the esterification reactions. Nmr spectra were recorded on the HCl salts in dimethyl-d_6 sulfoxide.

Hydrolysis of N-Methyl-3-Piperidyl Esters of Glycolic Acids.—The method of Biel and co-workers[10] was employed. A 0.45-g sample of the N-methyl-3-piperidyl glycolate HCl (**1**, **2**, **21**, or **22**, Table II) was heated vigorously for 1 hr in 30 ml of 33% H_2SO_4. The cooled solution was decanted from resinous material and was extracted three times with ether. The aqueous solution was cooled in an ice bath, made strongly alkaline with NaOH pellets, then was extracted repeatedly with ether. The combined ethereal extracts were dried (Na_2SO_4), and the ir spectrum was recorded. In each instance, the spectrum was superimposable upon a similar spectrum of an authentic sample of N-methyl-3-piperidinol.

Chemistry and Pharmacology of a Series of Substituted 4H-Pyrazino[1,2-*a*]pyrimidin-4-ones

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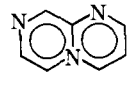
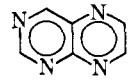
A series of 3-phenyl-2-(tertiary aminoalkoxy)-4H-pyrazino[1,2-*a*]pyrimidinones obtained by condensing ethyl phenylmalonate with aminopyrazine followed by base-catalyzed O-tertiary-amino alkylation of the resulting 2-hydroxy-3-phenyl-4H-pyrazino[1,2-*a*]pyrimidin-4-one was screened for CNS activity in mice. None showed significant activity in the maximal electroshock, oxotremorine, strychnine lethality, pentylenetetrazole seizure threshold, or *d*-amphetamine aggregate toxicity tests. Some potentiated the effect of hexobarbital and *d*-amphetamine in mice and antagonized the effect of reserpine.

A search of the chemical literature revealed that the pyrazino[1,2-*a*]pyrimidine system has not been reported. In fact, the only pyrazinopyrimidine system reported is that found in the pteridines, the pyrimido[4,5-*b*]pyrazine heterocycle. This prompted us to undertake a synthesis and pharmacological testing study in this area. This paper reports the results of this study.



pyrazino[1,2-*a*]pyrimidine



pyrimido[4,5-*b*]pyrazine

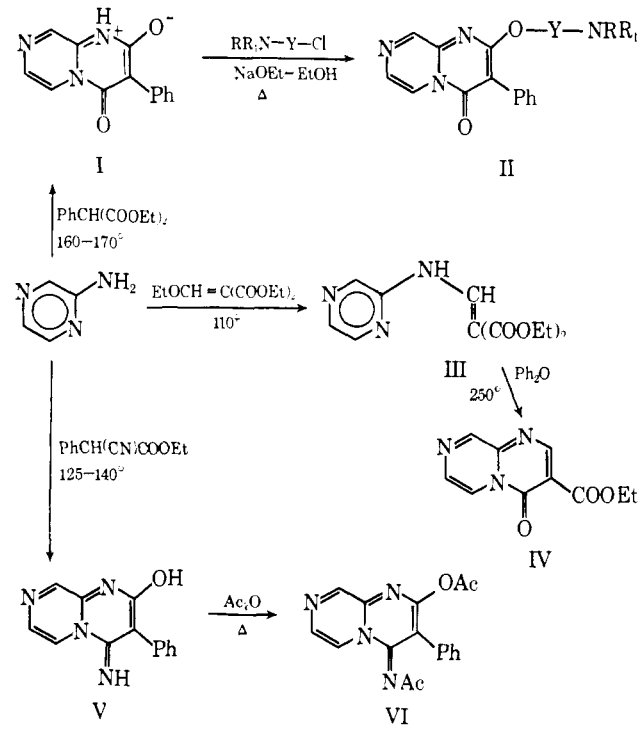
Because our primary interest was the uncovering of new structures with central nervous system activity, derivatives of the pyrazino[1,2-*a*]pyrimidine heterocycle that contained a phenyl or substituted phenyl in the 3 position and a tertiary aminoalkoxy chain in the 2 position were synthesized and evaluated for CNS activity. This type of derivative was selected for synthesis because it contains a type of phenethylamine moiety and a choline or choline-like side chain which increases the likelihood it will affect the CNS neurotransmitters, norepinephrine and acetylcholine.

