[Chem. Pharm. Bull. 24(12)2955—2960(1976)] UDC 547.857.7.02 : 576.85.098

Identification of a Novel Aminonucleoside Produced by *Enterobacter* sp. as 2′-Amino-2′-deoxyguanosine[1]

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(Received March 22, 1976)

A novel guanosine analog, aminonucleoside antibiotics (2AG), produced by *Enterobacter* sp., was identified as 9-(2′-amino-2′-deoxy-β-D-ribofuranosyl)-guanine (2′-amino-2′-deoxyguanosine) by proton magnetic resonance and carbon-13 nuclear magnetic resonance spectra of 2AG and acetylated 2AG, and also by the comparison of the aminosugar obtained by hydrolysis of 2AG with 2-amino-2-deoxypentoses appeared in the literatures. This is the first report on the occurrence of 2-amino-2-deoxy-D-ribose in nature.

The isolation and preliminary structure determination of a novel aminonucleoside produced by *Enterobacter* sp. were reported in the previous paper.[3] The aminonucleoside (named 2AG) showed antibacterial activity against only some *Escherichia coli* and antitumor activity.

Many nucleoside antibiotics have been discovered from microorganisms, but only puromycin and 3′-amino-3′-deoxyadenosine were known as aminoribosyl-nucleoside.[4] It was suggested that 2AG consisted of guanine and 2-amino-2-deoxypentose. The occurrence of 2-amino-2-deoxypentose in nature has not been reported. Therefore, we undertook a detailed study on the structure of 2AG. This paper describes the structure elucidation of 2AG.

Results and Discussion

Comparison of carbon-13 nuclear magnetic resonance (CMR) spectrum (25 MHz) of 2AG with that of guanosine[5] was shown in Table I. The chemical shift of 2′-C in 2AG (δ, 58.15)

TABLE I. Comparison of Carbon-13 Chemical Shifts in 2AG, and Guanosine and 2′-Deoxyguanosine

Carbon position	2AG[a]	Guanosine[b]	Deoxyguanosine[b]
C-2	154.93	154.06	154.18
C-4	152.51	151.82	151.18
C-5	117.85	117.02	117.15
C-6	160.42	157.25	157.63
C-8	139.11	136.35	136.13
C-1′	87.56	86.73	83.33
C-2′	58.15	71.02	39.90
C-3′	72.81	74.34	71.31
C-4′	89.21	86.87	88.27
C-5′	63.00	61.66	62.35

a) in ppm from TMS (external)
b) These data were cited from reference 5).

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[2] Location: *Asahi-machi, Machida, Tokyo, 194, Japan.*

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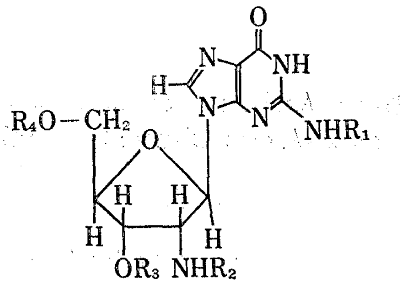

	R_1	R_2	R_3	R_4
2AG	H	H	H	H
N-Diacetyl 2AG	CH_3CO	CH_3CO	H	H
Tetraacetyl 2AG	CH_3CO	CH_3CO	CH_3CO	CH_3CO

Chart 1

was very different from that of guanosine (δ, 71.02). This result suggested that the amino group of the aminopentose in 2AG attached to 2′-C. To confirm the position of amino group, N-diacetyl-2AG and tetraacetyl-2AG were prepared. The proton magnetic resonance (PMR) spectra (100 MHz) of 2AG (in D_2O), N-diacetyl-2AG (in D_2O) and tetraacetyl-2AG (in $CDCl_3$–CD_3OD) were shown in Fig. 1. Each signal was assigned with spin decoupling. The chemical shifts and coupling constants of sugar moieties in these compounds were summarized in Table II. Downfield shift of anomeric proton and 2′-proton in N-diacetyl derivative showed that amino group attached to 2′-C.

