period of 40 min. The resulting solution (pH 8) was neutralized with acetic acid to ~pH 6 and the methanol was removed by evaporation *in vacuo* to give long light yellow needles. Three crops yielded 8.3 g. (87%) of IX. Recrystallization from hot water gave a pure, colorless material: m.p. 140–141°; $\lambda_{max}^{50\% EtOH}$ 202.5, 277.5, 315 mμ; minima at 237.5 and 292.5 mμ; shoulder at 220 mμ; ratio at 277.5/315 mμ = 0.79. The absence of acetyl groups in IX was verified by the absence of acetoxy resonances in the n.m.r. spectrum[23]; $[\alpha]^{23}D$ +219° (*c* 0.22, methanol).

Anal. Calcd. for $C_{10}H_{13}FN_2O_5S$: C, 41.08; H, 4.48; F, 6.50; N, 9.58; S, 10.97. Found: C, 40.99; H, 4.76; F, 6.82; N, 9.70; S, 10.82.

1-β-D-Arabinofuranosyl-5-fluorocytosine (FCA, X).—Compound IX (5.0 g., 0.017 mole) was left at room temperature in 25 ml. of anhydrous ammonia overnight. Ammonia was removed *in vacuo*. About 50 ml. of water was added and the residual ammonia was neutralized to ~pH 5 with acetic acid. The solution was evaporated to a crystalline residue, and 10 ml. of water was added to give X (3.4 g. in two crops), m.p. 232–233° dec. (effervescent with previous browning at 210°). X was purified on a column of Dowex 50 (H^+) 100–200 mesh, by first washing with water until free of ultraviolet-absorbing material, then eluting with 1 *N* NH_4OH. The ammonia eluates containing ultraviolet-absorbing material were combined and evaporated to a crystalline residue, which was recrystallized in 90% ethanol. Two crops of colorless needles yielded 3.2 g., m.p. 237–238° (to a brown liquid with previous browning at 225°), $[\alpha]^{23}D$ +163 ± 2 (*c* 0.18, in water). FCA was examined in thin layer chromatography using Brinkmann GF 254 silica gel and was found to be free from FUA, 5-fluorocytosine, and all other pyrimidine derivatives listed in Table I in two chromatographic systems.

Ultraviolet absorption properties of FCA are: in 1 *N* HCl, maxima at 221 and 290.5 mμ (ε 10,300 and 11,900), minimum at 246 mμ (ε 1160); at pH 5–7, maxima at 235 and 280 mμ (ε 7860 and 8240), minima at 225 and 257.5 mμ (ε 7550 and 5240); pK_a (spectrophotometrically determined)[24] = 2.33 ± 0.05.

Anal. Calcd. for $C_9H_{12}FN_3O_5$: C, 41.38; H, 4.63; F, 7.27; N, 16.08. Found: C, 41.53; H, 4.62; F, 7.32; N, 16.05.

(23) The authors are indebted to Dr. Floie Vane of Hoffmann-La Roche, Inc., Nutley, N. J., for the n.m.r. spectrum.

(24) J. J. Fox and D. Shugar, *Bull. soc. chim. Belges*, **64**, 44 (1952); D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).

TABLE I

THIN LAYER CHROMATOGRAPHY DATA

Compd.	R_f in solvent system[a] A	B
FCA (X)	0.06	0.11
Cytosine	0	0
5-Fluorocytosine	0.22	0.09
CA	0	0
5-Fluorocytidine	0.03	0
5-Fluorouracil	0.42	0.74
FUA (V)	0.31	0.69
5-Fluorouridine	0.21	0.04

[a] Solvent system A, ethyl acetate–acetic acid (22:3); B, 5% boric acid in ethanol–ethyl acetate (1:3). The authors are indebted to Dr. R. Duschinsky of Hoffmann-La Roche, Inc., for these solvent systems.

