same medium (10 to 15 mL). The washed cells, which were over 95% viable and over 99% free from erythrocytes, were then suspended in a volume of medium calculated to give a cell density of 5×10^5 cells/mL. The final medium contained dialyzed, heat-inactivated fetal bovine serum (10%), 100 units/mL penicillin, and 100 μg/mL streptomycin supplemented with 100 μg/mL glutamine and was readjusted to pH 7.2 ± 0.1 with 10 mM Hepes buffer.

Aliquots (1.0 mL) of P388 cell suspension were added to individual siliconized tubes and were incubated with test compounds dissolved in 0.1 mL of the MEM containing 10% fetal calf serum and 20 mM Hepes buffer, pH 7.2. Actinomycin D and analogues were assayed initially at concentrations of 39.1, 15.63, 6.26, 2.5, 1.0, 0.4, 0.16, 0.064, and 0.016 μM. The appropriate concentrations exhibiting ED_{50} were repeated. The samples were preincubated at 37 °C for 2 h in a shaking water bath under an atmosphere of 5% CO_2–95% oxygen. Control P388 cells containing no drug were included in each assay, as was actinomycin D as an internal standard. After this initial incubation, either 0.5 μCi of [^{3}H]-thymidine (20.2 Ci/mmol) or [2-^{14}C]uridine (18.1 mCi/mmol), both from New England Nuclear, Boston, MA, was added in 0.005 mL volume. The cells were then incubated for an additional 1 h; after this incubation, the assay solutions were made 10% (w/v) with TCA, chilled to 0 °C, and allowed to stand for 2 h at 0 °C. The resulting precipitates were collected on 1.2-μm millipore filters, which were prewet with 20% TCA and were washed thoroughly with 10% TCA. The filters were placed in scintillation vials with 10 mL of Liquiscint scintillation mixture (National Diagnostic, NJ) and allowed to stand for 16 h before measuring their radioactivity in a Packard Tri-Carb Model 3375 scintillation spectrometer.

All initial experiments were done in duplicate, and repeat experiments were in triplicate. The percent synthesis of DNA or RNA in test samples was calculated relative to the synthesis in the controls. The percentage of inhibition closest above and below 50% was plotted; by extrapolation the concentrations for 50% inhibition of either RNA or DNA were determined [ED_{50} (RNA) and ED_{50} (DNA)].

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New Antiarrhythmic Agents. 7. 2,3-Diaminopropionanilides

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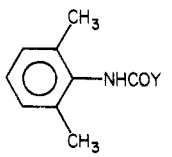
A series of 2,3-diaminopropionanilides was synthesized by acylation of mono- and disubstituted aniline derivatives with 2,3-dibromopropionyl chloride and subsequent amination with the appropriate secondary amines. The target compounds were evaluated in mice for antiarrhythmic efficacy against chloroform-induced tachycardia and for central nervous system toxicity. Several of the active agents were found to have much higher antiarrhythmic potencies than lidocaine, but they were also toxic. Evaluation of the target compounds for local anesthetic activity in the form of sciatic nerve block in rats showed that most compounds had durations of block similar to that of lidocaine; none exhibited the long duration of block seen with etidocaine.

Within the framework of a search for new orally active antiarrhythmic agents, we have described the synthesis and the pharmacological properties of primary amine analogues[2-4] of lidocaine (1). One representative, tocainide



1, Y = $CH_2N(C_2H_5)_2$
2, Y = $CH(CH_3)NH_2$

(2), had appropriate pharmacologic[2,5] and pharmacokinetic[6] parameters and was selected for clinical trials. Based on the relatively low potency of these primary amine analogues in general, and of tocainide specifically, we decided to search for more potent compounds with increased selectivity for antiarrhythmic vs. toxic effects. A series of primary and tertiary analogues of lidocaine with increased lipophilicity was synthesized,[7] yielding potent and selective agents as well as quantitative structure–activity relationships showing the usual positive correlation between partition coefficients and potency. In a parallel attempt at increasing potency, we decided to test aminoanilides with an additional amino group in the aminoacyl moiety. A series of 2,3-diaminopropionanilides carrying one alkyl, oxoalkyl, acyl, or aryl substituent on the aromatic ring had previously been synthesized[8] and evaluated for local anesthetic effects.[9] More recently the synthesis and local

