Synthetic Studies in the Isoxazolo[4,5-*b*]pyrazine System (1)

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The synthesis of 3-methylisoxazolo[4,5-*b*]pyrazine (**5**) and its 7-oxide (**6**) is reported. Nitration of **6** furnished the 6-nitro derivative (**8**) while reaction with phosphoryl chloride provided the chloro derivative (**7**). Efforts to functionalize the methyl group in **5** were not successful.

A number of novel heterocyclic systems which possess structures that resemble some natural nucleic acids are of interest in the search for potential antitumor agents. We have chosen the isoxazolo[4,5-*b*]pyrazine system as one that can be considered an analog of the purines or of the pyrazolo[4,3-*d*]pyrimidines that occur in the formycin antibiotics which have antitumor activity (2).

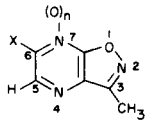
Our initial target was 3-methylisoxazolo[4,5-*b*]pyrazine (**5**) with the hope that the methyl group was a potential anionic center which could be elaborated to a variety of functional groups. The approach used was that of Desimoni and Minoli (3) for the preparation of 3-phenylisoxazolo[4,5-*b*]pyrazine. Thus, in a convenient new synthesis, 5-amino-3-methylisoxazole (**1**) was prepared by the reaction of 3-aminocrotononitrile and hydroxylamine hydrochloride. Acetylation of the amino group followed by nitration in acetic anhydride afforded an excellent yield of 5-acetamido-3-methyl-4-nitroisoxazole (**2**). Acid hydrolysis of **2** yielded 5-amino-3-methyl-4-nitroisoxazole (**3**) which could be reduced with aluminum amalgam (4) to the rather unstable 4,5-diamino-3-methylisoxazole, isolated as the hydrochloride (**4**). Alternative methods for reducing the nitro group such as the sodium borohydride/palladium reagent useful for the 3-phenyl compounds (3) or catalytic hydrogenation were not successful and led to intractable products.

Reaction of the diamine with glyoxal afforded a good yield of the desired heterocycle (**5**). Efforts to functionalize the 3-methyl group of **5** with a variety of reagents including sodamide, *N*-chlorosuccinimide, *N*-bromosuccinimide, bromine and selenium dioxide showed no evidence of useful reaction, in a manner analogous to that observed for the 3-methyl group of the isoxazole ring (5).

In an effort to functionalize **5**, it was oxidized with *m*-chloroperbenzoic acid in a mixture of chloroform and a phosphate buffer, which greatly improved the yield over that experienced with the peracid in chloroform alone. The product was the 3-methylisoxazolo[4,5-*b*]pyrazine 7-oxide (**6**) as shown by the insensitivity of the chemical shift of the methyl protons in **6** as compared to **5** (*cf.* Table I) and the expected upfield shift of H-6 in the course of the oxidation. The structure of **6** is that expected on the basis of the steric hindrance to oxidation at N-4 exerted by the methyl group and finds analogy in the oxidation of 5,6-dimethylquinoxaline which gives the 1-oxide because of steric hindrance by the 5-methyl group (6). Reaction of **6** with phosphoryl chloride gave a good yield of 6-chloro-3-methylisoxazolo[4,5-*b*]pyrazine (**7**), but efforts to replace the chlorine by ammonolysis at 120° were unsuccessful; under more drastic conditions no product could be isolated. Nitration of **6** with nitric acid in acetic anhydride occurred under vigorous conditions to give 3-methyl-6-nitroisoxazolo[4,5-*b*]pyrazine 7-oxide (**8**) but the yields were impractically low to use this as an approach to preparing the 6-aminoheterocycle. Such low yields seem to be characteristic in nitration of analogous *N*-oxides (7). These reactions are depicted in Scheme 1.

