release of the anticancer drug obtained in the present study may be applicable in transcatheter arterial embolization therapy[9] of cancers in kidney and liver.

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An Improved Procedure for the Synthesis of 1-Alkyl-5-(alkylamino)-imidazole-4-carboxamides, Synthetic Intermediates for 3,9-Dialkylpurine Derivatives[1]

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Heating N,N-diethyl-3,9-dialkyladeninium halides (Ig—j) in aqueous sodium hydroxide gave 1-alkyl-5-(alkylamino)imidazole-4-carboxamides (IV) together with minor amounts of 1-alkyl-5-(alkylamino)imidazole-4-carbonitriles (III), which were converted into IV on further heating. N,N-Dimethyl-3,9-dialkyladeninium halides (Ia—d) underwent hydrolysis more rapidly to provide IV selectively in 90—94% yields.

Keywords——imidazolecarboxamides; imidazolecarbonitriles; cleavage of purine ring; N,N,3,9-tetraalkyladeninium halides; hydrolysis of amidines; base-catalyzed elimination; dehydration

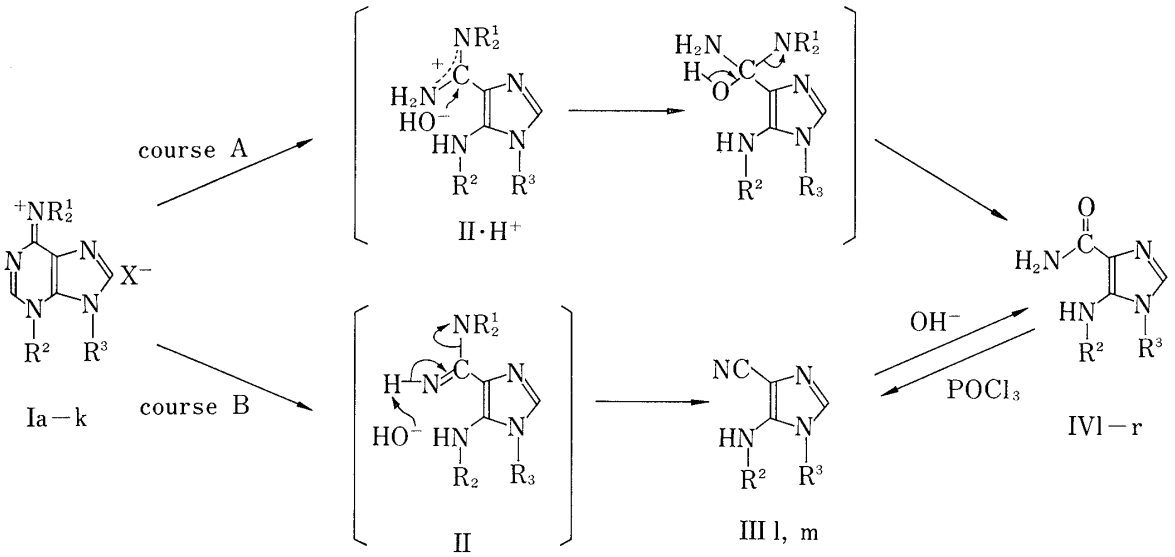
1-Methyl-5-(methylamino)imidazole-4-carboxamide (IVl) was first synthesized by Marsico and Goldman in 1965,[3] and compounds of this type were found to be useful in recent syntheses of various 3,9-dialkylpurine derivatives.[4] These authors obtained IVl in 54% yield with a minor amount of 1-methyl-5-(methylamino)imidazole-4-carbonitrile (IIIl) by heating N,N-diethyl-3,9-dimethyladeninium iodide (Ig) in 1 N aqueous sodium hydroxide at 100° for 2 hr. They suggested that IIIl had resulted from the initially formed N,N-diethyl-1-methyl-5-(methylamino)imidazole-4-carboxamidinium (type II·H^+), and hydrolysis of IIIl then produced IVl. We have investigated the alkaline hydrolysis of N,N,3,9-tetraalkyladeninium halides (I) and wish to describe here a general synthesis of 1-alkyl-5-(alkylamino)imidazole-4-carboxamides (IV).