1-β-D-Arabinofuranosyl-4-thio-5-fluorouracil Disulfide (VIII).—Crude VII (300 mg.) was allowed to stand overnight at room temperature in 20 ml. of methanol saturated with HCl. The solution was evaporated to dryness and azeotroped repeatedly with ethanol. The syrup did not crystallize. It was treated with 20 ml. of phosphate buffer (pH 6.8). Iodine solution (1 *N*, 1.3 ml.) was added dropwise to pH 4. Immediately a white amorphous material precipitated. The solid was collected and washed with water, ethanol, and ether (yield 120 mg., m.p. 199–203° dec.). Recrystallization from 50% ethanol gave minute crystals: 50 mg.; m.p. 213–214° (to amber liquid with effervescence, with previous browning at 210°); $\lambda_{max}^{50\% EtOH}$ 215, 260, 327.5 mμ; minima at 205, 234, 288 mμ; ratio at 260/327.5 mμ = 0.66.

Anal. Calcd. for $C_{18}H_{20}F_2N_4O_{10}S_2$: C, 39.13; H, 3.65; N, 10.14; S, 11.24. Found: C, 38.93; H, 3.98; N, 9.98; S, 11.32.

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3-Deaza-6-methylthiopurine Ribonucleoside[1]

JOHN A. MONTGOMERY AND KATHLEEN HEWSON

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama

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The synthesis and biologic evaluation of 4-methylthio-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine, the 3-deaza analog of 6-methylthiopurine ribonucleoside, a highly cytotoxic purine nucleoside, is described.

6-Mercaptopurine [purine-6(1*H*)-thione, I] is converted *in vivo* by inosine monophosphate–guanosine monophosphate (IMP–GMP) pyrophosphorylase to its ribonucleotide, thioinosinic acid (III),[2] a potent allosteric inhibitor of phosphoribosyl pyrophosphate (PRPP)–glutamine amidotransferase.[3] Furthermore, there is good evidence that inhibition of this enzyme is responsible for the cytotoxicity of 6-mercaptopurine.[4]

In an effort to learn more about the binding sites of all the enzymes involved in this process of growth inhibition by 6-mercaptopurine, we first synthesized its four deaza analogs.[5] None of these compounds are cytotoxic except at high levels. This lack of biologic activity could be due to the fact that the possible deaza analogs of thioinosinic acid do not bind to the allosteric site of phosphoribosyl pyrophosphate–glutamine amidotransferase, but it is equally likely that the deazapurines simply are not metabolized to ribonucleotides by cells.

Recently we have found that, although 6-mercaptopurine ribonucleoside is not a substrate for cellular purine kinase (presumably adenosine kinase), 6-methylthiopurine ribonucleoside (II) is, and is readily converted *in vivo* to 6-methylthiopurine ribonucleotide (IV) (see Chart I). As a consequence II is highly

(1) This work was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH-43-64-51.

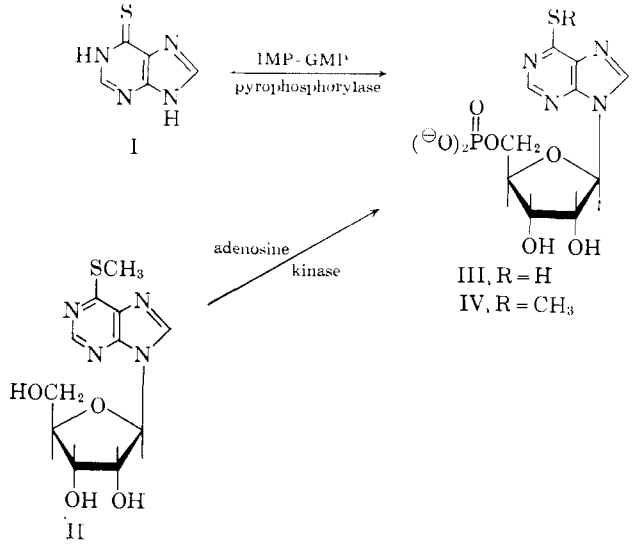
(2) For a review of this subject see J. A. Montgomery, *Progr. Drug Res.*, **8**, 433 (1965).

(3) R. J. McCollister, W. R. Gilbert, Jr., D. M. Asleton, and J. B. Wyngaarden, *J. Biol. Chem.*, **239**, 1560 (1964).

(4) L. L. Bennett, L. Simpson, J. Golden, and T. L. Barker, *Cancer Res.*, **23**, 1574 (1963).

(5) J. A. Montgomery and K. Hewson, *J. Med. Chem.*, **8**, 708 (1965).

