hydroxide in 40 ml of of water at 80–90° was added 4 ml of 30% hydrogen peroxide dropwise over a period of 0.5 hr with vigorous stirring. After the mixture had been stirred for an additional 1 hr at 80–90° and cooled, it was extracted with three 30-ml portions of chloroform. The chloroform extracts were washed consecutively with 20 ml of 5% hydrochloric acid, 20 ml of saturated sodium bicarbonate solution, and 20 ml of saturated sodium chloride solution. After the chloroform was removed, the residue was molecularly distilled at 220° (0.05 mm) to yield 1.50 g (85%) of a very viscous product: $\nu_{max}^{10\%\ CCl_4}$ 2240 (m, C≡N) cm^{-1}; δ 7.0–7.8 (cm, C_6H_5), 1.8 (CH_3), ratio 2:3.

Anal. Calcd for $C_{20}H_{20}N_2S_2$: C, 68.14; H, 5.72; S, 18.19. Found: C, 68.06; H, 5.67; S, 18.33.

3,3-Dimethyl-2-thianaphthenone (13).—A solution of 1.07 g (0.005 mole) of **9** in 40 ml of 6 *N* hydrochloric acid was heated under reflux for 2 hr. The mixture was cooled and extracted with three 25-ml portions of ether. The ether solution was washed with 20 ml of saturated sodium chloride solution, 20 ml of saturated sodium bicarbonate solution, and 20 ml of saturated sodium chloride solution. After final work-up, distillation of the residue yielded 0.75 g (84%): bp 54–55° (0.05 mm); $n^{25}D$ 1.5750; ν_{max} 1695 (w, thio lactone C=O) cm^{-1}; λ_{max}^{EtOH} 262 mμ (ϵ 4350); δ 7.1–7.3 (cm, C_6H_5), 1.4 (CH_3), ratio 2:3.

Anal. Calcd for $C_{10}H_{10}OS$: C, 67.38; H, 5.65; S, 17.99. Found: C, 67.56; H, 5.66; S, 17.88.

Registry No.—**1**, 13584-50-4; **3a**, 13584-51-5; **3b**, 13584-52-6; **4a**, 13584-53-7; **4b**, 13584-54-8; **6**, 13584-55-9; **6** hydrochloride, 13584-56-0; **7**, 13584-57-1; **8**, 13584-58-2; **9**, 13584-59-3; **10**, 13584-60-6; **11**, 13584-61-7; **12b**, 496-31-1; **13**, 13584-63-9; benzo[*b*]thiophene, 95-15-8; 2-aminobenzo[*b*]thiophene, 4521-30-6; 2-aminobenzo[*b*]thiophene hydrochloride, 13584-65-1.

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Synthesis of Guanosine and Its Derivatives from 5-Amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide. II. Ring Closure with Sodium Methylxanthate[1]

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A novel and convenient method for the synthesis of guanosine is described. The reaction of 5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide (AICA-riboside) with sodium methylxanthate gave 2-mercaptoinosine (I), in almost quantitative yield, which was oxidized with hydrogen peroxide to afford inosine-2-sulfonic acid (IV). Compound IV was readily aminated to give guanosine (VII) in excellent yield. In a similar fashion, the preparation of N^2-methylguanosine (VIII) and N^2,N^2-dimethylguanosine (IX), the minor constituents of transfer ribonucleic acid, was accomplished. Further, this method was extended to the synthesis of 2′,3′-O-isopropylideneguanosine (XV) and the isopropylidene derivatives of various N^2-substituted guanosines from 5-amino-4-carbamoyl-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)imidazole (Ip-AICA-riboside).

Although several methods for the preparation of guanosine (VII) have been reported by Davoll, *et al.*,[2–4] involving the condensation of a metallic salt of purine base with a blocked halo sugar followed by the variation of substituents of the purine ring, the over-all yield of VII obtained by such a procedure is very low. Furthermore, disodium guanosine 5′-phosphate (5′-guanylic acid as well as disodium inosine 5′-phosphate (5′-inosinic acid) has been found to be a useful seasoning agent.[5] This finding prompted us to investigate a new synthetic route to VII or 2′,3′-O-isopropylideneguanosine (XV) which is an important precursor for the synthesis of 5′-guanylic acid.

As the starting material, we employed the pure 5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide (AICA-riboside) which was accumulated in the culture broth of the mutant of *Bacillus subtilis* and purified by ion-exchange chromatography.[6]

In a preceding paper,[7] XV was shown to be obtained by the ring-closure reaction of 5-amino-4-carbamoyl-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)imidazole (Ip-AICA-riboside)[8,9] with benzoyl isothiocyanate. This method, however, proceeds with a concomitant formation of an equivalent amount of benzoic acid in the last step and the over-all yield of XV based on Ip-AICA-riboside was not satisfactory.

Shaw[10] reported that attempts to obtain guanine from 5-amino-4-imidazolecarboxamide (AICA) using ring-closing reagents such as guanidine, S-methyl isothiourea, and cyanamide, were unsuccessful. From these results, it seemed to be extremely difficult to convert AICA-riboside directly into VII.