(1) Address correspondence to Bristol-Myers Co., International Division, New York 10154.
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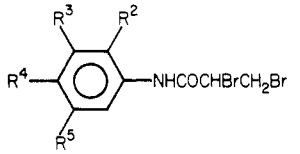
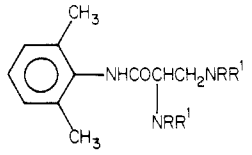
Table I. Structures, Melting Points, and Yields of 2,3-Dibromopropionanilides



no.	R^2	R^3	R^4	R^5	yield, %	mp, °C	recrystn solvent[a]	emp formula[b]
8	OCH_3	H	H	H	73	135-135.5	A	$C_{10}H_{11}NO_2Br_2$
9	H	OCH_3	H	H	62	116.5-117	B	$C_{10}H_{11}NO_2Br_2$
10	OC_2H_5	H	H	H	44	107-107.5	B	$C_{11}H_{13}NO_2Br_2$
11	H	H	OC_2H_5	H	56	137	B	$C_{11}H_{13}NO_2Br_2$
12	OC_4H_9	H	H	H	50	121.5-122	B	$C_{13}H_{17}NO_2Br_2$
13	CH_3	H	OCH_3	H	65	164-164.5	B	$C_{11}H_{13}NO_2Br_2$
14	OCH_3	H	H	CH_3	38	123-123.5	B	$C_{11}H_{13}NO_2Br_2$
15[c]	H	OC_2H_5	OC_2H_5	H	65	139-139.5 148-148.5	B	$C_{13}H_{17}NO_3Br_2$

[a] A = 2-propanol; B = 95% ethanol. [b] Experimental values of elemental analysis for C, H, and Br were within ±0.4% of theoretical values. [c] The two melting points refer to different crystal forms (differential scanning calorimetry).

anesthetic properties of diaminopropionanilides related to lidocaine (**26–32**) were reported from our laboratory.[10]



26, R = R^1 = CH_3
27, R = CH_3; R^1 = C_2H_5
28, R = CH_3; R^1 = $CH(CH_3)_2$
29, R = CH_3; R^1 = $CH_2CH{=}CH_2$
30, R = CH_3; R^1 = $CH_2C{\equiv}CH$
31, R = CH_3; R^1 = $(CH_2)_2OCH_3$
32, R, R^1 = $-(CH_2)_4-$

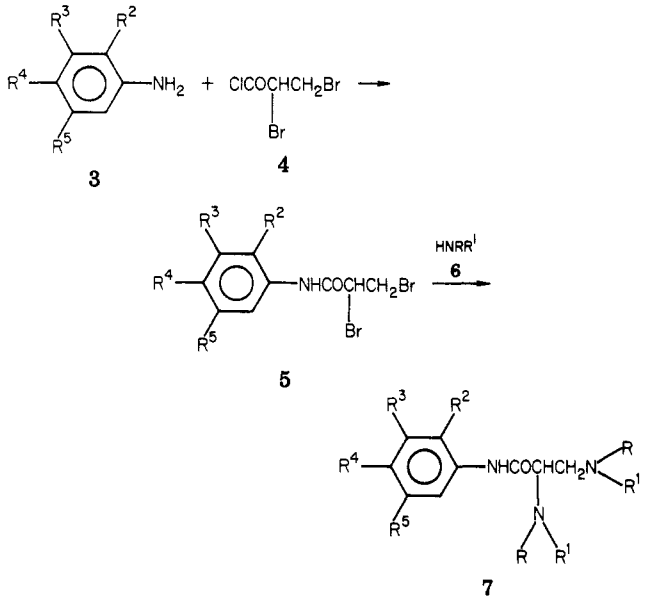
Some of these compounds produced long-lasting anesthesia comparable to tetracaine in the in vivo rat sciatic model. We now describe the synthesis of a new series of 2,3-diaminopropionanilides and the test results relating to antiarrhythmic, local anesthetic, and toxic properties.

Chemistry. The compounds reported here were prepared in two steps (Scheme I). The aromatic amines **3** were coupled with 2,3-dibromopropionyl chloride[11,12] (**4**) in an acetate buffer system.[13] The resulting 2,3-dibromopropionanilides **5** were refluxed with the appropriate secondary aliphatic amine **6** in anhydrous benzene, and the 2,3-diaminopropionanilides **7** thus obtained were isolated either as the base or as a suitable salt.

