anhyd. Na_2SO_4. The $CHCl_3$ solution was chromatographed on a silica gel column by using $CHCl_3$ as an eluent. The first fraction gave 190 mg (32%) of Ib, the second one gave 20 mg of a mixture of Ia and Ib, and the third one gave 81 mg (13%) of Ia.

4-Bromo-6-bis(2-chloroethyl)aminomethyl-3-hydroxypyridazine 1-Oxide Hydrobromide (IV)——In a hood, 2.45 g (0.01 mol) of bromine was added into a solution of 3.03 g (0.01 mol) of 6-bis(2-chloroethyl)aminomethyl-3-hydroxypyridazine 1-oxide hydrochloride[5] (III) in 20 ml of water, with vigorous stirring, and the reaction mixture was then stirred for 30 min. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was recrystallized from ethanol to give the hydrobromide, colorless leaflets, mp 185—186° (dec.). NMR (τ, DMSO-d_6): 6.68, 6.12 (a pair of doublet, $((ClCH_2CH_2)_2N)$, 5.78 (s, CH_2). Yield, 1.54 g (42%). *Anal.* Calcd. for $C_9H_{12}BrCl_2N_3O_2 \cdot HBr$: C, 25.38; H, 3.06; N, 9.86. Found: C, 25.84; H, 3.17; N, 10.07.

Screening Method——Complete details of the screening methods have been described in our previous report.[3]

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Electrophilic Substitution of 1,2-Benzisothiazol-3-acetic Acid

Hitoshi Uno and Mikio Kurokawa

Research Laboratories, Dainippon Pharmaceutical Co., Ltd.[1]

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The bromination, the nitration, the chlorosulfonation, the formylation and the Mannich reaction of 1,2-benzisothiaizol-3-acetic acid (1) were investigated and it was shown that the 3α-methylene group is also activated to the electrophilic substitutions as in 1,2-benzisoxazol-3-acetic acid.

Keywords——1,2-benzisothiazole; electrophilic substitution; bromination; nitration; chlorosulfonation; formylation; Mannich reaction

In the previous paper,[2] it has been demonstrated that 3α-methylene group of 1,2-benzisoxazol-3-acetic acid is strongly activated to the electrophilic substitution. As an extension of this study, several electrophilic substitution reactions of 1,2-benzisothiazol-3-acetic acid (1)[3] were investigated.

As the result, electrophilic substitution reactions occurred to 3α-methylene group of **1** and it was shown that 3α-methylene group of **1** is also activated to the electrophilic substitu tion as in the 1,2-benzisoxazole derivative.

When brominated with 2 molar equivalents of bromine in acetic acid, **1** gave 3α-bromo-1,2-benzisothiazol-3-acetic acid (2) expectedly. However, the reaction of **1** with 4 molar equivalents of N-bromosuccinimide (NBS) did not afford the expected compound, 3-tribromomethyl-1,2-benzisothiazole (3) but gave 3-dibromomethyl-1,2-benzisothiazole (4), the analogue of which, 3-dibromomethyl-1,2-benzisoxazole, was not obtained on the bromination of 1,2-benzisoxazole derivative under the same condition. Compound **3** was obtained by the reaction of **1** with 4 molar equivalent of bromine in acetic acid at 80°. The decarboxylation of **2** afforded 3-bromomethyl-1,2-benzisothiazole (5), which was converted to monoiodomethyl derivative (6) by the reaction with potassium iodide in acetone.

The nitration of **1** with fuming nitric acid on an ice-bath for 1 hour and then at room temperature for 2 hours afforded a mixture of three products, 5-nitro-3-trinitromethyl-1,2-

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1) Location: *33-94, Enoki-cho, Suita, Osaka.*
2) H. Uno and M. Kurokawa, *Chem. Pharm. Bull.* (Tokyo), **26**, 3498 (1978).
3) M. Giannella, F. Gualtieri and C. Melchiorri, *Phytochemistry*, **10**, 539 (1971).

benzisothiazole (**7**), 5-nitro-1,2-benzisothiazole-3-carboxaldehyde (**8**) and 5-nitro-1,2-benzisothiazole-3-carboxylic acid (**9**). Yields of each compounds were 8%, 20% and 34% respectively. Structures of these compounds were determined by data of elemental analyses and spectral measurements.

