New Local Anesthetics.[1] I. Esters of 1,2-Diethyl-3-hydroxymethylpyrazolidine

MILTON J. KORNET

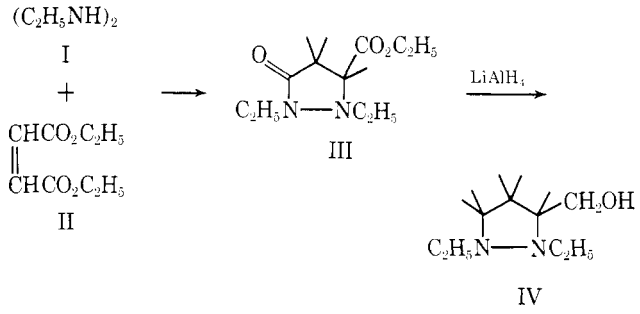
College of Pharmacy, University of Kentucky, Lexington, Kentucky

Received September 17, 1965

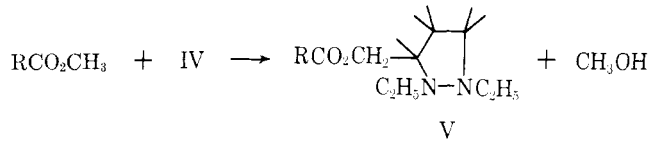
A new alcohol, 1,2-diethyl-3-hydroxymethylpyrazolidine, has been prepared in two steps from 1,2-diethylhydrazine and diethyl maleate. Transesterification of this alcohol with several methyl esters afforded eleven new esters embodying the pyrazolidine ring system. The hydrochloride salts of these esters possess local anesthetic activity.

Since the introduction of procaine by Einhorn,[2] several thousand ester-type local anesthetics have been synthesized. A great number of these arose by variation of the amino alcohol portion of the molecule.[3] In the present study ester-type compounds have been prepared in which the customary amino alcohol has been replaced by an alcohol incorporating an –N–N– linkage and in this series of compounds the –N–N– linkage is part of a pyrazolidine ring system. These esters bear structural resemblances to procaine, piperocaine, and other synthetic local anesthetics.

The alcohol embodying the pyrazolidine ring was constructed in the following way. Treatment of 1,2-diethylhydrazine (I) with diethyl maleate (II) gave a good yield of 1,2-diethyl-5-ethoxycarbonyl-3-pyrazolidinone (III). The latter compound may be visualized as arising by an addition of the hydrazine to the α,β-unsaturated ester, followed by closure to the five-membered ring with elimination of a molecule of ethanol. A somewhat similar reaction, that of hydrazine with ethyl methacrylate to give 4-methyl-3-pyrazolidinone has been reported.[4] The structure of III is supported by elemental analysis and its infrared spectrum (see Experimental Section). Reduction of the amido ester III by lithium aluminum hydride gave the alcohol, 1,2-diethyl-3-hydroxymethylpyrazolidine (IV). The latter was converted to a picrate derivative upon treatment with picric acid.



Reaction of IV under transesterification conditions with eleven different methyl esters afforded the basic esters (V) containing the pyrazolidine ring system (see Table I). Yields in the latter reaction were good with the possible exception of the reaction involving methyl *p*-aminobenzoate. This methyl ester polymerized to a considerable extent. Treatment of the basic esters V with HCl gave the hydrochloride salt derivatives (see Table II).



V

Local Anesthetic Activity.—The compounds prepared were evaluated according to the procedure of Block and co-workers using earthworms of genus *Lumbricus*.[5] Each compound was tested at four or five concentrations (0.015–0.8%) and five worms were used for each concentration. Straight line plots were obtained when the response time (min) was plotted against per cent concentration on log scale. The relative potencies were calculated using 10 min as the mean response time. The results are summarized in Table II. The most potent compound was assigned a relative anesthetic potency of 100%. By comparison, the standard piperocaine hydrochloride had a relative anesthetic potency of 17%.