When Ig was treated under conditions similar to those employed by Marsico and Goldman,[3] IVl was obtained in 73% yield. Brief treatment (15 min) of Ig under the same conditions afforded IIIl in 10% yield as well as IVl (68% yield). The nitrile IIIl gave IVl in 58% yield with a 26% recovery of IIIl on brief treatment.

N,N,9-Triethyl-3-methyladeninium iodide (Ih)[5] underwent hydrolysis similarly to give

1) A part of this work was reported in preliminary form.[4a]
2) Location: *13-1 Takara-machi, Kanazawa 920, Japan.*
3) J.W. Marsico and L. Goldman, *J. Org. Chem.*, **30**, 3597 (1965).
4) *a*) T. Itaya and K. Ogawa, *Heterocycles*, **6**, 965 (1977); *b*) *Idem*, *Tetrahedron Lett.*, **1978**, 2907; *c*) K. Ienaga and W. Pfleiderer, *ibid.*, **1978**, 1447.
5) T. Itaya, K. Ogawa, H. Matsumoto, and T. Watanabe, *Chem. Pharm. Bull.*, **28**, 2522 (1980).

1-ethyl-5-(methylamino)imidazole-4-carboxamide (IVm: 58% yield) and 1-ethyl-5-(methylamino)imidazole-4-carbonitrile (IIIm: 14% yield) on being heated in boiling 1 N aqueous sodium hydroxide for 15 min. Prolonged heating (2 hr) increased the yield of IVm to 74%. Similarly, 5-(ethylamino)- (IVo) and 5-(benzylamino)-1-methylimidazole-4-carboxamide (IVq) were obtained in 75% and 82% yields, respectively, from the corresponding N,N-diethyl-3,9-dialkyladeninium halides (Ii,j).[5] 1-Benzyl-5-(benzylamino)imidazole-4-carboxamide (IVr)[6] was prepared from N,N-diethyl-9-benzyladenine[7] through 3,9-dibenzyl-N,N-diethyladeninium bromide (Ik) in 28% overall yield. The structures of IIIm and IVm,o,q,r were assigned on the basis of ultraviolet (UV) spectral similarity to IIIl or IVl and by comparison of the proton magnetic resonance (PMR) and infrared (IR) spectra with those of IIIl or IVl. 1-Alkyl-5-(alkylamino)imidazole-4-carbonitriles (III) are also considered to be good synthetic intermediates for 3,9-dialkyl-6-aminopurine derivatives and could be easily obtained by treatment of IV with phosphorus oxychloride in the usual manner.[8] Therefore, improvement in the yields of IV was desirable.



a: $R^1=R^2=R^3=CH_3$; X=I
b: $R^1=R^2=CH_3$; $R^3=C_2H_5$; X=I
c: $R^1=R^2=CH_3$; $R^3=C_6H_5CH_2$; X=Br
d: $R^1=R^3=CH_3$; $R^2=C_2H_5$; X=I
e: $R^1=CH_3$; $R^2=R^3=C_2H_5$; X=I
f: $R^1=CH_3$; $R^2=R^3=C_6H_5CH_2$; X=Br
g: $R^1=C_2H_5$; $R^2=R^3=CH_3$; X=I
h: $R^1=R^3=C_2H_5$; $R^2=CH_3$; X=I
i: $R^1=R^2=C_2H_5$; $R^3=CH_3$; X=I
j: $R^1=C_2H_5$; $R^2=C_6H_5CH_2$; $R^3=CH_3$; X=Br
k: $R^1=C_2H_5$; $R^2=R^3=C_6H_5CH_2$; X=Br

l: $R^2=R^3=CH_3$
m: $R^2=CH_3$; $R^3=C_2H_5$
n: $R^2=CH_3$; $R^3=C_6H_5CH_2$
o: $R^2=C_2H_5$; $R^3=CH_3$
p: $R^2=R^3=C_2H_5$
q: $R^2=C_6H_5CH_2$; $R^3=CH_3$
r: $R^2=R^3=C_6H_5CH_2$

