4—5. After stirring for 30 min at 70—80°, the solution was cooled and extracted with $CHCl_3$ or AcOEt. The organic layer was washed with water and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave a yellow residue which was purified by distillation *in vacuo*. The nitrosation of N-butyl-N-(2-hydroxybutyl)amine and other amines with a secondary hydroxyl group was carried out in the same manner.

For elemental analysis and UV, IR or NMR spectral measurements, the compounds were further purified by column chromatography of Silica gel (E. Merck AG) using hexane–ether–CH_2Cl_2 (4 : 3 : 2) as eluting solvent. The yield, bp, and data of elemental analysis are indicated in Table II, and UV and IR spectral data are listed in Table III. Purification of the nitrosamines which were not distilled was made by column chromatography in the similar way.

Oxidation of N-Alkyl-N-(hydroxyalkyl)nitrosamine with a Secondary Hydroxyl Group to the Corresponding Oxo Compound——The oxidation was performed in the usual way using CrO_3–pyridine complex or CrO_3–AcOH. For example, BOBN-3 was prepared from BHBN-3 as follows: To a solution of BHBN-3 (10 g) in anhyd. CH_2Cl_2 (50 ml) was added a solution of CrO_3-pyridine complex (100 g) in the same solvent (1700 ml), and the mixture was allowed to stand at room temperature for 20 hr. After filtration of the precipitate, the solvent was removed under reduced pressure. The residue was extracted with EtOAc, and the organic layer was washed successively with 10% HCl, 5% Na_2CO_3 and water, and then dried over anhyd. Na_2SO_4. After evaporation of the solvent, an oily residue was distilled to give BOBN-3 as a pale yellow oil.

For elemental analysis and UV, IR or NMR spectral measurements, the compounds were further purified by column chromatography as described above. Purification of AOPN which was not distilled was also performed by column chromatography. Yield of the oxidation, bp, and data of elemental analysis are given in Table II, and UV and IR spectral data are shown in Table III.

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UDC 547.876.04.09 : 615.225.2.011.5.015.11

Synthesis and Reaction of Furazanobenzothiadiazole and Related Compounds

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Furazanobenzothiadiazole and related compounds were prepared by a new synthetic route starting from 4,7-dibromo-5-nitrobenzo-2,1,3-thiadiazole, which was obtained in good yield by nitration of 4,7-dibromobenzo-2,1,3-thiadiazole easily acessible. Some chemical reactions of this tricyclic ring system—ring opening reaction, photo-cleavage reaction, bromination and reduction—were discussed.

Keywords——furazanobenzothiadiazole; furoxanobenzothiadiazole; tricyclic ring system; vasodilatory and hypotensive activities; mass spectrum;

Synthesis of furazanobenzothiadiazole (I) and its N-oxide (furoxanobenzothiadiazole) (II) having vasodilatory and hypotensive activities[2] was reported by Ghosh.[3] His method consists of two independent routes, starting in each case from the appropriately substituted benzothiadiazole (Chart 1). We now describe a new synthetic route to I starting from easily ac-

1) Location: *a) 1-3-80, Tomobuchi-cho, Miyakojima-ku, Osaka; b) 4, Koishikawa, Bunkyo-ku, Tokyo.*
2) P.B. Ghosh and B.J. Everitt, *J. Med. Chem.*, **17**, 203 (1974).
3) P.B. Ghosh, *Tetrahedron Lett.*, **32**, 2999 (1971).

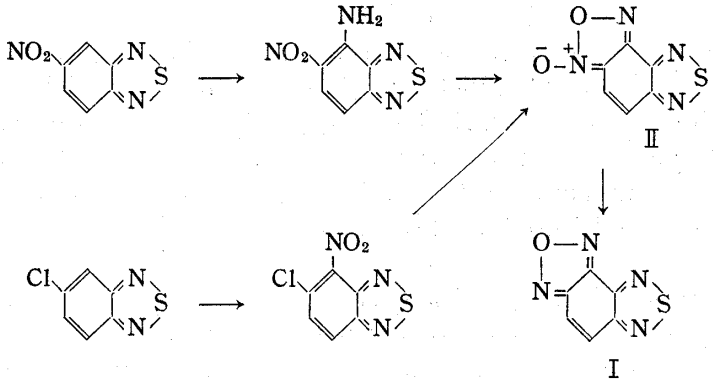


Chart 1

cessible 4,7-dibromobenzo-2,1,3-thiadiazole (III), and some reactions of this tricyclic ring system.