It has been reported that the amination of 2-methylthioinosine (V)[4] prepared by chloromercuri method[11] yielded VII. If 2-mercaptoinosine (I), that is, 2-thioxanthosine, could be obtained from AICA-riboside, a new route for preparing VII would be provided by methylation of I followed by amination. Accordingly, many attempts have been made to convert AICA-riboside into I with thiourea, thiophosgen, thiocyanic acid, O,S-dimethyl xanthate,[12] and carbon disulfide in pyridine,[13] but were found to be unsuccessful in all cases. When sodium methylxanthate was employed, however, a satisfactory result was obtained. By reaction of AICA-riboside with 5 equiv of sodium methyl-

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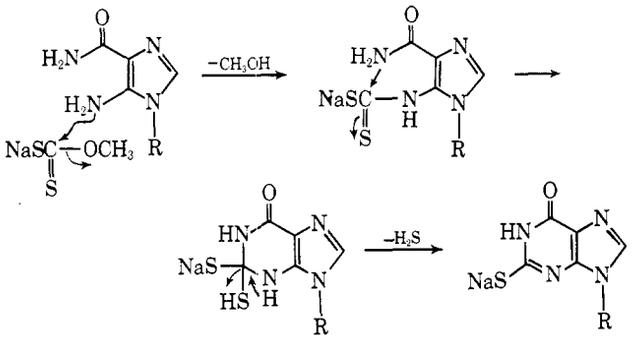
xanthate generated *in situ* in methanolic sodium hydroxide, I was easily obtained. In this case, AICA-riboside is almost insoluble in methanol but becomes soluble in the presence of sodium hydroxide. Therefore, after AICA-riboside was dissolved in methanolic sodium hydroxide, carbon disulfide was added and then the mixture was heated in an autoclave at 180° for 2–3 hr, giving I in nearly quantitative yield. It is very interesting that the ring-closure reaction of AICA-riboside proceeded almost quantitatively at such a high temperature. The structure of I was confirmed by desulfurization with Raney nickel to give inosine (II), by acid hydrolysis to 2-mercaptohypoxanthine (III),[14-16] and by methylation with methyl iodide to yield V which was in good agreement with that reported by Davoll.[4] Similar treatment of Ip-AICA-riboside with sodium methylxanthate in methanol furnished 2′,3′-O-isopropylidene-2-mercaptoinosine (XIII), in almost quantitative yield, which was isolated in 42% as ammonium salt. In addition, when AICA[17-21] and crystalline sodium methylxanthate were heated in N,N-dimethylformamide, III was obtained in good yield. A plausible mechanism for this condensation of AICA derivatives with xanthate is shown in Scheme I.

SCHEME I



Recently, several investigators[22-24] commented on the nucleophilic substitution reactions of 6-alkylthiopurine ribosides. In the substitution reaction of 2-alkylthiopurine riboside,[4] VII could be obtained by the amination of V at 130° for 18 hr in 24% yield; however, when the reaction time was shorter (2 hr), no formation of VII was detected on paper chromatogram.

In order to facilitate the nucleophilic displacement on methylthio group, the conversion of V into 2-methylsulfonylinosine (VI) was attempted. Previous workers have used oxidizing agents such as hydrogen peroxide[25] and chlorine[26] to oxidize a methylthio to methylsulfonyl group. N-Chlorosuccinimide, which was used for the preparation of 2-methylsulfonylhypoxanthine,[27] was found to be a suitable reagent for the oxidation of V to VI. Resulting VI was shown to be homogeneous on paper chromatogram but could not be obtained in the crystalline state. Subsequent reaction of VI with ammonia in a sealed tube at 120° for 2 hr provided VII in 56% yield based on V. Further, a facile route to VII from I was investigated. Compound I was found to be rapidly oxidized with 10 equiv of hydrogen peroxide at 5° to give the ammonium salt of inosine-2-sulfonic acid (IV) in good yield. The infrared absorption spectrum of IV exhibited absorption band at 1220 cm^{-1} for sulfonic acid group. When IV was allowed to react with ammonia at 120° for 2 hr, VII was obtained in excellent yield.

For the purpose of producing VII on a large scale, ring closure, oxidation, and amination reactions were carried out successively without isolating the intermediates. When 3 equiv of hydrogen peroxide was used in the oxidation of I, the paper chromatogram of the solution showed two spots, one of which was identical with that of IV. The other (minor) spot was proved to be xanthosine (X).[28] In the hydrogen peroxide reaction mixture, no bis(6-hydroxy-9-β-D-ribofuranosyl-2-purinyl)disulfide (XI) was present. After oxidation, the solution was treated with ammonia as usual to afford VII. Trace amounts of X and guanine were detected as by-products on the paper chromatogram. The measurement of the absorbancy of the extract from an excised spot corresponding to the desired product exhibited the formation of VII in 82% conversion based on AICA-riboside and it was isolated in 70% yield in a pure state (Scheme II).

Similarly, the ammonium salt of 2′,3′-O-isopropylidene-2-mercaptoinosine (XIII) was oxidized with 3 equiv of hydrogen peroxide[29] to give a mixture of the isopropylidene derivatives of IV and probably inosine-2-sulfinic acid,[30] whose R_f values in solvent B were 0.25 and 0.35, respectively. The latter spot was minor and, after being excised and then extracted, showed ultraviolet absorption spectra with a maximum at 255 mμ in 0.1 *N* hydrochloric acid and at 257 mμ in 0.1 *N* sodium hydroxide. Both the oxidation products were aminated with ammonia to afford XV, which was isolated in 76% yield based on XIII.

When the above oxidation of I was carried out in the presence of acetic acid, XI was formed. The ultraviolet absorption spectrum of the parent nucleoside I showed a maximum (EtOH) at 298 mμ. whereas XI showed a maximum (pH 7) at 261 mμ. Thus, a hypsochromic shift of *ca.* 30 mμ was observed. Moreover, when the ultraviolet absorption spectrum of I was compared with that of V in neutral solution, a hypsochromic

