A Simplified Procedure for the Synthesis of 6-Methylthiopurine Ribonucleoside 5′-Phosphates

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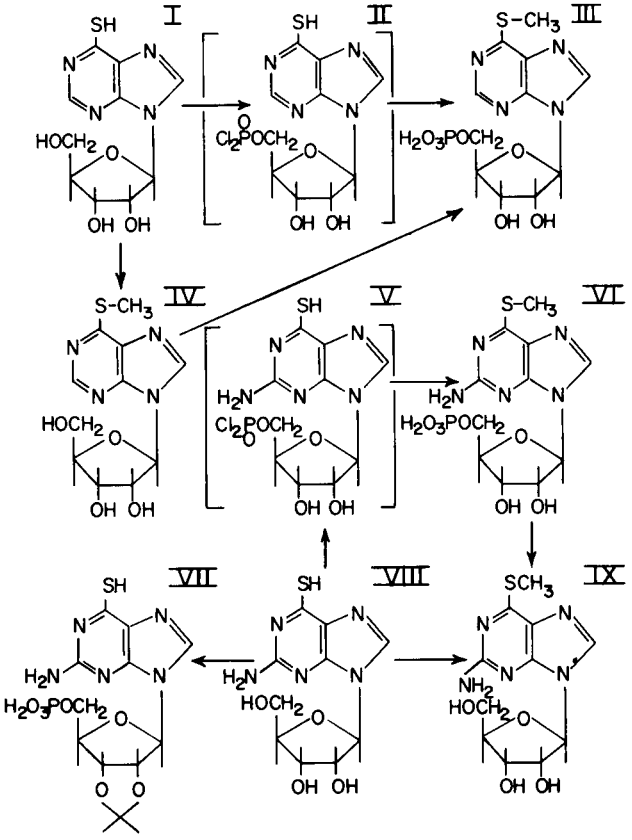
6-Methylthiopurine 5′-nucleotides can be anticipated to be useful intermediates for synthesis of a variety of 6-substituted purine 5′-nucleotides in view of the readiness with which 6-methylthiopurine ribonucleoside, IV (1,2,3), and its 2-amino derivative, IX (4), undergo nucleophilic attack at C-6 to produce 6-substituted purine nucleosides, and because of the relative inertness of the 5′ phosphate ester linkage toward nucleophilic reagents. This communication reports that 6-methylthiopurine ribonucleoside 5′-phosphate, III, and its 2-amino derivative, VI, can be easily synthesized directly from 6-mercaptopurine nucleoside, I, and 2-amino-6-mercaptopurine nucleoside, VIII, respectively. The 6-methylthiopurine nucleotide, III, is currently of chemotherapeutic interest as an intracellular metabolite of the antileukemic drug 6-mercaptopurine (5) and has been previously synthesized (6) by alkylation of 6-mercaptopurine 5′-ribonucleotide which in turn has been prepared from 6-mercaptopurine ribonucleoside by successive protection of the 2′ and 3′ hydroxyls, phosphorylation, and removal of sugar and phosphate blocking groups (7,8). Synthesis of the nucleotide VI has not hitherto been reported.

Application to 6-mercaptopurine ribonucleoside, I, of the phosphorus oxychloride-trimethyl phosphate procedure of Yoshikawa (9) for selective phosphorylation of a ribonucleoside 5′-hydroxyl group, followed by hydrolysis of the nucleoside phosphorodichloridate, II, at *p*H 9 for 1.5 hours gave a mixture of the expected 6-mercaptopurine 5′-nucleotide and 6-methylthiopurine 5′-nucleotide, III, in a 2:3 ratio. The *S*-alkylation took place principally during the *p*H 9 treatment, although it could also occur to some extent during the preceding phosphorylation since a slow, non acid-catalyzed conversion of 6-mercaptopurine nucleoside, I, to its 6-methylthio derivative, IV, did occur in trimethyl phosphate solution at 25°. At 100°, trimethyl phosphate completely converted I within 2 hours to a mixture of IV and another product of unknown structure. Trialkyl phosphates have been reported to alkylate both phenols (10) and aromatic amines (11) above room temperature, and alkylation of the considerably more nucleophilic mercaptide ion of I [pK_a 7.7 (3)] at a lower temperature is hence not surprising. When the foregoing *p*H 9 treatment was prolonged, 6-methylthiopurine 5′-ribonucleotide, III, was obtained in purified form in 60% yield. Compound III was obtained in 85% yield (in admixture with traces of the ribonucleoside 2′,5′- and 3′,5′-diphosphates) by phosphorylation of 6-methylthiopurine ribonucleoside, IV, by the Yoshikawa procedure (9). However, since IV is most conveniently obtained from 6-mercaptopurine ribonucleoside (3), the direct conversion of I to III is clearly preferable.



