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ACKNOWLEDGMENTS AND ADDRESSES

Received from the *Institut de Médecine et de Chirurgie Expérimentales, Université de Montréal, Montreal, Canada.*

This work was supported in part by the Ministère de la Santé, Quebec, and the Medical Research Council of Canada (Block Term Grant MT-1829).

The author wishes to express his special thanks to all the pharmaceutical companies (listed in the tables) that furnished the many drugs necessary for these investigations.

RESEARCH ARTICLES

Synthesis and Properties of Some Hypotensive *N*-Alkylaminopropionic Esters and *N,N*-Dialkylaminopropionic Esters and Their Hydroxamic Acids

R. T. COUTTS, J. W. HUBBARD, KAMAL K. MIDHA, and K. PRASAD

Abstract □ Syntheses of selected 3-(*N*-alkylamino)- and 3-(*N,N*-dialkylamino)propionic esters and hydroxamic acids, as well as some related compounds, are reported. The esters were prepared by the interaction of methyl acrylate or methyl methacrylate and an appropriate amine. In certain cases, amides were by-products of this reaction, and some hindered amines did not react with the acrylate. Some esters hydrolyzed to the corresponding carboxylic acids when stored even for a short time. The hydroxamic acids were prepared from the amino esters by the action of hydroxylamine. IR, proton magnetic resonance (PMR), and mass spectrometry were used to characterize these esters, carboxylic acids, and hydroxamic acids. A preliminary study was made of the effect of the esters, carboxylic acids, and hydroxamic acids on the blood pressure of anesthetized cats. The majority of the esters and hydroxamic acids produced a fall in blood pressure, but the carboxylic acids were inactive.

Keyphrases □ 3-(*N*-Alkylamino)propionic esters and hydroxamic acids—synthesis □ 3-(*N,N*-Dialkylamino)propionic esters and hydroxamic acids—synthesis □ Hypotensive activity—3-(*N*-alkylamino)propionic esters and 3-(*N,N*-dialkylamino)propionic esters, hydroxamic acids □ IR spectrophotometry—identification □ PMR spectroscopy—identification □ Mass spectroscopy—identification

In a previous communication (1), the preparation was reported of β-aminopropionohydroxamic acids and β-aminopropionic esters of general structure I which possessed hypotensive properties in rats and cats. These hydroxamic acids (I, R_3 = NHOH) and esters (I, R_3 = OMe) had R_2 = H or Me, and R_1 was a substituted piperidine ring or related ring structure. By changing

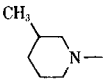
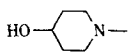
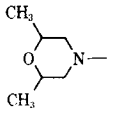
$$R_1CH_2CHR_2COR_3$$
I

the nature of the basic group R_1 in I, the magnitude and duration of the fall in blood pressure were affected significantly. This observation prompted the authors to prepare a large number of compounds and to evaluate them as hypotensive agents. In this communication, the synthesis, some physical properties, and the hypotensive properties of selected 3-(*N*-alkylamino)propionic esters and hydroxamic acids, 3-(*N,N*-dialkylamino)propionic esters and hydroxamic acids, and a few additional representative compounds, in which the basic center is a ring structure, are reported. The aliphatic esters are listed in Table I. All were prepared by the interaction of methyl acrylate or methyl methacrylate and an appropriate amine. Their hydrochlorides were obtained by passing dry hydrogen chloride through ether solutions of each ester.

With two exceptions, monoalkylamines and dialkylamines with unbranched alkyl chains reacted readily with methyl acrylate and methyl methacrylate in the absence of basic or acidic catalysts to give good yields of β-aminoesters. The exceptions were di-*n*-propylamine and phenethylamine, which reacted only slowly with methyl methacrylate. Attempts to react diisopropylamine with methyl methacrylate in boiling methanol or *n*-butanol for lengthy periods of time were unsuccessful, and only starting materials were recovered. These results are in agreement with Hughes' (2) findings but contrast with those of Suminov (3) who reported that he obtained methyl 2-methyl-3-(diisopropylamino)propionate by the interaction of diisopropylamine and methyl methacrylate under rela-