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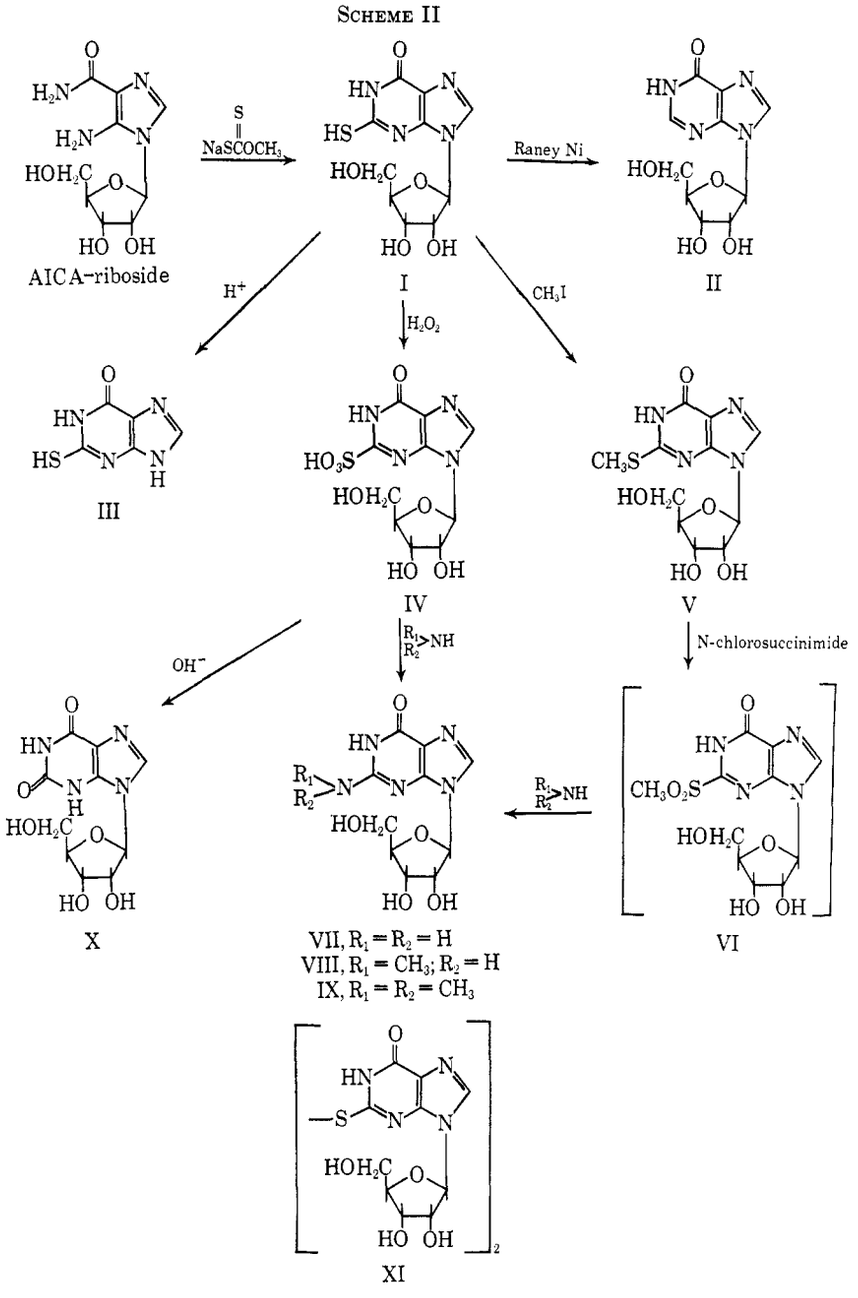
(28) The authors are indebted to Mr. S. Mori and Mr. G. Shionoya of Ajinomoto Co., Inc., for the information on the finding.
(29) The oxidation of XIII with potassium permanganate in alkaline solution gave only the isopropylidene derivative of IV (R_f 0.25, solvent B), which was aminated with ammonia to yield XV, although in relatively low yield (40–50%).
(30) It seems that, in the oxidation of I, inosine-2-sulfinic acid, which may have the same R_f value as IV in solvent A or B, is formed to some extent because the amount of hydrogen peroxide (3 equiv) is not enough for the complete oxidation.

SCHEME II



shift of *ca.* 37 mμ was also observed. From these results, it seems likely that I exists predominantly in the thiono form in neutral solution as well as in the cases of 6-mercaptopurine riboside[22,31] and 5-amino-2-mercaptothiazole.[32]

Oxidation of I with hydrogen peroxide followed by the treatment with aqueous sodium hydroxide afforded X in 47% yield. The ultraviolet and infrared absorption spectra of this compound were identical with those of an authentic sample.

The above-mentioned procedure was extended to the synthesis of various N^2-substituted guanosines. N^2-Methylguanosine (VIII) and N^2,N^2-dimethylguanosine (IX), the minor constituents of transfer RNA, were readily prepared in good yields by oxidation of I or V followed by reaction with methylamine and dimethylamine, respectively. The structures of both the methylated nucleosides were confirmed by the fact that these compounds were hydrolyzed to N^2-methylguanine[14] and N^2,N^2-dimethylguanine,[14] respectively, and that their ultraviolet absorption spectra were identical with those of naturally occurring nucleosides[33,34] isolated from transfer RNA.

Levene, *et al.*,[35] have reported that N^2-methyl-2′,3′,-5′-tri-O-methylguanosine was obtained by methylation of 2′,3′,5′-tri-O-acetylguanosine with dimethyl sulfate in a mixture of aqueous sodium hydroxide and acetone. Bredereck, *et al.*,[36] have also reported that the treatment of VII with dimethyl sulfate at pH 13–14 gave VIII. However, we could not obtain the desired compound by such direct methylation procedures. Although, recently, Robins, *et al.*,[37,38] succeeded in preparing VIII or IX by Schiemann reaction *via* 2-amino-6-chloro-9-β-D-ribofuranosylpurine or 6-thioguanosine, these methods

