SYNTHESES OF 9-β-D-RIBOFURANOSYLPURINE DERIVATIVES AS EFFECTIVE ANTIRADIATION AGENTS

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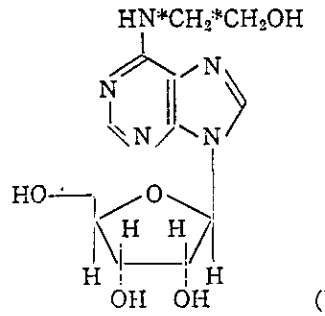
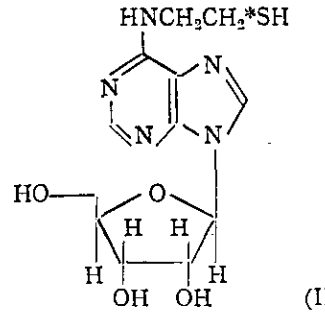
6-(2-Hydroxyethyl-1, 2-^{14}C) amino-9-β-D-ribofuranosylpurine (I) and 6-(2-mercaptoethyl-^{35}S) amino-9-β-D-ribofuranosylpurine (II) were synthesized for the investigation of their in vivo distribution in mice. All the reaction conditions were made by non-radioactive runs and it was found that thiol compound II was easily oxidized to form the disulfide IV in the purification procedure.

Some 9-β-D-ribofuranosylpurine derivatives were known as effective antiradiation agents[1-5]. Among these the radioprotective action of I (1,2) and II (3) was nearly comparable with that of a typical radioprotector β-mercaptoethylguanidine.

The present work is to synthesize these two 9-β-D-ribofuranosylpurine derivatives and their labeled compounds, 6-(2-hydroxyethyl-1, 2-^{14}C)amino-9-β-D-ribofuranosylpurine (I) and 6-(2-mercaptoethyl-^{35}S) amino-9-β-D-ribofuranosylpurine (II), for the investigation of their in vivo distribution in mice.



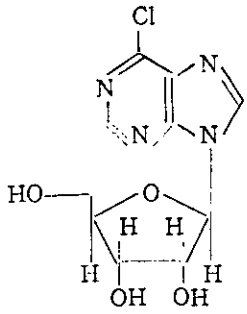
(I)



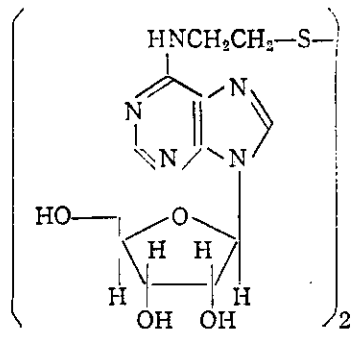
(II)

2′, 3′, 5′-Tri-O-acetylinosine (mp. 243-244.5°, lit. (6) mp. 236-238°) was prepared in 95% yield by treatment of inosine with acetic anhydride in pyridine and recrystalized from ethyl alcohol. The tri-O-acetylinosine was chlorinated with dimethylformamide-thionyl chloride complex[10]* and deacetylated with methanolic ammonia[6] to give 6-chloro-9-β-D-ribofuranosylpurine (III) (mp. 172.5° (decomp.), yield 57% from tri-O-acetylinosine). (lit. (7) mp. 170-171° (decomp.), lit. (8) mp. 168-170° (decomp.)).

* After the decomposition of reaction mixture, there were some crystals precipitated (mp. 228-230° from ethyl alcohol) which was assumed to be 6-chloro-9-(2′, 3′, 5′-tri-O-acetyl-β-D-ribofuranosyl)-purine by means of IR and NMR spectra. But further identification of this compound was not tried.



(III)



(IV)

Compound III was allowed to heat under reflux with ethanolamine-1,2-^{14}C hydrochloride, cysteamine-^{35}S or cystamine hydrochloride in a suitable solvent and in the presence of triethylamine to yield the corresponding N-alkylated adenosine derivatives I, II or IV respectively. The result was summarized in table 1 and all of these reaction conditions were made by non-radioactive runs.

Table 1.

Amine	Solvent	Reaction time (hrs)	Yield (%)
ethanolamine-1, 2-^{14}C HCl salt	isopropanol-ethanol	4	17.4
cysteamine-^{35}S*	isopropanol	9	24
cystamine HCl salt	isopropanol	8.5	50

* In non-radioactive run, the reaction product of III with cysteamine or its HCl salt was Bis-2, 2′-(N-adenosyl)diethyl disulfide (IV) after recrystalization. The presence of thiol group of II was confirmed only by positive test of sodium nitropursside. It seems that thiol group of II was very easily oxidized to form the disulfide IV in this reaction.

The biological activities and in vivo distribution of I and II will be reported in eleswhere.