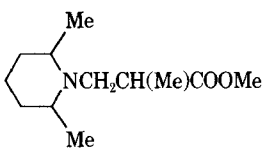
Table I—β-Aminopropionates

$R_1CH_2CHR_2CO_2R_3$
II

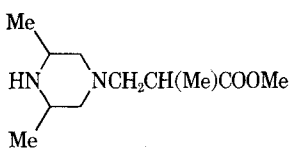
	R_1	R_2	R_3	Yield, %	B.p./mm.	Lit. b.p./mm.	Lit. Ref.	Formula	Anal., % Calcd.	Anal., % Found	Hydrochloride m.p.
a	CH_3NH—	H	CH_3	40	70–72°/20	43.3–43.8°/0.8	(8)	$C_5H_{11}NO_2$	C, 51.26 H, 9.47 N, —	C, 51.48 H, 9.58 N, —	Hygroscopic
b	CH_3CH_2NH—	H	CH_3	73	28°/0.5	27°/0.3	(9)	$C_6H_{13}NO_2$	C, 54.94 H, 9.99 N, 10.68	C, 54.72 H, 9.89 N, 10.84	70–71°
c	*n*-C_3H_7NH—	H	CH_3	70	86–88°/16	57°/3	(10)	$C_7H_{15}NO_2$	C, 57.48 H, 10.41 N, 9.65	C, 57.48 H, 9.94 N, 8.80	130–132°
d	*n*-C_3H_7NH—	CH_3	CH_3	58	—	—		$C_8H_{18}ClNO_2$	C, 49.10 H, 9.27 N, 7.16	C, 48.87 H, 9.51 N, 7.36	130–131°
e	*n*-C_4H_9NH—	H	CH_3	75	48–50°/0.5	75°/5	(11)	$C_8H_{17}NO_2$	C, 60.34 H, 10.76 N, —	C, 60.22 H, 10.53 N, —	174–176°
f	*n*-$C_6H_{13}NH$—	H	CH_3	62	80–84°/0.5	—		$C_{10}H_{21}NO_2$	C, 64.13 H, 11.30 N, 7.48	C, 64.13 H, 11.50 N, 7.11	190–192°
g[a]	$C_6H_5CH_2CH_2NH$—	CH_3	CH_3	65	124–126°/1	116–118°/0.6	(12)	$C_{13}H_{19}NO_2$	C, 70.55 H, 8.65 N, —	C, 70.77 H, 8.54 N, —	116–118°
h	$(CH_3)_2N$—	H	C_2H_5	—	—	—		—	C, — H, — N, —	C, — H, — N, —	137–138°[b]
i	$(CH_3)_2N$—	CH_3	CH_3	75	66–68°/30	70–71°/24	(13)	$C_7H_{15}NO_2$	C, 57.90 H, 10.41 N, 9.65	C, 57.81 H, 10.33 N, 9.33	123–125°
j	$(C_2H_5)_2N$—	CH_3	CH_3	68	98–99°/35	77–79°/15	(4)	$C_9H_{19}NO_2$	C, 62.39 H, 11.05 N, 8.09	C, 62.52 H, 10.61 N, 8.23	168–170°
k	$(n\text{-}C_3H_7)_2N$—	CH_3	CH_3	60	58–60°/1	—		$C_{11}H_{23}NO_2$	C, 65.63 H, 11.52 N, —	C, 65.81 H, 11.74 N, —	175–176°
l		CH_3	CH_3	80	122–124°/25	—		$C_{11}H_{21}NO_2$	C, 66.29 H, 10.62 N, —	C, 65.81 H, 10.81 N, —	Hygroscopic
m		CH_3	CH_3	90	58–60°/0.5	—		$C_{11}H_{21}NO_2$	C, 66.29 H, 10.62 N, —	C, 66.61 H, 10.71 N, —	129–130°
n		CH_3	CH_3	14	70°/0.1	—		$C_{12}H_{23}NO_2$	C, 67.56 H, 10.87 N, 6.57	C, 67.77 H, 10.68 N, 6.55	Hygroscopic
o		CH_3	CH_3	18	92°/0.03	—		$C_{13}H_{25}NO_2$	C, 68.68 H, 11.08 N, 6.16	C, 68.52 H, 10.84 N, 5.82	Hygroscopic
p		CH_3	CH_3	65	—	—		$C_{10}H_{20}ClNO_3$	C, 50.52 H, 8.48 N, 5.89	C, 49.53 H, 8.23 N, 6.55	71–73°
q		H	CH_3	89	—	—		$C_{10}H_{19}NO_3$	C, 59.68 H, 9.52 N, 6.96	C, 59.74 H, 9.59 N, 6.51	231–233°

