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SIMULTANEOUS PRODUCTION OF ISOMERIC DI- AND MONO-GLUCOPYRANOSYL HYPOXANTHINES. (SYNTHESIS IN NUCLEOSIDE ANTIBIOTICS, IV)

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In the course of the investigations on the synthesis of nucleoside antibiotics, we have closely examined the reaction products of bromomercuri derivative of hypoxanthine with acetobromoglucose, and found that two isomeric diglucosylhypoxanthines are produced simultaneously in considerable yields besides the expected monoglucosides.

It is the first time that diglucosyl purines have been isolated from the reaction of acetobromosugar with the halomercuri derivative of the base.

This finding gives an explanation for the low yield in the synthesis of N-9-β-D-glucopyranosyl hypoxanthine by the Davoll-Lowy method. In addition, this suggests an effective synthetic route for the diglycosyl purine such as N-1-(5'-phosphoribosyl)-ATP,[1] an intermediate of histidin biosynthesis and the first example of the naturally-occuring 1,9-diglycosyl purine.

When monobromomercuri derivative of hypoxanthine was allowed to react with equimolar amount of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosylbromide according to the Davoll-Lowy's original procedure,[2] thin-layer chromatography on alumina* (solvent system : ethyl acetate-methanol, 20 : 1 v/v) revealed that the reaction product contained at least four components which consisted of the base and sugar. These components were separated and isolated by column chromatography packed with neutral alumina* followed by rechromatography with silicic acid*. Elutions were conducted by using the solvent-systems, ethyl acetate, ethyl acetate-chloroform (3 : 1), ethyl acetate-chloroform-methanol (4 : 1 : 2) and ethyl acetate-ethanol (1 : 1). The detail will be reported later. These four components are named I, II, III, and IV, respectively, according to

* Purchased from E. Merck AG., Darmstadt

the order of elution. Their yields are shown in TABLE 1.

TABLE 1

Yields of four components by coupling of monobromomercuri hypoxanthine with acetobromoglucose in xylene

		molar ratio
Bromomercuri hypoxanthine	7.0 g	1
		:
Acetobromoglucose	6.9 g	1

compound	yield, g (%)
I	1.70 (12.7)
II	1.64 (12.3)
III	0.47 (6.0)
IV	0.80 (10.2)
total	(41.2)

(I + II) + (III + IV) = 2.6

The estimation of integral ratios between the two protons (C-2, C-8) of the purine ring (δ, 8.0-10.0 p.p.m.) and the acetyl protons of the glucosyl moieties (δ, 1.8-2.2 p.p.m.) in their n.m.r. spectra suggested that the components I and II corresponded to bis-(tetra-O-acetyl-D-glucosyl) hypoxanthines whereas both components III and IV corresponded to tetra-O-acetyl-D-glucosyl hypoxanthines. This was also supported by the result of elemental analysis of these compounds.

Deacetylation of these compounds with methanolic ammonia at 0° followed by recrystallization gave the compounds I'-IV' in the yields of 70-80 % which are listed in TABLE 2.

The infrared spectra of the compound IV' and those of the authentic N-9-β-D-glucopyranosyl hypoxanthine[3] were superimposable, and the compound showed no depression of melting point upon admixture. The identity was also confirmed by their ultraviolet and n.m.r. spectra.

In the compound III' the allocation of the glucosyl moiety to the position 7 of hypoxanthine was based on the ultraviolet spectral comparison with the corresponding N-alkylhypoxanthines, as shown in TABLE 3.

Anomeric configurations of III' and IV' were assigned as β- on the basis of Baker's trans rule. Their coupling constants for the $C_{1'}$ and $C_{2'}$ protons, $J_{1',2'}$ 9 c.p.s. as shown in TABLE 2, FIG. 1 and 2, support this assignment.[4]

TABLE 2

M.p. and n.m.r. spectral data of deacetylated coupling products

compound	m.p.	n.m.r. spectra anomeric protons (δ, p.p.m.)
I'	215.5-216.5°	5.72 doublet (J = 9 c.p.s.) 6.08 doublet (J = 9 c.p.s.)
II'	221.5-223.0°	5.98 doublet (J = 9 c.p.s.) 6.02 doublet (J = 9 c.p.s.)
III'	203.0-204.0°	5.90 doublet (J = 9 c.p.s.)
IV'	275.0-276.0°	5.71 doublet (J = 9 c.p.s.)

