by isoamyl nitrite (0.09 ml) and the mixture was stirred for 20 min until hydrazine test was negative. The mixture was neutralized with Et_3N (0.70 ml) at −60°. XII (276 mg) was treated with trifluoroacetic acid (1.3 ml) for 20 min as described above. The resulting tripeptide ester trifluoroacetate in DMF (3 ml) was neutralized with Et_3N (0.07 ml) and chilled in an ice bath. To a cold solution of the peptide ester, a cold solution of the dipeptide azide described above was added and stirred at 5° for 24 hr and at room temperature for 1 hr. The reaction mixture was diluted with saturated NaCl and EtOAc. The EtOAc layer was washed and treated as described for the preparation of XII. The resulting oily residue was scratched with ether to give fine powder, yield 251 mg (55%). For analysis a sample was reprecipitated from acetone and ether, mp 101—112°; $[\alpha]_D^{25}$ −18.3° (c=0.6, DMF); de-Boc derivative, *Rf*(A) 0.59, *Rf*(B) 0.81, single ninhydrin positive spot; *Anal.* Calcd. for $C_{32}H_{49}O_{12}N_9 \cdot 2H_2O$: C, 48.78; H, 6.78; N, 16.00. Found: C, 48.55; H, 6.26; N, 16.40.

H-Thr-Pro-Gly-Ser-Arg-OH (XVI)——XV (104 mg) was treated with trifluoroacetic acid (1.5 ml) for 20 min at room temperature. The mixture was diluted with dry ether. The precipitate thereby formed was collected by centrifugation, washed with dry ether and dried over KOH pellets in vacuum. The product was hydrogenated in a mixture of H_2O (25 ml) and AcOH (5 ml) for 24 hr in the presence of 5% Pd-C. The hydrogenated product was treated essentially in the same manner as described for the preparation of III. CMC column (2.0×10.0 cm) was eluted with a linear gradient elution from H_2O (300 ml) in mixing chamber to 0.13M pyridinium acetate (pH 5.1, 300 ml) in reservoir. The eluates in tubes No. 33 to 44 were pooled, evaporated in vacuum and lyophilized; colorless fluffy material, yield 51 mg (58%); mp 122—136°; $[\alpha]_D^{25}$ −48.5° (c=0.3, H_2O); *Rf*(A) 0.17, *Rf*(B) 0.30, single ninhydrin and Sakaguchi positive spot; amino acid ratios in the acid hydrolysate: Thr 0.98, Pro 1.02, Gly 1.02, Ser 1.00, Arg 0.98, (average recovery 74%).

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Purines. XVI.[1] One-Step Alkylation of Adenine 1-Oxide Leading to 1-Alkoxy-9-alkyladenine Hydriodide[2]

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Previous reports[4,5] from this laboratory described the facile synthesis of 1-alkoxy-9-alkyladenine salts (type III·HX) from adenine 1-oxide (I) *via* 1-alkoxyadenines (type II) or from 9-alkyladenine 1-oxides (type IV). The route starting with I consists of three steps (Chart 1),[4a,b] *i.e.*, alkylation of I with an alkyl halide in N,N-dimethylacetamide (DMAC) to give 1-alkoxyadenine salt (II·HX), conversion of II·HX into the corresponding free base (II), and the second alkylation of the base (II) at the 9-position under similar alkylation

1) Paper XV in this series, T. Fujii, F. Tanaka, K. Mohri, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **22**, 2211 (1974).
2) Presented in part at the 37th Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Toyama, October 27, 1973.
3) Location: *13-1 Takara-machi, Kanazawa 920, Japan.*
4) *a*) T. Fujii, T. Itaya, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **13**, 1017 (1965); *b*) T. Fujii and T. Itaya, *Tetrahedron*, **27**, 351 (1971); *c*) *Idem*, *Chem. Pharm. Bull.* (Tokyo), **19**, 1611 (1971).
5) *a*) T. Fujii, C.C. Wu, T. Itaya, and S. Yamada, *Chem. Ind.* (London), **1966**, 1598; *b*) T. Fujii, C.C. Wu, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **19**, 1368 (1971); *c*) T. Fujii, C.C. Wu, T. Itaya, S. Moro, and T. Saito, *ibid.*, **21**, 1676 (1973).

conditions. Although the reaction and after-treatment in each step are very easy and simple, one may feel that such a three-step synthesis is rather tedious. For this reason we investigated the one-step alkylation of I which directly leads to 1-alkoxy-9-alkyladenine hydriodides (type III·HI).