[a] Fourfold molar excess of amine used in preparation (*Reference 14*). [b] Sample obtained commercially.

tively mild conditions. Suminov also reported a successful preparation of methyl 2-methyl-3-(2,6-dimethylpiperidino)propionate (III) in 70% yield from methyl methacrylate and 2,6-dimethylpiperidine. Attempts to repeat this reaction under the same and then more vigorous conditions failed to give any III. Similarly, when 2 moles of methyl methacrylate and 1 mole of 2,6-dimethylpiperazine were heated under reflux in methanol, the only product obtained was methyl 2-methyl-3-(3,5-dimethyl-1-piperazinyl)propionate (IV). It seems probable that steric hindrance prevented reaction at the nitrogen atom that had the two methyl groups on adjacent carbon atoms.



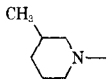
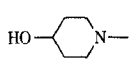
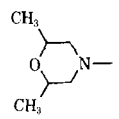
III



IV

Table II—β-Aminopropionohydroxamic Acids

$$R_1CH_2CHR_2\overset{\overset{O}{\|}}{C}NHOH\cdot HCl$$
XI

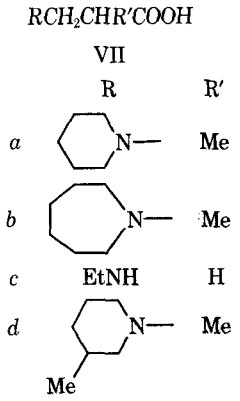
	R_1	R_2	Reaction Time, hr.	Method	Yield, %	M.p.	Formula	Anal., % Calcd.	Anal., % Found
a	CH_3NH—	H	60	A	65	114–115°	$C_4H_{11}ClN_2O_2$	C, 31.07 H, 7.17 N, 18.12	C, 31.10 H, 7.19 N, 17.91
b	C_2H_5NH—	H	40	B	64	44–45°	$C_5H_{13}ClN_2O_2\cdot H_2O$	C, 32.17 H, 8.10 N, 15.01	C, 32.22 H, 7.99 N, 15.36
c	*n*-C_3H_7NH—	H	48	A	72	78–79°	$C_6H_{15}ClN_2O_2$	C, 39.45 H, 8.27 N, 15.34	C, 39.36 H, 8.62 N, 15.34
d	*n*-C_3H_7NH—	CH_3	40	A	65	125–127°	$C_7H_{17}ClN_2O_2$	C, 42.74 H, 8.71 N, 14.24	C, 42.53 H, 8.45 N, 14.05
e	*n*-C_4H_9NH—	H	48	B	61	108–109°	$C_7H_{17}ClN_2O_2\cdot 1/2H_2O$	C, 40.87 H, 8.81 N, 13.62	C, 40.49 H, 8.68 N, 13.95
f	*n*-$C_6H_{13}NH$—	H	40	B	79	134–135°	$C_9H_{21}ClN_2O_2$	C, 48.10 H, 9.42 N, 12.47	C, 47.72 H, 9.49 N, 12.72
g	$C_6H_5CH_2CH_2NH$—	CH_3	48	B	75	126–127°	$C_{12}H_{19}ClN_2O_2$	C, 55.70 H, 7.40 N, 10.83	C, 55.77 H, 7.69 N, 10.94
h[a]	$(CH_3)_2N$—	H	10	A	71	86–88°	$C_5H_{13}ClN_2O_2$	C, 35.61 H, 7.77 N, 16.62	C, 35.59 H, 7.94 N, 16.77
i	$(CH_3)_2N$—	CH_3	10	A	77	140–142°	$C_6H_{15}ClN_2O_2$	C, 39.45 H, 8.27 N, 15.34	C, 39.29 H, 7.91 N, 15.32
j	$(C_2H_5)_2N$—	CH_3	10	A	73	118–119°	$C_8H_{19}ClN_2O_2$	C, 45.59 H, 9.08 N, 13.29	C, 45.82 H, 9.28 N, 13.09
k	$(n\text{-}C_3H_7)_2N$—	CH_3	10	A	62	152–153°	$C_{10}H_{23}ClN_2O_2$	C, 50.30 H, 9.71 N, 11.74	C, 50.60 H, 9.93 N, 11.30
l		CH_3	48	B	69	170–172°	$C_{10}H_{21}ClN_2O_2$	C, 50.73 H, 8.94 N, 11.84	C, 50.45 H, 9.31 N, 11.63
m		CH_3	18	B	86	190–193°	$C_{10}H_{21}ClN_2O_2$	C, 50.73 H, 8.94 N, 11.84	C, 50.23 H, 8.93 N, 11.81
n		CH_3	48	B	70	124–125°	$C_{11}H_{23}ClN_2O_2$	C, 52.68 H, 9.25 N, 11.17	C, 52.62 H, 9.10 N, 11.03
o		CH_3	48	B	67	167–168°	$C_{12}H_{25}ClN_2O_2$	C, 54.43 H, 9.52 N, 10.58	C, 54.34 H, 9.33 N, 10.60
p		CH_3	20	B	65	154–155°	$C_9H_{19}ClN_2O_3$	C, 45.28 H, 8.02 N, 11.74	C, 45.38 H, 8.02 N, 11.95
q		H	20	B	48	170–171°	$C_9H_{19}N_2O_3$	C, 45.28 H, 8.02 N, 11.74	C, 45.56 H, 8.24 N, 11.50