TABLE 3

UV spectra of monoalkylhypoxanthines

Hypoxanthine	λ_{max} (mμ)		
	pH 1-2	pH 5-7	pH 11-13
1-Methyl-[5]	249	251	260
1-Glucosyl-	249	250.5	260.5
3-Methyl-[5]	253	264	265
3-Benzyl-[6]	254	265	264 (277 sh)
6-Methyl-[5]	254	252	261
7-Metny -[5]	250	256	262
7-Ribosyl-[6,7]	251	257	263
7-Glucosyl-	253.5	253	254
9-Methyl-[5]	250	250	253
9-Ribosyl-[6]	249	248	253
9-Glucosyl-	249	248	254

Position of the attachment of the two D-glucosyl residues in the diglucosyl hypoxanthines (I' and II') were supposed preliminarily on the ultraviolet spectral comparison, as indicated in TABLE 4. However, since these data do not cover all the possibilities and not provide definite evidence, the following acid hydrolysis and recoupling (recondensation) were conducted.

Compounds I' and II' were dissolved in N-hydrochloric acid and heated on boiling water-bath for 1.5 hr, giving monoglucosyl hypoxanthine in the yield of about 50 %. Both monoglucosyl hypoxanthines obtained were identical and proved to be 1-β-D-glucopyranosyl hypoxanthine by comparison with those ultraviolet spectra of N-alkylhypoxanthines (TABLE 3). The β-configuration

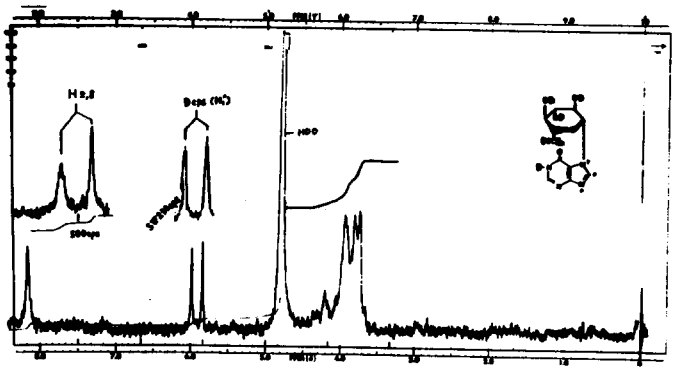

FIG. 1 N.m.r. spectrum of N-7-β-D-glucopyranosyl hypoxanthine in deuterium oxide

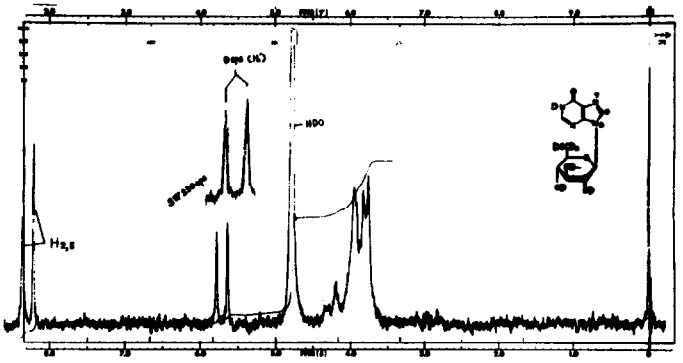

FIG. 2 N.m.r. spectrum of N-9-β-D-glucopyranosyl hypoxanthine in deuterium oxide

was assigned in similar manner described above from the coupling constant. Then, compounds III and IV previously identified were reacted with mercuric bromide in alkaline media, obtaining their bromomercuri derivatives, respectively. These derivatives were coupled with acetobromo-glucose under the usual conditions of the Davoll-Lowy method. The coupling product (yield, 23.3 %) obtained from the compound III was identical with the compound II, and the product (yield, 17.0 %) from the compound IV was identical with the compound I. Therefore, it is concluded that compounds I' and II' are bis-(1,9-β-D-glucopyranosyl) hypoxanthine and its 1,7-position isomer, and compounds I and II are their acetyl derivatives, respectively.