The structure–activity relationship results obtained in this work parallel findings previously made for benzoate esters.[6] Thus, introduction of a *p*-amino substituent results in a compound of higher potency than the unsubstituted benzoate. Although the *p*-methoxy-substituted derivative was less active than the *p*-amino-substituted derivative, the corresponding ethoxy derivative was more active (see Table II). The introduction of an *o*-fluoro substituent gives a compound less active than the *p*-amino derivative, also in agreement with previous work.[7] Noteworthy are compounds **4** and **6** of Table II; the former is approximately 6 times as potent as piperocaine hydrochloride and the latter is about 3 times as potent.

Experimental Section[8]

Most of the required methyl esters were obtained from commercial sources. Methyl *p*-ethoxybenzoate was prepared by the published procedure.[9]

(1) For recent reviews on local anesthetics see: (a) S. Wiedling and C. Tegner in "Progress in Medicinal Chemistry," Vol. 3, G. P. Ellis and G. B. West, Ed., Butterworth Inc., Washington, D. C., 1963, p 332; (b) S. Oldham, *Mfg. Chemist*, **35**, No. 12, 50 (1964).

(2) A. Einhorn and E. Uhlfelder, *Ann.*, **371**, 131 (1909).

(3) T. P. Carney in "Medicinal Chemistry," C. M. Suter, Ed., John Wiley and Sons, Inc., New York, N. Y., 1951, p 280.

(4) T. Lieser and K. Kemmner, *Chem. Ber.*, **84**, 4 (1951).

(5) B. P. Block, D. J. Potts, and R. S. H. Finney, *J. Pharm. Pharmacol. Suppl.*, **16**, 85T (1964).

(6) Reference 1a, p 354.

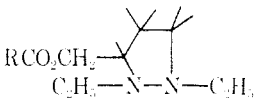
(7) G. A. Olah, A. E. Pavlath, J. A. Olah, and F. Herr, *J. Org. Chem.*, **22**, 879 (1957).

(8) Melting points were determined with the Fisher-Johns apparatus and are uncorrected. Infrared spectra were recorded on a Beckman IR 8 spectrophotometer using NaCl optics. Microanalyses were performed by Dr. Kurt Eder, Geneva, Switzerland.

(9) R. West, S. Ornstein, D. McKee, and R. Layzer, *J. Am. Chem. Soc.*, **74**, 3960 (1952).

TABLE I

ESTERS OF 1,2-DIETHYL-3-HYDROXYMETHYLPYRAZOLIDINE



Compd	R	Bp, °C (mm)	$n^{20}D$	Yield, %	Formula	% calcd C	% calcd H	% calcd N	% found C	% found H	% found N
1	C_6H_5	105 (0.05)	1.5135	64.9	$C_{15}H_{22}N_2O_2$	68.67	8.45	10.68	68.19	8.30	11.06
2	o-FC_6H_4	114 (0.12)	1.5070	74.5	$C_{15}H_{21}N_2O_2F$	64.26	7.55	9.99	64.46	7.45	10.07
3	p-$NH_2C_6H_4$	[a]	—	50.0	$C_{15}H_{23}N_3O_2$	64.95	8.36	15.15	65.08	8.27	15.04
4	p-n-$BuNHC_6H_4$	180 (0.03)	1.5539	78.9	$C_{19}H_{31}N_3O_2$	68.43	9.37	12.60	68.65	9.42	12.60
5	p-$CH_3OC_6H_4$	130 (0.07)	1.5245	82.5	$C_{16}H_{24}N_2O_3$	65.73	8.27	9.58	65.64	8.23	9.53
6	p-$CH_3CH_2OC_6H_4$	142 (0.1)	1.5212	82.3	$C_{17}H_{26}N_2O_3$	66.64	8.55	9.14	66.80	8.52	9.17
7	p-c-$C_6H_{11}OC_6H_4$	180 (0.1)	1.5290	75.0	$C_{21}H_{32}N_2O_3$	69.96	8.95	7.77	69.39	8.57	7.83
8	2-Furyl	99 (0.05)	1.4988	79.5	$C_{13}H_{20}N_2O_3$	61.88	7.99	11.10	61.99	8.05	11.16
9	$C_6H_5CH{=}CH$	137 (0.06)	1.5499	78.8	$C_{17}H_{24}N_2O_2$	70.80	8.39	9.71	70.70	8.31	9.84
10	o-$NH_2C_6H_4$	138 (0.1)	1.5525	72	$C_{15}H_{23}N_3O_2$	64.95	8.36	15.15	65.09	8.42	15.29
11	$(C_6H_5)_2CH$	157 (0.05)	1.5435	73	$C_{22}H_{28}N_2O_2$	74.97	8.01	7.95	75.13	7.85	8.07

