[Chem. Pharm. Bull. 19(3) 576—586 (1971)]

UDC 547.963.32.07.09 : 1.04

Synthesis of Compounds related to Inosine 5′-Phosphate and Their Flavor Enhancing Activity. IV.[1] 2-Substituted Inosine 5′-Phosphates

Kin-ichi Imai, Ryuji Marumoto, Kunio Kobayashi, Yoshio Yoshioka, Jun Toda, and Mikio Honjo

Research and Development Division, Takeda Chemical Industries, Ltd.[2]

(Received September 28, 1970)

Ring closure of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICA-riboside) with phenyl isothiocyanate afforded 2-mercaptoinosine (I) in good yield. Similarly, the ring closure of AICA-riboside 5′-phosphate (AICAR) led to the formation of 2-mercaptoinosine 5′-phosphate (II). Various 2-substituted inosine 5′-phosphates were prepared from I and II or starting with AICA-riboside. It was found that 2-furfurylthioinosine 5′-phosphate possessed a flavor enhancing activity of about 17-times that of inosine 5′-phosphate. The chemical structure-flavor enhancing activity relationship was presented.

In the previous paper,[1] we have reported that a flavor enhancing activity of 2-chloroinosine 5′-phosphate was stronger than that of inosine 5′-phosphate. We felt it desirable to examine the activity of other 2-substituted inosine 5′-phosphates.

Among a number of synthetic methods of 2-substituted inosine 5′-phosphate, a method which has been developed by Yamazaki, *et al.*[3] appeared most attractive, because of the use of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICA-riboside) as a starting material, which is easily accessible from the culture broth of Bacillus subtilis[4] or pumilus.[5]

We adopted their route to synthesize a variety of 2-substituted inosine 5′-phosphates required in the present investigation, with a considerable modification in the ring-closure step.

Synthesis of 2-Mercaptoinosine

Yamazaki, *et al.*[6] synthesized 2-mercaptoinosine (I) in 64% yield by heating AICA-riboside with sodium methylxanthate in a sealed tube. We subjected AICA-riboside to ring-closure with methyl isothiocyanate[7,8] to I in refluxing pyridine (6 hr). Paper electrophoresis (sodium borate buffer) and the ultraviolet (UV) determination of the reaction product demonstrated that I was produced in an approximately 60% yield. Prolonged heating of the reaction mixture resulted in intensive coloration and an increased production of undesirable by-products. When phenyl isothiocyanate was used in place of methyl derivative and the reaction was carried out for 3.5 hr, only slight coloration was observed and the product was readily obtained as crystals. The substance was proved to be the pyridinium salt of I on the basis of both UV and nuclear magnetic resonance (NMR) spectra and elemental analysis. Treatment of the pyridinium salt with aqueous potassium hydroxide afforded the potassium salt[6] of I in an overall yield of 85% based on AICA-riboside. When *p*-chlorophenyl isothiocyanate was used, I (potassium salt) was also obtained in 89% yield.

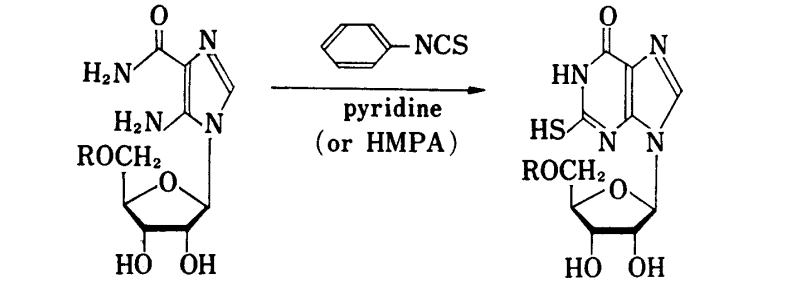
1) Part III: M. Honjo, K. Imai, Y. Furukawa, Y. Kanai, R. Marumoto, H. Honda, H. Aoki, and T. Hirata, *Takeda Kenkyusho Nempo*, **25**, 74 (1966).
2) Location: *Juso-Nishino-cho, Higashiyodogawa-ku, Osaka.*
3) A. Yamazaki, I. Kumashiro, and T. Takenishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 338 (1968).
4) T. Shiro, A. Yamanoi, S. Konishi, S. Okumura, and T. Takenishi, *Agr. Biol. Chem.* (Tokyo), **26**, 785(1962).
5) H. Shirafuzi, A. Imada, S. Yashima, and M. Yoneda, *Agr. Biol. Chem.* (Tokyo), **32**, 69 (1968).
6) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **32**, 3032 (1967).
7) A.H. Cook and E. Smith, *J. Chem. Soc.*, **1949**, 3001.
8) R.J. Rousseau, R.K. Robins, and L.B. Townsend, *J. Am. Chem. Soc.*, **90**, 2661 (1968).

The nucleoside (I) was also obtained in a satisfactory yield when AICA-riboside was refluxed in pyridine with alkali-metal salts of either alkyl or phenyl dithiocarbamate or with diphenylthiourea (an intermediate for the synthesis of phenyl isothiocyanate) (Table I).

Table I. Ring Closure of AICA-riboside

Reagent	Time (hr)	Yield of 2-mercapto-inosine (I)[a], %
CH_3NCS	6	60
C_6H_5NCS	3.5	quantitative (85)
p-Cl–C_6H_4NCS	3.5	quantitative (89)
$(C_2H_5)_2NCS_2Na$	18	65
n-$C_4H_9NHCS_2K$	18	43
$C_6H_5NHCS_2Na$	23	70 (34)
$C_6H_5NHCS_2K$	18	60 (32)
$(C_6H_5)_2CS$	3.5	30

a) Determined spectrophotometrically after paper electrophoresis (sodium borate buffer). The isolated yield was given in parentheses.



AICA−riboside : R=H

AICAR : R=−P(=O)(OH)OH

I : R=H

II : R=−P(=O)(OH)OH

Chart 1

Synthesis of 2-Mercaptoinosine 5′-Phosphate and Its S-Substituted Derivatives

1) The above-mentioned ring closure reaction with phenyl isothiocyanate was applied to 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide 5′-phosphate (AICAR).[9] Thus, AICAR (tri-n-butylammonium salt) was treated with phenyl isothiocyanate in pyridine or hexamethylphosphoramide (HMPA). The reaction mixture was purified by diethylaminoethyl (DEAE)-cellulose (HCO_3^- type) column chromatography to isolate the sodium salt of 2-mercaptoinosine 5′-phosphate (II) in 30—40% yields. The identity of this product was confirmed on the basis of the paper electrophoresis, paper chromatography, UV absorption spectrum and the elemental analysis (Chart 1). Attempts to prepare II by phosphorylation of 2-mercaptoinosine or its isopropylidene derivative were unsuccessful.[10] The reaction of AICAR with sodium methylxanthate[6] afforded 2-mercaptoinosine instead of the desired 5′-phosphate.