The dibromo compound III[4] (prepared from benzothiadiazole) was nitrated according to the procedure of Pesin *et al.*[5] to yield exclusively 4,7-dibromo-5-nitrobenzo-2,1,3-thiadiazole (IV), which on treatment with sodium azide in acetone-methanol formed 7-bromo-4,5-(2′,1′,3′-oxadiazole-1′-oxide)benzo-2,1,3-thiadiazole (V), presumably *via* an intermediate azido compound. On heating with triethyl phosphite in ethanol, V was converted into 7-bromo-4,5-(2′,1′,3′-oxadiazole)benzo-2,1,3-thiadiazole (VI), and finally the catalytic hydrogenation of VI in the presence of triethylamine, using 10% palladium on charcoal as a catalyst, afforded the desired 4,5-(2′,1′,3′-oxadiazole)benzo-2,1,3-thiadiazole (I) (furazanobenzothiadiazole, mp 135—136°; lit.[2], 130—131°) (Chart 2). The above synthetic route is considered to be an efficient access to I, taking into account the ease of obtaining the starting compound and the unequivocal reaction of each step. Proof of the structure of I rests upon infrared and mass spectral data, elemental analysis and subsequent chemical conversions (in Ghosh's report, there were no spectroscopic data in support of the proposed structure I).

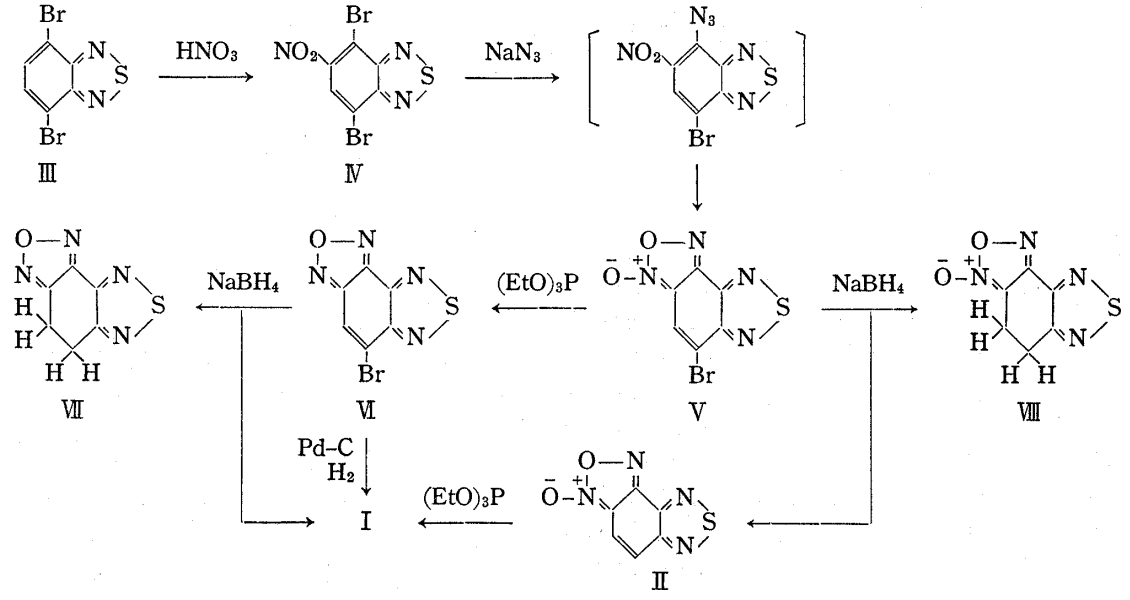


Chart 2

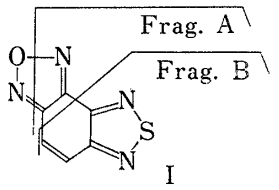
4) K. Pilgram, M. Zupan and R. Skiles, *J. Heterocycl. Chem.*, **6**, 629 (1970).
5) V.G. Pesin, A.M. Khaletskii and V.A. Sergeev, *Zh. Obshch. Khim.*, 33 1759 (1963) [*Chem. Abstr.*, **60** 1734 (1964)].

