as seen with decamethonium and hence belongs to the group of depolarizing neuromuscular blocking agents. The block seen with XII and XIII was similar to that observed with the antidepolarizer, (+)-tubocurarine.

Discussion.—Even the slightest modification of the decamethonium molecule usually either decreases the potency or changes it from a depolarizing drug to an antidepolarizer, or both.[21] Thus the introduction of the halogen atoms and the double bond in the 5,6 positions of the decamethylene chain, as in III, caused a change in potency and therapeutic index. Furthermore, on the isolated preparations, the ability of III to produce the characteristic biphasic block and initial contracture, together with fasciculations normally seen with depolarizers, was less marked as compared with decamethonium. In particular this was the case for the isolated guinea pig diaphragm, as III produced only a slight phase I block. The slowly developing block seen with III on this preparation is not typical of either the depolarizers or antidepolarizers. Presumably, this block is characteristic of compounds which have a very weak ability to depolarize the motor end plate.

Recently, tritiated decamethonium has become available and has proved to be useful for *in vitro* studies,[4e,22] However, III labeled with ^{131}I would still be particularly suited for *in situ* experiments where continuous external monitoring of its radioactivity is desirable, *e.g.*, in a muscle.

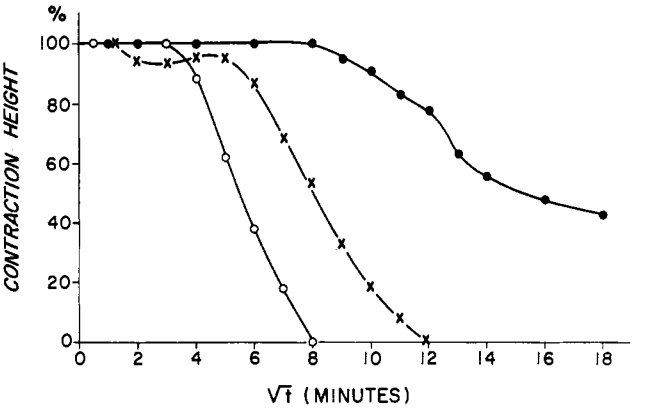

Figure 3.—The blocking effect on three isolated guinea pig diaphragm preparations of different concentrations of III: ●—●, 10^{-5} *M*; ×—×, 2.5×10^{-5} *M*; ○—○, 5.0×10^{-5} *M*. Ordinate: the response to nerve stimulation expressed as a percentage of the response to direct stimulation. Abscissa: the square root of time.

Acknowledgment.—The authors wish to express their appreciation to Professor D. J. Cram and Dr. L. Gaston, Department of Chemistry, U.C.L.A., for their assistance in some of the syntheses.

(21) R. B. Barlow, "Introduction to Chemical Pharmacology," Methuen and Co., London, 1964, pp 87–139.

(22) (a) O. A. Nedergaard, Ph.D. Dissertation, University of California, Los Angeles, 1964; *Dissertation Abstr.*, **25**, 1254 (1964); (b) D. B. Taylor, R. Creese, O. A. Nedergaard, and R. Case, *Nature*, **208**, 901 (1965); (c) O. A. Nedergaard and D. B. Taylor, *Experientia*, **22**, 521 (1966).

The Synthesis and Evaluation of the Local Anesthetic Activity of a Series of 4-(ω-Alkylaminoacylamino)salicylate Esters[1,2]

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A series of ω-alkylaminoacyl derivatives of 4-aminosalicylic acid esters (methyl through hexyl, plus 2-diethylaminoethyl) were synthesized and their hydrochlorides were tested for local anesthetic activity. The synthesis of the diethylaminoethyl ester series was examined in some detail since these compounds easily undergo alcoholysis and aminolysis. These reactions were ascribed to an intramolecular *o*-hydroxy catalysis. Only derivatives of the methyl, ethyl, and diethylaminoethyl esters exhibited significant local anesthetic activity. Compared to lidocaine, these compounds were generally more irritating, less toxic, and less active. When the compounds exhibiting local anesthetic activity were quaternized with methyl iodide, local anesthetic activity was lost while the toxicity increased.

Although Drill[3] states that, as a general rule, effective local anesthetics rarely contain either free carboxy or hydroxy groups, Clinton and co-workers[4] and Ludueña and Hoppe[5] have reported that a series of dialkylaminoalkyl 4-alkylaminosalicylates showed a high degree of infiltration and topical anesthetic activity. Keil and Rademacher[6] also reported that some alkylaminoethyl 4-aminosalicylates possessed local anesthetic activity similar to the corresponding esters of 4-aminobenzoic acid. Oxycaine, the 2-hydroxy analog of procaine synthesized by Grimme and Schmitz,[7] has been shown by

(1) The investigation at the University of Athens was supported by a research grant from the Royal Hellenic Research Foundation.

(2) A preliminary report of part of this work has been presented at the 21st International Congress of Pharmaceutical Sciences, Pisa, Italy, Sept. 4–8, 1961, by G. T. and C. S. Preliminary announcements have appeared: G. Tsatsas and C. Sandris, *Proc. Acad. Athens*, **35**, 372 (1960); G. Tsatsas, C. Sandris, and D. Kontonassios, *ibid.*, **37**, 54 (1962). This paper comprises a portion of a thesis presented by D. K. at the University of Athens.

(3) A. V. Drill, "Pharmacology in Medicine," 2nd ed, McGraw-Hill Book Co., Inc., New York, N. Y., 1958, p 98.

(4) R. O. Clinton, S. C. Laskowski, U. J. Salvador, and M. Wilson, *J. Am. Chem. Soc.*, **73**, 3674 (1951).

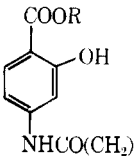
(5) F. P. Ludueña and J. O. Hoppe, *Federation Proc.*, **9**, 297 (1950).

(6) W. Keil and E. Rademacher, *Arzneimittel-Forsch.*, **1**, 154 (1951).

(7) W. Grimme and H. Schmitz, *Ber.*, **84**, 734 (1951).