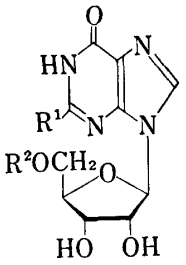
2) The nucleotide (II) was oxidized with an equivalent amount of aqueous hydrogen peroxide to bis(2-inosinylyl)disulfide (III), whereas II was oxidized with excess aqueous hydro-

9) M. Yoshikawa, T. Kato, and T. Takenishi, *Bull. Chem. Soc. Japan*, **42**, 3505 (1969).
10) M. Yoshikawa and T. Kato, *Bull. Chem. Soc. Japan*, **40**, 2849 (1967).

gen peroxide to inosine-2-sulfonic acid 5′-phosphate (IV) (Table II). Attempts at phosphorylation of bis(2-inosine)disulfide and inosine-2-sulfonic acid failed.

3) The reaction of nucleoside (I) (potassium salt) with alkyl and alkenyl halides in appropriate solvents afforded the corresponding S-substituted 2-mercaptoinosines (Tables III, IV-A and V). Reaction of I (potassium salt) with S-(1-adamantylthio) isothiourea hydrochloride[11] gave rise to 2-(1-adamantyldithio) inosine (Va). Oxidation of I (potassium salt) with

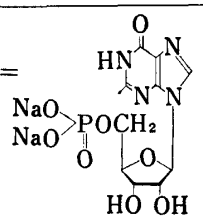
TABLE II. S-Substituted 2-Mercaptoinosine 5′-Phosphates (Part 1)



R^1	R^2		Formula		Analyses (%) C	H	N	S	P
-SK	H	I	$C_{10}H_{11}O_5N_4SK \cdot \frac{1}{2}H_2O$	Calcd.	34.57	3.46	16.15	9.16	
				Found	34.84	3.38	16.31	9.38	
-SNa	PO_3Na_2	II	$C_{10}H_{10}O_8N_4SNa_3P \cdot \frac{1}{2}C_2H_5OH \cdot \frac{1}{2}H_2O$	Calcd.	27.99	2.93	11.71	6.67	
				Found	28.19	3.18	11.41	6.91	
-S-S-R^3 [a]	PO_3Na_2	III							
$-SO_3Na$	PO_3Na_2	IV	$C_{10}H_{10}O_4N_4SNa_3P \cdot 2H_2O$	Calcd.	25.00	3.47	9.72	5.56	
				Found	24.64	3.47	9.69	5.88	
	H	Va	$C_{20}H_{26}O_5N_4S_2$	Calcd.	51.47	5.62	12.01		
				Found	51.42	5.59	11.70		
	PO_3Na_2	Vb	$C_{20}H_{25}O_8N_4S_2P \cdot \frac{1}{2}(CH_3)_2CO \cdot 3H_2O$	Calcd.	38.33	5.09	8.32		4.60
				Found	38.16	5.34	8.06		5.20

	mp (°C)	$[\alpha]_D$	UV absorption spectra λ_{max} mμ (ε) 0.1N HCl	H_2O	0.1N NaOH	Flavoring strengths[b]
I	160—165 (decomp.)					
II		$[\alpha]_D^{22} = -12.5°$ (c=1.0, H_2O)	230, 290 (17400)	285 (14700)	282 (15000)	2.8
III			258, 280	262	216, 268	0
IV		$[\alpha]_D^{23} = +4.7°$ (c=1.0, H_2O)	253 (9700)	254 (10300) 272 sh[c]	256 (10500) 270 sh	0
Va	190—192	$[\alpha]_D^{22} = -4.1°$ (c=0.75, $HCON(CH_3)_2$)	264 (13100) 278 (12300)	260 (13100) 286 (10700)	267 (12400)	
Vb				262, 285 sh		0.4

a) R^3 =



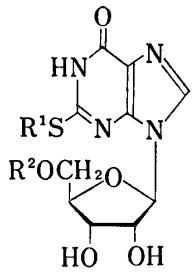
b) ratio of the synergistic strength of 5′-nucleotide with monosodium L-glutamate to that of inosine 5′-phosphate·2Na·$8H_2O$

c) shoulder

11) K. Shirakawa, O. Aki, T. Tsuzikawa, and T. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **18**, 235 (1970).

aqueous hydrogen peroxide and treatment of the oxidized product with *p*-dimethylaminothiophenol afforded 2-(*p*-dimethylaminophenylthio)inosine (Table IV-B). All these nucleosides were subjected to the selective phosphorylation[12] of the 5′-hydroxyl with pyrophosphoryl chloride in acetonitrile or *m*-cresol to give the corresponding 5′-phosphates. They were isola-

TABLE III. S-Substituted 2-Mercaptoinosine 5′-Phosphates (Part 2)

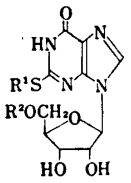


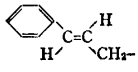
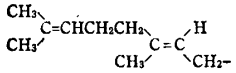
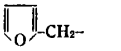
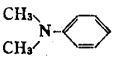
R^1	R^2		Formula		Analyses (%) C	H	N	S	P
$CH_3CH_2CH_2-$	H	VIa	$C_{13}H_{18}O_5N_4S$	Calcd.	45.60	5.30	16.36	9.36	
				Found	45.20	5.33	16.26	9.32	
	PO_3Na_2	VIb	$C_{13}H_{17}O_8N_4SNa_2P \cdot 1/5C_2H_5OH \cdot H_2O$	Calcd.	31.46	4.37	10.95		6.06
				Found	31.94	4.25	11.01		5.45
CH_3 CH-CH_2- CH_3	H	VIIa	$C_{14}H_{20}O_5N_4S \cdot H_2O$	Calcd.	44.92	5.88	14.97	8.56	
				Found	44.59	5.82	15.13	9.05	
	PO_3Na_2	VIIb	$C_{14}H_{19}O_8N_4SNa_2P \cdot 1\frac{1}{2}H_2O$	Calcd.	33.14	4.34	11.05	6.32	6.12
				Found	33.21	4.48	11.10	6.37	6.19
CH_3 $CHCH_2CH_2-$ CH_3	H	VIIIa	$C_{15}H_{22}O_5N_4S$	Calcd.	48.64	5.99	15.13	8.65	
				Found	48.62	5.94	15.82	8.54	
	PO_3Na	VIIIb	$C_{15}H_{21}O_8N_4SNa_2P \cdot H_2O$	Calcd.	35.15	4.54	10.93	6.26	6.08
				Found	35.36	5.24	10.95	5.52	5.84
$CH_3(CH_2)_3CHCH_2-$ C_2H_5	H	IXa	$C_{18}H_{28}O_5N_4S$	Calcd.	52.41	6.84	13.58	7.77	
				Found	52.20	6.72	13.70	8.02	
	PO_3Na_2	IXb	$C_{18}H_{27}O_8N_4SNa_2P \cdot 2H_2O$	Calcd.	37.76	5.44	9.79		5.42
				Found	37.44	5.17	9.81		5.83