When an attempt was made for the reductive elimination of bromine atom of VI in the last step using sodium borohydride, 4,5-(2′,1′,3′-oxadiazole)-6,7-dihydrobenzo-2,1,3-thiadiazole (VII) was obtained in 14% yield, in addition to I in 51% yield. Reduction of V with sodium borohydride gave 4,5-(2′,1′,3′-oxadiazole-1′-oxide)-6,7-dihydrobenzo-2,1,3-thiadiazole (VIII) in 20% yield and furoxanobenzothiadiazole (II) in 37% yield. The latter compound, which had been prepared by Ghosh[2,3] from 5-chloro-4-nitro- or 4-amino-5-nitrobenzothiadiazole, was converted into I by heating with triethyl phosphite according to Ghosh's method (Chart 2).

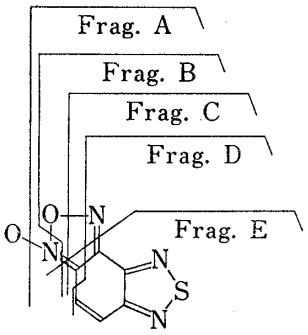
The mass spectroscopic data of I and II allow us to suppose the fragmentation patterns summarized in Table I, which are consistent with the proposed structures. Both compounds I and II show the intense molecular ions, and the fragmentation pattern of II has several intense peaks, among which the M-16$^{\neg+}$ (loss of oxygen), M-30$^{\neg+}$ (loss of NO) and M-60$^{\neg+}$ (loss of N_2O_2) are characteristic, as is frequently observed in furoxans[6] and bnezofuroxans.[7]

Table I. 75 eV Mass Spectra of I and II

m/e	Relative intensity (%)	Fragments
178	100	$C_6H_2N_4OS$ (M^+)
148	21	$C_6H_2N_3S$ (Frag. A)
110	4	$C_4H_2N_2S$ (Frag. B)
194	100	$C_6H_2N_4O_2S$ (M^+)
178	10	$C_6H_2N_4OS$ (Frag. A)
164	5	$C_6H_2N_3OS$ (Frag. B)
148	5	$C_6H_2N_3S$ (Frag. C)
136	14	$C_5H_2N_3S$ (Frag. D)
134	19	$C_6H_2N_2S$ (Frag. E)



I



II

Treatment of I in diluted sodium hydroxide solution at 135—140° in a sealed tube afforded 4,5-diaminobenzofurazan (IX)[8] resulting from a cleavage of the thiadiazole ring of I, whereas the reduction of I with sodium hydrosulfite in aqueous solution induced a cleavage of the oxadiazole ring to give 4,5-diaminobenzothiadiazole (X), which had already been synthesized by another route.[9] Reduction of I with sodium borohydride in ethanol led to the exclusive formation of the corresponding dihydro compound (VII). When I was treated with bromine in carbon disulfide under irradiation of light, an addition reaction took place to produce 6,7-dibromo-4,5-(2′,1′,3′-oxadiazole)-6,7-dihydrobenzo-2,1,3-thiadiazole (XI). Application of the photo-cleavage reaction to I in the presence of triethyl phosphite, according

6) A.J. Boulton, P. Hadjimihalakis, A.R. Katritzky and A. Majid Hamid, *J. Chem. Soc.*, (*C*), **1969**, 1901.
7) P.M. Hadjimihalakis, *J. Heterocycl. Chem.*, **13**, 1327 (1976).
8) W. Borche and H. Weber, *Ann. Chem.*, **489**, 270 (1931).
9) V.G. Pesin, A.M. Khaletskii and V.A. Sergeev, *Zh. Obshch. Khim.*, **32**, 181 (1962) [*Chem. Abstr.*, **57**, 13753 (1962)].

