**en-17-one and of the Corresponding Acetate (3a).**—The benzoate (0.10 mmol) in deuteriochloroform containing tetramethylsilane in an nmr tube was refluxed and irradiated after addition of N-bromosuccinimide (0.11 mmol). After 2 min, the doublet at 5.46 ppm (H-6) (7,7 $H_2$) had diminished while a doublet at $\delta$ 5.95 ppm ($J$ = 5.1 Hz), H-6 (7$\alpha$ Br) and a singlet at $\delta$ 5.72 ppm (H-6) (7$\beta$ Br), ratio 1.3:1, had appeared.

A similar bromination of 3$\beta$-acetoxyandrost-5-en-17-one (**3a**) in $CCl_4$ gave a ratio of 7$\alpha$ Br (**3b**) to 7$\beta$ Br (**3c**) product of 1.2:1.

**Registry No.**—Chloranil, 118-75-2; **1c**, 23668-15-7; **2b**, 23668-16-8; **2c**, 633-34-1; **3b**, 23668-18-0; **3e**, 748-37-8; **4a**, 23668-20-4; **4a** diacetate, 23668-21-5; **4b**, 23688-22-6.

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# The Addition of Coordinated Glycine to Acetaldehyde. Mechanism[1]

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The kinetics of the reaction of acetaldehyde with glycinatobis(ethylenediamine)cobalt(III) chloride in the presence of a tertiary amine in water solution to produce the threoninato complex ion has been studied. The rate of reaction is first order in aldehyde and one-half order in each the complex ion and the amine base. These results are consistent with a mechanism involving abstraction by base of an $\alpha$ proton of the glycine moiety followed by reaction of the resulting enolatelike ion with aldehyde.

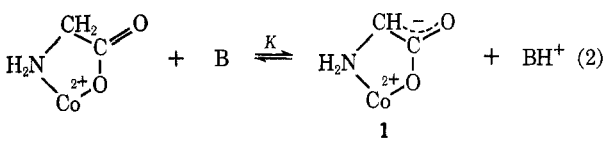
Aldehydes react with glycine coordinated with certain metal ions in the presence of base to produce hydroxy amino acids,[2-6] *e.g.*, eq 1, where en is ethylenediamine and gly and thr are glycine and threonine (allo and

$$(\text{Coen}_2\text{gly})^{2+} + \text{CH}_3\text{CHO} \xrightarrow[\text{H}_2\text{O}]{\text{base}} (\text{Coen}_2\text{thr})^{2+} \qquad (1)$$

threo) anions, respectively, coordinated with cobalt(III). If optically active glycinatobis(ethylenediamine)cobalt(III) ion is treated with acetaldehyde, an asymmetric synthesis of threonine and allothreonine can be effected.[6] The base most commonly used is sodium carbonate. The amino acids are obtained upon cleavage of the ligands from the metal ion.

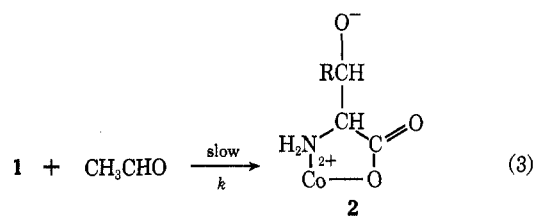
This paper reports the results of a kinetic study of reaction 1 with a tertiary amine, 1,4-diazabicyclo[2.2.2]-octane (dabco), serving as the base and glycinatobis-(ethylenediamine)cobalt(III) chloride and acetaldehyde serving as the reactants. Essentially complete conversion of glycine into the threonines occurs with this base. The results of this study are consistent with the mechanism of eq 2–4 where B is the base and the ethylenediamine ligands are omitted for clarity.