[a] Lit. (15) m.p. 90–91°.

When the duration of the reaction between some of the amines and the α,β-unsaturated esters was prolonged, amides were formed. Thus, 3-ethylaminopropionic ethylamide (V) was the major product of the reaction between ethylamine and methyl acrylate when the reactants were allowed to react at room temperature for 10 days. 2-Methyl-3-*n*-propylaminopropionic *n*-propylamide (VI) was prepared in a similar manner. Amides were also observed as being by-products of

$EtNHCH_2CH_2CONHEt$ (V) $\quad$ $nPrNHCH_2CH(CH_3)CONHnPr$ (VI)

the reaction between other primary amines (methylamine, *n*-propylamine, *n*-butylamine, and *n*-hexylamine) and methyl acrylate, especially when the reaction period was prolonged. Their presence was detected in the

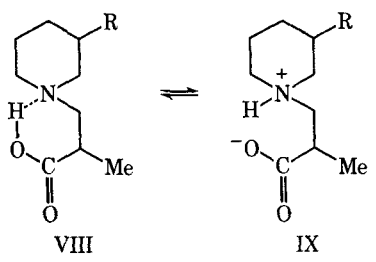


less volatile portion of the reaction mixture by means of IR spectra (strong carbonyl absorption in the 1645–1690 cm.$^{-1}$ region), but they were not isolated. In contrast, no amides were detected in the reactions between methyl methacrylate and secondary amines or cyclic amines.

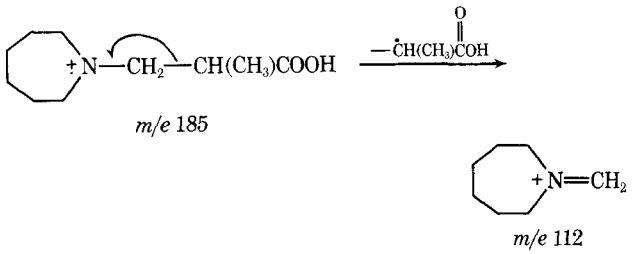
When some of the esters prepared in this and in the previous study (1) were stored for 6–8 weeks at room temperature, colorless crystalline solids slowly deposited. These products were characterized as 3-amino acids by means of elemental analysis, IR and proton magnetic resonance (PMR) spectra, and, in one instance (VII*b*), by mass spectrometry. In this way, 2-methyl-3-piperidinopropionic acid (VII*a*), 2-methyl-3-homopiperidinopropionic acid (VII*b*), 3-ethylaminopropionic acid (VII*c*), and 2-methyl-3-(3-methylpiperidino)propionic acid (VII*d*) were obtained from the corresponding esters, presumably the result of hydrolysis by trace quantities of moisture present. Bieber (4) has already commented on the ease with which methyl 2-methyl-3-piperidinopropionate hydrolyzes when shaken with cold water.

