Aromatization and Ring Cyclization: A better Understanding on the Ring Cyclization Mechanism of 3-Amino-6-hydrazino-1,2,4-triazin-5(2*H*)-one Reacted with Acetic Acid in *N*,*N*-Dimethylformamide

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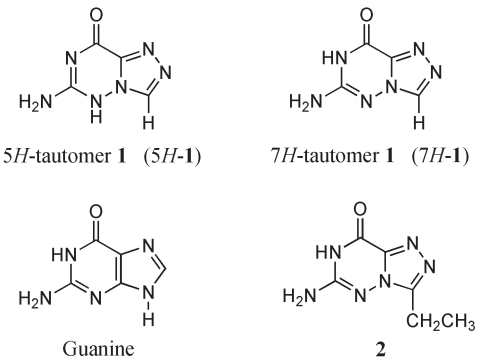
Received November 5, 2004

In this paper we report that the title compound (**3**) reacts with excess *N*,*N*-dimethylformamide (DMF) containing two equivalents of acetic acid to afford 6-amino-1,2,4-triazolo[3,4-*f*][1,2,4]triazin-8(7*H*)-one (**1**). When 3-amino-2-benzyl-6-hydrazino-1,2,4-triazin-5(2*H*)-one (**6**), the N-2 benzylated derivative of **3**, is treated under the same conditions, ring cyclization does not occur; instead, 3-amino-2-benzyl-6-(2-formylhydrazino)-1,2,4-triazin-5(2*H*)-one (**7**) is formed. Single-crystal X-ray analysis of a 3-ethyl derivative of compound **1** reveals the predominant tautomeric structure to be the 7*H*-tautomer (7*H*-**1**). From these results, we propose a reasonable cyclization mechanism that incorporates two important points: (1) the tautomerism of the N-2 hydrogen with the C-5 oxo group aromatizes the 1,2,4-triazine ring, and (2) the DMF is protonated by acetic acid on the nitrogen atom, then deamination occurs where DMF is attacked by the 6-hydrazino group of **3** or **6**.

J. Heterocyclic Chem., **42**, 851 (2005).

Introduction.

Many aza and deaza analogues of purine and their nucleosides have attracted considerable interest due to their biological activities. Compound **1**, 6-amino-1,2,4-triazolo[3,4-*f*][1,2,4]triazin-8(5*H*)-one (4,8-diaza-9-deazaguanine), the isosteric isomer of guanine, has been previously synthesized by Lovelette [1]. He treated 3-amino-6-hydrazino-1,2,4-triazin-5(2*H*)-one (**3**) with two equivalents of acetic acid in excess *N*,*N*-dimethylformamide (DMF) under reflux, and the ring closure at the N-1 nitrogen of the 1,2,4-triazine ring occurred to afford **1**. Lovelette suggested the heterobicyclic structure of **1** as the 5*H*-tautomer (5*H*-**1**) and described the probable mechanism shown in Figure 1 [1]. As a part of our ongoing program, we have recently described the prototropic tautomerism of 6-amino-3-ethyl-1,2,4-triazolo[3,4-*f*][1,2,4]-triazin-8(7*H*)-one (**2**), a 3-ethyl derivative of compound **1** [2]. In that study, the crystal structure of **2** was clarified by single-crystal X-ray analysis, revealing the predominant tautomeric structure as the 7*H*-tautomer, not the 5*H*-tautomer as speculated by Lovelette. This result inspired us to understand more about the actual mechanism of this ring cyclization.



Results and Discussion.

Initially, we worked backward through the mechanism from the 7*H*-tautomer **2** to the starting material compound **3**, which exists predominantly as the 2*H*-tautomer according to the X-ray crystallographic structure previously

