Facile Synthesis and Antifungal Activity of 3-Substituted 4-Amino-8-ethoxycarbonylpyrazolo[5,1-*c*][1,2,4]triazines and Pyrazolo[1′,5′:3,4][1,2,4]triazino[5,6-*b*][1,5]benzodiazepines [1]

Yoshihisa Kurasawa,* Megumi Kanoh, Yumiko Kamigaki,
Mari Okiyama and Atsushi Takada

School of Pharmaceutical Sciences, Kitasato University, Shirokane, Minato-ku, Tokyo 108, Japan

Yoshihisa Okamoto

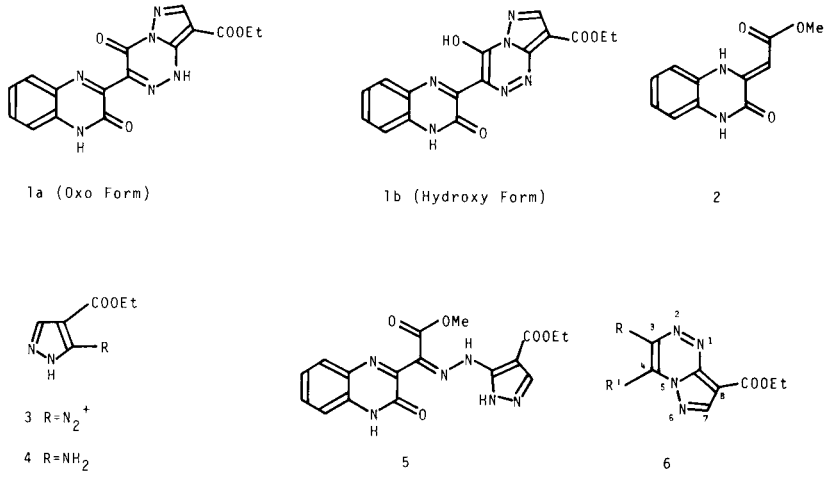
Division of Chemistry, College of Liberal Arts and Sciences, Kitasato University, Kitasato, Sagamihara-shi, Kanagawa 228, Japan

Received December 16, 1987

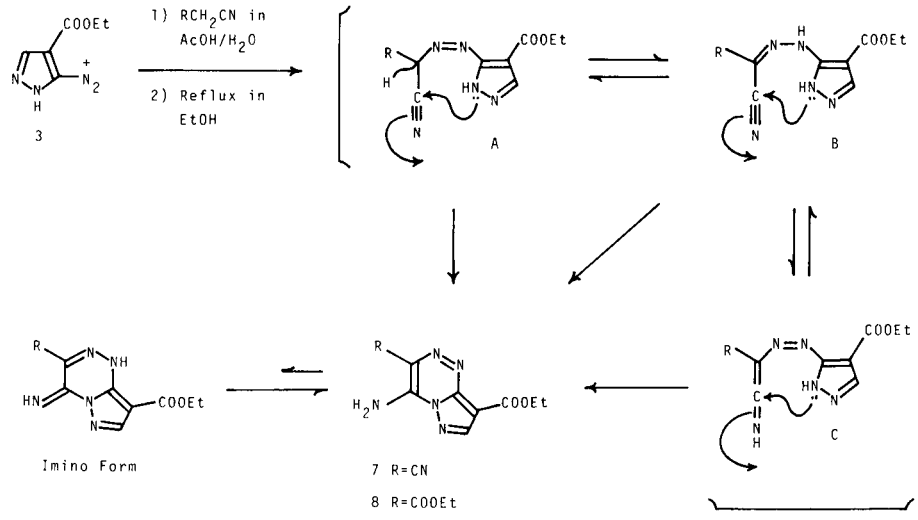
The reactions of the pyrazole-5-diazonium salt **3** with malononitrile and ethyl cyanoacetate gave 4-amino-3-cyano-8-ethoxycarbonylpyrazolo[5,1-*c*][1,2,4]triazine **7** and 4-amino-3,8-bisethoxycarbonylpyrazolo[5,1-*c*]-[1,2,4]triazine **8**, whose reactions with *p*-chloroaniline hydrochloride afforded 4-amino-8-ethoxycarbonyl-3-(*p*-chlorophenyl)amidinopyrazolo[5,1-*c*][1,2,4]triazine **9** and 4-amino-8-ethoxycarbonyl-3-(*p*-chlorophenyl)carbamoylpyrazolo[5,1-*c*][1,2,4]triazine **10**, respectively. The reactions of **7** and **8** with *o*-phenylenediamine dihydrochloride provided 9-ethoxycarbonyl-13*H*-spiro[benzimidazole-2′(3′*H*),6(5*H*)-pyrazolo[1′,5′:3,4][1,2,4]triazino[5,6-*b*][1,5]benzodiazepine] hydrochloride **11a** and 9-ethoxycarbonyl-6-oxo-13*H*-5,6-dihydropyrazolo-[1′,5′:3,4][1,2,4]triazino[5,6-*b*][1,5]benzodiazepine **12**, respectively. The antifungal activity of the above compounds was described.

