## 720. *The Preparation of 7- and 9-Ribofuranosyl Derivatives of 8-Azaxanthine. A Note on the Preparation of 9-Glucopyranosylxanthine.*

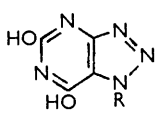
By J. Baddiley, J. G. Buchanan, and G. O. Osborne.

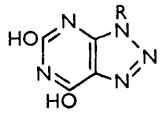
The 7- and the 9-β-D-ribofuranoside of 8-azaxanthine (I and II; R = ribofuranosyl) have been synthesised by the action of potassium hypobromite on 1-β-D-ribofuranosyl-1 : 2 : 3-triazole-4 : 5-dicarboxyamide (III; R = ribofuranosyl, R′ = $NH_2$).

In a re-examination of a similar reaction on glucosylglyoxaline-4 : 5-dicarboxyamide (V; R = glucopyranosyl) it is found that both the 7- and the 9-isomer of β-D-glucopyranosylxanthine (VI and IV; R = glucopyranosyl) are formed.

Since certain 8-azapurine bases function as purine antagonists in various biological systems, the synthesis and biological examination of the corresponding nucleosides is clearly of interest. The preparation of tri-*O*-benzoyl-β-D-ribofuranosyl azide in this Laboratory has led to a synthetic route to *N*-glycosyl derivatives (I and II; R = glycosyl) of 8-azaxanthine. The preparation by this route of 7- and 9-β-D-glucopyranosyl and β-D-xylopyranosyl derivatives of 8-azaxanthine has already been described.[1] The synthesis of the 7- and the 9-β-D-ribofuranosyl derivative of 8-azaxanthine is now reported.

Dimethyl acetylenedicarboxylate and 2 : 3 : 5-tri-*O*-benzoyl-β-D-ribofuranosyl azide[2] gave dimethyl 1-(2 : 3 : 5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1 : 2 : 3-triazole-4 : 5-dicarboxylate (III; R = ribofuranosyl, R′ = OMe). With methanolic ammonia at 0° this compound yielded 1-β-D-ribofuranosyl-1 : 2 : 3-triazole-4 : 5-dicarboxyamide (III; R = ribofuranosyl, R′ = $NH_2$). Reaction of the diamide with an excess of potassium hypobromite yielded the 7- and the 9-β-D-ribofuranosyl derivative of 8-azaxanthine (I and II; R = ribofuranosyl), separated by adsorption on a Dowex-2 (formate) column and elution with 0·2M-ammonium formate: complete removal of the 9-isomer required the passage of a large volume of eluate.


(I)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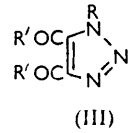
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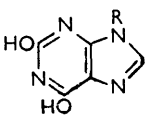
7-β-D-Ribofuranosyl-8-azaxanthine possessed spectroscopic properties similar to those recorded for the corresponding xylose and glucose derivatives;[1] the spectroscopic properties of 9-β-D-ribofuranosyl-8-azaxanthine resembled those of 9-β-D-glucopyranosyl-8-azaxanthine and of 9-β-D-xylopyranosyl-8-azaxanthine. The 7- and the 9-ribofuranosyl derivative had generally similar $R_F$ values in two chromatographic systems. In ultraviolet light the 7-isomer was detected as a blue-fluorescent spot after exposure of the chromatograms to ammonia. The 9-isomer was detected as a similar fluorescent spot after exposure to hydrochloric acid fumes.

Very recently, Davoll[3] reported the preparation of *N*-glycosyl derivatives of several 8-azapurine bases through chloromercuri-derivatives. 8-Azaxanthosine (II; R = ribofuranosyl) was synthesised and the position of the ribose established conclusively. A sample, kindly provided by Dr. J. Davoll, is indistinguishable from one of our isomers, to which we had assigned the 9-ribosyl structure, in its spectra and on paper chromatography. Our orientation scheme,[1] based on spectroscopic comparison with a methylated 8-azaxanthine, is thus confirmed. Davoll has prepared three *N*-ribosyl derivatives of

[1] Baddiley, Buchanan, and Osborne, *J.*, 1958, 1651.
[2] Baddiley, Buchanan, Hodges, and Prescott, *J.*, 1957, 4769.
[3] Davoll, *J.*, 1958, 1593.