The IR spectra of the three tertiary amino acids (VII*a*, *b*, and *d*) showed broad bands between 2120 and 2400 cm.$^{-1}$ (ν $\overset{+}{\text{N}}$H), whereas broad bands in the region 2660–2740 cm.$^{-1}$ (ν $\overset{+}{\text{N}}$H$_2$) were observed in the spectrum of VII*c*. Strong bands near 1620 and 1450 cm.$^{-1}$ (ν CO_2^-) were present in all four IR spectra. The PMR spectrum of VII*a* and VII*d* had deuterium-exchangeable singlets at 843 and 887 Hz., respectively, *i.e.*, considerably further downfield than is usually observed for either intermolecularly hydrogen-bonded carboxylic acid protons (5) or $\overset{+}{\text{N}}$H protons. These two acids apparently exist in tautomeric forms VIII $\rightleftharpoons$ IX (Scheme I).

The mass spectrum of the homopiperidino compound VII*b* was simple and consistent with its structure. The



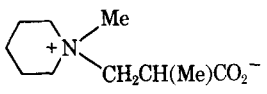
Scheme I



Scheme II

molecular ion, *m/e* 185, was present but of relatively low intensity. The only fragment ion of relative abundance greater than 30% was the base peak at *m/e* 112 which undoubtedly was formed in the manner illustrated in Scheme II (6).

In 1950, Vystrcil and Hudecek (7) reported their studies on the addition of amines to methyl methacrylate and the effect of the presence of water on the nature of the reaction products obtained. According to this study, aliphatic amines such as diethylamine, piperidine, and morpholine add to methyl methacrylate to form *N*-substituted-3-amino esters in nonaqueous media; but in an aqueous medium, a shift of the ester methyl group to the amino group was postulated which resulted in the formation of *N*-methylbetaines (*e.g.*, X) in very good yields. This particular betaine (X) was reported to melt at 106–108°, which is suspiciously close to the melting point of 2-methyl-3-piperidino-



X

propionic acid (VII*a*) (109–110°). For this reason, methyl methacrylate was reacted with piperidine in the presence of water, using the conditions described by Vystrcil and Hudecek (7). A betaine was not obtained; the product was VII*a*. It is probable that none of the products described by these workers was a betaine. None was isolated in the present study.

The hydroxamic acids (Table II) were prepared from the amino esters by the action of hydroxylamine and were converted to hydrochlorides for pharmacological evaluation. Some of the hydrochlorides were appreciably hygroscopic. All the hydroxamic acids gave a violet color with alcoholic ferric chloride, and each was characterized by elemental analysis and IR and PMR spectroscopy. In their IR spectra, all showed a carbonyl absorption band attributable to the hydroxamic acid group between 1635–1675 cm.$^{-1}$. The PMR study showed that acyclic hydroxamic acids of general structure XI (Table II), when examined in DMSO-d_6, gave three characteristic 1-proton broad signals within the 500–700 Hz. range, all of which exchange in D_2O. One of these signals was located between 521 and 540 Hz. and another between 603 and 635 Hz. These are due to the two protons of the NHOH group. The third signal was observed in all spectra between 645 and 663 Hz. and is ascribable to the $\overset{+}{\text{N}}$H proton; this signal was absent when the spectra of bases rather than hydrochlorides were examined.