The reaction between low-boiling amines, e.g., dimethylamine, and the dibromopropionanilides was sufficiently exothermic to suggest a slow mixing of the reagents and/or external cooling of the reaction vessel during additions. In the purification of the diamines, distillations were generally avoided because of the risk of β-elimination; it was, however, possible to distill one of the compounds under high vacuum without decomposition. For further details of the preparative work, see Tables I and II and the Experimental Section.

Pharmacology. Antiarrhythmic testing was performed in mice essentially according to the method described by

Scheme I



Lawson.[14] Antiarrhythmic effect was calculated[15] as ED_{50} for protection against chloroform-induced tachycardia. During the period between drug administration and exposure to chloroform, the mice were observed for signs of ataxia and convulsions, and ED_{50} values were calculated for these effects.

Local anesthetic activity, expressed as duration of block, was evaluated in rats by means of the sciatic nerve block method described by Camougis and Takman.[16] LD_{50} values were determined in mice.[17]

Results and Discussion

Table III presents the antiarrhythmic and toxic effects of 2,3-diaminopropionanilides in mice. In addition, test data for lidocaine (**1**), tocainide (**2**), and the β-homologue (**34**) of lidocaine are given for comparison. Most of the

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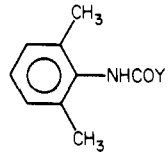
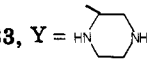


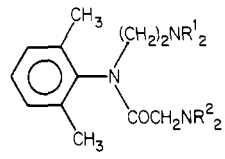
33, Y = 

34, Y = $(CH_2)_2N(C_2H_5)_2$
35, Y = $CH(C_2H_5)N(C_2H_5)C_3H_7$

compounds tested were active against chloroform-induced arrhythmias, and ED_{50} values were obtained for all but six representatives. Compounds **25**, **30**, and **33** were inactive at the doses tested, and compound **29** was too toxic for a determination [LD_{50} = 17 (14–21) mg/kg]. Compounds **18** and **24** were not fully tested, but based on the available results, their ED_{50} values may be estimated to be approximately 100 and 400 mg/kg, respectively.

Five compounds were found to be more potent than lidocaine and tocainide, the starting points of this study. Without exception, these potent compounds (**26–28**, **31**, and **32**) belong to the group of 2′,6′-dimethylanilide derivatives. On the other hand, anilides having only one ortho substituent were of moderate to low potency: **16**, **18**, **23**, and **24**. (The remaining compounds of this group, **17** and **19–22**, were not tested for antiarrhythmic effect.) These findings parallel earlier ones in the series of primary aminoanilides[2] and may reflect the steric influence of two ortho substituents preventing coplanarity of amide function and aromatic ring.[18] However, not all 2′,6′-xylidides had antiarrhythmic activity, thus demonstrating the influence of the diaminoacyl partial structure on biological properties. The piperazine-2-carboxy-2′,6′-dimethylanilide (**33**)[19] was inactive, possibly a consequence of steric restriction introduced by the ethylene bridge connecting the two amino groups. The dimorpholino compound **25** was also inactive, possibly because of low basicity. Earlier studies had similarly shown the relatively low antiarrhythmic activity of morpholino derivatives: 3-morpholinopropiono-2′,6′-dimethylanilide was found to have an ED_{50} of 351 mg/kg[20] and a pK_a of 6.16, compared with the analogous diethylamine **34** (ED_{50} = 69 mg/kg; pK_a = 8.83[7]). This finding is in agreement with a QSAR study by Ehrhardt et al.[21] in which the antiarrhythmic effects were shown to be positively correlated to pK_a. Finally, the propargylamine **30** was also inactive, although it had local anesthetic effects.[10]

In a related study[22] we have described the synthesis of other diamines (**36**) as well as their antiarrhythmic and



36

toxic effects in mice. Their structures are similar to those described here insofar as they also represent α-aminoacylanilides related to lidocaine and tocainide. No diamines of structure **36** were found with very high antiarrhythmic potencies (3.5 times that of lidocaine, on a molar basis, was the maximum observed[23]), whereas some diamines reported here had outstandingly high potencies [the most potent compound (**31**) being 35 times more potent than lidocaine]. Obviously the location of the second aminoalkyl group is of critical importance to good antiarrhythmic activity.