J. Heterocyclic Chem., **25**, 1015 (1988).

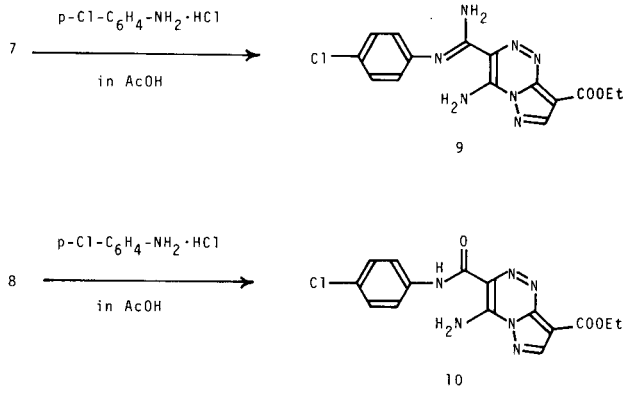
In a previous paper [2], we reported the synthesis of the 3-quinoxalinylpyrazolo[5,1-*c*][1,2,4]triazine **1a** from the reaction of the quinoxaline **2** with the pyrazole-5-diazonium salt **3**, produced from the pyrazole **4**, *via* the pyrazolylhydrazone **5** (Chart). This reaction provided a facile method for the synthesis of some substituted pyrazolo[5,1-*c*][1,2,4]-triazines, but it was not suitable for the construction of condensed pyrazolo[5,1-*c*][1,2,4]triazines. In order to prepare various condensed pyrazolo[5,1-*c*][1,2,4]triazines, it was indispensable to produce the 3,4-bifunctional pyrazolo[5,1-*c*][1,2,4]triazine such as **6** shown in the Chart. Therefore, we devised the synthesis of the 3,4-disubstituted pyrazolo[5,1-*c*][1,2,4]triazine using the pyrazole-5-diazonium salt **3** and active methylene compounds such as malononitrile and ethyl cyanoacetate [3-5]. As the result, we succeeded in the synthesis of 3-substituted 4-amino-8-ethoxycarbonylpyrazolo[5,1-*c*][1,2,4]triazines **7** and **8** (Scheme 1), which were found to be versatile intermediates to condensed pyrazolo[5,1-*c*][1,2,4]triazines [1,6]. This paper describes the synthesis of the 3-substituted 4-amino-8-ethoxycarbonylpyrazolo[5,1-*c*][1,2,4]triazines **7**, **8** (Scheme 1), **9** and **10** (Scheme 2) and the conversion of **7** and **8** into the pyrazolo[1′,5′:3,4][1,2,4]triazino[5,6-*b*][1,5]benzodiazepines **11** and **12** (Scheme 3) together with the screening data for the above compounds.