Table III—Effect of Methyl β-Aminopropionate Hydrochlorides and β-Aminopropionohydroxamic Acid Hydrochlorides on the Arterial Blood Pressure of Anesthetized Cats

Compound	Dosage, mg./kg.	Percent Fall in Blood Pressure[a]	Duration,[b] min.
II *a*	25	62	5
b	25	48	10
c	25	(27)[c]	50
d	25	21	10
e	25	13	10
f	25	87	80
g	5	18	8
	10	35	30
h	25	38	5
i	10	72	90
	25	67	115
j	25	39	5
k	25	28	5
l	—	—	—
m	25	43	5
n	25	58	3
o	25	60	3
p	25	31	2
q	25	13	3
XI *a*	25	(33)[c]	10
b	25	53	5
c	25	30	15
d	25	16	5
e	25	39	15
f	25	40	120
g	5	17	60
	10	39	80
	25	50	120
h	25	56	3
i	25	58	30
j	25	50	15
k	25	41	20
l	25	33	5
m	25	39	5
n	25	72	4
o	25	44	3
p	25	44	4
q	25	45	5

[a] Average of two experiments. [b] Time for blood pressure to return to control level. [c] Rise in blood pressure.

PHARMACOLOGY

A preliminary study was made of the effect of the β-aminopropionohydroxamic acid hydrochlorides (XI) and β-aminopropionate hydrochlorides (II) on the blood pressure of anesthetized cats.

Cats of either sex, weighing 2–3 kg., were anesthetized with pentobarbital sodium (35 mg./kg.). The blood pressure (carotid artery) was recorded by means of a force displacement transducer (E & M model 3000) coupled to an E & M physiograph (model IV). All drugs were dissolved in distilled water and administered *via* a cannula inserted into a femoral vein.

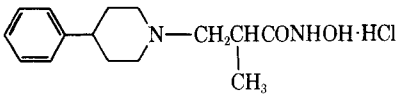
The results are summarized in Table III. The majority of the β-aminopropionohydroxamic acids and β-aminopropionates produced a fall in blood pressure. In certain cases, the hypotensive effect was prolonged (hydroxamic acids XI*f* and XI*g* and ester II*i*), but none of the compounds was as active as the 2-methyl-3-(4-phenylpiperidino)propionohydroxamic acid hydrochloride(XII) reported earlier (1, 16).

The four aminopropionic acids (VII*a*–VII*d*) were also tested and found to have no effect on blood pressure in anesthetized cats.

EXPERIMENTAL[1]

Melting and boiling points are uncorrected. IR spectra were measured with a Beckman IR-10 spectrophotometer. Mass spectra were measured at an ionizing potential of 70 ev. with an A.E.I. MS-9 spectrometer, using the direct insertion technique, and PMR spectra were recorded on a Varian A-60 spectrometer using trimethylsilane (TMS) as standard.

[1] Microanalyses were performed by Mr. W. Dylke, and Mrs. S. K. Li recorded the PMR spectra.



XII

Esters—The methods used to prepare and characterize the esters described in Table I were essentially the ones reported previously (1). The reaction time varied from 2 hr. to 15 days, depending on the amine used. Reaction was continued until the C=C stretching band near 1630 cm.$^{-1}$ (due to the acrylate starting material) disappeared from the IR spectrum of the crude product.

Ester hydrochlorides were prepared and characterized as described previously (1).

Methyl 2-Methyl-3-(3,5-dimethyl-1-piperizinyl)propionate (IV)—In an attempt to synthesize 1,4-bis(2-methoxycarbonylpropyl)-2,6-dimethylpiperazine, 2,6-dimethylpiperazine (14.25 g.) was dissolved in anhydrous methanol (50 ml.), and to this solution was added methyl methacrylate (25 g.). The mixture was boiled under reflux for 2 weeks. The methanol was removed *in vacuo*, and the resulting oil was distilled under reduced pressure. The first fraction collected was methyl methacrylate. The fraction b.p. 88–90°/4 mm. (19.1 g.) was the title compound. IR (film): 3320 (ν NH), 1740 (ν C=O) cm.$^{-1}$. PMR (CCl_4), methyl group characteristics: 60 Hz. [t, 9 (overlap of two doublets), ring methyl groups and side-chain methyl group]; 211 Hz. (s, 3, $COOCH_3$).

