-methylpyrrole with 37% yield. By acid hydrolysis under mild conditions the compound loses the *N* substituent and from the resulting unstable derivative 2-methylpyrrole is obtained (52% yield). It is concluded that derivatives of aminohexoses substituted at C-1 with a dioxopentyl chain are the precursors of 2-methylpyrrole in the Cessi & Serafini-Cessi (1963) modification of the Elson–Morgan reaction. As demonstrated previously, products of condensation of aminohexoses with pentane-2,4-dione at the amino group are not converted directly into 2-methylpyrrole, but this step provides protection of the amino group during condensation at C-1.

2-Methylpyrrole is an important chromogen in the Elson & Morgan (1933) reaction for the determination of aminohexoses. The reactions leading to production of this compound are fairly specific, and its purification from the mixture of chromogens obtained by condensation of aminohexoses with pentane-2,4-dione has proved of practical value in the analytical procedures (Cessi & Piliego, 1960; Cessi & Serafini-Cessi, 1963). Aminohexoses substituted at C-3 fail to give 2-methylpyrrole, and can be distinguished from unsubstituted parent compounds as demonstrated by Johansen, Marshall & Neuberger (1960).

The mechanisms of the reactions leading to 2-methylpyrrole are but little known. Cornforth & Firth (1958) suggested that this chromogen is formed by condensation between 2-amino-2-deoxy-D-glucose and pentane-2,4-dione via a pyrroline intermediate that is subsequently attacked by hydroxyl ions and loses both the acetyl group and the tetrahydroxybutyl chain by retroaldol reaction. The way in which the pyrroline intermediate is formed is not known. Condensation of the amino group of the aminohexose with the carbonyl group of pentane-2,4-dione and subsequent ring closure at C-1 may be ruled out by the following considerations. The product of condensation of 2-amino-2-deoxy-D-glucose was prepared in our laboratory, and the structure 2-deoxy-2-(1-methyl-3-

oxobut-1-enyl)amino-D-glucose was proved (Cessi & Serafini-Cessi, 1963). The compound cyclized readily to 3-acetyl-2-methyl-5-(*D*-arabino-tetrahydroxybutyl)pyrrole in neutral or alkaline aqueous solutions. García González, Gómez Sánchez & Goñi de Rey (1965) pointed out that 3-acetyl-2-methyl-5-(*D*-arabino-tetrahydroxybutyl)pyrrole may contribute chromogens other than 2-methylpyrrole.

An alternative route was considered. García González (1956) and García González & Gómez Sánchez (1965) discussed the reactions between carbonyl compounds and the aldehyde group of hexoses, and the possibility that such derivatives of aminohexoses might cyclize to pyrroles. The present paper deals with the isolation and identification of the intermediates formed by condensation of 2-amino-2-deoxy-D-galactose with pentane-2,4-dione in the modification proposed by Cessi & Serafini-Cessi (1963) of the Elson–Morgan reaction. Evidence is presented that pentane-2,4-dione condenses with the amino group of the aminohexose. The product undergoes reaction with a second molecule of pentane-2,4-dione, condensation occurring with the aldehyde group. The amino group is then probably made free by the attack of hydroxyl ions, and the formation of pyrroles ensues.

EXPERIMENTAL AND RESULTS

2-Amino-2-deoxy-D-galactose hydrochloride was obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Chemicals were reagent grade.

* Present address: Istituto di Patologia Generale, Università di Modena, via G. Campi 287, 41100 Modena, Italy.

(I)

(II)

(III)

2-Deoxy-2-(1-methyl-3-oxobut-1-enyl)amino-D-galactose (I) and 3-acetyl-2-methyl-5-(D-lyxo-tetrahydroxybutyl)pyrrole (II). The method described by Cessi & Serafini-Cessi (1963) for the corresponding derivatives of D-glucose was followed. Further purification of compound (I) was obtained by gel filtration. The sample was dissolved in 0.5 ml of anhydrous methanol and placed on a column (1 cm $\times$ 60 cm) of Sephadex LH-200 which had been equilibrated with and was eluted with the same solvent. One main peak corresponding to compound (I) and a minor peak corresponding to compound (II) appeared. The properties of the compounds were the same as reported by Ludowieg & Benmaman (1968).

