ties of Mg^{2+}–ATPase and 5′-nucleotidase in the liver plasma membrane are known to be affected by detergents.[9] Accrording to the report,[9] a 0.1% sodium lauryl sulfate solution inhibited the Mg^{2+}–ATPase and 5′-nucleotidase activities. A 0.1% Triton X–100 solution had no effect on ATPase but stimulated 5′-nucleotidase. Furthermore components in bile, *e.g.* bile salts and bilirubin, were reported to affect the activities of the enzymes.[9,10] Therefore the enzyme activity levels in this experiment are those after affected by these interfering substances. The effect of surfactants and bile components except Triton X–100 are, however, compensated because they exist in samples both before and after the treatment with Triton X–100. Only Triton X–100 might have an uncompensated effect because the initial bile sample does not contain Triton X–100 and 5′-nucleotidase was, as described above, reported to be stimulated with it. Accordingly the effect of Triton X–100 on 5′-nucleotidase was investigated by adding Triton X–100 to a new bile sample in a concentration of 0.1—1.0%. The activity of 5′-nucleotidase was not found to be changed significantly in this concentration range. Consequently the activity of 5′-nucleotidase was shown, as well as the other enzymes, to be elevated by the retrograde infusion of Triton X–100 irrespective of the interfering substances. The increase in protein concentration and the concomitant elevation of the activities of the canalicular enzymes after the treatment with Triton X–100 suggest that the part of the bile canalicular membrane was solubilized by this treatment.

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An Improved Procedure for the Synthesis of 1-Alkyladenines: Removal of the Ribofuranosyl Group from 1-Benzyl-, 1-(3-Methyl-2-butenyl)-, and 1-Allyladenosine Hydrobromide in Acetic Acid

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1-Benzyl- (IIIa), 1-(3-methyl-2-butenyl)- (IIIb), and 1-allyladenine (IIIc) were prepared in 77,% 44%, and 69% yield, respectively, by alkylation of adenosine (I) followed by heating the resulting 1-substituted adenosine hydrobromides (IIa,b,c) in acetic acid.

Keywords——1-alkyladenines; adenosine; alkylation; 1-alkyladenosines; acetolysis; depurinylation

1-Alkyladenines (type III) have assumed considerable importance with the finding that 1-methyladenine (type III: R=CH_3) and other 1-substituted adenines trigger the release of

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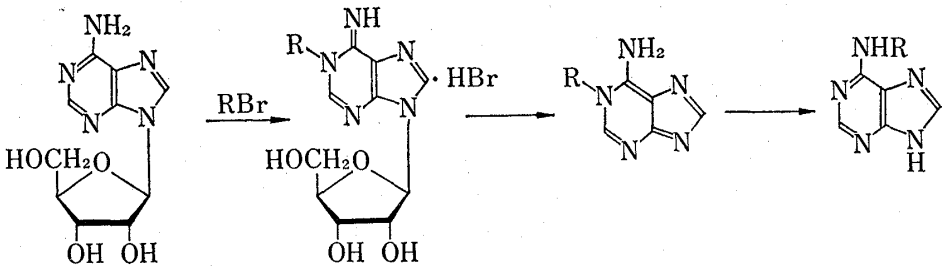
meiosis inhibition in *Marthasterias glacialis* and *Asterias rubens*[3] and with the knowledge that these compounds readily undergo the Dimroth rearrangement to produce N-alkyladenines (type IV), which include highly potent cytokinins in their family.[4,5] Convenient proce-



I IIa, b, c IIIa, b, c IVa, b, c

a: R=$C_6H_5CH_2$ b: R=$(CH_3)_2C{=}CHCH_2$ c: R=$CH_2{=}CHCH_2$

Chart 1

dures for preparation of 1-alkyladenines (type III) have been achieved by alkylation at the 1-position of 9-substituted adenines (type I) followed by removal of the 9-substituents from the resulting 1,9-disubstituted adenine salts (type II).[4,6] The syntheses of 1-benzyladenine (IIIa)[4] and 1-(3-methyl-2-butenyl)adenine (IIIb)[4,7d] according to this reaction sequence are representative of the use of adenosine (I) and hydrolysis of 1-alkyladenosine salts[7] with hydrochloric acid. The object of the present paper is to describe an improvement for the removal of the ribofuranosyl group from the 1-alkyladenosine hydrobromides (IIa,b,c).

When 1-benzyladenosine hydrobromide (IIa), which had been prepared from I in almost quantitative yield,[8] was heated in 0.5 N hydrochloric acid at 95—98° for 45 min, compound IIIa was provided in 52% yield (I→II→III) after neutralizing the aqueous solution of the resulting mixture. Although Brookes, *et al.* claimed that heating 1-benzyladenosine in hydrochloric acid did not yield IIIa,[7e] the result described above confirmed the reproducibility of the earlier reported procedure.[4] The yield was improved by the use of acetic acid as a solvent. Heating a solution of IIa in acetic acid (1 mmole per 4 ml) at 95—100° for 2 hr afforded 1-benzyladenine hydrobromide (IIIa·HBr) in 80% yield (I→II→III·HBr). The hydrobromide (IIIa·HBr) was then converted to the free base (IIIa) in 96% yield. Thus, compound IIIa can now be synthesized from I in 77% overall yield.