[a] Mp 100–101°.

TABLE II

HYDROCHLORIDE SALT DERIVATIVES

Compd	Mp, °C	Formula	% calcd C	% calcd H	% calcd Cl	% calcd N	% found C	% found H	% found Cl	% found N	Relative[a] anesthetic potency, %
1	104–105	$C_{15}H_{23}ClN_2O_2$	60.29	7.76	11.87	9.38	60.33	7.75	12.06	9.58	21
2	118.5–119.5	$C_{15}H_{22}ClFN_2O_2$	56.87	7.00	11.19	8.84	56.90	7.04	11.14	8.89	17
3[b]	165–166	$C_{15}H_{24}ClN_3O_2$	57.40	7.71	11.30	13.39	57.47	7.81	11.34	13.42	29
4	126.5–128	$C_{19}H_{32}ClN_3O_2$	61.69	8.72	9.58	11.36	61.60	8.66	9.71	11.44	100
5	129–130	$C_{16}H_{25}ClN_2O_3$	58.44	7.66	10.78	8.52	58.61	7.75	10.90	8.69	15
6	134–135	$C_{17}H_{27}ClN_2O_3$	59.55	7.94	10.34	8.17	59.74	8.00	10.51	8.08	55
7	105.5–107	$C_{21}H_{33}ClN_2O_3$	63.54	8.38	8.93	7.06	63.55	8.39	8.89	6.94	[c]
8	128–129	$C_{13}H_{21}ClN_2O_3$	54.07	7.33	12.28	9.70	54.07	7.52	12.30	9.74	4
9	123	$C_{17}H_{25}ClN_2O_2$	62.85	7.76	10.91	8.62	62.57	7.75	10.89	8.50	7
10	141–142	$C_{15}H_{24}ClN_3O_2$	57.40	7.71	11.30	13.39	57.60	7.60	11.40	13.36	8

[a] By comparison piperocaine hydrochloride has a relative anesthetic potency of 17%. [b] Recrystallized from isopropyl alcohol. [c] This compound was insoluble in worm "Ringer" solution.

Methyl p-(n-butylamino)benzoate was prepared by addition of sufficient ethereal diazomethane to 7.72 g (0.04 mole) of p-(n-butylamino)benzoic acid in 50 ml of ether to give a yellow color which persisted. The clear solution which resulted was evaporated to dryness *in vacuo*. On recrystallization of the residual solid from aqueous ethanol, 7.4 g (89.4%) of the crystalline ester was obtained, mp 107–109°, lit.[10] mp 104–105°.

Methyl p-cyclohexyloxybenzoate was prepared by addition of sufficient ethereal diazomethane to 8.8 g (0.04 mole) of p-cyclohexyloxybenzoic acid in 150 ml of ether to give a yellow color which persisted. The ethereal solution was washed once with water and dried ($MgSO_4$). The spent drying agent was filtered and the ether was distilled. Distillation of the residue afforded 8.14 g (87%) of the ester, bp 127° (0.55 mm), $n^{20}D$ 1.5421.

Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 71.78; H, 7.82.