1-C-(1-Acetylaceton-1-yl)-2-deoxy-2-(1-methyl-3-oxobut-1-enyl)amino-D-galactitol (III). A 50 mg portion of compound (I) was dissolved in 5 ml of methanol-pentane-2,4-dione-triethylamine (2:1:1, by vol.) and kept at 55°C for 16 h. After evaporation of reagents, the residue was dissolved in a small volume of anhydrous methanol and kept in the cold. A few crystals separated, which were identified as compound (II). The solution was fractionated by gel filtration as above. Again two peaks were obtained, containing compounds (III) and (II) respectively. The yield of amorphous compound (III) was 30 mg. The substance decomposed at 165°C. It had $[\alpha]_D^{22} -48 \pm 5^\circ$ (c 1.0 in methanol), and no mutarotation was observed. $\lambda_{\max}$ in water at 315 nm (ϵ 18000), i.r. spectrum (amorphous film from dried methanolic solution) 3195, 1708, 1602, 1545 cm^{-1} . N.m.r. gave a number of peaks around $\delta 7$ corresponding to 12 hydrogen atoms, which could be assigned to the four methyl groups of the molecule (Found: C, 52.6; H, 7.6; N, 3.9. Calc. for $\text{C}_{16}\text{H}_{27}\text{NO}_5$: C, 53.1; H, 7.5; N, 3.9%). Compound (III) is a C₆ ketose [3-C-acetyl-1,3,5-trideoxy-5-(1-methyl-3-oxobut-1-enyl)amino-D-galacto-nonulose] with two new asymmetric carbon atoms (C-3 and C-4). As their configuration is unknown, the above denomination is incomplete. It is also expected that a cyclic hemiacetal is formed, involving one ketone oxygen atom and C-2 and C-6 of the nonulose.

The same compound was obtained when 2-amino-2-deoxy-D-galactose replaced compound (I) in the above procedure.

Acid hydrolysis of 1-C-(1-acetylaceton-1-yl)-2-deoxy-2-(1-methyl-3-oxobut-1-enyl)amino-D-galactitol (III). Partial

hydrolysis of compound (III) was performed by dissolving 5 μmol in 1 ml of 0.1 M-HCl. The mixture was incubated for 1 h at 37°C. The extent of hydrolysis was followed by measuring the extinction at 315 nm, which is due to the enamine, and by determining the free pentane-2,4-dione by steam-distillation (extinction at 275 nm in 0.01 M-HCl and at 295 nm in 0.01 M-NaOH). The yield was 4.9 μmol .

The product of hydrolysis (IV) had lost the 315 nm band and gave 2-methylpyrrole in high yield on heating in borate buffer, pH 10.0, proving that an oxoalkyl chain was still present after the loss of the pentane-2,4-dione moiety bound to the amino group. The substance is fairly unstable and turns brown rapidly. The structure 1-C-(1-acetylaceton-1-yl)-2-amino-2-deoxy-D-galactitol (IV), a C₆ ketose, is tentatively proposed.

Paper chromatography. Ascending paper chromatograms were developed with butan-1-ol-ethanol-water (5:1:4, by vol.) and sprayed with alkaline permanganate or Ehrlich reagent (5 min, 110°C). All the compounds gave a yellow stain with permanganate. The following spots appeared with Ehrlich reagent: compound (I), faint yellow; compound (II), pink turning to grey; compound (III), strong orange-red; compound (IV), red-purple. R_F values were: compound (I), 0.58; compound (II), 0.51; compound (III), 0.61; compound (IV), 0.17. It is possible that the unstable compound (IV) is converted into a heterocyclic derivative during the development of the chromatograms, and the observed R_F refers to the latter.

Determination of 2-methylpyrrole. 2-Methylpyrrole was prepared by the modification of the procedure of Cornforth & Firth (1958). It had u.v. $\lambda_{\max}$ at 214 nm. After reaction with *p*-dimethylaminobenzaldehyde [0.8% (w/v) in ethanolic 3.5% (v/v) HCl] ϵ at 548 nm was found to be 82000.

