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Pyrido[2,3-*d*]pyrimidine Antibacterial Agents. IV.[1] Synthesis of Metabolites of Piromidic Acid

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Three metabolites of the antibacterial agent, piromidic acid (8-ethyl-5,8-dihydro-5-oxo-2-(1-pyrrolidinyl)pyrido[2,3-*d*]pyrimidine-6-carboxylic acid), were synthesized in order to confirm their structures, *i.e.*, 2-(3-hydroxy-1-pyrrolidinyl)- (**4**), 2-(2-hydroxy-1-pyrrolidinyl)- (**8**), and 2-(3-carboxypropylamino)-8-ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]-pyrimidine-6-carboxylic acids (**13**). Compounds **4**, **8**, and **13** were identified with the metabolites designated as A, B, and I, respectively, on the basis of their IR, UV, MS, and NMR spectra.

8-Ethyl-5,8-dihydro-2-(3- and 4-hydroxypiperidino)-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acids (**6a** and **6b**) as well as the *O*-formyl (**5a**), -acetyl (**5b**), -benzoyl (**5c**), and -methanesulfonyl (**5d**) derivatives of **4** were prepared and tested for antibacterial activity. Of the compounds discussed in this paper, **4** was the most active, particularly against gram-negative bacteria.

Keywords——pyrido[2,3-*d*]pyrimidine; piromidic acid; metabolite; synthesis; antibacterial activity; structural identification

One of a series of 5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acids widely investigated in our laboratories,[3] piromidic acid (**1**),[3a] was developed as an orally effective antibacterial agent and is now clinically used for the treatment of infections mainly due to gram-negative bacterial pathogens. In studies on the metabolism of piromidic acid,[4] unchanged piromidic acid and five metabolites designated as A, B, C, I, and N, as well as their glucuronides, were detected in the urine and blood of rats and humans receiving the drug orally. Since metabolite A, which was a major, active component among the metabolic products, proved to be more active than the parent drug, particularly against gram-negative organisms,[4a] it was of interest to determine the structures of metabolite A and the other metabolites.

The metabolites A, B, and I (but not metabolite C, which was too unstable to isolate) were provisionally assigned to 2-(3-hydroxy-1-pyrrolidinyl)- (**4**), 2-(2-hydroxy-1-pyrrolidinyl)- (**8**), and 2-(3-carboxypropylamino)-8-ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acids (**13**), respectively, on the basis of their infrared (IR), ultraviolet (UV), mass (MS), and nuclear magnetic resonance (NMR) spectra.[4b] The present study was undertaken to obtain conclusive proof for these structures of the metabolites through synthesis. The metabolite N was identified by direct comparison with an authentic specimen as 2-amino-8-ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (**3**), which had previously been prepared from 8-ethyl-5,8-dihydro-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (**2**).[3b]

1) Part III: J. Matsumoto and S. Minami, *J. Med. Chem.*, **18**, 74 (1975).
2) Location: *Enoki-cho 33-94, Suita, Osaka, 564, Japan.*
3) *a*) S. Minami, T. Shono, and J. Matsumoto, *Chem. Pharm. Bull.*, **19**, 1426 (1971); *b*) *Idem, ibid.*, **19**, 1482 (1971).
4) *a*) M. Shimizu, S. Nakamura, Y. Takase, Y. Sekine, H. Suzuki, and K. Nakamura, *Chemotherapy*, **19**, 387 (1971); *b*) Y. Sekine, M. Miyamoto, M. Hashimoto, and K. Nakamura, *Zenobiotica*, **6**, 185 (1976); *c*) *Idem, Chem. Pharm. Bull.*, **24**, 437 (1976).

