Synthesis and Reactions of 2-(5-Amino-4-carbamoylimidazol-1-yl)-2-deoxy-D-glucose, an Analogue of a Naturally Occurring Imidazole Nucleoside

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Treatment of D-glucosamine with ethyl *N*-[carbamoyl(cyano)methyl]formimidate (I) gave the title compound (IIa), an analogue of the nucleoside corresponding to AICAR (5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide 5′-phosphate), in which the linkage between sugar and base is not a glycosidic link.

RECOGNITION of the central importance in metabolism of the biosynthetic pathway to purine nucleotides has stimulated development of chemical syntheses for naturally occurring imidazole nucleosides and some of their analogues. Compounds possessing a variety of different substituents and arrangements in the aglycone part of the molecule have been prepared,[1,2] but in all cases where a sugar residue is attached to nitrogen in the heterocyclic ring it is by means of an *N*-glycosidic link. The synthesis of some related compounds which are not glycosides is now reported. These should be immune to the action of glycoside-splitting enzymes and may have interesting biochemical and pharmacological properties.

Treatment of 2-amino-2-deoxy-D-glucose at pH 8 with ethyl *N*-[carbamoyl(cyano)methyl]formimidate [3] (I) gave a crystalline product, $C_{10}H_{16}N_4O_6$, which had spectroscopic properties consistent with structure (IIa). Mutarotation was observed when recrystallised samples were dissolved in water, the compound gave a purple colour in the Bratton–Marshall test,[4] and it consumed more than 2 equiv. of periodate. The nucleoside (IIa) is unstable in both dilute acid (which appears to induce polymerisation) and dilute alkali; and, unlike D-glucosamine, it does not split at the sugar–nitrogen bond when treated with a phosphate–borate mixture [5] or undergo disproportionation during catalytic hydrogenation over Adams catalyst.[6] Reduction with sodium borohydride gave the polyol (III), identical with the product obtained by treatment of 2-amino-2-*deoxy*-D-glucitol with the imidate (I). All attempts to convert compound (IIa) into a glucosaminic acid by a variety of oxidising agents failed.

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[1] L. B. Townsend, *Chem. Rev.*, 1967, **67**, 533; I. E. Burrows, G. Shaw, and D. V. Wilson, *J. Chem. Soc.* (*C*), 1968, 40; M. Greenhalgh, G. Shaw, D. V. Wilson, and (in part) N. J. Cusack, *ibid.*, 1969, 2198.

[2] C. G. Beddows and D. V. Wilson, *J.C.S. Perkin I*, 1972, 1773.

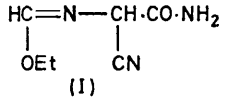
[3] G. Shaw, R. N. Warrener, D. N. Butler, and R. K. Ralph, *J. Chem. Soc.*, 1959, 1648.

[4] A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, 1939, **128**, 537.

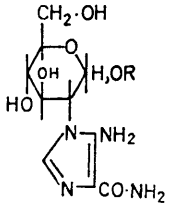
[5] M. V. Tracey, *Biochem. J.*, 1952, **52**, 265.

[6] P. A. Levene and C. C. Christman, *J. Biol. Chem.*, 1937, **120**, 575.

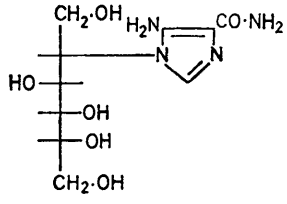
Prolonged action of methanolic hydrogen chloride on compound (IIa) did not give the expected methyl glycosides; however the methyl pyranosides (IIb) could be obtained directly by treating the appropriate anomer of methyl D-glycopyranoside [7] with the imidate (I).



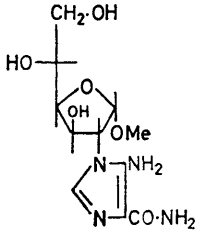
(I)



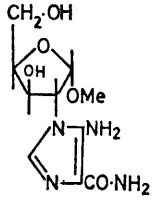
(II) a; R = H
b; R = Me



(III)



(IV)



(V)

The products were purified by formation and recrystallisation of their picrates, which were subsequently converted into free bases by the action of an anion exchange resin.