Most of the target compounds tested for antiarrhythmic effect evoked symptoms of CNS toxicity in the form of ataxia and convulsions and occasionally also of loss of righting reflex and death. In general, there was little or no difference between the ED_{50} for protection and those for ataxia and convulsions. This lack of separation between antiarrhythmic and toxic effects eliminated the target compounds from further consideration as antiarrhythmic agents despite the high potency observed in some examples.

Local anesthetic testing by means of the rat sciatic nerve block method showed that most of the compounds tested had durations of block similar to that of lidocaine; none exhibited the long durations of block seen with etidocaine.[17] Many of the compounds exhibited a sharp break in the dose–duration curve at some point: a twofold increase in concentration produced durations of block that were significantly longer than would have been predicted. In many cases, the "blocks" persisted for days. We have noted this phenomenon in previous studies and conclude that this may be an effect of the local anesthetic agent on the skeletal muscles of the injected limb rather than a block of the sciatic nerve trunk.

Based on the acute toxicity data available, all the compounds evaluated, except **2** and **21**, were more toxic in female mice than lidocaine but less toxic than etidocaine. Since none of the compounds exhibited durations of block comparable to that of etidocaine, they were not considered eligible for further local anesthetic testing at that time.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. IR (Perkin-Elmer 257) and NMR (JEOL C 60H or Varian T-60) spectra of all compounds were completely consistent with the assigned structures. Elemental analyses were performed by Alfred Bernhardt Microanalytical Laboratory, Elbach über Engelskirchen, West Germany, or by Schwarzkopf Micro-Analytical Laboratory, Woodside, NY. The differential scanning calorimeter used was a Perkin-Elmer DSC-1B.

2,3-Dibromopropionanilides (8–15). Structural formulas, yields, melting points and recrystallization solvents are listed in Table I. The following method was used in the preparation of compounds **8–15**.

The aromatic amine (0.2 mol) was dissolved in 170 mL of glacial acetic acid in a 1000-mL stoppered bottle and cooled to 10 °C; 0.22 mol of 2,3-dibromopropionyl chloride[11,12] was added and quickly mixed, whereupon a cooled (10 °C) solution of 66 g of $NaOAc·3H_2O$ in 275 mL of water was added. The mixture was immediately shaken vigorously by hand for 1–2 min and then mechanically for 30 min. The precipitated product was filtered by suction, carefully washed with water until the filtrate was chloride free, and dried, first in air for 6–7 h and then in a desiccator over silica gel and KOH flakes. Usually the product was sufficiently pure to be used for the next step but could be further purified by recrystallization.

2,3-Diaminopropionanilides (16–25). Structural formulas, yields, melting points and recrystallization solvents are listed in Table II. The following general method is a representative ex-

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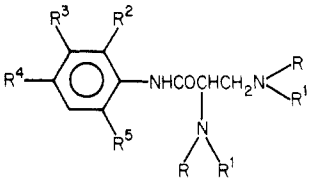
(23) One of the precursors to these diamines, *N*-[2-(diethylamino)ethyl]-2-phthalimidoaceto-2′,6′-xylidide, was 21 times more potent than lidocaine.