Reaction conditions (Table I) were chosen so that the reaction was pseudo first order in acetaldehyde and such that the competing aldol condensation[7] can be neglected. The spectrophotometric method developed for the determination of aldehyde concentration as a



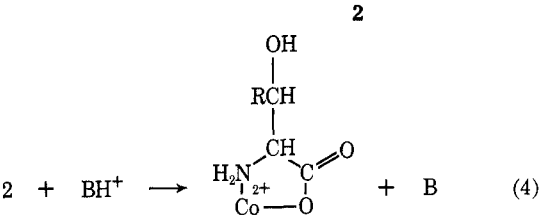
(2)

**1**



(3)

**2**



(4)

function of time is described in the Experimental Section. Equation 5 describes the observed rate law at essentially constant ionic strength and chloride ion concentration for the reaction conditions investigated (Table I).

$$\frac{-\text{d}[\text{CH}_3\text{CHO}]}{\text{d}t} = k_1[\text{dabco}]^{1/2}[(\text{Coen}_2\text{gly})\text{Cl}_2]^{1/2}[\text{CH}_3\text{CHO}] \qquad (5)$$

This rate law is related to the above mechanism as shown in eq 6–9; $K_B$ is the basic dissociation constant of

$$\frac{-\text{d}[\text{CH}_3\text{CHO}]}{\text{d}t} = k[\text{CH}_3\text{CHO}][1] \qquad (6)$$

$$= k[\text{CH}_3\text{CHO}]K[(\text{Coen}_2\text{gly})^{2+}][\text{B}]/[\text{BH}^+] \qquad (7)$$

$$[\text{BH}^+] = [^-\text{OH}] + [1] \qquad (8)$$

$$\text{or } [\text{BH}^+]^2 = [\text{B}]\{K_B + K[(\text{Coen}_2\text{gly})^{2+}]\} \qquad (9)$$

dabco. The contribution to $[BH^+]$ from the formation of $CH_3CH(OH)O^-$ can be neglected since the known values at 25° of the hydration constant[7] of acetaldehyde

(1) The support of this work by the U. S. Public Health Service, National Institute of Arthritis and Metabolic Diseases Grant AM 12262, is gratefully acknowledged.

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TABLE I

RATE DATA FOR REACTION 1 AT 35.00°

| Initial concentrations, $M$ | | | | | | |
|---|---|---|---|---|---|---|
| $[Coen_2gly]Cl_2$ | Dabco[b] | $CH_3CHO$ | KCl | $10^3k_1$,[a] $sec^{-1}$ | $10^3k_1/[dabco]^{1/2}$ | $10^3k_1/[(Coen_2gly)Cl_2]^{1/2}$ |
| 0.0797 | 0.379 | *Ca.* $1.6 \times 10^{-3}$ | | 2.50 | 41 | |
| 0.0797 | 0.733 | *Ca.* $1.6 \times 10^{-3}$ | | 3.22 | 38 | |
| 0.0797 | 1.36 | *Ca.* $1.6 \times 10^{-3}$ | | 5.52 | 47 | |
| | | | | | Av 42 | |
| 0.0797 | 1.36 | *Ca.* $1.6 \times 10^{-3}$ | 1.28 | 4.84 | | 17 |
| 0.0428 | 1.36 | *Ca.* $1 \times 10^{-3}$ | 1.28 | 3.33 | | 16 |
| 0.0214 | 1.36 | *Ca.* $1 \times 10^{-3}$ | 1.28 | 2.33 | | 16 |
| | | | | | | Av 16.3 |

[a] Average of two or three determinations. Maximum deviation from the average for runs without KCl is 6.7%; with KCl, 2.5%. [b] Calculated values of concentration based on density measurements accurate to 1 or 2%.

and the acid dissociation constant[7] of $CH_3CH(OH)_2$ lead to a value much smaller than that[8] of $K_B$ of eq 9. If $K_B$ can be neglected compared with the other term on the right-hand side of eq 9, combination of eq 7 and 9 leads to an equation identical in form with eq 5.

