in ethanolic NaOH under reflux for 30 min. After cooling, the reaction mixture was neutralized with dil. HCl to deposit a crystalline solid. The solid was washed with H_2O, dried and recrystallized from EtOH to give VI, mp 124°, as red needles (0.7 g). *Anal.* Calcd. for $C_{13}H_{12}O_2N_2S$: C, 59.99; H, 4.65; N, 10.77. Found: C, 59.60; H, 4.50; N, 10.68. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH).

1-Methylthio-7-chlorophenazine (V)——VI (1.7 g, 0.005 mole), powdered potassium hydroxide (1.4 g, 0.025 mole), and xylene (100 ml) was stirred and heated under reflux for 7 hr. The reaction mixture was filtered whilst hot and the residue was washed with hot xylene. The combined filtrates were evaporated to dryness and the residue was dissolved in $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and dried over anhydrous Na_2SO_4. The $CHCl_3$ solution was chromatographed on silica gel to isolate yellow crystals. Recrystallization of the crystals gave V, mp 189°, as yellow needles (0.13 g). *Anal.* Calcd. for $C_{13}H_9N_2SCl$: C, 59.88; H, 3.48; N, 10.74. Found: C, 59.59; H, 3.77; N, 10.50. NMR ($CDCl_3$) δ: 8.39—7.30 (6H, multiplet, aromatic protons), 2.65 (3H, singlet, SCH_3). V thus prepared was identical in infrared, nuclear magnetic resonance and Mass spectra with a sample obtained by irradiation of I followed by deoxygenation.

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A Convenient Synthesis of 1-(2-Halogenoethyl)-3-nitro-1-nitrosoguanidines

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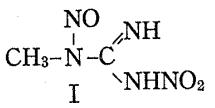
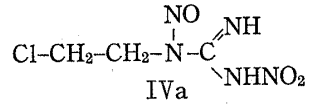
1-Methyl-3-nitro-1-nitrosoguanidine (I) has recently received much attention from the fact that, when continuously administered by dissolving in drinking water, it induces adenocarcinoma in the glandular stomach of rats with high frequency.[2] While, Skinner, *et al.*[3] reported that 1-(2-chloroethyl)-3-nitro-1-nitrosoguanidine (IVa) showed carcinostatic action against intraperitoneal L 1210.



I



IVa

In the preceding paper,[4] the present author reported that, among 1-alkyl(R=C_{1-9})-3-nitro-1-nitrosoguanidines, the chloroethyl compound IVa showed the most potent mutagenicity for bacteria, though the mutagenicity of I was remarkably high in comparison with those of other 1-alkyl derivatives.

This paper describes a convenient synthesis of 1-(2-halogenoethyl)-3-nitro-1-nitrosoguanidines (IVa, b, c). Since introduction of an N-(2-chloroethyl)-N-nitroso group [Cl-CH_2-CH_2-N(NO)-] into a molecule is sometimes difficult because of easy cyclization, this synthetic method involving an ethylenimine derivative as an intermediate will be useful for the synthesis of this type of compounds.

As shown in Chart 1, treatment of I with ethylenimine gave 1,1-dimethylene-3-nitroguanidine (II) in 82% yield, of which nuclear magnetic resonance (NMR) spectrum in hexa-

1) Location: *Kamiyoga 1-18-1, Setagaya, Tokyo.*
2) T. Sugimura, M. Nagao, and Y. Okada, *Nature*, **210**, 962 (1966); T. Sugimura and S. Fujimura, *ibid.*, **216**, 943 (1967); T. Sugimura, S. Fujimura, and T. Baba, *Cancer Res.*, **30**, 455 (1970).
3) W.A. Skinner, H.F. Gram, M.O. Green, J. Greenberg, and B.R. Baker, *J. Med. Pharm. Chem.*, **2**, 299 (1960).
4) S. Iwahara, K. Yanagimachi, S. Kamiya, M. Nakadate, and I. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **19**, 1914 (1971).