Table II. Structures, Yields, and Melting Points of 2,3-Diaminopropionanilides



no.	R	R^1	R^2	R^3	R^4	R^5	isolated as	yield, %	mp, °C	recrystn solvent[a]	emp formula	anal.[b]
16	C_2H_5	C_2H_5	OCH_3	H	H	H	oxalate	71	138.5-139[c]	C	$C_{20}H_{33}N_3O_6$	C, H, O
17	C_2H_5	C_3H_7	OCH_3	H	H	H	base	94[d]	e		$C_{20}H_{35}N_3O_2$	C, H, N
18	C_2H_5	C_2H_5	H	OCH_3	H	H	base	49	118-118.5[f]	D	$C_{18}H_{31}N_3O_2$	C, H, N
19	CH_3	C_2H_5	OC_2H_5	H	H	H	base	51	g		$C_{17}H_{29}N_3O_2$	C, H, N
20	C_2H_5	C_2H_5	OC_2H_5	H	H	H	HCl	93[d]	37-38[h]		$C_{19}H_{33}N_3O_2$	C, H, N
21	C_2H_5	C_2H_5	CH_3	H	OCH_3	H	base	53	i		$C_{19}H_{33}N_3O_2$	C, N; H[k]
22	C_2H_5	C_3H_7	CH_3	H	OCH_3	H	base	82		j	$C_{21}H_{37}N_3O_2$	C, H, N
23	C_2H_5	C_2H_5	H	CH_3	H	OCH_3	base			l	$C_{19}H_{33}N_3O_2$	C, H, N
							tartrate	55	137-138	B	$C_{23}H_{39}N_3O_8$	C, H, O
24	CH_3	CH_3	H	OC_2H_5	OC_2H_5	H	base	56	105-106.5	E	$C_{17}H_{29}N_3O_3$	C, H, O
25	$-C_2H_4OC_2H_4-$		CH_3	H	H	CH_3	base	79	209.5-210.5	B	$C_{19}H_{29}N_3O_3$	C, H, N

[a] B = 95% ethanol; C = acetonitrile; D = absolute ethanol; E = cyclohexane. [b] Experimental values of elemental analysis were within ±0.4% of theoretical values except where noted. [c] Melting point of the base, 47.5-48 °C. [d] Yield of unpurified base. [e] Melting point of the oxalate, 128.5-130 °C (recrystallized from absolute ethanol; boiling point of the base, ~145 °C (<0.1 mmHg). [f] Melting point of the oxalate, 164.5-165 °C (recrystallized from absolute ethanol). [g] Melting point of the oxalate, 151.5-152 °C (recrystallized from ethanol-ether). [h] The melting point refers to the solidifying oil obtained from the oxalate, mp 128.5-130 °C. [i] The base was distilled in vacuo, bp 150-157 °C (10^{-4} mmHg). [j] The base (oil) was purified by chromatography on Al_2O_3 (activity grade III), eluting with benzene. [k] H: calcd, 9.92; found, 9.46. [l] Base prepared from the purified tartrate.

Table III. Antiarrhythmic[a] and Toxic Effects of 2,3-Diaminopropionanilides

	ED_{50},[b] mg/kg		
no.	protection	ataxia	convulsion
16	81 (65-101)	c	65 (47-75)
18	d	d	d
23	106 (83-149)	96 (73-116)	137 (107-205)
24	e	199 (143-277)	317 (239-541)
25	inactive[f]	f	inactive[f]
26	17 (10-26)	16 (7-24)	23 (17-39)
27	13 (12-15)	g	12 (9-14)
28	6.1 (3.9-8.1)	6.0 (4.5-7.8)	9.3 (6-15)[h]
29	i	13 (8-17)	17 (13-20)
30	inactive[k]	k	k
31	2.6 (2.3-3.2)	4.3 (3.8-5.6)	5.1 (4.1-6.5)
32	14 (12-18)	12 (9-13)	12 (9-13)
33	inactive[l]	inactive[l]	inactive[l]
1[m]	61 ± 21[n]	42 (40-47)	
2[o]	250 ± 115[p]	146 (121-171)	
34	69 (52-94)	52 (37-64)	

[a] Protection against chloroform-induced tachycardia. Compounds 17 and 19-23 were not tested. [b] 95% Fieller limits in parentheses. Subcutaneous administration. [c] At 50.1 mg/kg: 40% ataxia. [d] At 100 mg/kg: 60% protection, 0% ataxia, 0% convulsions. [e] At 398 mg/kg: 50% protection, 100% ataxia, 70% convulsions. [f] At 79.4 mg/kg: 10% protected, 100% ataxia, 0% convulsions. [g] At 10 mg/kg: 100% ataxia. [h] Approximate confidence limits. [i] At 15.8 mg/kg: 30% protection. At 20 mg/kg: 80% dead; of the remaining 20%: 10% protected. [k] At 631 mg/kg: 0% protected, 40% ataxia, 40% convulsions, 40% dead. [l] At 200 mg/kg: 10% protected, 0% ataxia, 0% convulsions. [m] Lidocaine. [n] Mean and standard deviation of 72 determinations. [o] Tocainide. [p] Mean and standard deviation of 14 determinations.

ample for the preparation of compounds 16–25.

