TLC (silica gel, chloroform–methanol, 6:1) R_f 0.33; ^{1}H NMR ($CDCl_3$) δ 5.49 (dd, J = 3.5, 1.8 Hz, 1 H, H-2), 4.21 (bs, 2 H, H-9), 4.11 (bs, 1 H, H-8), 3.87 (ddt, J = 15.4, 4.6, 2.3 Hz, 1 H, H-3α), 3.33 (dtd, J = 15.3, 4.3, 2.1 Hz, 1 H, H-3β), 3.11 (dt, J = 9.8, 4.9 Hz, 1 H, H-5α), 2.49 (ddd, J = 9.7, 7.8, 6.5 Hz, 1 H, H-5β), 1.94 (m, 1 H, H-7α), 1.75 (m, 2 H, H-6), 1.51 (ddd, J = 14.6, 11.8, 7.8 Hz, 1 H, H-7β), 0.91 (s, 9 H, Si-t-Bu), 0.08 (s, 6 H, $SiMe_2$); IR (CCl_4) ν 2965, 2940, 2905, 2865, 1474, 1465, 1447, 1392, 1376, 1364, 1345, 1322, 1307, 1252, 1198, 1172, 1122, 1089, 1074, 1023, 1001, 948, 940, 909 cm^{-1}; EIMS m/e (relative intensity) 41 (22), 53 (12), 57 (15), 59 (12), 73 (24), 75 (16), 80 (15), 89 (12), 93 (23), 108 (24), 120 (47), 122 (100), 196 (37), 221 (12), 225 (11), 238 (25), 252 (36), 254 (M^+, 64).

9-*O*-Benzoylsupinidine (7D). This compound was obtained from **5D** and purified by preparative TLC on aluminia using a mixture of hexane and chloroform (1:1 by volume) as eluent for developing. **7D** was obtained as an oil in 64% yield: $[\alpha]^{24}_D$ = −10.56° (c 1.25, $CHCl_3$); TLC (alumina, ethyl acetate) R_f 0.48; ^{1}H NMR ($CDCl_3$) δ 8.05 (d, J = 8.2 Hz, 2 H, ortho aromatic), 7.57 (t, J = 7.3 Hz, 1 H, para aromatic), 7.45 nt, J = 7.7 Hz, 2 H, meta aromatic), 5.70 (d, J = 1.6 Hz, 1 H, H-2), 4.92 (bs, 2 H, H-9), 4.22 (bs, 1 H, H-8), 3.92 (bd, J = 15.8 Hz, 1 H, H-3α), 3.39 (ddd, J = 15.7, 4.8, 1.9 Hz, 1 H, H-3β), 3.14 (quintet, J = 5.0 Hz, 1 H, H-5α), 2.51 (ddd, J = 10.0, 8.2, 5.9 Hz, 1 H, H-5β), 2.02 (m, 1 H, H-7α), 1.77 (m, 2 H, H-6), 1.59 (ddd, J = 14.9, 11.7, 7.9 Hz, 1 H, H-7β); IR (CCl_4) ν 2970, 2940, 2875, 1730, 1453, 1316, 1270, 1198, 1178, 1110, 1070, 1027 cm^{-1}; EIMS m/e (relative intensity) 41 (11), 51 (13), 77 (39), 80 (23), 93 (57), 105 (45), 108 (42), 110 (37), 120 (100), 122 (80), 138 (26), 243 (M^+, 11); CIMS m/e (relative intensity) 93 (2), 105 (1), 108 (2), 110 (1), 120 (14), 122 (46), 244 (M^+ + 1, 100); high-resolution mass spectrum, m/e 243.1257 ($C_{15}H_{17}O_2N$ requires 243.1259).

Deprotection of Supinidine Derivatives. (−)-Supinidine (1): Method I. A mixture of 9-*O*-(*tert*-butyldimethylsilyl)supinidine (**7B**) (0.75 g, 2.96 mmol) and ammonium fluoride (0.22g, 5.92 mmol) in dry methanol (60 mL) was stirred and kept at 60 °C for 18 h. The solvent was removed under reduced pressure to give a pale-yellow residual oil that was purified by preparative TLC on 2-mm silica gel plates (three times), developing with a mixture of chloroform/methanol/concentrated ammonium hydroxide (10/4/1, respectively). Supinidine (1) was extracted from the silica gel with methanol, and the solvent was removed under reduced pressure. The residues were then taken up in chloroform or acetone, which resulted in the precipitation of small amounts of silica gel that were removed by filtration. Evaporation of the solvent under reduced pressure gave chromatographically homogeneous product 1 in 70% yield.