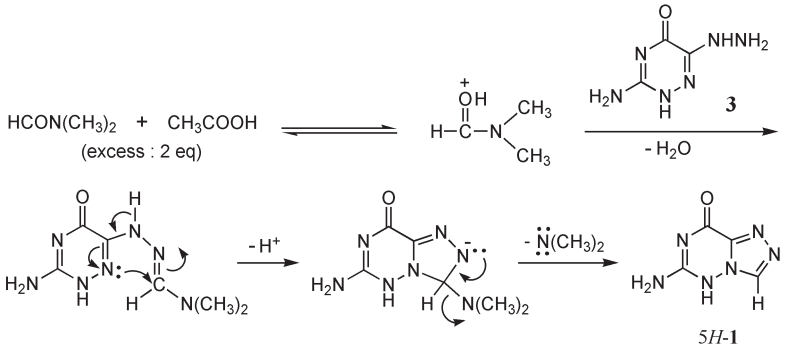
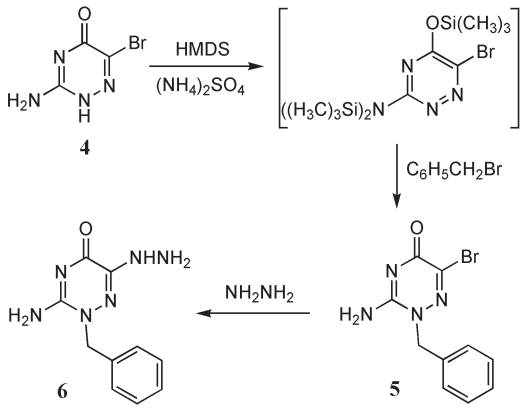


Figure 1

reported by us [3]. Pith *et al*. had presented similar results based on ultraviolet spectra and ionization constants [4]. From our mechanistic analysis we noted that the ring cyclization occurred by way of an aromatic 1,2,4-triazine ring, that is, the 2*H*-5-one aromatized to the 5-ol. Therefore, the cyclization was speculated to be dependent on the 1,2,4-triazine ring aromatization. To prove this hypothesis, our starting point was to replace the N-2-H of **3** with a benzyl group to prevent prototropic tautomerism to the aromatic intermediate. Therefore, we synthesized the N-2-benzyl derivative **6**, and investigated the reactions of **6** with acetic acid in DMF.

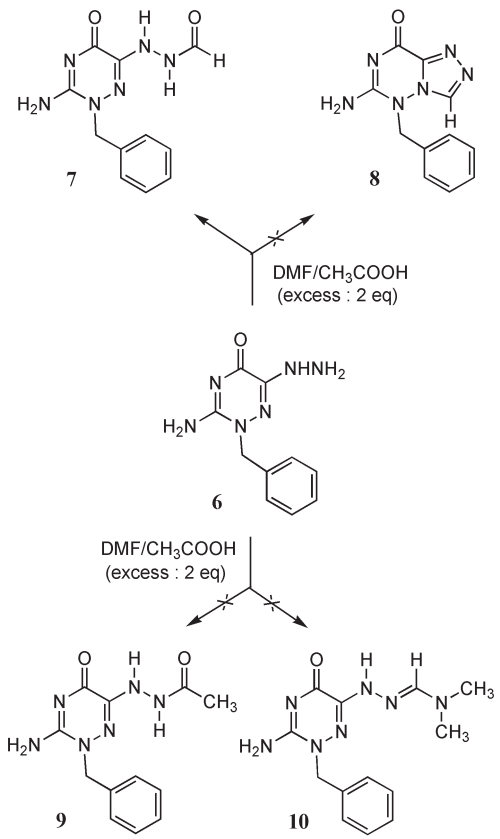
Scheme 1 shows our synthesis of **6** from 3-amino-6-bromo-1,2,4-triazin-5(2*H*)-one (**4**). Treatment of **4** with hexamethyldisilazane gave the silylated intermediate, which was then reacted with benzyl bromide to give 3-amino-2-benzyl-6-bromo-1,2,4-triazin-5(2*H*)-one (**5**). The ^{1}H nmr spectrum of **5** showed two singlets at δ_H 5.17 and 7.58 ppm corresponding to the methylene protons of the benzyl group and the 3-amino group, respectively. The remaining multiplets at δ_H 7.24 to 7.45 ppm were attributed to the aromatic protons of the benzyl group. The benzylation site of **5** was established as N-2 (not N-4) based on uv spectra [5-9] and 2D nmr [5,6]. The long-range ^{1}H-^{13}C heteronuclear correlation (HETCOR) nmr revealed the connectivity of methylene protons (δ_H 5.17 ppm) on the benzyl group to C-3 (δ_C 155.09 ppm) in the 1,2,4-triazine ring. Hydrazination of **5** by nucleophilic substitution with hydrazine monohydrate gave compound **6**. Characterization of **6** was done by the ^{1}H nmr spectrum which showed signals at δ_H 3.90 (s, 2H, NHNH_2) and 7.34 (s, 1H, N*H*NH$_2$) for hydrazino group, revealing that the bromine atom was displaced by hydrazine.

