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# The 2′(3′)-Phosphates of 6-Mercaptopurine Ribonucleoside and 8-Azaguanosine[1]

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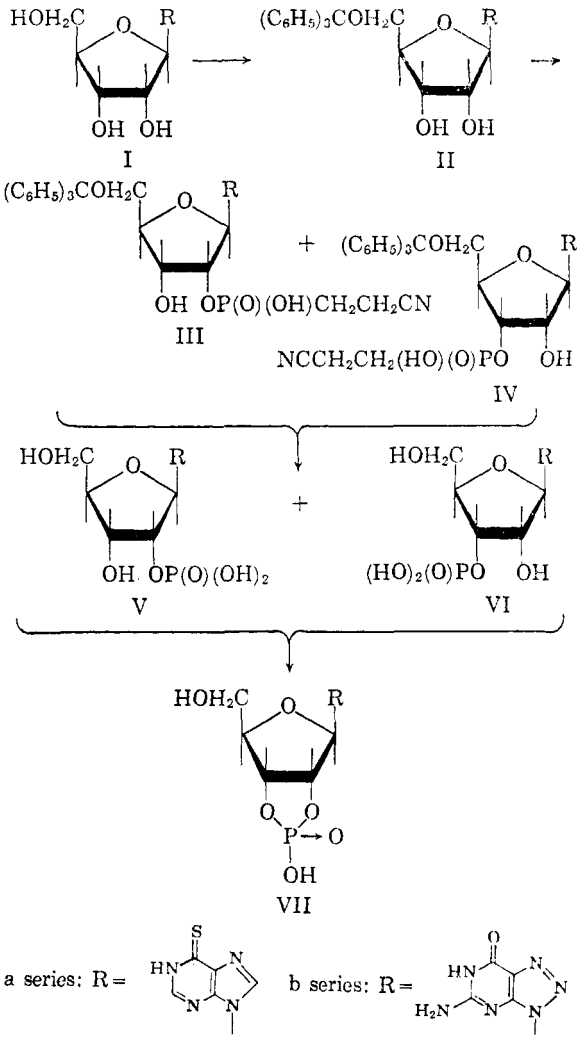
9-β-D-Ribofuranosyl-9*H*-purine-6(1*H*)-thione 2′(3′)-phosphates (Va and VIa) and 5-amino-3-β-D-ribofuranosyl-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-7-(6*H*)-one 2′(3′)-phosphates (Vb and VIb) have been prepared from the 5′-trityl nucleosides (IIa and IIb) by reaction with 2-cyanoethyl phosphate and dicyclohexylcarbodiimide followed by removal of the cyanoethyl and the trityl blocking groups. The properties of these compounds are described.

The chemical synthesis of the 5′-phosphates of 6-mercaptopurine ribonucleoside (Ia) and 8-azaguanosine (Ib) by the 2-cyanoethyl phosphate method of Gilham and Tener[2,3] has been described.[4] One of these compounds (Ia 5′-phosphate) has also been prepared[5] by the tetra-*p*-nitrophenyl pyrophosphate method,[6] but the procedure is quite lengthy and apparently useful for the preparation of small quantities of material only.

Although 6-mercaptopurine ribonucleotide inhibited the growth of Adenocarcinoma 755 markedly,[7] neither it nor 8-azaguanylic acid increased the life span of mice implanted with leukemias resistant[8] to the action of the 6-mercaptopurine and 8-azaguanine respectively.[10] This negative result led us to seek other nucleotide derivatives that might be effective in the treatment of resistant leukemias, and the 2′(3′)-phosphates of Ia and Ib were selected for synthesis.

Because of the excellent results already obtained[4] with the 2-cyanoethyl phosphate procedure, it appeared to be the method of choice for the preparation of the 2′(3′)-phosphates. It was first necessary to block the 5′- position of the nucleosides Ia and




Ib, and this was accomplished by the conventional method[11]—the preparation of the 5′-trityl compounds IIa and IIb. Reaction of 6-mercaptopurine ribonucleoside (Ia) with triphenylchloromethane gave a mixture of the desired 9-(5′-*O*-trityl-β-D-ribofuranosyl)-9*H*-purine-6-(1*H*)-thione (IIa) and the 9-(5′-*O*-trityl-β-D-ribofuranosyl)-6-tritylthio-9-*H*-purine. The two products were easily separated by the difference in their solubilities, and the ditrityl

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(2) P. T. Gilham and G. M. Tener, *Chem. & Ind.*, 542 (1959).