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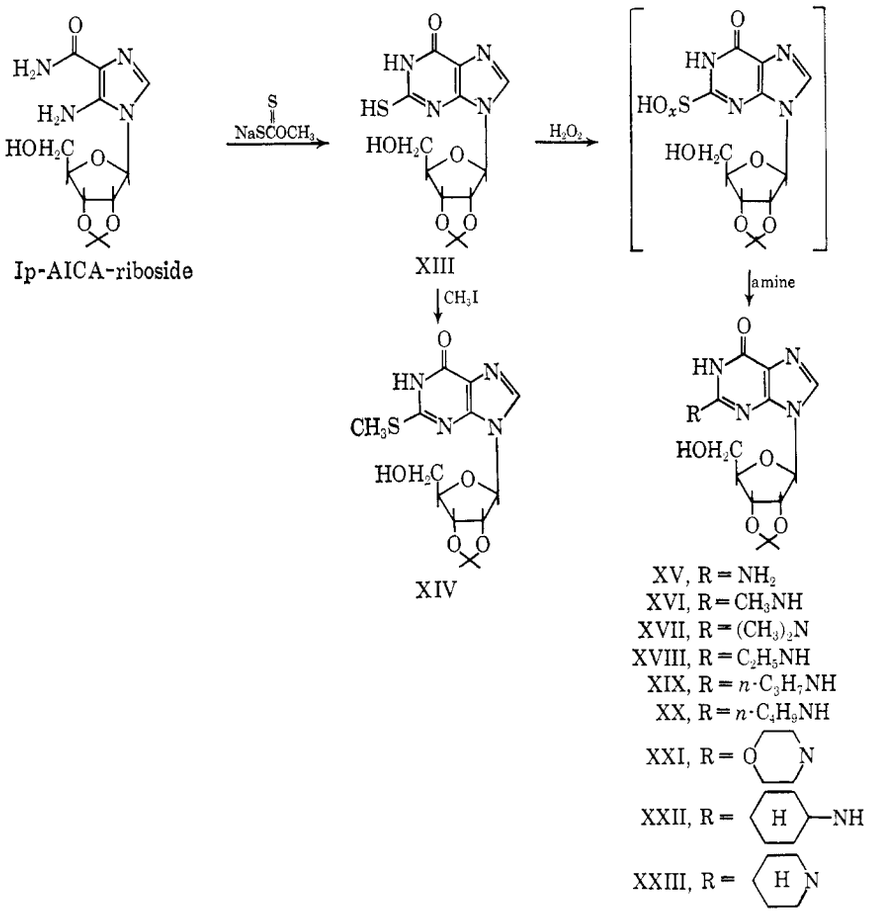
SCHEME III



were circuitous and the over-all yields were only about 5%. Consequently, it is apparent that the present procedure is superior for the preparation of N^2-substituted guanosines.

In order to obtain various N^2-substituted guanosine 5′-phosphates, a number of isopropylidene derivatives of 2-substituted 6-hydroxypurine ribosides, which were direct precursors for 5′-nucleotides, were synthesized (Scheme III). Firstly, 2′,3′-O-isopropylidene-2-methylthioinosine (XIV) was prepared by reaction of XIII with methyl iodide in 68% yield. By the same method as described for the preparation of XV, 2′,3′-O-isopropylidene-N^2-methylguanosine (XVI) and 2′,3′-O-isopropylidene-N^2,N^2-dimethylguanosine (XVII) were prepared in 67 and 53% yields, respectively. New compounds obtained are summarized in Table I. The phosphorylation study of these isopropylidene derivatives will be reported later.

Since it is well known that 6-mercapto-9-β-D-ribofuranosylpurine[39] has antitumor activity, it seems to be of interest to test compound I which is an analog of xanthosine. This testing is now in progress.

Experimental Section[40]

The Sodium Salt of 2-Mercaptoinosine (I).—To a solution of sodium hydroxide (7.76 g, 194 mmoles) in 70 ml of methanol was added 10 g (38.8 mmoles) of 5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide (AICA-riboside) on slight warming and then 14.7 g (194 mmoles) of carbon disulfide was added. The clear solution was heated in an autoclave in an oil bath regulated at 180° for 3 hr. After being cooled to room temperature, the separated product was collected by filtration and washed with a small amount of cold methanol. This product was recrystallized from water with charcoal treatment to give 8.2 g (64%) of analytically pure, colorless prisms: mp 186–198° dec; pK_{a1} 3.75, pK_{a2} 9.10; $[\alpha]^{27}D$ −48° (*c* 1, H_2O).

Anal. Calcd for $C_{10}H_{11}O_5N_4SNa \cdot {}^1/_2H_2O$: C, 36.25; H, 3.63; N, 16.92. Found: C, 36.35; H, 3.82; N, 16.95.

After ring-closure reaction of AICA-riboside, the reaction mixture was diluted with water and the solution showed a single spot on paper chromatogram. The ultraviolet absorbing spot was excised and eluted with 0.1 *N* sodium hydroxide. The measurement of the absorbancy of the eluate exhibited the formation of I in almost quantitative yield.

2-Mercaptoinosine (I).—The sodium salt of I (10 g) was dissolved in 100 ml of water and the solution was then adjusted to pH 3 with dilute hydrochloric acid. The precipitate thus formed was filtered, washed adequately with cold water, and dried to yield 6.9 g (74%) of a pure sample.

The Ammonium Salt of 2-Mercaptoinosine (I).—Compound I (20 g) was dissolved in 100 ml of 1 *N* ammonium hydroxide and the solution was concentrated *in vacuo* to give crude crystals, which were recrystallized from water to afford 11.2 g (53%) of colorless crystals, mp 183–186°.

Anal. Calcd for $C_{10}H_{15}O_5N_5S \cdot {}^1/_2H_2O$: C, 36.81; H, 4.91; N, 21.47. Found: C, 36.95; H, 4.98; N, 21.09.

