

## *The Syntheses of 5'-Deoxy-5'-methylthioadenine and its Analogues*

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9 $\beta$ -(5'-Deoxy-5'-methylthio-D-ribofuranosyl)-adenine (I), known as "adenyl thiomethyl pentoside" or "vitamin L<sub>2</sub>", was first isolated from yeast by Mandel and Dunhan<sup>1)</sup> in 1912. Recently this nucleoside was directly converted into S-(5'-deoxyadenosine-5')-methionine<sup>2)</sup>, "active methionine", which participates in the transmethylation reaction in living cells.

Baddiley et al.<sup>3)</sup>, Makino et al.<sup>4)</sup> and Weygand et al.<sup>5)</sup> have succeeded independently, in the synthesis of this nucleoside, using adenosine as the starting material. Their synthetic route analogously involved the introduction of a toluene-*p*-sulfonyl group into the 5'-position of 2',3'-*O*-isopropylidene-adenosine, followed by its replacement by a methylthio group.

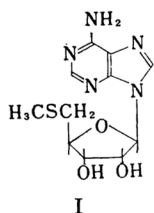
1) I. A. Mandel and E. K. Dunhan, *J. Biol. Chem.*, **11**, 85 (1912).

2) J. Baddiley and G. A. Jamieson, *J. Chem. Soc.*, **1954**, 4280.

3) J. Baddiley, *ibid.*, **1951**, 1343.

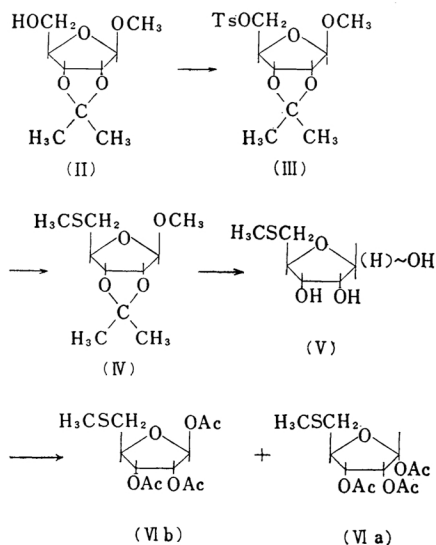
4) K. Makino and K. Sato, *Nature*, **167**, 238 (1951).

5) F. Weygand and O. Trauth, *Chem. Ber.*, **84**, 633 (1951).



In a previous short communication<sup>6)</sup> from this laboratory, a new synthetic method for obtaining compound I was described. In the present paper this new method and the synthesis of its analogues are described in detail.

In the authentic method, considerable difficulty was experienced in the formation of an intramolecular 3-5'-cyclic compound. So, to avoid the formation of this cyclic compound, D-ribose was converted into 5'-deoxy-5'-methylthio derivative and its halide was condensed with a purine base. It was reported by Overend and Parker<sup>7)</sup> and by Yoshimura and Sato<sup>8)</sup> that methyl 2,3-O-isopropylidene-β-D-ribofuranoside (II) was converted into 5-O-toluene-*p*-sulfonyl derivative (III), which reacted with sodium methyl sulfide in acetone to give methyl 2,3-O-isopropylidene-5-deoxy-5-methylthio-D-ribofuranoside (IV). The isolation of this compounds in its pure state was not, however, attempted by these authors.



This reaction was repeated by using anhydrous acetonitrile as a solvent in place of acetone. The yield of the product was in-

creased to 85% by this modification. The solution of the pure product from the reaction mixture was achieved by the use of distillation followed by column chromatography on alumina.

The methyl and isopropylidene residues of IV were removed by heating it in 0.01N sulfuric acid. The resultant syrup (V) could not be crystallized, but gave one spot on paper chromatography (with butanol-acetic acid-water 5:1:4 v/v). The acetylation of this product with pyridine-acetic anhydride in the usual manner afforded crystals, m. p. 66°C (yield 21%) (VIb), and an oily material (yield 48%) (VIa). Elemental analysis found no difference between these products. It was deduced from the values of the optical rotation of these compounds that VIa and VIb are α- and β-isomer respectively.