TABLE I

4-(ω-CHLOROACYLAMINO)SALICYLATES



R	n	Yield, %	Mp, °C	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Chlorine, % Calcd	Chlorine, % Found	Nitrogen, % Calcd	Nitrogen, % Found
CH_3	1	78	184–185	$C_{10}H_{10}ClNO_4$					14.49	14.58	5.75	5.70
CH_3	2	55	152	$C_{11}H_{12}ClNO_4$					13.76	13.60	5.44	5.57
C_2H_5	1	81	150–151	$C_{11}H_{12}ClNO_4$					13.76	13.58	5.44	5.58
C_2H_5	2	56	170	$C_{12}H_{14}ClNO_4$					13.05	12.95	5.15	5.28
n-C_3H_7	1	95	148	$C_{12}H_{14}ClNO_4$	53.05	53.03	5.20	5.25			5.15	5.12
n-C_3H_7	2	57	163	$C_{13}H_{16}ClNO_4$	54.64	54.83	5.65	5.83			4.91	4.87
n-C_4H_9	1	86	110	$C_{13}H_{16}ClNO_4$	54.64	54.82	5.65	5.72			4.91	5.12
n-C_4H_9	2	56	136	$C_{14}H_{18}ClNO_4$	56.10	56.41	6.06	6.16			4.67	4.69
n-C_5H_{11}	1	94	112	$C_{14}H_{18}ClNO_4$	56.10	56.00	6.06	6.11	11.83	12.06	4.67	4.83
n-C_5H_{11}	2	77	126	$C_{15}H_{20}ClNO_4$	57.41	57.51	6.42	6.56			4.46	4.57
n-C_6H_{13}	1	87	90	$C_{15}H_{20}ClNO_4$	57.41	57.43	6.42	6.41			4.46	4.63
n-C_6H_{13}	2	73	116	$C_{16}H_{22}ClNO_4$	58.62	58.65	6.77	6.75			4.28	4.31

Vinogradov[8] to possess toxicity approximately equal to procaine but with considerably greater local anesthetic activity. In addition, Epstein and Kaminsky[9,10] reported local anesthetic activity in a number of alkylaminoacylaminobenzoates. Examination of these studies suggested that an exploration of the activity of alkylaminoacyl derivatives of 4-aminosalicylic acid esters, might result in the discovery of potentially useful local anesthetic agents.

Chemistry.—The derivatives synthesized were: methyl through hexyl 4-(2-alkylaminoacetylamino)- and 4-(3-alkylaminopropionylamino)salicylates as the hydrochloride and, in some cases, as the methiodide salts (Tables II and III). Their preparation consisted of treating an ester of 4-aminosalicylic acid with chloroacetyl or 3-chloropropionyl chloride and subsequent heating of the intermediate 4-(ω-chloroacylamino) derivative (Table I) with an excess of amine in ethanol.

Synthesis of the 2-diethylaminoethyl 4-(2-alkylaminoacetylamino)salicylates was performed as outlined in Scheme I. 2-Diethylaminoethyl 4-aminosalicylic acid (I) was treated with chloroacetyl chloride to yield the intermediate chloroacetanilide hydrochloride II. Reaction of II with an excess of amine in a *polar* medium, *e.g.*, ethanol, gave the corresponding ethyl ester derivative III (R = C_2H_5)—equally obtained *via* the ethyl ester chloroacetanilide IV (R = C_2H_5)—instead of the desired diethylaminoethyl ester derivative V. Alcoholysis was found to occur in methanol, as well as in ethanol, irrespective of the amine used (Experimental Section and Table II) and was prevented by carrying out the reaction in a *nonpolar* solvent, such as benzene (Table III). Heating II with a low-boiling amine, in the *absence* of solvent, resulted in the formation of the corresponding diethylaminoethyl ester derivatives V. However, with high-boiling amines, salicylamide derivatives VI constituted the main product of the reaction (Table IV). Synthesis *via* the 4-chloroacetylaminosalicylic acid chloride (VII) proved the structure of the aminolysis products.

(8) V. M. Vinogradov, *Farmakol. Zh. SSSR*, **43**, 508 (1957); *Chem. Abstr.*, **52**, 1473*a* (1958).

(9) E. Epstein, and D. Kaminsky, *J. Am. Chem. Soc.*, **79**, 5814 (1957).

(10) E. Epstein and D. Kaminsky, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 150 (1959).

The observed facile alcoholysis and aminolysis of the diethylaminoethyl ester series may be compared to the intramolecular *o*-hydroxy catalysis in the hydrolysis of *p*-nitrophenyl salicylates studied by Bender and coworkers.[11] The reported reactions of 2-diethylaminoethyl 4-chloroacetylaminobenzoate with an excess of amine, either in ethanol[12] or in the absence of solvent,[9] resulting only in substitution of the chlorine atom, agree with such a description. On the other hand, no comparable large rate enhancement of the *o*-hydroxy group could be detected in the case of the ethyl salicylate series.[11] This might account equally well for the isolation of the ethyl ester derivatives III as products of alcoholysis, as well as for the observed lack of alcoholysis in the alkyl ester series (IV → III, R = methyl through hexyl), the reaction with the amine being run in ethanol solution.

Experimental Section[13]

The **N-alkylaminoacylanilines** obtained by the various methods described below were transformed to their salts, hydrochlorides and methiodides, without further purification. Accordingly, the free bases reported in the tables correspond to nonpurified products. Salts were purified by recrystallization from absolute ethanol or from absolute ethanol–anhydrous ether. Hydrochlorides were prepared in absolute alcohol (and ether, where necessary). Quaternary ammonium salts were prepared by heating a solution of the amine in absolute ethanol or anhydrous acetone with an excess (2–4 moles) of methyl iodide under reflux for 2 hr. In most cases the salt crystallized upon cooling; otherwise, it was precipitated by adding anhydrous ether. Colors, ranging from intense yellow to orange, were formed by adding 1% $FeCl_3$ solution.

Esters of 4-Aminosalicylic Acid.—The alkyl esters, methyl through *n*-amyl, were prepared by standard methods.[11] The ***n*-hexyl ester**, not previously reported in the literature, was obtained by direct esterification of the acid as follows. A mixture of 4-aminosalicylic acid (25 g, 0.18 mole), 125 ml of purified *n*-hexyl alcohol, and 50 ml of concentrated H_2SO_4 was heated on a water bath for 8 hr. After standing overnight, the mixture was