The methyl aminoimidazolyl-α-D-glucofuranoside (IV) was prepared and purified in the same way. Periodate oxidation of this compound followed by reduction with sodium borohydride and purification on AG 1 × 8 (formate) resin gave the xylofuranoside (V).

EXPERIMENTAL

The general experimental procedures used in the present work are those described in detail in an earlier paper.[2] Thin-layer chromatograms were run on glass plates coated with CC41 cellulose, with one of the following as developing solvent: (A) n-butanol–water (87 : 13), (B) 5% ammonium citrate (pH 4·4)–ethanol (18 : 83), (C) n-butanol–ethanol–water (4 : 1 : 5; upper layer), (E) propan-2-ol–0·2N-ammonia solution (4 : 1), (G) saturated ammonium hydrogen carbonate solution, (H) n-butanol–glacial acetic acid–water (4 : 2 : 5), (I) propan-2-ol–water–ammonia (*d* 0·880) (6 : 2 : 3).

Freeze-drying was carried out with an Edwards model 30P2 freeze-dryer. U.v. spectra were recorded with a Unicam SP 800 spectrophotometer; values ' in acid ' refer to solutions adjusted to pH 1·5—2·0 with hydrochloric acid and those ' in alkali ' to solutions adjusted to pH 10·5 with sodium hydroxide solution.

2-(5-*Amino*-4-*carbamoylimidazol*-1-*yl*)-2-*deoxy*-D-*glucose* (IIa).—D-Glucosamine hydrochloride (0·9 g) in potassium hydrogen carbonate solution (8 ml) was shaken thoroughly with a freshly prepared solution of ethyl *N*-[carbamoyl-(cyano)methyl]formimidate (0·9 g) in ether (10 ml). The aqueous phase was separated, extracted with ether (10 ml), and evaporated to 3 ml. Crystals (290 mg) appeared when the solution was kept at 4 °C for 3 days. Recrystallisation from water gave the *nucleoside analogue* as needles, m.p. 198—200 °C, $[\alpha]_D^{16}$ +56·2 (*c* 0·1 in H_2O) initially, changing to +30·1° after 24 h (Found: C, 41·9; H, 5·6; N, 19·3. $C_{10}H_{16}N_4O_6$ requires C, 41·7; H, 5·6; N, 19·4%), $\lambda_{max.}$ (H_2O) 264—266 nm (ε 10,000), $\lambda_{max.}$ (in acid) 267 nm (ε 7800), $\lambda_{max.}$ (in alkali) 265 nm (ε 10,450). The compound gave a purple colour ($\lambda_{max.}$ 545 nm) in the Bratton–Marshall test and consumed 1·95 mol. equiv. of periodate in 2 days, 2·15 mol. equiv. in 3 days, and 2·43 mol. equiv. in 8 days.

Reactions of the Nucleoside Analogue (IIa).—(i) *With methanolic hydrogen chloride.* The compound (21 mg) and a solution containing 0·2% hydrogen chloride in anhydrous methanol (0·75 ml) were heated in a sealed tube at 60 °C for 46 h. T.l.c. of the syrup remaining after evaporation of the tube contents in system (A) showed only starting material and a spot at the origin; no material resembling the glycosides described later was detected.

(ii) *Attempted catalytic hydrogenation.* The compound (208 mg) and Adams catalyst (22 mg) in water (5 ml) were shaken for 2 h in hydrogen at room temperature and atmospheric pressure. Most of the starting material was recovered unchanged; t.l.c. revealed only a trace product with the same R_F values as the glucitol derivative described later.

(iii) *Oxidation.* Unidentified products which gave no colour in the Bratton–Marshall test were obtained when the compound was treated with any of the following reagents: bromine water in the presence of barium carbonate at room temperature, bromine water alone, mercuric oxide at 100 °C for 1 h, Tollens reagent at room temperature, a mixture of equal volumes of glacial acetic acid and hydrogen peroxide (100 vol.) for 1 h at 50 °C, concentrated nitric acid at 100 °C for 4 min, and acidified potassium permanganate solution.