Anal.—Calcd. for $C_{11}H_{22}N_2O_2$: C, 61.6; H, 10.3. Found: C, 61.1; H, 9.9.

Hydroxamic Acids—They were prepared by two methods.

Method A—To a constantly stirred solution of hydroxylamine hydrochloride (0.18 mole in 50 ml. of anhydrous methanol) at 0° was added a cold methanolic solution of potassium hydroxide (0.12 mole in 40 ml. of anhydrous methanol); after 3 min., the precipitated potassium chloride was filtered off. To the filtrate was added a solution of the appropriate ester (0.03 mole) in anhydrous methanol (20 ml.). The temperature of the stirred solution was maintained at 0 to −5° for 1 hr. and then allowed to come to room temperature. Stirring was continued for 18–48 hr., and then the methanol was removed *in vacuo*. To the resulting viscous oil, sufficient acetone was added to give a turbidity. On refrigeration, the hydroxamic acid hydrochloride separated as a colorless solid which was recrystallized from absolute ethanol.

Method B—This method has been described previously (1).

*3-Ethylaminopropionic Acid (VII*c)—When methyl 3-ethylaminopropionate (II*b*) was stored at room temperature for 4 weeks, a white crystalline solid slowly separated. It was collected, recrystallized from ethanol–acetone, and characterized as 3-ethylaminopropionic acid, m.p. 188° [lit. (17) m.p. 174–176°]. IR (KBr): 2740–2660 and 2500–2420 (broad bands, ν $\overset{+}{N}H_2$); 1630, 1450 (ν CO_2^-) cm.$^{-1}$. PMR (D_2O): 78 Hz. (t, 3, $J=7$, CH_3CH_2); 154 Hz. (t, 2, $J=6.6$, CH_2CO); 177–201 Hz. (m, 4, CH_2NHCH_2).

Anal.—Calcd. for $C_5H_{11}NO_2$: C, 51.3; H, 9.5. Found: C, 51.0; H, 9.3.

*2-Methyl-3-(3-methylpiperidino)propionic Acid (VII*d)—This acid, m.p. 122–124°, was obtained from the corresponding ester (II*m*) in a manner similar to that just described. IR (KBr): 2260–2120 (broad, ν $\overset{+}{N}H$); 1610, 1450 (ν CO_2^-) cm.$^{-1}$. PMR (D_2O): 57 Hz. (d, 3, $J=6$, ring CH_3); 69 Hz. (d, 3, $J=7$, CH_3CHCO); 93–230 Hz. (m, 12, remaining protons). PMR ($CDCl_3$): 887 Hz. (s, 1, COOH, exchanged in D_2O).

Anal.—Calcd. for $C_{10}H_{19}NO_2$: C, 64.8; H, 10.3. Found: C, 64.6; H, 10.2.

*2-Methyl-3-piperidinopropionic Acid (VII*a)—This acid, m.p. 109–110° [lit. (4) m.p. 108°], was obtained from the corresponding ester (1) in the manner described for the previous two acids. IR (KBr): 2300–2120 (broad, ν $\overset{+}{N}H$); 1610, 1450 (ν CO_2^-) cm.$^{-1}$. PMR ($CDCl_3$): 67 Hz. (d of d, 3, CH_3CHCO); 98 Hz. (m, 6, three ring CH_2 groups remote from N atom), 130–190 Hz. (m, 7, $\begin{matrix}CH_2\\CH_2\end{matrix}\!\!>\!NCH_2CH$), 843 Hz. (s, 1, COOH, exchanged in D_2O).

Anal.—Calcd. for $C_9H_{17}NO_2$: C, 63.2; H, 9.9. Found: C, 63.4; H, 9.9.