The chemistry of the diamine, derived from **4** showed clearly the large difference in basicity between the two amino groups. Previously Boulton and Katritzky (8) reported that 4-aminoisoxazole possessed a pK_a 3.8 units higher than that of 5-aminoisoxazole. This difference is explicable on the basis of the much-diminished ability of the 4-amino group to delocalize its unshared electron pair. Thus, reaction of the diamine with a limited amount of ethyl oxalyl chloride gave 5-amino-4-ethyloxalylamido-3-methylisoxazole (**9**); with excess reagent 4,5-diethyloxalylamido-3-methylisoxazole (**10**) was easily prepared. The latter compounds were visualized as providing an

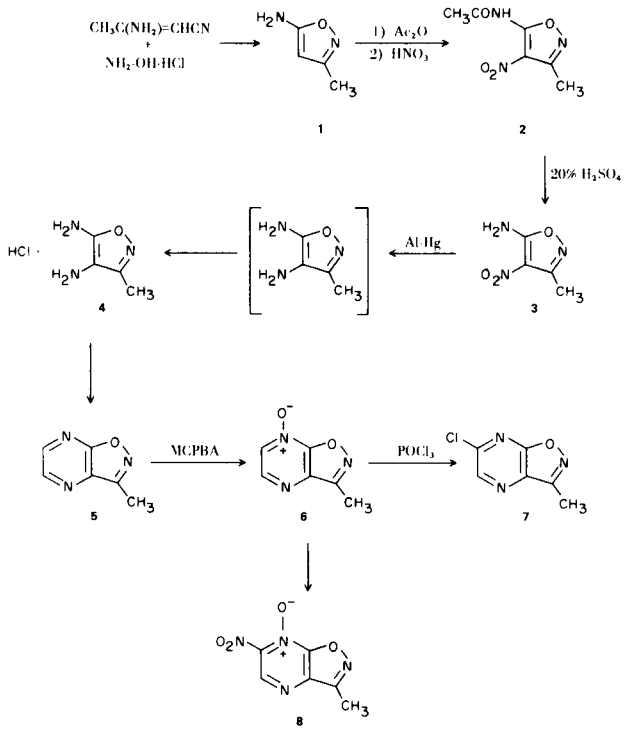
TABLE I



Compound	n=	X=	δ ppm H_5	δ ppm H_6	δ ppm CH_3
5	0	H	8.6 or 8.76 (d) ($J = 2H_z$)	8.76 or 8.6 (d) ($J = 2H_z$)	2.73 (s)
6	1	H	8.5 (d) ($J = 4H_z$)	8.2 (d) ($J = 4H_z$)	2.82 (s)
7	0	Cl	8.62 (s)		2.68 (s)
8	1	NO_2	9.45 (s)		2.7 (s)

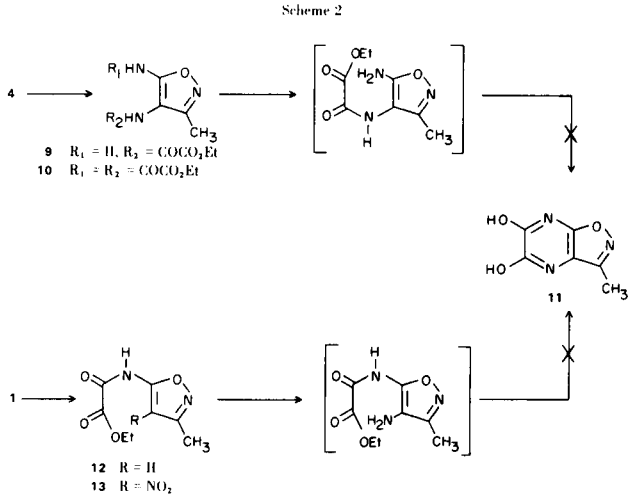
alternative approach to functionalized derivatives of **5**. One could anticipate that heating of **9** would lead spontaneously to the 5,6-dihydroxy-3-methylisoxazolo[4,5-*b*]-pyrazine (**11**), or that selective saponification of **10** would give **9**, *in situ*, in a form that would lead to **11**. In neither case was there evidence for such a ring closure. The 5-amino-3-methylisoxazole (**1**) was then converted to 5-

Scheme 1

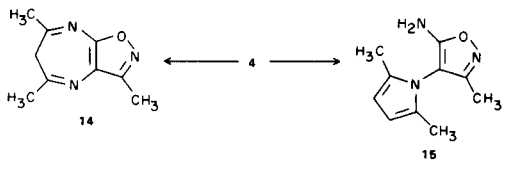


ethyloxalylamido-3-methylisoxazole (**12**) which was nitrated to give 5-ethyloxalylamido-3-methyl-4-nitroisoxazole (**13**). The reduction of **13** with aluminum amalgam was studied, with the expectation of forming the 4-amino derivative, *in situ*, which would spontaneously cyclize to **11**. Again no evidence for the dihydroxy derivative (**11**) was found. Scheme 2 summarizes these reactions.