Treatment with neutral potassium permanganate solution or catalytic oxidation in potassium hydrogen carbonate solution over Adams catalyst, gave mainly unchanged starting material.

(iv) *Reactions with acid.* After storage at room temperature for 3 days a solution of the compound in N-hydrochloric acid gave no colour in the Bratton–Marshall test. During this time the u.v. maximum shifted from 267 to 277 nm.

In aqueous 20% acetic acid solution at 100 °C Bratton–Marshall activity was lost within 30 min; the main product ($\lambda_{max.}$ 291 nm in acid) remained at the origin on t.l.c. in systems (A) and (E).

(v) *Reactions in sodium hydroxide solution.* A dilute solution of the nucleoside in N-sodium hydroxide gave no colour in the Bratton–Marshall test after it had been kept at room temperature for less than 2 days.

2-(5-*Amino*-4-*carbamoylimidazol*-1-*yl*)-2-*deoxy*-D-*glucitol* (III).—(a) *From compound* (IIa). A solution of sodium borohydride (100 mg) in water (5 ml) was added with stirring during 10—15 min to 2-(5-amino-4-carbamoylimidazol-1-yl)-2-deoxy-D-glucose (IIa) (200 mg) in water (10 ml) and the mixture was set aside at room temperature overnight. Acetone (5 ml) was added and, after 2 h at room temperature, the solution was evaporated to 1 ml and placed on a

[7] Y. Matsushima and T. Miyazaki, *Bull. Chem. Soc.* (*Japan*), 1965, **38**, 325.

column (1·8 × 20 cm) of CM52 cellulose (H^+). The column was washed with water at 48 ml h^{-1}. The main u.v.-absorbing fraction emerged after 710 ml and was eluted in the next 1700 ml of eluate; this was freeze-dried, redissolved in water (1 ml) and rechromatographed on a column (1 × 8 cm) of the same modified cellulose. The main fraction was again freeze-dried to give the *glucitol derivative* (III) as a white solid (68 mg) (Found: C, 41·1; H, 6·3; N, 19·5. $C_{10}H_{18}N_4O_6$ requires C, 41·4; H, 6·2; N, 19·3%), $\lambda_{max.}$ (in acid) 267—268 (ε 10,250) and 245 nm (8150), $\lambda_{max.}$ (neutral) 267—268 nm (ε 10,500), $\lambda_{max.}$ (in alkali) 267 nm (ε 13,150); $\lambda_{max.}$ in the Bratton–Marshall test was 542 nm, and R_F values of 0·13, 0·50, 0·37, and 0·45 were observed in systems (A), (B), (C), and (E), respectively. The compound consumed 3·08 mol. equiv. of periodate.

(b) *From 2-amino-2-deoxy-*D*-glucitol.* A solution of 2-amino-2-deoxy-D-glucitol hydrochloride (270 mg) in water (20 ml) was neutralised with potasium hydrogen carbonate and then shaken for 2 min with ethyl *N*-[carbamoyl(cyano)-methyl]formimidate (300 mg) in ether (25 ml). The ether layer was removed, extracted with water (10 ml), and discarded. The aqueous layers were combined, then boiled under reflux for 30 min, cooled, and treated with small portions of ZeoKarb 225 resin (H^+) until gas evolution ceased. The resin was filtered off and the filtrate was evaporated to a solid which was dissolved in water (2 ml) and chromatographed on a column of AG 1 × 8 (200—400 mesh) resin (HCO_2^-) with water as eluant. The main u.v.-absorbing fraction emerged between 110 and 160 ml, and was identical [t.l.c. in systems (A), (B), (C), and (E)] with the product from the foregoing reaction.