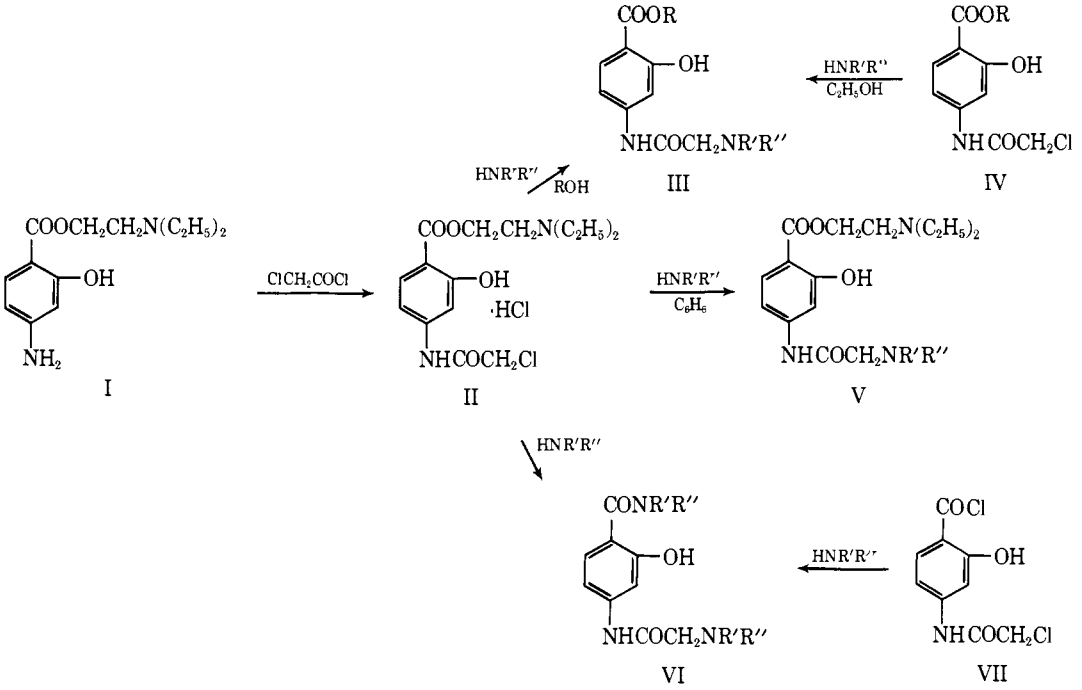
(11) M. C. Bender, F. J. Kezdy, and B. Zerner, *J. Am. Chem. Soc.*, **85**, 3017 (1963).

(12) E. Profft and A. Jonas, *Arch. Pharm.*, **289**, 90 (1956).

(13) Melting points of the intermediates were determined by the capillary tube method; those of the hydrochlorides and methyl iodide salts by means of the Maquenne block. All melting point values are corrected.

(14) D. J. Brain, D. W. Mitchell, D. E. Seymour, and F. S. Spring, *J. Chem. Soc.*, 1438 (1949).

SCHEME I



diluted with an equal volume of cold water and the precipitate which formed was filtered and washed successively (cold H_2O, 20% NH_4OH, cold water) yielding 20 g (52%) of a colorless product, mp 62°. Upon recrystallization from absolute ethanol, a crystalline solid, mp 64°, was obtained.

Anal. Calcd for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 66.00; H, 8.10; N, 6.07.

This procedure may be applied to the preparation of the alkyl esters, methyl through *n*-butyl, with yields ranging from 65 (*n*-butyl) to 80% (methyl). The 2-diethylaminoethyl ester hydrochloride was obtained in 84% yield according to details given in a patent.[15]

Alkyl 4-(ω-Chloroacylamino)salicylates.—Alkyl 4-aminosalicylate (0.15 mole) was dissolved in 300 ml of glacial acetic acid and 0.165 mole of the corresponding chloride (chloroacetyl or 3-chloropropionyl) was added in small portions with continuous stirring. Generally, a voluminous, colorless precipitate formed immediately. The mixture was then diluted with 150 ml of glacial acetic acid and stirred for 1 hr; water (500 ml) was added and the stirring continued for an additional hour. Yields of crude product, melting point, and analytical data, after recrystallization from ethanol, for the chloroanilides prepared are given in Table I.

2-Diethylaminoethyl 4-Chloroacetylaminosalicylate.—A stirred solution of 2-diethylaminoethyl salicylate hydrochloride (65 g, 0.23 mole) in 260 ml of glacial acetic acid was treated with a solution of chloroacetyl chloride (28 g, 0.25 mole) in 30 ml of glacial acetic acid, added in small portions. Stirring was continued for 1 hr after the last addition of chloride and the excess of acetic acid was evaporated *in vacuo*. The residual acetic acid was then just neutralized by adding a saturated $NaHCO_3$ solution to the heavy syrupy residue. The crystalline hydrochloride which formed weighed 60.1 g (73%), mp 99–103°. After recrystallization from ethanol, mp 105–106°.

Anal. Calcd for $C_{15}H_{22}Cl_2N_2O_4$: Cl, 19.42; N, 7.67; mol wt, 365.25. Found: Cl, 19.27; N, 7.85; mol wt, 363.0 (acidimetry).

If the addition of bicarbonate solution is continued until the medium is strongly alkaline, the hydrochloride initially formed is transformed to the free base; this is extracted with ether and obtained as an amorphous solid in a 60% yield, mp 75–80°. After crystallization from ligroin, it is obtained in a crystalline form, mp 81–82°. When the crystalline material is allowed to stand for a few hours in the air or even under vacuum, it transforms into a glutinous mass,[16] melting in a wide range over 160°. However, the free base gives the same reactions as its stable hydrochloride if used immediately after its isolation.

(15) Dr. A. Wander A.-G., British Patent 739,210 (1955); *Chem. Abstr.*, **50**, 10779*f* (1956).

Alkyl 4-(ω-Alkylaminoacylamino)salicylates.—A suspension of the chloroamide (0.05 mole) in 200 ml of absolute ethanol was refluxed for 2 hr with an excess of amine (0.15 mole). In two cases the aminoacylaniline crystallized after cooling and was filtered, washed with water, and dried (method A). Otherwise, the ethanol was removed by distillation, and the residue was treated with 50 ml of a saturated $NaHCO_3$ solution and 50 ml of water; the aminoacylaniline separated either as a solid, or as an oil. In the first case it was filtered, washed, and dried (method B); in the second, it was extracted with ether (method C). Following this same procedure, reaction of 2-diethylaminoethyl 4-chloroacetylaminosalicylate hydrochloride with different amines in methanol or ethanol, gave the corresponding methyl or ethyl esters (methods A′, B′, and C′, respectively). Mixtures of the products obtained by the two methods described showed no melting point depression. The constants of the aminoacylanilines prepared and their salts are given in Table II.