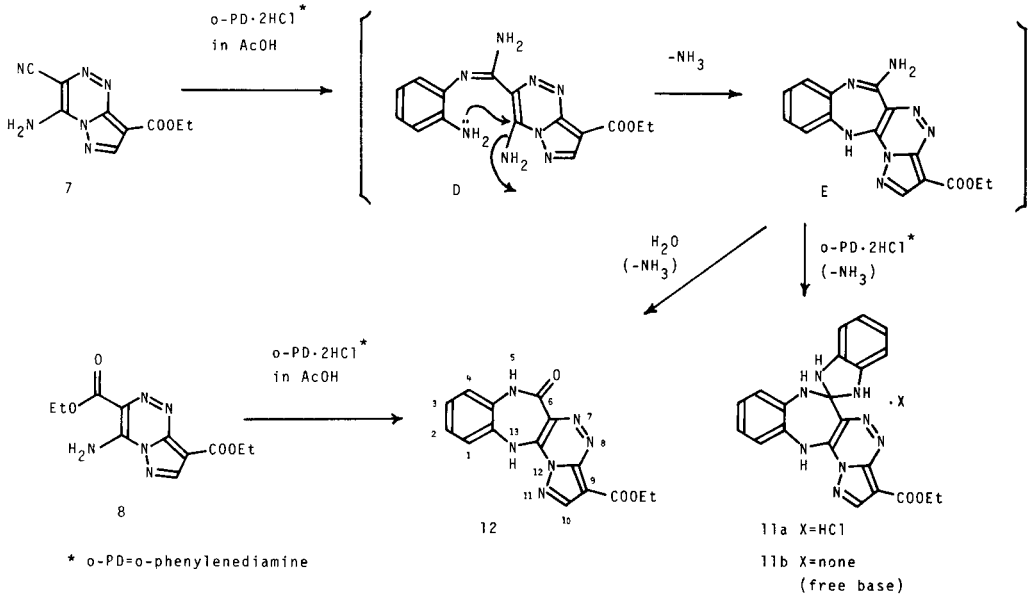
Chart



Scheme I



Scheme II



Scheme III

The reactions of the pyrazole-5-diazinium salt **3** with malononitrile and ethyl cyanoacetate in acetic acid/water and then in ethanol resulted in diazotization and cyclization to give 4-amino-3-cyano-8-ethoxycarbonylpyrazolo-[5,1-*c*][1,2,4]triazine **7** as an ethanol complex (85%) and 4-amino-3,8-bisethoxycarbonylpyrazolo[5,1-*c*][1,2,4]triazine **8** (65%), respectively, presumably *via* an intermediate **A** [3], **B** [7] or **C** (Scheme 1). Compounds **7** and **8** were found to exist in the amino form, but not in the imino form, from the pmr spectral data in deuteriodimethylsulfoxide, while compound **1** occurred in the oxo form **1a**, but not in the hydroxy form **1b**, in the solid state [2].

Compounds **7** and **8** did not react with *p*-chlorobenzaldehyde, acetophenone, *p*-chloroaniline and *o*-phenylenediamine in some solvents, but they easily reacted with hydrochlorides of *p*-chloroaniline and *o*-phenylenediamine in acetic acid.

The reaction of **7** and **8** with *p*-chloroaniline hydrochloride in acetic acid provided 4-amino-8-ethoxycarbonyl-3-(*p*-chlorophenyl)amidinopyrazolo[5,1-*c*][1,2,4]triazine **9** (63%) and 4-amino-8-ethoxycarbonyl-3-(*p*-chlorophenyl)-carbamoylpyrazolo[5,1-*c*][1,2,4]triazine **10** (67%), respectively (Scheme 2), indicating that the substituent at the 3-position is susceptible for the reaction.

The reaction of **7** with a 3-fold molar amount of *o*-phenylenediamine dihydrochloride in acetic acid furnished 9-ethoxycarbonyl-13*H*-spiro[benzimidazole-2'(3'*H*),6(5*H*)-pyrazolo[1',5':3,4][1,2,4]triazino[5,6-*b*][1,5]benzodiazepine] hydrochloride **11a** (53%) and 9-ethoxycarbonyl-6-oxo-13*H*-5,6-dihydropyrazolo[1',5':3,4][1,2,4]triazino[5,6-*b*][1,5]-benzodiazepine **12** (28%), presumably *via* intermediates **D** and **E** (Scheme 3). The reaction of **7** with an equimolar amount of *o*-phenylenediamine dihydrochloride in acetic acid gave the hydrochloride **11a** in a poor yield (18%). Since the reaction of **8** with *o*-phenylenediamine dihydrochloride in acetic acid produced **12** (56%), the formation of an intermediate **E** was supported. Treatment of **11a** with 10% sodium hydroxide solution gave the free base **11b**. The spiro ring structure of **11b** was assigned by the pmr and ^{13}C-nmr spectral data. Namely, the spiro carbon signal was observed at δ 96 ppm.

Table

Antifungal Activity of Compounds **7-10** at Concentration of 100 ppm.