*Methyl 2-(5-Amino-4-carbamoylimidazol-1-yl)-2-deoxy-α-*D*-glucopyranoside* (IIb).—Methyl 2-amino-2-deoxy-α-D-glucopyranoside [7] (0·9 g) in methanol (25 ml) was treated with a solution of ethyl *N*-[carbamoyl(cyano)methyl]formimidate (0·9 g) in ether (25 ml). The solution was concentrated to 25 ml, then methanol (15 ml) was added. The mixture was kept at room temperature for 24 h then heated under reflux for 15 min, cooled, and evaporated to a syrup, which did not crystallise. A portion (100 mg) of the syrup in water (0·5 ml) was cooled to 0 °C and saturated aqueous picric acid solution was added slowly until no more precipitation occurred. The precipitate was collected, washed with water, and dried at room temperature *in vacuo* (P_2O_5) to give *methyl 2-(5-amino-4-carbamoylimidazol-1-yl)-2-deoxy-α-*D*-glucopyranoside picrate* (42 mg), m.p. 238—240 °C (Found: C, 38·6; H, 3·8; N, 18·6. $C_{17}H_{21}N_7O_{13}$ requires C, 38·4; H, 4·0; N, 18·5%), R_F 0·30 (A), 0·78 (E). A suspension of this picrate (147 mg) in water (2 ml) and AG 1 × 8 (20—50 mesh) resin (OH^-) were stirred together until a colourless solution was obtained (usually 2—3 h). This was filtered and the filtrate freeze-dried to give the *free base* (68 mg) $[\alpha]_D^{20}$ +114·4° (*c* 0·1 in H_2O) (Found: C, 43·5; H, 5·7; N, 18·3. $C_{11}H_{18}N_4O_6$ requires C, 43·7; H, 6·0; N, 18·5%), $\lambda_{max.}$ (in acid) 265—266 nm (ε 9300), $\lambda_{max.}$ (neutral) 266 nm (ε 11,200), $\lambda_{max.}$ (in alkali) 265—266 nm (ε 11,600). It gave a purple colour in the Bratton–Marshall test [$\lambda_{max.}$ 548 nm (ε 15,600)] and consumed 0·94 mol. equiv. of periodate during 3 days. It had R_F values of 0·30, 0·54, 0·78, 0·95, 0·79, and 0·36 in systems (A), (C), (E), (G), (H), and (I), respectively.

*Methyl 2-(5-Amino-4-carbamoylimidazol-1-yl)-2-deoxy-β-*D*-glucopyranoside* (IIb).—Ethyl *N*-[carbamoyl(cyano)-methyl]formimidate (0·3 g) in ether (5 ml) was added to methyl 2-amino-2-deoxy-β-D-glucopyranoside [7] (300 mg) in absolute ethanol (5 ml). The mixture was kept at room temperature overnight then heated under reflux for 10 min, cooled, and evaporated to a syrup which was dissolved in the minimum volume of water (*ca.* 0·5 ml). A picrate (330 mg) was prepared as described for the α-compound; m.p. 229—230 °C (from water) (Found: C, 38·5; H, 4·05; N, 18·5. $C_{17}H_{21}N_7O_{13}$ requires C, 38·4; H, 4·0; N, 18·5%). The picrate, water (2 ml), and AG 1 × 8 (20—50 mesh) resin (OH^-) were stirred together until a colourless solution was formed. The resin was filtered off and the solution freeze-dried to give the *β-glycoside* (69 mg), $[\alpha]_D^{16}$ +6·0° (*c* 0·1 in H_2O) (Found: C, 43·6; H, 6·2; N, 18·5. $C_{11}H_{18}N_4O_6$ requires C, 43·3; H, 6·0; N, 18·5%), $\lambda_{max.}$ (in acid) 240 (ε 9980) and 265—266 nm (ε 9500); $\lambda_{max.}$ (neutral) 266 nm (ε 11,400); $\lambda_{max.}$ (in alkali) 265—266 nm (ε 11,900). It gave a purple solution [$\lambda_{max.}$ 550 nm (ε 17,450)] in the Bratton–Marshall test, consumed 0·97 mol. equiv. of periodate during 5 days, and had R_F values 0·27, 0·65, and 0·62 in systems A, C, and E, respectively.