Chart 1

To find optimum conditions for the formation of IV, we followed the reactions of Ih by paper chromatography. Fig. 1 shows the time courses of the amounts of IIIm and IVm in the reactions at 80° and at various concentrations of sodium hydroxide. Fig. 2 shows

6) J.A. Montgomery, K. Hewson, S.J. Clayton, and H.J. Thomas, *J. Org. Chem.*, **31**, 2202 (1966).
7) T. Itaya, H. Matsumoto, and K. Ogawa, *Chem. Pharm. Bull.*, **28**, 1920 (1980).
8) Conversion of IVl into IIIl and synthesis of 3,9-dimethylisoguanine from IIIl have been reported.[4c]

the reaction features in 0.2 N aqueous sodium hydroxide at various temperatures. We can see from these figures that IVm is the main product in every case and that the changes in the concentration of sodium hydroxide or the reaction temperature have little effect on the yield of IVm.

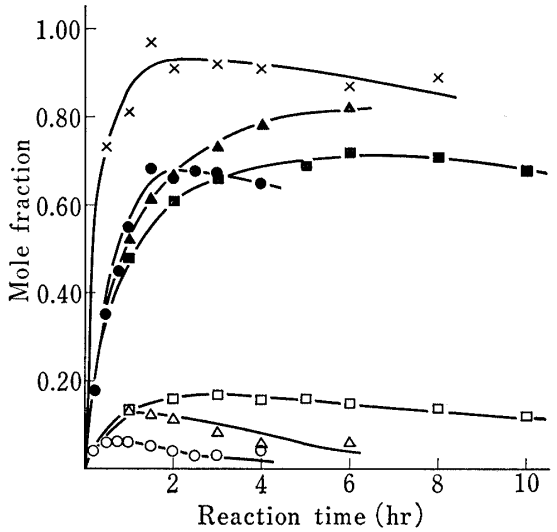


Fig. 1. Variation of the Concentrations of the Products (IIIm and IVm) with Time in the Reactions of 9-Ethyl-N,N,3-trimethyladeninium Iodide (Ib) and N,N,9-Triethyl-3-methyladeninium Iodide (Ih) in Aqueous Sodium Hydroxide at 80°

○ and ● : IIIm and IVm, respectively, in the hydrolysis of Ih in 3 N aq. NaOH,
△ and ▲ : IIIm and IVm, respectively, in the hydrolysis of Ih in 1 N aq. NaOH,
□ and ■ : IIIm and IVm, respectively, in the hydrolysis of Ih in 0.2 N aq. NaOH,
× : IVm in the hydrolysis of Ib in 0.2 N aq. NaOH.

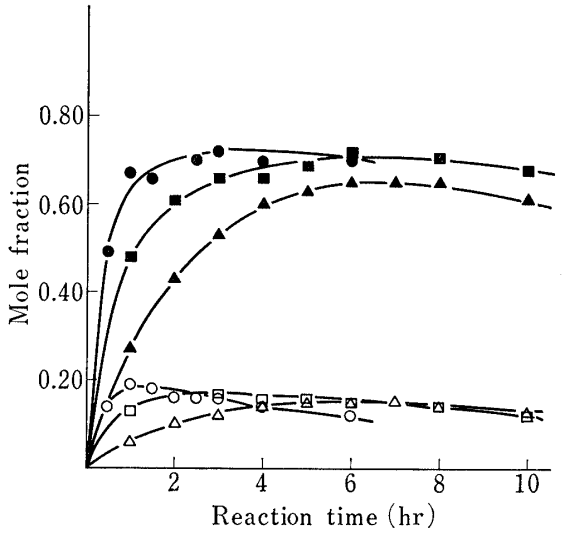


Fig. 2. Variation of the Concentrations of the Products (IIIm and IVm) with Time in the Reaction of N,N,9-Triethyl-3-methyladeninium Iodide (Ih) in 0.2 N Aqueous Sodium Hydroxide at Various Temperatures

○ and ● : IIIm and IVm, respectively, at 90°,
□ and ■ : IIIm and IVm, respectively, at 80°,
△ and ▲ : IIIm and IVm, respectively, at 70°.

