Agric. Biol. Chem., **42** (2), 467～469, 1978

Reinvestigation of the Synthesis of 2-Methylthio-*trans*-ribosylzeatin

Tamizi SUGIYAMA, Mimiko IMURA and Takeshi HASHIZUME

Laboratory of Bioorganic Chemistry, Tokyo Institute of Agriculture and Technology, Fuchu, Tokyo 183, Japan

Received July 19, 1977

2-Methylthio-*trans* and *cis*-ribosylzeatin isolated from the plant tRNA were characterized by comparing the mass and ultraviolet spectra with those of their synthesized specimen.[1,2] The *trans* isomer was synthesized in 18% yield by the condensation of 2,6-dimethylthio-9-β-D-ribofuranosylpurine and 4-hydroxy-3-methyl-*trans*-2-butenylamine.[1] The *cis* isomer was prepared by the condensation of 2,6-dichloro-9-β-D-ribofuranosylpurine and 4-hydroxy-3-methyl-*cis*-2-butenylamine, followed by treatment with sodium methyl mercaptide.[2] The yield was calculated to be 12.8%. These methods, however, are unsuitable for synthesis of labeled cytokins which are required for the metabolic and quantitative studies. We, therefore, have reinvestigated the preparation of 6-chloro-2-methylthio-9-β-D-ribofuranosylpurine[3] which is an important intermediate in the synthesis of the both isomers. This paper dealed with an improved synthetic method of 2-methylthio-*trans*-ribosylzeatin from 6-chloro-2-methylthiopurine.

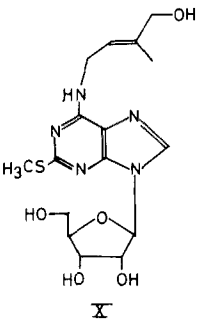
When 6-chloro-2-methylthiopurine[4] was fused with tetra-O-acetyl-D-ribofuranose[5] at 145～150°C for 20 min in the presence of concentrated sulfuric acid, three products (I, II and III) which gave a parent ion peak at *m/e* 458 were detected. These products were separated by silica-gel column chromatography and characterized after deacetylation.

Deacetylation of I, II and III affored IV, V and VI, respectively. Melting point of the compound VI agreed with that of authentic 6-chloro-2-methylthio-9-β-D-ribofuranosylpurine,[3] and no depression was observed on admixing. The structure was also supported by the NMR and UV spectra.

The binding position of sugar moiety in the compound IV was assigned to be N-9, as the UV spectra were similar to those of VI.[3] The anomeric configuration was assigned as α on the basis of NMR spectrum ($J_{1'2'}$=9 Hz).[6] Thus, compound IV was determined as 6-chloro-2-methylthio-9-α-D-ribofuranosylpurine.

The binding position of sugar moiety in the compound V was also inferred to be N-9, because the UV spectra were similar to those of IV and VI.[3] In order to determine further the binding position of sugar moiety, amination was performed to give 2-methylthio-9-pentosyladenine (VII) whose UV spectra were similar to those of 2-methylthioadenosine (VIII).[7] It is unlikely that the sugar moiety of V is ribofuranose, since the admixture of V and VI showed a melting point depression. Thus, 6-chloro-2-methylthio-9-β-D-ribopyranosylpurine (IX) was synthesized by the fusion method, but its physicochemical properties were not identical to those of V. At present the compound V is assumed to be 6-chloro-2-methylthio-9-pentosylpurine since the nucleosides of 6-chloro-2-methylthiopurine were not available for identification.

This fusion procedure was originally reported by Sato who, however, characterized only VI.[3] We now observed the formation of IV, V and VI in this reaction. Although these products were difficult to separate because of the close *Rf* value on tlc, their fully acetylated products (I, II and III) were able to separate.



V

In this manner, 2-methylthio-*trans*-ribosylzeatin (X)[1] was synthesized in 51.4% yield by the condensation of VI and 4-hydroxy-3-methyl-*trans*-2-butenylamine.[8] The NMR spectrum was in agreement with the structure assigned. The overall yield was approximately 14% which is almost twice as much as the conventional procedures, starting from 6-chloro-2-methylthiopurine.

EXPERIMENTAL

All mps were measured on a Yanagimoto melting-point apparatus. UV and NMR spectra were recorded with a Hitachi EPS-3T automatic spectrophotometer and a Hitachi R-24 instrument, respectively. MS spectra were obtained by a Shimadzu LKB 9000, equipped with a direct inlet system.