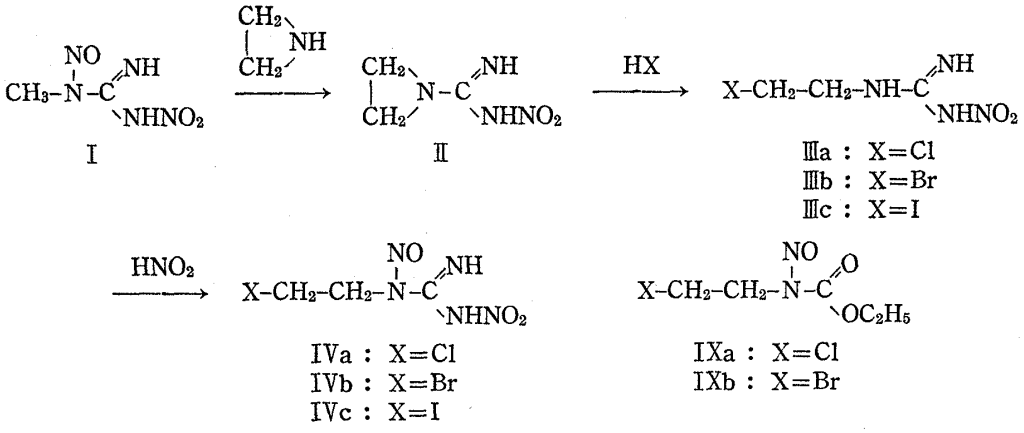


Chart 1

deuterodimethylsulfoxide (DMSO-d_6) indicated a 4H singlet at 7.78 τ, due to the ethylenimine moiety, and a broad 2H signal at 0.73 τ, due to two guanidine NH. The structure of II was further confirmed by its catalytic hydrogenation in the presence of palladium on charcoal. Namely, on absorption of one molar equivalent of hydrogen, compound II afforded 1-ethyl-3-nitroguanidine (V) together with a small amount of 1-ethyl-3-nitrosoguanidine (VI) (Chart 2).

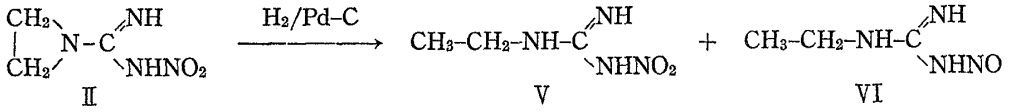


Chart 2

When II was treated with 20% hydrochloric acid at room temperature, 1-(2-chloroethyl)-3-nitroguanidine (IIIa) was obtained in 82% yield. 1-(2-Bromoethyl)-3-nitroguanidine (IIIb) and 1-(2-iodoethyl)-3-nitroguanidine (IIIc) were analogously prepared from the reaction of II with the corresponding halogenic acids, in 85 and 92% yields, respectively.

Treatment of IIIa with sodium nitrite and nitric acid under ice-cooling afforded 1-(2-chloroethyl)-3-nitro-1-nitrosoguanidine (IVa) in 72% yield. The NMR spectrum of IVa showed an A_2B_2 pattern at 5.92 and 6.43 τ, due to the Cl-CH_2-CH_2-N(NO)- group. The same treatment of IIIb and IIIc gave 1-(2-bromoethyl)-3-nitro-1-nitrosoguanidine (IVb) and 1-(2-iodoethyl)-3-nitro-1-nitrosoguanidine (IVc) in 58 and 41% yields, respectively.

This method was then successfully applied to the synthesis of N-(2-chloroethyl)-N-nitrosourethan (IXa) and the corresponding bromo compound IXb, both in good yields (Chart 1).

Experimental[5]

1,1-Dimethylene-3-nitroguanidine (II)——A solution of 2.15 g (0.05 mole) of ethylenimine in 5 ml of water was added dropwise into a suspended solution of 5.88 g (0.04 mole) of 1-methyl-3-nitro-1-nitrosoguanidine[6] (I) in 30 ml of water under ice-cooling, with stirring. After the addition of the ethylenimine solution was completed, the stirring was continued for further 30 min. The leaflets separated were filtered, washed with ice-water and dried in an evacuated desiccator. This product was recrystallized from a mixture of methanol and ethanol to give colorless prisms, mp 138—139°. Yield, 4.59 g (82%). *Anal.* Calcd. for $C_3H_6O_2N_4$: C, 27.69; H, 4.65; N, 43.07. Found: C, 28.07; H, 5.00; N, 43.06.