	mp (°C)	$[\alpha]_D$	UV absorption spectra λ_{max} mμ (ε) 0.1N HCl	H_2O	0.1N NaOH	Flavoring strengths
VIa	193—196	$[\alpha]_D^{23}$ +9.5° (c=1.0, CH_3OH)	269 (16300)	262 (15400) 280 sh	228 (19200) 272 (15200)	
VIb		$[\alpha]_D^{22}$ −16.0° (c=1.0, H_2O)	272 (16300)	199 (25600) 262 (15100) 280 sh	228 (18400) 271 (15500)	8.6
VIIa	195	$[\alpha]_D^{24}$ +7.9° (c=1.0, H_2O)	270 (15600)	262 (14400) 282 sh	227 (19400) 272 (14900)	
VIIb		$[\alpha]_D^{23}$ −10.9° (c=1.0, H_2O)	272 (16200)	263 (14800) 280 sh	228.5 (18200) 272 (15300)	7.1
VIIIa	162—163	$[\alpha]_D^{24}$ +16.6° (c=1.0, CH_3OH)	271 (15600)	263 (14400) 285 sh	227 (18000) 272 (14800)	
VIIIb		$[\alpha]_D^{23}$ −9.2° (c=1.0, H_2O)	272 (15600)	262 (14300) 280 sh	228 (17600) 272 (13000)	6.1
IXa	150	$[\alpha]_D^{23}$ +19.0° (c=1.0, CH_3OH)	273 (15600)	263 (14300) 283 sh	229.5 (17500) 273 (15000)	
IXb		$[\alpha]_D^{24}$ −7.4° (c=1.0, CH_3OH)	272 (16100)	263 (16000) 282 sh	229 (17700) 272 (15100)	1.2

12) K. Imai, S. Fujii, K. Takanohashi, Y. Furukawa, T. Masuda, and M. Honjo, *J. Org. Chem.*, **34**, 1547 (1969).

TABLE IV. S-Substituted 2-Mercaptoinosine 5′-Phosphates (Part 3)



	R¹	R²		Formula	Analyses (%) Calcd. C	H	N	S	P	Found C	H	N	S	P
		H	Xa	$C_{13}H_{16}O_5N_4S$	45.87	4.74	16.46	9.42		45.76	4.69	16.34	9.25	
A	$CH_2=CHCH_2-$	PO_3Na_2	Xb	$C_{13}H_{15}O_8N_4SPNa_2 \cdot 3\frac{1}{2}H_2O$	29.61	4.21	10.63	6.08	5.88	29.50	4.67	10.62	6.44	5.84
	$CH_2=C(CH_3)-CH_2-$	H	XIa	$C_{14}H_{18}O_5N_4S$	47.46	5.08	15.81	9.04		47.20	4.92	15.70	9.03	
		PO_3Na_2	XIb	$C_{14}H_{17}O_8N_4SPNa_2 \cdot 2H_2O$	32.68	4.09	10.51	6.23	6.03	32.76	4.45	10.87	6.59	6.27
	$CH_3(H)C=C(H)-CH_2-$	H	XIIa	$C_{14}H_{18}O_5N_4S$	47.46	5.08	15.81	9.04		47.44	4.93	15.53	9.31	
		PO_3Na_2	XIIb	$C_{14}H_{17}O_8N_4SPNa_2 \cdot 1\frac{1}{2}H_2O$	33.27	3.74	11.09	6.34	6.16	33.55	4.10	10.98	6.24	6.53
	$(CH_3)_2C=CHCH_2-$	H	XIIIa	$C_{15}H_{20}O_5N_4S$	48.90	5.47	15.21	8.70		48.72	5.50	14.98	8.77	
		PO_3Ca	XIIIb	$C_{15}H_{19}O_8N_4SPCa \cdot 1\frac{1}{2}H_2O$	35.08	4.32			6.04	35.17	4.65			5.79
		H	XIVa	$C_{19}H_{20}O_5N_4S$	54.80	4.84	13.45	7.70		55.05	4.78	13.22	7.55	
		PO_3Na_2	XIVb	$C_{19}H_{19}O_8N_4SPNa_2 \cdot 2H_2O$	39.56	4.02	9.72	5.56	5.38	39.57	4.05	9.74	5.54	5.33
		H	XVa	$C_{20}H_{28}O_5N_4S$	55.05	6.42	12.84	7.34		55.31	6.55	12.66	6.92	
		PO_3Na_2	XVb	$C_{20}H_{27}O_8N_4SPNa_2 \cdot 1\frac{1}{2}H_2O$	40.89	5.11	9.54	5.45	5.28	41.00	5.33	9.57	5.55	5.67
		H	XVIa	$C_{15}H_{16}O_6N_4S$	47.36	4.24	14.73	8.43		47.18	4.13	14.74	8.40	
		PO_3Na_2	XVIb	$C_{15}H_{15}O_9N_4SPNa_2 \cdot H_2O$	34.48	3.28	10.73	6.14	5.94	34.65	3.55	10.76	6.49	5.73
	$H(H)C=C(Br)-$	H	XVIIa	$C_{12}H_{13}O_5N_4SBr$	35.56	3.21	13.83	7.90		34.88	3.08	13.41	7.73	
		PO_3Na_2	XVIIb	$C_{12}H_{12}O_8N_4SPBrNa_2 \cdot 5H_2O$			9.05		5.00			9.34		4.65
B		H	XVIIIa	$C_{18}H_{22}O_5N_5S$	51.43	5.24	16.67	7.62		51.27	5.32	16.64	7.66	
		PO_3Na_2	XVIIIb	$C_{18}H_{20}O_8N_5SPNa_2$	39.68	3.68	12.87		5.52	39.50	3.98	12.51		6.17