Method II. A mixture of 9-*O*-benzoylsupinidine (**7D**) (0.21 g, 0.86 mmol) and lithium aluminum hydride (0.066 g, 1.73 mmol) in dry ether (10 mL) was heated under reflux for 30 min. To the resulting solution was sequentially added 0.062 mL of water, 0.062 mL of 3 N aqueous NaOH, and 0.088 mL of water. The mixture was filtered, and the filtrate was dried (anhydrous $MgSO_4$) and concentrated in vacuo to give a pale-yellow liquid, which was purified by preparative TLC as described above to yield **1** as a chromatographically homogeneous product supinidine (1) in 59% yield: mp (picrate) 139–141 °C (MeOH) [lit.[13b] mp 143–144 °C (EtOH)]; $[\alpha]^{26}_D$ = −10.4° (c 2.64, EtOH),[28] after distillation at reduced pressure to remove last traces of solvent; TLC (silica gel, chloroform–methanol–concentrated ammonium hydroxide, 10:4:1) R_f 0.49; ^{1}H NMR ($CDCl_3$) δ 5.52 (bs, 1 H, H-2), 4.31 (bs, 1 H, H-8), 4.25 and 4.16 (d + d, J = 14.3 Hz, 2 H, H-9), 3.98 (bd, J = 15.3 Hz, 1 H, H-3α), 3.36 (ddd, J = 15.4, 4.3, 2.1 Hz, 1 H, H-3β), 3.19 (td, J = 10.9, 5.0 Hz, 1 H, H-5α), 2.58 (td, J = 10.3, 6.9 Hz, 1 H, H-5β), 2.03 (ddd, J = 13.7, 12.2, 6.0 Hz, 1 H, H-7α), 1.81 (quintet, 2 H, H-6), 1.57 (ddd, J = 14.5, 11.8, 7.1 Hz, 1 H, H-7β); IR ($CHCl_3$) ν 3620, 3350, 2970, 2880, 1605, 1450, 1385, 1290, 1190, 1115, 1085, 1040; EIMS m/e (relative intensity) 39 (16), 41 (31), 43 (14), 45 (12), 53 (16), 55 (13), 68 (21), 80 (100), 94 (12), 108 (41), 110 (28), 120 (11), 122 (53), 138 (18), 139 (M^+, 69); high-resolution mass spectrum, m/e 139.1013 ($C_8H_{13}ON$ requires 139.0997).

Acknowledgment. This investigation was supported by the National Cancer Institute, National Institutes of Health (Grant RO1 CA31490).

Registry No. **1**, 551-59-7; **2**, 480-85-3; **3**, 520-63-8; **4A**, 82485-07-2; **4B**, 126253-89-2; **4C**, 95363-37-4; **4D**, 126191-51-3; **5A**, 126191-48-8; **5B**, 126191-52-4; **5C**, 126191-53-5; **5D**, 126191-54-6; **6A**, 126191-49-9; **6C**, 126191-55-7; **7B**, 126191-50-2; **7D**, 126191-56-8.

(28) Several different values have been reported for the specific rotation of synthetic $[[\alpha]^{25}_D$ = −9.7° (c 2.5, EtOH);[13a] $[\alpha]^{18}_D$ = −8.3° (c 2.0, EtOH)[13b]] and natural $[[\alpha]^{18}_D$ = −10.3° (c 1.65, EtOH)[29]] supinidine. The NMR spectrum of our synthetic (−)-supinidine run in the presence of the chiral shift reagent Eu(hfc)$_3$ did not reveal any splitting of peaks. Requests for authentic samples of (±)-supinidine were unfruitful.

(29) Culvenor, C. C. J. *Aust. J. Chem.* **1954**, *7*, 287.

Intermolecular Decomposition of *N*-Acylcyanamides

Chul-Hoon Kwon*,† and Herbert T. Nagasawa‡

Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, New York 11439, and Department of Medicinal Chemistry, University of Minnesota, and Medical Research Laboratories, VA Medical Center, Minneapolis, Minnesota 55417

Received July 25, 1989

In efforts to synthesize acyl derivatives as prodrugs of cyanamide (1, $H_2NC{\equiv}N$),[1] a potent aldehyde dehydrogenase inhibitor which is used therapeutically as an alcohol deterrent agent,[2] we discovered that certain *N*-acylcyanamides, viz., *N*-(benzyloxycarbonyl)cyanamide (**2a**) and *N*-acetylcyanamide (**2b**), the latter a major urinary metabolite of cyanamide in rodents, dog, and humans,[3] were unstable in their free states and gradually decomposed at room temperature. We now report our investigations on the nature of the decomposition products of these *N*-acylcyanamides, and by deduction, propose a pathway for these reactions.