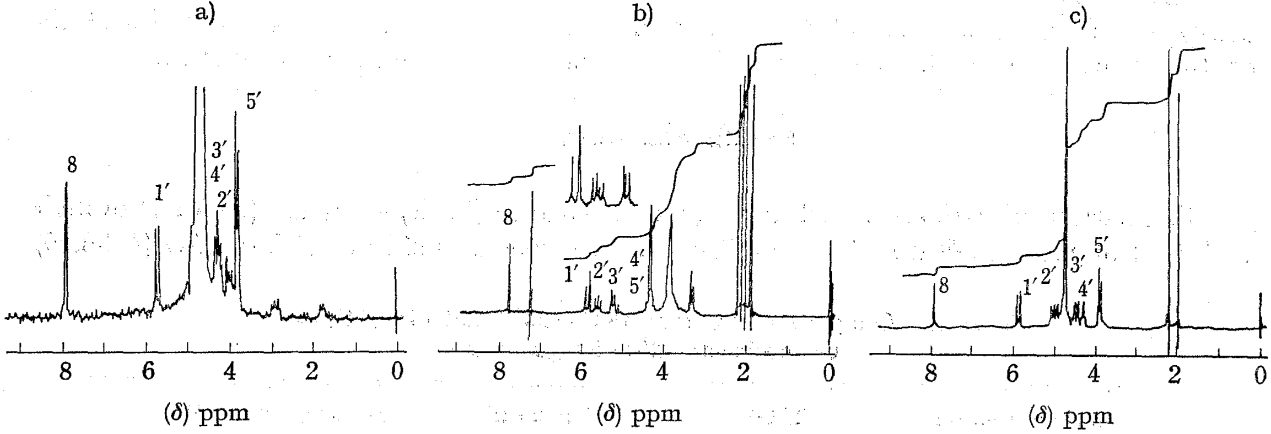

Fig. 1. PMR Spectra of 2AG and Its Acetylated Analogs (100 MHz)

a) 2AG in D_2O
b) 2,2′,3′,5′-tetraacetyl-2AG in $CDCl_3$–CD_3OD
c) 2,2′-N-diacetyl-2AG in D_2O

After hydrolyzing 2AG with 1N hydrochloric acid, 2-amino-2-deoxypentose was isolated as hydrochloride by cation exchange column chromatography. This amino sugar was crystallized from methanol–acetone, in the similar manner as reported.[7a] The optical rotation and melting point of eight types of 2-amino-2-deoxypentose hydrochloride were summarized

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Table II. PMR Chemical Shifts and Coupling Constants for Sugar Moieties in 2AG Related Compounds

Position	Guanosine[a] (ppm) J(Hz)	2AG[a] (ppm) J(Hz)	N-Ac-2AG[a] (ppm) J(Hz)	Tetra-Ac-2AG[b] (ppm) J(Hz)
1′	5.88	5.72	5.91	5.94
	$J_{1'2'}=7$	$J_{1'2'}=8$	$J_{1'2'}=8.5$	$J_{1'2'}=8$
2′	—	3.98	5.01	5.68
	$J_{2'3'}=6$	$J_{2'3'}=6$	$J_{2'3'}=5.5$	$J_{2'3'}=5.8$
3′	4.39	4.2—4.4	4.48	5.32
	$J_{3'4'}=3.5$	—	$J_{3'4'}=2$	$J_{3'4'}=2$
4′	4.19	4.2—4.4	4.32	
	$J_{4'5'}=4$	—	$J_{4'5'}=3.7$	4.40
5′	3.76	3.98	3.91	brs
		$J_{4'5'}=4$		

a) 100 MHz, in D_2O
b) 100 MHz, in $CDCl_3$-CD_3OD

in Table III. The data of the isolated amino sugar completely agreed with those of 2-amino-2-deoxy-α-D-ribose hydrochloride. Furthermore, 2-N-acetyl-2-deoxypentose, obtained by N-acetylation of isolated amino sugar, gave a similar value in optical rotation with 2-N-acetyl-2-deoxy-D-ribose.[6]

Table III. Physical Properties of Isolated Amino Sugar and 2-Amino-2-deoxypentoses Appeared in the Literatures

2-Amino-2-deoxy-phentose·HCl	mp (decomp.) (°C)	$[\alpha]_D$ (°C)	References
Isolated aminosugar	143—150	+12° → −4.5° (27)	
D-Arabinose β	154—157	−174° → −124° (23)	7*b*)
L-Arabinose β	153—155	+174° → +115° (20)	7*c*)
D-Lyxose α	148—155	+54° → −36° (25)	7*a*)
L-Lyxose α	150—160	−16° → +5.2° (25)	7*d*)
D-Ribose α	144—149	+14.1° → −2.8° (23)	7*a*)
	153—155	+18.6° → −5.8° (24)	7*b*)
L-Ribose α	142—148	−15.6° → +6.7° (22)	7*e*)
D-Xylose α	165—167	+82° → +44.9° (22)	7*b*)
L-Xylose α	164—168	−68.6° → −46.4° (25)	7*f*)