VIb was chlorinated with hydrogen chloride in absolute ether. Freshly prepared 1-chloro derivative (VII) was condensed with the silver salt of theophylline, which is known to react easily with sugar halide in general. The condensation product was deacetylated by methanolic ammonia to give white needles, m. p. 74~76°C (IX<sub>1</sub>). The position of the sugar residue at the purine base and the configuration of the glucosidic linkage of this compound were confirmed as follows. The authentic sample of 7β-(5'-deoxy-5'-iodo-D-ribofuranosyl)-theophylline (X) was treated with sodium methyl sulfide to introduce a methylthio group. The product was identical to IX<sub>1</sub>. Thus, the structure was confirmed to be 7β-(5'-deoxy-5'-methylthio-D-ribofuranosyl)-theophylline.

Chloromercuri-2,8-dichloroadenine and chloromercuri-2-methylthioadenine were also condensed with VII, in the manner described above, to give the corresponding derivatives with a methylthio group at position 5' (VIII<sub>2</sub> and VIII<sub>3</sub> respectively). VIII<sub>2</sub> and VIII<sub>3</sub> were deacetylated with methanolic ammonia, and structures of the resultant compounds (IX<sub>2</sub> and IX<sub>3</sub>) were confirmed by derivations from XI and XII to be 9β-(5'-deoxy-5'-methylthio-D-ribofuranosyl)-2,8-dichloroadenine and 9β-(5'-deoxy-5'-methylthio-D-ribofuranosyl)-2-methylthioadenine respectively. Furthermore, the results of elemental analysis, ultraviolet absorption measurement and Fehling's test supported this structure.

Compound I was not obtained from IX<sub>2</sub> or IX<sub>3</sub> by partial reduction in either cases.

It has been reported<sup>9-11)</sup> that the yield of

6) T. Kanazawa, *J. Chem. Soc. Japan, Pure Chem. Sec. (Nippon Kagaku Zasshi)*, **81**, 516 (1960).

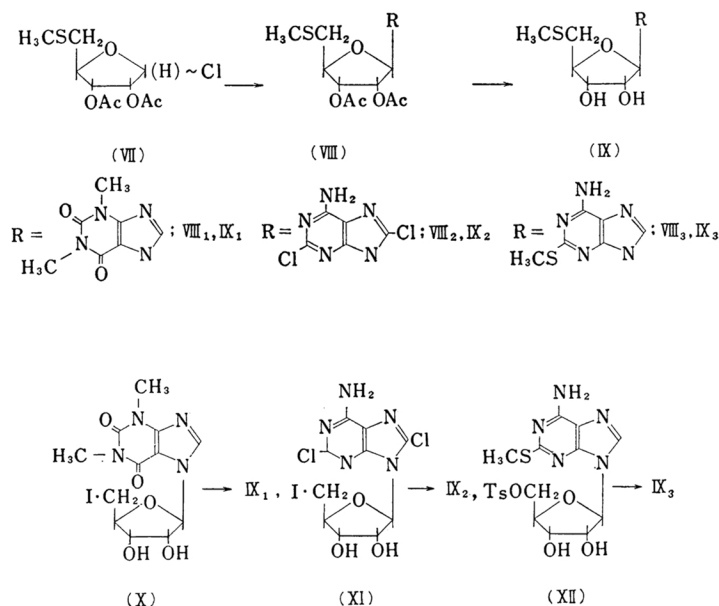
7) W. G. Overend and L. E. J. Parker, *Nature*, **167**, 527 (1951).

8) J. Yoshimura and T. Sato, *J. Chem. Soc. Japan, Pure Chem. Sec. (Nippon Kagaku Zasshi)*, **73**, 350 (1952).

9) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).

10) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

11) H. M. Kissman and M. J. Weiss, *J. Org. Chem.*, **21**, 1053 (1956).



adenine with a sugar halide of the condensation reaction of metallic salt is less than 2~3%; 6-acetaminopurine and 6-chloropurine, however, were easily condensed with sugar halides, and the substituents at position 6 were converted into amino groups by treatment with methanolic ammonia. These facts suggest that chloromercuri-6-acetaminopurine and chloromercuri-6-chloropurine might be condensed in a good yield with methylthio sugar derivatives and converted into compound I. The test of the condensation showed that 9 $\beta$ -(2',3'-di-O-acetyl-5'-deoxy-5'-methylthio-D-ribofuranosyl)-6-acetaminopurine and 9 $\beta$ -(2',3'-di-O-acetyl-5'-deoxy-5'-methylthio-D-ribofuranosyl)-6-chloropurine are obtainable in 41% and 63% yields respectively. In either case, the products were treated with methanolic ammonia to afford I, m. p. 205~206°C. (The yields from VIb were 17.5 and 35% respectively.)

The synthetic substance and natural "adenylthiomethyl pentoside" were proved to be identical by measurement of their melting points and paper chromatography.

### Experimental

**Methyl 2,3-O-isopropylidene-5-deoxy-5-methylthio- $\beta$ -D-ribofuranoside (IV).**—A mixture of III (30 g., 0.084 mol.) and potassium methyl sulfide (15 g., 0.175 mol.) in dry acetonitrile (150 ml.) was heated in a sealed tube for 7 hr. at 100°C. After the solution had been chilled, the insoluble materials were filtered off and the filtrate was evaporated to dryness. The residue was dissolved in a mixture of ice cold water and chloroform. The chloroform layer was washed with water. After being dried with anhydrous sodium sulfate, it was

evaporated under reduced pressure. A colorless syrup (24 g.) was obtained by distillation under 0.03 mmHg (b. p. 0.03 82°C, b. p. 0.005 62°C). Yield, 94%. This product (15 g.) was chromatographed on a column of active alumina (150 g.). A colorless syrup (13.7 g., 91%) was obtained when it was eluted with 600 ml. of benzene-petroleum ether (1:2).

Found: C, 51.31; H, 7.75; S, 13.99. Calcd. for  $\text{C}_{10}\text{H}_{18}\text{O}_5\text{S}$  (234.3): C, 51.28; H, 7.69; S, 13.70%.  $R_f(a) = 0.57^*$ .

**5-Deoxy-5-methylthio-D-ribofuranose (V).**—A mixture of IV (13.7 g.), dioxane (80 ml.) and 0.1 N sulfuric acid (200 ml.) was heated at 100°C for 2 hr. The acid was then neutralized with barium carbonate. The insoluble materials were filtered off, and the filtrate was evaporated to dryness. An oily product was obtained. Yield, 9.7 g., 92%.

**1,2,3-Tri-O-acetyl-5-deoxy-5-methylthio- $\beta$ -D-ribofuranose (VIb) and its  $\alpha$ -Anomer (VIa).**—Compound V (6 g.) was acetylated with acetic anhydride in pyridine (50 ml.). By fractional distillation of the acetyl derivatives, the following three fractions were obtained. No. 1: b. p. 114~118°C/0.01 mmHg (2.95 g.); No. 2: b. p. 118~125°C/0.01 mmHg (3.5 g.); No. 3: b. p. 130~134°C/0.01 mmHg (3.2 g.). Ether (2 ml.) was added to fraction No. 3, and the solution was left to stand for 1 day. 1.6 g. of crystals were obtained, m. p. 64~66°C. Yield, 50%. The same crystals (0.1 g.) were obtained from No. 2 (3%). These crystals were collected and recrystallized from ether, m. p. 66°C.

Found: C, 46.95; H, 5.87; S, 10.47. Calcd. for  $\text{C}_{12}\text{H}_{18}\text{O}_7\text{S}$  (306.32): C, 47.06; H, 5.92; S, 10.45%.  $[\alpha]_D^{20} -26.7^\circ$  (c 0.99, ethanol).