8-azaguanine. One was 8-azaguanosine. In the absence of appropriate reference compounds the others were described provisionally as 8- and 7-ribosyl-8-azaguanine. Treatment with nitrous acid converted the latter into a product spectroscopically similar to our 7-glycosyl derivatives of 8-azaxanthine.* The structures assigned provisionally are thus confirmed.

Xanthosine (IV; R = ribofuranosyl),[4] 9-D-ribopyranosylxanthine,[5] 9-D-mannopyranosylxanthine,[5] and 9-D-xylopyranosylxanthine [6] have been prepared from the appropriate 1-glycosylglyoxaline-4 : 5-dicarboxyamide (V; R = glycosyl) by treatment with potassium hypobromite. Attempts to prepare 9-D-glucopyranosylxanthine in a similar way were unsuccessful [6] and the 9-D- and 9-L-arabinopyranosyl diamide yielded only traces of glycosyl-purines. In the 8-azapurine field we have observed that the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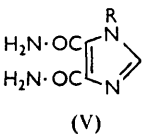
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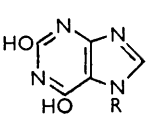
(VI)

1-β-D-glucopyranosyl as well as the 1-β-xylopyranosyl and 1-β-D-ribofuranosyl derivative of 1 : 2 : 3-triazole-4 : 5-dicarboxyamide give *N*-glycosyl derivatives of 8-azaxanthine on treatment with potassium hypobromite. In view of this, and of the probable similarity of the cyclisation step in the purine series, the failure of the route to the glucopyranosylpurine was surprising. Consequently, the reaction of 1-β-D-glucopyranosylglyoxaline-4 : 5-dicarboxyamide with an equivalent of potassium hypobromite was re-examined.

In small-scale experiments 1-D-xylopyranosyl- † and 1-D-glucopyranosyl-glyoxaline-4 : 5-dicarboxyamide were treated with potassium hypobromite (approx. 1 mol.). The reaction conditions were those previously employed for the preparation of 9-D-xylopyranosylxanthine.[6]

Products were examined by paper chromatography in propan-1-ol–ammonia (*d* 0·88)–water (6 : 3 : 1). On chromatograms, the xyloside reaction mixture displayed a prominent spot, detected by its ultraviolet absorption, at $R_F$ 0·34. The glucoside reaction mixture displayed a counterpart at $R_F$ 0·33. The areas corresponding to these spots were cut out, the materials eluted, and the ultraviolet absorption spectra in aqueous solution at pH 7 and in 0·1N-sodium hydroxide were recorded. The spectra of the two compounds were identical, the pairs of maxima in 0·1N-alkali being similar to those recorded for 9-D-xylopyranosylxanthine. The major products represented by the spots thus appeared to be 9-D-xylopyranosyl- and 9-D-glucopyranosyl-xanthine, respectively.

9-D-Glucopyranosylxanthine was isolated from a larger-scale preparation by ion-exchange chromatography on a Dowex-2 (formate) column, elution being carried out with ammonium formate at pH 5·8. The major product yielded xanthine and glucose (both identified by paper chromatography) on hydrolysis in acid. It ran as a single compound on paper in several solvents and showed absorption maxima in the ultraviolet region typical of a 9-substituted xanthine.[4-7] Its mode of preparation from 1-D-glucopyranosylglyoxaline-4 : 5-dicarboxyamide, itself prepared from 2 : 3 : 4 : 6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, implies that it is a β-glucosyl compound.[8]

Two minor components were eluted from the column in the above experiment. One of these showed only general ultraviolet absorption over the range 220—280 mμ and was not further investigated. The other component was of considerable interest. In two

* Communication from Dr. J. Davoll.
† Prepared by Dr. J. Stewart.