The figures also suggest that a major part of IVm was formed competitively with IIIm. Accordingly, the reaction of I in aqueous sodium hydroxide could be depicted as in Chart 1. Since the nucleophilic attack of OH^- on the carbon atom of the amidino group (course A) of II·H^+ ($R^1=C_2H_5$) would suffer steric hindrance to a greater extent than the hydrogen abstraction by OH^- from the amidino group of II (course B), replacement of the diethylamino group of II by the smaller dimethylamino group would favor course A. In fact, the maximum yield of IVm was estimated to be more than 90% in 1.5 hr when 9-ethyl-N,N,3-trimethyladeninium iodide (Ib)[5] was heated at 80° in 0.2 N aqueous sodium hydroxide (Fig. 1). The yield of IIIm was not more than 1% even though the reaction conditions were optimum for the formation of IIIm in the case of the reaction of Ih. On a preparative scale, Ib was heated under reflux in 1 N aqueous sodium hydroxide for 15 min to give IVm in 90% yield. Similarly, the yields of IVl,o,r were improved by the use of the corresponding N,N-dimethyl derivatives (Ia,d,f)[5] as the starting materials. 1-Benzyl-5-(methylamino)- (IVn) and 1-ethyl-5-(ethylamino)imidazole-4-carboxamide (IVp) were also prepared in this way.

The present work, combined with our previously reported studies[5,7] has established an efficient synthesis of IV from N,N-dimethyladenine through 3-alkyl-N,N-dimethyladenines and I, and should permit the syntheses of various 3,9-dialkylpurine derivatives.

Experimental[9]

1-Methyl-5-(methylamino)imidazole-4-carboxamide (IVl)[10]——i) A solution of Ia (20.0 g, 62.7 mmol) in 1 N aq. NaOH (500 ml) was refluxed for 15 min. After cooling, the resulting precipitate was filtered off, washed with cold H_2O (2×10 ml), then dried to give colorless prisms (7.80 g), mp 210—213°. The filtrate and the washings were combined, brought to pH 8 with conc. aq. HCl, concentrated *in vacuo* to a small volume, then extracted with chloroform. The chloroform extracts were dried and chloroform was removed by evaporation. The residue was washed with ethyl acetate–ethanol (1: 1, v/v; 10 ml) then dried to give a second crop (0.90 g). Total yield, 90%. Recrystallization from ethanol gave colorless prisms, mp 211—213°[11] (lit.[3] mp 211—213°). UV $\lambda_{max}^{95\%\ EtOH}$ 270 nm (ε 9700); $\lambda_{max}^{H_2O\ (pH\ 1)}$ 253 (7100), 270 (6600); $\lambda_{max}^{H_2O\ (pH\ 7)}$ 269 (9100); $\lambda_{max}^{H_2O\ (pH\ 13)}$ 269 (9100). PMR δ: 2.84 (3H, bs), 3.55 (3H, s), 5.74 (1H, b), 6.84 (2H, b), 7.18 (1H, s).

ii) A mixture of Ig (11.50 g, 33.1 mmol) and 1 N aq. NaOH (350 ml) was refluxed for 2 hr. The mixture was concentrated to *ca.* 100 ml *in vacuo* then cooled with ice-water. The resulting precipitate was filtered off, washed with cold H_2O, then dried to give colorless prisms (699 mg), mp 211—213°. The filtrate and the washing were combined and extracted continuously with chloroform. The chloroform extracts were dried and evaporated to dryness *in vacuo*. The residue was washed with chloroform (3×5 ml) to give a second crop (2.795 g), mp 211—213°. The washings were evaporated to dryness and the residue was purified on a silica gel (75 g) column. Elution with chloroform–ethanol (8: 1, v/v) afforded a final crop (246 mg), mp 211—213°. Total yield, 3.74 g or 73%.

iii) A mixture of IIIl (136 mg, 1 mmol) and 1 N aq. NaOH (10 ml) was refluxed for 15 min. After cooling, the mixture was brought to pH 8 and the resulting precipitate was filtered off, washed with a little H_2O, then dried to give recovered IIIl (20 mg). The filtrate and the washing were combined and evaporated to dryness *in vacuo*. The solid residue was extracted with hot ethanol (3×10 ml). The extracts were combined and evaporated to dryness after adding silica gel (1 g). The solid was placed on top of a 5-g silica gel column. The column was eluted with ethyl acetate–ethanol. A second crop of IIIl (15 mg) was obtained as the less polar product. Compound IVl (89 mg, 58%) was obtained as the more polar product.