The chlorosulfonation and successive amination of **1** afforded 3-sulfamoylmethyl-1,2-benzisothiazole (**10**) in a yield of 14%. A byproduct, which was detected on thin-layer chromatogram of the reaction mixture, was supposed to be 3-sulfamoylmethyl-5-sulfamoyl-1,2-benzisothiazole (**11**) by an analogy of products on the reaction of 1,2-benzisoxazol-3-acetic acid.

By the reaction with sodium hydride and ethyl formate, **1** afforded 1,2-benzisothiazol-3-acetaldehyde (**12**). The structure of **12** was confirmed by the formation of its oxime (**13**).

The Mannich reaction of **1** with an excess of morpholine and formalin afforded the expected 3-(1-morpholinomethyl)-vinyl-1,2-benzisothiazole (**14**).[4]

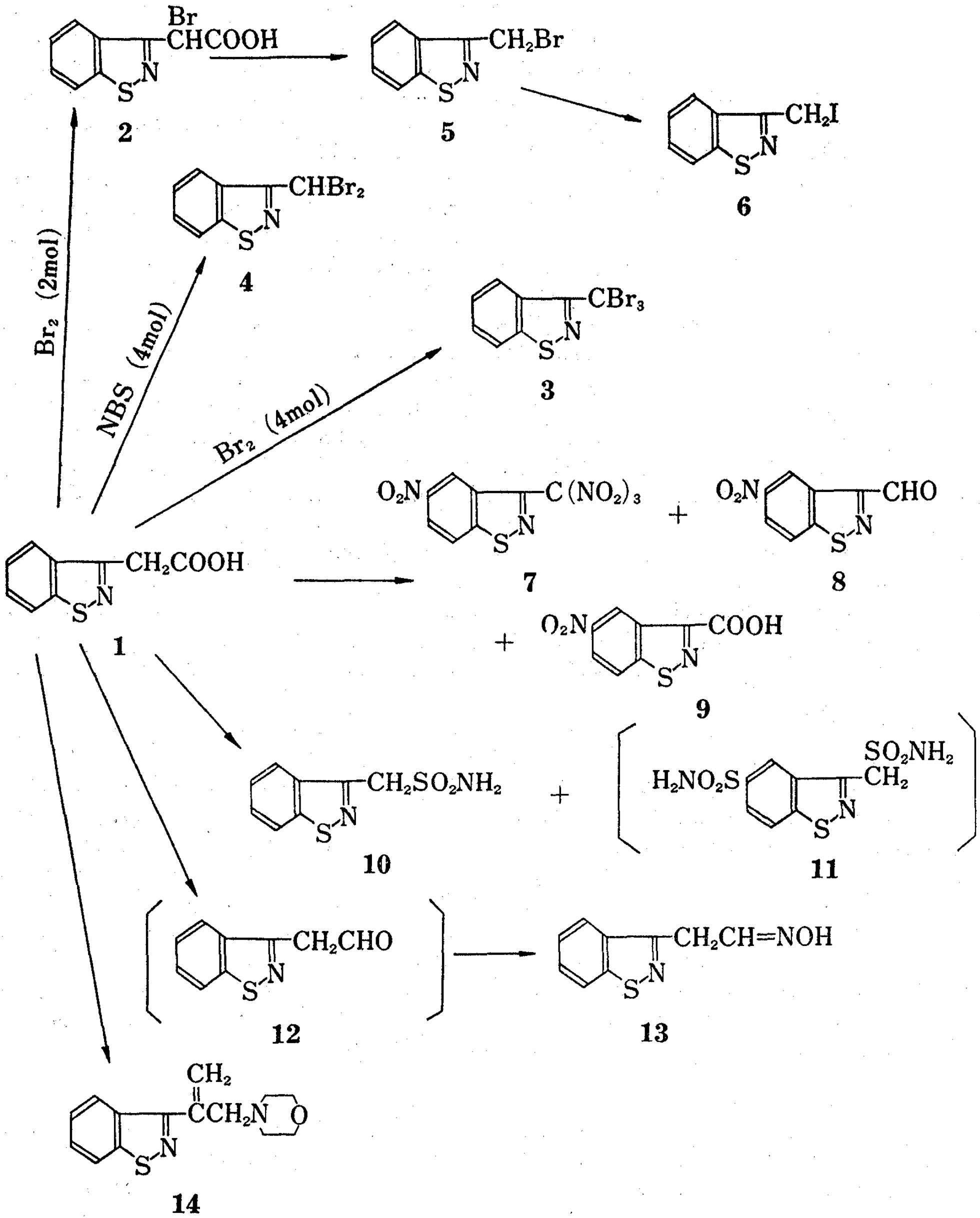

Chart 1

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[4] H. Uno and M. Kurokawa, *Chem. Pharm. Bull.* (Tokyo), **26**, 312 (1978).

Experimental[5]

3α-Bromo-1,2-benzisothiazol-3-acetic Acid (2)——To the solution of **1** (3.0 g) on AcOH (50 ml) was added a solution of bromine (5.0 g) in AcOH (10 ml). The mixture was stirred for 3.5 hr. The mixture was poured into H_2O (300 ml) and extracted with benzene. The solvent was removed *in vacuo* and the residue was crystallized from benzene to give 1.0 g of **2**, mp 110—113° (dec). NMR (δ in $CDCl_3$) ppm: 6.01 (1H, singlet). *Anal.* Calcd. for $C_9H_6BrNO_2S$: C, 39.72; H, 2.22; Br, 29.37; N, 5.15; S, 11.78. Found: C, 39.94; H, 2.20; Br, 29.09; N, 5.22; S, 12.07.