[4] Howard, McLean, Newbold, Spring, and Todd, *J.*, 1949, 232.
[5] Baxter, McLean, and Spring, *J.*, 1948, 523.
[6] Baxter and Spring, *J.*, 1947, 378.
[7] Gulland, Holiday, and Macrae, *J.*, 1934, 1639.
[8] Haynes and Newth, *Adv. Carbohydrate Chem.*, 1955, **10**, 207.

chromatographic systems it had the same $R_F$ as 9-β-D-glucopyranosylxanthine. Moreover, as in the latter case, treating the paper chromatogram with hydrogen chloride fumes before viewing it in ultraviolet light led to the appearance of a blue fluorescent spot in place of the opaque spot previously observed. On acid hydrolysis, xanthine and glucose were produced (both detected on paper chromatograms) but rather more slowly than from 9-β-D-glucopyranosylxanthine. The absorption spectrum in 0·1N-hydrochloric acid consisted of a single maximum at 267 mμ, and in 0·1N-sodium hydroxide of a maximum at 290 mμ. These values agree well with those recorded for 7-methylxanthine.[7] The product was present in insufficient quantity to permit isolation of a pure sample, but it was clearly a 7-glucosylxanthine (VI; R = glucopyranosyl).

Although the reaction between glycosylglyoxaline-4 : 5-dicarboxyamides and hypobromite is potentially capable of yielding both 7- and 9-glycosides of xanthine, the preparation only of 9-glycosyl compounds has been recorded. In the light of the present work it is clear that 7-glycosyl compounds may also be produced, but in small quantity. A rough spectrophotometric estimation indicated a yield of 7-glucosylxanthine of about 2%. The yield of 9-β-D-glucopyranosylxanthine, also estimated spectrophotometrically, was 25%.

Since 5-amino-1-glucosylglyoxaline-4-carboxyamide and its isomers are also possible reaction products, the aqueous washings from the Dowex-2 column were examined. The complex mixture present appeared to contain no appreciable amount of these substances.

## Experimental

*Dimethyl* 1-(2 : 3 : 5-*Tri*-O-*benzoyl*-β-D-*ribofuranosyl*)-1 : 2 : 3-*triazole*-4 : 5-*dicarboxylate*.—Dimethyl acetylenedicarboxylate (2·5 g., 1·25 mol.) in dry benzene (100 c.c.) was heated under reflux for 5 hr. with 2 : 3 : 5-tri-*O*-benzoyl-β-D-ribofuranosyl azide [2] (6·7 g., 1 mol.), and the solution poured into an excess of light petroleum (b. p. 40—60°). The precipitated gum (9·7 g.) was chromatographed in benzene on neutral alumina, 4 : 1 benzene–chloroform being used for elution. Fractions 5—19 (each of 100 c.c.) contained the triazole derivative. Fractions 7—19 were evaporated to a colourless gum (6·5 g.). For analysis the 1 : 2 : 3-*triazole derivative* was converted into a foam (Found: C, 61·3; H, 4·2; N, 6·9. $C_{32}H_{27}O_{11}N_3$ requires C, 61·0; H, 4·3; N, 6·7%).

1-β-D-*Ribofuranosyl*-1 : 2 : 3-*triazole*-4 : 5-*dicarboxyamide*.—Dimethyl 1-(2 : 3 : 5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1 : 2 : 3-triazole-4 : 5-dicarboxylate (6·5 g.) in dry methanol (180 c.c.) saturated with ammonia was kept at 0° for 4 days. After concentration of the solution to half its volume, crystals (1·75 g.) separated on cooling. Further evaporation followed by the addition of an excess of ether precipitated a further quantity of solid (0·4 g.). Recrystallised from methanol the *diamide* had $[\alpha]_D^{20}$ −64° (*c* 1·03 in water) (Found: C, 37·8; H, 4·8; N, 24·3. $C_9H_{13}O_6N_5$ requires C, 37·6; H, 4·6; N, 24·4%). It consumed 0·94 mol. of periodate.

*Reaction with hypobromite*. 1-β-D-Ribofuranosyl-1 : 2 : 3-triazole-4 : 5-dicarboxyamide (0·621 g.) was treated with freshly prepared potassium hypobromite [5] (12 c.c.) at 2° for 20 hr. and at 70° for 15 min. The product was percolated down a Dowex-2 resin column (18 × 1 cm., 200—400 mesh) in the formate form, which was washed with water and then eluted with 0·2M-ammonium formate at pH 7. Fractions 58—90 (each of 25 c.c.), containing the 7-isomer, were combined. Fractions 112—300, containing the 9-isomer, were also combined.