1-Ethyl-5-(methylamino)imidazole-4-carboxamide (IVm)[10]——A solution of Ib (16.6 g, 49.8 mmol) in 1 N aq. NaOH (420 ml) was treated in a similar manner to that described for IVl [method (i)] to give 7.51 g (90%) of IVm. Recrystallization from ethanol gave colorless plates, mp 176—177°. *Anal.* Calcd for $C_7H_{12}N_4O$: C, 49.98; H, 7.19; N, 33.31. Found: C, 49.89; H, 7.22; N, 33.30. UV $\lambda_{max}^{95\%\ EtOH}$ 270 nm (ε 9300); $\lambda_{max}^{H_2O\ (pH\ 1)}$ 254 (6800), $\lambda_{shoulder}$ 267 (6500); $\lambda_{max}^{H_2O\ (pH\ 7)}$ 268 (8900); $\lambda_{max}^{H_2O\ (pH\ 13)}$ 268 (8900). PMR δ: 1.32 (3H, t, J=7 Hz), 2.83 (3H, d, J=6 Hz), 3.95 (2H, q, J=7 Hz), 5.75 (1H, bq, J=6 Hz), 6.86 and 6.95 (1H each, b), 7.30 (1H, s).

1-Benzyl-5-(methylamino)imidazole-4-carboxamide (IVn)——This compound was prepared from Ic[5] (17.41 g, 50 mmol) by refluxing in 1 N aq. NaOH (400 ml) for 15 min in 94% yield. Recrystallization from ethanol gave colorless needles, mp 182—183°. *Anal.* Calcd for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33. Found: C, 62.55; H, 6.19; N, 24.55. UV $\lambda_{max}^{95\%\ EtOH}$ 270 nm (ε 10500); $\lambda_{max}^{H_2O\ (pH\ 1)}$ 257 (7800); $\lambda_{max}^{H_2O\ (pH\ 7)}$ 269 (9900); $\lambda_{max}^{H_2O\ (pH\ 13)}$ 269 (9900). PMR δ: 2.70 (3H, d, J=6 Hz), 5.19 (2H, s), 5.77 (1H, bq, J=6 Hz), 6.85 and 6.95 (1H each, b), 7.32 (6H, m).

5-(Ethylamino)-1-methylimidazole-4-carboxamide (IVo)[10]——A solution of Id (20.0 g, 60 mmol) in 1 N aq. NaOH (500 ml) was treated as described for IVl [method (i)], giving 9.19 g (91%) of IVo. Recrystallization from ethyl acetate gave colorless prisms, mp 195—196°. *Anal.* Calcd for $C_7H_{12}N_4O$: C, 49.98; H, 7.19; N, 33.31. Found: C, 49.89; H, 7.37; N, 33.32. UV $\lambda_{max}^{95\%\ EtOH}$ 269 nm (ε 9500); $\lambda_{max}^{H_2O\ (pH\ 1)}$ 252 (6800), $\lambda_{shoulder}$ 266 (6100); $\lambda_{max}^{H_2O\ (pH\ 7)}$ 267 (9300); $\lambda_{max}^{H_2O\ (pH\ 13)}$ 267 (9300). PMR δ: 1.09 (3H, t, J=7 Hz), 3.16 (2H, m), 3.54 (3H, s), 5.66 (1H, b), 6.86 and 6.95 (1H each, b), 7.27 (1H, s).