Application of the same phosphorylation-alkylation procedure to 2-amino-6-mercaptopurine ribonucleoside, VIII, yielded 42% of 2-amino-6-methylthiopurine ribonucleoside 5′-phosphate, VI, together with traces of the corresponding nucleoside 2′,5′- and 3′,5′-diphosphates;

no attempts were made to optimize conditions for the phosphorylation (unchanged VIII was observed in the above experiment), and higher yields of VI could conceivably be attained. Structural assignments for the products of the reaction were made on the basis of ultraviolet and infrared spectra, paper chromatographic and electrophoretic properties and the action on VI of purified 5′-nucleotidase of *Crotalus adamanteus* which gave a product chromatographically indistinguishable from 2-amino-6-methylthiopurine ribonucleoside, IX. The latter nucleoside was prepared by treatment of VIII with methyl iodide. Methylation of VIII has previously given poor yields of IX (4,12,13) due, at least partly, to difficulty in separation of IX from inorganic salts formed in the reaction. In the present studies it was found that IX forms a stable crystalline complex with sodium iodide and that IX could be isolated in 50% yield subsequent to its adsorption onto the acidic form of a cation exchange resin.

While treatment of free ribonucleosides with phosphorus oxychloride in trimethylphosphate solution (9) is a useful and efficient method for the synthesis of nucleoside 5′-phosphates, the present findings indicate that a limitation of the procedure may be the difficulty of preventing *S*-methylation of thiol-substituted nucleosides. A preferable procedure for such nucleosides could be *in situ* formation of the 2′,3′-*O*-isopropylidene nucleoside in acetone-acetonitrile-phosphorus oxychloride followed by pyridine-catalyzed phosphorylation (14), since a preliminary trial showed that 2′,3′-*O*-isopropylidene-2-amino-6-mercaptopurine nucleoside 5′-phosphate, VII, is obtainable in 40% yield from the corresponding ribonucleoside, VIII, by this procedure. Compound VII was not obtained in significant yield by phosphorylation of 2′,3′-*O*-isopropylidene-2-amino-6-mercaptopurine nucleoside (7) with phosphorus oxychloride and a trace of water, although this procedure converts isopropylidene guanosine and other isopropylidene nucleosides in high yields to their respective 5′-phosphates (15).

EXPERIMENTAL

Infrared spectra were determined on a Perkin-Elmer 137 spectrophotometer and ultraviolet absorption spectra with a Cary 15 spectrophotometer. Paper chromatograms were run by the ascending method on Whatman No. 3 MM paper in the following solvent systems: (A) 2-propanol-concentrated ammonia-water (7:1:2, v/v); (B) 93.8% aqueous *n*-butanol-44% aqueous propionic acid (1:1, v/v); (C) ethanol-1 *M* ammonium acetate (7:3, v/v); (D) 2-propanol-saturated ammonium sulfate-water (2:79:19, v/v); (E) *n*-butanol-acetic acid-water (5:2:3, v/v/v). Elemental analyses were by J. F. Alicino. Melting points (capillary method) are uncorrected.

6-Methylthio-9-β-D-ribofuranosylpurine 5′-Phosphate Barium Salt (III).