No. 1 and mother liquors of No. 2 and No. 3 (6.3 g.) were dissolved in a mixture of benzene

\* Solvent systems of paper chromatography:  $R_f(a)$  *n*-Butanol-water (saturated) (upper layer):  $R_f(b)$  *n*-Butanol-acetic acid-water, 5:2:3 (upper layer).

and petroleum ether, and the solution was fractionated on a magnesol-celite column (80 g. of 5:1 mixture). The solvents used for elution were mixtures of benzene and petroleum ether with successively increasing concentrations of benzene. The effluents were examined as to the weight of the eluates and their rotation. As is shown in Fig. 1, 0.8 g. of crystals (the  $\beta$ -anomer) and 4.4 g. of the  $\alpha$ -anomer were obtained. The  $\alpha$ -anomer had  $[\alpha]_D^{25} + 26.1^\circ$  ( $c$  1.015, ethanol).

Found: C, 47.15; H, 6.29. Calcd. for  $C_{12}H_{18}O_7S$  (306.3): C, 47.05; H, 5.99%.

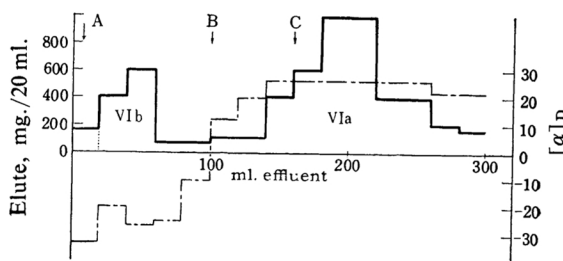


Fig. 1. Chromatographic separation of VI on a magnesol-celite column.

A, B and C are used for elution in which the ratio of benzene to petroleum ether are 0.3, 0.6 and 1 respectively. —, weight of elute; — — —, rotation of elute; VIb, the  $\beta$  anomer; VIa, the  $\alpha$  anomer.

**2,3-Di-O-acetyl-5-deoxy-5-methylthio-D-ribofuranosyl Chloride (VII).**—VIb (4 g.) was dissolved in absolute ether (160 ml.), and the solution was saturated with dry hydrogen chloride at  $-7^\circ\text{C}$  and left to stand for 4 days at  $0^\circ\text{C}$ .

**7 $\beta$ -(5'-Deoxy-5'-methylthio-D-ribofuranosyl)-theophylline (IX<sub>1</sub>).**—From Theophylline Silver.—A solution of VII, freshly prepared as above from 2.4 g. of VIb in 30 ml. of toluene, was added to an azeotropically-dried suspension of 2.5 g. of theophylline silver in 30 ml. of toluene. The mixture was allowed to reflux for 2 hr. (kept from moisture), and the insoluble materials were filtered off. The filtrate was then evaporated, and the residual syrup was treated with methanolic ammonia. The product (1.4 g.) was recrystallized twice from water, m. p.  $66\sim 68^\circ\text{C}$ . Yield, 1.2 g.

Found: C, 45.22; H, 5.30; N, 16.61. Calcd. for  $C_{13}H_{18}O_5N_4S$  (342.3): C, 45.61; H, 5.35; N, 16.37%.  $R_f(a)=0.66$ ,  $R_f(b)=0.08$ .  $\lambda_{\text{max}}$ : 275  $m\mu$ ,  $\epsilon=9300$  (water).

**From X.**—A mixture of 1 g. of X and 2 g. of dry potassium methyl sulfide was added to 5 ml. of anhydrous dimethylformamide, and the mixture was heated at  $100^\circ\text{C}$  for 2 hr. After the solvent had been evaporated in vacuo, the residue was added to water. 0.5 g. of crystals were obtained. This was recrystallized twice from water, m. p.  $73\sim 75^\circ\text{C}$ , undepressed on admixture with IX<sub>1</sub>.