(3) G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).

(4) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **26**, 1926 (1961); J. K. Roy, D. C. Kram, J. L. Dahl, and R. E. Parks, Jr., *J. Biol. Chem.*, **236**, 1158 (1961).

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

(6) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 3741 (1957).

(7) This inhibition may well be due to the *in vivo* cleavage of the nucleotides back to the parent heterocycles.

(8) The resistance of these leukemias to the action of 6-mercaptopurine and 8-azaguanine is now widely believed to be due to their inability to convert these fraudulent purines to their 5′-ribonucleotides.[9]

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(10) J. A. Montgomery, F. M. Schabel, Jr., R. W. Laster, and H. E. Skipper, to be published.

(11) D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 44 (1952).

TABLE I

| Compound | $R_f$ Values, Solvent System[b] A | B | C | D | E | $M_{Ia}$[a] Values, Solvent System F | G |
|---|---|---|---|---|---|---|---|
| IIa | 0 | 0.92 | 0.92 | 0.81 | — | — | — |
| IIb | 0 | 0.78 | 0.86 | 0.77 | — | — | — |
| Va, VIa | 0.79 | 0 | 0.21 | 0 | 0.32, 0.25 | 93 | 111 |
| Vb, VIb | 0.83 | 0 | 0.20 | 0 | 0.53, 0.44 | 93 | 105 |
| VIIa | 0.71 | 0 | 0.24 | 0.27 | 0.16 | 102 | 73 |
| VIIb | 0.75 | 0 | 0.19 | 0.19 | 0.27 | 107 | 74 |

[a] Migration relative to that of inosinic acid (assigned a value of 100). [b] A, potassium acetate buffer (*p*H 6.1); B, water–saturated butyl alcohol; C, butyl alcohol–acetic acid–water (5:2:3); D, isopropyl alcohol–ammonium hydroxide–water (14:1:5); E, saturated ammonium sulfate in water, *M* sodium formate, isopropyl alcohol (80:18:2); F, 0.05*M* ammonium formate buffer (*p*H 3.5); G, 0.05*M* phosphate buffer (*p*H 7.4).

compound was readily converted to IIa by mild acid hydrolysis. Furthermore, the ditrityl compound or the mixture can be utilized in the subsequent step. 8-Azaguanosine (Ib) was converted in good yield to its 5′-trityl derivative (IIb). The trityl compounds IIa and IIb reacted smoothly with 2-cyanoethyl phosphate to give the corresponding 2-cyanoethyl phosphate esters, which were deblocked without isolation by treatment first with 80% aqueous acetic acid and then with 0.5*N* lithium hydroxide. A 21% yield (based on IIa) of the 2′(3′)-phosphates of 6-mercaptopurine ribonucleoside (Va and VIa) and a 44% yield (based on IIb) of the 2′(3′)-phosphates of 8-azaguanosine (Vb and VIb) were obtained.

Each 2′(3′)-mixture traveled as one spot electrophoretically and chromatographically (in the four solvent systems[12] we normally employ). However, it was possible to separate them chromatographically into the 2′- and the 3′-isomers by use of a solvent system which is known to separate the 2′- and 3′- isomers of the natural nucleotides.[13] Elution of the two spots in the case of Va and VIa from their paper chromatogram gave solutions whose ultraviolet absorption spectra were practically identical indicating a 1:1 mixture as was expected. All four spots gave a positive phosphate test.[14]

Treatment of the two 2′(3′)-mixtures with dicyclohexyl carbodiimide[15] gave the cyclic phosphates VIIa and VIIb, which were isolated as their salts. The identity of the cyclic phosphates was established by their chromatographic and electrophoretic behavior (see Table I), which corresponds exactly to the behavior of the 2′(3′)-phosphates and 2′,3′-cyclic phosphates of the natural nucleosides such as adenosine.[16]

The biological evaluation of these compounds will be reported later.