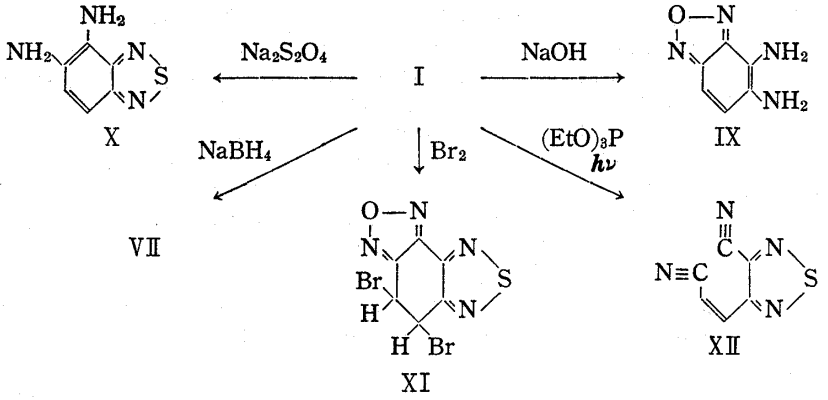


Chart 3

to the method of Mukai and Nitta[10] described for benzofurazan itself, resulted in the formation of 3-cyano-4-cyanovinyl-1,2,5-thiadiazole (XII) (Chart 3).

Satisfactory analytical data were obtained for all new compounds described (V, VI, VIII, XI and XII) and their spectral properties were also consistent with the assigned structures.

Experimental[11]

4,7-Dibromo-5-nitrobenzothiadiazole (IV)——According to the procedure of Pesin *et al*,[5] 4,7-dibromobenzothiadiazole (III)[4] (20 g) was nitrated with 100 ml of HNO_3 (*d.* 1.42) in conc. H_2SO_4 (200 ml) to obtain IV. Recrystallization from ethanol gave 16.5 g (71%) of yellow needles, mp 161—162° (lit.[5] 154—156°). *Anal.* Calcd. for $C_6HBr_2N_3O_2S$: C, 21.26; H, 0.30; N, 12.39. Found: C, 21.51; H, 0.39; N, 12.34.

7-Bromo-4,5-(2′,1′,3′-oxadiazole-1′-oxide)benzo-2,1,3-thiadiazole (V)——To a solution of IV (2.33 g) in acetone-methanol (1: 1, 200 ml) was added a solution of NaN_3 (500 mg) in 28 ml of acetone–methanol–water (1: 2: 1). The reaction mixture was maintained at 60—65° for 1.5 hr and concentrated to about 100 ml. Dilution with water (100 ml) gave the crude product of V, which was filtered, washed with water, dried *in vacuo* and recrystallized from 95% ethanol to obtain 1.25 g (66%) of yellow needles, mp 190—191°. *Anal.* Calcd. for $C_6HBrN_4O_2S$: C, 26.38; H, 0.37; N, 20.51. Found: C, 26.37; H, 0.29; N, 20.36. UV λ_{max}^{EtOH} nm(ε); 217 (16800), 277 (26300), 337 (11700). IR ν_{max}^{KBr} cm^{-1}: 3061 (arom. C–H), 1613 (C=N), 1568 (C=C), 1432, 1405 (N-O). MS *m/e*: 272 (M^+), 274.

7-Bromo-4,5-(2′,1′,3′-oxadiazole)benzo-2,1,3-thiadiazole (VI)——V (2.0 g) in ethanol (40 ml) was heated with triethyl phosphite (4.5 ml) in a sealed tube at 130—140° for 13 hr. The reaction mixture was evaporated to dryness *in vacuo*. The residual black oil was chromatographed on silica gel (240 g). Elution with benzene (750 ml) gave crude product of VI which was recrystallized from ethanol to yield colorless needles (1.4 g, 76%), mp 148—149°. *Anal.* Calcd. for C_6HBrN_4OS: C, 28.03; H, 0.39; N, 21.71. Found: C, 28.09; H, 0.51; N, 21.78. UV λ_{max}^{EtOH} nm(ε): 263 (18900), 292 (6300), 302 (6000). IR ν_{max}^{KBr} cm^{-1}; 3081, 3041 (arom. C-H), 1615 (C=N), 1530 (C=C). MS *m/e*: 256 (M^+), 258.