Scheme 1



When **6** was reacted with excess DMF containing two equivalents of acetic acid, **7** was isolated (Scheme 2). According to the reports of Lovelette [1] and Becker *et al*. [10], the intense infrared absorption at 1725 cm^{-1} is diagnostic for the carbonyl absorption of fused triazines. However, our ir spectrum of **7** showed an intense absorption at 1666 cm^{-1}, which suggested that ring closure didn't occur. The ^{1}H nmr spectrum of **7** showed two singlets at δ_H 4.98 and 6.97 ppm corresponding to the methylene protons of the benzyl group and the 3-amino group, respectively. The multiplets at δ_H 7.22 to 7.37 ppm corresponded to aromatic protons of the benzyl group, and one singlet at δ_H 7.98 ppm to the *N*-1 hydrogen of the 6-formylhydrazino group. The doublets at δ_H 8.26 and 9.71 ppm (both with J = 1.2 Hz) corresponded to *N*-2 hydrogen and the proton of the formyl group, respectively. Additional supporting evidence for **7** was found in the high-resolution mass spectrum showing the molecular ion at m/z 260.1021, confirming the precise molecular formula as $C_{11}H_{12}N_6O_2$ (calcd. 260.1022). Taken together, **7** was assigned as 3-amino-2-benzyl-6-(2-formylhydrazino)-1,2,4-triazin-5(2*H*)-one; the ring closed compound **8**, the acetylhydrazino compound **9**, and the 2-(*N*,*N*-dimethylaminomethylene)-hydrazino compound **10** were not observed (Scheme 2).

Scheme 2



A reasonable mechanism explaining the deamination of DMF by **6** to form **7** is shown in Figure 2. We proposed that the DMF was protonated by acetic acid on the nitrogen atom, not the oxygen atom, in the first step. The carbonyl

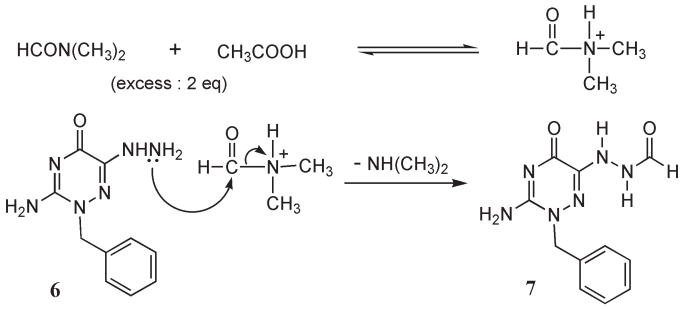
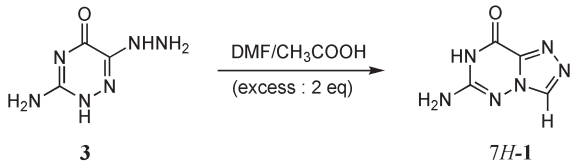


Figure 2

carbon of the protonated DMF was then attacked by the 6-hydrazino group of **6**. Finally the dimethylamine was lost and **7** formed.

We then repeated the reaction with compound **3** (Scheme 3) to afford **1**, which was characterized by the ^{1}H nmr spectrum [δ_H 6.36 (s, 2H, NH_2), 9.00 (s, 1H, 3-H), 11.55 (br s, 1H, NH)]. The proton-decoupled ^{13}C nmr and DEPT of **1** indicated three resonances (with δ_C of both C-3 and C-8a at 139.70 ppm), including one methylidene (C-3) and three

Scheme 3



quarternary carbons (δ_C of C-8a, C-6 and C-8 at 139.70, 151.07 and 152.10 ppm, respectively). Additionally, the occurrence of ring closure was confirmed by the intense infrared absorption at 1719 cm^{-1} corresponding to the carbonyl of similar fused triazines, as reported by Lovelette [1]. The high-resolution mass spectrum showed a molecular ion at m/z 152.0446 corresponding to the molecular formula $C_4H_4N_6O$ (calcd.152.0447). From these spectral and analytical data, the product structure was assigned as 6-amino-1,2,4-triazolo[3,4-*f*][1,2,4]triazin-8-one (**1**). The major prototropic tautomer of **1** was conjectured through the X-ray structure of **2** as the 7*H*-tautomer [2]. Accordingly, the nomenclature of compound **1** is 6-amino-1,2,4-triazolo[3,4-*f*][1,2,4]triazin-8(7*H*)-one.