	mp (°C)	$[\alpha]_D$	UV absorption spectra λ_{max} mμ (ε) 0.1N HCl	H_2O	0.1N NaOH	Flavoring strengths
Xa	192—194	$[\alpha]_D^{23}$ +10.5° (c=1.0, CH_3OH)	271 (17000)	264 (16000) 283 sh	227 (20300) 273 (16500)	
Xb		$[\alpha]_D^{25}$ −12.8° (c=1.0, H_2O)	271 (15200)	263 (13900) 283 sh	226.5 (19400) 272 (14500)	9.8
XIa	179—180	$[\alpha]_D^{25}$ +10.2° (c=1.0, CH_3OH)	268 (14400)	261 (13500) 280 sh	226.5 (18600) 271 (14200)	
XIb		$[\alpha]_D^{25}$ −18.0° (c=1.0, H_2O)	270 (15600)	262 (14200) 280 sh	226.5 (19300) 271 (15200)	9.5
XIIa	191—193	$[\alpha]_D^{24}$ +10.8° (c=1.0, CH_3OH)	271 (15500)	261 (13900) 280 sh	226.5 (19400) 272 (14800)	
XIIb		$[\alpha]_D^{23}$ −15.5° (c=1.0, H_2O)	271 (16000)	262 (14400) 280 sh	227 (20400) 272 (15300)	9.7
XIIIa	193—195	$[\alpha]_D^{24}$ −12.5° (c=1.0, CH_3OH)	275 (17100)	264 (15300) 283 sh	227, 274 (16400)	
XIIIb			275 (17600)	264 (14600) 283 sh	227.5 (22500) 273 (15800)	11.0
XIVa	198—201	$[\alpha]_D^{22}$ +32.0° (c=1.0, CH_3OH)	256 (27700) 284 sh	254 (28000) 284 sh	230 (20000) 257 (28400)	
XIVb		$[\alpha]_D^{25}$ +3.1° (c=1.0, H_2O)	256 (28800) 280 sh	256 (29500) 280 sh	230 infl.,[a] 257 (30100)	5.5
XVa	170—172	$[\alpha]_D^{24.5}$ +23.0° (c=1.0, CH_3OH)		261 (13600)[b] 280 (13200)		
XVb		$[\alpha]_D^{26.1}$ −5.9° (c=1.0, H_2O)	273 (15300)	264 (13600) 280 sh	227 (19800) 272 (14700)	1.0
XVIa	212—213	$[\alpha]_D^{24}$ +14.7° (c=1.0, CH_3OH)	267 (14600)	261 (13600) 280 sh	225 (25100) 270 (14300)	
XVIb		$[\alpha]_D^{25}$ −6.1° (c=0.43, H_2O)	269 (15100)	262 (13900) 282 sh	224.5 (25400) 270 (14600)	17.3
XVIIa	174—176 (decomp.)	$[\alpha]_D^{25}$ +14.8° (c=0.5, $HCON(CH_3)_2$)	237 (9700) 283 (16600)	232 (10700) 270 infl., 284 (15400)	238 (19800) 278 (17800)	
XVIIb		$[\alpha]_D^{25}$ −6.0° (c=1.0, H_2O)				3.4
XVIIIa	208—210	$[\alpha]_D^{25}$ +18.1° (c=1.0, CH_3OH)	264 (13500) 281 (13500)	264 (22100) 288 (25200)	270 (27000)	
XVIIIb		$[\alpha]_D^{25}$ +6.5° (c=1.0, H_2O)	278 (13300)	287 (25900)	271 (27300)	2.8

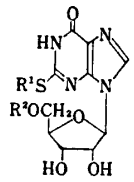
a) inflexion b) CH_3OH solution

ted as the sodium or calcium salts after recrystallization or purification by DEAE-cellulose column chromatography.

Synthesis of O^2-Substituted Xanthosine 5′-Phosphates and N^2-Substituted Guanosine 5′-Phosphate

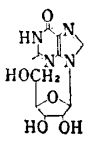
The nucleoside (I) was converted to 2-chloroinosine according to a reported method.[13] Reaction of 2-chloroinosine with sodium phenoxide in an aqueous solution gave rise to 2-phenoxyinosine.

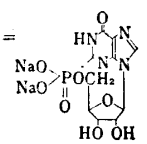
Table V. S-Substituted 2-Mercaptoinosine 5′-Phosphates (Part 4)



R^1	R^3		Formula	Analyses (%) Calcd. C	H	N	S	P	Found C	H	N	S	P
$CH_3OCH_2CH_2-$	PO_3Na_2	XIX	$C_{12}H_{17}O_9N_4SPNa_2 \cdot 4H_2O$	28.16	4.51	10.10		5.60	28.35	4.04	9.55		5.30
$C_2H_5OCH_2CH_2-$	H	XXa	$C_{14}H_{20}O_6N_4S \cdot \frac{1}{2}H_2O$	44.09	5.51	14.69	8.40		44.01	5.29	14.93	8.78	
	PO_3Na_2	XXb	$C_{14}H_{19}O_9N_4SPNa_2 \cdot H_2O$	32.69	4.09	10.89		6.04	32.59	4.35	10.65		5.87
	H	XXIa	$C_{15}H_{20}O_6N_4S$			14.57	8.34				14.63	8.10	
	PO_3Na_2	XXIb	$C_{15}H_{19}O_9N_4SPNa_2 \cdot H_2O$	34.24	4.02	10.64	6.09	5.89	33.95	4.10	10.61	5.54	5.51
$C_2H_5OCOCH_2CH_2-$	H	XXIIa	$C_{15}H_{20}O_7N_4S$	45.00	5.00	14.00	8.00		45.06	4.99	13.83	8.00	
	PO_3Na_2	XXIIb	$C_{15}H_{19}O_{10}N_4SPNa_2 \cdot 2H_2O$	32.14	4.11	10.00	5.54		32.41	4.31	9.72	5.30	
$H_2NCH_2CH_2-$	H	XXIIIa	$C_{12}H_{17}O_5N_5S$	41.97	4.99	20.40	9.34		41.81	5.10	20.35	9.45	
	PO_3Na_2	XXIIIb	$C_{12}H_{18}O_8N_5SPNa_2 \cdot 2H_2O$	28.63	4.00	13.92	6.37	6.16	28.86	4.07	13.40	6.17	5.94
$-CH_2-S-R^3$ [a]	H	XXIVa	$C_{21}H_{24}O_{10}N_8S_2 \cdot \frac{1}{4}HCON(CH_3)_2 \cdot \frac{1}{2}H_2O$	38.72	4.06	17.23	9.50		39.01	4.11	16.77	9.12	
$-CH_2-S-R^4$ [b]	PO_3Na_2	XXIVb	$C_{21}H_{22}O_{16}N_8S_2P_2Na_4 \cdot \frac{1}{2}C_2H_5OH \cdot \frac{1}{2}H_2O$	29.48	2.91		7.17	7.21	29.22	3.22		7.06	7.34
$-(CH_2)_3S-R^3$	H	XXVa	$C_{23}H_{28}O_{10}N_8S_2$	43.13	4.37	17.50			43.17	4.36	17.51		
$-(CH_2)_3S-R^4$	PO_3Na_2	XXVb	$C_{23}H_{28}O_{16}N_8S_2P_2Na_4 \cdot \frac{1}{2}C_2H_5OH \cdot H_2O$	31.00	3.34	12.06		6.67	30.62	3.71	11.36		6.93