3-Dibromomethyl-1,2-benzisothiazole (4)——To 15 ml of CCl_4 were added NBS (1.85 g) and **1** (0.5 g) and the solution was refluxed for 3 hr and filtered. The filtrate was evaporated *in vacuo*. The redidue was chromatographed on silicagel. The fraction eluted with benzene–hexane (1: 3) was evaporated to give **4** (0.5 g, 63%). The recrystallization from MeOH gave pure **4**, mp 87—90°. NMR (δ in $CDCl_3$) ppm: 7.02 (1H, singlet). *Anal.* Calcd. for $C_8H_5Br_2NS$: C, 31.29; H, 1.64; Br, 52.06; N, 4.56; S, 10.45. Found; C, 31.09; H, 1.49; Br, 51.98; N, 4.37; S, 10.38.

3-Tribromomethyl-1,2-benzisothiazole (3)——To the solution of **1** (0.5 g) in AcOH (5 ml) was added a solution of bromine (1.66 g) in AcOH (1 ml). The solution was heated at 50—60° for 2 hr and then at 80° for 1 hr. The cooled solution was poured into H_2O and extracted with benzene. The benzene solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The residual oil was chromatographed on silicagel. The fraction eluted with benzene–hexane (1: 3) was evaporated and the residue was recrystallized from MeOH to give 0.3 g of **3**, mp 105—107°. *Anal.* Calcd. for $C_8H_4Br_3NS$: C, 24.90; H, 1.04; Br, 62.12; N, 3.63; S, 8.31. Found: C, 24.91; H, 1.15; Br, 61.96; N, 3.52; S, 8.28.

Nitration of 1——To cooled HNO_3 ($d=1.5$, 30 ml) was added **1** (1.4 g). The mixture was stirred for 1 hr in an ice-bath and then at room temperature for 2 hr. The mixture was poured onto ice and resulting precipitate was extracted with AcOEt. The solution was washed with H_2O, dried over Na_2SO_4 and evaporated. The residue was washed with benzene and recrystallized from AcOEt to give **9** (0.6 g, 34%), mp 197—200°. NMR (δ in DMSO-d_6) ppm: 8.42 (1H, double doublet, $J=2$ and 9 Hz), 8.60 (1H, doublet, $J=9$ Hz), 9.40 (1H, doublet, $J=2$ Hz). MS *m/e*: 224 (M^+). *Anal.* Calcd. for $C_8H_4N_2O_4S \cdot H_2O$: C, 39.67; H, 2.50; N, 11.50; S, 13.24. Found: C, 40.22; H, 2.12; N, 11.56; S, 13.21.