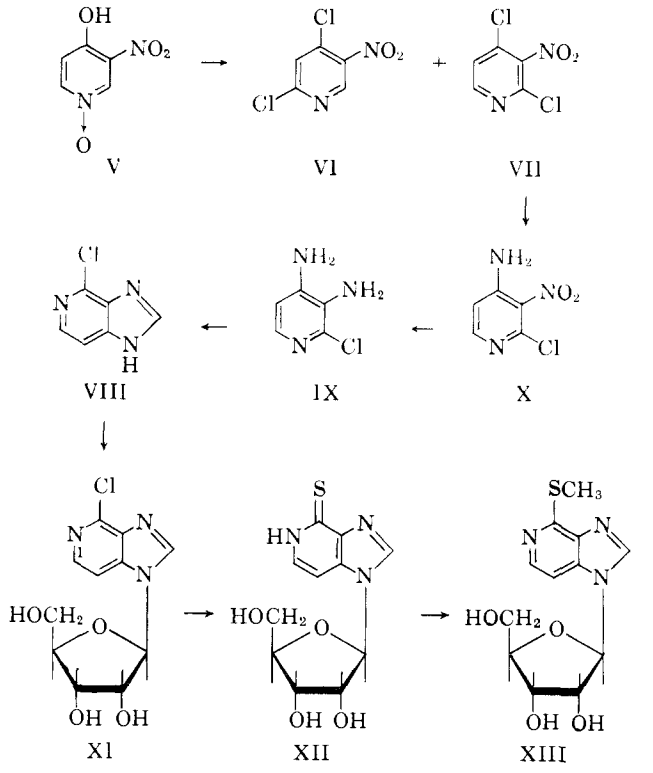
CHART I



inhibitory to cell lines resistant to 6-mercaptopurine (I).[6]

We now wish to report the synthesis of 3-deaza-6-methylthiopurine ribonucleoside (4-methylthio-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine, XIII) (see Chart II). 2,4-Dichloro-3-nitropyridine (VII), along

CHART II



with 4,6-dichloro-3-nitropyridine (VI), was prepared directly from 4-hydroxy-3-nitropyridine *N*-oxide (V) by a modification of the method of Mizuno, *et al.*[7] It was possible by the use of mild conditions to obtain a monoamination product from the reaction of VII with alcoholic ammonia. This compound, shown by subsequent conversions to known compounds to be 4-amino-2-chloro-3-nitropyridine (X),[8] was catalytically reduced to 3,4-diamino-2-chloropyridine (IX). Treatment of IX with diethoxymethyl acetate gave 3-deaza-6-chloropurine (4-chloro-1*H*-imidazo[4,5-*c*]pyridine, VIII)[7,9] in good yield. Fusion[10] of VIII with tetra-*O*-acetylribofuranose gave the desired nucleoside, 4-chloro-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine (XI).[11]

The chlorine of XI was found to be less reactive than that of 6-chloropurine ribonucleoside, since XI failed to react with sodium hydrosulfide in boiling methanol, conditions under which 6-chloropurine ribonucleoside was readily converted to 6-mercaptopurine ribonucleoside.[12] Reaction of XI with sodium hydrosulfide in methanol took place at 90° in 18 hr. in a pressure vessel, and a 48% conversion to the 3-deaza-6-mercaptopurine ribonucleoside [1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine-4(5*H*)-thione, (XII)] was obtained. Methylation of XII with methyl iodide gave the nucleoside XIII.

Biologic Activity.—Table I shows a comparison of the cytotoxicity of 3-deaza-6-methylthiopurine ribonucleoside (XIII) with that of 6-methylthiopurine ribonucleoside (II) and 6-mercaptopurine (I) in HEp-2 cell lines sensitive and resistant to 6-mercaptopurine. It is apparent that 6-methylthiopurine ribonucleoside (II) is 1000 times as inhibitory as its 3-deaza analog XIII. The 3-nitrogen of the ribonucleoside is obviously necessary for binding either to adenosine kinase or to the allosteric site of PRPP–glutamine amidotransferase. Experiments in progress should tell which enzyme is not interacting with the deaza analog.