**9 $\beta$ -(2', 3'-Di-O-acetyl-5'-deoxy-5'-methylthio-D-ribofuranosyl)-2,8-dichloroadenine (VIII<sub>2</sub>).**—A solution of sugar halide, prepared as above from 2 g. of VIb in 30 ml. of xylene, was added to chloromercuri-2,8-dichloroadenine on celite (from

2 g. of 2,8-dichloroadenine) in 200 ml. of xylene, and the mixture was allowed to reflux for 2.5 hr. There was obtained 0.9 g. of crystals, m. p.  $187\sim 188^\circ\text{C}$  (34% yield).

Found: C, 39.92; H, 3.84; N, 15.37. Calcd. for  $C_{15}H_{17}O_5N_5Cl_2S$ : C, 40.01; H, 3.78; N, 15.55%.  $R_f(a)=0.88$ ,  $R_f(b)=0.92$ .

**9 $\beta$ -(5'-Deoxy-5'-methylthio-D-ribofuranosyl)-2,8-dichloroadenine (IX<sub>2</sub>).**—From VIII<sub>2</sub>.—To a solution of 0.9 g. of VIII<sub>2</sub> in 30 ml. of absolute methanol was added 35 ml. of absolute methanol saturated with ammonia at  $0^\circ\text{C}$ , and the mixture was left to stand overnight at room temperature. The solution was evaporated in vacuo, and the residual syrup was dissolved in 50 ml. of hot water. After being treated with activated charcoal, the solution was concentrated to 10 ml. and was chilled. White needles were obtained, m. p.  $89\sim 92^\circ\text{C}$ . Yield, 0.7 g. 90%. Recrystallization from water, m. p.  $94\sim 96^\circ\text{C}$ .

Found: C, 34.61; H, 4.03; N, 18.16. Calcd. for  $C_{11}H_{13}O_3N_5S\cdot H_2O$  (313.3): C, 34.39; H, 3.91; N, 18.23%.  $R_f(a)=0.79$ ,  $R_f(b)=0.67$ .  $\lambda_{\text{max}}$ : 267  $m\mu$ ,  $\epsilon=19500$  (water).

**From XI.**—A solution of 0.5 g. of XI in 15 ml. of acetonitrile was added to 1 g. of sodium methyl sulfide in a sealed tube at  $0^\circ\text{C}$ , and the shaking mixture was kept at  $100^\circ\text{C}$  for 7 hr. After the solution had been chilled, the insoluble materials were filtered off and the filtrate was evaporated in vacuo. The residue was dissolved in chloroform and washed with water. The solution was concentrated and dissolved in 30 ml. of hot water and passed through a column of Amberlite IR-120 resin (H form). After washing of the column with 20% alcohol, the product was eluted with a large volume of ammonia (equal parts of water and ammonia of  $d=0.880$ ). The effluent was evaporated to a small volume in vacuo, and the crystalline nucleoside (0.2 g.) was filtered off. Recrystallized from water, m. p.  $94\sim 96^\circ\text{C}$ , undepressed on admixture with IX<sub>2</sub>.

**9 $\beta$ -(2', 3'-Di-O-acetyl-5'-deoxy-5'-methylthio-D-ribofuranosyl)-2-methylthioadenine (VIII<sub>3</sub>).**—A mixture of VII (prepared from 1.4 g. of VIb) and chloromercuri-2-methylthioadenine (from 1.8 g. of 2-methylthioadenine) was heated under the reflux condition described above. 0.3 g. of crystals was obtained, m. p.  $172\sim 174^\circ\text{C}$ .

Found: C, 44.50; H, 5.10; N, 16.18. Calcd. for  $C_{16}H_{21}O_5N_5S_2$  (427.5): C, 44.90; H, 4.95; N, 16.38%.  $R_f(a)=0.72$ ,  $R_f(b)=0.78$ .

