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SYNTHESIS OF GUANINE NUCLEOSIDES BY FUSION, AND A MECHANISTIC ASPECT OF THE REACTION

HAJIME IWAMURA*, MASAKAZU MIYAKADO, AND TAKESHI HASHIZUME
Department of Food Science and Technology, Kyoto University, Kyoto 606 (Japan)
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ABSTRACT

N^2-Acetylguanine (**1**) was condensed by fusion with the fully acetylated derivatives of the following sugars: β-D-ribofuranose (**2**), β-D-ribopyranose (**3**), α-D-xylopyranose (**4**), β-D-xylopyranose (**5**), α-D-glucopyranose (**6**), and β-D-glucopyranose (**7**). The reaction of **1** with either **2** or **3** gave a mixture of 7-β, 9-α, and 9-β isomers, whereas only the 7-β and 9-β isomers, and virtually no 9-α isomer, were obtained when **4**, **5**, **6**, and **7** were used. When each isomeric acetylated ribofuranosylguanine was heated in the presence of an acidic catalyst, a mixture of 7-β, 9-α, and 9-β nucleosides was formed. Close examination of the product ratios showed that the ratio of 7:9 isomers remained unchanged throughout the reactions, but the anomeric nature of the 9-substituted nucleoside was dependent on the sugar used.

INTRODUCTION

The first direct synthesis of guanine nucleosides was accomplished by condensing the chloromercuri derivative of acetylguanine with acylated glycosyl bromides[1,2]. Subsequently, several groups used the fusion method to obtain guanine nucleosides directly from acylguanines[3–5]. In all of these instances, however, a mixture of 7- and 9-substituted nucleosides, in their anomeric isomers, was formed. From these results, the formation of isomeric mixtures seems to be an inevitable disadvantage. However, a considerable amount of information has been gained on the mechanism of the fusion reaction from close examination of the reaction products[6–9]. The reactions dealt with in previous reports were limited to those giving anomeric isomers only. As already mentioned, we are now able to examine the fusion reaction to determine both position isomers and anomeric isomers and, therefore, obtain more information about the mechanism. In this report, we describe the glycosylation of acetylguanine with various fully acetylated sugars by fusion in the presence of bis(*p*-nitrophenyl) phosphate[10] as catalyst, together with detailed studies on the product distribution in the reaction.

*To whom inquiries should be addressed.

RESULTS AND DISCUSSION

The fusion reaction was performed for 30 min at 170–180° under diminished pressure with N^2-acetylguanine (**1**) and the respective fully acetylated sugars listed in Table I. The reaction products were isolated by column chromatography on silicic acid and by preparative t.l.c. The total yields of the isolated nucleosides were in the range of 39–58%.

Assignment of the position of attachment of the sugar moiety at N-7 or N-9 in the guanine nucleosides was made on the basis of a comparison of their u.v. absorption spectra with those of authentic *N*-methylguanines[17]. The anomeric configuration of the glycopyranosyl nucleosides was deduced from p.m.r. spectra. The anomeric H-1′ signals in the β-D-ribo-, β-D-xylo-, and β-D-glucopyranosyl derivatives appeared as wide doublets having $J_{1',2'} = 9.0$ Hz, indicating a 1,2-*trans*-diaxial configuration of protons with a projected valence angle of ~180° between H-1′ and H-2′. The H-1′ signal of 9-α-D-ribopyranosylguanine was a narrow doublet having $J_{1',2'} = 3.0$ Hz, indicative of an axial–equatorial orientation[18]. Structural elucidation of the ribofuranosylguanines was effected by direct comparison of their p.m.r. spectra and physicochemical data with those of authentic 7-β-D-ribofuranosylguanine, 9-α-D-ribofuranosylguanine, and guanosine[3,19].

For the determination of isomeric ratios, the band of each protected nucleoside in the t.l.c. chromatogram was excised and extracted, the absorbance of the extract at the respective u.v. absorption maximum was determined, and the product ratio was calculated from the ε values. The ratio was determined at least twice.