TABLE 4

UV spectra of dialkylhypoxanthines

Hypoxanthine	λ_{max} (mμ) pH 1-2	pH 5-7	pH 11-13
1,7-Dibenzyl-[6]	255	256	256
1,7-Diglucosyl-	254	254	256
1,9-Dibenzyl-[6]	253	252	252
1-Benzyl-9-ribosyl-[6]	251	249	249 (243 sh)
1,9-Diglucosyl-	251	251	252
3,7-Dibenzyl-[6]	256	266	267
3-Benzyl-7-ribosyl-[6]	255	266	266
6-Methyl-7-benzyl-[8]	256.5	258	261
6-Methyl-9-benzyl-[8]	254	252	256

The infrared spectra of compounds I'-IV' show interesting features in the region of 1500 - 1600 cm^{-1}. The spectra of I' and IV' show three medium absorption at 1510, 1550, and 1590 cm^{-1},

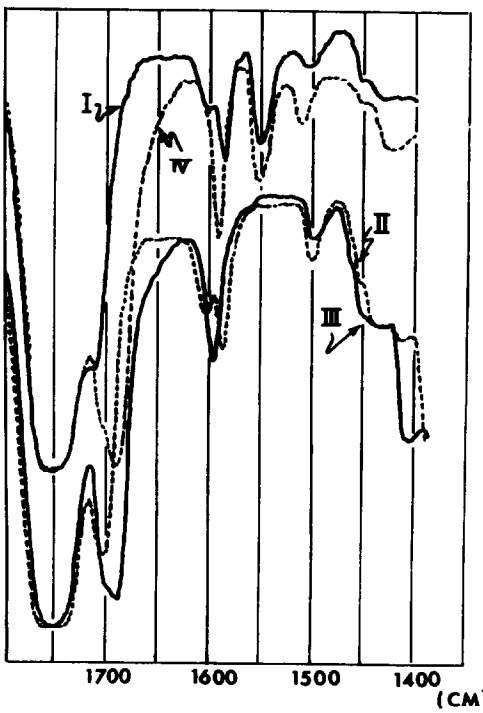

FIG. 3 IR spectra of isomeric diglucosyl hypoxanthines in chloroform

whereas those of II' and III' show two medium absorption at 1510 and 1590 cm^{-1}. Those features were particularly conspicuous in the spectra of their acetate. (see FIG. 3) It is noteworthy that this 1550 cm^{-1} band is characteristic of 9-substituted hypoxanthine regardless of the substitution on pyrimidine ring moiety.

When two molar equivalents of acetobromoglucose was coupled with the bromomercuri derivative of hypoxanthine, which was prepared by reacting two molar quantities of mercuric bromide with a molar hypoxanthine in the presence of two molar sodium hydroxide, the ratio of the yield of di-glucosides against that of monoglucoside was increased by 2.5 times as shown in TABLE 5.

TABLE 5

Yields of four components by coupling of bis-(bromomercuri) hypoxanthine with acetobromoglucose in xylene

		molar ratio
Bis-(bromomecuri) hypoxanthine	7.0 g	1
		:
Acetobromoglucose	6.9 g	2

compound	yield, g (%)
I	2.46 (15.7) (30.3)*
II	1.86 (11.5) (22.7)*
III	0.28 (2.9) (6.0)*
IV	0.40 (4.2) (8.4)*
total	(33.8) (67.4)
(I + II) + (III + IV) =	6.4

* based on bis-(bromomercuri) hypoxanthine

The result suggested that the composition and/or structure of the bromomercuri derivative of hypoxanthine is a key factor for such simultaneous production of mono- and di-glucosyl hypoxanthines. Recent titration studies elucidated that the binding of mercury occurs at the N-1 O-6 grouping of uridine and guanosine.[9] Accordingly, it can be reasonably inferred that the position of the attachment of mercury to hypoxanthine would be the N-1 O-6 grouping, besides the known N-7 or N-9. The attachment of D-glucosyl residue to the N-1 position can be interpreted on this assumption.

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