Compound	Activity [a] P.d.	R.s.	P.o. [b]
7	30	63	0
8	30	80	30
9	84	45	0
10	59	28	0

[a] Growth inhibition (%). [b] P.d.: *Pythium debaryanum;* R.s.: *Rhizoctonia solani;* P.o.: *Pyricularia oryzae.*

Compounds **7**, **8**, **9** and **10** showed a weak antifungal activity (28-80% growth inhibition) against *Pythium debaryanum, Rhizoctonia solani* and *Pyricularia oryzae* at a concentration of 100 ppm (Table), while compounds **11** and **12** exhibited no antifungal activity against the above three fungi. All the above compounds represented no antibacterial activity against *Xanthomonas oryzae, Erwinia carotovora* and *Pseudomonas lachrymans.*

EXPERIMENTAL

All melting points were determined on a Ishii melting point BY-2 apparatus and are uncorrected. The ir spectra (potassium bromide) were recorded with a JASCO IRA-1 spectrophotometer. The pmr and ^{13}C-nmr spectra were measured in deuteriodimethylsulfoxide with an EM 390 and an XL-400 spectrometers at 90 and 400 MHz, respectively, using tetramethylsilane as an internal reference. Chemical shifts are given in the δ scale, relative to the internal reference. The mass spectra (ms) were determined with a JEOL JMS-01S spectrometer. Elemental analyses were performed on a Perkin-Elmer 240B instrument.

4-Amino-3-cyano-8-ethoxycarbonylpyrazolo[5,1-*c*][1,2,4]triazine **7** (Ethanol Complex) and 4-Amino-3,8-bisethoxycarbonylpyrazolo[5,1-*c*][1,2,4]-triazine **8**.

A solution of sodium nitrite (4.45 g, 64.5 mmoles) in water (50 ml) was added dropwise to a solution of the pyrazole **4** (10 g, 64.5 mmoles) in acetic acid (150 ml) with stirring for 10 minutes in an ice-water bath to give a clear solution, to which malononitrile (6.38 g, 96.8 mmoles) or ethyl cyanoacetate (10.94 g, 96.8 mmoles) was added portionwise or dropwise, respectively. Stirring was continued for an additional 20 minutes to precipitate colorless crystals, and the mixture was heated on a boiling water bath for 10 minutes in order to accomplish the diazotization. Then, ethanol (200 ml) was added to the reaction mixture to dissolve the colorless crystals under reflux (about 40 minutes) on a boiling water bath with intermittent stirring.

When malononitrile was used, the solution obtained above was allowed to stand at room temperature to precipitate analytically pure colorless needles **7** as ethanol complex, which were collected by suction filtration (15.09 g, 85%).

When ethyl cyanoacetate was employed, the solvent was evaporated *in vacuo* to give colorless crystals, which were dissolved in ethanol (500 ml) under reflux on a boiling water bath, and refluxing was continued for an additional 1 hour to give a clear solution. The hot clear solution was filtered, and the filtrate was allowed to stand at room temperature to precipitate analytically pure colorless needles **8**, which were collected by suction filtration (11.7 g, 65%).

Compound **7** had mp 270-275° dec; ir: ν cm^{-1} 3490, 2960, 2230, 1660; ms: m/z 232 (M^+); pmr: 9.63 (s, 2H, NH_2), 8.73 (s, 1H, C_7-H), 4.37 (q, J = 7 Hz, 2H, CH_2), 4.27 (s, 1H, OH), 3.44 (q, J = 7 Hz, 2H, CH_2), 1.33 (t, J = 7 Hz, 3H, CH_3), 1.04 (t, J = 7 Hz, 3H, CH_3).

Anal. Calcd. for $C_9H_8N_6O_2 \cdot C_2H_5OH$: C, 47.48; H, 5.07; N, 30.20. Found: C, 47.18; H, 4.94; N, 30.32.

Compound **8** had mp 201-202°; ir: ν cm^{-1} 3340, 3260, 3200, 2970, 1685, 1630; ms: m/z 279 (M^+); pmr: 9.14 (brs, 2H, NH_2), 8.73 (s, 1H, C_7-H), 4.44 (q, J = 7 Hz, 2H, CH_2), 4.36 (q, J = 7 Hz, 2H, CH_2), 1.42 (t, J = 7 Hz, 3H, CH_3), 1.34 (t, J = 7 Hz, 3H, CH_3).