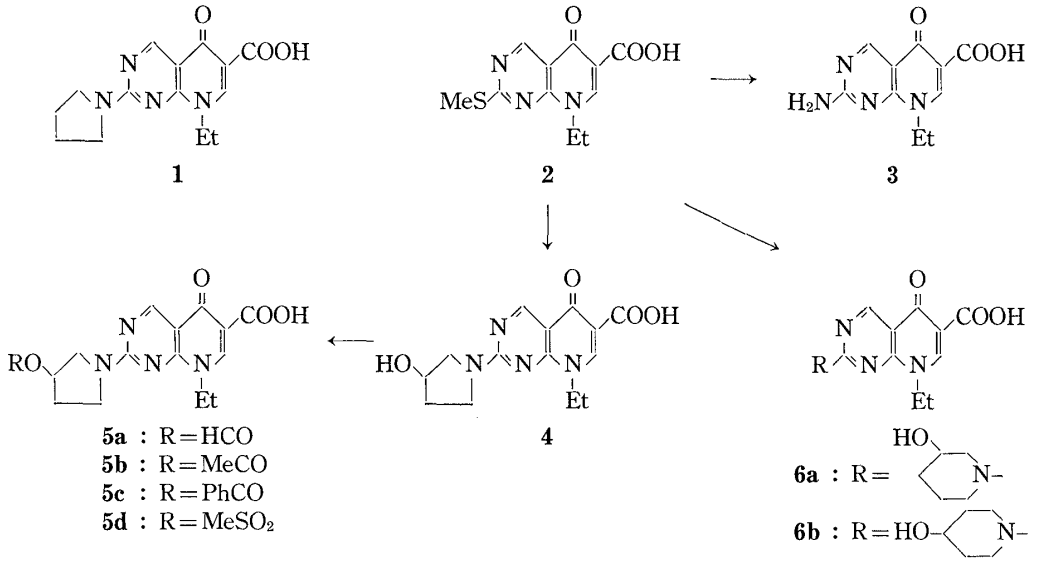


Chart 1

1. Metabolite A (4) and Related Compounds (5 and 6)

The methylthio group of **2** has been found to be readily displaced by nucleophiles in an aprotic polar solvent.[3a] Treatment of **2** with *dl*-3-hydroxypyrrolidine in dimethyl sulfoxide gave **4** in 73% yield.

In the NMR spectrum of **4** (in DMSO-d_6 at 31°), a nuclear Overhauser effect (22% enhancement in the integrated intensity) on the one-proton signal at δ 8.92 was observed on irradiating the N-8 methylene signal at δ 4.39 (2H, q, J=7 Hz), permitting assignment of the former to the C-7 proton. A signal at δ 9.15 due to the C-4 proton appeared with the small splitting of 0.7 Hz. On scanning the spectrum at 70°, however, the split signal collapsed to a sharp singlet. This was attributable to restricted rotation of the 3-hydroxypyrrolidine ring about the C–N bond, which caused a difference in the anisotropic effect on C-4 H. Similar effects were observed in the NMR spectra (Table II) of the compounds discussed later, in which the substituent at position 2 was unsymmetrical; the intensities of the nonequivalent proton signals corresponding to C-4 H and, in some cases, C-2′ H and C-2′ OCH_3 on the pyrrolidine ring varied with changes in the proportions of the rotamers.

Compound **4** thus prepared was identical with metabolite A in their MS, UV, IR, and NMR spectra, except as regards optical rotation; the specific rotation of metabolite A was $[\alpha]_D^{25}$ +6.7 (c=2, 1 M NaOH).[4b] Attempted optical resolution of the synthetic compound **4** was unsuccessful. The antibacterial activity of **4**, however, was essentially identical with that of metabolite A isolated from animal subjects. This finding indicated that the configuration of the hydroxyl group did not influence the activity.

As described above, the antibacterial activity of metabolite A (**4**) was higher than that of the parent drug (**1**). The fact that the introduction of a hydrophilic function (hydroxyl group) into the pyrrolidine ring in **1** caused an increase in activity may be suggestive for future studies on structural modifications in this series of compounds. In connection with this finding, an interest in structure-activity relationships led us to prepare 8-ethyl-2-(3- and 4-hydroxypiperidino)-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (**6a** and **6b**). Treatment of **2** with 3- and 4-hydroxypiperidines gave **6a** and **6b**, respectively. Increasing the ring size of the 2-substituent caused a decrease in activity; thus, the minimal inhibitory concentrations (MIC) of **6a** and **6b** were 10 and 3 μg/ml, respectively, against, for example, *Escherichia coli* K-12, whereas **4** showed an MIC of 1 μg/ml against the same strain. The acyl derivatives **5a**—**d** were also prepared by treating **4** with an appropriate acylating reagent as described in "Experimental." No compounds possessing higher activity than **4** were

found, although **5a** and **5b** showed the same level of activity as **4**.