The Ammonium Salt of 2′,3′-O-isopropylidene-2-mercaptoinosine (XIII).—5-Amino-4-carbamoyl-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)imidazole (Ip-AICA-riboside)[9] (9 g, 3 mmoles) and 24 g (15 mmoles) of crystalline sodium methylxanthate were dissolved in 90 ml of hot methanol. The mixture was heated in an autoclave at 180° for 2 hr. After the solution was cooled to room temperature, aeration was continued until the evolution

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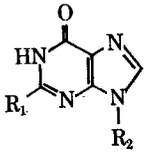
(40) All melting points are uncorrected. Ultraviolet absorption spectra were taken with a Hitachi Type EPS-2 automatic recording spectrophotometer and infrared absorption spectra were measured with Jasco Model IR-S spectrophotometer. The nmr spectra were measured with a Varian A-60 using tetramethylsilane as internal standard. Chemical shifts are given in τ values. The pK_a values were determined potentiometrically.

TABLE I



Compd	R_1	R_2	Mp, °C	R_f values in solvents[c] A	B	[α]D	λmax (ε), μ pH 1	pH 7	pH 13	Empirical formula	Calcd, % C	H	N	Found, % C	H	N
I	HS	Rf[a]	186–198 dec	0.50	0.16		231.5 (6,200) 294 (20,500)	226 sh[d] 296 (12,800)	234 sh 289 (12,200)	$C_{10}H_{12}O_5N_4S \cdot H_2O$	37.74	4.40	17.07	37.62	4.39	17.06
IV	HSO_3	Rf	148–153	0.30	0.03	$[\alpha]^{26}D$ −15.3° (c 1, H_2O)	255 (10,100) 280 sh	255 (10,000) 277 (6,500)	258 (8,900) 280 sh	$C_{10}H_{12}O_5N_4S \cdot NH_3 \cdot {}^1/_2H_2O$	32.09	4.28	18.72	32.07	4.58	18.79
XIII	HS	Ip-Rf[b]	241–243 dec	0.68	0.66	$[\alpha]^{21}D$ −52.2° (c 1, H_2O)	232 (10,400) 294 (22,700)	295 (17,900)	234 (13,700) 289 (17,700)	$C_{13}H_{16}O_5N_4S \cdot NH_3$	43.70	5.32	19.61	44.00	5.79	19.92
XIV	CH_3S	Ip-Rf	212–213	0.72	0.74	$[\alpha]^{23}D$ −22.6° (c 1, 0.1 N NaOH)	270 (13,900)	260 (8,700) 280 sh	273 (14,300)	$C_{14}H_{18}O_5N_4S$	47.45	5.12	15.81	47.71	5.32	15.59
XVI	CH_3NH	Ip-Rf	267–268	0.72	0.66	$[\alpha]^{23}D$ −40° (c 1, 0.1 N NaOH)	260 (16,300) 290 sh	255 (13,600) 282 sh	262 (18,500) 276 sh	$C_{14}H_{19}O_5N_5$	49.84	5.68	20.76	49.62	5.59	20.64
XVII	$(CH_3)_2N$	Ip-Rf	151–152 dec	0.76	0.72	$[\alpha]^{23}D$ −19.6° (c 1, 0.1 N NaOH)	267 (16,200) 295 sh	261 (14,700) 290 sh	266 (13,900) 285 sh	$C_{15}N_{21}O_5N_5 \cdot {}^1/_2H_2O$	49.99	6.17	19.43	50.41	6.34	19.40
XVIII	C_2H_5NH	Ip-Rf	258–259	0.84	0.74	$[\alpha]^{23}D$ −21.5° (c 1, 0.1 N NaOH)	263 (14,900) 287 sh	257 (12,000) 284 sh	264 (13,700) 280 sh	$C_{15}H_{21}O_5N_5$	51.28	5.98	19.94	51.17	6.09	19.69
XIX	n-C_3H_7NH	Ip-Rf	253	0.86	0.83	$[\alpha]^{23}D$ −12.5° (c 1, 0.1 N NaOH)	263 (14,400) 290 sh	257 (13,200) 287 sh	263 (12,400) 280 sh	$C_{16}H_{23}O_5N_5$	52.59	6.35	19.17	52.73	6.47	19.11
XX	n-C_4H_9NH	Ip-Rf	235	0.90	0.87	$[\alpha]^{21}D$ −16.6° (c 0.84, 0.1 N NaOH)	263 (14,500) 290 sh	256 (10,600) 290 sh	262 (15,800) 280 sh	$C_{17}H_{25}O_5N_5$	53.81	6.64	18.46	53.80	6.79	18.79
XXI		Ip-Rf	243	0.75	0.67	$[\alpha]^{23}D$ −12.7° (c 1, 0.1 NaOH)	268 (18,300)	263 (15,900) 285 sh	268 (14,600)	$C_{17}H_{23}O_5N_5$	51.90	5.89	17.80	52.24	6.07	17.67
XXII		Ip-Rf	266–267	0.91	0.83	$[\alpha]^{23}D$ +8.95° (c 1, 0.1 N NaOH)	263 (7,100) 293 sh	257 (13,700) 285 sh	263 (13,600) 280 sh	$C_{19}H_{27}O_5N_5 \cdot H_2O$	53.89	6.92	16.54	54.50	7.01	16.20
XXIII		Ip-Rf	196	0.85	0.87		270 (17,000)	268 (16,600)	268 (14,300)	$C_{18}H_{25}O_5N_5 \cdot {}^1/_2H_2O$	53.99	6.56	17.49	53.88	6.43	17.42

[a] Rf = β-D-ribofuranosyl. [b] Ip-Rf = 2′,3′-O-isopropylidene-β-D-ribofuranosyl. [c] All chromatographies were performed on Toyo filter paper no. 51A by the ascending technique. Solvent systems: A, n-propyl alcohol–ammonia (28%)–water, 20:12:3 v/v; B, n-butyl alcohol–acetic acid–water, 4:1:1 v/v; C, ethanol–water, 1:2 v/v. [d] Shoulder.

of hydrogen sulfide ceased (*ca.* 30 min). To this solution was added 150 ml of water and it was subjected to paper chromatography. A single spot was observed in solvent B. After the spot was excised and eluted with 0.1 *N* sodium hydroxide, the measurement of the absorbancy of the eluate showed that XIII had been formed in almost quantitative yield. The aqueous solution was brought to pH 3 by addition of about 60 ml of Amberlite IR-120 (H^+) with stirring. The resin was removed by filtration and washed with water. The filtrate and washings were combined and to this solution was added 150 ml of concentrated ammonium hydroxide. The yellow solution was treated with charcoal, filtered, and evaporated under reduced pressure to give the crude product, which was crystallized from water to afford the ammonium salt of XIII as yellow prisms: 4.5 g (42%); pK_{a1} 3.80, pK_{a2} 9.80.