Fusion of 6-chloro-2-methylthiopurine with tetra-O-acetyl-D-ribofuranose. A mixture of 6-chloro-2-methylthiopurine[4] (1 g) and tetra-O-acetyl-D-ribofuranose[5] (1.6 g) was fused at 145～150°C for 20 min under the diminished pressure (water pump) in the presence of conc. H_2SO_4 (5 mg). The reaction mixture was extracted with chloroform. The chloroform was evaporated, and the residue was subjected to the silica-

TABLE I. UV ABSORPTION SPECTRAL DATA OF TRISUBSTITUTED PURINES

Compounds	0.1 N-HCl in 95% ethanol λ_{max} nm ($\varepsilon\times10^{-3}$)	95% ethanol λ_{max} nm ($\varepsilon\times10^{-3}$)	0.1 N-NaOH in 95% ethanol λ_{max} nm ($\varepsilon\times10^{-3}$)
	235 (17.1)	235.5 (17.6)	265 (14.2)
IV	264 (11.9)	264 (11.9)	285[s] (9.8)
	307 (7.9)	307 (7.8)	305[s] (8.0)
	236.5 (16.4)	236 (17.5)	262 (16.8)
V	264 (11.2)	264 (11.2)	282[s] (13.4)
	307 (8.1)	307 (7.4)	306[s] (9.5)
	238 (17.4)	238 (18.0)	264 (12.8)
VI	265.5 (11.0)	265 (11.0)	285[s] (13.4)
	307 (7.4)	307 (7.4)	306[s] (6.9)
VII	270 (14.2)	235.5 (21.0)	276 (17.4)
		276 (14.5)	
VIII[3,7]	270 (16.0)	237 (24.3)	235 (21.2)
		277 (14.5)	277 (14.7)
	235 (16.1)	235.5 (17.0)	264 (22.3)
IX	265 (12.2)	265 (11.7)	282[s] (18.8)
	307 (7.9)	308 (7.4)	305[s] (11.7)

s=shoulder

gel column (2.1×40 cm). The column was eluted stepwise with 13% (1.5 liter), 15% (0.5 liter), 17% (0.5 liter) and 19% (0.5 liter) ethyl acetate in benzene. The effluent was collected in 20 ml fractions and examined by analytical tlc. I and III were obtained from the fractions 37~49 and 59~120, respectively. II was isolated from the fractions 50~58 containing trace amount of III by preparative tlc.

I. Yield, 335 mg (14.6%). MS *m/e* 458 (M^+). UV λ_{max}^{EtOH} 265 nm. NMR ($CDCl_3$) δ: 8.24 (1H, s, C_8-H), 2.64 (3H, s, SCH_3), 1.83, 2.05, 2.27 (3H, 3H, 3H, s, acetyl protons). *Rf*, 0.32 (benzene: ethyl acetate=2: 1, v/v).

II. Yield, 149 mg (6.5%). MS *m/e* 458 (M^+). UV λ_{max}^{EtOH} 262.5 nm. NMR ($CDCl_3$) δ: 8.23 (1H, s, C_8-H), 2.58 (3H, s, SCH_3), 1.85, 2.05, 2.13 (3H, 3H, 3H, s, acetyl protons). *Rf*, 0.28 (benzene: ethyl acetate=2: 1, v/v).

III. Yield, 882 mg (38.5%). MS *m/e* 458 (M^+). UV λ_{max}^{EtOH} 264.5 nm. NMR ($CDCl_3$) δ: 8.17 (1H, s, C_8-H), 2.63 (3H, s, SCH_3), 2.03, 2.08, 2.13 (3H, 3H, 3H, s, acetyl protons). *Rf*, 0.21 (benzene: ethyl acetate=2: 1, v/v).

6-Chloro-2-methylthio-9-α-D-ribofuranosylpurine (IV). I was dissolved in methanolic ammonia (50 ml). The solution was kept at 4°C for overnight, followed by removal of the solvent. The residue was crystallized from ethanol to give colorless needles. Yield, 162 mg (66.4%). mp, 248~252°C. NMR (DMSO-d_6+D_2O) δ: 8.53 (1H, s, C_8-H), 5.67 (1H, d, J=9 Hz, $C_{1'}$-H), 2.57 (3H, s, SCH_3). MS *m/e* 332 (M^+). *Anal.* found: C, 39.96; H, 3.99; N, 17.18; calcd. for $C_{11}H_{13}O_4N_4SCl$: C, 39.69; H, 3.91; N, 16.84%. *Rf*, 0.19 (ethyl acetate: ethanol=10: 1, v/v).

6-Chloro-2-methylthio-9-pentosylpurine (V). Deacetylation of II in 40 ml of methanolic ammonia yielded V, as described above. Yield, 65 mg (61.7%). mp, 205~208°C. NMR (DMSO-d_6+D_2O) δ: 8.63 (1H, s, C_8-H), 6.44 (1H, d, J=6 Hz, $C_{1'}$-H), 2.62 (3H, s, SCH_3). MS *m/e* 332 (M^+). *Anal.* found: C, 39.59; H, 3.94; N, 16.56; calcd. for $C_{11}H_{13}O_4N_4SCl$: C, 39.69; H, 3.91; N, 16.84%. *Rf*, 0.30 (ethyl acetate: ethanol=10: 1, v/v).