2. Metabolite B (8)

Treatment of **2** with 4-aminobutyraldehyde diethyl acetal afforded 2-(4,4-diethoxybutylamino)-8-ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (**7**). It was rather troublesome to hydrolyze the acetal **7** into the 2-hydroxypyrrolidinyl derivative **8**, because of concomitant formation of an enamine **10**. The successful preparation of **8** involved dissolution of **7** in ethanol (or methanol) in the presence of a catalytic amount of sulfuric acid followed by treatment of the intermediate 2-alkoxypyrrolidinyl derivative **9** with water in dioxane.

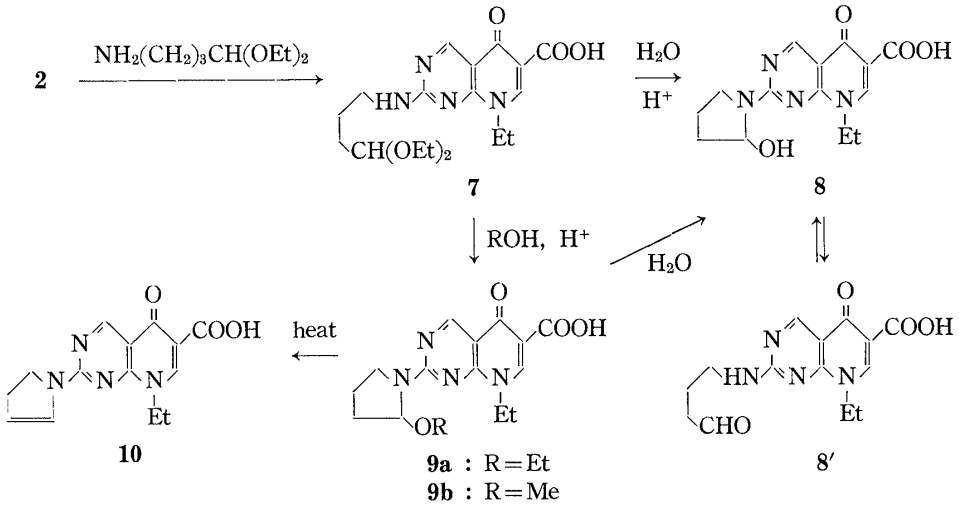


Chart 2

Compound **8** was identical in all respect with metabolite B. The NMR spectrum of **8** showed a signal at δ 5.80 (1H, d, J=3 Hz) due to C-2′ H on the pyrrolidine ring but no signal due to an aldehyde proton which would be expected for the tautomeric structure **8′**, indicating that the equilibrium favored the closed structure **8** under these conditions.

When **9a** or **9b** was heated near its melting point, 8-ethyl-5,8-dihydro-2-(2-pyrrolin-1-yl)-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (**10**) was formed with loss of the corresponding alcohol from the 2-alkoxypyrrolidine moiety.

3. Metabolite I (13)

Compound **13** was derived from the displacement reaction of 2-chloro-8-ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (**11**) with ethyl 4-aminobutyrate in ethanol followed by alkaline hydrolysis of the ester **12**, and was found to be identical with metabolite I by spectral comparison.

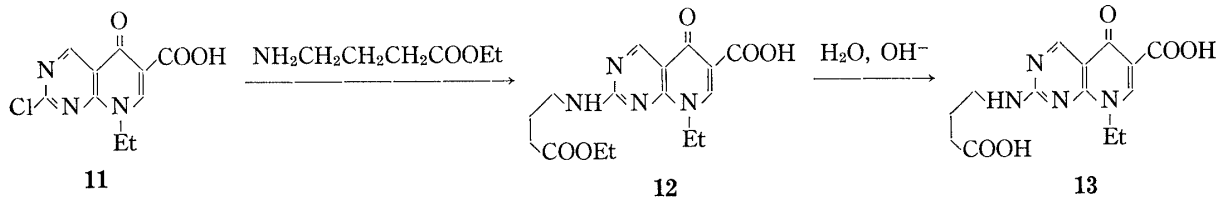
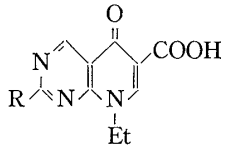