Neglect of $K_B$ in eq 9 requires that the acid dissociation constant for ionization of an $\alpha$ hydrogen of the glycine moiety be two or three orders of magnitude greater than the ion product constant of water. This is reasonable since the dipositive charge on the cobalt atom would be expected to increase the acidity compared with the neutral free ligand.

The concentrations in eq 5 refer to stoichiometric concentrations. Thus the observed pseudo-first-order rate constant will be a function of the hydration constant of acetaldehyde, the ion-pairing constant for glycinatobis(ethylenediamine)cobalt(III) chloride, and the chloride ion concentration. The ion-pair association constant for chloropentaamminecobalt(III) chloride at 25° and zero ionic strength is ten.[9] The same form for the rate equation will be obtained whether or not the ion pair undergoes reaction; this will also be true if hydroxide ion generated from the tertiary amine base and water serves as the base in eq 2.

Ultraviolet spectroscopy indicates that there is no interaction between dabco and the carbonyl group of acetaldehyde. The absorption due to the carbonyl group is the same for 0.019 $M$ acetaldehyde in water containing no, 0.10 $M$, and 0.73 $M$ dabco, respectively.

Additional evidence for intermediate **1** is found in previous reports by other workers. Nmr deuterium-exchange experiments[10,11] and mutarotation studies[11] have shown the lability of the $\alpha$ hydrogens of the amino acid moiety of $(Coen_2aa)^{2+}$ where aa is the amino acid anion of glycine and other compounds. The rate of exchange of the $\alpha$ hydrogen of alaninatobis(ethylenediamine)cobalt(III) ion in $D_2O$ with NaOD at 34.3° was found[11] to be first order in $^-OD$ ion and first order in complex ion. These data may be used as follows to show that process 3 is slower than process 2 in the proposed mechanism. If the only function of the dabco were to generate hydroxide ion which then serves as the base in eq 2, then the calculated half-life for the exchange (based on the literature data for the basicity[8] of dabco and the exchange experiments using the alaninato moiety as a model for the glycinato moiety) is less than half of the half-life for the overall reaction with acetaldehyde (Table I). Dabco itself is an effective base for proton removal;[8] *e.g.*, the ratio of the second-order catalytic constants for deuteron abstraction from isobutyraldehyde-2-*d* is $k_{-OH}/k_{dabco} = 4.8$ in water solution at 35°. Since the dabco is in large excess to hydroxide ion in the present system, the half-life for exchange of the $\alpha$ hydrogen of the glycine moiety is expected to be even smaller than that calculated above.

## Experimental Section

**Materials.**—Acetaldehyde was distilled and stored under nitrogen in a refrigerator. It was checked for absence of polymers and acetic acid by infrared spectroscopy before each use. 1,4-Diazabicyclo[2.2.2]octane (Aldrich Chemical Co.) was crystallized from 1:1 methanol–ethyl ether and stored in a desiccator over potassium hydroxide pellets: mp 155.3–158.1° (lit.[12] mp 155–157°). Glycinatobis(ethylenediamine)cobalt(III) chloride monohydrate, a gift of Dr. D. W. Cooke, was crystallized from aqueous ethanol. *Anal.*[13] Calcd for $(Coen_2gly)Cl_2 \cdot H_2O$: C, 21.06; N, 20.47; H, 6.48. Found: C, 21.14; N, 20.67; H, 6.49. Doubly distilled water was used throughout. The concentrations listed in Table I are for ambient temperature and are corrected for volume changes occurring upon mixing.