Figure 3 shows the proposed cyclization mechanism for **3** refluxed with acetic acid in DMF to form the 7*H*-tautomer **1**. Lovelette [1] had previously described the probable ring cyclization mechanism for 5*H*-tautomer **1** (Figure 1). He suggested that DMF was protonated by acetic acid on the oxygen atom in the first step, partly based upon the report by Fraenkel *et al*. [11], who suggested that DMF was monoprotonated on the oxygen atom in strong acids (*e.g.* 100% sulfuric acid). However, the chemical reaction from **3** to **1** (Scheme 3) was done in a weaker acid (acetic acid in DMF). Moreover, when **6** was reacted with acetic acid in DMF, no prototropic tautomerism from the 2*H*-5-one to the 5-ol was possible and, therefore, no ring cyclization occurred (Scheme 2). This observation suggested that DMF was protonated on the nitrogen atom. These findings corresponded well with our previous hypothesis, that is, the ring cyclization was dependent on the resonance of the 2*H*-5-

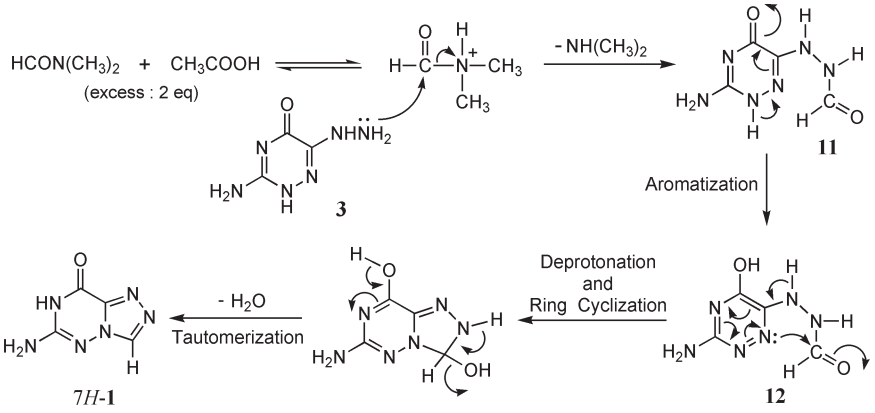


Figure 3

one to the 5-ol in the 1,2,4-triazine ring, which aromatized the system. Aromatization placed more electron density in the 1,2,4-triazine ring of 3-amino-6-(2-formylhydrazino)-1,2,4-triazin-5(2*H*)-one (**11**). In such a case, the sequential deprotonation of the *N*-1 hydrogen of 6-formylhydrazino group gave the electron pair into 1,2,4-triazine ring *via* π-electron resonance. The 1,2,4-triazine ring then served as a nucleophile, allowing for the intramolecular nucleophilic cyclization at N-1 of 3-amino-6-(2-formylhydrazino)-1,2,4-triazin-5-ol (**12**), followed by dehydration and tautomerization to the predominant form 7*H*-tautomer **1**.

Conclusions.

As we know, aromaticity has ubiquitous influence in molecular stability and reactivity, the reaction products to be expected, the symmetry and geometry of molecules, and various physicochemical properties [12,13]. In this research two important ideas were presented: (1) that the tautomerism of the N-2 hydrogen with the C-5 oxo group induced aromatization in the 1,2,4-triazine ring, and (2) that DMF was protonated by acetic acid on the nitrogen atom, promoting deamination upon attack by the 6-hydrazino group of **3** or **6**. Our investigations revealed that the aromatization and resonance influenced the reactivity of 1,2,4-triazine π-deficient heterocyclic molecule. Evidence was presented to suggest that the cyclization is dependent on the aromatization of the 1,2,4-triazine ring. Additionally, studying and expounding the correct dominant tautomeric structure makes it possible to properly interpret the detailed mechanisms of reactions of tautomeric heterocycles [14].