## EXPERIMENTAL

The melting points were determined on a Kofler Heizbank and are corrected. The ultraviolet spectra were determined in aqueous solution (except where indicated) with a Cary Model 14 or a Beckman DK-2 (optical densities at the maxima with a DU). The infrared spectra were determined in pressed potassium bromide disks with a Perkin-Elmer Model 221. Electrophoresis and chromatographic studies were carried out as previously described.[4]

*9-(5′-O-Trityl-β-D-ribofuranosyl)-9H-purine-6(1H)-thione* (IIa) *and 9-(5′-O-trityl-β-D-ribofuranosyl)-6-tritylthio-9H-purine.* A solution of 9-β-D-ribofuranosyl-9*H*-purine-6(1*H*)-thione (1.5 g., 5.3 mmoles) and triphenylchloromethane (3.1 g., 11.1 mmoles) in dry pyridine (100 ml.) was held at 56° for 3 days. The light amber solution was concentrated *in vacuo* to one-sixth volume before it was poured in a thin stream into an ice-cold sodium bicarbonate solution (0.93 g., 11.1 mmoles in 500 ml. of water). The resulting mixture was stirred until the gummy material that precipitated solidified to a curdy solid. This solid was collected by filtration, washed with water, and dried; the mixture of mono-$O^{5'}$ and $O^{5'}$, *S* ditrityl derivatives of Ia and triphenylcarbinol weighed 4.6 g. Ether extraction of this material removed the triphenylcarbinol and ditrityl derivative of Ia leaving 1.35 g. (48%) of crude IIa which was dissolved in concentrated ammonium hydroxide. The solution was filtered and then evaporated to give a solid which was dissolved in a minimum of boiling ethanol (7 ml.) containing a drop of 0.1*N* hydrochloric acid. The slow addition of ether (14 ml.) to the alcohol solution gave the analytical sample; yield 260 mg. (10%); m.p. 160°. $\lambda_{max}$ in mμ ($\epsilon \times 10^{-3}$): *p*H 1—322.5 (24.1); *p*H 7—319.5 (21.5); *p*H 13—230.5 (21.8), 312 (22.9).

*Anal.* Calcd. for $C_{29}H_{24}N_4O_4S \cdot 1/2\ H_2O$: C, 65.41; H, 4.75; N, 10.53. Found: C, 65.27; H, 5.28; N, 10.93.

Concentration of the ether filtrate from the isolation of the crude IIa gave 1.23 g. of the $O^{5'}$, *S* ditrityl compound, 409 mg. of which was recrystallized from acetone; yield 120 mg. (9%); m.p. 258°; $\lambda_{max(m\mu)}^{ethanol}$ 296–297 (16.3).

*Anal.* Calcd. for $C_{48}H_{40}N_4O_4S$: C, 74.98; H, 5.24. Found: C, 74.15; H, 5.27.

*5-Amino-3-(5′-O-trityl-β-D-ribofuranosyl)-3H-v-triazolo[4,5-d]pyrimidin-7(6H)-one* (Ib). To a solution of 5-amino-3-β-D-ribofuranosyl-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-7(6*H*)-one[17] (1 g., 3.5 mmoles) in dry pyridine (100 ml.) was added triphenylchloromethane (2.06 g., 7.3 mmoles), and the mixture was heated at 60° for 3 days. The cooled, concentrated (35 ml.) yellow solution was poured into sodium bicarbonate solution (620 mg., 7.39 mmoles in 150 ml. of ice water). The bicarbonate solution was decanted from the gum that formed and the gum was triturated with water and then boiling hexane (300 ml. in several portions). The insoluble residue (1.91 g.) was further purified by recrystallization from a 1:1 mixture of ethyl alcohol and hexane; yield 1.14 g. (61%). The analytical sample was obtained by a second recrystallization from alcohol-hexane, after which the material was dried for 16 hr. over phosphorus pentoxide *in vacuo* at 110°; m.p. 200–202°. $\lambda_{max}$ in mμ ($\epsilon \times 10^{-3}$): *p*H 1—257 (13.9); *p*H 7—257 (12.4); *p*H 13—278 (10.4).

*Anal.* Calcd. for $C_{28}H_{23}N_5O_5$: C, 63.85; H, 4.98; N, 15.96. Found: C, 63.76; H, 5.00; N, 15.74.