Results and Discussion

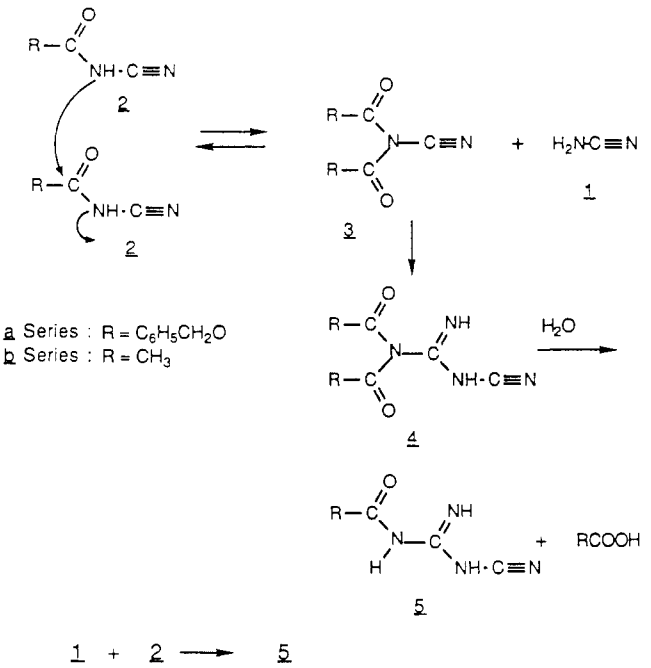
When *N*-(benzyloxycarbonyl)cyanamide (**2a**) was stored at room temperature for 1 week, the originally clear liquid was converted to a semisolid. By TLC analysis, the residue was found to contain unchanged **2a**, **1**, and some other UV-quenching substances. Two crystalline compounds were isolated from the above mixture by solvent extractions followed by column chromatography, etc. The spectral data for the first suggested it to be *N*-(benzyloxycarbonyl)-*N'*-cyanoguanidine (**5a**), and this was confirmed by acylation of cyanoguanidine (dicyanodiamide) with (benzyloxy)carbonyl chloride in aqueous base to give a product with physicochemical and spectral properties identical with those of **5a**. The IR and NMR spectral data for the second compound suggested it to be *N*,*N*-bis(benzyloxycarbonyl)cyanamide (**3a**), and this was confirmed by its elemental analysis.

N-Acetylcyanamide (**2b**) was similarly converted to a semisolid under the same conditions. By TLC analysis, the residue was shown to contain unchanged **2b**, cyanoguanidine, and a number of UV-quenching substances. The spectral data for a minor product isolated from the above mixture by preparative TLC suggested it to be *N*-acetyl-*N'*-cyanoguanidine (**5b**), again proven by acetylation of cyanoguanidine to a product with identical physicochemical and spectral properties.

† St. John's University.
‡ University of Minnesota and VA Medical Center.

0022-3263/90/1955-3403$02.50/0

Scheme I



1 + 2 → 5

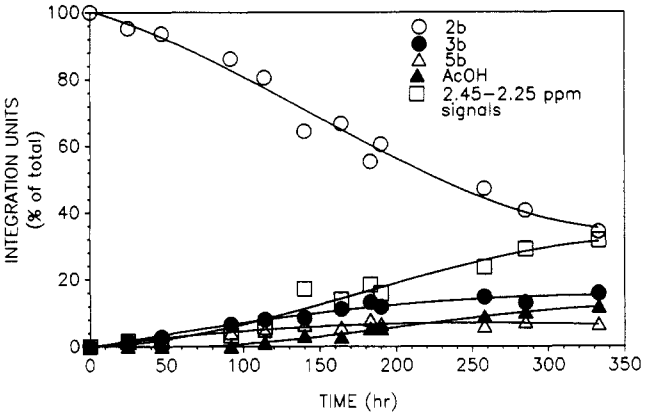


Figure 1. Decomposition of *N*-acetylcyanamide (**2b**) in $CDCl_3$ at room temperature as monitored by ^1H NMR spectroscopy over time.

The observation that the above *N*-acylcyanamides produced decomposition products with a common structure, viz., the corresponding *N*-acyl-*N*-cyanoguanidines (**5**), suggested a mechanism for their formation. The starting *N*-acylcyanamides **2** were free of **1** and water, and since *N*,*N*-bis(benzyloxycarbonyl)cyanamide (**3a**) was also isolated from the decomposition mixture of **2a**, we surmised that the starting *N*-acylcyanamide (**2**) must have initially undergone an intermolecular reaction to produce *N*,*N*-diacylcyanamide (**3**) and an equimolar amount of **1** (Scheme I). The liberated **1** could then react at the nitrile carbon of the starting *N*-acylcyanamide (**2**) to give **5** directly, or with the product, *N*,*N*-diacylcyanamide (**3**) to give the unstable adduct, *N*,*N*-bis(acyl)-*N'*-cyanoguanidine (**4**), which then could lose an acyl group by transfer to another cyanamide molecule, or by solvolysis, to produce