To an opaque solution of 6-mercapto-9-β-D-ribofuranosylpurine (3) (0.113 g., 0.40 mmole) in dry trimethyl phosphate (2.80 ml., 25.0 mmoles) at 0° phosphorus oxychloride (110 μl., 1.21 mmoles) was added slowly, followed by water (7 μl., 0.40 mmole). The clear, yellowish solution was stirred at 0-2° for 3 hours and was then poured into 16 ml. of ice water. To this stirred solution saturated lithium hydroxide solution (total, 2.5 ml.) was added dropwise to maintain the *p*H at 9. Trimethyl phosphate (0.5 ml.) was added every 2 hours, and the mixture was stirred for 10 hours at 25°. Ultraviolet spectral monitoring indicated that a 92% conversion of the 6-mercaptopurine nucleotide (max., 319 mμ) to its 6-methylthio derivative (max., 292 mμ) had taken place. Lithium phosphate was centrifuged off, 16 ml. of water was added and the solid re-centrifuged. The combined supernatants were extracted with 2 x 15 ml. of chloroform to remove trimethyl phosphate and the aqueous layer was diluted to 50 ml. To this solution barium acetate (0.205 g., 0.80 mmole) was added and the mixture (*p*H 8.5) was let stand overnight. The precipitated barium phosphate was removed by centrifugation, washed with 10 ml. of water, and the combined supernatants were evaporated to 15 ml. *in vacuo*. Additional barium phosphate was centrifuged down, and to the supernatant was added 30 ml. of ethanol. The colorless precipitate of the barium nucleotide was washed in turn with 15 ml. portions of 2:1 ethanol-water, ethanol, acetone and ether, respectively. The salt was reprecipitated from 12 ml. of water with 24 ml. of ethanol, and dried over phosphorus pentoxide at 78° (0.04 mm.) to give 0.148 g. of product (72%). Paper chromatography in solvent B showed two minor spots in addition to that of the major product, of which one corresponded to 6-mercapto-9-β-D-ribofuranosylpurine 5′-phosphate and the other probably to the 6-methylthio-9-β-D-ribofuranosylpurine 2′(3′),5′-diphosphates. An aqueous solution of the barium salt mixture (24 mg.) was applied to an Avicel F (Analtech) 800 μ layer plate and developed with solvent B. The major band (tlc R_f, 0.53) was scraped off, washed with a minimum volume of water, and the eluate treated with 2 volumes of ethanol to give the colorless barium nucleotide (20 mg., 83% recovery), which was dried as above. This compound was identical in all respects to the major product obtained by similar phosphorylation of 6-methylthio-9-β-D-ribofuranosylpurine, and on paper chromatograms it exhibited in ultraviolet light a single, blue fluorescent spot, which gave a positive reaction to a molybdate spray reagent for phosphates. R_f values: solvent A, 0.25; solvent B, 0.31 [lit. (5, 16), 0.30-0.33]; solvent C, 0.35; solvent D, 0.23; λ max (Nujol), 3.03 μ (OH), 3.15 (CH), 6.12, 6.37 (C=C, C=N), 9.15 (P—O—C); λ max (*p*H 1), 293 mμ (ε, 16,950) [lit. (6), 293; ε, 16,600]; λ max (*p*H 6), 292 (ε, 18,150); λ max (*p*H 12), 292 (ε, 18,300) [lit. (6), 291; ε, 19,100].

2-Amino-6-methylthio-9-(β-D-ribofuranosyl)purine (IX).

A stirred suspension of 2-amino-6-mercapto-9-(β-D-ribofuranosyl)purine (299 mg., 1 mmole) in water (4 ml.) containing sodium hydroxide (0.55 mmole) was treated dropwise at 3° with methyl iodide (1 mmole). Additional sodium hydroxide (0.45 mmole) followed by methyl iodide (1 mmole) was added. The solution was kept at 3° for 15 hours, when paper chromatography (solvent E) showed that complete (> 97%) conversion of the thiol VIII (R_f 0.41) to its *S*-methyl derivative (R_f 0.70) and a minor component (R_f 0.79) had occurred. Volatiles were removed *in vacuo*. To a solution of the residue in water (40 ml.) was added an excess of ice and 5 ml. of Dowex 50 ion-exchange resin (H^+ form, 200-400 mesh, 8% cross-linkage). The mixture was stirred for 15 minutes and the resin immediately filtered off, washed with ice water (10 ml.) and transferred to 80 ml. of ice and water. Ammo-

nium hydroxide (30 ml. of 0.5 *M*) was added gradually, with stirring, and after 1 hour the resin was removed by filtration. Vacuum evaporation of the filtrate gave a colorless gum. This ion-exchange treatment removed sodium iodide which co-crystallizes with the product from a variety of solvents. Two crystallizations of the product from ethanol-acetonitrile (1:3) (by slow evaporation at 25°) afforded 160 mg. (51%) of white needles, m.p. 179-180° [reported (12), 195-198° dec., (13), 182°]. The product contained *ca.* 1% of a second ultraviolet absorbing component of R_f 0.79 in solvent E. In aqueous solution, *p*H 1, it showed absorption maxima at 246 mμ (ε, 12,200) and 315 mμ (ε, 11,000); at *p*H 7-12 the maxima were at 246 mμ (ε, 14,500) and 311 mμ (ε, 13,100).