1-Ethyl-5-(ethylamino)imidazole-4-carboxamide (IVp)[10]——A mixture of 3-ethyl-N,N-dimethyladenine[7] (10.0 g, 52.3 mmol), ethyl iodide (12.5 ml), and N,N-dimethylacetamide (DMAc) (140 ml) was kept at 40° for 168 hr, then evaporated to dryness *in vacuo*. The solid residue was washed with benzene (70 ml), dried, then dissolved in 1 N aq. NaOH (350 ml). The solution was refluxed for 15 min and treated in a manner similar to that described for IVl [method (i)] to give 5.41 g (57% overall yield) of IVp, mp 143—144°. Recrystallization from ethyl acetate gave colorless pillars, mp 143—144°. *Anal.* Calcd for $C_8H_{14}N_4O$: C,

9) Melting points are corrected. Spectra reported herein were measured with a Hitachi 323 UV spectrophotometer, a JEOL JNM-PS-100 or a JEOL JNM-FX 100 NMR spectrometer, or a JASCO IRA-2 IR spectrophotometer. UV spectra were measured using solutions in 95% ethanol, 0.1 N aq. HCl (pH 1), 0.005 M phosphate buffer (pH 7), and 0.1 N aq. NaOH (pH 13). PMR spectra were determined in 0.25 M solutions in hexadeuterated dimethyl sulfoxide at 23—25° using tetramethylsilane as an internal standard. The following abbreviations are used: b=broad, d=doublet, m=multiplet, q=quartet, s=singlet, t=triplet.

10) A new synthesis of this compound was reported recently (T. Fujii, T. Saito, and M. Kawanishi, *Tetrahedron Lett.*, **1978**, 5007).

11) Routine C, H, N analyses were consistent with the calculated values within ±0.3% for this compound.

52.73; H, 7.74; N, 30.75. Found: C, 52.75; H, 7.90; N, 30.66. UV $\lambda_{max}^{95\%\ EtOH}$ 269 nm (ε 9200); $\lambda_{max}^{H_2O\ (pH\ 1)}$ 253 (6600), $\lambda_{shoulder}$ 267 (6100); $\lambda_{max}^{H_2O\ (pH\ 7)}$ 267 (9200); $\lambda_{max}^{H_2O\ (pH\ 13)}$ 267 (9200). PMR δ: 1.09 (3H, t, J=7 Hz), 1.32 (3H, t, J=7 Hz), 3.11 (2H, m), 3.88 (2H, q, J=7 Hz), 5.59 (1H, bt, J=7 Hz), 6.80 and 6.94 (1H each, b), 7.28 (1H, s).

5-(Benzylamino)-1-methylimidazole-4-carboxamide (IVq)[10]——Treatment of Ij (3.00 g, 7.97 mmol) in a manner similar to that described for IVl [method (ii)] gave IVq (1.50 g, 82%). Recrystallization from ethyl acetate gave colorless needles, mp 161—162°. *Anal.* Calcd for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33. Found: C, 62.42; H, 6.18; N, 24.31. UV $\lambda_{max}^{95\%\ EtOH}$ 270 nm (ε 9900); $\lambda_{max}^{H_2O\ (pH\ 1)}$ 253 (7200); $\lambda_{max}^{H_2O\ (pH\ 7)}$ 268 (8900); $\lambda_{max}^{H_2O\ (pH\ 13)}$ 268 (8800). PMR δ: 3.55 (3H, s), 4.41 (2H, d, J=7 Hz), 6.36 (1H, bt, J=7 Hz), 6.89 and 6.97 (1H each, b), 7.21 (1H, s), 7.36 (5H, m).

1-Benzyl-5-(benzylamino)imidazole-4-carboxamide (IVr)——A mixture of 3-benzyl-N,N-dimethyladenine[7] (2.026 g, 8 mmol), benzyl bromide (4.1 g, 24 mmol), and DMAc (8 ml) was kept at 40° for 72 hr and evaporated to dryness. The resulting heavy oil was washed with ether (2×10 ml) and then heated under reflux in 1 N aq. NaOH (50 ml) for 30 min with vigorous stirring. After cooling, the mixture was extracted with chloroform (2×40 ml). The chloroform extracts were dried and evaporated to dryness to leave a slightly yellow semisolid. Crystallization from ethanol (5 ml) gave a colorless solid (863 mg, 35% overall yield), mp 154—155°. Recrystallization from ethanol gave colorless plates, mp 155—156°[11] (lit.[6] mp 153°). UV $\lambda_{max}^{95\%\ EtOH}$ 268 nm (ε 10000); $\lambda_{max}^{H_2O\ (pH\ 1)}$ 257 (7700); $\lambda_{max}^{H_2O\ (pH\ 7)}$ 268 (8800); $\lambda_{max}^{H_2O\ (pH\ 13)}$ 268 (8800). PMR δ: 4.26 (2H, d, J=7 Hz), 5.17 (2H, s), 6.22 (1H, bt, J=7 Hz), 6.88 and 7.04 (1H each, b), 7.27 (11H, m).