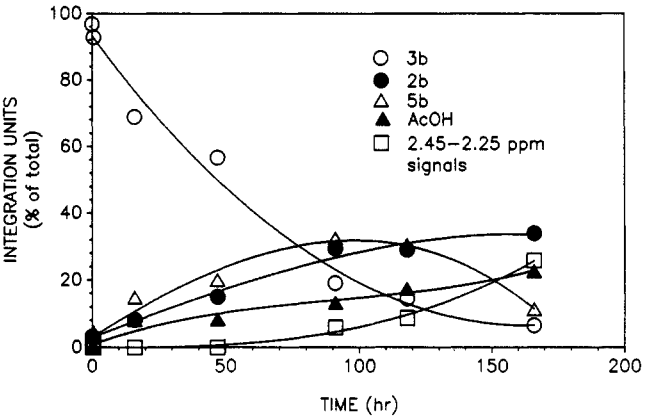


Figure 2. Reaction of *N*,*N*-diacetylcyanamide (**3b**) with cyanamide (**1**) in $CDCl_3$ at room temperature as monitored by ^1H NMR spectroscopy over 166 h.

N-acyl-*N'*-cyanoguanidine (**5**).

To verify this mechanism, the decomposition of *N*-acetylcyanamide (**2b**) was monitored over time by ^1H NMR spectrometry, quantifying its decomposition products by peak intensities (Figure 1). Compound **2b** (singlet, 2.22 ppm) was stable for several hours, but after 25 hs, two additional singlets (each approximately 2% of the total) corresponding to *N*,*N*-diacetylcyanamide (**3b**) and *N*-acetyl-*N'*-cyanoguanidine (**5b**) appeared at 2.52 and 2.12 ppm, respectively. The intensity of the peak for **2b** gradually decreased over time with concomitant increases in the signals for **3b** and **5b**. In addition, multiplets at 2.45–2.25 ppm and a singlet at 2.09 ppm due to acetic acid appeared after 47 and 114 h, respectively. The signals for **3b** and **5b** appeared to level off as the signal for acetic acid and the multiple bands at 2.45–2.25 ppm became significant (after approximately 120 h). The latter became the dominant signals at 335 h and represented decomposition end products. Heating of **2b** on the steam bath accelerated the decomposition, but essentially the same products were obtained after 10 min of heating as in the room-temperature reaction over several days.

The intermediacy of **3** as the precursor for **5** was next investigated. *N*,*N*-Diacetylcyanamide (**3b**) containing a small amount (approximately 3%) of *N*-acetylcyanamide (**2b**) was stable in $CDCl_3$ at room temperature for at least 7 days. When **3b** was allowed to react at room temperature with 1 molar equiv of **1** in $CDCl_3$, disappearance of **3b** (singlet, 2.52 ppm) was relatively fast with $t_{1/2}$ = 48 h (Figure 2). The signals for **2b** (2.22 ppm) and **5b** (2.12 ppm) appeared within 30 min. The concentrations of **2b** and **5b** steadily increased over time, but the latter started to diminish as the presence of the acetic acid became apparent. These data suggested that **1** had attacked the carbonyl carbon of **3b** to product **2b**. Compound **5b** could be formed by the addition of **1** directly on the nitrile moiety of **2b**, or by the reaction of **1** with the chemically more reactive **3b**, followed by solvolysis of one of the acetyl groups from the resulting *N*,*N*-bis(acetyl)-*N'*-cyanoguanidine (**4b**) as depicted in Scheme I. Despite the fact that **4b** was not isolated or detected by NMR, its role as an intermediate is likely, since **2b** was considerably more stable than **3b** in the presence of **1**, and the initial rate of appearance of **5b** from the reaction of **1** and **3b** was faster than the appearance of **2b** (Figure 2).

Cyanamide (**1**) was not detected by TLC or by colorimetric assay (see the Experimental Section for details) at any time during the decomposition of *N*-acetylcyanamide (**2b**). This is consistent with the decomposition pathway proposed, since **1** generated from **2b** would be expected

(1) Kwon, C.-H.; Nagasawa, H. T.; DeMaster, E. G.; Shirota, F. N. *J. Med. Chem.* **1986**, *29*, 1982.
(2) (a) Reynolds, J. E. F. *Martindale, The Extra Pharmacopeia*; London Pharmaceutical Press: London, 1982; p 580. (b) *Japta List*; *Japanese Drug Director*; Japanese Pharmaceutical Trader's Association: Tokyo, 1973; p 182.
(3) Shirota, F. N.; Nagasawa, H. T.; Kwon, C.-H.; DeMaster, E. G. *Drug. Metabol. Disp.* **1984**, *12*, 337.

to react immediately with the **3b** produced by attacking either the nitrile carbon or one of the carbonyl groups.