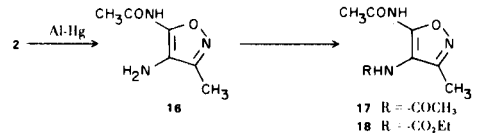
Scheme 2



By analogy with Desimoni and Minoli's latter work with 3-phenylisoxazolo[4,5-*b*]pyrazine (9), the diamine derived from **4** was allowed to react with 2,4-pentanedione to give 3,5,7-trimethylisoxazolo[4,5-*b*][1,4]diazepine (**14**) and with 2,5-hexanedione to afford 5-amino-4-[2,5-dimethyl-1-pyrryl]-3-methylisoxazole (**15**), derived from the more basic 4-amino group. The nmr data (see Experimental) offer conclusive evidence for the structural assignment of these compounds.



In the course of the synthetic work, 5-acetamido-4-nitro-3-methylisoxazole (**2**) was reduced to the corresponding 5-acetamido-4-amino-3-methylisoxazole (**16**) using aluminum amalgam. This was converted to 4,5-diacetamido-3-methylisoxazole (**17**) and to ethyl 5-acetamido-3-methylisoxazolo-4-carbamate (**18**).



EXPERIMENTAL

Uv absorption spectra were measured on a Cary Model 15 spectrophotometer. Ir spectra were determined on a Beckman IR 8 spectrophotometer. Nmr spectra were recorded on either a Varian A-60 or Jeolco C-60-HL spectrometer with tetramethylsilane as the internal standard. Mass spectra were obtained on a Perkin Elmer RMV-6E or CEC-104 spectrometer. Microanalyses were performed by Micro-Analysis, Inc., Marshallton, Delaware. Melting points were taken by the capillary method in a Thomas Hoover capillary melting point apparatus at a rate of heating of 2°/minute without correction. All thin layer chromatographic plates were Brinkmann Silplate-F-52. Unless otherwise indicated, all organic solutions of products were dried over anhydrous magnesium sulfate and evaporated under reduced pressure.

5-Amino-3-methylisoxazole (**1**) (10a-c).

Hydroxylamine hydrochloride (84.0 g., 1.2 moles) was dissolved in 10% sodium hydroxide solution (450 ml.) and the *p*H was adjusted to 9. This was added to a stirred cold solution of 3-aminocrotononitrile (82.0 g., 1 mole, Aldrich) in 95% ethanol (200 ml.). Stirring was continued for 30 minutes after which the solution was extracted with ether (100 ml. x 3). The ether extracts were dried and evaporated to yield a dark liquid from which a yellow solid (81.0 g.) crystallized out upon cooling. Recrystallization from benzene gave white needles, m.p. 80-82°.

5-Acetamido-3-methyl-4-nitroisozole (**2**).

Synthesis of this compound could be conveniently achieved from **1** without the isolation of the intermediate acetamide. Thus, crude 5-amino-3-methylisoxazole (81.0 g.) was dissolved in acetic anhydride (200 ml.) and then heated on the steam bath for one hour. Cooling resulted in the formation of a precipitate. A 10% solution of concentrated nitric acid (65 ml.) in acetic anhydride (650 ml.) was carefully prepared by dropwise addition of nitric acid to cold (ice bath) acetic anhydride while stirring. This cold solution was carefully added to the above suspension of the acetamide in acetic anhydride with cooling and stirring. Upon complete addition a clear solution resulted which after several hours of standing in an ice bath furnished the product as a white solid (59.5 g.). The *p*H of the mother liquor was adjusted to 5 using 10% sodium hydroxide solution. This was followed by chloroform extraction. Drying and evaporation afforded a second crop (32.5 g.) with a total yield of 46%. The analytical sample was obtained by recrystallization from a mixture of DMSO and water, m.p. 136-138°.

Anal. Calcd. for $C_6H_7N_3O_4$: C, 38.94; H, 3.78; N, 22.69. Found: C, 38.87; H, 3.58; N, 22.75.

5-Amino-3-methyl-4-nitroisoxazole (**3**).

The nitroacetamide (**2**) (9.25 g., 0.05 mole) was suspended in 20% sulfuric acid (150 ml.) and heated on the steam bath for one hour. The product crystallized out upon cooling in quantitative yield, m.p. 149-151°.