1-Methyl-5-(methylamino)imidazole-4-carbonitrile (IIIl)——i) A mixture of Ig (6.94 g, 20 mmol) and 1 N aq. NaOH (200 ml) was refluxed for 15 min, brought to pH 8, concentrated to *ca.* 50 ml, then extracted with chloroform using a continuous extractor. The chloroform extracts were dried and evaporated to dryness. The solid residue was recrystallized from ethanol to give IVl (1.32 g), mp 211—213°. The mother liquor was evaporated to dryness and the residue was recrystallized from H_2O, giving IIIl (242 mg), mp 242—243°. The mother liquor was evaporated to dryness again and the residue was purified on a silica gel column (22×300 mm) in a manner similar to that described for the preparation of IVl [method (iii)] to give 30 mg of IIIl, mp 241—243°, and 773 mg of IVl, mp 210—212°. Recrystallization of crude IIIl from H_2O gave colorless plates, mp 243—244.5°[11] (lit.[3] mp 244—245°). UV $\lambda_{max}^{95\%\ EtOH}$ 251 nm (ε 12700); $\lambda_{max}^{H_2O\ (pH\ 1)}$ 241 (10000), 261 (7700); $\lambda_{max}^{H_2O\ (pH\ 7)}$ 251 (11200); $\lambda_{max}^{H_2O\ (pH\ 13)}$ 251 (11200). PMR δ: 2.94 (3H, d, J=5 Hz), 3.35 (3H, s), 6.29 (1H, bq, J=5 Hz), 7.20 (1H, s).

ii) Triethylamine (3 ml) and $POCl_3$ (1.10 g, 7.2 mmol) were added to a solution of IVl (925 mg, 6 mmol) in chloroform (100 ml). The solution was allowed to stand at room temp. for 1 hr, then evaporated to dryness *in vacuo*. The residue was neutralized with 10% aq. NaOH. The resulting precipitate was filtered off, washed with H_2O (10 ml), then dried to give a solid (686 mg, 84%), mp 240—242.5°. Recrystallization from H_2O gave colorless plates, mp 243—244.5°, identical (IR spectrum and mixed melting-point test) with those from (i).

1-Ethyl-5-(methylamino)imidazole-4-carbonitrile (IIIm)——i) A solution of Ih (3.61 g, 10 mmol) in 1 N aq. NaOH (100 ml) was refluxed for 15 min, concentrated *in vacuo* to *ca.* 30 ml after being brought to pH 8, then extracted with chloroform (5×30 ml). The combined chloroform extracts were dried and concentrated to 10 ml. This was chromatographed on a column of Merck alumina 90 (100 g). The column was eluted with ethyl acetate (300 ml) then with mixed solvents of ethyl acetate and ethanol (200 ml: 10 ml, 200 ml: 20 ml, then 200 ml: 40 ml). Compound IIIm (0.21 g, 14%), mp 164—165.5°, as the less polar material, and IVm (0.97 g, 58%), mp 176—177°, as the more polar material, were obtained. Recrystallization of crude IIIm from benzene gave colorless pillars, mp 165—166°. *Anal.* Calcd for $C_7H_{10}N_4$: C, 55.98; H, 6.71; N, 37.31. Found: C, 55.92; H, 6.64; N, 37.37. IR ν_{max}^{Nujol} cm^{-1}: 3210, 3150, 3070, and 3030 (NH), 2190 (C≡N). UV $\lambda_{max}^{95\%\ EtOH}$ 251 nm (ε 13600); $\lambda_{max}^{H_2O\ (pH\ 1)}$ 242 (10600), 262 (8300); $\lambda_{max}^{H_2O\ (pH\ 7)}$ 252 (12000); $\lambda_{max}^{H_2O\ (pH\ 13)}$ 252 (12000). PMR δ: 1.22 (3H, t, J=7 Hz), 2.96 (3H, d, J=5 Hz), 3.79 (2H, q, J=7 Hz), 6.28 (1H, bq, J=5 Hz), 7.26 (1H, s).