**Reaction Products.**—A solution of glycinatobis(ethylenediamine)cobalt(III) chloride monohydrate (0.40 g, 0.0012 mol), 1,4-diazabicyclo[2.2.2]octane (0.22 g, 0.0020 mol), and acetaldehyde (0.67 ml, 0.012 mol) in 25 ml of water was allowed to stand at room temperature for 3 days. Hydrogen sulfide was bubbled through the mixture until the filtrate from filtration of the resulting mixture was no longer orange. The filtrate was concentrated at reduced pressure and placed on a Dowex 50 (100–200 mesh, H form) column. The amino acids were eluted with 1:5 pyridine–water by volume. The eluate totaled 125 ml, the last 50-ml portion of which was alkaline. The eluate was evaporated to dryness by use of reduced pressure and heat. The residue was further purified by redissolving it in water and placing it on an ion-exchange column as above. Elution of the amino acids with 2 $N$ ammonia was begun after the eluate from water elution was no longer colored. The alkaline eluate (50 ml) was evaporated to dryness as before. An nmr spectrum (Varian A-60 spectrometer) of the residue dissolved in deuterium oxide containing sodium 2,2-dimethyl-2-silapentanesulfonate as an internal standard was obtained. The spectrum corresponds to that of a mixture of authentic threonine and allothreonine. No glycine signals are present.[14]

**Spectrophotometric Method for Acetaldehyde.**—2,4-Dinitrophenylhydrazine (0.025 g), water (5 ml), and concentrated hydrochloric acid (0.1 ml) were dissolved in 95% ethanol to make a final volume of 50 ml to prepare the standard reagent (DNPH reagent). The reagent contained added amounts of concentrated hydrochloric acid (and water) required for neutraliza-

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(12) S. D. Ross, J. J. Bruno, and R. C. Petersen, *ibid.*, **85**, 3999 (1963).

(13) Galbraith Laboratories, Inc., Knoxville, Tenn.

(14) J. C. Dabrowiak of this department has shown that the ratio of authentic glycine:threonine:allothreonine remains unchanged upon placement on and elution from the ion-exchange column.

tion of base contained in the aliquots from kinetic runs. The reagent is stable for several days. One milliliter of the DNPH reagent was pipetted into each of 25-ml volumetric flasks. An aliquot of the aldehyde containing solution (for amounts, see kinetic procedure) was added and the solution was allowed to stand for 1 day before dilution with 1:1 95% ethanol–water by volume. The absorbance of the solution at 374 mμ was determined with 1 ml of the DNPH reagent diluted to 25 ml as above serving as the blank solution with a Beckman Model DU spectrophotometer. A small decrease in the absorbance with time occurs; however, if a constant time interval passes from addition of the aldehyde solution until determination of the absorbance, the ratio of aldehyde concentrations in different samples is the same as the corresponding absorbance ratios.

**Kinetic Procedure.**—Ten milliliters (20 ml for runs listed in last two rows of Table I) of the appropriate dabco solution was pipetted into a polypropylene tube containing the glycinatobis(ethylenediamine)cobalt(III) chloride monohydrate and the resulting solution was equilibrated at 35.00 ± 0.01°. Cold acetaldehyde was transferred by a micropipet to 10 ml of cold dabco solution. One milliliter of this was added to the above equilibrated solution. After 15–20 min of further equilibration, a 1-ml aliquot (2 ml for runs listed in last two rows of Table I) of the reaction mixture was pipetted into a 25-ml volumetric flask containing 1 ml of DNPH reagent and the time was taken as zero time. Aliquots were then taken periodically and added to the DNPH reagent in volumetric flasks, and the absorbance was determined as described above.

Observed "infinity" absorbances were within experimental error of those calculated from the spectra of the complex ions involved. The visible spectrum is changed only slightly when glycine is replaced by threonine;[15,16] since the $(Coen_2gly)^{2+}$ is in large excess to the aldehyde under the reaction conditions, the difference is negligible. Calculated infinity values[17] were used to determine rate constants. The pseudo-first-order rate constants were calculated from the following equation: $\log (A - A_\infty) = -k_1t/2.303 + \text{constant}$. The slope was calculated by the method of least squares. The reaction was followed to two-thirds complete reaction.