The infrared (IR) spectrum of isolated amino sugar was shown in Fig. 2, together with the data reported by Rebello *et al.*[8] The IR spectra of the isolated amino sugar and 2-amino-2-deoxy-D-ribose hydrochloride agreed completely. From the above results, it was proved that 2AG had 2-amino-2-deoxy-D-ribose as a sugar moiety.

It had been reported[9] that the signal assigned to the anomeric proton of a C-1′-C-2′-*trans*-nucleoside (that is, a β-D-ribofuranosyl nucleoside) appeared at higher field (usually at $\delta \sim 0.5$ ppm) than the peak observed for the anomeric proton of corresponding α-D-anomer (C-1′-C-2′-*cis*-nucleoside). In Table II, the chemical shift assigned to the anomeric proton was 0.16 ppm higher than that of guanosine (C-1′-C-2′-*trans*-nucleoside). Therefore, 2AG was suggested to have a C-1′-C-2′-*trans*-configuration, even if effects of amino group were taken into consideration.

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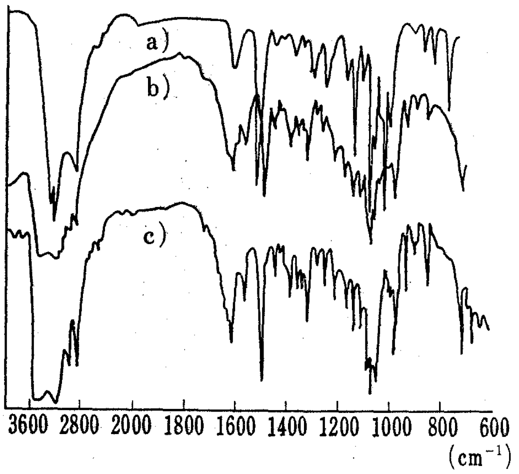

Fig. 2. IR Spectra of 2-Amino-2-deoxypentoses (KBr)

a) 2-amino-2-deoxy-D-arabinose hydrochloride[8]
b) 2-amino-2-deoxy-D-ribose hydrochloride[8]
c) 2-amino-2-deoxypentose hydrochloride isolated from 2AG

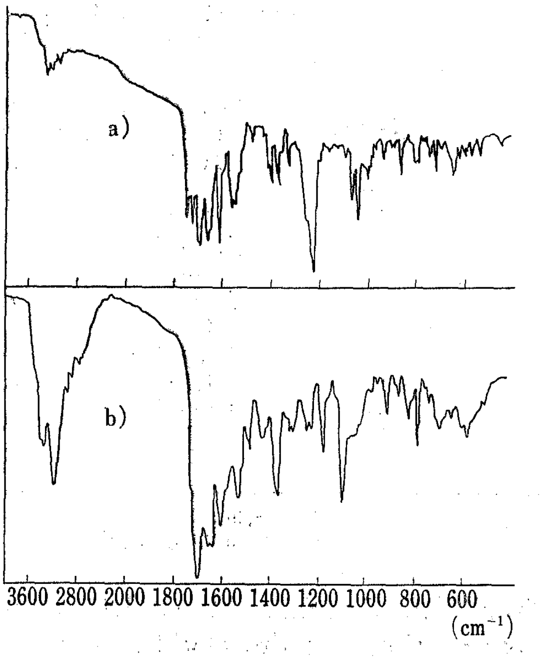

Fig. 3. IR Spectra of Acetylated 2AG Analogs (KBr)

a) 2,2',3',5'-tetraacetyl-2AG
b) 2,2'-N-diacetyl-2AG

Usually, coupling constants were a valuable tool in the determination of stereochemistry of sugar. But, in furanose, the dihedral angles of neighboring *cis*-hydrogen atoms and that of neighboring *trans*-hydrogen atoms might vary from 0—45° and 75—165°, respectively. The Karplus equation, or a modification thereof, made possible to predict that the observed coupling constants ($J_{1',2'}$) would be in the approximate region of 3.5—8.0 Hz for the *cis*- and 0.0—8.0 Hz for the *trans*-configuration.[9] Consequently, an assignment of anomeric configuration on the basis of these data could be made only for the *trans*-arrangement for the protons on C-1' and C-2'.