As shown in Table I, the reaction of **1** with either **2** or **3** gave a mixture of 7-β, 9-α, and 9-β nucleosides, whereas the reactions with **4**, **5**, **6**, and **7** afforded 7-β and 9-β nucleosides. In the latter cases, an isolable amount of the 9-α isomer could not be obtained, although its formation was observed by t.l.c. as a faint spot. This fact indicates that the product distribution is influenced not only by the properties of the base but also by those of the sugar. A similar observation has been made by Lee *et al.*[20] in employing a different technique for nucleoside synthesis, where N^2-acylguanine was made to react with either ribose or arabinose in chlorobenzene, with aluminum chloride as catalyst.

It is note worthy that, in the pyranose examples, a significant proportion of the 9-α isomer was produced only with the ribopyranose. One of the possible α-D conformers of the xylo- and glucopyranosyl nucleosides is the *C1*(D) conformation, in which the acetoxyl groups are equatorially oriented but the bulky guanine group is in the axial disposition. In the alternative *1C* conformation, all of the acetoxyl groups are in the supposedly unfavored axial disposition, although the bulky guanine group is oriented equatorially. The foregoing results seem to show, therefore, that the α-D form is very unstable in either the *C1* or the *1C* conformation. On the other hand, one of the two possible α-D conformers (the *C1* and the *1C* conformation) of the ribopyranose derivative may be rather favored, despite the fact that the sugar

portion has 2,3-*cis*-disposed acetoxyl groups*. Further comparative studies on related compounds are desirable, however, before rationalizations in terms of sugar conformation are attempted.

The anomeric configuration of the sugar had no effect on the product distribution, as may be seen in cases of α- and β-D-xylopyranose and of α- and β-D-glucopyranose. On the other hand, the constancy of the ratio of N-7 to N-9 isomers (40–45:55–60) was observed through all examples. This constancy was also apparent when the reaction of **1** with **2** was conducted with *p*-toluenesulfonic acid or zinc chloride as catalyst (Table II). These observations suggest that, under the fixed

TABLE I

FORMATION OF GUANINE NUCLEOSIDES BY FUSION

Acetylated sugar precursor	*Product ratio* (%)		
	7β	*9α*	*9β*
β-D-Ribofuranose[11] (2)	43	28	29
β-D-Ribopyranose[12] (3)	41	11	48
α-D-Xylopyranose[13] (4)	47	—	53
β-D-Xylopyranose[14] (5)	45	—	55
α-D-Glucopyranose[15] (6)	45	—	55
β-D-Glucopyranose[16] (7)	46	—	54

TABLE II

COMPARISON OF THE PRODUCT RATIOS IN THE REACTION OF **1** WITH **2**

Catalyst	*Product ratio* (%)		
	7β	*9α*	*9β*
Bis(*p*-nitrophenyl) phosphate	43	28	29
p-Toluenesulfonic acid	38	26	36
Zinc chloride	44	22	34

*In the p.m.r. spectrum of the acetylated ribopyranosylguanine in chloroform-*d*, the H-1′ signal appears as a doublet having $J_{1',2'} = 4.0$ Hz, and the H-2′ and H-3′ signals are observed at τ 4.1–4.7 as partially overlapping triplets ($J_{2',3'} = 4.0$ Hz and $J_{3',4'} = 3.5$ Hz). These first-order coupling constants indicate[18] that the projected valence angles between H-1′ and H-2′, H-2′ and H-3′, and H-3′ and H-4′, are approximately 60°. Unfortunately the H-4′ and H-5′ signals are incompletely resolved, but the H-4′ band is relatively narrow (~10 Hz) indicating small vicinal coupling-constants[21]. This result appears to support the *1C*(D) conformation, as the values of $J_{3',4'}$, $J_{4',5a'}$, and $J_{4',5e'}$ should be small in the *1C*(D) conformation. The alternative *C1*(D) conformation would have given a wider band because of a large $J_{4',5a'}$ coupling. The acetoxy-group signals provide further support; one signal occurs at relatively high field in the region indicative of the equatorial orientation and the other two signals are observed at lower field in the region for axial acetoxyl groups[18].

reaction conditions, the ratio of N-7 to N-9 substitution is dependent on the base derivative but is not influenced by the sugar or the catalyst.