5) All melting points are uncorrected. Infrared (IR) spectra were measured on a JASCO Model-S spectrophotometer. NMR spectra were measured on a Japan Electron Optics JNM-C-60H spectrometer, and tetramethylsilane was used as an internal standard for DMSO-d_6 and $CDCl_3$.

6) Purchased from Aldrich Chemical Co. Inc., Milwaukee, U.S.A.

1-(2-Halogenoethyl)-3-nitroguanidines (IIIa, b, c)——A general procedure for these 1-(2-halogenoethyl)-3-nitroguanidines is described with IIIa. To 4 ml of 20% hydrochloric acid was added 0.52 g (0.004 mole) of I at room temperature, and the mixture was stirred for 15 min. The colorless leaflets separated were filtered, washed with ice-water, and dried in an evacuated desiccator. This compound melted at 105—107° with resolidification, finally melting again at 185° with decomposition (Reported,[7] 116—117°, remelted at 185° (decomp.)). IR ν_{max}^{Nujol} cm^{-1}: 3400, 3180 (NH), 1252 (NNO_2). Yield, 0.55 g (82%). Since this compound gave a cyclic product on recrystallization from ethanol or methanol, these compounds IIIa, b, c were all nitrosated without purification.

1-(2-Bromoethyl)-3-nitroguanidine (IIIb): Colorless leaflets, mp 105—106° with resolidification, then remelted at 173° with decomposition (Reported,[7] 102—103°, remelted at 179—180° (decomp.)). Yield, 85%.

1-(2-Iodoethyl)-3-nitroguanidine (IIIc): Brownish leaflets, mp 108—109° with resolidification, then melted at about 140° with decomposition. Yield, 92%. *Anal.* Calcd. for $C_3H_7O_2N_4I$: C, 13.96; H, 2.73; N, 21.71. Found: C, 13.72; H, 2.61; N, 21.42.

Catalytic Hydrogenation of 1,1-Dimethylene-3-nitroguanidine (II): Formation of 1-Ethyl-3-nitroguanidine (V) and 1-Ethyl-3-nitrosoguanidine (VI)——A solution of 0.65 g (0.005 mole) of II in 40 ml of methanol was hydrogenated in the presence of palladium on charcoal prepared from 15 ml of a 1% palladium chloride solution and 0.2 g of charcoal. After one molar equivalent of hydrogen was absorbed, the catalyst was filtered off, and the filtrate was concentrated under nitrogen atmosphere. The crystals separated was filtered and dried. Colorless needles, mp 148—149° (decomp.). The IR spectrum of this product was identical with that of the authentic 1-ethyl-3-nitroguanidine (V). Yield, 0.48 g (73%). The filtrate was again concentrated under nitrogen atmosphere, the yellow prisms separated were filtered, and carefully recrystallized from methanol under nitrogen atmosphere. Yellow prisms, mp 125° (decomp.). This product was identical with the authentic 1-ethyl-3-nitrosoguanidine in usual criteria. *Anal.* Calcd. for $C_3H_8ON_4$: C, 31.03; H, 6.94; N, 48.25. Found: C, 30.83; H, 6.78; N, 48.38. Yield, 0.03 g (5%).

1-(2-Halogenoethyl)-3-nitro-1-nitrosoguanidines (IVa, b, c)——A general procedure for the synthesis of these nitrosoguanidines is described with IVa. To a solution of 0.33 g (0.002 mole) of 1-(2-chloroethyl)-3-nitroguanidine (IIIa) in a mixture of 5 ml of nitric acid and 3 ml of water, was added in portions 0.35 g (0.005 mole) of sodium nitrite with stirring at −5°, and the reaction mixture was stirred for 30 min. The yellow leaflets separated were filtered, washed with ice-water and dried in an evacuated desiccator. Recrystallization from methanol gave yellow leaflets, mp 110—111° (decomp.) (Reported,[4,7] 114.5° (decomp.)). IR ν_{max}^{Nujol} cm^{-1}: 3420, 3300 (NH), 1240 (NNO_2), 1540, 870 (NNO). Yield, 0.28 g (72%). *Anal.* Calcd. for $C_3H_6O_3N_5Cl$: C, 18.35; H, 3.08; N, 35.81. Found C, 18.41; H, 3.03; N, 36.22.