EXPERIMENTAL

Melting points were measured on a YANACO micromelting point apparatus and were uncorrected. The ir spectra were taken with potassium bromide (KBr) discs on a Perkin-Elmer FTIR 1650 spectrophotometer and the uv absorption spectra were recorded on a Shimadzu UV-Visible spectrophotometer. The ^{1}H nmr and ^{13}C nmr spectra were obtained in dimethylsulfoxide-d_6 (DMSO-d_6) on a Varian Gemini 200 (200 MHz) or a Varian Unity 400 (400 MHz) spectrometer. Chemical shifts are expressed in ppm (δ) with tetramethylsilane (Me_4Si) as an internal standard. Coupling constants J are given in Hertz (Hz); multiplicities were recorded as broad peaks (br), singlet (s), doublets (d), triplets (t), quartets (q), and multiplets (m). Thin layer chromatography (tlc) analyses were performed on silica gel plates (Merck 60 F_{254}, 0.2 mm thickness), and the components were detected by uv light (254 nm). MS and hrms spectra were obtained on a Quattro VG-5022 spectrometer and VG 70-250S GC/MS, respectively, with an ionization potential of 70 eV. Elemental analyses were performed on a Heraeus CHN-O-Rapid elemental analyzer. All the solvents used were dried and distilled under argon prior to use.

3-Amino-2-benzyl-6-bromo-1,2,4-triazin-5(2*H*)-one (**5**).

Compounds **3** and **4** were prepared as described by Lovelette [1]. Compound **4** (1.90 g, 10 mmoles) was suspended in hexamethyldisilazane (HMDS; 25 mL, 118 mmoles), and a catalytic amount of ammonium sulfate (60 mg, 0.45 mmoles) was added. The mixture was heated under reflux for 4 hours with the exclusion of moisture until a clear solution was obtained. The excess HMDS was removed under reduced pressure to give the silylated intermediate as an oil, which was then dissolved in dry toluene (30 mL). To this stirred solution was added a solution of benzyl bromide (3.42 g, 20 mmoles) in dry toluene (15 mL). The reaction mixture was refluxed for 16 hours (monitored by tlc). The solvent was evaporated to afford the crude product as an oil, which was then applied to a silica gel (230-400 mesh) column. The column was eluted with $CHCl_3$ and MeOH (60:1) and the proper fractions were combined and evaporated. The residue thus obtained was recrystallized from CH_2Cl_2 and EtOH (5:1) to give **5** (0.90 g, 32%) as bright white crystals, mp 227-229° (from CH_2Cl_2-EtOH); ir (KBr): (ν, cm^{-1}) 3338, 3122, 1635, 1521, 1441, 1149, 1046; uv: (0.1 *N* HCl): λ_{max} nm (logε) 208 (3.53), 270 (2.83); (H_2O): λ_{max} nm (logε) 206 (3.51), 271 (2.81); (0.1 *N* NaOH): λ_{max} nm (logε) 231 (3.93), 260 (3.56); ^{1}H nmr (200 MHz): δ 5.17 (s, 2H, CH_2), 7.24-7.45 (m, 5H, C_6H_5), 7.58 (s, 2H, NH_2); ^{13}C nmr (200 MHz): δ 55.59 (CH_2), 135.52 (C-6), 155.09 (C-3), 157.85 (C-5); ms: m/z, 282 (M^++1, 5.3), 280 (5.6), 175 (25), 106 (41), 91 (100), 69 (35), 65 (48).

Anal. Calcd. for $C_{10}H_9N_4OBr$: C, 42.73; H, 3.23; N, 19.93. Found: C, 42.59; H, 3.20; N, 19.96.

3-Amino-2-benzyl-6-hydrazino-1,2,4-triazin-5(2*H*)-one (**6**).

A solution of compound **5** (0.84 g, 3 mmoles) and hydrazine monohydrate (0.3 g, 6 mmoles) in ethanol (20 mL) was refluxed for 16 hours (monitored by tlc). The solvent was evaporated to 5 mL, and then cooled. The precipitate was collected and washed with water, followed by recrystallization from ethanol to give **6** (0.48 g, 69%), mp 245-247° (from EtOH); ir (KBr): (ν, cm^{-1}) 3419, 3326, 1562, 1496, 1056; uv: (0.1 *N* HCl): λ_{max} nm (logε) 208 (4.24), 280 (3.73); (H_2O): λ_{max} nm (logε) 220 (3.23), 292 (2.76); (0.1 *N* NaOH): λ_{max} nm (logε) 226 (3.19), 290 (2.69); ^{1}H nmr (400 MHz): δ 3.90 (s, 2H, NHN*H*$_2$), 5.01 (s, 2H, CH_2), 6.83 (s, 2H, NH_2), 7.28-7.38 (m, 5H, C_6H_5), 7.34 (s, 1H, N*H*NH_2); ^{13}C nmr (200 MHz): δ 54.96 (CH_2), 147.60 (C-6), 154.05 (C-3), 158.69 (C-5); ms: m/z 232 (M^+, 45), 141 (85), 106 (16), 91 (100), 69 (21), 65 (33).