2-Diethylaminoethyl 4-Alkylaminoacetylaminosalicylates.—A suspension of 2-diethylaminoethyl 4-chloracetylaminosalicylate hydrochloride (0.025 mole) in 100 ml of anhydrous benzene was refluxed for 4 hr with an excess of amine (0.125 mole). The benzene was then distilled, the residue was treated with 80 ml of a saturated $NaHCO_3$ solution, and the aminoacylaniline was extracted with ether (method D). The diethylamino and isopropylamino derivatives were also prepared by method E (*vide infra*). The constants of the derivatives and their salts are given in Table III.

4-Alkylaminoacetylaminosalicylamides.—A mixture of 2-diethylaminoethyl 4-chloroacetylaminosalicylate hydrochloride and an excess of amine (*ca.* 5 moles) was heated on a steam bath for 4 hr. After cooling, the excess amine was removed under vacuum, and the residue was extracted with ether to remove the diethylaminoethyl ester derivative and then with chloroform which dissolves the amide derivative (method E). The reaction with diethylamine and isopropylamine resulted in the corresponding ester derivatives (Table III), while all other amines gave the corresponding amides (Table IV); in these cases a low yield (10–15%) of the corresponding ester derivative could be isolated from the ether extracts.

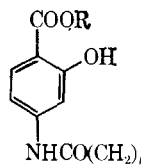
Starting with 4-chloroacetylaminosalicylic acid[17] the same amide derivatives were obtained as follows. The acid (0.02 mole) was transformed to its chloride by refluxing with excess $SOCl_2$. After elimination of the excess reagent under vacuum,

(16) W. A. Jacobs and M. Heidelberger, *J. Biol. Chem.*, **21**, 139 (1915), reported that in a similar manner the 2-(diethylaminoethyl) ester of 4-chloroacetylamino benzoic acid was unstable and did not keep well in the crude state.

(17) A. J. Quick and R. Adams, *J. Am. Chem. Soc.*, **44**, 816 (1922).

TABLE II

ALKYL 4-(ω-ALKYLAMINOACYLAMINO)SALICYLATES



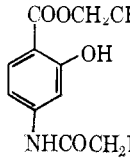
No.	R	n	NR′R″	Method[a]	Free amine[b] mp, °C	Salt	Yield,[c] %	Mp, °C	Formula	C Calcd	C Found	H Calcd	H Found	Halogen Calcd	Halogen Found	N Calcd	N Found	Approx LD50, mg/kg iv or po	Local anesthetic activity[d]
1	CH_3	1	Dimethylamino	C[e]	86–88	HCl	74	230 dec	$C_{12}H_{17}ClN_2O_4$					12.28	12.04	9.70	9.88	80	+
2						CH_3I	80	264 dec	$C_{12}H_{19}IN_2O_4$					32.19	31.99	7.10	7.12	38	−
3	CH_3	1	Diethylamino	C	32–34	HCl	74	171 dec	$C_{14}H_{21}ClN_2O_4$					11.20	11.27	8.85	8.92	150	+
4						CH_3I	73	132	$C_{15}H_{23}IN_2O_4$					30.06	29.13	6.63	6.49	18	−
5	CH_3	1	Isopropylamino	B	75–76	HCl	73	257 dec	$C_{13}H_{19}ClN_2O_4$					11.71	11.48	9.26	9.49	>800[f]	−
6	CH_3	1	Piperidino	A, A′	137	HCl	64, 46	226 dec	$C_{15}H_{21}ClN_2O_4$					10.79	10.66	8.53	8.52	115	+
7						CH_3I	73, 54	214 dec	$C_{16}H_{23}IN_2O_4$					29.23	29.10	6.45	6.51	>1000[f]	g
8	CH_3	1	Morpholino	A	163–164	HCl	72	229 dec	$C_{14}H_{19}ClN_2O_5$					10.72	10.56	8.47	8.38	115	+
9						CH_3I	61	201 dec	$C_{15}H_{21}IN_2O_5$					29.09	28.55	6.42	6.23	40	−
10	CH_3	1	Benzylamino	B	62–63	HCl	88	258 dec	$C_{17}H_{19}ClN_2O_4$					10.11	9.93	7.98	8.10	>1000[f]	g
11	CH_3	1	Cyclohexylamino	B	106–108	HCl	94	296 dec	$C_{16}H_{23}ClN_2O_4$					10.35	10.02	8.17	7.94	>1000[f]	g
12	CH_3	2	Dimethylamino	B[e]	95	HCl	78	232 dec	$C_{13}H_{19}ClN_2O_4$					11.71	11.58	9.26	8.95	150	+
13						CH_3I	66	210 dec	$C_{14}H_{21}IN_2O_4$					31.09	31.01	6.86	6.72	35	−
14	CH_3	2	Diethylamino	B	42–45	HCl	68	155	$C_{15}H_{23}ClN_2O_4$					10.72	10.62	8.47	8.50	85	+
15						CH_3I	56	159	$C_{16}H_{25}IN_2O_4$					29.09	29.00	6.42	6.30	18	−
16	CH_3	2	Isopropylamino	C	Oil	HCl	61	195 dec	$C_{14}H_{21}ClN_2O_4$					11.20	10.74	8.85	8.66	>50	+
17	CH_3	2	Piperidino	B	119	HCl	76	217 dec	$C_{16}H_{23}ClN_2O_4$					10.35	10.27	8.17	8.43	80	+
18						CH_3I	68	165	$C_{17}H_{25}IN_2O_4$					28.31	27.94	6.25	6.39	18	+
19	CH_3	2	Morpholino	B	125	HCl	90	230 dec	$C_{15}H_{21}ClN_2O_5$					10.29	10.02	8.12	8.21	>100[f]	+
20						CH_3I	80	185 dec	$C_{16}H_{23}IN_2O_5$					28.18	27.84	6.23	6.28	>1000[f]	g
21	CH_3	2	Benzylamino	B	65–70	HCl	82	231 dec	$C_{18}H_{21}ClN_2O_4$					9.72	9.49	7.68	7.82	>1000[f]	g
22	CH_3	2	Cyclohexylamino	C	62	HCl	80	220 dec	$C_{17}H_{25}ClN_2O_4$					9.93	10.01	7.85	7.91	>1000[f]	g
23	C_2H_5	1	Dimethylamino	B, C[e]	73–75	HCl	79, 91	205 dec	$C_{13}H_{19}ClN_2O_4$					11.71	11.31	9.26	9.51	>1000[f]	g
24						CH_3I	65, 67	282 dec	$C_{14}H_{21}IN_2O_4$					31.09	30.72	6.86	6.85	>1000[f]	g
25	C_2H_5	1	Diethylamino	C, C′	Oil	HCl	86, 80	172 dec	$C_{15}H_{23}ClN_2O_4$					10.72	10.88	8.47	8.64	80	+
26						CH_3I	80, 64	130	$C_{16}H_{25}IN_2O_4$					29.09	28.72	6.42	6.61	18	−
27	C_2H_5	1	Isopropylamino	B, B′	60	HCl	75, 55	281 dec	$C_{14}H_{21}ClN_2O_4$					11.20	11.03	8.85	9.04	>1000[f]	g
28	C_2H_5	1	Piperidino	B, B′	76–77	HCl	78, 62	206 dec	$C_{16}H_{23}ClN_2O_4$					10.35	10.11	8.17	8.11	80	+
29						CH_3I	67, 64	197 dec	$C_{17}H_{25}IN_2O_4$					28.31	28.65	6.25	6.04	>1000[f]	g
30	C_2H_5	1	Morpholino	B, B′	116–117	HCl	78, 62	215 dec	$C_{15}H_{21}ClN_2O_5$					10.29	9.88	8.12	8.16	>200	−
31						CH_3I	65, 58	155	$C_{16}H_{23}IN_2O_5$					28.18	27.94	6.23	6.22	>1000[f]	g
32	C_2H_5	1	Benzylamino	C, C′	47–49	HCl	92, 61	261 dec	$C_{18}H_{21}ClN_2O_4$					9.72	10.02	7.68	7.66	>1000[f]	g