Montgomery *et al.*[22] have shown that N→N alkyl migration occurs in the alkylation of *N*-benzylhypoxanthine at a high reaction-temperature. More recently, Miyaki and Shimizu[23] have reported a similar, intermolecular N→N glycosyl migration in purine derivatives. In line with these experiments, an attempt was made to keep each acetylated ribofuranosylguanine under the fusion conditions in the presence of a catalytic amount of bis(*p*-nitrophenyl) phosphate. It was observed that each blocked nucleoside gave 7-β, 9-α, and 9-β nucleosides, and the product ratios were similar to those observed for the fusion reaction (Table III). The same result was obtained when an equimolar amount of N^2-acetylguanine (**1**) was added to the reaction mixture, but no rearrangement was observed in the absence of catalyst. These results support the conclusion that the product distribution in the fusion reaction is determined by the thermodynamic stability of the products; this would explain the apparent exception of the fusion reaction to Baker's *trans* rule.

TABLE III

FORMATION OF RIBOFURANOSYLGUANINES IN THE REARRANGEMENT REACTION

Acetylated ribofuranosylguanine	*Product ratio* (%)		
	7β	*9α*	*9β*
7β	40	29	31
7β+**1**	45	25	30
9α	34	21	45
9α+**1**	38	27	35
9β	35	30	35
9β+**1**	42	26	32

Relevant to our results, Imai *et al.*[3] reported that the 7-α isomer was formed, in addition to the 7-β, 9-α, and 9-β nucleosides, in the fusion of diacetylguanine with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose. In this case, the benzoyl-blocked 7-α nucleoside is believed to be as thermodynamically stable as the other isomers, whereas, in our work with the acetates, the 7-α isomer seems to be thermodynamically unfavored. Onodera *et al.*[24] reported that an anomeric mixture was formed when 6-benzamidopurine was fused with penta-*O*-acetyl-β-D-glucopyranose in the presence of *p*-toluenesulfonic acid as catalyst, but only the β-isomer was obtained when zinc chloride was used. This discrepancy with our results may be explained on the assumption that the glucopyranosyladenine derivatives are thermodynamically very stable and, therefore, that the product distribution reflects a kinetically controlled process. In fact, no anomerization was observed by those investigators[24] when the 9-β derivative of adenine was heated in the presence of *p*-toluenesulfonic acid.

EXPERIMENTAL

General. — U.v. absorption spectra were determined with a Hitachi EPS-3T automatic spectrophotometer; p.m.r. spectra, with a Hitachi R-20 spectrometer; and i.r. spectra, with a Hitachi EPI-G3 spectrometer. Melting points are corrected.

Fusion reaction. — N^2-Acetylguanine and the respective fully acetylated sugar were mixed in a molar ratio of 1:1.3 and the mixture was heated for 30 min at 170–180° under diminished pressure (water pump) in the presence of a catalyst (5% by wt of the reactants).

Chromatographic separation. — The reaction mixture was applied to a column of silicic acid (Mallinckrodt, 100 mesh) and the column was eluted with chloroform–ethanol (98:2, v/v) or with chloroform–propyl alcohol (97:3, v/v). The fractions were examined by t.l.c. Silica Gel G (Merck), and the appropriate fractions were pooled. When preparative t.l.c. (Silica Gel G, Merck) was used, the reaction mixture was streaked onto a plate, and the plate was developed several times with chloroform–methanol (97:3, v/v). The nucleoside bands were eluted with chloroform–methanol (90:10, v/v) from the silica gel.