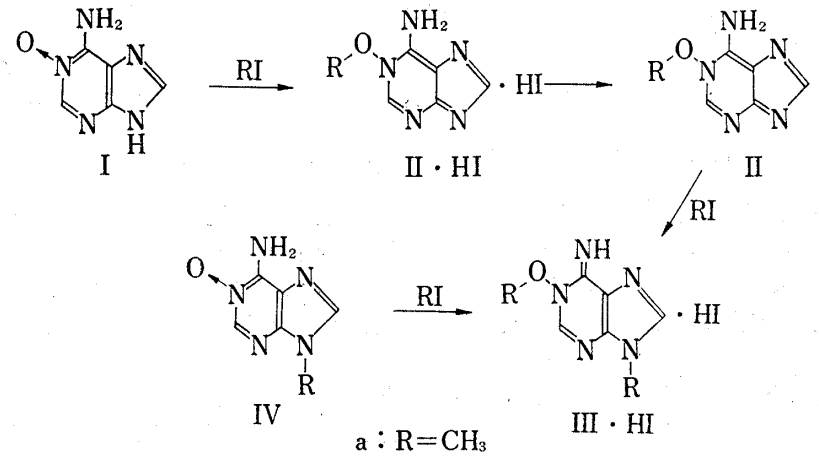

Chart 1

The alkylation examined first was the methylation of I. At first glance the use of two or more equivalents of methyl iodide with one equivalent of an appropriate inorganic base would be a very favorable choice. This scheme, however, was abandoned for the following reasons. First, the orientation of the incoming methyl groups under such basic conditions was as yet uncertain. Secondly, the inorganic base present should cause the 1-methoxy derivatives, if any, to decompose.[6] In the third place, separation of the two possible main products, an inorganic iodide salt and 1-methoxy-9-methyladenine hydriodide (IIIa·HI), might be troublesome. Thus, we tried to convert the intermediate hydriodide (IIa·HI) into the free base (IIa) during the methylation by carrying out the reaction in the presence of an oxidizing agent which is neutral or weakly acid. The principle of the desired conversion was based on the equation:

$$2\,\mathrm{IIa{\cdot}HI} \xrightarrow{[O]} 2\,\mathrm{IIa} + \mathrm{H_2O} + \mathrm{I_2}$$

When the N-oxide (I) was treated with 10 equivalents of methyl iodide and one equivalent of 30% aqueous hydrogen peroxide in DMAC at room temperature for 89 hr, it was possible to obtain IIIa·HI[4a,b] in 70% yield. Since the free base (IIIa) prepared from that sample of the hydriodide (IIIa·HI) by the use of Amberlite IRA-410 (HCO_3^-) did not respond to the Beilstein test for halogen, it became clear that the salt was free from contamination by iodinated derivatives, if any, which might be formed by the action of the iodine liberated. In order to learn the effect of amount of hydrogen peroxide on yield of IIIa·HI, the same methylation was run with a variable amount of the oxidizing agent. It may be seen from Table I that the use of 1.3 equivalents of hydrogen peroxide should be recommendable for this reaction, giving IIIa·HI in 82% yield. The yield of the salt is better than the overall yield recorded in the previous stepwise synthesis.[4a,b] Other oxidizing agents such as peracetic acid and *m*-chloroperbenzoic acid were also found to be effective, but with less satisfactory results.

6) *a*) T. Fujii, T. Itaya, C.C. Wu, and F. Tanaka, *Tetrahedron*, **27**, 2415 (1971); *b*) T. Fujii, T. Sato, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **19**, 1731 (1971); *c*) T. Itaya, F. Tanaka, and T. Fujii, *Tetrahedron*, **28**, 535 (1972); *d*) T. Fujii, T. Itaya, and S. Moro, *Chem. Pharm. Bull.* (Tokyo), **20**, 958 (1972); *e*) *Idem*, *ibid.*, **20**, 1818 (1972).

Table I. Methylation of Adenine 1-Oxide (I) with Methyl Iodide and H_2O_2

Expt. No.[a]	H_2O_2 Amt. (equiv.)	IIIa·HI Yield (%)
1	1.0	70
2	1.3	82
3	1.5	55
4	1.75	38

a) For details of the reaction conditions, see Experimental part.