No.	R	n	NR′R″	Method[a]	Free amine[b] mp, °C	Salt	Yield,[c] %	Mp °C	Formula	% C Calcd	% C Found	% H Calcd	% H Found	% halogen Calcd	% halogen Found	% N Calcd	% N Found	Approx LD_{50}, mg/kg iv or *po*	Local anesthetic activity
33	C_2H_5	1	Cyclohexylamino	B, C′	70	HCl	94, 72	302 dec	$C_{17}H_{25}ClN_2O_4$					9.93	9.68	7.85	7.64	>1000[f]	*g*
34	C_2H_5	2	Dimethylamino	B[e]	95	HCl	91	222 dec	$C_{14}H_{21}ClN_2O_4$					11.20	11.04	8.85	8.57	>200	+
35						CH_3I	77	185 dec	$C_{15}H_{23}IN_2O_4$					30.06	30.01	6.63	6.83	18	−
36	C_2H_5	2	Diethylamino	B	46–48	HCl	75	155 dec	$C_{16}H_{25}ClN_2O_4$					10.29	10.01	8.12	7.94	43	+
37						CH_3I	68	145	$C_{17}H_{27}IN_2O_4$					28.18	28.43	6.22	6.27	18	−
38	C_2H_5	2	Isopropylamino	C	42–45	HCl	70	214 dec	$C_{15}H_{23}ClN_2O_4$					10.72	10.78	8.47	8.58	80	+
39	C_2H_5	2	Piperidino	B	84	HCl	87	212 dec	$C_{17}H_{25}ClN_2O_4$					9.93	10.05	7.85	7.84	150	+
40						CH_3I	68	147	$C_{18}H_{27}IN_2O_4$					27.45	26.94	6.06	5.88	9	+
41	C_2H_5	2	Morpholino	B	97	HCl	83	236 dec	$C_{16}H_{23}ClN_2O_5$					9.88	9.95	7.81	7.92	>100	−
42						CH_3I	72	161	$C_{17}H_{25}IN_2O_5$					27.33	27.44	6.04	5.94	20	−
43	C_2H_5	2	Benzylamino	B	63	HCl	79	230 dec	$C_{19}H_{23}ClN_2O_4$					9.36	9.12	7.39	7.60	>1000[f]	*g*
44	C_2H_5	2	Cyclohexylamino	B	65	HCl	75	203 dec	$C_{18}H_{27}ClN_2O_4$					9.56	9.55	7.55	7.28	>1000[f]	*g*
45	*n*-C_3H_7	1	Diethylamino	C	Oil	HCl	82	137 dec	$C_{16}H_{25}ClN_2O_4$	55.73	55.65	7.30	7.38			8.12	7.93	60	−
46	*n*-C_3H_7	1	Piperidino	C	65–68	HCl	73	215 dec	$C_{17}H_{25}ClN_2O_4$	57.23	57.43	7.06	7.02			7.85	7.60	87	−
47	*n*-C_3H_7	2	Diethylamino	B	66–68	HCl	75	114	$C_{17}H_{27}ClN_2O_4$		56.90		7.58				7.80	90	−
48	*n*-C_3H_7	2	Isopropylamino	C	Oil	HCl	77	197 dec	$C_{16}H_{25}ClN_2O_4$	55.73	55.55	7.30	7.21			8.12	8.16	115	−
49	*n*-C_3H_7	2	Piperidino	B	77–82	HCl	82	199 dec	$C_{18}H_{27}ClN_2O_4$	58.29	58.02	7.34	7.35			7.55	7.69	85	−
50	*n*-C_3H_7	2	Morpholino	B	85–88	HCl	89	235 dec	$C_{17}H_{25}ClN_2O_5$	54.77	54.67	6.76	6.85			7.51	7.52	80	−
51	*n*-C_4H_9	1	Diethylamino	C	Oil	HCl	65	132 dec	$C_{17}H_{27}ClN_2O_4$	56.90	56.66	7.58	7.56			7.80	8.05	115	−
52	*n*-C_4H_9	1	Piperidino	C	68	HCl	74	185 dec	$C_{18}H_{27}ClN_2O_4$	58.29	58.26	7.34	7.16			7.55	7.49	67	−
53	*n*-C_4H_9	2	Diethylamino	B	52–55	HCl	70	132–133	$C_{18}H_{29}ClN_2O_4$	57.98	58.11	7.84	7.91			7.51	7.41	80	−
54	*n*-C_4H_9	2	Isopropylamino	B	56–62	HCl	62	201 dec	$C_{17}H_{27}ClN_2O_4$	56.90	56.74	7.58	7.67			7.80	7.84	>1000[f]	*g*
55	*n*-C_4H_9	2	Piperidino	B	85–90	HCl	69	199 dec	$C_{19}H_{29}ClN_2O_4$	59.29		7.59				7.28		68	−
56	*n*-C_4H_9	2	Morpholino	B	55–60	HCl	77	206 dec	$C_{18}H_{27}ClN_2O_5$	55.88	55.80	7.03	7.11			7.24	7.32	800[f]	*g*
57	*n*-C_5H_{11}	1	Diethylamino	C	Oil	HCl	64	125	$C_{18}H_{29}ClN_2O_4$	57.98	57.72	7.84	7.69			7.51	7.52	80	−
58	*n*-C_5H_{11}	1	Piperidino	C	65–69	HCl	74	196 dec	$C_{19}H_{29}ClN_2O_4$	59.29	59.58	7.59	7.63			7.28	7.38	68	−
59	*n*-C_5H_{11}	2	Diethylamino	C	Oil	HCl	60	110	$C_{19}H_{31}ClN_2O_4$	58.96	59.11	8.07	7.86			7.24	7.34	64	−
60	*n*-C_5H_{11}	2	Isopropylamino	C	54–60	HCl	56	199 dec	$C_{18}H_{29}ClN_2O_4$	57.98	58.20	7.84	7.87			7.51	7.41	20	−
61	*n*-C_5H_{11}	2	Piperidino	C	67	HCl	67	201 dec	$C_{20}H_{31}ClN_2O_4$	60.22	60.07	7.83	7.90			7.02	7.04	35	−
62	*n*-C_5H_{11}	2	Morpholino	C	60–63	HCl	81	206 dec	$C_{19}H_{29}ClN_2O_5$	56.92	57.09	7.29	7.48			6.99	6.81	40	−
63	*n*-C_6H_{13}	1	Diethylamino	C	Oil	HCl	73	122	$C_{19}H_{31}ClN_2O_4$	58.96	58.98	8.07	8.02			7.24	7.24	95	−
64	*n*-C_6H_{13}	1	Piperidino	C	57–62	HCl	76	188 dec	$C_{20}H_{31}ClN_2O_4$	60.22	59.99	7.83	7.70			7.02	6.96	60	−
65	*n*-C_6H_{13}	2	Diethylamino	B	55–60	HCl	78	130 dec	$C_{20}H_{33}ClN_2O_4$	59.91	60.00	8.30	8.52			6.99	6.80	37	−
66	*n*-C_6H_{13}	2	Isopropylamino	C	Oil	HCl	59	195 dec	$C_{19}H_{31}ClN_2O_4$	58.96	59.07	8.07	7.89			7.24	7.27	1000[f]	*g*
67	*n*-C_6H_{13}	2	Piperidino	C	Oil	HCl	75	189 dec	$C_{21}H_{33}ClN_2O_4$	61.08	60.84	8.05	7.87			6.78	6.97	1000[f]	*g*
68	*n*-C_6H_{13}	2	Morpholino	C	52	HCl	82	195 dec	$C_{20}H_{31}ClN_2O_5$	57.89	57.94	7.53	7.72			6.75	6.64	60	−