Whereas *N*-(*n*-butyryl)cyanamide was similarly unstable (products not identified), *N*-benzoylcyanamide (**6**) and *N*-pivaloylcyanamide were found to be stable cyanamide derivatives.[1] Attempts to prepare *N,N*-dibenzoylcyanamide by reacting **6** with benzoyl chloride/pyridine gave instead an intermediate which had an IR absorption band at 2165 cm^{-1}, suggesting it to be *N,N'*-dibenzoylcarbodiimide (**7**). Indeed, solvolysis of this intermediate gave rise to *N,N'*-dibenzoylurea (**8**). This behavior of *N*-acylcyanamides is reminiscent of the reaction of alkylcyanamides (RNHCN) with trimethylsilyl chloride; e.g., when R was small (CH_3, C_2H_5), silylation occurred on the amino group, whereas when R was large (*t*-Bu, C_6H_5), the corresponding silylated carbodiimides were formed.[4] Thus, the relative stability of acylcyanamides is dependent on steric factors. It should be emphasized that the resonance stabilized sodium salts of **2a** or **2b** are stable indefinitely.[1]

Experimental Section

Melting points were determined on a Fisher-Johns or Thomas Hoover capillary melting point apparatus and are uncorrected. Microanalysis were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY, or Galbraith Laboratories, Knoxville, TN. The following instruments were used: IR, Perkin-Elmer Model 281; Colorimetry, Milton Roy Spectronic 301; HPLC, Waters equipped with a Model 501 pump, a Model 481 detector, and a Model U6K loop injector; NMR, Varian EM-360L CW and JEOL-FX90Q FT spectrometers (Me_4Si as internal standard); CI-MS, Finnegan 4000; FAB-MS, VG7070E-HF (Xe, 8 kV, 1 mA). Cyanamide and its derivatives were detected on TLC plates (silical gel GF) by use of a ferricyanide/nitroprusside (FCNP) spray reagent.[5] All chemicals and solvents were reagent grade unless otherwise specified and were purchased from commercial vendors.

***N*-Acetylcyanamide (2b) and *N*-benzoylcyanamide (6)** were prepared as described previously.[3]

Isolation of *N,N*-Bis(benzyloxycarbonyl)cyanamide (3a) from *N*-(Benzyloxycarbonyl)cyanamide (2a). To a cooled (ice bath) stirred solution of cyanamide (**1**, 6.30 g, 0.15 mol) in 60 mL of distilled water were added simultaneously and dropwise (benzyloxy)carbonyl chloride (8.50 g, 0.050 mol) and 10% NaOH (40 mL, 0.10 mol). After 3 h the reaction mixture (pH 10.2) was extracted with Et_2O (2 × 50 mL) and EtOAc (2 × 50 mL). The aqueous layer was acidified with 10% HCl to pH 1.5 and extracted with methylene chloride (3 × 50 mL). The combined extract was dried (Na_2SO_4) and filtered, and the filtrate was evaporated in vacuo to give 7.59 g (86.3% yield) of crude **2a** as a pale yellow oil. This product (3 g) was applied to a silica gel column (15 × 2.5 cm, 230–400 mesh) and eluted with EtOAc/petroleum ether (1:2) at 15 psi. The fractions containing the desired product were pooled and the solvent was evaporated in vacuo to give 2.4 g (69.0% yield) of *N*-(benzyloxycarbonyl)cyanamide (**2a**) as a clear liquid: TLC R_f = 0.67 in EtOAc/petroleum ether/AcOH (50:100:1), detected by UV quenching and orange color with FCNP spray reagent; IR (neat, cm^{-1}) 3250–3400 (NH), 2260 (C≡N), 1760 (C=O); NMR ($CDCl_3$, δ from TMS) 7.26 (s, C_6H_5), 5.2 (s, $CH_2C_6H_5$). This liquid gradually converted to a semisolid when stored at room temperature for 7 days. This material was dissolved in CH_2Cl_2 and washed with 5% aqueous citric acid, whereupon white precipitates formed in both the organic and the aqueous phases. The collected solid was crystallized from ethyl acetate to yield **5a** whose physicochemical and spectral data were identical with those of **5a** prepared below. The methylene chloride filtrate was evaporated in vacuo to near dryness and applied to a silica gel column, and the major UV-quenching substance was separated by flash chromatography. The isolated thick liquid was crystallized from EtOAc/petroleum ether to give **3a** (fluffy, colorless): mp 85–86 °C; TLC R_f = 0.37 (UV quenching) in EtOAc/petroleum ether (1:4); IR (KBr, cm^{-1}) 3090, 3060, and 3040 (C_6H_5), 2980 (CH_2), 2260 (C≡N), 1830 and 1750 (C=O); NMR ($CDCl_3$, δ from TMS) 7.26 (s, $OCH_2C_6H_5$). Anal. Calcd for $C_{17}H_{14}N_2O_4$: C 65.80; H, 4.55; N, 9.02. Found: C, 65.98; H, 4.65; N, 8.77.