2,3-Bis(diethylamino)-2′-methoxypropionanilide (16). 2,3-Dibromo-2′-methoxypropionanilide (8; 46.7 g, 0.139 mol) was added at a slow rate to a cooled solution (5–10 °C) of diethylamine (64 mL, 45.6 g, 0.623 mol) in anhydrous benzene (100 mL) in a three-necked flask equipped with a reflux condenser and a thermometer. The mixture was allowed to reflux for 5 h and then cooled. Ethylammonium bromide (99%) was filtered off, and the filtrate was evaporated to dryness. The residue was dissolved in 4 M HCl, washed with ether, and based out with 7 M NaOH to pH 11. The freed amine was extracted into ether, and the ether extract was dried over anhydrous Na_2SO_4, filtered, and evaporated. From the resulting oil (96% yield) was prepared the oxalate by dissolving the oil in absolute ethanol and adding a hot solution of oxalic acid dihydrate (mole ratio 1:2). The oxalate was recrystallized from acetonitrile to constant melting point (138.5–139 °C). The base was prepared by dissolving the salt in water, alkalizing (7 M NaOH), and extracting into ether. On evaporation of the ether, 26.0 g (0.081 mol, 58%) of pure base was obtained, mp 47.5–48 °C.

Compounds **17**, **19**, and **20** were prepared in a similar manner via the oxalate, whereas **23** was purified as the tartrate. Compounds **18** and **24** could be recrystallized as bases, and the base **21** was distilled in a short-path high-vacuum system.

Compound **22** did not easily form salts and was purified by chromatography on Al_2O_3 (activity grade III).[24] The base was eluted with benzene, the solvent was evaporated, and the residue was dissolved in 1 M HCl. The solution was washed twice with ether, adjusted to pH 6 with NaOH and washed twice with ether. After adjustment to pH 11 (7 M NaOH), the aqueous phase was extracted with ether (four portions). The ether extracts were dried and the ether was evaporated in vacuo. The residue was dissolved in a small amount of absolute methanol (1:1–1.5) and filtered. On complete evaporation of the methanol from the filtrate, an analytically satisfactory product was obtained.

Because of solubility problems, compound **25**[25] was purified in a different way. After the reaction was complete, the benzene was evaporated *without filtering*, and the residue was dissolved in 4 M HCl and washed with ether. CH_2Cl_2 or $CHCl_3$ was added to the aqueous phase, and an excess of 7 M NaOH was added slowly with stirring. The organic phase was removed, and the water phase was further extracted with CH_2Cl_2 or $CHCl_3$. The combined organic extracts were dried (Na_2SO_4), the solvent was evaporated, and the base was recrystallized.

Pharmacology. Antiarrhythmic testing was performed in mice essentially according to the method described by Lawson.[14] Drugs, dissolved in 0.9% saline, were injected subcutaneously to

(24) Hardegger, E. "Einführung in das Organisch-Chemische Praktikum, Allgemeiner und Analytischer Teil", 2nd ed.; Verlag der VCS: Zürich, Switzerland, 1958; p 43.

(25) The 2,3-dibromo-2′,6′-propionoxylidide used in the synthesis of this compound has been described.[10]

Table IV. Local Anesthetic Activity and Acute Toxicity of 2,3-Diaminopropionanilides

	duration of block,[a] min					LD_{50},[b] mg/kg	
no.	concn:[c] 0.125%	0.25%	0.5%	1.0%	2.0%	ip	iv
16		91 ± 11	66 ± 13	178 ± 54	184 ± 12[d]	70 (41-93)	
17	80 ± 13	116 ± 13	260 ± 52	days[e]	days[e]		11 (9-13)
18	77 ± 4	87 ± 6	100 ± 14	145 ± 19	265 ± 39		20 (16-23)
19	78 ± 3	105 ± 17	136 ± 18	189 ± 16	days[e]	94 (73-120)	
20	140 ± 19	138 ± 11	176 ± 14	days[e]	days[e]	81 (64-101)	
21	162 ± 5	151 ± 4	157 ± 10	150 ± 31	184 ± 24	102 (72-143)	
22	96 ± 1	107 ± 22	227 ± 49	days[e]	days[e]	66 (50-83)	
23	94 ± 9	125 ± 5	166 ± 15	273 ± 35	days[e]	81 (53-113)	
24	NB[f]	NB[f]	83 (2/10)	133 ± 22	147 ± 19		
25	72 ± 8	72 ± 8	102 ± 30	138 ± 25	193 ± 38		
1		102 ± 15	123 ± 10	162 ± 39		102 (73-142)	26 (23-33)
2			44 ± 8	79 ± 19			94 (81-106)
34		110 ± 5	146 ± 4	184 ± 10	days[e]		
35	156 ± 41	222 ± 54	279 ± 16			56 (47-65)	7 (6-8)