Next this procedure was applied to the ethylation of I. Since it had been known[4b] that the progress of the reaction with ethyl iodide is slower than that with methyl iodide, the N-oxide (I) was first ethylated, in the absence of hydrogen peroxide, with 10 equivalents of ethyl iodide in DMAC at 80° for 3 hr. The resulting mixture was treated with 1.3 equivalents of 30% aqueous hydrogen peroxide at room temperature for 7 days to produce 1-ethoxy-9-ethyladenine hydriodide (IIIb·HI)[4a,b] in 56% yield.

In an attempted benzylation of I with benzyl bromide under similar oxidizing conditions, we failed in obtaining the desired dibenzylated derivative (III·HBr: R=$C_6H_5CH_2$). This is probably owing to the incapability of hydrogen peroxide to transform the intermediate monobenzylated hydrobromide salt (II·HBr: R=$C_6H_5CH_2$) into the corresponding free base (II: R=$C_6H_5CH_2$) under the reaction conditions used.

Returning to the methylation study I→IIIa·HI, the employment of a larger excess of hydrogen peroxide to effect this transformation led to a significant decrease of the desired product (IIIa·HI) as shown in table I. This suggests the possibility that the dimethylated hydriodide salt (IIIa·HI) is further oxidized to form the free base (IIIa), which is in turn methylated to give a trimethylated product. In practice 1-methoxy-N,9-dimethyladenine as its perchlorate (V: X=ClO_4)[1] (4%) and its ring-opened product, 5-formamido-N′-methoxy-N,1-dimethylimidazole-4-carboxamidine (VI)[1] (16%), could be isolated from the reaction mixture in which I had been treated with 15 equivalents of methyl iodide in the presence of *ca.* 5 equivalents of hydrogen peroxide at 30° for 72 hr. In the light of the recently reported, facile ring opening of the free base of V in water,[1] it is reasonable to assume that VI was derived during the after-treatment from the trimethylated derivative (V) once formed (Chart 2). This finding indicates that the N^6-position is one of the possible sites of alkylation of 1-alkoxy-9-alkyladenines (type IIIa), and the alkylation study to establish the generality is now in progress.

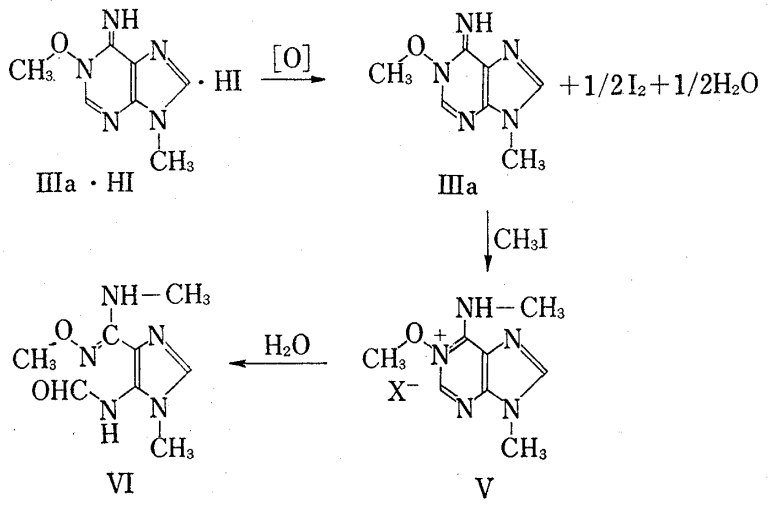

Chart 2

In conclusion, the present alkylation of I with alkyl iodide in the presence of hydrogen peroxide has provided a convenient one-step procedure for preparation of 1-alkoxy-9-alkyladenine hydriodides (type III·HI).

Experimental[7]

Methylation Study (Table I)——A mixture of adenine 1-oxide monohydrate[4b] (I·H_2O: 1.01 g, 6 mmoles), methyl iodide (8.52 g, 60 mmoles), N,N-dimethylacetamide (DMAC) (8 ml), and an appropriate amount of 30% aq. H_2O_2 was stirred at room temperature for 89 hr. The dimethylated product (IIIa·HI) was isolated from the reaction mixture and identified with an authentic sample[4a,b] in a manner similar to that described below for the preparation of IIIa·HI. The results are summarized in Table I.