[a] See Experimental Section. [b] Melting points of free amines are given for nonpurified products. [c] Yields of the salts are based on the starting chloroacylaminosalicylates; where a double set of values is indicated, these correspond to the two methods mentioned. [d] Qualitative screening for local anesthetic activity was conducted using the Bianchi Method.[18] Compounds exhibiting local anesthetic activity in this test were further quantitatively evaluated using the method of Bulbring and Wajda[19] (Table V). [e] For the preparation of this compound a 33% solution of dimethylamine in absolute ethanol was used. [f] Approximate LD_{50} *po*. [g] These compounds were too insoluble in water to test by the Bianchi procedure and thus unsuitable for use as local anesthetics as per criteria for selection of local anesthetics (L. S. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," W. B. Saunders Co., Philadelphia, Pa., 1965.

TABLE III

2-(DIETHYLAMINOETHYL) 4-(2-ALKYLAMINOACETYLAMINO)SALICYLATES



No.	NR'R''	Method[a]	Free amine[b] mp, °C	Salt	Yield,[c] %	Mp, °C dec	Formula[e]	Halogen, % — Calcd	Halogen, % — Found	Nitrogen, % — Calcd	Nitrogen, % — Found	Approx LD_{50}, mg/kg iv or ip	Local anesthetic activity[d]
69	Diethylamino	D, E	Oil	HCl	91, 78	201	$C_{19}H_{33}Cl_2N_3O_4$	16.17	16.09	9.58	9.70	85	+
70				CH_3I	86, 82	238	$C_{24}H_{42}I_2N_3O_4$	39.09	39.15	6.48	6.51	1.5	-
71	Isopropylamino	D, E	Oil	HCl	72, 62	255	$C_{18}H_{31}Cl_2N_3O_4$	16.71	16.54	9.90	9.96	70	+
72	Piperidino	D	56–58	HCl	59	242	$C_{20}H_{33}Cl_2N_3O_4$	15.74	15.53	9.33	9.39	50	+
73				CH_3I	52	232	$C_{22}H_{37}I_2N_3O_4$	38.38	37.89	6.36	6.39	*f*	
74	Morpholino	D	85–89	HCl	55	206	$C_{19}H_{31}Cl_2N_3O_5$	15.68	15.82	9.29	9.36	175	+
75				CH_3I	48	247	$C_{21}H_{35}I_2N_3O_5$	38.26	37.92	6.34	6.38	*f*	
76	Benzylamino	D	Oil	HCl	47	204	$C_{22}H_{31}Cl_2N_3O_4$	15.01	14.87	8.89	8.78	*f*	
77	Cyclohexylamino	D	53	HCl	78	227	$C_{24}H_{35}Cl_2N_3O_4$	15.27	15.45	9.05	9.27	80	+

[a–d] As in Table II. [e] Corresponding to dihydrochlorides and bismethiodides. [f] These compounds were not submitted for pharmacological evaluation.

the residue was heated with 0.2 mole of the amine for 2 hr and the amide was isolated according to method E (method F) (Table IV). Mixtures of the amides prepared by methods E and F showed no melting point depression.

Pharmacology. Local Anesthetic Screening.—Preliminary qualitative screening was done by subcutaneous injection of the test compounds into the mouse tail according to the method described by Bianchi.[18] A 2% solution of each compound was prepared in distilled water. Five mice were injected subcutaneously with 0.1 ml of test solution in the tail about 1 cm from the base and an artery clip was applied periodically for about 15 min. Untreated mice attempted to remove the artery clip within 5–6 sec. The end point of the procedure indicating local anesthesia was the failure of the mouse to attempt to dislodge the clip within 30 sec. The compounds which exhibited local anesthetic activity using the Bianchi method were in addition quantitatively evaluated for local anesthetic potency in guinea pigs using the Bulbring and Wajda[19] method. All of the compounds were tested at 1% concentration and the relative potency on a molar basis was compared with 1% lidocaine. A minimum of six guinea pigs was used for each compound shown in Table V. No more than two compounds were tested on any single guinea pig. The tissue irritancy of the compounds was determined in rabbits by the trypan blue method of Hoppe, *et al.*[20] The numerical estimation of TIC (tissue irritant concentration) was made according to the scoring system described by these authors.