***N*-(Benzyloxycarbonyl)-*N'*-cyanoguanidine (5a).** (Benzyloxy)carbonyl chloride (3.40 g, 2.85 mL, 0.020 mol) was allowed to react with cyanoguanidine (1.68 g, 0.020 mol) and KOH (1.12 g, 0.020 mol) in 100 mL of water overnight at room temperature. The reaction mixture (pH 7.9) was extracted with EtOAc (50 mL), and the extract was dried (Na_2SO_4) and concentrated in vacuo. The product was precipitated by addition of petroleum ether to give crude **5a** (100 mg, 2.3% yield), which was crystallized from EtOAc/petroleum ether: mp 187–188 °C; TLC R_f = 0.66 (detected by UV quenching) in EtOAc/petroleum ether/AcOH (100:100:1); IR (Nujol, cm^{-1}) 3260 and 3160 (NH), 3030 (C_6H_5), 2200 (C≡N), 1740 (C=O), 1670 and 1610 (C=N); NMR [$CDCl_3$/DMSO-d_6 (10:1), δ from TMS] 8.5 (broad, NH), 7.4 (s, C_6H_5), 5.22 (s, $OCH_2C_6H_5$). Anal. Calcd for $C_{10}H_{10}N_4O_2$: C, 55.04; H, 4.62; N, 25.68. Found: C, 55.17; H, 4.58; N, 25.63.

Isolation of *N,N'*-Dibenzoylurea (8) by Benzoylation of Benzoylcyanamide (6). Benzoyl chloride (1.40 g, 0.010 mol) in 30 mL of anhydrous Et_2O was added dropwise to a solution of *N*-benzoylcyanamide (**6**, 1.52 g, 0.010 mol) in 5 mL of pyridine and 70 mL of anhydrous Et_2O at ice-bath temperature. After overnight reaction at room temperature, the mixture was filtered and the solvent was evaporated in vacuo to give a pale yellow solid with an IR band at 2165 cm^{-1}. A water wash was followed by crystallization from EtOAc/hexane to give 1.9 g (71% yield) of a white, fluffy crystalline **8**: mp 212–216 °C (reported[6] mp 216–217 °C); IR (KBr, cm^{-1}) 3250–3550 (NH), 3080 (aromatic C-H), 1755 and 1670 (C=O). Anal. Calcd for $C_{15}H_{12}N_2O_3$: C, 67.16; H, 4.51; N, 10.44. Found: C, 67.32; H, 4.52; N, 10.38.

***N,N*-Diacetylcyanamide (3b).** Acetyl chloride (7.85 g, 7.11 mL, 0.10 mol) in 10 mL of acetonitrile was added dropwise to a suspension of sodium cyanamide (3.20 g, 0.050 mol) and triethylamine (5.06 g, 6.97 mL, 0.050 mol) in 150 mL of acetonitrile at ice bath temperature. After overnight reaction at room temperature the mixture was filtered, and the filtrate was evaporated in vacuo to give 9.56 g of a clear liquid. A portion (3.0 g) was applied to a silica gel column (15 × 5 cm, 40 μm particle diameter) and eluted with EtOAc/hexane/AcOH (100:200:1) at 15 psi. The fractions containing the desired compound were pooled and the solvent was evaporated in vacuo to give 1.15 g of crystalline **3b** (58.1% yield): mp 37–38 °C (reported[7] mp 65 °C); TLC R_f = 0.63 in EtOAc/hexane/AcOH (100:50:1), detected by orange color with FCNP spray; IR (Nujol, cm^{-1}) 2250 (C≡N), 1770 and 1740 (C=O); NMR ($CDCl_3$, δ from TMS) 2.55 (s, CH_3); CI-MS (CH_4) *m/z* 127 (MH^+), *m/z* 85 ($MH^+ - CH_2{=}C{=}O$).