The benzene washings were evaporated and the residue was chromatographed on silicagel. The fraction eluted with benzene–hexane (1: 3) was evaporated to give **7** (0.2 g, 8%). The recrystallization from benzene–hexane gave pure **7**, mp 188—191°. NMR (δ in CD_3COCD_3) ppm: 8.63 (1H, double doublet, $J=2$ and 9 Hz), 8.83 (1H, double doublet, $J=1$ and 9 Hz), 9.21 (1H, double doublet, $J=1$ and 2 Hz). *Anal.* Calcd. for $C_8H_3N_5O_8S$: C, 29.19; H, 0.92; N, 21.28; S, 9.74. Found: C, 29.10; H, 1.28; N, 21.40; S, 9.85.

The fraction eluted with benzene gave 0.3 g (20%) of crude **8**, which was recrystallized from benzene–hexane to give pure product, mp 149—150°. NMR (δ in $CDCl_3$) ppm: 8.40 (1H, doublet, $J=9$ Hz), 8.55 (1H, double doublet, $J=2$ and 9 Hz), 9.70 (1H, doublet, $J=2$ Hz), 13.33 (1H, singlet, $C\underline{H}O$) *Anal.* Calcd. for $C_8H_4N_2O_3S$: C, 46.15; H, 1.93; N, 13.45; S, 15.39. Found: C, 46.42; H, 1.86; N, 13.33; S, 15.25.

3-Sulfamoylmethyl-1,2-benzisothiazole (10)——To 15 ml of $ClSO_3H$ was added **1** (3.0 g) and the mixture was heated at 60° for 7 hr and poured into ice-H_2O. The resulting oil was separated by decantation and cold NH_4OH (28%, 30 ml) was added to the oil. The mixture was stirred for 1 hr at room temperature and extracted with AcOEt. The extracts were washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was recrystallized from AcOEt to give **10** (0.50 g, 14%), mp 181—184°. *Anal.* Calcd. for $C_8H_8N_2O_2S_2$: C, 42.09; H, 3.52; N, 12.27; S, 28.09. Found: C, 42.16; H, 3.34; N, 12.20; S, 28.40.

1,2-Benzisothiazol-3-acetaldoxime (13)——To 30 ml of benzene were added **1** (1.93 g), ethyl formate (8 ml) and NaH (50%, 1.4 g) under cooling. The mixture was stirred at room temperature for 1.5 hr, stored overnight and poured into H_2O. The benzene layer was removed and the aqueous layer was neutralized with conc. HCl and extracted with ether. To the ether solution was added a solution of NH_2OH (prepared from 6.9 g $NH_2OH \cdot HCl$, Na 2.3 g and EtOH 70 ml). The mixture was stirred at room temperature for 30 min and concentrated. To the residue was added H_2O and the solution was extracted with $CHCl_3$. The organic layer was washed with H_2O, dried and evaporated. The residue was chromatographed on silica gel. The fraction eluted with $CHCl_3$ was recrystallized from ether–hexane to give **13** (0.3 g, 16%), mp 107—110°. *Anal.* Calcd. for $C_9H_8N_2OS$: C, 56.23; H, 4.19; N, 14.58; S, 16.68. Found C, 56.26; H, 3.89; N, 14.24; S, 16.78.