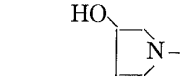
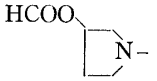
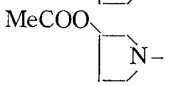
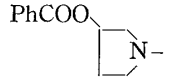
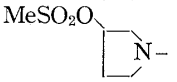
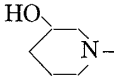
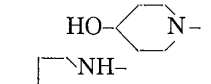
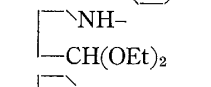
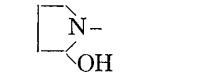
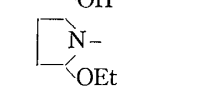
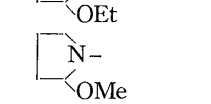
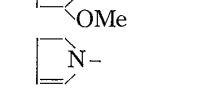
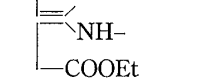
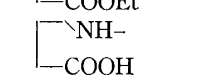
Chart 3

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded in KBr discs on a Hitachi model 215 spectrophotometer, and UV spectra were

Table I. 2-Substituted 8-Ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acids



Compd.	R	mp (°C)	Recryst. solvent	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N
4		281—282 (dec.)	DMF	73	$C_{14}H_{16}N_4O_4$	55.25 (55.46	5.30 5.28	18.41 18.20)
5a		231—232	EtOH	76	$C_{15}H_{16}N_4O_5$	54.21 (54.33	4.85 4.78	16.86 16.95)
5b		230—232	DMF	78	$C_{16}H_{18}N_4O_5$	55.48 (55.36	5.24 5.17	16.18 16.08)
5c		238—241	EtOH	67	$C_{21}H_{20}N_4O_5$	61.76 (61.90	4.94 4.64	13.72 13.57)
5d		207—209	Me_2CO	63	$C_{15}H_{18}N_4O_6S$[a]	47.11 (46.92	4.76 4.58	14.65 14.52)
6a		281—282 (dec.)	EtOH–DMF	79	$C_{15}H_{18}N_4O_4$	56.59 (56.54	5.70 5.93	17.60 17.35)
6b		243—245	EtOH–DMF	81	$C_{15}H_{18}N_4O_4$	56.59 (56.85	5.70 5.61	17.60 17.64)
7		176—178	Dioxane	94	$C_{18}H_{26}N_4O_5$	57.13 (57.40	6.93 7.06	14.81 14.86)
8		237—240 (dec.)	DMF	42	$C_{14}H_{16}N_4O_4$	55.25 (55.42	5.30 5.09	18.41 18.50)
9a		181—184	EtOH	86	$C_{16}H_{20}N_4O_4$	57.82 (57.85	6.07 6.17	16.86 16.77)
9b		186—188	MeOH	81	$C_{15}H_{18}N_4O_4$	56.59 (56.72	5.70 5.57	17.60 17.63)
10		242—245	MeCN	25	$C_{14}H_{14}N_4O_3$	58.73 (58.79	4.93 4.84	19.57 19.36)
12		180—181	Benzene	81	$C_{16}H_{20}N_4O_5$	55.16 (55.21	5.79 5.79	16.08 15.93)
13		241—243	MeCN	61	$C_{14}H_{16}N_4O_5$	52.49 (52.53	5.04 4.97	17.49 17.53)

a) *Anal.* Calcd for S: 8.39. Found: S, 8.09.

taken with a Shimadzu MPS-5000 spectrophotometer. NMR spectra were recorded on a Varian HA-100D spectrometer with Me_4Si as an internal standard. Mass spectra were recorded on a Hitachi RMU-6 mass spectrometer using the direct inlet system at 70 eV ionization potential.

8-Ethyl-5,8-dihydro-2-(3-hydroxy-1-pyrrolidinyl)-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (4)——A mixture of **2**[3b] (2.65 g) and *dl*-3-hydroxypyrrolidine[5] (1.0 g) in DMSO (40 ml) was heated at 110° for 4 hr and then cooled. The precipitate was collected by filtration and the filtrate was concentrated to about half the original volume under reduced pressure, giving an additional crop of the precipitate. Recrystalliza-

5) Yu. A. Arbuzov and Yu. A. Ovchinnikov, *Dokl. Acad. Nauk. SSSR*, **117**, 813 (1957) [*C.A.*, **52**, 8120 (1958)].