1-(2-Bromoethyl)-3-nitro-1-nitrosoguanidine (IVb): Yellow leaflets, mp 101—102° (decomp.) (Reported,[7] 101—103°). Yield, 58%. This compound should be recrystallized from a mixture of methanol and water without heating.

1-(2-Iodoethyl)-3-nitro-1-nitrosoguanidine (IVc): Straw yellow leaflets, mp 103—104° (decomp.). This compound was recrystallized from a mixture of methanol and water, without heating. Yield, 41%. *Anal.* Calcd. for $C_3H_6O_3N_5I$: C, 12.55; H, 2.10; N, 24.40. Found: C, 12.38; H, 1.88; N, 23.88.

N,N-Dimethyleneurethan (VII)——To 2.15 g (0.05 mole) of ethylenimine was added dropwise 6.60 g (0.05 mole) of N-methyl-N-nitrosourethan with vigorous stirring under ice-cooling. After the reaction had ceased, the reaction mixture was stirred for 1 hr at room temperature, and extracted with ether. The ether extract was washed with water twice, and dried over anhyd. potassium carbonate. The ether was evaporated, and the residue was distilled twice under reduced pressure. Colorless oil, bp 35—39°/6 mmHg. IR $\nu_{max}^{liq.}$ cm^{-1}: 1738, 1203 ($COOC_2H_5$). NMR τ ($CDCl_3$): 7.82 (s, $(CH_2)_2N$), 8.73 (t, CH_3), 5.85 (q, CH_2). Yield, 3.69 g (66%). *Anal.* Calcd. for $C_5H_9O_2N$: N, 12.17. Found: 12.02.

N-(2-Halogenoethyl)urethans (VIIIa, b)——A general procedure for the synthesis of VIIIa, b is described with VIIIa. To a mixture of 2 ml of conc. hydrochloric acid and 2 ml of water was added dropwise 2.30 g (0.02 mole) of N,N-dimethyleneurethan (VII) with stirring, and the mixture was stirred for 30 min. The reaction mixture was extracted with ether, the ether extract was washed with water twice, and dried over anhyd. calcium chloride. The ether was evaporated, and the residue was distilled under reduced pressure. Colorless oil, bp 80—82°/5 mmHg (Reported,[9] bp 76—77°/3 mmHg). IR $\nu_{max}^{liq.}$ cm^{-1}: 3310 (NH), 1700, 1250 ($COOC_2H_5$). Yield, 1.87 g (61%).

N-(2-Bromoethyl)urethan (VIIIb): Colorless oil, bp 87—89°/6 mmHg. IR $\nu_{max}^{liq.}$ cm^{-1}: 3320 (NH), 1705, 1255 ($COOC_2H_5$). Yield, 74%. *Anal.* Calcd. for $C_5H_{10}O_2NBr$: N, 7.15. Found: N, 7.12.

N-(2-Halogenoethyl)-N-nitrosourethans (IXa, b)——A general procedure for the synthesis of these nitrosourethans, is described with IXa. To an ice-cooled solution of 15.2 g (0.1 mole) of N-(2-chloroethyl)-urethan (VIIIa) in 50 ml of 40% sulfuric acid was added in portions 16.0 g (0.4 mole) of sodium nitrite with stirring during 30 min. The reaction mixture was stirred for 1 hr, and extracted with chloroform. The chloroform extract was washed with water twice, dried over anhyd. calcium chloride, and the chloroform

7) A.F. McKay and J.E. Milks, *J. Am. Chem. Soc.*, **49**, 2303 (1929).