The stability of the reactants under the reaction conditions was tested as follows. Acetaldehyde (*ca.* $2.9 \times 10^{-3}$ *M*) in 0.96 *M* dabco solution was sampled periodically as described above (except 0.5-ml aliquots were used); an 8% decrease in absorbance occurred after 5 hr. Glycinatobis(ethylenediamine)cobalt(III) chloride monohydrate (0.190 *M*) in 1.36 *M* dabco was held at 35° for 3 days. The visible spectrum of a 1-ml aliquot diluted to 25 ml was identical with that of authentic material.

**Registry No.**—Acetaldehyde, 75-07-0; glycinatobis(ethylenediamine)cobalt(III) chloride, 14408-57-2.

(15) C. T. Liu and B. E. Douglas, *Inorg. Chem.*, **3**, 1356 (1964).
(16) S. K. Hall and B. E. Douglas, *ibid.*, **8**, 372 (1969).
(17) Determined from spectra obtained by use of a Cary Model 14 spectrophotometer.

# Pyrimidines. IX. A New Synthesis of 8-Azapurines and v-Triazolo[4,5-b]pyridines[1]

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The 5-nitropyrimidines **1–5** and the 5-nitropyridines **6** react with sodium azide to furnish the 8-azapurines **14–18** and the *v*-triazolo[4,5-*b*]pyridines **19**, respectively. The first step of this new reaction leading to *v*-triazoles is probably attack of azide ion at position 6 of the 5-nitropyrimidines and -pyridines followed by cyclization and subsequent elimination of the nitro function as nitrous acid. With hydroxide, deuteroxide, and ethoxide ions as the nucleophiles, N-1 substituted derivatives of some of these nitroheterocycles form stable Meisenheimer-type adducts by reaction at position 6. A reaction with deuterium oxide in DMSO-$d_6$/$D_2O$ concurrent with adduct formation is H–D exchange at position 6 of the N-1 substituted 5-nitro-2-oxo-pyrimidines and -pyridines, **1–3** and **6**. A carbanion mechanism is postulated for these H–D exchange reactions.

The chemistry of *v*-triazolo[4,5-*d*]pyrimidines[2] (8-azapurines) has developed in conjunction with biological studies on the antimetabolite activity of analogs of the nucleic acid purines.[3] Such compounds have been prepared previously by the action of nitrous acid on 4,5-diaminopyrimidines[4,5] and from substituted *v*-triazoles.[6,7] This report describes a new and facile synthesis of some 8-azapurines and 5-oxo-*v*-triazolo[4,5-*b*]-pyridines. The procedure consists of the treatment of certain 5-nitrooxopyrimidines and -pyridines with sodium azide, which results, overall, in the addition of the three-nitrogen fragment of the *v*-triazole ring to the 5,6 positions of the nitropyrimidine or -pyridine followed by elimination of the nitro function as nitrous acid. A preliminary report[8] on this reaction has appeared. The extent and mechanism of this process as well as its practical value are now further elaborated.

## Results

The reaction with azide ion was achieved with the following types of compounds (Scheme I): 5-nitrouracils (**1**), 5-nitrocytosines (**2**, Y = $NR_2$), 4-ethoxy-1-methyl-2-oxo-5-nitropyrimidine (**2h**), 2-oxo-5-nitropyrimidines (**3**), 4-oxo-5-nitropyrimidine (**4**), 2-amino-4-oxo-5-nitropyrimidine (**5**), and 2-oxo-5-nitropyridines (**6**). With compounds **1–3** and **6**, which are not alkylated at N-1, only salt formation between these acidic nitro compounds and the reagent is observed. Therefore ammonium chloride (in slight molar excess relative to the sodium azide) was added to these reaction

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant CA 08748).

(2) For recent reviews on the chemistry of *v*-triazolo[4,5-*d*]pyrimidines, see (a) J. Gut, *Advan. Heterocycl. Chem.*, **1**, 238 (1963); (b) R. K. Robins in "Heterocyclic Compounds," Vol. 8, R. C. Elderfield, Ed., John Wiley & Sons, Inc., New York, N. Y., 1967, p 434.

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