Anal. Calcd. for $C_{11}H_{13}N_5O_4$: C, 47.31; H, 4.69; N, 25.08. Found: C, 47.18; H, 4.58; N, 25.11.

4-Amino-8-ethoxycarbonyl-3-(*p*-chlorophenyl)amidinopyrazolo[5,1-*c*]-[1,2,4]triazine **9**.

A solution of **7** (2 g, 7.19 mmoles) and *p*-chloroaniline hydrochloride (1.77 g, 10.79 mmoles) in acetic acid (100 ml) was refluxed in an oil bath for 2 hours to give a clear solution. Evaporation of the solvent *in vacuo* gave crystals, whose recrystallization from *N,N*-dimethylformamide/etha-

nol afforded yellow needles (1.63 g, 63%), mp 303-304°; ir: ν cm^{-1} 3430, 3240, 2980, 1700; ms: m/z 359 (M^+), 361 ($M^+ + 2$); pmr: 10.67 (s, 2H, NH_2), 9.57 (s, 2H, NH_2), 8.71 (s, 1H, C_7-H), 7.63-6.90 (m, 4H, aromatic), 4.33 (q, J = 7 Hz, 2H, CH_2), 1.33 (t, J = 7 Hz, 3H, CH_3).

Anal. Calcd. for $C_{15}H_{14}ClN_7O_2$: C, 50.08; H, 3.92; Cl, 9.85; N, 27.25. Found: C, 49.94; H, 3.86; Cl, 10.04; N, 26.98.

4-Amino-8-ethoxycarbonyl-3-(*p*-chlorophenyl)carbamoylpyrazolo[5,1-*c*]-[1,2,4]triazine **10**.

A solution of **8** (5 g, 17.9 mmoles) and *p*-chloroaniline hydrochloride (6.55 g, 39.9 mmoles) in acetic acid (250 ml) was refluxed in an oil bath for 3 hours to give a clear solution. Evaporation of the solvent *in vacuo* provided an oily residue, which was triturated with hot water to furnish yellow crystals. Recrystallization from *N,N*-dimethylformamide/ethanol afforded yellow needles (4.30 g, 67%), mp above 330°; ir: ν cm^{-1} 3400, 3180, 3090, 1710, 1610; ms: m/z 360 (M^+), 362 ($M^+ + 2$); pmr: 11.20 (brs, 1H, NH), 9.83 (s, 2H, NH_2), 8.45 (s, 1H, C_7-H), 7.73-7.40 (m, 4H, aromatic), 4.31 (q, J = 7 Hz, 2H, CH_2), 1.33 (t, J = 7 Hz, 3H, CH_3).

Anal. Calcd. for $C_{15}H_{13}ClN_6O_3$: C, 49.94; H, 3.63; Cl, 9.83; N, 23.30. Found: C, 49.97; H, 3.60; Cl, 10.04; N, 23.54.

9-Ethoxycarbonyl-13*H*-spiro[benzimidazole-2'(3'*H*),6(5*H*)-pyrazolo-[1',5':3,4][1,2,4]triazino[5,6-*b*][1,5]benzodiazepine] Hydrochloride **11a** and 9-Ethoxycarbonyl-6-oxo-13*H*-5,6-dihydropyrazolo[1',5':3,4][1,2,4]triazino-[5,6-*b*][1,5]benzodiazepine **12**.

A suspension of **7** (5 g, 17.99 mmoles) and *o*-phenylenediamine dihydrochloride (9.77 g, 53.97 mmoles) in acetic acid (300 ml) was refluxed in an oil bath for 1 hour to give a clear solution, and then yellow needles **11a** precipitated. Refluxing was carried out for an additional 4 hours. The yellow needles of **11a** were collected by suction filtration, triturated with hot ethanol (500 ml) and then collected by suction filtration to give analytically pure yellow needles of **11a** (4.28 g, 53%), mp 308-309°; ir: ν cm^{-1} 3160, 2620, 1720, 1610; ms: m/z 414 (M^+); pmr: 9.00-6.33 (br, NH and H_2O), 8.07-7.67 (m, 4H, aromatic), 7.92 (s, 1H, C_{10}-H), 7.60-7.27 (m, 4H, aromatic), 4.48 (q, J = 7 Hz, 2H, CH_2), 1.40 (t, J = 7 Hz, 3H, CH_3).