The desired 3-phenyl-2-(tertiary aminoalkoxy)pyrazino[1,2-*a*]pyrimidin-4-ones (II) were obtained by condensing ethyl phenylmalonate with aminopyrazine followed by base-catalyzed O-alkylation of the resulting 2-hydroxy-3-phenyl-4H-pyrazino[1,2-*a*]pyrimidin-4-one (I) (Scheme I).

In order to obtain more diverse structural modifications pyrazinopyrimidinone I was O-alkylated with α-chloro esters and chloracetonitrile, and aminopyrazine was condensed with diethyl ethoxymethylenemalonate and diethyl phenylcyanoacetate.

When aminopyrazine was allowed to react with diethyl ethoxymethylenemalonate neat at 110° a 63% yield of diethyl pyrazinylaminomethylenemalonate

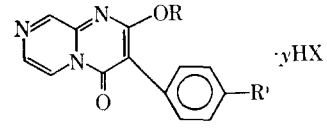
SCHEME I



(III) was obtained. That the *exo*-amino nitrogen and not one of the ring nitrogens was alkylated was indicated by the pmr spectrum of III. The vinyl proton and the D_2O exchangeable proton on the *exo* nitrogen appeared as doublets (J = 12.5 cps) at 543 and 672 cps, respectively. The pmr spectrum of the alternative structure, alkylation of a ring nitrogen, would not show a vinyl proton coupled with an exchangeable NH proton. Ring closure of III to 3-carboethoxy-4H-pyrazino[1,2-*a*]pyrimidin-4-one (IV) was accomplished in 89% yield by heating III at 250° in Dowtherm.