By the chemical shift of anomeric proton in PMR spectrum of 2AG, the C-1'-C-2'-*trans*-configuration of 2AG was suggested and the 2AG had 2-amino-2-deoxy-D-ribose. Thus this amino sugar must be attached to guanine in β-configuration. Furthermore, the optical rotation of 2AG ($[\alpha]_D^{26}$ $-56.6°$ (c=0.5, H_2O)) strongly supported that it had a β-configuration, because that of guanosine showed $[\alpha]_D^{25}$ $-60°$.

From all above results, it appeared well established that the structure of 2AG was 9-(2'-amino-2'-deoxy-β-D-ribofuranosyl)-guanine. This compound can be called 2'-amino-2'-deoxyguanosine. This is a new aminonucleoside, which has antibacterial and antitumor activity. Recently, this compound has been synthesized chemically by Ikehara *et al.* (personal communication). 5'-Amino-5'-deoxyguanosine[10] and 3'-amino-3'-deoxyguanosine[11] have been synthesized and these guanosine analogs had not a biological activity. Aminonucleosides comprised 2-amino-2-deoxy-D-ribose such as 2'-amino-2'-deoxyadenosine,[12] 2'-amino-2'-deoxyuridine,[13] 2'-amino-2'-deoxycytidine,[14] 2'-amino-2'-deoxy-5-fluorouridine[15] and 9-(2'-

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amino-2′-deoxy-D-ribofuranosyl)-6-dimethyl-aminopurine[16] have been synthesized and some of them showed biological activities. The compound comprised 2-amino-2-deoxypentose is discovered first in nature.

Experimental

PMR and CMR spectra were measured with a JNM–PS 100 spectrometer. The chemical shifts were described in δ units (part per million) relative to TMS (tetramethylsilane) or DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) as an internal standard. IR spectra were recorded in potassium bromide discs with a Shimadzu IR 27G spectrometer. Ultraviolet (UV) spectra were determined with a Hitachi 124 spectrophotometer. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter. UV absorbance of effluent of column chromatography was recorded with a Hitachi 034 UV–VIS Effluent Monitor. All melting point were not corrected.

Peracetylation of 2AG——Acetic anhydride (10 ml) was poured into a suspension of 2AG (90 mg) in 10 ml of pyridine with stirring in an ice bath. Then the reaction mixture was stirred for 18 hr at room temperature. Methanol (15 ml) was added to this mixture cooled in an ice bath and after the evaporation of the mixture to dryness, the residue was dissolved in chloroform. This solution was chromatographed on a column (0.9×20 cm) of silica gel (Wako gel C-200). The column was eluted stepwise with chloroform, 2% methanol in chloroform and 4% methanol in chloroform. Each 10 ml fractions were collected. Three main peaks of UV-absorption were obtained and one of them which was eluted in fraction numbers 89—140 was concentrated *in vacuo* to dryness, giving tetraacetyl-2AG (30 mg) as a white powder (mp 252—257° (decomp.)).

Anal. Calcd. for $C_{18}H_{22}O_8N_6$ (2,2′,3′,5′-tetraacetyl-2AG): C, 48.00; H, 4.92; N, 18.65. Found: C, 47.82; H, 5.07; N, 18.33. UV $\lambda_{max}^{H_2O(neutral)}$ mμ (ε): 258 (16.2×10^3). UV $\lambda_{max}^{(0.1N\ HCl)}$ mμ (ε): 261 (16.2×10^3). UV $\lambda_{max}^{(0.1N\ NaOH)}$ mμ (ε): 262 (11.8×10^3). IR ν_{max}^{KBr} cm^{-1}: 1220 (C–O), 1560, 1615, and 1690 (CONH), 1745 (C=O) as shown in Fig 2-a. PMR (in $CDCl_3$–CD_3OD) δ: 7.85 (1H, singlet, 8-CH), 2.25 (3H, singlet, 2-$NHCOCH_3$), 2.17 (3H, singlet, 3′-$COCH_3$), 2.10 (3H, singlet, 5′-$COCH_3$), 1.94 (3H, singlet, 2′-$COCH_3$). Others were shown in Table II. PNR spectrum was shown in Fig. 1-b.