	mp (°C)	$[\alpha]_D$	UV absorption spectra λ_{max} mμ (ε) 0.1N HCl	H_2O	0.1N NaOH	Flavoring strengths
XIX		$[\alpha]_D^{25}$ −16.8° (c=1.0, H_2O)				8.1
XXa	178—179	$[\alpha]_D^{25}$ −26.2° (c=1.0, 0.1N NaOH)	266 (18300)	261 (14700) 280 infl.	226 (19700) 270 (15200)	
XXb		$[\alpha]_D^{25}$ −9.8° (c=1.0, H_2O)				11.8
XXIa		$[\alpha]_D^{24.5}$ +6.5° (c=0.55, H_2O)	267 (13800)	262 (13000) 280 sh	226 (17400) 271 (13500)	
XXIb		$[\alpha]_D^{25}$ −13.8° (c=0.73, H_2O)	268 (14900)	261 (13900) 280 sh	226 (18400) 270 (14200)	7.6
XXIIa	158—160	$[\alpha]_D^{25}$ +7.0° (c=0.5, $HCON(CH_3)_2$)	266 (15100)	261 (14400) 280 infl.	227 (19200) 271 (14800)	
XXIIb		$[\alpha]_D^{25}$ −7.8° (c=1.0, H_2O)				11.8
XXIIIa	232 (decomp.)	$[\alpha]_D^{25}$ +11.0° (c=1.0, 0.1N HCl)	262 (14800) 276 sh	219 (15200) 266 (13800)	227 (18700) 272 (14800)	
XXIIIb		$[\alpha]_D^{25}$ −27.5° (c=1.0, H_2O)	263 (14800)	261 (13600)	226 (18700) 271 (14900)	2.3
XXIVa	218—220	$[\alpha]_D^{25}$ +186.3° (c=1.0, $HCON(CH_3)_2$)	270 sh, 280		228, 273	
XXIVb		$[\alpha]_D^{25}$ −40.4° (c=1.0, H_2O)	275 (27100)	265 (25000) 280 sh	228 (40400) 273 (29900)	1.4
XXVa	203—204	$[\alpha]_D^{25}$ +36.8° (c=1.0, 0.1N NaOH)	270 (31700)	263 (29600) 280 infl.	228 (38100) 272 (30800)	
XXVb		$[\alpha]_D^{25}$ +8.8° (c=1.0, H_2O)				1.3

a) R^3 = 

b) R^4 = 

13) A.G. Beaman and R.K. Robins, *J. Appl. Chem.*, **12**, 432 (1962).

The potassium salt of I was oxidized with aqueous hydrogen peroxide and treatment of the resulting 2-sulfonic acid derivative with furfuryl amine afforded 2-furfurylaminoinosine.

These nucleosides were converted to the corresponding 5′-phosphates by the selective phosphorylation[12] of the 5′-hydroxyl group.

2′,3′-O-Isopropylidene 2-chloroinosine was allowed to react with sodium salts of either saturated or unsaturated alcohols and the reaction products were phosphorylated with pyrophosphoryl chloride and then deacetonized to afford the corresponding 5′-phosphates (Table VI).

Synthesis of 2-Arylinosine 5′-Phosphates

The synthesis of 2-alkylinosine has already been published by Yamazaki, *et al.*[14] The method consists in heating AICA-riboside with ethyl alkylcarboxylate in the presence of sodium ethoxide. They reported that the analogous synthesis of 2-phenylinosine was unsuccessful and the reaction resulted in the recovery of AICA-riboside. We repeated their method and found that 2-phenylinosine (XXXIIIa) could be successfully synthesized by the use of methyl benzoate in 22% yield, and that a considerable amount of N^5-benzoyl-AICA-riboside (XXXV) could be also isolated from the reaction mixture. Similarly, 2-thienylinosine (XXXIVa) was obtained by the reaction with ethyl 2-thenoate in 15% yield, the reaction being accompanied by the formation of N^5-theonyl-AICA-riboside (XXXVI). The identity of these compounds was established on the basis of the paper electrophoresis, UV and NMR spectra and the elemental analyses. These 2-substituted inosines were phosphorylated[12] to the corresponding 5′-phosphates (Table VII) (Chart 2).

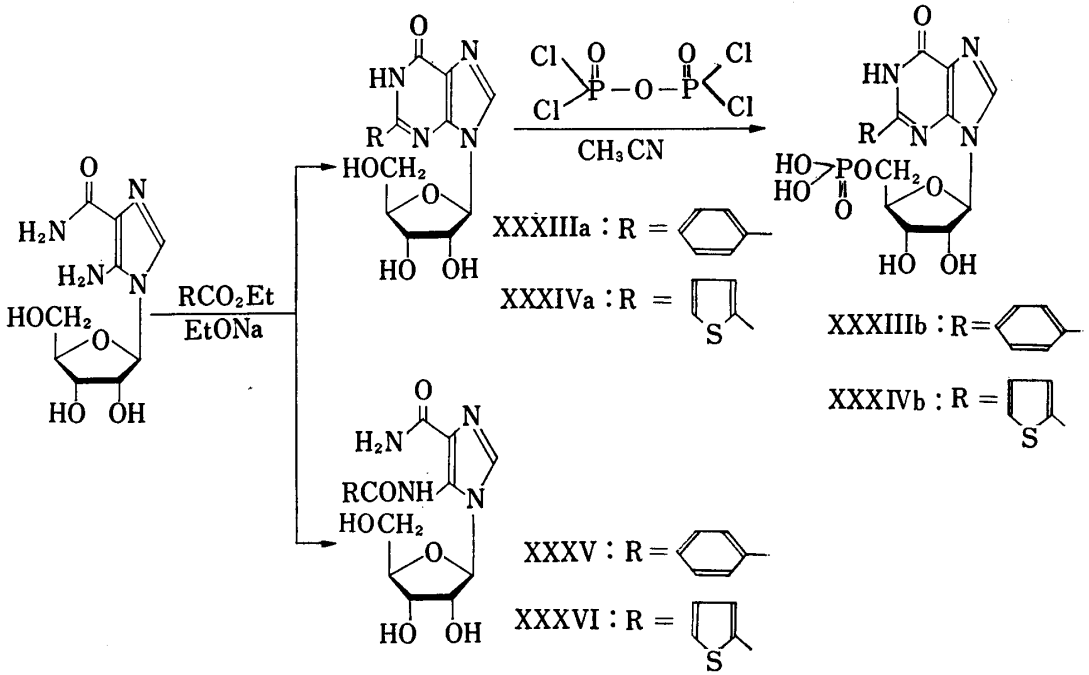


Chart 2

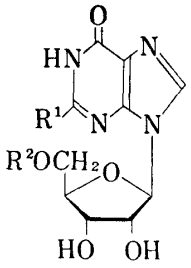
The Chemical Structure–Flavor Enhancing Activity Relationship