The rates of conversion of 0.1 μmol of compounds (III) and (IV) into 2-methylpyrrole were determined by heating them in borate buffer (cf. Cessi & Serafini-Cessi, 1963), pH 7.0–11.0, for 30 min in sealed tubes in a boiling-water bath. The yields of 2-methylpyrrole from 0.1 μmol of 2-amino-2-deoxy-D-galactose were determined under the conditions described by Cessi & Serafini-Cessi (1963), except for the pH values (7.0–11.0) of borate buffer. 2-Methylpyrrole was separated by steam-distillation and the extinction at 548 nm was determined after reaction with the above *p*-dimethylaminobenzaldehyde reagent.

Fig. 1. Yields of 2-methylpyrrole from compound (III) (○) and from compound (IV) (□) after heating them at 100°C in borate buffers at different pH values. The yields from 2-amino-2-deoxy-D-galactose (●) when it is subjected to the method of Cessi & Serafini-Cessi (1963) modified for the pH values are also shown. The amount of 2-methylpyrrole is indicated by values of extinction at 548nm.

Fig. 2. Absorption spectra of the products of condensation with Ehrlich reagent of 0.05 μmol of 2-methylpyrrole (○), 0.1 μmol of compound (III) (●) and 0.05 μmol of 2-methylpyrrole plus 0.1 μmol of compound (III) (□).

pyrrole were 37% at pH 8.0 from compound (III) and 52% at pH 10.0 from compound (IV).

Different spectra were observed on reaction of the pyrroles (2ml of aqueous solution) with Ehrlich reagent (8ml). Compound (II) gave a faint colour (ϵ_{525} 120). 2-Methylpyrrole (0.05 μmol) gave a broad curve around $\lambda_{\max}$ at 548nm. An unknown pyrrole derived from compound (III) (0.1 μmol) and from compound (IV) on incubation with Ehrlich reagent (1 h at 70°C) had $\lambda_{\max}$ at 525nm. A mixture of compound (III) (0.1 μmol) and 2-methylpyrrole (0.05 μmol) gave a spectrum resulting from both curves, with $\lambda_{\max}$ at 530nm as in the original procedure by Elson & Morgan (1933) (see Fig. 2).

DISCUSSION

2-Amino-2-deoxy-D-galactose may react with two molecules of pentane-2,4-dione. The product, 1-C-(1-acetylacetyl)-2-deoxy-2-(1-methyl-3-oxo-but-1-enyl)amino-D-galactitol (III), shows the properties of both the enamine and the diketone side chains. The presence of an enamine structure is suggested by the occurrence of a u.v. $\lambda_{\max}$ at 315nm and by the i.r. bands typical of the intramolecularly bonded -CO- and -NH- groups of α -amino $\alpha\beta$ -unsaturated ketones (Cromwell, Miller, Johnson, Frank & Wallace, 1949; Weinstein & Wyman, 1958). The enamine is easily split by hydrolysis in dilute acid and approx. 1 mol of pentane-2,4-dione/mol of compound (III) is released.

The presence of a diketone side chain is suggested by the presence of the i.r. band at 1708 cm⁻¹ (see Rasmussen, Tunnicliff & Brattain, 1949). Evidence of the presence of four methyl groups is obtained from the results of n.m.r. Compound (III) gives 2-methylpyrrole almost certainly after the N-substituent has been released. The process can be accomplished in two stages, acid hydrolysis followed by cyclization at pH 10.0, or in a single stage at pH 8.0. The yield of 2-methylpyrrole from the product of hydrolysis is greater the more alkaline the medium. Maximum yield of 2-methylpyrrole is at pH 8.0 for compound (III), the enamine structure of which needs to be split before cyclization. pH 8.0 may represent a compromise between the conditions for fastest rate of hydrolysis of enamine, which is better catalysed by acid, and fastest rate of reaction of the carbonyl with the amino group, which is favoured by alkali.