Desulfurization of I with Raney Nickel.—To a solution of the ammonium salt of I (2 g) in 70 ml of water, Raney nickel[41] (3 g) was added with stirring. The solution was heated at 60° for 2 hr. After the catalyst was filtered off, the filtrate was evaporated to dryness *in vacuo*. The residue was crystallized from water to provide 0.8 g of pure product, which was identified by comparison with an authentic II.

2-Mercaptohypoxanthine (III). **A.**—A solution of I (5 g) in 100 ml of 1 *N* hydrochloric acid was refluxed for 1 hr. The resulting precipitate was collected by filtration, washed with water, and purified by reprecipitation from aqueous ammonium hydroxide with dilute acetic acid, yield 2.5 g (89.3%). This compound was identified with an authentic sample prepared by the method of Mizuno, *et al.*[15]

B.—A mixture of 3 g (18.6 mmoles) of 5-amino-4-imidazolecarboxamide (AICA) hydrochloride and 4.84 g (37.2 mmoles) of crystalline sodium methylxanthate in 180 ml of N,N-dimethylformamide was refluxed for 1 hr. In several minutes, it became clear and turned to dark green. The separated product was filtered and dissolved in 100 ml of 0.5 *N* sodium hydroxide. The solution was treated with charcoal, filtered, and acidified with glacial acetic acid. The light yellow precipitate was collected by filtration, washed with water, and dried to yield 2.1 g (64%) of pure product. This compound was proved to be identical with the sample described in A.

2-Methylthioinosine (V).—The sodium salt of I (10 g, 31 mmoles) was dissolved in 150 ml of water and to this solution was added 5.3 g (37.2 mmoles) of methyl iodide. The mixture was stirred vigorously at room temperature for 2 hr. The resulting precipitate was filtered and crystallized from water to afford 14.3 g (73.3%) of colorless needles. The physical properties of this compound were in good agreement with those reported by Davoll[4] and its nuclear magnetic resonance spectrum in pyridine indicated a singlet at τ 7.57 due to methyl group.

The Ammonium Salt of Inosine-2-sulfonic Acid (IV).—To a stirred solution of 5 g (15.8 mmoles) of the ammonium salt of I in 30 ml of water was added 18 g (158 mmoles) of 30% hydrogen peroxide dropwise at 5°. During the addition of hydrogen peroxide, it is necessary to keep the reaction temperature at 5° because a by-product, xanthosine (X), increases over 5°. After stirring was continued for 1 hr, 30 ml of ethanol was added and the mixture was allowed to stand overnight in the refrigerator. The resulting product was filtered and washed with water. Crystallization from 50% aqueous ethanol gave white crystals, yield 2.7 g (47%).

Guanosine (VII). **A.**—To a suspension of V (2 g, 6.25 mmoles) in 60 ml of water was added 2.54 g (18.8 mmoles) of N-chlorosuccinimide.[42] The mixture was warmed at 40° for 1 hr with stirring. Gradually the solution became clear. Paper chromatogram of this solution showed a single spot in solvent B. The spot was excised and eluted with 0.1 *N* sodium hydroxide and 0.1 *N* hydrochloric acid. The ultraviolet absorption spectra of the extracts showed $\lambda_{max}^{pH\ 13}$ 260 mμ and $\lambda_{max}^{pH\ 1}$ 256 mμ. As 2-methylsulfonylinosine (VI) could not be obtained in a crystalline state, subsequent amination was carried out. The above reaction mixture was saturated with ammonia at 0° and heated in an autoclave at 120° for 2 hr. After removal of the solvent under reduced pressure, the residue was dissolved in 200 ml of water. Then, about 80 ml of Amberlite IR-120 (H^+) ion-exchange resin was added portionwise and with stirring until the solution was free from ultraviolet absorbing material. After the resin was filtered and washed with water, the product absorbed on resin was eluted with 500 ml of 1 *N* ammonium hydroxide. Concentration of the eluate gave a crude product, which was recrystallized from water to give 1 g (56%) of a pure sample. The infrared absorption spectrum of this compound was identical with that of an authentic sample.

B.—The ammonium salt of IV (2 g) was added to 50 ml of ethanol saturated with ammonia at 0° and the mixture was heated in an autoclave at 120° for 2 hr. The reaction mixture was concentrated to dryness *in vacuo* and the residue was crystallized from water to give 1.4 g (90%) of pure product.

C.—To a stirred solution of the ammonium salt of I (5 g, 15.8 mmoles) in 25 ml of water cooled to 0° was added 5.37 g (47.4 mmoles) of 30% hydrogen peroxide dropwise over a period of 5 min. The reaction temperature should be maintained at 5° to avoid the formation of X. After 1 hr, the examination of this solution by paper chromatogram showed two spots. The major spot was that of IV and the other (minor) spot was identical with that of X. After being diluted with 35 ml of water, the reaction mixture was saturated with ammonia at 0° and amination was carried out as described above. The solution showed three spots on paper chromatogram. The major spot was that of VII and the minor two spots were proven to be those of guanine and X by spectral examination. A spot corresponding to VII was excised and eluted with 0.1 *N* sodium hydroxide. The measurement of the absorbancy of the eluate showed the formation of VII in 85% yield. The reaction mixture was concentrated *in vacuo* to precipitate a crude product, which was recrystallized from water to give 3.4 g (75%) of pure sample.