Toxicity in Mice.—Male CF strain mice weighing between 20 and 30 g were used for acute toxicity determination. An approximate intravenous LD_{50} was determined in five groups of mice (five mice per dose level). Water-insoluble compounds were suspended in 1% tragacanth and given orally to obtain approximate oral LD_{50}'s. The acute intraperitoneal toxicity (24 hr) of the selected compounds presented in Table V was conducted with five groups of mice (ten animals per dose level). The median lethal dose ($LD_{50} \pm SE$) with 95% confidence limits was estimated according to the method of Litchfield and Wilcoxon.[21]

Results and Discussion

In Tables II–IV, 58 compounds are listed which were tested for local anesthetic activity and toxicity as described under Methods. The following correlations between local anesthetic activity, acute toxicity, and chemical structure can be made.

(1) Of the various esters tested, only the methyl, ethyl, and diethylaminoethyl derivatives exhibited local anesthetic activity. The propyl, butyl, amyl, and hexyl esters were generally more toxic and did not show local anesthetic activity.

(2) In the acyl-substituted portion of the molecule, the benzylamino, isopropylamino, and cyclohexylamino derivatives were very insoluble and could not be tested for local anesthetic activity by the procedure of Bianchi.[18] The oral toxicity of these compounds was quite low which may be due, in part, to their low solubility. The dimethylamino, diethylamino-, piperidino-, and morpholinoacyl derivatives of the methyl and ethyl esters generally showed local anesthetic activity and, with the exception of the dimethylaminoacyl derivatives of the ethyl ester, were also appreciably soluble in water. These compounds apparently possess the balance which, as Quevauviller[22] has pointed out, must exist between the lipophilic and hydrophilic portions of a molecule in order to have a local anesthetic effect. The acute toxicities of the methyl and ethyl esters containing these four groups were in the same range varying from 80 to 150 mg/kg.

(3) The number of methylene groups (n) in the acyl chain was either one or two. In general, the compounds with two methylene groups exhibit a higher local anesthetic activity but also show increased toxicity and irritancy.

In Table II, 17 compounds are listed which were synthesized as the methiodide salts. The quaternization of the tertiary amine by the methyl group destroyed the local anesthetic activity of compounds which were active as the hydrochlorides. This is in agreement with the findings of Nador, *et al.*,[23] and Löfgren and Fischer[24] who reported a similar loss of activity after the methyl quaternization of active compounds. In addition, these compounds are much more toxic than the corresponding hydrochlorides.

In Table V, the toxicity (intraperitoneal), irritancy, and local anesthetic potency of ten of the derivatives which exhibited significant activity are compared to lidocaine. Although 19 compounds of the series showed a qualitative local anesthetic response, only

(18) C. Bianchi, *Brit. J. Pharmacol.*, **11**, 104 (1956).

(19) E. Bulbring and I. Wajda, *J. Pharmacol. Exptl. Therap.*, **85**, 78 (1945).

(20) J. O. Hoppe, E. B. Alexander, and L. C. Miller, *J. Am. Pharm. Assoc., Sci. Ed.*, **39**, 147 (1950).

(21) J. T. Litchfield, Jr., and F. J. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 113 (1949).

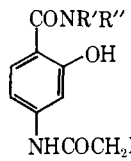
(22) A. Quevauviller, *Proc. Pharm.*, **7**, 533, 585 (1952).

(23) K. Nador, F. Herr, G. Pataky, and J. Borsy, *Nature*, **171**, 788 (1953).

(24) N. Löfgren and I. Fischer, *Svensk Kem. Tidskr.*, **58**, 219 (1946).

TABLE IV

N-ALKYL-4-(2-ALKYLAMINOACETYLAMINO)SALICYLAMIDES



No.	NR′R″		Method[a]	Yield,[b] %	Mp, °C	Formula	Carbon, % Calcd	Carbon, % Found	Hydrogen, % Calcd	Hydrogen, % Found	Nitrogen, % Calcd	Nitrogen, % Found	Approx LD_{50}, mg/kg iv or ip	Local anesthetic activity[d]
78	Piperidino	Free amine[c]	E, F	49, 58	162	$C_{19}H_{27}N_3O_3$	66.05	66.16	7.88	7.82	12.17	12.38	*e*	
79		Hydrochloride			240 dec	$C_{19}H_{28}ClN_3O_3$	59.76	59.70	7.39	7.25	11.00	11.14	200	—
80		Methiodide			230 dec	$C_{20}H_{30}IN_3O_3$					8.62	8.77	*e*	
81	Morpholino	Free amine[c]	E, F	45, 33	160								*e*	
82		Hydrochloride			205 dec	$C_{17}H_{24}ClN_3O_5$	52.91	53.14	6.27	6.10	10.89	10.58	200	—
83	Benzylamino	Free amine[c]	E, F	86, 55	170	$C_{23}H_{23}N_3O_3$	70.93	70.93	5.95	5.71	10.79	11.22	*e*	
84		Hydrochloride			258 dec	$C_{23}H_{24}ClN_3O_3$	64.86	64.96	5.68	5.83	9.87	9.79	*e*	
85	Cyclohexyamino	Free amine[c]	E, F	76, 23	154								*e*	
86		Hydrochloride			290 dec	$C_{21}H_{32}ClN_3O_3$	61.52	61.41	7.87	7.81	10.25	10.05	1000[f]	*g*

[a] See Experimental Section. [b] The two values indicated correspond to the two methods mentioned. [c] Free amines were recrystallized from ethanol. [d] See footnote *d*, Table II. [e] These compounds were not evaluated pharmacologically. [f] See footnote *f*, Table II. [g] See Footnote *g*, Table II.