Anal. Calcd. for $C_{10}H_{12}N_6O$: C, 51.72; H, 5.21; N, 36.19. Found: C, 51.61; H, 5.19; N, 35.97.

3-Amino-2-benzyl-6-(2-formylhydrazino)-1,2,4-triazin-5(2*H*)-one (**7**).

Compound **6** (0.70 g, 3 mmoles) in acetic acid (0.36 g, 6 mmoles) and *N*,*N*-dimethylformamide (15 mL) were refluxed for 12 hours. Cooling gave white crystals which were recrystallized from ethanol to afford **7** (0.48 g, 62%), mp 245-246° (from EtOH); ir (KBr): (ν, cm^{-1}) 3296, 3194, 1666, 1509, 1374; uv: (0.1 *N* HCl): λ_{max} nm (logε) 207 (4.09), 291 (3.59) ; (H_2O): λ_{max} nm (logε) 222 (4.04), 284 (3.51); (0.1 *N* NaOH): λ_{max} nm (logε) 230 (4.27), 266 (3.97), 329 (3.85); ^{1}H nmr (400 MHz): δ 4.98 (s, 2H, CH_2), 6.97 (s, 2H, NH_2), 7.22-7.37 (m, 5H, C_6H_5), 7.98 (s, 1H, N*H*NHCO), 8.26 (d, 1H, J = 1.2 Hz, NHN*H*CO), 9.71 (d, 1H, J = 1.2 Hz, C*H*O); ^{13}C nmr (200 MHz): δ 54.95 (CH_2), 145.04 (C-6), 154.51 (C-3), 159.99 (C-5), 167.55 (CO); ms: m/z 260 (M^+, 3), 141 (10), 91 (100), 69 (15), 65 (27); hrms: m/z Calcd. for $C_{11}H_{12}N_6O_2$: 260.1022. Found: 260.1021.

Anal. Calcd. for $C_{11}H_{12}N_6O_2$•1/2 H_2O: C, 49.07; H, 4.83; N, 31.21. Found: C, 49.14; H, 4.75; N, 31.19.

6-Amino-1,2,4-triazolo[3,4-*f*][1,2,4]triazin-8(7*H*)-one (**1**).

Compound **3** (0.71 g, 5 mmoles) in acetic acid (10 mmoles) and *N*,*N*-dimethylformamide (15 mL) were gently refluxed for 12 hours. Cooling gave compound **1** (0.51 g, 67%) as white crystals, mp >300° (from H_2O); ir (KBr): (ν, cm^{-1}) 3331, 3186, 1719, 1663, 1351, 1293, 1090; uv: (0.1 *N* HCl): λ_{max} nm (logε) 213 (3.38), 302 (2.89); (H_2O): λ_{max} nm (logε) 217 (3.57), 296 (3.01); (0.1 *N* NaOH): λ_{max} nm (logε) 225 (4.20), 282 (3.65); ^{1}H nmr (200 MHz): δ 6.36 (s, 2H, NH_2), 9.00 (s, 1H, 3-H), 11.55 (br s, 1H, NH); ^{13}C nmr (200 MHz): δ 139.70 (C-3 and C-8a), 151.07 (C-6), 152.10 (C-8); ms: m/z 152 (M^+, 3), 128 (100), 100 (34), 69 (12); hrms: m/z Calcd. for $C_4H_4N_6O$: 152.0447. Found: 152.0446.

Anal. Calcd. for $C_4H_4N_6O$: C, 31.58; H, 2.65; N, 55.25. Found: C, 31.43; H, 2.68; N, 55.07.

Acknowledgement.

We are grateful to the National Science Council, Taiwan, ROC, for support under grant NSC 88-2113-M-037-013.

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