TABLE I

2-AMINOALKOXY-3-PHENYL-4H-PYRAZINO[1,2-*a*]PYRIMIDIN-4-ONES



No.	R	R'	*y*	X	Mp, °C	Recrystn solvent	% yield	Method[a]	Formula[b]	Toxicity LD_{50}, mg/kg	Screening dose, mg/kg	Hexobarbital sleep time, ratio T/C	Reserpine ptosis (es)[c]	Potentiate *d*-amphetamine toxicity test[d]
1	H	H	0		278–279 dec	AcOH	60	A	$C_{13}H_{13}N_3O_2$	>1000	215	2.7	0/10	6/10
2	$(CH_3)_2NCH_2CH_2CH_2$	H	1	Cl	213–214 dec	EtOH	32	B	$C_{18}H_{19}N_4O_2 \cdot HBr$	147	44	1.3	0/10	3/10
3	$(C_2H_5)_2NCH_2CH_2$	H	1	Br	210–211	EtOH	64	B	$C_{19}H_{22}N_4O_2 \cdot HBr$	159	48	1.3	0/10	2/10
4	$(C_2H_5)_2NCH_2CH_2CH_2$	H	1	Br	197–198	EtOH	31	B	$C_{20}H_{24}N_4O_2 \cdot HBr$	147	44	1.9	2/10	6/10
5	$(CH_3)_2NCH_2CH_2$	H	1	Br	200–201	EtOH	19	B	$C_{17}H_{18}N_4O_2 \cdot HBr$	159	48	1.2	2/10	0/10
6	$(CH_3)_2NCH_2CH(CH_3)$	H	1	Br	193–195	EtOH	12	B	$C_{18}H_{20}N_4O_2 \cdot HBr$	159	48	1.4	18 (10–32)	8/10
7	$[(CH_3)_2CH]_2NCH_2CH_2$	H	1	Br	197–198 dec	EtOH	19	B	$C_{21}H_{26}N_4O_2 \cdot HBr$	150	45	1.6	0/10	2/10
8	$C_4H_8NCH_2CH_2$	H	1	Br	188–190 dec	*i*-PrOH	12	B	$C_{19}H_{20}N_4O_2 \cdot HBr$	121	36	1.5	10 (4–25)	0/10
9	$C_5H_{10}NCH_2CH_2$	H	1	Br	216–217 dec	*i*-PrOH–EtOH	74	C	$C_{20}H_{22}N_4O_2 \cdot HBr$	150	45	4.6	0/10	0/10
10	$O(C_2H_4)_2NCH_2CH_2$	H	1	Br	240–241 dec	EtOH	23	C	$C_{19}H_{20}N_4O_3 \cdot HBr$	562	169	5.5	7.7 (3.5–17)	4/10
11	$(CH_3)_2NCH_2CH(CH_3)CH_2$	H	1	Br	214–215	*i*-PrOH	30	C	$C_{19}H_{22}N_4O_2 \cdot HBr$	100	30	1.5	0/10	0/10
12	$(CH_2)_6NCH_2CH_2$	H	1	Br	197–198	*i*-PrOH	25	C	$C_{21}H_{24}N_4O_2 \cdot HBr$	147	36	1.3	0/10	0/10
13	$3\text{-}C_2H_5NC_5H_7$	H	1	Br	220–221 dec	*i*-PrOH	18	C	$C_{20}H_{22}N_4O_2 \cdot HBr$	147	44	2.1	0/10	0/10
14	$C_2H_5OOCCH_2$	H	0		102–103	*i*-PrOH	41	D	$C_{17}H_{15}N_3O_4$	>1000	1000	1.6	0/10	3/10
15	CH_2CN	H	0		164–165	Et_2O–hexane	12	D	$C_{13}H_{10}N_4O_2$	316	95	3.9	1/10	*e*
16	H	Cl	0		281–282 dec	AcOH	35	A	$C_{13}H_8ClN_3O_2$	681	204	1.3	0/10	1/10
17	$(C_2H_5)_2NCH_2CH_2CH_2$	Cl	1	Br	184–185	*i*-PrOH	13	C	$C_{20}H_{23}ClN_4O_2 \cdot HBr$	147	44	1.1	32 (18–54)	*e*
18	$C_2H_5OOCCH(CH_3)$	H	0		119–120	*i*-PrOH	30	D	$C_{18}H_{17}N_3O_4$	>1000	464	1.7	121 (83–178)	*e*
19	$C_2H_5OOCCH(C_2H_5)$	H	0		127–128	*i*-PrOH	10	D	$C_{19}H_{19}N_3O_4$	>1000	464	1.5	2/10	*e*
20	$C_2H_5OOCCH_2CH_2$	H	0		124–125	*i*-PrOH	17	D	$C_{18}H_{17}N_3O_4$	>1000	464	1.2	0/10	1/10
21	$O(C_2H_4)_2NCH_2CH_2$	Cl	1	Br	230–231 dec	MeOH–H_2O	39	C	$C_{19}H_{19}ClN_4O_3 \cdot HBr$	681	204	1.3	2/10	2/10

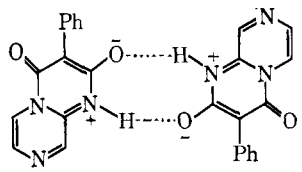
[a] See Experimental Section. [b] All compounds were analyzed for C, H, N. [c] Results are expressed either as a ratio of the number of mice protected to number of mice treated or as ED_{50} values and their 95% confidence limits. [d] Results are expressed as ratio of the number of mice dead to number of mice treated. [e] Not measured.

3-Carboethoxy-4H-pyrazino[1,2-*a*]pyrimidin-4-one (IV) was allowed to react with N,N-diethylethylenediamine, phenylmagnesium bromide, and sodium β-dimethylaminoethoxide in an attempt to introduce moieties that are present in known CNS active compounds. However, IV is sensitive enough to these basic reagents that in our hands these reactions all yielded intractable tars.

Ethyl phenylcyanoacetate reacted with aminopyrazine to give a product tentatively assigned the structure 4-imino-2-hydroxy-3-phenyl-4H-pyrazino-[1,2-*a*]pyramidine (V) based on spectral analysis of V and elemental and spectral analysis of its diacetate derivative VI. However, the spectral data do not exclude other protomeric forms nor do they exclude the structural isomer of V in which the imino group and the hydroxyl group are interchanged.

Because "malonylaminopyrazine" has not been reported and because various structures have been formulated[1] for "malonyl-α-aminopyridine," pyrazinopyrimidinone (I) is described in the Experimental Section.