TABLE I

Compound	ED_{50}, μM[a] HEp-2/S	HEp/MP
6-Mercaptopurine	1.4	1760
6-Methylthiopurine ribonucleoside	0.34	0.24
3-Deaza-6-methylthiopurine ribonucleoside	>340	>340

[a] ED_{50} is that concentration of compound inhibiting cell growth to 50% of controls. Cells were grown on glass and growth was measured by the determination of protein content [V. I. Oyama and H. Eagle, *Proc. Soc. Exptl. Biol. Med.*, **91**, 305 (1956)] after 4 days growth in the presence of the compound.

Experimental Section

The melting points reported were determined on a Kofler hot stage and are corrected. The ultraviolet spectra were determined in aqueous solution with a Cary Model 14 spectrophotometer, and the infrared spectra were determined in pressed KBr disks with a Perkin-Elmer Model 521 spectrophotometer. The optical rotations were determined in methanol solution with a Rudolph Model 80 polarimeter.

2,4-Dichloro-3-nitropyridine (VII).[7,9]—A solution of 3-nitro-4-hydroxypyridine *N*-oxide[13] (22.4 g., 144 mmoles) in $POCl_3$

(6) L. L. Bennett, R. W. Brockman, H. P. Schnebli, S. Chumley, G. J. Dixon, F. M. Schabel, Jr., E. A. Dulmage, H. E. Skipper, J. A. Montgomery, and H. J. Thomas, *Nature*, **205**, 1276 (1965).

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(220 ml.) was heated at 80° for 3 hr. The solution was concentrated *in vacuo* and 50 ml. of $POCl_3$ was added to the residue. This solution was refluxed for 24 hr. After concentration *in vacuo* the solution was poured into ice water (800 ml.) and the aqueous mixture was extracted with chloroform. The $CHCl_3$ extract was washed with water, dried ($MgSO_4$), and then evaporated to dryness *in vacuo*. The residue was dissolved in ether and the ether solution was filtered before concentration. The crystals that deposited were removed by filtration, washed with ether, and dried; yield of VII, 2.4 g., m.p. 57° (lit.[7,9] m.p. 61–62°). A thin layer chromatogram on silica gel H (Merck) using benzene–chloroform (2:1) as the eluent showed only one spot. The ether filtrate from the procedure described above was evaporated to dryness and the residue was distilled *in vacuo;* yield of a mixture of VI and VII, 9.3 g., b.p. 102–105° (2.5 mm.). Fractional crystallization of this mixture from petroleum ether (b.p. 30–60°) gave 3.5 g. of VII, m.p. 55–61°. Recrystallization of the combined crops of VII gave 5.2 g. (19%) of pure material: m.p. 59–61°; λ_{max}, mμ ($\epsilon \times 10^{-3}$), at pH 1, 7, and 13—264 (1.94) and 274 (sh) (1.90); $\bar{\nu}_{max}$, cm.$^{-1}$, 3080 and 2900 (CH), 1560 (sh) and 1545 (C=C, C=N), 850 and 825 (pyridine ring).

4-Chloro-1*H*-imidazo[4,5-*c*]pyridine (VIII).[7,9]—A solution of 4-amino-2-chloro-3-nitropyridine (X, 780 mg., 4.5 mmoles) in ethanol (100 ml.) was hydrogenated at 1 atm. pressure in the presence of PtO_2 catalyst (75 mg.). After the theoretical amount of hydrogen had been consumed, the catalyst was removed by filtration in a nitrogen atmosphere and the filtrate was evaporated to dryness *in vacuo*. The resulting diaminopyridine was dissolved in diethoxymethyl acetate (10 ml.), and the solution was allowed to stand at room temperature for 4 hr. before it was evaporated to dryness. Ethanol solutions of the residue were evaporated to dryness several times before the solid was dissolved in ether. The solid that precipitated from the ether solution was collected in several crops to give a crude yield of 515 mg. Recrystallization of the crude product from boiling water with Norit treatment gave chromatographically pure material (390 mg., 56%), m.p. 264° with sublimation above 200°. Thin layer chromatography on silica gel H (Merck) using chloroform–ethyl acetate (1:1) as the eluent showed a single spot; λ_{max}, mμ ($\epsilon \times 10^{-3}$), at pH 1—234 (sh), 240 (3.3), 248 (sh), 268 (6.1), 274 (sh), at pH 7—244 (sh), 250 (4.9), 258 (sh), 265 (6.0), 273 (5.1), 283 (sh), and at pH 13—272 (sh), 278 (5.7), 285 (sh); $\bar{\nu}_{max}$, cm.$^{-1}$, 3070, 3000, 2960, and 2800 (CH), 2700–2500 (acidic H), 1615 and 1575 (C=C, C=N). The ultraviolet data are in agreement with the literature values.[7]