**93-(5'-Deoxy-5'-methylthio-D-ribofuranosyl)-2-methylthioadenine (IX<sub>3</sub>).**—From VIII<sub>3</sub>.—VIII<sub>3</sub> (2 g.) was treated with methanolic ammonia as described in section for IX<sub>2</sub>. 1.4 g. of crystals, were obtained m. p.  $165\sim 166^\circ\text{C}$ .

Found: C, 41.50; H, 5.10; N, 19.86. Calcd. for  $C_{12}H_{17}O_5N_5S_2$  (343.4): C, 41.96; H, 4.98; N, 20.39%.  $R_f(a)=0.65$ ,  $R_f(b)=0.78$ .  $\lambda_{\text{max}}$ : 235.5  $m\mu$ ,  $\epsilon=22900$  and 275  $m\mu$ ,  $\epsilon=15000$ ,  $\lambda_{\text{min}}$ : 250  $m\mu$ ,  $\epsilon=8000$  (methanol).

**From XII.**—A mixture of 0.3 g. of XII and 0.5 g. of potassium methyl sulfide in 12 ml. of dimethylformamide was heated at  $100^\circ\text{C}$  for 1.5 hr. and evaporated in vacuo. The residue was dissolved



in chloroform. After the insoluble materials had been filtered off, the solution was washed with water, dried evaporated in vacuo. The obtained syrup was dissolved in 10 ml. of dioxane. 1 N Sulfuric acid (20 ml.) was added, and the solution kept at room temperature for 24 hr. The acid was neutralized with a calculated amount of barium hydroxide solution. Barium sulfate was removed by centrifugation and washed with water, and the combined supernatant liquid and washings were evaporated to dryness in vacuo. The residue was dissolved in 20% ethanol and treated with Amberlite IR-120 as described above. A crystal was obtained m. p. 165~166°C, undepressed on admixture with IX<sub>3</sub>.

**9β - (5' - Deoxy - 5' - methylthio-D-ribofuranosyl)-adenine (I).—**From 6-Acetaminopurine.—A mixture of chloromercuri-6-acetaminopurine (prepared from 2.1 g. of 6-acetaminopurine) and VII (prepared from 2 g. of VIb) was treated as described above. 9β - (2', 3'-Di-O-acetyl-5'-deoxy-5'-methylthio-D-ribofuranosyl)-6-acetaminopurine (1 g.) was obtained. This product was dissolved in 30 ml. of absolute methanol and was added to 35 ml. of methanolic ammonia at 2~4°C. After 24 hr., the solution was evaporated and the residue was dissolved in hot water. After 3 days a crystalline solid was obtained, m. p. 199~201°C. This was recrystallized from water, m. p. 205~206°C, undepressed on

admixture with naturally occurring "adenyl thio-methyl pentoside". The mother liquors of the crystallization and recrystallization were purified with Amberlite IR-120; 0.15 g. of the crystals was obtained.

*From 6-Chloropurine.*—Chloromercuri-6-chloropurine (prepared from 1.5 g. of 6-chloropurine) was condensed with VII (prepared from 1.7 g. of VIb). 1.4 g. of a glass-like material were obtained. Yield from VIb, 63%. This substance was added to 40 ml. of methanol saturated with ammonia at 0°C in a sealed tube, and the mixture was kept at 100°C for 5 hr. The solution was treated as described above. 0.67 g. of the crystals was obtained, m. p. 204~206°C, undepressed on admixture with the natural substance.

Found: C, 44.09; H, 5.29; N, 23.25. Calcd. for C<sub>11</sub>H<sub>15</sub>O<sub>5</sub>N<sub>5</sub>S (297.3): C, 44.43; H, 5.04; N, 23.25%.

Synthetic nucleoside:  $R_f(a)=0.45$ ,  $R_f(b)=0.59$

Natural nucleoside:  $R_f(b)=0.42$ ,  $R_f(b)=0.59$ .

We are grateful to Dr. Katushi Makino for the gift of a sample of natural "adenyl thio-methyl pentoside".

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