2-Methyl-3-homopiperidinopropionic Acid (*VII*b)—This acid, m.p. 100°, was prepared in the same way as the acids just mentioned. IR (KBr): 2400–2120 (broad, ν $\overset{+}{N}H$); 1620, 1450 (ν CO_2^-) cm.$^{-1}$. PMR (D_2O): 69 Hz. (d, 3, $J=6.5$, CH_3CHCO); 105 Hz. (s, 8, four ring CH_2 groups remote from N atom); 138–181 Hz. (m, 7, $\begin{matrix}CH_2\\CH_2\end{matrix}\!\!>\!NCH_2CH$). Mass spectrum: (percent relative abundance) *m/e* 185 (6.9%), 112 (100%).

Anal.—Calcd. for $C_{10}H_{19}NO_2 \cdot 0.5\ H_2O$: C, 61.8; H, 10.3. Found: C, 61.7; H, 10.6.

3-Ethylaminopropionic Ethylamide (*V*)—In an attempt to prepare methyl 3-ethylaminopropionate by reacting ethylamine and methyl methacrylate at room temperature for 10 days, using the general method for the preparation of esters, the reaction mixture was fractionally distilled and yielded the title compound (46% yield), b.p. 113–114°/2 mm. IR (film): 3300 (ν NH); 1645 (ν C=O) cm.$^{-1}$. PMR (CCl_4): 63 Hz. (t, 3, $J=7$, $CH_3CH_2NHCH_2$) overlapping with 66 Hz. (t, 3, $J=7$, $CONHCH_2CH_3$); 88 Hz. (s, 1, CH_3CH_2NH); 498 Hz. (s, 1, CONH), both singlets exchanged in D_2O.

Anal.—Calcd. for $C_6H_{14}N_2O$: C, 55.3; H, 10.8. Found: C, 54.9; H, 10.8.

*2-Methyl-3-*n*-propylaminopropionic* n-*Propylamide* (*VI*)—The title compound was obtained in 40% yield in a manner similar to that described for the preparation of V.

This compound was characterized as the hydrochloride, m.p. 130–131.5°. IR (mineral oil) 3270 (amide ν NH); 2900–2410 (ν $\overset{+}{N}H_2$); 1660 (C=O).

Anal.—Calcd. for $C_{10}H_{23}ClN_2O$: C, 53.92; H, 10.41. Found: C, 53.83; H, 10.41.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 13, 1970, from the *Faculty of Pharmacy and Pharmaceutical Sciences*, and the *Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada.*

Accepted for publication July 20, 1970.

Abstracted in part from a thesis by K. K. M. in partial fulfillment of the Doctor of Philosophy degree requirements.

This research was supported by the Defence Research Board of Canada (Grant No. 8875-05) and by the Medical Research Council of Canada (Grant No. MA-2993).

The authors acknowledge the assistance of Mrs. A. G. MacArthur in this project.

Structure–Activity Relationships of Tetracyclines I: Inhibition of Cell Division and Protein and Nucleic Acid Syntheses in *Escherichia coli* W

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Abstract □ The kinetics of inhibition of *Escherichia coli* in a peptone broth as a function of the concentration of 18 tetracyclines were determined. Viable and total cell count methods were used to measure rates of cell division. Rates of protein and nucleic acid syntheses were determined simultaneously by a membrane filter technique in conjunction with the Folin–Lowry assay and the orcinol reaction, respectively. The relationship of these rate constants to antibiotic concentration is an accurate estimate of activity under the test conditions. Different times before onset of inhibition of protein synthesis, cell division, and nucleic acid synthesis are observed in kinetic experiments. These differences are interpreted as being consistent with theories of primary inhibition of protein-synthesizing systems.

Keyphrases □ Tetracyclines—structure–activity relationships □ Structure–activity relationships, tetracyclines—*E. coli* inhibition □ Rate constants, *E. coli* cell division—tetracyclines □ Protein, nucleic acid syntheses, *E. coli*—tetracycline effect □ Viable, total cell counts—*E. coli* division rates □ Concentration, tetracyclines—*E. coli* inhibition

Quantitative biological activities of a large number of compounds obtained under identical conditions in a precise manner are required to establish structure–activity relationships. Ideally, these activities should be directly related to the mechanism of action of the compounds and be free of extraneous competing equilibria. Presently available activities for tetracycline antibiotics were summarized by Barrett (1). Many of these activities were determined under conditions such that the results parallel clinical activity, a type of activity