Furazanobenzothiadiazole (I)——To a solution of VI (3 g) in ethanol (200 ml) and acetone (20 ml) was added 10% Pd-C (1 g) and triethylamine (2 ml). The solution was hydrogenated at room temperature under atmospheric pressure. After 30 min, absorption of hydrogen ceased (*ca.* 270 ml). The catalyst was filtered off and washed with ethanol. The filtrate and washing were combined and evaporated to dryness *in vacuo*. The residue was extracted with benzene and the benzene solution was washed with sat. $NaHCO_3$ solution and water, dried (anhyd. Na_2SO_4) and evaporated to yield a yellow residue. Column chromatography on silica gel (150 g) with benzene (750 ml) as an eluent afforded I, which was recrystallized from *n*-hexane to yield 1.8 g (87%) of colorless crystals, mp 135—136° (lit.[2] 130—131°). *Anal.* Calcd. for $C_6H_2N_4OS$: C, 40.46; H, 1.13; N, 31.46. Found: C, 40.68; H, 1.26; N, 31.46. UV λ_{max}^{EtOH} nm(ε): 253 (20500), 259 (18500), 290 (7300), 298 (7400). IR ν_{max}^{KBr} cm^{-1}: 3084 (arom. C–H), 1605 (C=N), 1548 (C=C), 869. MS *m/e*: 178 (M^+).

10) T. Mukai and M. Nitta, *Chem. Commun.*, **1970**, 1192.

11) Melting points were measured with a Yanagimoto Micro Melting Point Apparatus. Melting points are uncorrected. IR spectra were measured with a JASCO-IRG; NMR spectra with a JEOL-PS-100 using TMS as an internal standard; Mass spectra (MS) with a JEOL-JMS-OISG; and UV spectra with a Hitachi Model 323.

Reduction of VI with $NaBH_4$——A solution of VI (100 mg) and $NaBH_4$ (50 mg) in ethanol (10 ml) was heated at 65° for 5 min and then kept at room temperature for 20 min with stirring. Evaporation of the solvent *in vacuo* gave an orange residue, which was extracted with benzene. The benzene layer was washed with water, dried (anhyd. Na_2SO_4) and concentrated *in vacuo* to give a pale yellow product (71 mg). Column chromatography on silica gel (90 g) with benzene (340 ml) as an eluent gave I. Recrystallization from *n*-hexane afforded 35 mg (51%) of pure crystals. Further elution with benzene (280 ml) gave VII, which was recrystallized from 50% ethanol to yield 10 mg of colorless needles (14%), mp 136—138°. *Anal.* Calcd. for $C_6H_4N_4OS$: C, 40.00; H, 2.24; N, 31.09. Found: C, 40.09; H, 2.13; N, 31.04. UV λ_{max}^{EtOH} nm (ε): 232 (2500), 240.5 (sh. 2100), 249.5 (sh. 1100), 290 (15500), 294 (15300). IR ν_{max}^{KBr} cm^{-1}: 2921, 2846 (C-H), 1604 (C=N), 858. MS *m/e*: 180 (M^+). NMR (10% $CDCl_3$) δ: 3.45 (singlet).

Reduction of V with $NaBH_4$——A solution of V (256 mg) and $NaBH_4$ (227 mg) in ethanol (30 ml) was heated at 65° for 5 min and then kept at room temperature for 1 hr with stirring. Evaporation of the solvent *in vacuo* gave an orange residue, which was extracted with benzene. The benzene layer was washed with water, dried (anhyd. Na_2SO_4) and concentrated *in vacuo* to give a yellow product (214 mg). The crude product (TLC 2 spots, silica gel, benzene) was purified by preparative Thin-Layer Chromatography (Silica gel GF_{254}: 50 g, benzene).