was distilled off. The residue was distilled under reduced pressure to give yellow orange oil, bp 78—81°/2 mmHg. IR $\nu_{max}^{liq.}$ cm^{-1}: 1745, 1145 ($COOC_2H_5$), 1395, 903 (NNO). NMR τ ($CDCl_3$): 8.53 (t, CH_3), 5.53 (q, CH_2), 6.60 (t, $ClCH_2$), 5.98 (t, CH_2N). Yield, 16.25 g (90%). *Anal.* Calcd. for $C_5H_9O_3N_2Cl$: C, 33.25; H, 5.02; N, 15.51. Found: C, 32.89; H, 4.88; N, 15.47.

N-(2-Bromoethyl)-N-nitrosourethan (IXb): Orange red oil, bp 86°/2 mmHg. IR $\nu_{max}^{liq.}$ cm^{-1}: 1750, 1140 ($COOC_2H_5$), 1380, 855 (NNO). NMR ($CDCl_3$): 8.50 (t, CH_3), 5.45 (q, CH_2), 6.72 (t, $BrCH_2$), 5.88 (t, CH_2N). Yield, 91%. *Anal.* Calcd. for $C_5H_9O_3N_2Br$: C, 26.68; H, 4.03; N, 12.45. Found: C, 26.78; H, 4.02; N, 12.30.

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UDC 547.597'457.1.04 : 542.98

Soil Bacterial Hydrolysis leading to Genuine Aglycone. VI.[1] On Stevioside

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As reported previously,[1,3] the soil bacterial hydrolysis method developed in this laboratory has been shown to be a useful procedure in the structure elucidation of the genuine triterpenoid sapogenols. As an extention of these studies, we have applied the microbiological method to the diterpenoid glycoside stevioside (I). The usefulness of the method is demonstrated in this paper.[4]

The structure of the aglycone steviol (II), obtained from stevioside (I), was established by Mossetig, *et al.*,[5] and the sugar portion of the glycoside was elucidated by Fletcher and his co-workers.[6] On acid treatment, both stevioside and steviol afforded isosteviol (III), possessing a rearranged skeleton, while by snail enzyme hydrolysis, stevioside and steviolbioside (IV) were shown to give steviol as the sole hydrolysate. Also, it was noted that stevioside was unattacked by emulsin, rhamnodiastase, air-dried brewer's yeast, and powder of *Aspergillus niger*. Afterwards, however, it was found[7] that a commercial pectinase preparation is suitable for the hydrolysis of the glycoside to give steviol.

The culture broth obtained by cultivation of a soil bacterial strain (YSB-9), which was selected as described before,[8] was extracted with ether and *n*-butanol successively. From the ether extract was obtained a minor hydrolysate, mp 207—208°, whose infrared (IR) spectrum and behavior on thin-layer chromatography (TLC) resembled that of steviol. A direct comparison of the substance with steviol showed the sample to be identical to the authentic compound.

1) Part V: I. Yosioka, T. Sugawara, K. Imai, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 2418 (1972).
2) Location: *a) Toneyama, Toyonaka, Osaka; b) Bethesda, Maryland, U.S.A.*
3) I. Yosioka, S. Saijoh, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 564 (1972), and the literature cited therein.
4) Presented at the 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya April 1969, Abstract Papers, p. 356.
5) *a)* E. Mossetig and W.R. Nes, *J. Org. Chem.*, **20**, 884 (1955); *b)* E. Mossetig, U. Beglinger, F. Dolder, H. Lichti, P. Quitt, and J.A. Waters, *J. Am. Chem. Soc.*, **85**, 2305 (1963).
6) H.R. Wood, Jr., R. Allerton, H.W. Diehl, and H.G. Fletcher, Jr., *J. Org. Chem.*, **20**, 875 (1955).
7) *a)* M. Ruddat, E. Heftmann, and A. Lang, *Arch. Biochem. Biophys.*, **110**, 496 (1965); *b)* R.D. Bennett, E.R. Lieber, and E. Heftmann, *Phytochemistry*, **6**, 1107 (1967).
8) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagwa, *Tetrahedron Letters*, **1966**, 6303.