EXPERIMENTAL**

2′, 3′, 5′-Tri-O-acetylinosine

A mixture of 100 *g* of inosine (0.373 mole), 220 *ml* of acetic anhydride (2.24 mole) and 300 *ml* of pyridine was stirred under reflux for 3 hours. The reaction mixture was evaporated in vacuo and the residue was recrystalized from ethyl alcohol to give tri-

** All melting points were not corrrected, IR spectra were taken with Hitachi-Perkin Elmer model 225 spectrophotometer and NMR spectra were taken with Varian HA-100 spectrometer. Radioactivities were measured with liquid scientilation counter (Nuclear Chicago Mark I). Thin layer chromatography (TLC) was carried out over Kieselgel GF, Merck, in a solvent system of $CHCl_3$: MeOH=7:3.

O-acetylinosine 140 *g* (95.3%) mp. 243-244.5° (lit. (6) mp. 236-238°). IR (KBr, cm^{-1}): 1740 (ester C=O), 1680 (amide C=O). NMR (τ value, in $CDCl_3$): 7.88 (3H, singlet, $-CO-CH_3$), 7.85 (6H, singlet, $-CO-CH_3$), 7.20 (1H, broad singlet, —NH—), 5.56 (3H, singlet, $-\overset{\diagdown}{\underset{\diagup}{C}}-CH_2-O-$ and $O-\overset{|}{C}H-\overset{\diagup}{\underset{\diagdown}{C}}-$), 4.37 (1H, triplet, J=5Hz, $-N-\overset{|}{C}H-\overset{\diagup}{\underset{\diagdown}{C}}-$), 1.89 (1H, singlet, $-N-\overset{|}{C}H-N-$), 1.68 (1H, singlet, $-N-\overset{|}{C}H-N-$).

6-Chloro-9-β-D-ribofuranosylpurine (III)

To a mixture of 27 *g* of thionyl chloride (0.225 mole), 7.5 *ml* of dimethylformamide (0.09 mole) and 450 *ml* of chloroform was added 28.8 *g* of tri-O-acetylinosine (0.073 mole) and the solution was heated under reflux for 6 hours. After standing over night at room temperature, the reaction mixture was poured into ice-water (500 *ml*) and extracted with 100 *ml* of chloroform for 3 times. The organic layer was washed with 1% sodium bicarbonate solution and water to nutral. The organic layer was dried with anhydrous sodium sulfate. After evaporation in vacuo, the residue was dissolved in 100 *ml* of methyl alcohol. The alcohol solution was added to 300 *ml* of dry ammonia saturated methyl alcohol and the whole was stirred at room temperature for 6 hours. After evaporation, the residue was dissolved in 60 *ml* of methyl alcohol and curde III was precipitated after standing over night at room temperature. Curde III was washed with small portion of methyl alcohol then with isopropyl ether to give the brownish crystals of III (10.8 *g*, 51.6%) mp. 172.5° (decomp.) (lit. (7) mp. 170-171° (decomp.), lit. (8) mp. 168-170° (decomp.)). IR (KBr, cm^{-1}): 1115, 1095, 1040 (alcoholic C—O), 820 (C—Cl). NMR (τ value, in DMSO-d_6): 6.30 (2H , doublet, J=5Hz, $-CH_2-O-$), 5.96 (1H, broad multiplet, $-O-\overset{|}{C}H-\overset{\diagup}{\underset{\diagdown}{C}}-$), 5.75 (1H, broad multiplet, $-O-\overset{|}{C}H-\overset{\diagup}{\underset{\diagdown}{C}}-$), 5.36 (1H, broad multiplet, $-O-\overset{|}{C}H-\overset{\diagup}{\underset{\diagdown}{C}}-$), 4.90 (1H, broad singlet, $-\overset{\diagdown}{\underset{\diagup}{C}}-\overset{|}{C}(OH)-\overset{\diagup}{\underset{\diagdown}{C}}-$), 4.75 (1H, broad singlet, $-\overset{\diagdown}{\underset{\diagup}{C}}-\overset{|}{C}(OH)-\overset{\diagup}{\underset{\diagdown}{C}}-$), 4.45 (1H, broad singlet, $-\overset{\diagdown}{\underset{\diagup}{C}}-OH$), 3.90 (1H, doublet, J=5Hz, $-N-\overset{|}{C}H-N-$), 1.18 (1H, singlet, $-N-\overset{|}{C}H-N-$), 1.05 (1H, singlet, $-N-\overset{|}{C}H-N-$).

6-Chloro-9-(2′, 3′, 5′-tri-O-acetyl-β-D-ribofuranosyl)purine ((III)-Tri-O-acetyl compouud)

After the decomposition of excess thionyl chloride with water, as mentioned above, there were some crystals precipitated (2.4 *g*, mp. 228-230° from ethyl alcohol). Structure of which was confirmed by comparison of its IR, NMR spectra and TLC R_f value with III. The R_f value of III and its tri-O-acetyl compound was 0.70 and 0.85 respectively.