ii) A mixture of IVm (672 mg, 4 mmol), $POCl_3$ (0.74 g, 4.8 mmol), triethylamine (2 ml), and chloroform (40 ml) was treated in the same way as IIIl [method (ii)] to give 450 mg (75%) of slightly yellow prisms, mp 164—165°. Recrystallization from benzene gave colorless pillars, mp 165—166°, identical (IR spectrum and mixed melting-point test) with those from (i).

Determination of Amounts of IIIm and IVm in the Hydrolysis of Ib,h——Reactions in 1 N and 3 N aq. NaOH at 80°: A solution of an exact amount of Ih (80—100 mg) in aq. NaOH (3.00 ml) was kept at 80±0.05°. At intervals a portion of the solution was withdrawn and chilled with ice-water to room temp., then 0.20 ml of this solution was mixed with acetic acid (0.20 ml). A portion (10 μl) of this solution was applied to Toyo Roshi No. 51A filter paper. The chromatogram was developed by the ascending method in a solvent system of 1-butanol: conc. aq. NH_4OH: H_2O (4: 1: 1, v/v). A spot whose *Rf* value corresponded to that of IIIm or IVm was located under a UV lamp, excised, and extracted with 0.1 N aq. HCl (5.0 ml) by shaking at room temp. overnight. The optical density of the extracts at 262 nm (for IIIm) or 267 nm (for IVm) was determined, and the concentrations of IIIm and IVm were estimated from calibration curves which had been constructed using analytically pure samples.

Reactions in 0.2 N aq. NaOH at 70°, 80°, and 90°: A solution of Ib (92.3 mg) or Ih (100.0 mg) in 0.2 N aq. NaOH (6.00 ml) was allowed to react at the requisite temperature. At intervals a portion (0.50 ml) of the solution was mixed with acetic acid (0.20 ml) to give a sample solution for paper chromatography. Concentrations of IIIm and IVm were determined as described above.

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Significance of Stomach-Emptying-Controlled Rabbits for GI Absorption Studies of Water-Soluble Drugs

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The usefulness of stomach-emptying- (SE-) controlled rabbits for GI absorption studies of relatively water-soluble drugs was investigated, employing aminopyrine and sulfisoxazole as model drugs.

When aminopyrine was administered, similar plasma level-time curves were obtained in both SE-controlled and conventionally fasted rabbits. In the case of sulfisoxazole, the time required to reach the peak plasma level was much longer in conventionally fasted rabbits than in the SE-controlled group.

It was concluded that use of conventionally fasted rabbits for GI absorption studies should be limited to work on water-soluble drugs.

Keywords——stomach-emptying-controlled rabbit; bioavailability; water-soluble drug; aminopyrine; sulfisoxazole

There is considerable evidence that drug bioavailability may be influenced by the presence of food in the GI tract.[2,3] The idea that prohibiting the coprophagy of a rabbit may avoid the delay of gastric emptying rate (GER) by gastric contents led to a series of studies using stomach-emptying-(SE-) controlled rabbits,[4–8] employing practically insoluble drugs such as griseofulvin,[4,5] indomethacin,[4] nalidixic acid,[4] indoxole,[6] and chloramphenicol palmitate[8] as models.

On the other hand, using radioactive tracer techniques the GERs of both the solid and the liquid components of a meal were measured simultaneously; it was demonstrated that liquid left the stomach more rapidly than solids.[9,10] This suggests that conventionally fasted rabbits may also be available for GI absorption studies of water-soluble drugs.

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