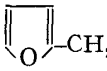
Disodium or monocalcium salts of 33 kinds of 2-substituted inosine 5′-phosphates were assessed with regard to the flavor enhancing activity synergistic with monosodium L-glutamate. The activity of the compounds was compared to that of disodium salt of inosine 5′-

14) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **32**, 3258 (1967).

phosphate, which contains 8 molecules of water in its crystals. The constant method[15] was used, and the data obtained were analyzed by the probit analysis.[16] The results are given in Tables II—VII. It should be noted that the following 9 nucleotides, VIb, Xb, XIb, XIIb, XIIIb, XVIb, XIX, XXb, and XXIIb possess comparable or stronger activity than that of 2-methylthioinosine 5′-phosphate.[3]

TABLE VI. O^2-Substituted Xanthosine 5′-Phosphates and N^2-Substituted Guanosine 5′-Phosphate



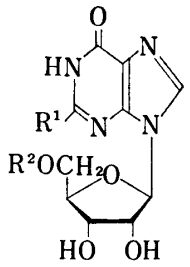
	R^1	R^2		Formula		Analyses (%) C	H	N	P
A		PO_3Ba	XXVI	$C_{16}H_{15}O_{19}N_4BaP \cdot H_2O$	Calcd.	32.38	2.89	9.44	
					Found	32.70	3.08	8.78	
B	C_2H_5O–	PO_3Ba	XXVII	$C_{12}H_{15}O_9N_4BaP \cdot 4H_2O$	Calcd.	24.04	3.84	9.35	
					Found	24.44	3.26	8.98	
	$CH_3CH_2CH_2O$–	PO_3Ba	XXVIII	$C_{13}H_{17}O_9N_4BaP \cdot 2H_2O$	Calcd.	27.03	3.66	9.70	5.36
					Found	27.17	3.57	9.05	5.20
	$(CH_3)_2CHO$–	PO_3Ba	XXIX	$C_{13}H_{17}O_9N_4BaP \cdot 2H_2O$	Calcd.	27.03	3.66	9.70	5.36
					Found	27.22	4.14	10.01	5.53
	$C_2H_5OCH_2CH_2O$–	PO_3Na_2	XXX	$C_{14}H_{19}O_{10}N_4Na_2P \cdot 2H_2O$	Calcd.	32.59	4.48	10.86	6.01
					Found	33.12	4.97	10.16	6.24
	$CH_2=CHCH_2O$–	H	XXXIa	$C_{13}H_{16}O_6N_4$	Calcd.	48.14	4.97	17.28	
					Found	47.87	5.04	17.29	
		PO_3Ca	XXXIb	$C_{13}H_{15}O_9N_4CaP \cdot \frac{1}{2}H_2O$	Calcd.	34.62	3.58	12.42	6.88
					Found	34.53	3.60	12.21	6.94
C		PO_3Na_2	XXXII	$C_{15}H_{16}O_9N_5Na_2P$	Calcd.	36.95	3.31	14.37	6.37
					Found	36.71	3.57	14.75	6.03

	mp (°C)	$[\alpha]_D$	UV absorption spectra λ_{max} mμ (ε) 0.1N HCl	H_2O	0.1N NaOH	Flavoring strengths
XXVI			250 (12100)			0.6[a] ($\frac{1}{2}H_2O$)
XXVII			245 (12000)			4.6[a]
XXVIII			253 (9900)	250 (10000)	261.5 (11700)	2.1[a]
XXIX			254 (9800)	249 (10600)	261.5 (11600)	4.3[a] (H_2O)
XXX		$[\alpha]_D^{24}$ −23.6° (c=1.0, H_2O)	247 (12200) 257 infl.	250 (11600)	259 (13400)	5.5
XXXIa	190—193	$[\alpha]_D^{21}$ −17.2° (c=1.0, H_2O)	251 (12700)	249 (13300)	261 (14500)	
XXXIb			250 (10200)	247 (10700) 260 sh	259 (11800)	5.9
XXXII		$[\alpha]_D^{23}$ −6.4° (c=1.0, H_2O)	259 (14100) 282 sh	254 (15000) 276 sh	259 (12200)	6.6

a) Flavoring strength was tested by using the disodium salts, two of which contained amounts of water given in parentheses.

15) J.P. Guilford, "Psychometric Methods," 2nd ed., McGraw-Hill Book Company, New York, 1954, p. 118.
16) T. Haga, H. Harano, S. Kuroki, S. Ito, T. Indow, M, Masuyama, S.Miura, R. Mori, S. Sato, S. Ura, M. Yoshida, and S. Yoshikawa, "Kogyo ni okeru kan'nokensa handbook (Sensory inspection handbook for industry)," ed. by Nikkagiren Kan'no Kensa I' inkai, Nikkagiren Shuppansha, Tokyo, 1962, p. 341.

TABLE VII. 2-Phenyl- and 2-Thienylinosine 5′-Phosphates



R^1	R^2		Formula		Analyses (%) C	H	N	P
	H	XXXIIIa	$C_{16}H_{16}O_5N_4$	Calcd.	55.81	4.68	16.27	
				Found	55.26	4.63	16.28	
	PO_3Na_2	XXXIIIb	$C_{16}H_{15}O_8N_4Na_2P \cdot 3H_2O$	Calcd.	36.78	4.02	10.73	5.94
				Found	36.95	4.56	10.96	6.27
	H	XXXIVa	$C_{14}H_{14}O_5N_4S$	Calcd.	48.00	4.00	16.00	
				Found	47.63	4.31	15.57	
	PO_3Na_2	XXXIVb	$C_{14}H_{13}O_8N_4SNa_2P \cdot 3H_2O$	Calcd.	31.82	3.60	10.61	5.88
				Found	32.07	3.81	10.32	5.44

	mp (°C)	$[\alpha]_D$	UV absorption spectra λ_{max} mμ (ε) 0.1N HCl	H_2O	0.1N NaOH	Flavoring strengths
XXIIIa	225—230	$[\alpha]_D^{22} = -13.0°$ (c=1.0, 0.1N NaOH)	261 (12700) 289 (13900)	260 (12300) 290 (13300)	234 (27000) 264 (14800) 283 (13400)	
XXIIIb		$[\alpha]_D^{23} = -12.4°$ (c=1.0, H_2O)				3.6
XXXIVa	209—212	$[\alpha]_D^{22} = -19.4°$ (c=1.0, 0.1N NaOH)	265 (11300) 317 (14600)	261 (11300) 317 (13900)	248 (15500) 306 (13000)	
XXXIVb		$[\alpha]_D^{23} = +2.4°$ (c=1.0, H_2O)				2.2

The comparison of the 2-S-substituted derivatives to that of the 2-O- or 2-N-substituted derivatives suggests that the former group possesses a stronger activity, as can be seen in each pair of Xb and XXXIb, and XVIb and XXXII. However, the nucleotides (III, IV and Vb) having sulfonic acid and dithio group have a faint or no detectable activity.