The physical and spectral properties of I indicate that, similarly to "malonyl-α-aminopyridine," it exists predominantly as the mesomeric betaine. Because of the strength of the $^{+}N-H\cdots O^{-}$ hydrogen bond, in the solid state, the betaine would most likely be intermolecularly hydrogen bonded so that I is most accurately formulated as follows. Also, the precise position of the acidic proton is a matter of conjecture as it was not found in the pmr spectrum and, although the ir spectrum indicates it is certainly bonded between N and O, the degree of association to either O or N is not indicated.



Chemically, pyrazinopyrimidinone (I) differed from "malonyl-α-aminopyridine" in that it failed to give a chloride when treated with $POCl_3$ and base-catalyzed alkylation resulted in isolation of only O-alkylated product. In contrast, "malonyl-α-aminopyridine" gives a chloride when allowed to react with $POCl_3$ and base-catalyzed alkylation has given N-alkylated or O-alkylated products or mixtures of both.[1]

Pharmacology.—These pyrazinopyrimidinones were evaluated for CNS activity using a battery of screening methods; the data are summarized in Table I. None showed significant activity in the maximal electroshock, oxotremorine, strychnine lethality, pentamethylenetetrazole seizure threshold, or the hydrochloric acid writhing tests except **10** and **15** which were active in HCl writhing test (ED_{50}'s of 31.6 and 76.1 mg/kg, respectively).

Some of the pyrazinopyrimidinones in this series showed activity in the hexobarbital sleep time test. The more active compounds (**1**, **9**, **10**, **15**) were selected for testing for reinduction of sleep in hexobarbital-treated mice. They were also active in this test which suggests a CNS rather than a metabolic mechanism of action.

To determine possible antidepressant activity of these compounds they were tested for ability to prevent reserpine-induced ptosis in mice and potentiate *d*-amphetamine toxicity in aggregated mice. When test compounds were given intraperitoneally 30 min before reserpine (1 mg/kg) and the mice checked for presence or absence of ptosis 45 min after the reserpine, ptosis was absent only in the group of mice that had been pretreated with **6**. However, when the test compounds were given 3 hr before reserpine, **6**, **8**, **10**, **17**, and **18** all prevented ptosis. Thus four compounds that were inactive when administered 30 min prior to reserpine were active in the delayed test.

Compounds **1**, **4**, **6**, and **10** potentiated *d*-amphetamine toxicity in aggregated mice.

In summary, this series of pyrazinopyrimidinones shows some CNS activity which is manifested in potentiating the effect of hexobarbital and *d*-amphetamine in mice and antagonizing the effect of reserpine. In these three tests some of these compounds behaved similarily to the antidepressants imipramine and amitriptyline.

Experimental Section

The melting points were obtained in an open capillary tube with the Thomas-Hoover Uni-Melt and are uncorrected. The elemental analyses were done by Midwest Microlabs., Inc., Indianapolis, Ind. The pmr spectra were obtained at 60 Mc with a Varian A-60 spectrophotometer, for 10% $CDCl_3$ or saturated d_6-DMSO solutions containing TMS as an internal standard. Ir spectra were obtained with a Perkin-Elmer 337 grating spectrophotometer. Uv spectra were scanned on the Cary recording spectrophotometer Model 15 in a 1.00-cm cell. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

Preparation of Compounds Listed in Table I. Method A. 2-Hydroxy-3-phenyl-4H-pyrazino[1,2-*a*]pyrimidin-4-one (I).—A mixture of 47.5 g (0.50 mole) of aminopyrazine and 118 g (0.50 mole) of diethyl phenylmalonate under N_2 was heated gradually with an oil bath to 160°. At this temperature an aspirator was attached and the system was evacuated and kept at approximately 70 mm. After 2 hr at 160–170°, an additional 30 g of diethyl phenylmalonate was added and the mixture was kept at 160–170° for 2 hr and the another 30-g portion of diethyl phenylmalonate was added and the heating was resumed for another 2 hr. The mixture was allowed to cool to ambient temperature, and the brown mass was crushed in a mortar with a pestle, triturated with ether, suction filtered, and recrystallized from glacial HOAc. Compound I is a high-melting (278° dec), yellow-gold solid insoluble in common organic solvents, except DMF or DMSO. Its ir spectrum (Nujol) does not show OH stretch in the 3200–3600-cm^{-1} region but rather exhibits a broad absorption from 2300 to 3200 cm^{-1} with a maximum at 2650 cm^{-1} that indicates intramolecular bonded OH or NH^{+}. The carbonyl region has five peaks located at 1760, 1735, 1680, 1660, and 1600 cm^{-1}. The uv spectrum of the conjugate acid of I (spectrum obtained in 5 *N* H_2SO_4) exhibited λ_{max} at 231, 267, 346, and 379 mμ with 10^{-3} ε of 23.7, 8.78, 4.11, and 4.09, respectively. Even though I has an extra ring N and a phenyl substituent its physical (high melting point, low solubility in nonpolar solvents) and spectral properties closely resemble those of "malonyl-α-aminopyridine."[1] Both compounds have five peaks in the C=O region of the ir and four absorption maxima in the ultraviolet. As would be expected the ir peaks in the C=O region of I are each shifted to a higher frequency and the uv maxima are shifted to a higher wavelength.