It has not been possible to isolate compound (IV), the product of acid hydrolysis of compound (III), because it is a fairly unstable substance, spontaneously passing to a pyrrole. Some properties have nevertheless been demonstrated, such as the absence of the absorption band at 315nm and the production in alkaline buffers of 2-methylpyrrole in a yield that is the highest observed for any amino-hexose or derivative. These properties require that

The dependence of the yield on pH of the buffer is summarized in Fig. 1. Compounds (I) and (II) gave no 2-methylpyrrole, as previously reported (Cessi & Serafini-Cessi, 1963) for the isomers derived from 2-amino-2-deoxy-D-glucose. The maximum yields of 2-methyl-

the ketone chain deriving from pentane-2,4-dione is bound to C-1, the only position useful for cyclization after the removal of the substituent from the amino group. Compound (IV) gives rise to the formation of a non-volatile pyrrole in acid solution. The product formed by the condensation of this pyrrole with *p*-dimethylaminobenzaldehyde had $\lambda_{\max}$ at 525nm.

The ability of the 1-*C*-acetylacetonyl derivatives of 2-amino-2-deoxy-D-galactose to give 2-methylpyrrole is much higher than that of aminohexoses under the conditions of the analytical reactions (10–18% yield, depending on the method; Cessi & Piliago, 1960; Cessi & Serafini-Cessi, 1963). On the other hand 2-deoxy-2-(1-methyl-3-oxobut-1-enyl)-amino-D-galactose (I) and the isomeric derivative of D-glucose give 3-acetyl-2-methylpyrrole in water. In hot alkaline buffer they do not give 2-methylpyrrole, but the corresponding 3-acetyl-2-methyl-5-tetrahydroxybutylpyrroles. These compounds are stable and are not transformed into 2-methylpyrrole in alkali. They are weak chromogens, although García González *et al.* (1965) have pointed out that they may contribute to the colour of the Elson–Morgan reaction at 530nm.

Condensation of aminohexoses with pentane-2,4-dione might be expected to give an acetylated pyrrole ring, and 3-acetyl-2-methylpyrrole has indeed been found among the products. The way in which the acetyl group is released in the formation of 2-methylpyrrole is not clear (see Cornforth & Firth, 1958). The mechanism based on the condensation of pentane-2,4-dione with the aldehyde group of aminohexoses provides a new approach. Unlike the *N*-methyloxobutenyl chain, the C-1 acetylacetonyl residue preserves the β -diketone structure, which is unstable in alkali and might release an acetyl group in the buffer used.

It is concluded that in the modification of the Elson–Morgan reaction proposed by Cessi &

Serafini-Cessi (1963) condensation of pentane-2,4-dione with the aldehyde group of aminohexoses is the condition for the formation of 2-methylpyrrole. Condensation at the amino group probably facilitates the reaction, the resulting enamine being subsequently hydrolysed. Ring closure occurs easily. The sequence is probably analogous to the reaction of *N*-acetylneuraminic acid in alkali investigated by Gottschalk (1955). The mechanism in this case involves the ring closure between the carbonyl group and the amino group after hydrolysis of the acetamido linkage. Loss of the side chain leads to pyrrole-2-carboxylic acid via a pyrroline intermediate.

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REFERENCES

- Cessi, C. & Piliago, F. (1960). *Biochem. J.* **77**, 508.
Cessi, C. & Serafini-Cessi, F. (1963). *Biochem. J.* **88**, 132.
Cornforth, J. W. & Firth, M. E. (1958). *J. chem. Soc.* p. 1091.
Cromwell, N. H., Miller, F. A., Johnson, A. R., Frank, R. L. & Wallace, D. J. (1949). *J. Am. chem. Soc.* **71**, 3337.
Elson, L. & Morgan, W. T. J. (1933). *Biochem. J.* **27**, 1824.
García González, F. (1956). *Adv. Carbohydr. Chem.* **11**, 97.
García González, F. & Gómez Sánchez, A. (1965). *Adv. Carbohydr. Chem.* **20**, 203.
García González, F., Gómez Sánchez, A. & Goñi de Rey, M. I. (1965). *Carbohydr. Res.* **1**, 261.
Gottschalk, A. (1955). *Biochem. J.* **61**, 298.
Johansen, P. G., Marshall, R. D. & Neuberger, A. (1960). *Biochem. J.* **77**, 239.
Ludowieg, J. J. & Benmaman, J. D. (1968). *Carbohydr. Res.* **8**, 185.
Rasmussen, R. S., Tunnicliff, D. D. & Brattain, R. R. (1949). *J. Am. chem. Soc.* **71**, 1068.
Weinstein, J. & Wyman, G. M. (1958). *J. org. Chem.* **23**, 1618.