[a] Rat sciatic nerve block, mean plus or minus standard deviation. [b] Intraperitoneal or intravenous injection into female mice, LD_{50}, mg/kg (measured as base), with 95% confidence limits in parentheses. [c] Concentration as base. All solutions contained 1:100 000 epinephrine. [d] 5/10 legs, 5 legs "blocked" for days. [e] Required days for complete recovery of normal motor function. [f] No blocks (0/10).

groups of 10 mice. During a 20-min period the mice were observed for overt signs of toxicity (ataxia, convulsions, loss of righting reflex, or death). The mice were then placed individually in an atmosphere saturated with chloroform vapor until respiration ceased. Immediately thereafter, the thorax was opened and the presence or absence of tachycardia was determined visually. If coordinated ventricular contractions were observed, the mouse was considered to be "protected" from the arrhythmogenic effects of chloroform. At least three doses of drug were chosen to give low, intermediate, and high degrees of protection against fibrillation. From these data, the ED_{50} and the 95% Fieller limits for protection, ataxia, and convulsion were calculated according to the logit chi-square method of Berkson.[15]

Local anesthetic activity was evaluated in rats by means of the sciatic nerve block method described by Camougis and Takman.[16] Most compounds were tested at three to five concentrations with 1:100 000 epinephrine. Precisely 0.2 mL of test solution was injected into the mid-thigh region so as to deposit the solution around the sciatic nerve trunk. Each animal was examined at frequent intervals to ascertain onset of and recovery from motor block. Five animals were used at each concentration of test compound. Since both hind limbs were injected, there was a possible maximum of 10 blocks in each group of five rats.

LD_{50} values were determined[17] by administering the compounds intravenously or intraperitoneally to groups of female CRCD mice weighing between 20 and 25 g. Test compounds were dissolved in isotonic saline or distilled water and injected into a tail vein or intraperitoneally. LD_{50} values and 95% confidence limits were calculated by means of the minimum logit chi-square method.[15]

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2-(Aminomethyl)phenols, a New Class of Saluretic Agents. 3. Effects of Functional Group Reorientation and Modification[1]

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A series of modified 2-(aminomethyl)phenols was synthesized and tested orally in rats for saluretic and diuretic effects. Intravenous dog data are included as supplementary material to show that the diuretic responses, or lack thereof, may be obtained in a second species. Reorientation of the 2-(aminomethyl) group either meta or para to the hydroxyl substituent resulted in loss of diuretic effects. Similarly, replacement of either the phenolic hydroxyl or the aminomethyl group with other functional moieties substantially diminished saluretic effects.

Recently, we reported[3] on a series of 2-(aminomethyl)-phenols which were shown to possess a high order of diuretic activity in rats and dogs. This report describes the effects of (1) reorientation of the 2-(aminomethyl) group relative to that of the phenolic hydroxyl group and (2) replacement of either the phenolic hydroxyl or the 2-

(1) Portions of this work were presented in August, 1977, at the 174th National Meeting of the American Chemical Society. See "Abstracts of Papers", 174th National Meeting of the American Chemical Society, Chicago, Il, 1977; American Chemical Society: Washington, DC, 1977, and *ACS Symp. Ser.* **1978**, *83*, 93–124.

(2) Deceased, May 31, 1977.

(3) For part 1, see Stokker, G. E.; Deana, A. A.; deSolms, S. J.; Schultz, E. M.; Smith, R. L.; Cragoe, E. J., Jr.; Baer, J. E.; Ludden, C. T.; Russo, H. F.; Scriabine, A.; Sweet, C. S.; Watson, L. S. *J. Med. Chem.* **1980**, *23*, 1414.

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