The effect of substituents in the 2-S-substituted derivatives on the flavor enhancing activity would be summarized as follows: i) as to alkyl groups, the increased carbon number causes a decrease in the activity (Table II), ii) an alkenyl-substituted derivative has a stronger activity than the corresponding alkyl-substituted derivative (Xb>VIb, XIb>VIIb and XIIIb>VIIIb), and iii) the introduction of an aliphatic ether or a carboxylic ester at the end of the alkyl group enhances the activity (XIX, XXb and XXIIb). The fact that XVIb possesses the strongest activity, about 17-fold as high activity as that of inosine 5′-phosphate, may be reasonable explained by the effect of introduction of both an alkenyl and an ether substituent.

Experimental[17]

2-Mercaptoinosine (I)——A mixture of AICA-riboside·H_2O (60 g, 0.22 moles) and pyridine (400 ml) was heated with stirring, to which C_6H_5NCS (79 ml, 0.66 moles) was added dropwise. The solution was heated

17) All melting points were uncorrected. NMR spectra were measured in DMSO-d_6 solutions using tetramethylsilane as internal standard. Chemical shifts were expressed in δ values.

for 3.5 hr, while it was bubbled with N_2 gas, to give almost colorless crystals (the pyridinium saltof I). After cooling they were collected by filtration and washed with C_6H_6 and $(C_2H_5)_2O$ successively. mp 190—192°. *Anal.* Calcd. for $C_{10}H_{12}O_5N_4S \cdot C_5H_5N$: C, 47.48; H, 4.52; N, 18.46; S, 8.45. Found: C, 47.65; H, 4.50; N, 18.00; S, 8.84. UV λ_{max} mμ (ε): 232, 256, 262, 291 (23000) [0.1N HCl]; 256 (sh), 262 (sh), 289 (18200) [H_2O]; 232, 250, 256 (sh), 262 (sh), 285 (18800) [0.1N NaOH]. NMR: 5.97 (H_1': d, J=6.0 Hz), 6.5—8.0 (2'-, 3'- and 5'-OH, pyridine-H_3, H_4 and H_5; m), 8.09 (H_8; s), 8.8—9.1 (pyridine-H_2 and H_6; m). This compound was dissolved by warming in a solution of KOH (14.5 g) in H_2O (100 ml). The solution was decolorized with activated charcoal (100 mg) and concetrated *in vacuo* (80 ml), to which MeOH (100 ml) was added and kept in the refrigerator to afford colorless prisms (the potassium salt of I, 65 g, 85%). Paper chromatography [isobutyric acid, 0.5N NH_4OH (10: 6), ascending method]: *Rf* 0.29. Paper electrophoresis (PE) [0.05M sodium borate buffer (pH 9.2), 22 v/cm, 1 hr]: $M_{AICA\text{-}riboside}$[18] 2.0.

2-Mercaptoinosine 5'-Phosphate(II)——1) One and two tenths ml of (n-Butyl)$_3$ N was added to a suspension of AICAR (842 mg, 2.5 mmoles) in MeOH (5 ml). The mixture was stirred to become clear and evaporated to distill off MeOH. Pyridine (5 ml) was added to the residue and the solution concentrated to dryness *in vacuo*. This procedure was repeated twice and the residue dissolved in pyridine (25 ml) and, after the addition of C_6H_5NCS (1.5 ml, 12.5 mmoles), refluxed for 1 hr. The reaction mixture was evaporated *in vacuo* to distill off pyridine and the residue dissolved in H_2O and extracted with $CHCl_3$. One fourth volume of the aqueous layer was put onto the column of DEAE-cellulose (HCO_3^- type, 50 ml). The column was washed with water, and eluted with i) 0.1M $NaHCO_3$ (300 ml, 2010 OD_{270} units) and ii) 0.1M $NaHCO_3$ (875 ml, 3765 OD_{286} units). The latter eluate was adjusted to pH 5 by the addition of Amberlite IR-120 (H^+ type), which was removed by filtration. The filtrate was passed through the column of IR-120 (H^+ type, 5 ml) to remove Na^+ completely, adjusted to pH 13 by the addition of NH_4OH and passed through the column of IR-120 (Na^+ type, 20 ml). The column was washed with water, and the effluent and the washings were combined and concentrated *in vacuo*. EtOH was added to the residue to afford a white powder (114 mg, 38%). PE (sodium borate buffer): M_{AICAR} 1.35.

2) The tri-n-butylammonium salt of AICAR (17 g, 50 mmoles) was dissolved in HMPA (500 ml), to which was added C_6H_5NCS (60 ml, 0.5 moles). The solution was heated with stirring at 100—120° for 4 hr, to which, after cooling was added H_2O (1 liter). The mixture was extracted with $CHCl_3$ (2 liters) to remove HMPA, the aqueous layer concentrated *in vacuo* and the residue treated in a manner similar to that described in 1) to afford a white powder (7.9 g, 33%).

2-Furfurylthioinosine (XVIa)——An ether solution (40 ml) of furfuryl chloride (7.5 g, 64 mmoles) was added dropwise to the ice-cooled suspension of the potassium salt of I (13.6 g, 40 mmoles) in pyridine (200 ml). The mixture was stirred at room temperature for 1.5 hr and evaporated to dryness *in vacuo*. The syrupy residue was dissolved in H_2O (50 ml) and the solution evaporated again to dryness *in vacuo*. The residue was crystallized after repeating twice this procedure. Pure colorless needles (12.4 g, 81%) were obtained by recrystallization from water (700 ml).