Table II. Spectral Data for 2-Susbtituted 8-Ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acids

Compd.	NMR (DMSO-d_6) δ (J in Hz) C-7H	C-4H	Others	IR ν_{max}^{KBr} cm^{-1}	UV λ_{max}^{EtOH} nm (log ε)	MS m/e
4	8.92(1H, s)	9.15(1H, s)	4.43(HC–OH)[a] 5.08(1H, d, J=1.8, HC–OH)	1720, 1640, 1620	219(4.11), 280(4.70), 326(3.91)	304(M^+) 260(M^+-CO_2)
7	8.92(1H, s)	9.10(4/5H) 9.18(1/5H)	4.50(1H, s, O–CH–O) 8.61(1H, br.t., J=6, NH)	1730, 1630	214(4.01), 274(4.62), 325(5.85)	378(M^+) 334(M^+-CO_2)
8	8.96(1H, s)	9.21(5/9H) 9.23(4/9H)	5.80(1/2H, d, J=3, C-2′H) 5.88(3/2H, C-2′H and OH)	1720—1710, 1620	218(4.09), 275(4.67), 325(3.95)	304(M^+) 286(M^+-H_2O) 260(M^+-CO_2)
9a	8.96(1H, s)	9.18(2/5H) 9.21(3/5H)	5.65, 5.78(each 1/2H, d, J= 3, C-2′H)	1720, 1620	219(4.08), 274(4.69), 325(4.02)	332(M^+) 288(M^+-CO_2)
9b	8.97(1H, s)	9.20(4/9H) 9.22(5/9H)	5.57, 5.69(each 1/2H, d, J= 3, C-2′H); 3.33 and 3.37 (OCH_3)[b]	1720, 1630—1620	219(4.08), 274(4.69), 325(4.02)	318(M^+) 274(M^+-CO_2)
10	8.95(1H, s)	9.15(5/9H) 9.21(4/9H)	5.53(1H, m, –CH=CH–N–)[c] 7.26(1H, m, –CH=CH–N–)[d]	1720, 1630	227(4.10), 304(4.62)	286(M^+) 242(M^+-CO_2)
12	8.92(1H, s)	9.10(3/4H) 9.18(1/4H)	8.63(1H, t, J=5.5, $NHCH_2$)	1730, 1630	219(4.03), 271(4.69), 323(4.05)	348(M^+) 304(M^+-CO_2)
13	8.90(1H, s)	9.10(3/4H) 9.18(1/4H)	8.62(1H, t, J=6, $NHCH_2$)	1745, 1730, 1630	215(3.98), 273(4.63), 324(3.84)	320(M^+) 276(M^+-CO_2)

a) Overlapped with a signal at δ 4.39 (2H, q, J=7, NCH_2CH_3).
b) The peak-height ratio was 1: 1 for these signals.
c) Observed as a doublet (J=5 Hz) on irradiation at δ 2.80 and 7.26.
d) Observed as a singlet on irradiation at δ 5.53 and 2.80.

tion of the combined precipitate gave **4** (2.22 g) as colorless fine needles (see Table I). The NMR, IR, UV, and MS spectral data are given in Table II.

8-Ethyl-2-(3-formyloxy-1-pyrrolidinyl)-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (5a)——A mixture of **4** (1.0 g) and 98% formic acid (5.0 ml) was heated on a steam bath for 30 min and then water (20 ml) was added. The resulting precipitate was collected by filtration and recrystallized to give **5a** (0.83 g) as colorless fine needles (see Table I).

2-(3-Acetoxy-1-pyrrolidinyl)-8-ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (5b)——A mixture of **4** (1.0 g), acetic anhydride (2.0 ml), and dried pyridine (2.0 ml) was stirred at room temperature for 4 hr. After removal of excess acetic anhydride and pyridine by distillation, the residual solid was washed with water and recrystallized, giving **5b** (0.89 g) as colorless fine needles (see Table I).

2-(3-Benzoyloxy-1-pyrrolidinyl)-8-ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (5c)——A mixture of **4** (1.0 g) and benzoic anhydride (0.6 g) was heated under reflux for 2 hr. After cooling the mixture, the precipitate was collected by filtration and recrystallized, giving **5c** (0.90 g) as colorless scales (see Table I).

8-Ethyl-5,8-dihydro-2-(3-methanesulfonyloxy-1-pyrrolidinyl)-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (5d)——A mixture of **4** (1.0 g) and methanesulfonyl chloride (3.0 ml) was heated under reflux for 2 hr. Excess reagent was distilled off and the residual solid was washed with water, then recrystallized, giving **5d** (0.54 g) as pale yellow prisms (see Table I).