4-Amino-2-chloro-3-nitropyridine (X).[8]—A solution of 2,4-dichloro-3-nitropyridine (VII, 5 g., 25.9 mmoles) in ethanolic NH_3 (150 ml. of absolute ethanol saturated at 5° with dry NH_3) was allowed to stand at room temperature for 7 hr. before it was refrigerated overnight. The reaction solution was evaporated to dryness *in vacuo*, and the residue was triturated with boiling chloroform (three 30-ml. portions). The resulting insoluble solid was dried *in vacuo* to give 2.38 g. (53%) of impure X. The crystallization of this material from ethyl acetate (50 ml.) gave essentially pure product suitable for use as an intermediate: yield 1.86 g. (41%), m.p. 214–215°. Thin layer chromatography on silica gel H (Merck) using chloroform–ethyl acetate (1:1) showed the presence of only traces of 2,4-diamino-3-nitropyridine and 2-amino-4-chloro-3-nitropyridine as the only contaminants. An analytical sample of X was isolated by means of preparative thin layer chromatography; m.p. 215–216° (lit.[8] m.p. 205–207°); λ_{max}, mμ ($\epsilon \times 10^{-3}$), at pH 1—239 (14.0), 260 (sh), 340 (1.7), at pH 7—239 (14.3), 278 (1.7), 362 (2.0), and at pH 13—238 (14.4), 278 (1.7), 362 (2.0); $\bar{\nu}_{max}$, cm.$^{-1}$, 3440, 3300, and 3150 (NH, CH), 1635, 1615, 1600, and 1525 (NH, C=C, C=N).

4-Chloro-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine (XI).[11]—A mixture of 4-chloro-1*H*-imidazo[4,5-*c*]pyridine (VIII, 154 mg., 1 mmole), 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (636 mg., 2 mmoles), and *p*-toluenesulfonic acid catalyst was fused at 160° (25 mm.) for 30 min. The reaction melt was dissolved in $CHCl_3$ (15 ml.), and the solution was washed with $NaHCO_3$ solution and water before it was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The resulting residue was triturated with ether and the insoluble solid that formed was collected by filtration and dried *in vacuo;* yield 166 mg. (40%), m.p. 150°. Thin layer chromatography on silica gel H (Merck) using chloroform–ethyl acetate (1:1) as the eluent showed one spot; λ_{max}, mμ ($\epsilon \times 10^{-3}$), at pH 1—255 (6.1), 264 (5.8), 273 (5.2), 283 (sh), at pH 7—254 (6.5), 264 (5.7), 273 (4.4), 285 (sh), and at pH 13—257 (6.4), 266 (6.1), 273 (4.9), 285 (sh); $\bar{\nu}_{max}$, cm.$^{-1}$, 3150 and 2980–2920 (CH), 1770, 1755, and 1750 (C=O), 1605 and 1565 (C=C, C=N), 1235, 1220, and 1210 (COC).

The chromatographically pure acetylated ribonucleoside (250 mg., 0.6 mmole) was dissolved in anhydrous ethanolic NH_3 (30 ml.) and the solution was refrigerated for 48 hr. The solution was evaporated to dryness, the residue was dissolved in water, and the solution was extracted with chloroform. Evaporation of the water solution to dryness gave chromatographically pure material; yield 170 mg. (98%), m.p. 188–190° (lit.[11] m.p. 189–190°), $[\alpha]^{19.5}D$ −38.7 ± 0.2 (*c* 1.25, methanol) [lit.[11] $[\alpha]^{19.5}D$ −41.6 (*c* 1.25, methanol)]. Thin layer chromatography on silica gel H (Merck) using chloroform–methanol (9:1) showed one spot which gave a positive Schiff metaperiodate test; λ_{max}, mμ ($\epsilon \times 10^{-3}$), at pH 1—207 (34.0), 257 (5.2), 265 (5.5), 273 (5.3), at pH 7—255 (6.3), 264.5 (5.7), 273 (4.5), and at pH 13—257 (6.4), 265 (6.0), 273 (4.7); $\bar{\nu}_{max}$, cm.$^{-1}$, 3340–3300, 3250–3220, 3120, 2940, 2910, and 2860 (OH, CH), 1610 and 1565 (C=C, C=N), 1120, 1100, 1085, and 1070 (COC).