IR (KBr, cm^{-1}): 1740 (ester C=O), 1230 (ester C—O—C), 820 (C—Cl). NMR (τ value, in DMSO-d_6): 7.95 (6H, singlet, —CO—CH_3), 7.88 (3H, singlet, —CO—CH_3), 6.58 (1H, broad singlet, —O—CH—C—), 5.63 (2H, doublet, J=5Hz, —O—CH_2—), 4.45 (1H, triplet, J=5Hz, —O—CH—), 4.10 (1H, triplet, J=5Hz, —O—CH—), 3.77 (1H, doublet, J=5Hz, —N—CH—), 1.76 (1H, doublet, J=5Hz, —N—CH—N—), 1.50 (1H, singlet, —N—CH—N—).

6-(2-Hydroxyethyl-1, 2-^{14}C)amino-9-β-D-ribofuranosylpurine (I)

To a mixture of 38.1 *mg* (0.0664 *m*M) of III and 54 *mg* (0.266 *m*M) of triethylamine in 2.7 *ml* of isopropyl alcohol was added 12.9 *mg* (0.0664 *m*M, in ethyl alcohol 2 *ml*, specific activity 1.5*m*Ci/*m*M, total activity 200 micro Ci) of ethanolamine-1, 2-^{14}C hydrochloride. The solution was refluxed under stirring for 4 hours. After evaporation, 10% aqueous isopropyl alcohol was added to the residue and stand for over night in refrigerator. The crystals was collected with centrifugal machine and washed twice with isopropyl alcohol to give I (7.2 *mg*, 17.4%). The R_f value of I was identical with non-radioactive I in TLC and the radiochemical purity was checked with radio activity scanner and autoradiography. The specific activity of I was 5.8 micro Ci/*mg*.

Non-radioactive I was prepared by the same manner and the yield was 74%, mp. 190-191.5° (lit. (2) mp. 198°, mixed melting point 190-192°). TLC gave one spot and its R_f value (0.75) was the same as the authentic sample (2).

6-(2-Mercaptoethyl-^{35}S) amino-9-β-D-ribofuranosylpurine (II)

52 *mg* (0.674 *m*M, specific activity 1.17 *m*Ci/*m*M, total activity 805 micro Ci) of cysteamine-^{35}S was added to the mixture of 193.2 *mg* (0.674 *m*M) of III and triethylamine (68.2 *mg*, 0.674 *m*M) in 20 *ml* of isopropyl alcohol. The reaction mixture was refluxed for 9 hours under stirring. II was collected with centrifugal machine after cooling and washed with isopropyl alcohol and water. Thiol test was positive and the yield was 55 *mg* (25%). TLC R_f value of II was identical with non-radioactive II and its radiochemical purity was checked with radio activity scanner and autoradiography. The specific activity of II was 4.3 micro Ci/*mg*.

Non-radioactive II was prepared by the same manner and the yield was 24%, mp. 171-173° (from aqueous ethanol). It showed negative thiol test after recrystalization. The structure of this compound was confirmed by comparison of its physicochemical data with authentic sample IV, which was prepared by amination of III with cystamine.

Bis-2, 2′-(N-adenosyl)diethyl disulfide (IV)

Cystamine hydrochloride (mp. 201-203°, 213° (decomp.)) was prepared by the iodine oxidation of cysteamine hydrochloride[9]. To a mixture of 115 *mg* (0.5 *m*M) of cystamine hydrochloride and 300 *mg* (3 *m*M) of triethylamine in 50 *ml* of isopropyl alcohol III was added and the reaction mixture was refluxed under stirring for 8.5 hours. After

evaporation of the solvent, 10 *ml* of water was added to the residue and curde IV was precipitated. It was recrystalized from aqueous ethyl alcohol to give IV as a white crystals (155.4 *mg* (50%), mp. 177-178°). Thiol test was negative. IR (KBr, cm^{-1}): 1620 (C—C or C—N double bond), 1120, 1080, 1050 (alcoholic C—O), NMR (τ value, in DMSO-d_6): 6.95 (4H, broad multiplet, $-CH_2-O-$), 6.60 (2H, broad singlet, $\diagdown\!\!\diagup C-OH$), 6.35 (2H, broad singlet, $-O-\underset{|}{C}H-$), 6.18 (4H, broad multiplet, $-CH_2-X$(X=S or N)), 5.80 (2H, broad multiplet, $-O-\underset{|}{C}H-$), 4.63 (4H, broad singlet, $\diagdown\!\!\diagup C-\underset{|}{C}(OH)-C\diagdown\!\!\diagup$), 4.06 (2H, doublet, J=5Hz, $-N-\underset{|}{C}H-$), 2.02 (2H, broad singlet, —NH—), 1.75 (2H, singlet, $-N-\underset{|}{C}H-N-$), 1.62 (singlet, $-N-\underset{|}{C}H-N-$). Anal. Calcd. for $C_{24}H_{32}O_8N_{10}S_2$: C, 44.17; H, 4.94; N, 21.47. Found: C, 44.02; H, 4.76; N, 21.18.

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