D.—To a solution of sodium hydroxide (7.76 g, 194 mmoles) in 70 ml of methanol was added AICA-riboside (10 g, 38.8 mmoles) on slight warming and then carbon disulfide (14.7 g, 194 mmoles) was added. The mixture was heated in an autoclave at 180° for 3 hr. The solvent was removed *in vacuo*, the residue was dissolved in 120 ml of water, and 22 g (194 mmoles) of 30% hydrogen peroxide was added at 5° with stirring. After 1 hr, the solution was saturated with ammonia at 0° and heated in an autoclave at 120° for 2 hr. Paper chromatography showed three spots (R_f 0.50, 0.62, and 0.76; system C) which, on elution, corresponded spectrophotometrically to guanine (trace), XII, and X. The yields of VII and X were 79 and 5%, respectively (paper chromatographically determined). The reaction mixture was concentrated *in vacuo* to precipitate a crude product. Recrystallization from water with charcoal afforded 7.7 g (70%) of pure material, which showed a single spot on paper and thin layer chromatograms. This procedure was found to be suitable for a large-scale preparation of VII. The average yield of VII isolated was 68–70%.

N^2-Methylguanosine (VIII).—To a solution of 10 g (31.6 mmoles) of the ammonium salt of I in 100 ml of water was added 10.4 g (94.8 mmoles) of 30% hydrogen peroxide at 5° with stirring. After 1 hr, 180 ml of 30% aqueous methylamine was added and the mixture was heated in an autoclave at 120° for 2 hr. Concentration of the solution under reduced pressure gave the crude crystals, which were recrystallized from water to afford 6.2 g (62.4%) of analytically pure, light yellow needles, mp 233° dec. The nuclear magnetic resonance spectrum in deuterium oxide indicated a singlet at τ 7.0 due to the methylamino group. The other physical properties were identical with those reported by Dunn, *et al.*,[32] and by Robins, *et al.*[36]

Anal. Calcd for $C_{11}H_{15}O_5N_5 \cdot H_2O$: C, 41.90; H, 5.39; N, 22.24. Found: C, 41.97; H, 5.39; N, 22.44.

N^2,N^2-Dimethylguanosine (IX). **A.**—To a cooled solution of the ammonium salt of I (10 g, 31.6 mmoles) in 100 ml of water, 30% hydrogen peroxide (10.4 g, 94.8 mmoles) was added with stirring. Then the mixture was worked up in the same manner as described for VIII using 30% aqueous dimethylamine as aminating agent. After crystallization from water, 5.1 g (52%) of colorless crystals were obtained. The nuclear magnetic resonance spectrum in deuterium oxide showed a singlet at τ 6.76 due to the dimethylamino group. The other physical properties of this compound were identical with those previously reported.[32,36]

B.—Compound V (1 g, 3.18 mmoles) was treated with 1.28 g (9.55 mmoles) of N-chlorosuccinimide as described for the preparation of VII. After oxidation, the reaction mixture was neutralized with aqueous dimethylamine and concentrated to dryness *in vacuo*. The residue was dissolved in 30 ml of 30% aqueous dimethylamine and heated in an autoclave at 120° for 2 hr. Concentration of the solution gave a semisolid, which was diluted with a small amount of water and allowed to stand at 5° over-

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night. The resulting crude product was filtered and recrystallized from water to yield 0.49 g (49.6%) of a pure sample, which was identical with that prepared by method A in all respects.

Anal. Calcd for $C_{12}H_{17}O_5N_5$: C, 46.30; H, 5.50; N, 22.50. Found: C, 45.61; H, 5.57; N, 22.50.

Xanthosine (X).—A solution of the ammonium salt of I (5 g, 15.8 mmoles) in 30 ml of water was adjusted to pH 9.5 with concentrated ammonium hydroxide. To this solution was added 18 g (158 mmoles) of 30% hydrogen peroxide with stirring at room temperature. After 2 hr, 100 ml of 1 *N* sodium hydroxide was added and the mixture was refluxed for 1 hr. Th pH of the solution was adjusted to 4 with Amberlite IR-120 (H^+). The resin was filtered off and the filtrate was evaporated to about 10 ml *in vacuo*. The mixture was allowed to stand in the refrigerator overnight. The precipitate that formed was collected by filtration and recrystallized from water to give a pure sample, which was identified with an authentic sample of X, yield 2.1 g (46.8%).

Bis(6-hydroxy-9-β-D-ribofuranosyl-2-purinyl)disulfide (XI).—A solution of the ammonium salt of I (5 g, 15.8 mmoles) in 50 ml of water containing 1 g (16.7 mmoles) of glacial acetic acid was cooled at 5°. To this stirred solution was added 1.8 g (15.8 mmoles) of 30% hydrogen peroxide dropwise. In a few minutes, white crystals precipitated. After stirring was continued for 2 hr, the precipitate was collected by filtration and washed with water. Purification of this compound was accomplished by reprecipitation from aqueous ammonium hydroxide with dilute acetic acid: yield 1.4 g (53%); mp 188–196° dec; $[\alpha]^{27}D$ −35.8° (*c* 1, 0.1 *N* sodium hydroxide); paper chromatography, R_f 0.46 (solvent A) and 0.10 (solvent B); ultraviolet absorption properties, $\lambda_{max}^{pH\ 1}$ 259 mμ (ε 26,600) and 280 mμ sh, $\lambda_{max}^{pH\ 7}$ 261 mμ (ε 32,800) and $\lambda_{max}^{pH\ 13}$ 266 mμ (ε 31,800).

Anal. Calcd for $C_{20}H_{22}O_{10}N_8S_2$: C, 40.14; H, 3.68; N, 18.72. Found: C, 39.66; H, 3.92; N, 18.46.