Anal. Calcd. for $C_{11}H_{15}N_5O_4S$: C, 42.2; H, 4.8; N, 22.3; S, 10.2. Found: C, 42.4; H, 5.0; N, 22.2; S, 10.3.

Phosphorylation-Alkylation of VIII.

The general procedure followed in the preparation of III was used. To a suspension of 2-amino-6-mercapto-9-β-D-ribofuranosylpurine (3) (0.299 g., 1.00 mmole) in dry trimethyl phosphate (3.40 ml., 30.3 mmoles), stirred at 0°, phosphorus oxychloride (275 μl., 3.00 mmoles) was slowly added, followed by water (13.5 μl., 0.75 mmole). After 3 hours another 275 μl. of phosphorus oxychloride was added, and the yellow solution was stirred at 0-2° for a total of 6 hours. The subsequent lithium hydroxide treatment was carried out for 1 day at *p*H 9 (25°) and the precipitated barium nucleotide was obtained as described for III and desiccated over phosphorus pentoxide (0.04 mm.) to yield 0.221 g. of VI (42% yield) contaminated with small amounts of 2-amino-6-methylthio-9-β-D-ribofuranosylpurine 2′(3′),5′-diphosphates. These diphosphates migrated 32% faster than adenosine 5′-phosphate upon paper electrophoresis at *p*H 8.2 in 0.04 *M* ammonium bicarbonate, and gave rise to violet fluorescent spots on paper chromatograms (R_f values 0.01, 0.05 and 0.04 in solvents A, B and C, respectively). Compound VI produced a violet fluorescent spot in ultraviolet light after paper chromatography; R_f values: 0.13, 0.11, 0.16 in solvents A, B and C, respectively; λ max (Nujol), 3.06 μ (OH), 3.22 (CH), 6.38 (C=C, C=N), 9.0 (P—O—C); λ max (*p*H 1), 246, 317 mμ; λ max (water), (*p*H 6 and 12), 245, 311; the electrophoretic mobility was 91% that of adenosine 5′-phosphate. A 5 m*M* solution (1 ml.) of the product in 0.1 *N* tris(hydroxymethyl)aminomethane buffer, *p*H 9.0, containing magnesium chloride (10 m*M*) and 0.25 mg. of purified 5′-nucleotidase of *Crotalus adamanteus* (Sigma Chemical Co.) was kept at 37° for 15 minutes, then at 90° for 10 minutes. Paper chromatography showed that the predominant component (R_f 0.73, 0.57, 0.75 in solvents A, B and C, respectively) corresponded to 2-amino-6-methylthiopurine ribonucleoside (IX).

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REFERENCES

(1) A. Hampton, J. J. Biesele, A. E. Moore, and G. B. Brown, *J. Am. Chem. Soc.*, 78, 5695 (1956).

(2) A. Hampton, *ibid.*, 79, 503 (1957).

(3) J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *ibid.*, 80, 1669 (1958).

(4) T. Naito, K. Ueno, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), 12, 951 (1964).

(5) P. W. Allan, H. B. Schnebli and L. L. Bennett, Jr., *Biochim. Biophys. Acta*, 114, 647 (1966).

(6) H. J. Thomas and J. A. Montgomery, *J. Med. Chem.*, 11, 44 (1968).

(7) A. Hampton and M. H. Maguire, *J. Am. Chem. Soc.*, 83, 150 (1961).

(8) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, 26, 1926 (1961).

(9) M. Yoshikawa, T. Kato and T. Takenishi, *Tetrahedron Letters*, 5065 (1967).

(10) C. R. Noller and G. R. Dutton, *J. Am. Chem. Soc.*, 55, 424 (1933).

(11) D. G. Thomas, J. H. Billman, and C. E. Davis, *ibid.*, 68, 895 (1946) and references cited therein.

(12) M. Ikehara, A. Yamazaki and T. Fujieda, *Chem. Pharm. Bull.* (Tokyo), 10, 1075 (1962).

(13) C. W. Noell and R. K. Robins, *J. Med. Pharm. Chem.*, 5, 1074 (1962).

(14) Y. Fujimoto and M. Naruse, *Yakugaku Zasshi*, 87, 270 (1967).

(15) M. Yoshikawa and T. Kato, *Bull. Chem. Soc. Japan*, 40, 2849 (1967).

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

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