Anal. Calcd. for $C_4H_5N_3O_3$: C, 33.59; H, 3.50; N, 29.36. Found: C, 33.67; H, 3.30; N, 29.24.

4,5-Diamino-3-methylisoxazole hydrochloride (**4**).

The aluminum amalgam was prepared in the following manner: shredded aluminum foil (about 1 cm^2) was treated with dilute sodium hydroxide to the point of strong hydrogen evolution. The liquid was then decanted and the metal was washed once with water and then treated with 0.5% mercuric chloride solution for one minute and the entire process was repeated. The shiny amalgamated metal was then washed rapidly and in turn with water, ethanol and ether was used at once.

The nitroamine (**3**) (14.3 g., 0.1 mole) was dissolved in 4% aqueous tetrahydrofuran (300 ml.). Aluminum amalgam (about 5.0 g.) was added (exothermic reaction). When gas evolution ceased the residue was filtered off, and concentrated hydrochloric acid (12.3 ml.) was added to the filtrate. The solid formed was filtered off (7.95 g., 54% yield).

Anal. Calcd. for $C_4H_8N_3ClO$: C, 32.11; H, 5.35; N, 28.09; Cl, 23.75. Found: C, 32.40; H, 5.55; N, 27.77; Cl, 23.69.

3-Methylisoxazolo[4,5-*b*]pyrazine (**5**).

Freshly prepared diamine hydrochloride (**4**) (0.74 g., 5 mmoles) was dissolved in water (50 ml.) and cooled to 0°. The bisulfite adduct of glyoxal was prepared by adding a 40% solution (3.75 g., 25 mmoles) of glyoxal in water to a solution (50 ml.) of sodium bisulfite (5.2 g., 0.05 mole) in water. After cooling this adduct was added to the diamine solution and was kept under refrigeration overnight. Extraction with ether followed by the usual work-up yielded a yellowish solid (0.346 g., 51% yield). The analytical sample was prepared by filtration through silica gel using benzene as the eluant, m.p. 61-62°; nmr (see Table I), uv (cyclohexane): λ max 293 nm (ϵ, 10,000), 287 nm (ϵ, 9,600), 241 nm (ϵ, 2,160); mass spectrum, m/e (relative intensity): 135 (50) (M^+), 107 (12.5), 94 (37), 67 (45), 66 (15), and 40 (100).

Anal. Calcd. for $C_6H_5N_3O$: C, 53.35; H, 3.70; N, 31.10. Found: C, 53.23; H, 3.83; N, 30.83.

3-Methylisoxazolo[4,5-*b*]pyrazine 7-Oxide (**6**).

The isoxazolopyrazine (**5**) (1.0 g., 7.4 mmoles) was dissolved in chloroform (25 ml.). To this solution was added a phosphate buffer (*p*H = 7.5) (25 ml.) followed by *m*-chloroperbenzoic acid (2.5 g.). This two layer system was refluxed on the steam bath for one hour. An additional amount of the peracid was added (2.5 g.) and the reaction mixture was refluxed overnight. The layers were separated and the organic phase was washed with 5% sodium hydroxide, dried and evaporated to furnish a yellow solid. This was crystallized from ether/hexane to yield the *N*-oxide as white needles (0.67 g., 60%), m.p. 95-96°; nmr (see Table I); ir (chloroform) 1290 cm^{-1} (N → O).

Anal. Calcd. for $C_6H_5N_3O_2$: C, 47.68; H, 3.31; N, 27.81. Found: C, 47.50; H, 3.56; N, 27.52.

6-Chloro-3-methylisoxazolo[4,5-*b*]pyrazine (**7**).

The *N*-oxide (**6**) (2.8 g., 18.5 mmoles) was carefully added to phosphoryl chloride (50 ml.) while stirring. The reaction mixture was heated at 120° overnight. The cooled reaction mixture was

poured over ice (50.0 g.). The resulting solution was neutralized with a sodium bicarbonate powder followed by chloroform extraction. Subsequent work-up gave a crude liquid product (3.0 g.) from which the analytical sample (2.14 g., 68%) was obtained as a colorless liquid by filtration on silica gel eluting with chloroform; nmr (see Table I).

Anal. Calcd. for $C_6H_4N_3OCl$: C, 42.48; H, 2.36; N, 24.78. Found: C, 42.66; H, 2.63; N, 24.84.