(12) See Table I.

(13) R. Markham and J. D. Smith, *Biochem. J.*, 49, 401 (1951).

(14) C. S. Hanes and F. A. Isherwood, *Nature*, 164, 1107 (1949).

(15) M. Smith, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, 80, 6204 (1958).

(16) R. Markham and J. D. Smith, *J. Chem. Soc.*, 52 (1952).

(17) J. Davoll, *J. Chem. Soc.*, 1593 (1958).

*9-β-D-Ribofuranosyl-9H-purine-6(1H)-thione 2′(3′)-phosphates* (Va and VIa). To a solution of 4.0 mmoles of 2-cyanoethyl phosphate (from 1.15 g. of its barium salt) in 30 ml. of anhydrous pyridine was added 3.30 g. (16.0 mmoles) of dicyclohexylcarbodiimide and 1.05 g. (2.00 mmoles) of 9-(5′-*O*-trityl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione. The resulting solution, in a tightly sealed flask, was allowed to stand for 2 days at room temperature. Upon filtration, the light orange reaction mixture yielded 1.62 g. of crystalline 1,3-dicyclohexylurea. To the filtrate was added 8 ml. of water and the solution set aside for 1 hr. A second precipitate of the urea (970 mg.) was obtained.

The filtrate was evaporated to dryness *in vacuo*, the residue suspended in 80 ml. of 80% acetic acid (v./v.), and the suspension heated for 20 min. in a 100° oil bath. During the heating period a cloudy solution was obtained. The reaction solution, after cooling, was filtered to remove a mixture of the urea and some triphenylcarbinol, and then evaporated to dryness. The residue was treated with several additions of water followed by evaporation *in vacuo* to remove the last traces of acetic acid.

A solution of the residue in 90 ml. of 0.5*N* lithium hydroxide was heated for 15 min. at 100°. After filtration, the solution was stirred with 30 g. of Amberlite IR-120 (H) ion-exchange resin. The resin was removed by filtration and the solution neutralized with aqueous barium hydroxide, then filtered, and finally evaporated to 45 ml. After diluting the solution with 90 ml. of ethanol, a precipitate formed which was collected by centrifugation; yield 650 mg.

This material was purified by placing 200 mg. of it on a column of Dowex 1-X2 (formate) ion-exchange resin (1 cm. × 12 cm. long). The product was obtained when the column was eluted with 2.5*N* formic acid. Evaporation of the formic acid solution gave a residue which was dissolved in 10 ml. of water and the solution neutralized with aqueous barium hydroxide. The barium salt of Va and VIa was precipitated by the addition of 20 ml. of ethyl alcohol and dried for 24 hr. at 110°/0.07 mm. over phosphorus pentoxide; yield 74 mg. (21%). $\lambda_{max}$ in mμ ($\epsilon \times 10^{-3}$): *p*H 1—321 (20.2); *p*H 7—319 (20.4); *p*H 13–310 (19.5). $\bar{\nu}_{max}$ in cm.$^{-1}$: 3400 (broad) (OH), 1600, 1570 (shoulder), and 1510 (shoulder) (C=C, C=N), 1100 (broad) (P—O—C).

*Anal.* Calcd. for $C_{10}H_{11}N_4O_7PSBa \cdot H_2O$: C, 23.20; H, 2.53; P, 5.98. Found: C, 23.14; H, 2.53; P, 6.02.

*5-Amino-3-β-D-ribofuranosyl-3H-v-triazolo[4,5-d]pyrimidin-7-(6H)-one 2′(3′)-phosphates* (Vb and VIb). To a solution of 2.0 mmoles of 2-cyanoethyl phosphate (from 575 mg. of its barium salt) in 14 ml. of anhydrous pyridine were added 1.65 g. (8.0 mmoles) of dicyclohexylcarbodiimide and 526 mg. (1.0 mmole) of 5-amino-3-(5′-*O*-trityl-β-D-ribofuranosyl)-3*H*-*v*-triazolo-[4,5-*d*]pyrimidin-7(6*H*)-one. The resulting solution was allowed to stand for 2 days at room temperature in a tightly sealed flask. Filtration of the dark orange reaction solution gave 780 mg. of 1,3-dicyclohexylurea. One hour after the addition of 2 ml. of water to the filtrate, another crop of the urea, 1.02 g., was obtained.