The following acetylated nucleosides were recrystallized from methanol, ethanol, or water* to give analytically pure samples: N^2-acetyl-7-(2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl)guanine (**9**), N^2-acetyl-9-(2,3,4-tri-*O*-acetyl-α-D-ribopyranosyl)-guanine (**10**), N^2-acetyl-9-(2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl)guanine (**11**), N^2-acetyl-7-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)guanine (**12**), N^2-acetyl-9-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)guanine (**13**), N^2-acetyl-7-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)guanine (**14**), N^2-acetyl-9-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-guanine (**15**). The acetylated ribofuranosylguanines did not crystallize from solvents.

Deacetylation. — Each protected nucleoside was treated with methanolic ammonia for 8 h at 0° and for 5 h at room temperature. Removal of the volatile products left a solid, which was recrystallized from water. The following guanine nucleosides were obtained in analytically pure state: 7-β-D-ribofuranosylguanine (**16**), 9-α-D-ribofuranosylguanine (**17**), 9-β-D-ribofuranosylguanine (**18**), 9-β-D-ribopyranosylguanine (**21**), 7-β-D-xylopyranosylguanine (**22**), 9-β-D-xylopyranosylguanine (**23**), 7-β-D-glucopyranosylguanine (**24**), and 9-β-D-glucopyranosylguanine (**25**)†.

The experimental results are summarized in Tables IV–VII.

Product ratio. — A portion of the reaction mixture was separated by t.l.c. by using multiple development with chloroform–methanol (97:3, v/v) as solvent. The

*In practice, water was added with heating to a solution of the sample in a small amount of alcohol. This treatment effected crystallization of acetylated nucleosides that could not be crystallized from organic solvents.

†Difficulties arose in obtaining satisfactory elemental microanalyses for 7-β-D-ribopyranosylguanine (**19**) and 9-α-D-ribopyranosylguanine (**20**), probably because of "bound water", and these two compounds were characterized mainly as their acetates (**9** and **10**) (Tables IV and VI). To obtain u.v. absorption and p.m.r.-spectral data for **19** and **20**, the pure acetates were saponified by methanolic ammonia, and the free nucleosides were used without further purification. Absorption spectra presented for **19** and **20** in Table VIII are qualitative only.

nucleoside bands were eluted with chloroform–methanol (90:10, v/v) and the eluates were evaporated to dryness. The residues were dissolved in a fixed amount of ethanol, their absorbances at λ_{max} were determined, and the product ratios were calculated by using the averages of ε_{max} values in Table VI. The product ratios listed in Tables I, II, and III are the averages of at least two determinations.

TABLE IV

PROPERTIES OF ACETYLATED GUANINE NUCLEOSIDES[a]

Compd.	*M.p.*	*Formula*	*Anal.* (%)					
			Calc.			*Found*		
			C	*H*	*N*	*C*	*H*	*N*
9	257–259°	$C_{18}H_{21}N_5O_9$	47.89	4.69	15.52	47.75	4.89	15.40
10	281–282°	$C_{18}H_{21}N_5O_9 \cdot H_2O$	46.05	4.95	14.92	46.07	4.97	15.15
11	162–164°	$C_{18}H_{21}N_5O_9 \cdot CH_3OH$	47.20	5.22	14.49	46.96	5.04	14.79
12	225–226°	$C_{18}H_{21}N_5O_9 \cdot 4/3H_2O$	45.47	5.02	14.73	45.45	5.23	14.75
13	295–300° (dec.)	$C_{18}H_{21}N_5O_9$	47.89	4.69	15.52	47.68	4.97	15.47
14	299–301° (dec.)	$C_{21}H_{25}N_5O_{11} \cdot 2/3H_2O$	47.10	4.96	13.08	47.17	4.92	13.11
15	298–300°	$C_{21}H_{25}N_5O_{11} \cdot 2/3H_2O$	47.10	4.96	13.08	46.99	4.82	13.07

[a]The samples for elemental microanalyses were dried over phosphorus pentaoxide at ambient temperature. Heating sometimes caused variable results, probably because of partial loss of water of crystallization. The presence of water of crystallization was detected in the p.m.r. spectra by integration, and was further confirmed by the addition of one drop of deuterium oxide to the sample solutions in chloroform-*d*.