*Methyl 2-(5-Amino-4-carbamoylimidazol-1-yl)-2-deoxy-α-*D*-glucofuranoside* (IV).—Methyl 2-amino-2-deoxy-α-D-glucofuranoside [7] (0·95 g) was dissolved in methanol (40 ml) and treated with ethyl *N*-[carbamoyl(cyano)methyl]formimidate (0·9 g) in ether (10 ml). The mixture was set aside at room temperature for 2 days then evaporated to a syrup, which was dissolved in the minimum volume of water (*ca.* 2 ml). Addition of a slight excess of saturated aqueous picric acid at 4 °C gave *methyl 2-(5-amino-4-carbamoylimidazol-1-yl)-2-deoxy-α-*D*-glucofuranoside picrate* (223 mg) as prisms, m.p. 184—186 °C (Found: C, 38·4; H, 3·9; N, 18·6. $C_{17}H_{21}N_7O_{13}$ requires C, 38·4; H, 4·0; N, 18·5%).

The picrate was converted into the free base by treatment with AG 1 × 8 (20—50 mesh) resin (OH^-) as described for the corresponding pyranoside. Alternatively, the picrate (16 mg) was dissolved in a large volume of water (40 ml) and the solution was passed through a column (1 × 5 cm) of DE 52 cellulose (elution with water). The eluate was freeze-dried to give the *furanoside* (IV) [8·9 mg after drying at room temperature (P_2O_5) *in vacuo*] as an amorphous fluffy solid, $[\alpha]_D^{16}$ +113·2° (*c* 0·1 in H_2O) (Found: C, 43·4; H, 6·2; N, 18·3. $C_{11}H_{18}N_4O_6$ requires C, 43·7; H, 6·0; N, 8·5%), $\lambda_{max.}$ (in acid) 265—266 nm (ε 8100), $\lambda_{max.}$ (neutral) 265—266 nm (ε 10,000), $\lambda_{max.}$ (in alkali) 265—266 nm (ε 10,700), $\lambda_{max.}$ (Bratton–Marshall test) 546—547 nm (ε 15,700), R_F values 0·44 and 0·68 in systems (A) and (C), periodate uptake 1·03 mol. equiv. during 3 days.

*Methyl 2-(5-Amino-4-carbamoylimidazol-1-yl)-2-deoxy-α-*D*-xylofuranoside* (V).—A solution of potassium periodate (0·184 g) in water (1 ml) was added to a cooled solution of methyl 2-(5-amino-4-carbamoylimidazol-1-yl)-2-deoxy-α-D-glucofuranoside (100 mg) in water (9 ml). Initially the solution had $[\alpha]_D^{16}$ +159·0°, after 5 min +127·5°, and after 20 min from the start of the reaction +127·3°. At this time 1·07 mol. equiv. of periodate had been consumed.

The solution was placed on a column (10 × 2 cm) of DE 52 cellulose and eluted with water (120 ml). The eluate was evaporated at 28 °C to a syrup, a sample of which reduced Fehlings solution. Sodium borohydride (203 mg) was added and the mixture was kept at room temperature overnight then treated with acetone (5 ml) and glacial acetic acid (4 drops). The syrup which remained after evaporation was dissolved in water (1 ml) and chromatographed on a column (2·5 × 52 cm) of AG 1 × 8 (200—400 mesh) resin (HCO_2^-) (elution with water at 29 ml^{-1}). The major fraction (116—159 ml) was rechromatographed on a

column (3·5 × 35 cm) of the same resin (eluted between 214 and 395 ml). Freeze-drying gave the *xylofuranoside* (V) (8·9 mg) as an amorphous solid (Found: C, 44·2; H, 5·8; N, 20·5. $C_{10}H_{16}N_4O_5$ requires C, 44·1; H, 5·9; N, 20·6%), $\lambda_{max.}$ (in acid) 243 and 266 nm (ε 9200), $\lambda_{max.}$ (neutral) 265—266 nm (ε 10,200), $\lambda_{max.}$ (in alkali) 265—266 nm (ε 10,900), R_F 0·45 (A) and 0·57 (C).

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