Rf 0.64 gave II (72 mg, 37.1%) which was recrystallized from 95% ethanol to give colorless needles, mp 175—178°. UV λ_{max}^{EtOH} nm(ε): 218.5 (8900), 270 (15900), 274 (sh. 15500). 328 (7600), 336 (7500). IR ν_{max}^{KBr} cm^{-1}: 3071 (arom. C-H), 1629 (C=N), 1584 (C=C). MS *m/e*: 194 (M^+). High Resolution MS *m/e*: 193.991 Calcd. for $C_6H_2N_4O_2S$: 193.990.

Rf 0.34 gave VIII (40 mg, 20.4%) which was recrystallized from 95% ethanol to give colorless needles, mp 147.5—148.5°. UV λ_{max}^{EtOH} nm (ε): 215 (6700), 262 (8100), 308 (sh. 12300). 314 (13000), 320 (12500). IR ν_{max}^{KBr} cm^{-1}: 2976, 2911 (C-H), 1632 (C=N), 1450. MS *m/e*: 196 (M^+). NMR (10% $CDCl_3$) δ: 3.37 (4H, triplet, J=2.5 Hz). High Resolution MS *m/e*: 196.008. Calcd. for $C_6H_4N_4O_2S$: 196.005.

Formation of I from II——According to Ghosh's method,[2] II (80 mg) was refluxed for 1 hr with $(EtO)_3P$ (5 ml). Recrystallization from ethanol afforded 46 mg (60%) of I, mp 134—135° (lit.[3] 130—131°), identical to I obtained by the debromination of VI.

4,5-Diaminobenzofurazan (IX)——A solution of I (176 mg) in ethanol (1 ml) and 2 N NaOH (4 ml) was heated in a sealed tube at 135—140° for 2 hr. After cooling, the solution was neutralized with acetic acid and extracted with ethyl acetate. The organic layer was dried (anhyd. Na_2SO_4) and evaporated to dryness *in vacuo* to yield the crude product of IX. Column chromatography on silica gel (100 g) with benzene–ethyl acetate (1: 1, 300—400 ml) as an eluent afforded a brown product, which was recrystallized from benzene–cyclohexane to yield brown needles of IX (84 mg, 56%), mp 151—153° (lit.[8] 151°). *Anal.* Calcd. for $C_6H_6N_4O$: C, 48.00; H, 4.03; N, 37.32. Found: C, 47.84; H, 4.13; N, 37.60. UV λ_{max}^{EtOH} nm (ε): 238 (20700), 290 (2600), 440 (4000), IR ν_{max}^{KBr} cm^{-1}: 3302, 3224 (N–H), 1630 (C=N), 1511 (C=C). MS *m/e*: 150 (M^+).

4,5-Diaminobenzothiadiazole (X)——To a solution of $Na_2S_2O_4$ (200 mg) in 5 ml of water, which was adjusted to pH 13 with 30% KOH solution, was added I (100 mg) in 5 ml of ethanol. The reaction mixture was heated at 100° for 5 min. After cooling, red needles were collected by filtration, washed with a small amount of water and dried *in vacuo*. Recrystallization from water gave 65 mg (70%) of X, mp 166—167° (lit.[9] 168—170°). UV λ_{max}^{EtOH} nm (ε): 264 (23200), 324 (8200), 450 (2800). IR ν_{max}^{KBr} cm^{-1}: 3381, 3321 (N–H), 1622 (C=N), 1535 (C=C). MS *m/e*: 166 (M^+).

Reduction of I with $NaBH_4$——A solution of I (100 mg) and $NaBH_4$ (100 mg) in 20 ml of ethanol was heated on a water bath for 4 hr. The reaction mixture was evaporated to dryness *in vacuo* and the yellow residue was extracted with benzene. The benzene solution was chromatographed on silica gel (90 g). Elution with benzene (350 ml) gave 75 mg of the starting compound I. Furter elution with benzene (260 ml) gave VII. Recrystallization from 95% ethanol gave 16 mg (16%) of colorless needles, mp 139°, identical to VII obtained by treatment of the bromo derivative VI with $NaBH_4$.