A pmr spectrum of I in d_6-$DMSO_4$ showed the five phenyl protons as a complex multiplet centered at 450 cps, ring proton at

(1) A. R. Katritzky and A. J. Waring, *J. Chem. Soc.*, 1544 (1962).

position 5 as a doublet at 535 cps (J = 1.5 cps), ring proton at position 6 as a doublet of doublets at 522 cps (J = 1.5 and 4.5 cps), and ring proton at position 8 as a doublet at 492 cps (J = 4.5 cps). The acidic proton was not found.

Method B. 2-Dimethylaminopropoxy-3-phenyl-4H-pyrazino[1,2-*a*]pyrimidin-4-one.—To a stirred solution of NaOEt in EtOH, prepared by adding 0.92 g of Na to 250 ml of absolute EtOH, was added, portionwise, over a period of 0.5 hr, 7.2 g (0.030 mole) of 2-hydroxy-3-phenyl-4H-pyrazino[1,2-*a*]pyrimidin-4-one. The mixture was stirred and heated to the reflux temperature. A solution of 4.8 g (0.040 mole) of γ-dimethylaminopropyl chloride in 40 ml of dry toluene was added, dropwise, over a period of 1.5 hr. After the addition was completed, the mixture was stirred and heated at the reflux temperature for 3 hr. It was allowed to cool to room temperature and suction filtered, and the filtrate was concentrated *in vacuo* to 75 ml. The residual mixture was diluted with 300 ml of cold H_2O, treated with 20 ml of 10 *N* NaOH, and extracted thoroughly with ether. The dried ($MgSO_4$) ether extract was treated with ethereal HCl until the precipitation of the hydrochloride was complete. The hydrochloride was removed by suction filtration, washed (Et_2O), and recrystallized from an appropriate solvent.

Method C.—The same as method B except that the reaction mixture was stirred and heated at reflux temperature overnight instead of 3 hr.

Method D.—The same as method C except that the product was isolated as the base instead of as a hydrochloride or hydrobromide.

Diethyl Pyrazinylaminomethylenemalonate (III).—A mixture of 4.4 g (0.046 mole) of aminopyrazine and 10 g (0.040 mole) of diethyl ethoxymethylenemalonate was stirred and heated at 105° for 1.5 hr. EtOH distilled from the mixture during this time. After cooling to ambient temperature, the solidified mixture was recrystalized from 95% EtOH to give 7.4 g (63%) of white crystals: mp 120–122°; pmr 80 and 82 (triplets, $COOCH_2CH_3$), 257 and 259 (quartets, $COOCH_2CH_3$), 499 (multiplet, three ring protons), 543 (doublet, J = 12.5 cps, $-NHCH{=}C<$), and 672 (broad doublet, J = 12.5 cps, $-NHCH{=}<$) cps. Deuterium exchange caused the doublet at 672 to disappear and the doublet at 543 to become a singlet. *Anal.* ($C_{12}H_{15}N_3O_4$) C, H, N.