***N*-Acetyl-*N'*-cyanoguanidine (5b).** Cyanoguanidine (1.68 g, 0.02 mol) in 50% aqueous THF (100 mL) was acetylated with acetyl chloride (1.42 mL, 0.020 mol) in THF (25 mL) and 10% NaOH (8.0 mL, 0.020 mol) at ice bath temperature for 4 h. After evaporation of the solvent in vacuo the residue was extracted with EtOAc (25 mL × 5). The EtOAc extract was dried over anhydrous Na_2SO_4 and filtered, and the filtrate was evaporated in vacuo to give 0.26 g (10% yield) of crude **5b**. A portion (0.16 g) of this product was applied to a silica gel column (10 × 2 cm, 40 μm particle diameter) and eluted with EtOAc/hexane/AcOH (100:100:1) at 15 psi. The fractions containing the desired product were pooled and the solvent was evaporated in vacuo to give 0.11 g of white crystalline **5b** (6.9% yield): mp 219 °C starts to decompose, turns dark brown >240 °C [reported[8] mp 240 °C (dec)]; TLC R_f = 0.49, 0.47, and 0.75 in EtOAc/hexane/AcOH (200:100:1), CH_2Cl_2/EtOH (15:1), and EtOAc, respectively (detected by UV quenching and appearance of pale pink color with FCNP spray); IR (Nujol, cm^{-1}) 3400 and 3180 (NH), 2180 (C≡N), 1715 (C=O), 1635 and 1580 (C=N); NMR ($CDCl_3$, δ from TMS) 2.10 (s, CH_3); HPLC (t_R = 6.8 min), eluent MeOH/AcOH/10 mM aqueous ammonium acetate (60:20:920); FAB-MS (glycerol) (MH^+) calcd 127, found 127.

Isolation of *N*-Acetyl-*N'*-cyanoguanidine (5b) from *N*-Acetylcyanamide (2b). Freshly prepared **2b** (100 mg) was al-

(4) Ruppert, I. *Tetrahedron Lett.* **1977**, 1987.
(5) Bolliger, H. R.; Brenner, M.; Ganshirt, H.; Mangold, H. K.; Seiler, H.; Stahl, E.; Waldi, D. In *Thin Layer Chromatography*; Stahl, E., Ed.; Academic: New York, 1965; p 497.
(6) Terss, R. H.; McEwen, W. E. *J. Am. Chem. Soc.* **1954**, *76*, 580.
(7) Mertens, L. *J. Prakt. Chem.* **1878**, *17*, 1.
(8) Kaiser, D. W.; Thurston, J. T. *Chem. Abstr.* **1947**, *41*, 481a.

lowed to stand in a capped amber vial for one week at room temperature. The resulting semisolid was dissolved in methanol, applied to a preparative silica gel plate (1000 μm, PreAdsorbant Uniplate, Analtech), and the plate was developed with EtOAc. The band which corresponded to that of coeluted authentic *N*-acetyl-*N'*-cyanoguanidine (**5b**) prepared above was scraped off and extracted with THF. The pale yellow liquid obtained by evaporation of the solvent in vacuo was further purified by additional preparative TLC using CH_2Cl_2/EtOH (15:1): TLC R_f = 0.49, 0.47, and 0.72 in EtOAc/hexane/AcOH (200:100:1), CH_2Cl_2/EtOH (15:1), and EtOAc, respectively; the product was UV quenching and gave a pale pink color with FCNP spray; IR (neat, cm^{-1}) 2180 (C≡N), 1720 (C=O), 1640 and 1570 (C=N); HPLC (t_R = 7.0 min), eluent MeOH/AcOH/10 mM aqueous ammonium acetate (60:20:920); FAB-MS (glycerol) (MH^+) calcd 127, found 127.

Colorimetric Assay for Cyanamide (1). All experiments were conducted in triplicate except as otherwise noted. A standard stock solution (10 mM) of **1** was prepared either from **1** or from sodium cyanamide. A standard curve was prepared as follows: 10 μL of the stock solution was mixed with 100 μL of FCNP spray solution[3] [10% NaOH/10% sodium nitroprusside/10% potassium ferricyanide/distilled water (1:1:1:3)] and 890 μL of distilled water. Serial dilutions were made and after 30 min their maximum absorption at 535 nm was measured against the reagent blank. The lower limit of sensitivity was about 1 μg, which is essentially the same as that of a procedure[9] using pentacyanoamine ferroate. *N*-Acetylcyanamide (**2b**), urea, cyanoguanidine, or melamine did not give any detectable absorbance at this wavelength when present in concentrations equal to that of **1**.

Typically, stock solutions of **2b** were made by dissolving 40 mg of **2b** in 50 mL of THF. Twenty-five sealed test tubes, each containing 2 mL of stock solution, were allowed to stand at room temperature for up to 1 week. At varying time intervals, the presence of cyanamide was measured colorimetrically: 100 μL of the test solution was mixed with 100 μL of FCNP solution and 800 μL of distilled water, and the absorbance was measured at 535 nm. There were no detectable levels of cyanamide observed at any time. Cyanamide was also not detected by TLC analysis using the FCNP reagent.