Mannich Reaction of 1——To a solution of morpholine (3.4 g) and formalin (40% aq. sol., 3.0 g) in AcOH (6 g) was added **1** (1.5 g). The mixture was stirred at 50° for 2 hr, made alkaline with NaOH sol. and extracted with AcOEt. The extracts was washed with H_2O, dried over Na_2SO_4 and evaporated. The residual oil was chromatographed on silicagel. The oily **14** was eluted with MeOH–$CHCl_3$ (3: 97) and converted to its oxalate. The oxalate was recrystallized from EtOH to give the product of mp 159—162°. NMR (δ

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[5] All melting points are uncorrected. NMR spectra were taken with Varian A-60 spectrometer using TMS as an internal standard, and mass spectra with Hitachi RMU-6L mass spectrometer.

in DMSO-d_6) ppm: 3.95 (2H, singlet, -CH_2-), 6.02 (2H, singlet, CH_2=). MS *m/e*: 260 (M^+). *Anal.* Calcd. for $C_{14}H_{16}N_2OS \cdot C_2H_2O_4 \cdot 1/2H_2O$: C, 53.47; H, 5.33; N, 7.80; S, 8.92. Found: C, 53.52; H, 5.10; N, 7.88; S, 8.90.

3-Bromomethyl-1,2-benzisothiazole (5)——Compound **2** (0.5 g) was heated at 95 ° for 30 min and chromatographed on silicagel. The fraction eluted with benzene–hexane (1:3) was evaporated and the residue was recrystallized from MeOH to give **5** (0.4 g, 95%), mp 44—46°. NMR (δ in $CDCl_3$) ppm: 4.85 (2H, singlet, $C\underline{H}_2Br$). MS *m/e*: 227, 229 (M^+). *Anal.* Calcd. for C_8H_6BrNS: C, 42.12; H, 2.65; Br, 35.03; N, 6.14; S, 14.06. Found: C, 42.07; H, 2.87; Br, 34.72; N, 5.93; S, 14.06.

3-Iodomethyl-1,2-benzisothiazole (6)——The solution of **5** (0.20 g) and KI (0.35 g) in acetone (10 ml) was refluxed for 2 hr and filtrated. The filtrate was evaporated and the residue was chromatographed on silicagel. The fraction eluted with benzene–hexane (3:7) was evaporated and the residue was recrystallized from MeOH to give **6** (0.23 g, 96%), mp 66—67°. *Anal.* Calcd. for C_8H_6INS: C, 34.92; H, 2.20; I, 46.13; N, 5.09; S, 11.66. Found: C, 35.01; H, 2.27; I, 45.71; N, 4.92; S, 11.76.

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Syntheses of N-Alkyl-N-(hydroxy- or oxo-alkyl)nitrosamines related to N-Butyl-N-(4-hydroxybutyl)nitroamine, a Potent Bladder Carcinogen

MASASHI OKADA, EMAKO SUZUKI, and MINORU IIYOSHI

Tokyo Biochemical Research Institute[1]

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Nineteen N-alkyl-N-(hydroxy- or oxo-alkyl)nitrosamines structurally related to N-butyl-N-(4-hydroxybutyl)nitrosamine, a potent bladder carcinogen, were synthesized for the purpose of investigating their carcinogenic effects in rats. They are N-alkyl-N-(4-hydroxybutyl)nitrosamines, N-alkyl-(3-hydroxypropyl)-nitrosamines, N-alkyl-N-(2-hydroxyalkyl)nitrosamines, and N-alkyl-N-(2- or 3-oxoalkyl)nitrosamines. They were mostly prepared by the nitrosation of the corresponding N-alkyl-N-(hydroxyalkyl)amines. The nitrosamines with an oxo group were prepared by chromium trioxide oxidation of the corresponding N-alkyl-N-(hydroxyalkyl)nitrosamines with a secondary hydroxyl group.

Keywords——N-nitrosamine; N-alkyl-N-(ω-hydroxyalkyl)nitrosamine; N-alkyl-N-(oxoalkyl)nitrosamine; nitrosation; bladder carcinogen; organotropic carcinogen; structure-carcinogenicity relationship

A great number of N-nitroso compounds are now known to be carcinogenic in experimental animals,[2–4] largely because of the extensive studies of Druckrey and his colleagues.[3] One of the most intriguing findings by these workers was that of 65 compounds examined in rats, only two, namely N,N-dibutylnitrosamine (DBN) and its ω-hydroxylated derivative, N-butyl-N-(4-hydroxybutyl)nitrosamine (butyl-butanol-(4) nitrosamine: BBN), induced

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