8-Ethyl-5,8-dihydro-2-(3- and 4-hydroxypiperidino)-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acids (6a and 6b)——A mixture of **2** (2.65 g) and 3- or 4-hydroxypiperidine (1.21 g) in DMSO (40 ml) was heated at 110° for 4 hr. After removal of the solvent by distillation, the resulting solid was collected, washed with water, and recrystallized, giving the corresponding compounds **6a** (2.51 g) and **6b** (2.58 g) (see Table I).

2-(4,4-Diethoxybutylamino)-8-ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (7)——A solution of **2** (1.85 g) and 4-aminobutyraldehyde diethyl acetal (1.61 g) in DMSO (20 ml) was heated at 120° for 2.5 hr with stirring and then cooled. Recrystallization of the precipitate gave **7** (2.49 g) as colorless needles (see Table I). The spectral data are given in Table II.

8-Ethyl-5,8-dihydro-2-(2-hydroxy-1-pyrrolidinyl)-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (8)——a) Aqueous 10% H_2SO_4 (0.2 ml) was added with stirring to a suspension of **7** (3.0 g) in a mixture of dioxane (20 ml) and water (10 ml). The reaction mixture was heated on a steam bath, becoming clear and immediately precipitating a yellowish solid. Heating was continued for several minutes and then the reaction mixture was cooled. The solid formed was collected by filtration, and washed with hot dioxane. Recrystallization of the crude product gave **8** (1.0 g) as yellowish fine needles (see Table I). The spectral data are given in Table II.

b) Water (10 ml) was added to a hot solution of **9a** (2.82 g) in dioxane (20 ml) with stirring. The mixture was heated on a steam bath for 5 min, during which period yellowish crystals separated out. The crystals collected by filtration and recrystallized, giving **8** (1.91 g).

8-Ethyl-2-(2-ethoxy- and -methoxy-1-pyrrolidinyl)-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acids (9a and 9b)——Aqueous 10% H_2SO_4 (15 ml) was added to a suspension of **7** (4.0 g) in EtOH or MeOH (200 ml). The mixture was heated on a steam bath for 5 min. The resulting precipitate was collected by filtration and the filtrate was concentrated to about 70 ml under reduced pressure, giving an additional crop of the precipitate. Recrystallization of the combined precipitate gave the corresponding 2-alkoxy derivatives **9a** (3.02 g) and **9b** (2.73 g) as yellowish fine needles (see Table I). The spectral data are given in Table II.

8-Ethyl-5,8-dihydro-2-(2-pyrrolin-1-yl)-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (10)——Compound **9a** (500 mg) was heated to *ca.* 200° under reduced pressure (30 mmHg), melting into a yellow liquid which then solidified. The solid was taken up in $CHCl_3$ and chromatographed on silica gel (10 g) with $CHCl_3$ as an eluent. Recrystallization of the crude product gave **10** (106 mg) as yellow needles (see Table I). Compound **9b** was treated in a similar manner to give **10**. The spectral data are given in Table II.

2-(3-Ethoxycarbonylpropylamino)-8-ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (12)——2-Chloro-8-ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (**11**)[3b] (4.0 g) was added to a solution of ethyl 4-aminobutyrate hydrochloride (3.02 g) and triethyl amine (3.8 g) in EtOH (200 ml). The mixture was heated under reflux for 2 hr. After removal of the EtOH by distillation, the residue was taken up in $CHCl_3$. The $CHCl_3$ solution was washed with water, dried, and the solvent was distilled off to leave the crude product, which was recrystallized, giving **12** (3.4 g) (see Table I). The spectral data are given in Table II.

2-(3-Carboxypropylamino)-8-ethyl-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (13)——A solution of **12** (1.74 g) in aqueous 5% NaOH (20 ml) was heated on a steam bath for 10 min. The mixture was adjusted to pH 4 with AcOH under cooling, giving precipitates which were collected, washed with water, and recrystallized to afford **13** (0.98 g) as colorless fine needles (see Table I). The spectral data are given in Table II.

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Reversible Ring-opening Reactions of Triazolobenzo- and Triazolothienodiazepines in Acidic Media at around Body Temperature

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Hydrolytic reactions of triazolobenzo- and triazolothienodiazepines (estazolam and etizolam) as well as a thienodiazepine (clotiazepam) were studied spectrophotometrically. Cleavage reactions of the azomethine bonds of estazolam and etizolam were reversible and the open-ring compounds were in equilibrium with the closed-ring compounds (protonated forms of the parent drugs). However, little spectral change was observed in

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