N-Acetylation of 2AG——Acetic anhydride (2 ml) was poured into a suspension of 100 mg of 2AG in methanol (6 ml) with stirring. The reaction mixture was stirred for 24 hr at room temperature. After the evaporation of the mixture to dryness, the residual acetic anhydride was removed by repeating dissolution in methanol and evaporation twice. This was dissolved in 3 ml of water and the solution was adjusted to pH 7. The solution was applied on a column (1.0×25 cm) of neutral polystyrene resin (Diaion HP-20). Eluting with water, two peaks of UV absorption were obtained, the latter peak was evaporated to dryness, giving N-diacetyl-2AG (90 mg) as a white powder.

Anal. Calcd. for $C_{14}H_{18}O_6N_6$ (2,2′-N-diacetyl-2′-deoxyguanosine): C, 45.90; H, 4.95; N, 22.94. Found: C, 46.15; H, 4.81; N, 22.68. UV $\lambda_{max}^{H_2O(neutral)}$ mμ(ε): 253 (15.4×10^3). UV $\lambda_{max}^{0.1N\ HCl}$ mμ(ε): 258 (14.0×10^3). UV $\lambda_{max}^{0.1N\ NaOH}$ mμ(ε): 265 (13.6×10^3). IR ν_{max}^{KBr} cm^{-1}: 1690, 1650 (CONH), 1090, 3280 (–OH) as shown in Fig. 2-b. PMR (in D_2O): 7.94 (1H, singlet, 8-CH), 2.25 (3H, singlet, 2-$COCH_3$), 2.01 (3H, singlet, 2′-$COCH_3$). Other signals were shown in Table II. PMR spectrum was shown in Fig. 2-c.

Isolation of the Sugar Moiety——A solution of 2AG (450 mg) in 1N HCl (100 ml) was heated in a boiling water bath for 1 hr. After removal of precipitated guanine, the reaction mixture was applied on a column (150 ml) of cation exchange resin (Dowex 50w X4, H^+ form, 200—400 mesh). The column was eluted with 0.33 N HCl and an amino sugar appeared at the fraction numbers 75—80 (fraction volume; 10 ml). The amino sugar was detected with ninhydrin reaction and Elson-Morgan reaction.[17] These fractions were concentrated and gave crude crystallines. Recrystallization from methanol–acetone afforded pure 2′-amino-2′-deoxy-α-D-ribose hydrochloride (99 mg). mp 143—150° (decomp.). $[\alpha]_D^{27}$ $+12°$ (initial, extrapolated)$\rightarrow-4.5°$ ($c=0.75$ H_2O).

Anal. Calcd. for $C_5H_{12}O_4NCl$ (2-amino-2-deoxy-D-ribose HCl): C, 32.36; H, 6.52; N, 7.55; Cl, 19.10. Found: C, 32.60; H, 6.56; N, 7.35; Cl, 19.15. IR spectrum of this amino sugar was shown in Fig. 3.

N-Acetylation of 2-Amino-2-deoxy-α-D-ribose——In order to obtain a free amino sugar, the amino sugar hydrochloride (90 mg) was applied on an anion exchange resin (Diaion PA-208, OH^- form). The amino sugar fraction was concentrated and lyophilyzed. To this methanol solution (9 ml) acetic anhydride (3 ml) was added and the reaction mixture was stirred for 20 hr at room temperature. The reaction mixture was subjected to two cycles of evaporation and dissolution in methanol. The residue was dissolved in ethanol and precipitated by addition of chloroform. The aqueous solution of the precipitate was chromatographed on neutral polystyrene resin (Diaion HP-20), and by elution with water, the positive fractions with Morgan-Elson reaction modified by Reissing *et al.*[18] were pooled. These fractions were evaporated and dissolved in metha-

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nol. Addition of ether to the solution gave a hygroscopic powder (12.5 mg). On the cellulose thin-layer chromatography, this material showed a single spot ($Rf=0.50$, *n*-butanol: acetic acid: water=4:1:2) by *p*-anisidine hydrochloride reaction[19] or Morgan-Elson reaction, but was not detected with ninhydrin reaction. $[\alpha]_D^{24}$ $-34°$ ($c=0.1$, H_2O).

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