3-Methyl-6-nitroisoxazolo[4,5-*b*]pyrazine 7-Oxide (**8**).

A 20% solution of nitric acid in acetic anhydride (10 ml.) was added dropwise to a stirred cool suspension of the *N*-oxide (**6**) (0.75 g., 4.5 mmoles) in acetic anhydride (10 ml.). The reaction mixture was then heated (50-80°) for two hours. After cooling and evaporation, water was added followed by chloroform extraction. The crude product obtained after the usual work-up was filtered on silica gel eluting with chloroform to give the analytical sample (48 mg., 5%), m.p. 187°; nmr (see Table I); ir (potassium bromide): 3080, 1550, 1340 ($-NO_2$), and 1250 cm^{-1} ($N \rightarrow O$).

Anal. Calcd. for $C_6H_4N_4O_4$: C, 36.74; H, 2.04. Found: C, 36.58; H, 2.20.

5-Amino-4-ethyloxalylamido-3-methylisoxazole (**9**).

Freshly prepared **4** (3.8 g., 25.4 mmoles) was dissolved in chloroform (200 ml.) and triethylamine (8 ml.). This was rapidly mixed with a solution of ethyloxalyl chloride (5.0 g., 36.6 mmoles) in chloroform (250 ml.), and the reaction mixture was immediately poured into water (50 ml.). The chloroform layer was separated and the aqueous phase was extracted once with chloroform. The combined extracts were dried and evaporated to give the product (3.66 g., 86%). The analytical sample was obtained by crystallization from chloroform, m.p. 147°.

Anal. Calcd. for $C_8H_{11}N_3O_4$: C, 45.07; H, 5.16; N, 19.72. Found: C, 45.30; H, 5.26; N, 19.42.

4,5-Diethyloxalylamido-3-methylisoxazole (**10**).

Freshly prepared **4** (1.5 g., 0.01 mole) was dissolved in chloroform (20 ml.) and triethylamine (5 ml.). To this, ethyloxalychloride (4.0 g., 0.029 mole) was added dropwise with stirring. After the addition was completed, the mixture was stirred for one hour followed by washing with water (20 ml., 3 times). The chloroform layer was dried, concentrated and treated with ether (20 ml.) to offer white solids (1.4 g., 45%). The analytical sample was prepared by crystallization from chloroform/ether, m.p. 141-142°.

Anal. Calcd. for $C_{12}H_{15}N_3O_7$: C, 46.02; H, 4.79; N, 13.42. Found: C, 46.08; H, 4.72; N, 13.66.

5-Ethyloxalylamido-3-methylisoxazole (**12**).

Compound **1** (0.98 g., 0.01 mole) was dissolved in chloroform (30 ml.) and pyridine (1 ml.). A solution of ethyloxalyl chloride (1.48 g., 0.015 mole) in chloroform (10 ml.) was added dropwise while stirring. After one hour the reaction mixture was washed several times with water, dried, filtered and evaporated to give a gum which crystallized from ether (1.4 g., 72%), m.p. 133-135°.

Anal. Calcd. for $C_8H_{10}N_2O_4$: C, 48.48; H, 5.05; N, 14.14. Found: C, 48.72; H, 5.07; N, 14.41.

5-Ethyloxalylamido-3-methyl-4-nitroisoxazole (**13**).

The procedure was similar to that used for the preparation of **2**. Thus compound **12** (12.84 g., 0.065 mole) was nitrated with nitric acid/acetic anhydride and subsequent work-up furnished the product (10.7 g., 65%), m.p. 119-121°.

Anal. Calcd. for $C_8H_9N_3O_6$: C, 39.51; H, 3.70; N, 17.28. Found: C, 39.63; H, 3.70; N, 17.51.

3,5,7-Trimethylisoxazolo[4,5-*b*][1,4]diazepine (**14**).

A 25% aqueous solution of 2,4-pentanedione (10 ml.) was added to a solution of the diamine (**4**) (2.6 g., 17.3 mmoles) in water (20 ml.) with stirring, resulting in immediate formation of a purple color. After stirring for two hours, the reaction mixture was extracted with ether (30 ml. x 3). The organic extracts were dried, filtered, evaporated and the residue was crystallized from ether/cyclohexane to give the product (0.9 g., 30%), m.p. 76-78°; nmr (deuteriochloroform): δ 2.31 (3H, s), 2.37 (3H, s), 2.4 (3H, s), 3.05 (2H, s).