2-Furfurylthioinosine 5-Phosphate (XVIb)——Pyrophosphoryl chloride (1.1 ml, 7.9 mmoles) was added at 0—5° to a suspension of 2-furfurylthioinosine (600 mg, 1.6 mmoles) in CH_3CN (70 ml). The mixture was stirred at this temperature for 2 hr and poured to ice-water (500 ml). The solution was adjusted to pH 2 by the addition of 4N NaOH, passed through a column of activated charcoal (9 g), and the column washed with water, eluted with a mixture of EtOH, 28% NH_4OH and H_2O (50:2:48 v/v). The eluate was concentrated *in vacuo* and passed through the column (4×12 cm) of DEAE-cellulose (Cl^- type). The column was washed with water, and eluted with 0.003N HCl+0.01M NaCl (1730 ml, 12500 OD_{260} units). The eluate was desalted with activated charcoal (3 g), added with 1N NaOH (2.2 ml) and evaporated to dryness *in vacuo*. Addition of EtOH to the syrupy residue afforded white powder (603 mg, 72%). PE [0.05M sodium phosphate buffer (pH 7.5), 22 v/cm, 50 min]: $M_{2\text{-}furfurylthioinosine}$ 3.5.

2-(1-Adamantyldithio)inosine (Va)——A solution of S-(1-adamanthylthio)isothiourea hydrochloride[11] (4.1 g, 14.7 mmoles) in MeOH (40 ml) was added to the solution of the potassium salt of I (5 g, 14.7 mmoles) in H_2O (70 ml), and the mixture was adjusted to pH 7 by the addition of 28% NH_4OH (0.3 ml). After cooling, the solid was collected by filtration and recrystallized from EtOH (30 ml) to afford colorless crystals (2.3 g, 34%).

Ammonium Salt of 2-Chloroinosine——A mixture of MeOH (100 ml) and conc. HCl (70 ml) was cooled at 0°, and saturated with HCl gas. To this was added the potassium salt of I (20 g) and the mixture bubbled with Cl_2 gas at 5—10° for 2 hr. The solution was poured into ice-water (2.5 liters), made neutral by the addition of 28% NH_4OH, evaporated to distill off MeOH and desalted with activated charcoal (200 g). The eluate was evaporated to dryness and the syrupy residue was recrystallized from MeOH (60 ml) to afford colorless crystals (9.0 g, 48%). mp 180—183°. *Anal.* Calcd. for $C_{10}H_{14}O_5N_5Cl$: C, 37.57; H, 4.41; N, 21.91; Cl; 11.09. Found: C, 37.46; H, 4.39; N, 21.29; Cl, 11.37. UV λ_{max} mμ (ε): 253 (12400) [0.1N HCl]; 256 (13700) [H_2O]; 257.5 (14100) [0.1N NaOH].

18) Ratio of the migration distance of the sample to that of AICA-riboside.

2-Phenylinosine (XXXIIIa)——AICA-riboside·H_2O (2.5 g, 9.1 mmoles) was dissolved in hot 1N EtONa (100 ml). The solution was added with methyl benzoate (10 ml, 80 mmoles) and heated at 120—130° (bath temp.) for 3 hr. The reaction mixture was evaporated to distill off EtOH, added with H_2O (100 ml), adjusted to pH 3 with conc. HCl and extracted with $(C_2H_5)_2O$ (100 ml). The aqueous layer was neutralized and concentrated *in vacuo* to afford colorless needles (750 mg, 22%). NMR: 6.00 (H_1'; d, J=6.0 Hz), 7.6—8.1 (aromatic protons; m), 8.35 (H_8; s).

N^5-Benzoyl-AICA-riboside (XXXV)——The mother liquor of 2-phenylinosine was adjusted to pH 3 and desalted with activated charcoal (25 g) to afford crude crystals (850 mg, 22%). Recrystallization of the crystals gave colorless needles. mp 142—143° (decomp.), *Anal.* Calcd. for $C_{16}H_{18}O_6N_4\cdot H_2O$: C, 50.52; H, 5.30; N, 14.73. Found: C, 50.57; H, 5.19; N, 14.71. $[\alpha]_D^{22}=+6.8°$ [c=1.0, $HCON(CH_3)_2$]. UV λ_{max} mμ (ε): 230 (20800) [pH 2]; 230 (19400) [pH 6]; 235 (18800) [pH 12]. NMR: 5.53 (H_1'; d, J=3.0 Hz), 7.0—7.4 (4-$CONH_2$; broad d), 7.6—8.0 (aromatic protons; m), 8.07 (H_2; s), 10.07 (5-NH–CO–; s).

2-(2-Thienyl)inosine (XXXIVa)——AICA-riboside·H_2O (5 g, 18.1 mmoles) was dissolved in hot 1N EtONa (150 ml). The solution was added with ethyl 2-thenoate (19 ml, 122 mmoles) and refluxed for 2 hr. After cooling, the solid was filtered off, the filtrate concentrated *in vacuo*, added with H_2O (300 ml) and adjusted to pH 3. The solution was mixed with $(C_2H_5)_2O$ (300 ml) and the aqueous layer was kept in the refrigerator to afford needles (1 g, 15%). NMR: 5.86 (H_1'; d, J=6.0 Hz), 7.18 (thienyl-H_4; m), 7.82 (thienyl-H_3; d, J=4.0 Hz), 8.19 (thienyl-H_5; d, J=4.0 Hz), 8.30 (H_8; d), 12.62 (N^1–H; s).

N^5-Thenoyl-AICA-riboside (XXXVI)——The mother liquor of 2-(2-thienyl)inosine was desalted with activated charcoal (50 g) to yield colorless needles (2.5 g, 35%). mp 128—131°. *Anal.* Calcd. for $C_{14}H_{16}O_6N_4S$: C, 45.65; H, 4.35; N, 15.22. Found: C, 45.97; H, 4.48; N, 14.97. $[\alpha]_D^{22}=+3°$ [c=0.5, $HCON(CH_3)_2$]. UV λ_{max} mμ (ε): 250 (11400), 278 (10500) [pH 1]; 244 (13200), 276 (11000) [pH 5]; 251 (16600) [pH 13]. NMR: 5.47 (H_1'; d, J=4.0 Hz), 7.0—7.3 (4-$CONH_2$; m), 7.28 (thienyl-H_4; t, J=4.0 Hz), 7.8—8.0 (thienyl-H_3 and H_5; m), 8.02(H_2;s), 10.14 (5-NH–CO–; s).

Acknowledgement The authors thank Prof. Y. Mizuno of Hokkaido University for his reviewing the manuscript, and Drs. S. Tatsuoka, Y. Abe and K. Tanaka for their encouragement throughout the work. They are grateful to Drs. K. Morita, M. Nishikawa and H. Sugibayashi for their useful suggestions. Thanks are also due to the members of analysis section for physico-chemical measurements and elemental analyses.