1-Methoxy-9-methyladenine Hydriodide (IIIa·HI)——A stirred mixture of I·H_2O[4b] (2.53 g, 15 mmoles), methyl iodide (21.3 g, 150 mmoles), DMAC (20 ml), and 30% aq. H_2O_2 (1.15 g, 10 mmoles) was kept at room temperature for 89 hr. The precipitates that resulted were filtered off, washed successively with abs. ethanol and ether, and dried to give IIIa·HI (3.76 g, 82%) as an almost colorless solid, mp 211—213° (decomp.), shown to be homogeneous by paper chromatography (PPC). Recrystallization from 50% aq. ethanol produced colorless pillars, mp 213—215° (decomp.) [lit.[4b] mp 214—215° (decomp.)], identical (by means of PPC and infrared (IR) spectrum) with authentic IIIa·HI.[4b]

A small sample of the hydriodide was treated with Amberlite IRA-410 (HCO_3^-) according to the previously reported procedure,[4b] and the resulting free base (IIIa) showed a negative Beilstein test.

1-Ethoxy-9-ethyladenine Hydriodide (IIIb·HI)——A mixture of I·H_2O[4b] (18.6 g, 110 mmoles), ethyl iodide (171.6 g, 1.1 moles), and DMAC (150 ml) was stirred at 80° for 3 hr. After cooling, 30% aq. H_2O_2 (8.1 g, 71.5 mmoles) was added. The mixture was then stirred at room temperature for 7 days. The precipitates that deposited were collected by filtration, washed successively with abs. ethanol and ether, and dried to furnish IIIb·HI (14.2 g) as a pale yellow solid, mp 175—178° (decomp.), shown to be homogeneous by PPC. The filtrate and washings were combined and from this mixture low boiling substances were removed by vacuum distillation. To the residual solution was added ether (*ca.* 500 ml), and the resulting precipitates were filtered off, washed successively with abs. ethanol and ether, and dried to give a second crop (6.5 g) of IIIb·HI. Total yield, 20.7 g (56%). Recrystallization from H_2O yielded colorless prisms, mp 185—186° (decomp.) [lit.[4b] mp 186° (decomp.)], which were identified with authentic IIIb·HI by means of PPC and IR spectrum.

1-Methoxy-N,9-dimethyladenine Perchlorate (V: X=ClO_4) and 5-Formamido-N′-methoxy-N,1-dimethylimidazole-4-carboxamidine (VI)——A stirred mixture of I·H_2O[4b] (507 mg, 3 mmoles), methyl iodide (6.39 g, 45 mmoles), DMAC (10 ml), and 30% aq. H_2O_2 (860 mg, 7.59 mmoles) was kept at 30° for 72 hr. To the resulting mixture was added a saturated solution (30 ml) of picric acid in abs. ethanol. The precipitates that resulted were filtered off, washed with abs. ethanol, and suspended in 10% aq. HCl (50 ml). The suspension was then extracted with benzene in order to remove picric acid. The aq. layer was separated, neutralized with 10% aq. Na_2CO_3, and evaporated to dryness *in vacuo*. The resulting residue was triturated with hot abs. ethanol (60 ml) and the mixture was filtered. Evaporation of the filtrate left a brownish solid, which was dissolved in H_2O (4 ml) and 24% (w/v) aq. $NaClO_4$ (1 ml) was added. The precipitates that resulted were filtered off, and washed with a little abs. ethanol to give V (X=ClO_4) (34 mg, 3.9%) as a colorless solid, mp 230—231° (decomp.) [lit.[1] mp 235—238° (decomp.)], shown to be identical (by PPC and IR spectrum) with authentic specimen.[1] The aq. filtrate and ethanolic washings, which were obtained on isolation of V (X=ClO_4), were combined and evaporated to dryness *in vacuo*. The residue was triturated with hot abs. ethanol (10 ml) and an insoluble solid was removed by filtration. Evaporation of the filtrate left a reddish solid, which was washed with a little abs. ethanol and recrystallized from abs. ethanol to afford VI (101 mg, 16%) as colorless prisms, mp 187—190° (decomp.) [lit.[1] mp 190—191° (decomp.)], identical (by PPC and IR spectrum) with an authentic sample.[1]

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7) All melting points are corrected. See ref. 6*d* for details of paper chromatography, instrumentation, and measurement.