2′,3′-O-Isopropylidene-2-methylthioinosine (XIV).—The ammonium salt of XIII (15 g, 42 mmoles) was dissolved in 130 ml of water on warming. To this solution was added 7.7 g (54 mmoles) of methyl iodide portionwise and the mixture was shaken vigorously at room temperature for 2 hr. The resulting light yellow crystals were filtered, washed with cold water, and recrystallized from ethanol to provide 10 g (67.5%) of a pure product.

2′-3′-O-Isopropylideneguanosine (XV).—The ammonium salt of XIII (5 g, 14 mmoles) was dissolved in 40 ml of water on slight warming and the solution was cooled to 5° with stirring. To this solution, 4.8 g (42 mmoles) of 30% hydrogen peroxide was added portionwise at 5° over a 10-min period. Stirring was continued for 1 hr to afford a colorless solution. An aliquot from the solution showed two spots in solvent B. The major spot with R_f 0.25 was found to be a sulfonic acid derivative by ultraviolet absorption spectral comparison with IV. The minor spot with R_f 0.35 was excised and eluted with acidic and basic solutions. Ultraviolet absorption spectra of the eluates showed $\lambda_{max}^{pH\ 1}$ 255 mμ and $\lambda_{max}^{pH\ 13}$ 257 mμ. This substance seemed to be sulfinic acid derivative, because its spot disappeared and only a single spot (R_f 0.25) was detected when a 10 *M* excess of hydrogen peroxide was employed in the oxidation. The above mixture was diluted with 20 ml of water and saturated with ammonia at 0° and heated in an autoclave at 120° for 2 hr. Paper chromatographic examination indicated two spots of XV and a trace of 2′,3′-O-isopropylidenexanthosine. No formation of guanine was observed. Concentration of the solution gave a crystalline product. Recrystallization from water with charcoal afforded a pure sample as a sole product, yield 3.4 g (76%). This compound was identical with an authentic sample[43] by comparison of ultraviolet and infrared absorption spectra. When Ip-AICA-riboside was treated successively as described above on a large scale, XV was isolated in 70–72% yield as a sole product.

2′,3′-O-Isopropylidene-N^2-methylguanosine (XVI).—To a stirred solution of the ammonium salt of XIII (10 g, 28 mmoles) in 100 ml of water was added 30% hydrogen peroxide (9.5 g, 84 mmoles) at 5° dropwise and with stirring. After 1 hr, 150 ml of 30% aqueous methylamine was added and the mixture was heated in an autoclave at 120° for 2 hr. Evaporation of the solvent gave a crude product, which was crystallized from a small amount of dilute ammonium hydroxide and water, yield 6.3 g (66.7%).

2′,3′-O-Isopropylidene-N^2,N^2-dimethylguanosine (XVII).—The ammonium salt of XIII (10 g) was oxidized with 3 equiv of hydrogen peroxide and then treated with 150 ml of 30% aqueous dimethylamine in a manner similar to that described for XVI to yield a crude product. An analytically pure sample was obtained by recrystallization from water, yield 5.8 g (59%).

The following compounds were obtained from 3 g of the ammonium salt of XIII by the same procedure as described for XVI.

2′,3′-O-Isopropylidene-N^2-ethylguanosine (XVIII).—A pure sample was obtained by crystallization from water, yield 1.44 g (49%).

2′,3′-O-Isopropylidene-N^2-(*n*-propyl)guanosine (XIX).—A crude product was recrystallized from water to give 1.7 g (56%) of pure sample.

2′,3′-O-Isopropylidene-N^2-(*n*-butyl)guanosine (XX).—An analytically pure sample was obtained by recrystallization from water, yield 1.2 g (38%).

2′,3′-O-Isopropylidene-2-morpholinoinosine (XXI).—After the ammonium salt of XIII (3 g) was treated with hydrogen peroxide, 50 ml of morpholine was added and the mixture was refluxed for 2 hr. Concentration of the solution *in vacuo* afforded a gummy substance, which was triturated with water, collected by filtration, and crystallized from water to give 1.2 g (40%) of a pure sample.

2′,3′-O-Isopropylidene-2-cyclohexylaminoinosine (XXII).—A solution of the ammonium salt of XIII (4 g, 11.2 mmoles) in 40 ml of water was cooled to 0° and then 30% hydrogen peroxide (3.9 g, 34.6 mmoles) was added dropwise at such a rate that the reaction temperature was maintained at 5°. After being stirred for 1 hr, 40 g of cyclohexylamine was added and the mixture was refluxed for 2 hr. Concentration of the solution *in vacuo* gave a gummy product, which showed a single spot on paper chromatogram. This product was chromatographed on alumina and eluted with ethanol. Ultraviolet absorbing fractions were evaporated to dryness and the residue was crystallized from aqueous ethanol to give 1.2 g (25%) of a pure sample.

2′,3′-O-Isopropylidene-2-piperidinoinosine (XXIII).—This compound was prepared by the same procedure as described for XXII. The ammonium salt of XIII (4 g) was oxidized and then allowed to react with 50 g of piperidine. After the solvent was removed by concentration, the residue was purified by alumina chromatography. A gummy residue obtained from ultraviolet absorbing fractions was crystallized from water to give 0.7 g (21%) of product.

Registry No.—I, 13428-23-4; I sodium salt, 13389-80-5; I ammonium salt, 13391-78-1; IV, 13391-49-6; VII, 85-30-3; VIII, 2140-77-4; IX, 2140-67-2; XI, 13421-60-8; XIII, 13428-07-4; XIII ammonium salt, 13391-52-1; XIV, 5356-65-0; XV, 362-76-5; XVI, 5391-07-1; XVII, 5391-12-8; XVIII, 5391-08-2; XIX, 5391-09-3; XX, 5391-10-6; XXI, 5566-97-2; XXII, 5391-11-7; XXII, 5391-13-9; AICA-riboside, 2627-69-2 sodium methylxanthate, 6370-03-2.

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