6,7-Dibromo-4,5-(2′,1′,3′-oxadiazole)-6,7-dihydrobenzo-2,1,3-thiadiazole (XI)——A solution of I (178 mg) and bromine (310 mg) in 25 ml of carbon disulfide was irradiated with a W-lamp (150 W). After 2.5 hr, the reaction mixture was concentrated to dryness *in vacuo* to yield the pale yellow residue. Column chromatography on silica gel (21 g) with benzene–cyclohexane (1: 1, 135 ml) as an eluent afforded XI, which was recrystallized from *n*-hexane to yield 242 mg (64%) of colorless needles. mp 120—121°. *Anal.* Calcd. for $C_6H_2Br_2N_4OS$: C, 21.32; H, 0.60; N, 16.58. Found: C, 21.68; H, 0.42; N, 16.31. UV λ_{max}^{EtOH} nm (ε): 286 (14100), 291 (13700). IR ν_{max}^{KBr} cm^{-1}: 2996, 2916 (C–H), 1611 (C=N), 1539, 1483. MS *m/e*: 336 (M^+), 338, 340. NMR (10% $CDCl_3$) δ: 6.00 (C_7-H, doublet, J=2.5 Hz), 5.93 (C_6-H, doublet, J=2.5 Hz). Further elution with benzene–cyclohexane (1: 1, 60 ml) gave 27 mg (15%) of the starting compound I.

3-Cyano-4-cyanovinyl-1,2,5-thiadiazole (XII)——A solution of I (178 mg) and triethyl phosphite (1.3 g) in benzene (10 ml) was left in bright daylight for 8 hr. The reaction mixture was chromatographed on silica gel (150 g) with benzene as an eluent (530 ml) to yield 134 mg (75%) of the starting material I. Further elution with benzene (170 ml) gave XII, which was recrystallized from *n*-hexane to yield 30 mg (18.4%) of colorless needles, mp 81—83°. *Anal.* Calcd. for $C_6H_2N_4S$: C, 44.45; H, 1.24; N, 34.56. Found: C, 44.38;

H, 1.41; N, 34.37. UV λ_{max}^{EtOH} nm (ε): 251 (8600), 259 (9100), 294 (5600). IR ν_{max}^{KBr} cm^{-1}: 3062 (arom. C-H), 2237. 2217 (C≡N), 1629 (C=N), 1413, 851, 794. MS *m/e*: 162 (M^+).

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Synthesis of the Nonadecapeptide corresponding to Porcine δ-Endorphin[1]

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The nonadecapeptide corresponding to δ-endorphin, a plasmin hydrolysate of porcine β-lipotropin, was synthesized using protecting groups removable by methanesulfonic acid. Analgesic activity of the synthetic peptide was estimated as active as Leu-enkephalin by the tail pinch method.

Keywords——synthesis of porcine δ-endorphin; synthesis of human β-endorphin; analgesic peptide; methanesulfonic acid deprotection; tail pinch method

In 1976, Gráf *et al.*[3] isolated, as one of the plasmin hydrolysates of porcine lipotropin (LPH), a new analgesic peptide corresponding to positions 61—79 of the parent hormone. This nonadecapeptide fragment, LPH-(61—79), was analogically termed as δ-endorphin, since other fragments, LPH-(61—91), LPH-(61—76) and LPH-(61—77), were proposed to designate as β, α and γ-endorphins respectively by Li and Chung[4] and Guillemin *et al.*[5]

We wish to report the synthesis of the nonadecapeptide corresponding to the entire amino acid sequence of δ-endorphin. This segment of porcine LPH is common to those of other mamalian LPHs so far known.[6] Thus available five peptide fragments employed for our previous synthesis[7] of human β-endorphin[8] served as a building blocks to construct the entire peptide chain in a conventional manner as shown in Fig. 1.

1) Amino acids and peptides are of the L-configuration. Following abbreviations were used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bzl=benzyl, DCC=dicyclohexylcarbodiimide, HOBT=1-hydroxybenzotriazole, MSA=methanesulfonic acid, DMF=dimethylformamide, DMSO=dimethylsulfoxide, THF=tetrahydrofuran. TFA=trifluoroacetic acid.
2) Location: *a) Minamifunabori-cho, Edogawa-ku, Tokyo, 132 Japan*; *b) Sakyo-ku, Kyoto, 606 Japan.*
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