TABLE V

PHARMACOLOGICAL PROPERTIES

No.[a]	R	*n*	NR′R″	Mol wt	Toxicity (mice), mg/kg ip	Threshold irritant concn[b] mM	Molar lidocaine ratio	Local anesthesia[d] Relative activity[c]	Local anesthesia[d] Duration, min
Liodaine	...	...	...	270.8	134 ± 11.5	55.4	1.00	1.0	27.5
3	CH_3	1	Diethylamino	316.7	505 ± 75.2	31.6	1.75	0.46	12.5
17	CH_3	2	Piperidino	342.2	280 ± 29.1	14.5	3.81	0.43	11.7
19	CH_3	2	Morpholino	344.7	765 ± 72.5	29.0	1.91	0.56	16.0
28	C_2H_5	1	Piperidino	342.7	458 ± 19.15	7.3	7.58	0.49	13.5
34	C_2H_5	2	Dimethylamino	316.7	433 ± 29.0	3.9	14.20	0.42	12.5
36	C_2H_5	2	Diethylamino	344.7	322 ± 49.4	0.9	61.50	0.986	26.7
38	C_2H_5	2	Isopropylamino	330.7	253 ± 37.7	0.24	230.00	0.84	22.5
39	C_2H_5	2	Piperidino	356.7	290 ± 45.5	3.5	15.80	0.64	15.0
72	$CH_2CH_2N(C_2H_5)_2$	1	Piperidino	450.4	116 ± 20.6	33.2	1.62	0.92	16.7
18	CH_3	2	Piperidino	448.2	54 ± 9.4	22.4	2.47	0.52	12.5

[a] As hydrochlorides, except compound **18** which is a methiodide salt. [b] Threshold irritant concentrations expressed on a molar basis and in terms of lidocaine ratio (lidocaine = 1), determined by the method of Hoppe, *et al.*[20] [c] Relative activity expressed in terms of lidocaine = 1. [d] Quantitative local anesthetic activity was determined by the method of Bülbring and Wajda.[19]

those compounds having a relative activity greater than 0.4 when compared to lidocaine are included in the table. Of the compounds tested, only **36**, **38**, and **72** have an activity (on a molar basis) approaching that of lidocaine. However, **36** and **38** are extremely irritating which rules out their use as local anesthetics. Compound **72** appears to be almost as effective as lidocaine on a molar basis but it is more irritating slightly more toxic, and has a shorter duration of action. It is of interest that **38**, the only secondary amine in the series with significant activity, is also the most irritating.

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6-Glycine-8-phenyllactic Acid Bradykinin. Its Synthesis, Biological Activity, and Splitting by Kininase (Carboxypeptidase N)

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The synthesis of a new depsipeptide analog of bradykinin is described. The biological activity of the analog has been studied. The vasodepressive effects in the rat and rabbit and the effects on isolated rat uterus and capillary permeability in rabbit skin of the depsipeptide were evaluated as 4, 2, 0.5, and 0.04 times, respectively, the potency of bradykinin. In the rat the analog has three phases of action: primary hypotension, partial restoration of the arterial blood pressure level, and secondary hypotension. The analog has no antibradykinin action. The rate of human and rabbit sera kininase induced degradation of the analog *in vitro* is about 20–35% slower than that of bradykinin; the enhanced and prolonged vasodepressive action of the depsipeptide may be explained on this basis.

In recent years considerable information has accumulated on the relation between the activity of biologically active peptides and the nature, configuration, sequence, and number of their amino acid residues. However, there is practically no knowledge as to the part played by the characteristic structural element of the polypeptide chain, the amide group, in particular, the necessity of its presence for the compounds to manifest their specific biological properties.

In 1964–1965 the depsipeptide analogs of a number of biologically active peptides, namely ophthalmic acid,[1] glutathione,[2] and bradykinin[2,3] in which one or more of the amide groups are replaced by an ester group, were synthesized and it was shown[2-5] that in a number of tests 6-glycolic acid bradykinin practically does not differ from bradykinin,[6] whereas the activity of 4-glycolic acid bradykinin is three to four orders of magnitude lower.

It was thus found that the replacement of an amide group by the spatially and electronically similar ester group does not abolish biological activity, although the degree of its retention does depend on the position of the replaced amide group in the peptide chain.

(1) L. A. Shchukina, A. L. Zhuze, E. P. Semkin, and S. N. Krasnova, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 685 (1964).

(2) L. A. Shchukina, G. A. Ravdel, M. P. Filatova, and A. L. Zhuze, *Acta Chim. Acad. Sci. Hung.*, **44**, 205 (1965).

(3) L. A. Shchukina, G. A. Ravdel, and M. P. Filatova, *Khim. Prirodn. Soedin.* 265 (1966).

(4) M. S. Surovikina, T. P. Egorova, and T. S. Paskhina, *Farmakol. Toksikol.*, in press.

(5) M. M. Shemyakin, L. A. Shchukina, E. I. Vinogradova, G. A. Ravdel, and Yu. A. Ovchinnikov, *Experientia*, **22**, 535 (1966).

(6) Since the replacement of serine by glycine caused no marked change in activity of bradykinin [M. Bodanszky, J. T. Sheehan, M. A. Ondetti, and S. Lande, *J. Am. Chem. Soc.*, **85**, 991 (1963); E. Schröder, *Ann.*, **673**, 186 (1964)] the more readily available 6-glycolic acid bradykinin was used for comparison.

In a continuation of our investigations into the depsipeptide analogs of bradykinin we have undertaken the synthesis of 6-glycine-8-phenyllactic acid bradykinin (I).[7]

H-Arg-Pro-Pro-Gly-Phe-Gly-Pro-PhLac-Arg-OH
1 2 3 4 5 6 7 8 9
I

The biological investigation of this analog was of particular interest because the replacement of phenylalanine by other amino acids is known to cause the greatest change in the biological activity of bradykinin.[8] On the other hand, Erdos has shown[9] that the main path of the inactivation of bradykinin in an organism is the splitting of the C-terminal Phe-Arg bond by blood plasma kininase (carboxypeptidase N). It could be expected that modification of the molecule in the direct proximity of the grouping undergoing attack would markedly affect its behavior toward this enzyme.

Chemistry.—The synthesis of the nonadepsipeptide I was carried out according to Scheme I, the protected intermediate pentadepsipeptide X being prepared in two ways, a and b. The initial compound for both ways was *t*-butyl benzyloxycarbonyl-L-prolyl-L-phenyllactate (II), obtained by condensation of benzyloxycarbonyl-L-proline with *t*-butyl phenyllactate[10] with the aid of benzenesulfonyl chloride.

(7) The abbreviations of the amino acids and their derivatives are those adopted by the 5th European Peptide Symposium, Oxford, Sept 1962. Additional abbreviations are: PhLac = L-3-phenyllactic acid, Glyc = glycolic acid.

(8) E. Schröder and R. Hempel, *Experientia*, **20**, 529 (1964).

(9) E. Y. Erdos and E. M. Sloane, *Biochem. Pharmacol.*, **11**, 585 (1962).

(10) L. A. Shchukina, G. F. Gromova, and G. A. Ravdel, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 519 (1966).