Anal. Calcd. for $C_9H_{11}N_3O$: C, 61.02; H, 6.21; N, 23.73. Found: C, 60.94; H, 6.28; N, 23.56.

5-Amino-4-[2,5-dimethyl-1-pyrryl]-3-methylisoxazole (**15**).

Acetonylacetone (3 ml.) was added dropwise to a freshly prepared solution of **4** (1.9 g., 12.6 mmoles) in water (25 ml.). The reaction mixture was stirred for two hours and was then extracted with chloroform. Drying and evaporation of the extracts followed by crystallization of the residue from cyclohexane gave the title compound (0.67 g., 45%), m.p. 125-127°; nmr (deuteriochloroform): δ 1.80 (3H, s), 1.85 (6H, s), 4.72 (2H, broad, deuterium oxide exchangeable), 6.24 (2H, s).

Anal. Calcd. for $C_{10}H_{13}N_3O$: C, 62.78; H, 6.91; N, 21.99. Found: C, 62.83; H, 6.75; N, 22.09.

5-Acetamido-4-amino-3-methylisoxazole (**16**).

The procedure was similar to that used for the preparation of the diamine **4**. Thus, the nitroacetamide (**2**) (5.55 g., 0.03 mole) upon treatment with aluminum amalgam and subsequent work-up furnished the product (2.2 g., 55%), m.p. 141-143°; mass spectrum, m/e: 155 (M^+).

Anal. Calcd. for $C_6H_9N_3O_2$: C, 46.45; H, 5.81; N, 27.10. Found: C, 46.19; H, 5.97; N, 26.82.

4,5-Diacetamido-3-methylisoxazole (**17**).

The monoacetamide (**16**) (1.0 g., 6.5 mmoles) was dissolved in acetic anhydride (20 ml.). Upon standing the product crystallized out (1.02 g., 78%), m.p. 176-178°.

Anal. Calcd. for $C_8H_{11}N_3O_3$: C, 48.75; H, 5.58; N, 21.31. Found: C, 48.57; H, 5.83; N, 21.58.

Ethyl-5-acetamido-3-methylisoxazolo-4-carbamate (**18**).

The monoacetamide (**16**) (0.18 g., 1.2 mmoles) was dissolved in ice cold water (20 ml.). Ethylchloroformate (0.5 ml.) was added with stirring. After one hour, the product was filtered off (0.2 g., 70%), m.p. 130-132°.

Anal. Calcd. for $C_9H_{13}N_3O_4$: C, 47.58; H, 5.73; N, 18.50. Found: C, 47.48; H, 5.78; N, 18.59.

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REFERENCES

(1) This investigation was carried out under the auspices of the Division of Cancer Treatment, National Cancer Institute,

National Institutes of Health, Public Health Service Contract No. NIH-71-2312.

(2) M. Ishizuka, T. Takeuchi, K. Nitta, G. Koyama, M. Hori, and H. Umezawa, *J. Antibiotics (Tokyo)*, 17A, 124 (1964).

(3) G. Desimoni and G. Minoli, *Tetrahedron*, **24**, 4907 (1968).

(4) C. Musante, *Gazz. Chim. Ital.*, **76**, 131 (1946).

(5) A. Katritzky, Ed., "Advances in Heterocyclic Chemistry", Vol. 2, Academic Press, New York, N. Y., 1963, pp. 396-397.

(6) H. V. Euler and H. Hosselquist, *Arkiv Kimi.*, **12**, 559 (1958).

(7) A. Katritzky and J. Lagowski, "Chemistry of the Heterocyclic *N*-oxides", Academic Press, New York, N. Y., 1971, pp. 236, 240-241.

(8) A. Boulton and A. Katritzky, *Tetrahedron*, **12**, 51 (1961).

(9) G. Desimoni and G. Minoli, *ibid.*, **26**, 1393 (1970).

(10) Other methods of preparation for this compound are: (a) I. Iwai and N. Kakamura, *Chem. Pharm. Bull.*, **14**, 1277 (1966); (b) G. Shaw and G. Sugowdz, *J. Chem. Soc.*, 665 (1954); (c) E. Haruki, Y. Hirai, and E. Imoto, *Bull. Chem. Soc. Japan*, **41**, 267 (1968).