7-β-D-*Ribofuranosyl*-8-*azaxanthine* (5 : 7-*Dihydroxy*-1-β-D-*ribofuranosyl*-v-*triazolo*[4 : 5-d]-*pyrimidine*).—Fractions 58—90 from the previous experiment were evaporated at 35° (rotary evaporator) and ammonium formate was sublimed under reduced pressure at 65°. The residual gum was dissolved in water and adsorbed on a column of " Norit " charcoal. This was washed with water (to remove inorganic impurities) and the product eluted with ethanol–ammonia (*d* 0·88)–water (40 : 1 : 50). Removal of the solvent left a gum which crystallised readily (47 mg.). Recrystallised from aqueous methanol, the 7-β-D-*ribofuranosyl derivative* had m. p. 185°, $[\alpha]_D^{20}$ −92° (*c* 0·5 in water) (Found: C, 37·9; H, 4·3. $C_9H_{11}O_6N_5$ requires C, 37·9; H, 3·9%). It consumed 0·94 mol. of periodate. The total yield of material estimated spectrophotometrically was 191 mg.

9-β-D-*Ribofuranosyl*-8-*azaxanthine* (5 : 7-*Dihydro*-3-β-D-*ribofuranosyl*-v-*triazolo*[4 : 5-d]-*pyrimidine*).—Fractions 112—300 from the earlier experiment were evaporated at 35°. The

considerable quantity of ammonium formate remaining was extracted with small quantities of dry methanol. Removal of solvent from these extracts, followed by sublimation of ammonium formate, gave a brown gum. This was adsorbed on charcoal and eluted with ethanol–ammonia (*d* 0·88)–water as before. Removal of solvent left a colourless gum. This was dissolved in ethanol, and ether was added to precipitate a white solid (about 10 mg.) (Found: C, 34·7; H, 4·8. $C_9H_{11}O_6N_5,1\frac{1}{2}H_2O$ requires C, 34·6; H, 4·5%). The amount of *product* present initially was 82 mg., estimated spectrophotometrically.

*Hydrolysis.* N-Hydrochloric acid (0·2 c.c.) was added to the ribofuranosyl derivative (0·5 mg.). Hydrolysis to 8-azaxanthine and ribose was complete in 1 hr. at 100°. Hydrolysates were examined by paper chromatography in the systems: *A*, propan-1-ol–ammonia (*d* 0·88)–water (6 : 3 : 1); and *B*, the water-poor phase from butan-1-ol–acetic acid–water (4 : 1 : 5).

| | $R_F$ values in solvents | | Conditions for fluorescence |
|---|---|---|---|
| | A | B | |
| 7-β-D-Ribofuranosyl-8-azaxanthine | 0·35 | 0·28 | Alkaline |
| 9-β-D-Ribofuranosyl-8-azaxanthine | 0·38 | 0·31 | Acid |
| ,, ,, ,, (from Davoll) | 0·38 | 0·31 | Acid |
| 8-Azaxanthine | 0·31 | 0·45 | Acid |
| Ribose | 0·59 | 0·29 | — |

8-Azaxanthine and its derivatives were detected by their fluorescence under the appropriate conditions. Ribose was detected by the aniline phthalate spray.

*Determination of the Ring Size.*—The method previously described [9] was employed in the examination of 1-β-D-ribofuranosyl-1 : 2 : 3-triazole-4 : 5-dicarboxyamide, 7-β-D-ribofuranosyl-8-azaxanthine, and 9-β-D-ribofuranosyl-8-azaxanthine. Periodate oxidation, followed by reduction with sodium borohydride and acid hydrolysis, gave glycerol (detected on paper chromatograms) and no ethylene glycol. This confirms the ribofuranosyl structure for these compounds.