Martin and Reese obtained 1-(3-methyl-2-butenyl)adenine hydrobromide (IIIb·HBr) from the crude products of the reaction between 3-methyl-2-butenyl bromide and adenosine in 8.5% yield, when set aside in aqueous solution.[7d] They found that IIIb was completely degraded to adenine by 1 N hydrochloric acid at 95° in 40 min.[7d] In our hands, crude IIb was heated in acetic acid, resulting in an improvement in yield of IIIb to 44%.

The use of acetic acid was also effective in the analogous synthesis of 1-allyladenine (IIIc). This compound was obtained in 69% yield by treatment of the intermediate with

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acetic acid, but in 42% yield by treatment with 0.2 N hydrochloric acid. Since the conventional hydrolysis of 1-methyladenosine and 1-ethyladenosine with hydrochloric acid does not encounter difficulties,[7c,9,10] the additional acetic acid procedure ensures the generality and practical value of the synthetic route (I→II→III).

Experimental[11]

1-Benzyladenine Hydrobromide (IIIa·HBr)——A mixture of I (6.68 g, 25 mmoles), benzyl bromide (12.8 g, 75 mmoles), and N,N-dimethylacetamide (DMAC) (25 ml) was stirred at 28—35° for 45 hr. The resultant solution was evaporated at 3 mmHg to a viscous sirup, which was washed with ether (100 ml). The product was then heated in acetic acid (100 ml) at 95—100° for 2 hr. The precipitate was filtered (5.47 g), mp 238—239° (decomp.). A second crop of identical material (0.65 g, total yield 80%) was obtained from the mother liquor after evaporation and trituration in a mixture of ethanol and ether (1 : 1, v/v). Recrystallization from 90% (v/v) aq. ethanol (after decolorization with charcoal) gave colorless needles, mp 238—239° (decomp.) (lit.[7e] mp 248—250°);[12] UV $\lambda_{max}^{95\%\ EtOH}$ 267 nm (ε 11800); $\lambda_{max}^{H_2O}$ (pH 1) 261 (12800); $\lambda_{max}^{H_2O}$ (pH 7) 269 (11900); $\lambda_{max}^{H_2O}$ (pH 13) 273 (14700). *Anal.* Calcd. for $C_{12}H_{12}N_5Br$: C, 47.07; H, 3.95; N, 22.88. Found: C, 47.14; H, 4.06; N, 22.88.

1-Benzyladenine (IIIa)——i) A sample of IIIa·HBr (1.53 g, 5 mmoles) was dissolved in warm H_2O and the solution was brought to pH 8—9 with conc. aq. NH_4OH. The resulting solid was collected by filtration, washed successively with H_2O (5 ml) and ethanol (5 ml), and dried to give colorless needles (1.08 g, 96%), mp 235—238° (decomp.). Two recrystallizations from ethanol gave an analytically pure sample, mp 235—238° (decomp.) (lit.[4] mp 244—246°);[12] UV $\lambda_{max}^{95\%\ EtOH}$ 229 nm (ε 24100), 274 (12400); $\lambda_{max}^{H_2O}$ (pH 1) 261 (12700); $\lambda_{max}^{H_2O}$ (pH 7) 269 (12000); $\lambda_{max}^{H_2O}$ (pH 13) 273 (14800). *Anal.* Calcd. for $C_{12}H_{11}N_5$: C, 63.98; H, 4.92; N, 31.09. Found: C, 64.08; H, 5.11; N, 31.18.

ii) A mixture of I (3.34 g, 12.5 mmoles), benzyl bromide (6.4 g, 37 mmoles), and DMAC (12.5 ml) was stirred at 29—30° for 48 hr. The solution was evaporated at 3 mmHg to a sirup, which was washed with two 50-ml portions of ether. A solution of the residue in 0.5 N HCl (30 ml) was heated at 95—98° (bath temperature) for 45 min. The ice-cold solution was brought to pH 9 with conc. aq. NH_4OH. The crystals which separated were collected, washed with H_2O (20 ml), and dried to provide 1.48 g (52%) of IIIa, mp 217—221° (decomp.). A single recrystallization from ethanol gave colorless prisms, mp 234—238° (decomp.).

1-Benzyladenine Picrate——This compound was prepared from a portion of IIIa by dissolving it in ethanol and adding a saturated solution of picric acid in ethanol. Recrystallization from 50% (v/v) aq. ethanol gave an analytically pure sample as yellow needles, mp 238—241° (decomp.). *Anal.* Calcd. for $C_{18}H_{14}O_7N_8$: C, 47.58; H, 3.10; N, 24.66. Found: C, 47.54; H, 3.20; N, 24.70.