Anal. Calcd. for $C_{21}H_{18}N_8O_2 \cdot HCl$: C, 55.94; H, 4.25; Cl, 7.86; N, 24.85. Found: C, 55.83; H, 4.13; Cl, 8.15; N, 24.75.

After the yellow needles of **11a** were collected by suction filtration, the filtrate (acetic acid solution) was evaporated *in vacuo* to give yellow crystals of **12**, which were triturated with hot water and then collected by suction filtration (1.83 g, 28%). Recrystallization from *N,N*-dimethylformamide/ethanol afforded yellow needles of **12** as a half hydrate, mp above 330°; ir: ν cm^{-1} 1710, 1640, 1615; ms: m/z 324 (M^+); pmr 8.70 (s, 1H, C_{10}-H), 7.83-7.57 (m, 2H, aromatic), 7.45-7.10 (m, 2H, aromatic), 6.67-4.67 (br, NH and H_2O), 4.35 (q, J = 7 Hz, 2H, CH_2), 1.37 (t, J = 7 Hz, 3H, CH_3).

Anal. Calcd. for $C_{15}H_{12}N_6O_3 \cdot 1/2\ H_2O$: C, 54.05; H, 3.93; N, 25.21. Found: C, 54.35; H, 3.72; N, 25.19.

Free Base **11b**.

A slight excess of 10% sodium hydroxide solution was added dropwise to a suspension of **11a** in ethanol with stirring on a boiling water bath to dissolve **11a**. Then a solution was filtered, and acetic acid (0.5 ml) was added to precipitate yellow needles of **11b**, mp 339-340°; ir: ν cm^{-1} 1700, 1600, 1560, 1540; ms: m/z 414 (M^+); pmr: 8.00-7.63 (m, 4H, aromatic), 7.85 (s, 1H, C_{10}-H), 7.53-7.13 (m, 4H, aromatic), 6.50-3.33 (br, NH and H_2O), 4.48 (q, J = 7 Hz, 2H, CH_2), 1.40 (t, J = 7 Hz, 3H, CH_3).

Anal. Calcd. for $C_{21}H_{18}N_8O_2$: C, 60.86; H, 4.38; N, 27.04. Found: C, 60.65; H, 4.35; N, 26.78.

Synthesis of **12** from **8**

A suspension of **8** (3 g, 10.75 mmoles) and *o*-phenylenediamine dihydrochloride (2.92 g, 16.13 mmoles) in acetic acid (200 ml) was refluxed in an oil bath for 5 hours to give a clear solution. The solvent was evaporated *in vacuo* to afford yellow crystals of **12**, which were triturated with hot ethanol/water and then collected by suction filtration (2.18 g, 56%).

REFERENCES AND NOTES

[1] Preliminary report: Y. Kurasawa, Y. Okamoto and A. Takada, *J. Heterocyclic Chem.*, **24**, 1799 (1987).

[2] Y. Kurasawa, A. Satoh, S. Ninomiya, H. Arai, K. Arai, Y. Okamoto and A. Takada, *J. Heterocyclic Chem.*, **24**, 1229 (1987).

[3] M. W. Partridge and M. F. G. Stevens, *J. Chem. Soc. (C)*, 1127 (1966).

[4] H. Neunhoeffer and P. F. Wiley, "The Chemistry of Heterocyclic Compounds; Chemistry of 1,2,3-Triazines and 1,2,4-Triazines, Tetrazines, and Pentazines", A. Weissberger and E. C. Taylor, eds, John Wiley and Sons, New York, Chichester, Brisbane, Toronto, 1978, pp 858-861, and references cited therein.

[5] Partridge and Stevens [3] have already reported the synthesis of pyrazolo[5,1-*c*][1,2,4]triazines using pyrazole-3-diazonium salts, while our method adopts a simplified one-pot synthesis. Compounds **7** and **8** prepared by us are not reported in the compound list [3,4] and in the computer on-line system between 1966 and 1987.

[6] Y. Kurasawa, M. Okiyama, Y. Kamigaki, M. Kanoh, Y. Okamoto and A. Takada, *J. Heterocyclic Chem.*, **24**, 1805 (1987).

[7] J. Slouka, V. Bekarek and J. Kubata, *Monatsh. Chem.*, **105**, 535 (1974).