TABLE V

PROPERTIES OF GUANINE NUCLEOSIDES[a]

Compd.	*M.p.*	*Formula*	*Anal.* (%)					
			Calc.			*Found*		
			C	*H*	*N*	*C*	*H*	*N*
16	>300°	$C_{10}H_{13}N_5O_5 \cdot 1/5H_2O$	41.87	4.71	24.42	41.66	4.12	24.61
17	261° (dec.)	$C_{10}H_{13}N_5O_5$	42.40	4.63	24.73	42.43	4.74	24.91
18	248–250° (dec.)	$C_{10}N_{13}H_5O_5 \cdot 1/5H_2O$	41.87	4.71	24.42	41.85	4.98	24.69
21	281° (dec.)	$C_{10}H_{13}N_5O_5 \cdot 1/2H_2O$	41.09	4.84	23.97	41.05	4.77	24.19
22	174–175° (dec.)	$C_{10}H_{13}N_5O_5 \cdot 1/3H_2O$	41.52	4.76	24.21	41.56	4.98	24.42
23	~180° (dec.)	$C_{10}H_{13}N_5O_5 \cdot 1/3H_2O$	41.52	4.76	24.21	41.63	5.00	24.20
24	299–301° (dec.)	$C_{11}H_{15}N_5O_6$	42.17	4.83	22.36	41.97	5.05	22.32
25	289–292° (dec.)	$C_{11}H_{15}N_5O_6$	42.17	4.83	22.36	41.91	5.07	22.15

[a]The samples for elemental microanalyses were dried over phosphorus pentaoxide at ambient temperature (see footnote *a* to Table IV).

TABLE VI

ULTRAVIOLET ABSORPTION SPECTRA OF ACETYLATED GUANINE NUCLEOSIDES

Compd.	λ_{max}^{EtOH}, *nm* (ε)
9	265 (13,200), 285 (sh) (9,600)
10	257 (15,700), 277 (sh) (11,000)
11	257 (17,000), 282 (sh) (12,100)
12	263 (13,250), 283 (sh) (10,010)
13	256 (16,200), 280 (sh) (11,120)
14	263 (13,500), 283 (sh) (10,100)
15	256 (15,800), 282 (sh) (11,100)

TABLE VII

ULTRAVIOLET ABSORPTION AND PROTON MAGNETIC RESONANCE DATA OF GUANINE NUCLEOSIDES

Compd.	$J_{1',2'}$ (Hz) (D_2O–NaOD)	λ_{max}, *nm* (ε)		
		H_2O	0.1M *HCl*	0.1M *NaOH*
16	6.0	287 (7,490)	251 (8,820)	283 (6,270)
17	4.5	253 (13,900)	256 (12,100)	263 (br) (10,800)
		276 (sh) (9,800)	275 (sh) (8,730)	
18	6.5	254 (14,150)	257 (12,700)	263 (br) (11,110)
		270 (sh) (9,980)	276 (sh) (8,830)	
19*	9.0	286	254	283
20*	3.0	253	258	263 (br)
		272 (sh)	275 (sh)	
21	9.0	253 (13,700)	257 (12,850)	263 (br) (11,500)
		270 (sh) (10,000)	280 (sh) (8,500)	
22	9.0	286 (7,400)	255 (7,730)	283 (6,140)
23	9.0	253 (13,370)	258 (12,030)	263 (br) (10,740)
		270 (sh) (9,420)	275 (sh) (8,550)	
24	9.0	287 (7,500)	256 (7,910)	284 (6,290)
25	9.0	253 (13,910)	257 (12,760)	263 (br) (11,340)
		270 (sh) (9,730)	275 (sh) (8,860)	

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