The filtrate was evaporated to dryness *in vacuo*, the residue dissolved in 20 ml. of 80% acetic acid (v./v.) and the resulting solution heated in a 100° oil bath for 20 min. and finally evaporated to dryness *in vacuo* with several additions of water to remove the last traces of acetic acid.

The residue was then dissolved in 45 ml. of 0.5*N* lithium hydroxide solution and heated in a 100° oil bath for 15 min. After cooling, the solution was filtered and stirred for 15 min. with 30 ml. of Amberlite IR (120) (H) ion-exchange resin. The resin was removed by filtration, and the solution neutralized with aqueous barium hydroxide and again filtered. The aqueous solution, now 50 ml., was diluted with 150 ml. of ethyl alcohol whereupon a precipitate formed immediately. The white solid was collected by centrifugation and washed with cold ethyl alcohol and then ether; yield 395 mg.

This barium salt was purified by placing 271 mg. of it on a column of Dowex 1-X2 (formate) ion-exchange resin (1 cm. × 14 cm. long). The product was obtained by elution of the column with 2.5*N* formic acid. Evaporation of the formic acid solution gave a residue which was dissolved in 10 ml. of water, and the solution was neutralized with aqueous barium hydroxide and diluted with 20 ml. of ethyl alcohol. The barium salt was collected by centrifugation, washed with ethyl alcohol, then ether, and dried at 110°/0.07 mm. over phosphorus pentoxide for 24 hr.; yield 163 mg. (44%). $\lambda_{max}$ in mμ ($\epsilon \times 10^{-3}$): *p*H 1—254 (12.8); *p*H 7—255 (12.2); *p*H 13—278 (11.1). $\bar{\nu}_{max}$ in cm.$^{-1}$: 3395 (OH), 1690 (C=O), 1635 (NH), 1600 and 1535 (shoulder) (C=C, C=N), 1100 (P—O—C).

*Anal.* Calcd. for $C_9H_{11}N_6O_8PBa \cdot 2H_2O$: C, 20.18; H, 2.82; N, 15.69; P, 5.78. Found: C, 20.62; H, 2.88; N, 15.09; P, 6.06.

*9-β-D-Ribofuranosylpurine-6(1H)-thione 2′,3′-cyclic phosphate* (VIIa) *and 5-amino-3-β-D-ribofuranosyl-3H-v-triazolo[4,5-d]pyrimidin-7(6H)-one 2,3′-cyclic phosphate* (VIIb). To a solution of 125 mg. (0.34 mmoles) of purine-6(1*H*)-thione ribonucleoside 2′(3′)-phosphates in 8.6 ml. of 2*N* ammonium hydroxide and 8.6 ml. of formamide was added a solution of 354 mg. (1.72 mmoles) of dicyclohexylcarbodiimide in 20 ml. of *tert*-butyl alcohol. After refluxing for 2.5 hr., the *tert*-butyl alcohol was evaporated and the solution was diluted with 80 ml. of water and washed with ether (3 × 100 ml.). The aqueous layer was evaporated to a thick syrup. The formamide was removed by evaporation. Trituration of the residue with acetone produced a yellow, gummy solid. A solution of the solid was stirred with 2 ml. of Amberlite IR-120 (cyclohexylammonium) ion-exchange resin. After removal of the resin, the solution was evaporated to dryness and the residue triturated with acetone. The cyclohexylammonium salt of VIIa was obtained as a light yellow solid; yield 60 mg. (39%). This material was chromatographically and electrophoretically homogeneous (See Table I) but was shown by its ultraviolet spectrum and its combustion residue to be contaminated with inorganic salts.

In a similar manner the 2′,3′-cyclic phosphate VIIb was prepared from 5-amino-3-β-D-ribofuranosyl-3*H*-*v*-triazolo-[4,5-d]pyrimidin-7(6*H*)-one 2′(3′)-phosphates and isolated as its barium salt.

Treatment of the 2′,3′-cyclic phosphates VIIa and VIIb with 0.1*N* hydrochloric acid[16] converted them back to the 2′(3′)-phosphates.

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BIRMINGHAM, ALA.