Ethyl 4-Oxo-4H-pyrazino[1,2-*a*]pyrimidine-3-carboxylate (IV).—To 50 ml of Dowtherm A preheated to 250–255° was added, portionwise, over a period of 5 min, 5 g (0.02 mole) of diethyl pyrazinylaminomethylenemalonate and the mixture was stirred and heated at 255° for 10 min. The mixture was cooled rapidly to ambient temperature, 60 ml of hexane was added, and the precipitated yellow solid was collected by suction filtration, washed with hexane, and recrystallized from *i*-PrOH to give 3.7 g (89%) of yellow crystals: mp 164–165°; pmr 85 (triplet, $COOCH_2CH_3$), 266 (quartet, $COOCH_2CH_3$), 503 (doublet, J = 5 cps, H at C-5 coupled with H at C-6), 534 (two doublets, H at C-6 coupled with H at C-5, J = 5 cps, coupled with H at C-8, J = 1.5 cps), 544 (singlet, H at C-2), and 553 (doublet, J = 1.5 cps, H at C-8 coupled with H at C-6) cps. *Anal.* ($C_{10}H_9N_3O_3$) C, H, N.

Reaction of Aminopyrazine with Ethyl Phenylcyanoacetate.—A mixture of 13.9 g (0.13 mole) of aminopyrazine and 25 g (0.13 mole) of ethyl phenylcyanoacetate was heated under N_2 to 125–140°. The system was attached to an aspirator and evacuated to 70 mm, and the heating was continued for 2 hr or until no further distillate was collected. After the solidified reaction mixture cooled to ambient temperature, it was triturated with ether, and collected by suction filtration to give 27.7 g (88%) of green solid, mp 202–207° dec. This material was too insoluble in the common solvents to allow recrystallization. Ir (Nujol) showed absence of C≡N and ester C=O and presence of considerable OH and NH stretch absorption at 3460, 3340, 3275, and 3160 cm^{-1}, intramolecular bonded OH by a broad absorption from 3200 to 2500 cm^{-1}, and C=N stretch at 1660 cm^{-1}.

A 5-g sample of the green solid (mp 202–207° dec) was added to 35 ml of Ac_2O and the mixture was heated at reflux temperature for 1 hr, cooled, poured onto crushed ice, stirred until all the acetic anhydride had reacted, and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed (Na_2CO_3, H_2O), dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The brown residue was recrystallized twice from EtOH to give 0.75 g of tan crystals, mp 250–252° dec; ir showed absence of NH and OH and the presence of two carbonyl peaks at 1715 and 1745 cm^{-1}; pmr in CF_3COOH showed the two methyl groups of the acetate functions as singlets at 134 and 138 cps. The rest of the spectrum was poorly resolved.

Pharmacology.—The acute toxicity,[2] maximal electroshock,[3] oxotremorine,[4] strychnine lethality,[5] pentylenetetrazole seizure threshold,[4] *d*-amphetamine aggregate toxicity,[6] HCl writhing,[7] hexobarbital sleeping time,[8] and reinduction of hexobarbital sleep[8] effects in mice were all investigated by the techniques previously described.

Antagonism to reserpine-induced ptosis in mice was measured in the following manner. Adult male mice were given the test compound intraperitoneally 3 hr prior to a reserpine (1 mg/kg ip) challenge. Observation for ptosis was made 45 min after reserpine. Results are given as a ratio of number of mice protected to number of mice tested. When 6/10 or more mice were protected additional tests were made to determine the ED_{50}. In these cases, the ED_{50} values and their 95% confidence limits (calculated according to the method of Litchfield and Wilcoxon) are listed instead of the protection ratios. The rest was also done with only a 30-min time lapse between administration of test compound and the reserpine challenge. In this case, only one compound was active so the test results are not tabulated.

(2) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).

(3) E. A. Swinyard, W. C. Brown, and L. S. Goodman, *ibid.*, **106**, 319 (1952).

(4) D. L. Trepanier, P. E. Krieger, and J. N. Eble, *J. Med. Chem.*, **8**, 802 (1965).

(5) D. L. Trepanier, V. Spranemanis, and J. N. Eble, *ibid.*, **9**, 753 (1966).

(6) J. H. Mennear and A. D. Radzik, *Life Sci.*, **4**, 1425 (1965).

(7) E. T. Eckhardt, F. Cheplovitz, M. Lipa, and W. M. Grovier, *Proc. Soc. Exp. Biol. Med.*, **98**, 186 (1958).

(8) D. L. Trepanier, P. E. Krieger, J. H. Mennear, and J. N. Eble, *J. Med. Chem.*, **10**, 1085 (1967).