6-Chloro-2-methylthio-9-β-D-ribofuranosylpurine (VI). III was deacetylated with methanolic ammonia (50 ml) to give VI. Recrystallization from ethanol gave a pure product. Yield, 448 mg (70%). mp, 183~184°C (lit. 181°C[5]). NMR (DMSO-d_6+D_2O) δ: 8.70 (1H, s, C_8-H), 5.97 (1H, d, J=6 Hz, $C_{1'}$-H), 2.60 (3H, s, SCH_3). MS *m/e* 332 (M^+). *Anal.* found: C, 39.81; H, 4.01; N, 16.86; calcd. for $C_{11}H_{13}O_4N_4SCl$: C, 39.69; H, 3.91; N, 16.84%. *Rf*, 0.31 (ethyl acetate: ethanol=10: 1, v/v).

2-Methylthio-9-pentosyladenine (VII). A solution of V (20 mg) in methanolic ammonia (5 ml) was heated in a sealed tube at 100°C for 5 hr. The reaction mixture was evaporated to dryness, and the residue was crystallized from ethanol. Yield, 8 mg (42%). mp, 222~223°C. MS *m/e* 313 (M^+). *Anal.* found: C, 42.76; H, 4.77; N, 35.36; calcd. for $C_{11}H_{15}O_4N_5S$: C, 43.08; H, 4.62; N, 35.90%.

6-Chloro-2-methylthio-9-β-D-ribopyranosylpurine (IX). 6-Chloro-2-methylthiopurine[4] (0.4 g) was fused with tetra-O-acetyl-D-ribopyranose[5] (0.7 g) at 145~150°C for 30 min in the presence of conc. H_2SO_4 (3 mg). The reaction mixture was extracted with chloroform. The chloroform was evaporated, and residue was subjected to silica-gel column (2.1×40 cm). The column was eluted with benzene: ethyl acetate (5: 1, v/v). The effluent was collected in 20 ml fractions and examined by analytical tlc. The fractions containing product were combined and evaporated to dryness. The residue was dissolved in methanolic ammonia. The solution was kept at 4°C for overnight, followed by removal of the solvent. The residue was crystallized from ethanol. Yield, 178 mg (27%). mp, 239~246°C. NMR (DMSO-d_6+D_2O) δ: 8.65 (1H, s, C_8-H), 5.71 (1H, d, J=8 Hz, $C_{1'}$-H), 2.60 (3H, s, SCH_3). MS *m/e* 332 (M^+). *Anal.* found: C, 39.71; H, 3.98; N, 16.41; calcd. for $C_{11}H_{13}O_4N_4SCl$: C, 39.69; H, 3.91; N, 16.84%.

2-Methylthio-trans-ribosylzeatin (X) [*6-(4-hydroxy-3-methyl-trans-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine*]. A mixture of VI (100 mg), 4-hydroxy-3-methyl-*trans*-2-butenylamine[8] (150 mg) and

triethylamine (500 mg) in *n*-butanol (10 ml) was refluxed for 4 hr. After cooling, the reaction mixture was evaporated to dryness under the diminished pressure. The residue was subjected to the silica-gel column (1.9 × 22 cm). The column was eluted with a solution of ethyl acetate and ethanol (10: 1, v/v). The fractions containing product were combined and evaporated to dryness. The residue (101 mg) was crystallized from 50% aqueous ethanol to give colorless needles. Yield, 61 mg (51.4%). mp, 154.5~157°C (lit. 155~156°C[1]). MS *m/e* 397 (M^+). *Anal.* found: C, 48.57; H, 5.82; N, 17.58; calcd. for $C_{16}H_{23}O_5N_5S$: C, 48.36; H, 5.79; N, 17.63%.

REFERENCES

1) W. J. Burrows, D. J. Armstrong, M. Kaminek, F. Skoog, F. M. Bock, S. M. Hecht, L. G. Damman, N. J. Leonard and J. Occolowitz, *Biochemistry*, **9**, 1867 (1970).
2) H. J. Verman, R. Y. Schmitz, F. Skoog, A. J. Playtis, C. R. Frihart and N. J. Leonard, *Phytochemistry*, **13**, 31 (1974).
3) T. Sato, "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, ed. by W. W. Zorbach and R. S. Tipson, Wiley-Interscience, 1968, p. 264.
4) C. W. Noell and R. K. Robins, *J. Org. Chem.*, **24**, 320 (1959).
5) G. B. Brown, J. Davoll and B. A. Lowy, *Biochem. Prepn.*, **4**, 70 (1955).
6) L. B. Townsend, "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 2, ed. by W. W. Zorbach and R. S. Tipson, Wiley-Interscience, 1973, p. 267.
7) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **74**, 1563 (1952).
8) G. Show, B. M. Smallwood and D. V. Wilson, *J. Chem. Soc.*, **1966**, 921.