*Spectra of Azaxanthine Derivatives.*—These maxima (mμ) were as tabulated. The figures in parentheses are extinction coefficients (ε).

| | In HCl, pH 2·2 | pH 7·0 | In 0·1N-NaOH |
|---|---|---|---|
| 7-β-D-Ribofuranosyl-8-azaxanthine | 278(5·3 × $10^3$) | 278(5·0 × $10^3$) | 310(5·1 × $10^3$) |
| 9-β-D-Ribofuranosyl-8-azaxanthine | (*a*) 255(8·9 × $10^3$) | 252(8·6 × $10^3$) | 250(5·6 × $10^3$) |
| | 240(6·7 × $10^3$) | 277(7·9 × $10^3$) | 280(8·4 × $10^3$) |
| | (*b*) 256(9·5 × $10^3$) | 252(9·7 × $10^3$) | 251(7·1 × $10^3$) |
| | 240(5·9 × $10^3$) | 277(8·8 × $10^3$) | 280(9·5 × $10^3$) |

(*a*) Prepared from sugar azide; determination carried out on 1 mg. sample. (*b*) Reported by Davoll.[3]

9-β-D-*Glucopyranosylxanthine.*—1-Glucopyranosylglyoxaline-4 : 5-dicarboxyamide [6] (1·0 g.) was treated with freshly prepared potassium hypobromite [5] (6 c.c.; approx. 1 mol.) at 2° for 1 hr., then at 70° for 10 min. The product was diluted with water (50 c.c.) and percolated down a Dowex-2 column (15 × 1 cm.; 200—400 mesh) in the formate form. The column was washed with water (500 c.c.), and products were eluted with 0·1M-ammonium formate solution at pH 5·8. Fractions 5—7 (each of 50 c.c.) and 14—17 contained minor components. Fractions 23—32, containing the major component, were combined and evaporated to dryness at 35°. Ammonium formate was sublimed at 60° under reduced pressure. The residue was dissolved in water (5 c.c.), and the solution filtered. Addition of an excess of ethanol led to the precipitation of a cream-coloured powder (149 mg.). This material was redissolved in water, followed by reprecipitation with ethanol. The 9-*glucoside* was dried for 18 hr. at 70° (Found: C, 39·7; H, 5·3; N, 16·9. $C_{11}H_{14}O_7N_4,H_2O$ requires C, 39·8; H, 4·9; N, 16·9%).

Ultraviolet absorption maxima were at 234 (ε 7·1 × $10^3$) and 262 mμ (ε 7·6 × $10^3$) in 0·1N-hydrochloric acid, and at 248 (ε 8·7 × $10^3$) and at 277 mμ (ε 7·6 × $10^3$) in 0·1N-sodium hydroxide.

7-*Glucopyranosylxanthine.*—Fractions 5—7 from the earlier experiment were combined and evaporated, and ammonium formate was sublimed as previously described. A small amount of coloured material remained. This had a maximum at 267 mμ in 0·1N-hydrochloric acid and at 290 mμ in 0·1N-sodium hydroxide.

*Hydrolysis of Glucopyranosylxanthine.*—Milligram quantities of the 7- and the 9-glucoside

[9] Viscontini, Hoch, and Karrer, *Helv. Chim. Acta*, 1955, **38**, 642.

were treated with N-hydrochloric acid (1 c.c.) at 100° for 1 hr., hydrolysis being followed by paper chromatography. The 9-glucoside was completely hydrolysed. The 7-glucoside also yielded xanthine and glucose, but unchanged starting material was observed. After treatment with N-acid at 100° for a further 3 hr., hydrolysis was still not quite complete.

*Paper Chromatography.*—Ascending chromatograms (see Table) were run on Whatman No. 4 paper in the following systems: *A*, propan-1-ol–ammonia (*d* 0·88)–water (6 : 3 : 1); *B*, the water-poor phase from butan-1-ol–acetic acid–water (4 : 1 : 5). Xanthine and the glucosylxanthines were detected by their ultraviolet absorption, or the latter by their fluorescence in ultraviolet light after exposure of chromatograms to hydrogen chloride fumes. Glucose was detected by the aniline phthalate spray.

| | $R_F$ in solvents | |
|---|---|---|
| | *A* | *B* |
| 9-β-D-Glucopyranosylxanthine | 0·34 | 0·17 |
| 7-Glucopyranosylxanthine | 0·34 | 0·17 |
| Xanthine | 0·45 | 0·40 |
| Glucose | 0·61 | 0·23 |

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