1-β-D-Ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine-4(5*H*)-thione (XII).—To a solution of 4-chloro-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine (XI, 275 mg., 0.96 mmole) in absolute methanol (8 ml.) was added a solution of 1 *N* sodium methoxide saturated at 5° with dry H_2S (2 ml.). The reaction solution was heated (90°) in a glass-lined bomb for 18 hr. before it was evaporated to dryness *in vacuo*. The residue was dissolved in water (5 ml.), and the solution was acidified with glacial acetic acid and filtered to remove precipitated sulfur. The filtrate was evaporated to dryness, the residue was triturated with water (1 ml.), and the insoluble solid was collected by filtration, washed with water (two 0.5-ml. portions), and dried *in vacuo* to give the crude product (185 mg.), which was recrystallized from 75% aqueous ethanol (15 ml.) with Norit treatment. The pure material was collected in two crops and dried *in vacuo;* yield 144 mg. (48%), m.p. 155–160°. Thin layer chromatography on silica gel H (Merck) using chloroform–methanol (3:1) as the eluent showed a single spot which gave a positive Schiff metaperiodate test; λ_{max}, mμ ($\epsilon \times 10^{-3}$), at pH 1—224 (11.8), 290 (sh), 332 (13.5), at pH 7—226 (12.3), 295 (sh), 324 (15.5), and at pH 13—226.5 (12.0), 302 (15.1); $\bar{\nu}_{max}$, cm.$^{-1}$, 3300, 3080, 3020, 2940, and 2900 (OH, CH), 1605, 1585, and 1535 (C=C, C=N), 1125, 1090, 1070, and 1050 (COC).

Anal. Calcd. for $C_{11}H_{13}N_3O_4S \cdot 1.75H_2O$: C, 42.01; H, 5.29; N, 13.36. Found: C, 42.12; H, 4.91; N, 13.54.

4-Methylthio-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine (XIII).—A solution of 1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]-pyridine-4(5*H*)-thione (XII, 149 mg., 0.47 mmole) in water (1 ml.) containing 1 *N* NaOH (0.47 ml.) was vigorously stirred during the dropwise addition of methyl iodide (0.18 ml., 1.9 mmoles). The reaction mixture was stoppered and allowed to stir at room temperature for 3 hr. (pH 5–6) before it was evaporated to dryness *in vacuo*. The residue was dissolved in 50% aqueous ethanol, the solution was decolorized with Norit and filtered, and the filtrate was evaporated to dryness *in vacuo*. The residue was triturated with ethyl acetate (20 ml.). The crystals that formed were collected in two crops and dried *in vacuo*. Recrystallization of the crude product from water (5 ml.) gave the pure material, yield 90 mg. (65%), m.p. 195°, $[\alpha]^{25}D$ −41.3 ± 0.2 (*c* 1.00, methanol). Thin layer chromatography on silica gel H (Merck) using chloroform–methanol (3:1) as eluent showed a single spot which gave a positive Schiff metaperiodate test; λ_{max}, mμ ($\epsilon \times 10^{-3}$), at pH 1—222 (sh), 235 (sh), 275 (sh), 300 (sh), 307 (16.9), at pH 7—216 (16.8), 284 (13.5), 291 (sh), and at pH 13—284 (13.7), 291 (sh); $\bar{\nu}_{max}$, cm.$^{-1}$, 3275, 3125, and 2940 (OH, CH), 1595 and 1575 (C=C, C=N), 1135, 1120, and 1060 (COC).

Anal. Calcd. for $C_{12}H_{15}N_3O_4S$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.08; H, 5.07; N, 13.98.

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