1-(3-Methyl-2-butenyl)adenine (IIIb)——A mixture of I (1.34 g, 5 mmoles), 3-methyl-2-butenyl bromide (1.54 g, 10.3 mmoles), and DMAC (16 ml) was stirred at 30—31° for 20 hr. The resulting solution was poured into dry ether (200 ml) and the mixture was kept in a refrigerator overnight. The ether solution was decanted and the oily product was heated in acetic acid (16 ml) at 85—88° (bath temperature) for 40 min. The acetic acid was removed under reduced pressure and the resulting sirup was washed with two 20-ml portions of dry ether. The sirup was then dissolved in H_2O (6 ml) and the solution was brought to pH 9 with conc. aq. NH_4OH. After standing overnight in a refrigerator, the precipitate that separated was collected, washed with a little H_2O, and dried to afford 380 mg of IIIb. A second crop (70 mg, total yield 44%) was obtained on concentration of the mother liquor to a small volume. Recrystallization from ethanol gave colorless pillars, mp 233—235° (decomp.) [lit.[4] mp 237—239° (decomp.)];[12] UV $\lambda_{max}^{95\%\ EtOH}$ 228 nm (ε 25400), 273 (12600); $\lambda_{max}^{H_2O}$ (pH 1) 260 (12600); $\lambda_{max}^{H_2O}$ (pH 7) 267 (11800); $\lambda_{max}^{H_2O}$ (pH 13) 272 (15100). *Anal.* Calcd. for $C_{10}H_{13}N_5$: C, 59.09; H, 6.45; N, 34.46. Found: C, 59.10; H, 6.64; N, 34.34. This sample was identified by IR spectrum with that obtained by the hydrolysis with 0.2 N HCl.[4]

1-Allyladenine (IIIc)——A stirred mixture consisting of I (1.34 g, 5 mmoles), allyl bromide (1.74 g, 14.4 mmoles), and DMAC (10 ml) was heated at 55—57° for 12 hr. To the resulting solution was added dry ether (100 ml). The partly crystallized oily precipitate that resulted was separated by decantation and washed with two 20-ml portions of dry ether. The crude product was then heated at 100—105° (bath temperature) with acetic acid (15 ml) for 70 min. After cooling, the solid that separated was collected to give

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11) All melting points are corrected. Ultraviolet (UV) spectra were measured with a Hitachi EPS-2U or a Hitachi Model 323 spectrophotometer on solutions in 95% aq. ethanol, 0.1 N aq. HCl (pH 1), 0.005 M phosphate buffer (pH 7), and 0.1 N aq. NaOH (pH 13). Elemental analyses were performed by Mr. Y. Itatani and Misses M. Imai and Y. Arano at Kanazawa University.
12) Since the 1-alkyladenines (IIIa,b,c) and the hydrobromide (IIIa·HBr) gradually decompose below the melting points, their melting points usually vary widely with the rate of temperature rise.

the hydrobromide of IIIc (876 mg). This was dissolved in H_2O (20 ml) and passed through a column which was packed with Amberlite IRA-402 (HCO_3^-) (8 ml). The column was further eluted with H_2O (70 ml). The eluate was concentrated to a small volume, providing IIIc (603 mg, 69%). Recrystallization from 70% (v/v) aq. ethanol gave colorless needles, mp 214—216° (decomp.) (lit.[6c] mp 227—228°)[12]; UV $\lambda_{max}^{95\%\ EtOH}$ 228 nm (ε 20700), 273 (11800); $\lambda_{max}^{H_2O}$ (pH 1) 260 (12200); $\lambda_{max}^{H_2O}$ (pH 7) 267 (11300); $\lambda_{max}^{H_2O}$ (pH 13) 272 (14500). *Anal.* Calcd. for $C_8H_9N_5$: C, 54.84; H, 5.18; N, 39.98. Found: C, 54.62; H, 5.23; N, 40.08.

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Determination of Total and Free Cholesterol by using Cholesterol Oxidase from *Streptomyces*

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Total and free serum cholesterol was determined by using a new cholesterol oxidase preparation obtained from *Streptomyces*. Cholesterol esters were hydrolyzed by cholesterol esterase, and free cholesterol was oxidized to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide, which oxidatively coupled with 4-aminoantipyrine and phenol to form the quinoneimine dye. The produced color was measured by its absorption at 500 nm.

The results of serum cholesterol determination correlate well with those obtained by Zak-Henly and Kiliani method. Moreover, this enzymatic method is simpler and more sensitive than the conventional method.

Keywords——total cholesterol determination; free cholesterol determination; cholesterol oxidase; cholesterol esterase; quinoneimine dye

Recently, the assay method of serum cholesterol using cholesterol dehydrogenase isolated from *Nocardia* has been reported by Flegg.[2] Afterward, Allain, *et al.*[3] and Masamichi, *et al.*[4] reported the procedures using cholesterol esterase, cholesterol oxidase and peroxidase to determine the total serum cholesterol.

We devised a new method to screen anticholesterol substances produced by microbes on a basis of the phenomenon that polyene antibiotics lost antiyeast activity by cholesterol.[5] In the course of screening, an active substance was found in the culture filtrate of *Streptomyces violascens*. This active substance was identified as a new cholesterol oxidase and purified.[5,6] Through a study of *Streptomyces* cholesterol oxidase, we investigated the possibility to determine serum cholesterol by using our enzyme, and found that total and free serum cholesterol could be estimated in a similar manner to the reported enzymatic method.

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