Acknowledgment. We thank Susie Kim and Soyoun Han (St. John's University) for technical assistance with the colorimetric assay.

Registry No. **1**, 420-04-2; **2a**, 86554-53-2; **2b**, 5634-51-5; **3a**, 19245-33-1; **3b**, 126297-12-9; **5a**, 126297-13-0; **5b**, 63071-29-4; **6**, 15150-25-1; **8**, 965-04-8; (benzyloxy)carbonylchloride, 501-53-1; cyanoguanidine, 461-58-5; sodium cyanamide, 19981-17-0.

(9) Buyske, D. A.; Downing, V. *Anal. Chem.* **1960**, *32*, 1798.

Hydrolysis of Adenosine Triphosphate by Conventional or Microwave Heating

Edwin G. E. Jahngen,[‡] Ronald R. Lentz,*[,†] Peter S. Pesheck,[†] and Patricia Holt Sackett[†]

The Pillsbury Company, Research and Development Laboratories, 311 2nd St., S.E., Minneapolis, Minnesota 55414, and Department of Chemistry, University of Lowell, Lowell, Massachusetts 01854

Received December 11, 1989

Sun et al.[1] recently reported that the hydrolysis rate of adenosine-5'-triphosphate (ATP) was 25 times greater during microwave heating than during conventional heating at comparable temperatures (100–105 °C). This remarkable rate increase was both attributed to and cited as evidence for a nonthermal microwave effect. Attempts to replicate those findings, however, lead to the conclusion that the hydrolysis rate is instead related solely to temperature. There is no need to postulate a nonthermal microwave effect. Accurate temperature measurement within the microwave-heated sample has been found to be crucial and not trivial.

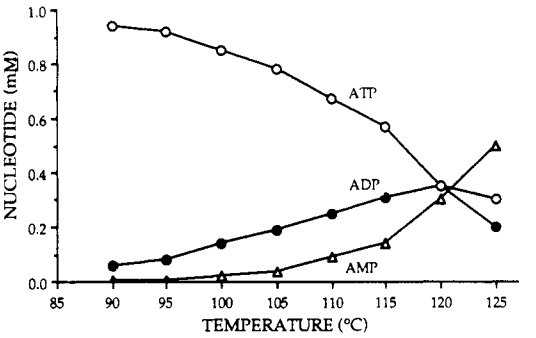


Figure 1. HPLC-determined nucleotide concentrations following 10-min conventional heating.

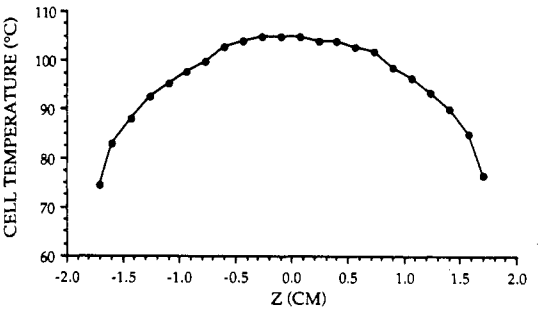


Figure 2. Sample temperature during microwave heating as a function of axial distance from middle of exposed sample.

In the present paper, ATP hydrolysis reaction kinetics determined from conventionally heated samples have been used in conjunction with temperatures measured in microwave-heated samples to calculate predicted final concentrations of ATP in the microwave-heated samples. These calculations, based only on temperature, accurately predicted the measured ATP concentrations in microwave-heated samples.

Conventional reaction kinetics were determined by exposing a series of samples for 10 min to fixed oil bath temperatures between 90 and 125 °C at 5 °C intervals. As the temperature increased, a regular decrease in ATP was seen along with a gradual increase in adenosine-5'-diphosphate (ADP) with an eventual plateau and dropoff, and a steady increase of adenosine-5'-monophosphate (AMP) concentration (Figure 1). Attention has been mainly confined to the decay of ATP to ADP by first-order reaction kinetics.

Using the oil bath data, the ATP hydrolysis rate k_1 was found to follow the Arrhenius law with regard to temperature T:

$$k_1(T) = A \exp\left(-\frac{E_a}{RT}\right)$$

with

$$E_a = 107.774 \times 10^3 \text{ J/mol}$$

$$A = 3.18217 \times 10^{11} \text{ s}^{-1}$$

$$R = 8.314 \text{ J/(K mol)}$$

[†] The Pillsbury Co.
[‡] University of Lowell.

(1) Sun, W.-C.; Guy, P. M.; Jahngen, J. H.; Rossomondo, E. F.; Jahngen, E. G. E. *J. Org. Chem.* **1988**, *53*, 4414-4416.

0022-3263/90/1955-3406$02.50/0