1,2-Diethyl-5-ethoxycarbonyl-3-pyrazolidinone.—To a solution of 46.5 g (0.528 mole) of 1,2-diethylhydrazine[11] in 25 ml of absolute ethanol was added a solution of 86.0 g (0.500 mole) of diethyl maleate in 65 ml of absolute ethanol dropwise, with stirring and ice-bath cooling. After stirring at room temperature overnight, the reaction mixture was refluxed on the steam bath for 8.25 hr. The ethanol was removed *in vacuo* and the residue was distilled and amounted to 95.7 g (89.5%) of a colorless oil, bp 94° (0.05 mm), $n^{20}D$ 1.4646, λ^{CHCl_3} 5.79 (ester C=O), 5.98 μ (amide C=O).

Anal. Calcd for $C_{10}H_{18}N_2O_3$: C, 56.05; H, 8.46; N, 13.08. Found: C, 55.91; H, 8.51; N, 12.94.

(10) A. R. Surrey and H. F. Hammer, *J. Am. Chem. Soc.*, **66**, 2127 (1944).
(11) R. Renaud and L. C. Leitch, *Can. J. Chem.*, **32**, 545 (1954).

1,2-Diethyl-3-hydroxymethylpyrazolidine.—A solution of 50.7 g (0.228 mole) of 1,2-diethyl-5-ethoxycarbonyl-3-pyrazolidinone in 60 ml of anhydrous ether was added dropwise with stirring to a solution of 13.2 g (0.348 mole) of $LiAlH_4$ in 150 ml of anhydrous ether. After completion of the addition the reaction mixture was refluxed for 13 hr. Decomposition of the complex was effected by 40% aqueous KOH with ice-bath cooling. The ether layer was separated and the inorganic salts were extracted twice with 100-ml portions of ether. The combined ether extracts were dried ($MgSO_4$). After removal of the spent drying agent, the ether was distilled on a water bath. The residue was distilled and yielded 25.8 g (71.6%) of a viscous, colorless liquid, bp 45° (0.24 mm), $n^{20}D$ 1.4700, λ^{CHCl_3} 2.95–3.15 (H-bonded OH), 2.78 μ (free OH).

Anal. Calcd for $C_8H_{18}N_2O$: C, 60.72; H, 11.47; N, 17.70. Found: C, 60.62; H, 11.54; N, 17.75.

A crystalline picrate derivative was prepared and recrystallized from absolute ethanol, mp 114–115°.

Anal. Calcd for $C_{14}H_{21}N_5O_8$: C, 43.41; H, 5.46; N, 18.08. Found: C, 43.58; H, 5.39; N, 18.05.

Esters of 1,2-Diethyl-3-hydroxymethylpyrazolidine. General Method.—To a solution of 1,2-diethyl-3-hydroxymethylpyrazolidine (0.05 mole) in 60 ml of dry heptane was added a 1-mm³ piece of freshly cut sodium. The resulting solution was heated on an oil bath until the sodium dissolved. The mixture was cooled, an appropriate methyl ester (0.05 mole) was added, and the reaction mixture was refluxed under a Dean–Stark tube until the theoretical amount of methanol had separated (10–40 hr). After washing twice with 5-ml portions of water, the heptane solution was dried ($MgSO_4$). The spent drying agent was filtered and the heptane was removed by distillation at atmospheric pressure. The residue was then distilled under reduced pressure (see Table I).

Hydrochloride Salts.—The monobasic esters were dissolved in ether and treated with dry HCl. The precipitated hydrochloride salts were filtered, washed with a small amount of ether, and recrystallized from absolute ethanol–ether. The dibasic esters were dissolved in 95% ethanol and treated with a calculated amount of 1 *N* HCl to form the monohydrochloride salts. The solvents were evaporated on a water bath *in vacuo* and the residue was dried (P_2O_5) in a desiccator before recrystallization from absolute ethanol–ether (see Table II).

Acknowledgment.—The author wishes to thank the University of Kentucky for a summer research fellowship during the summer of 1964 to initiate this work. Thanks are also extended to Mr. Hugh Frazier for assistance in the testing of the compounds and to Dr. Arthur C. Glasser for calling the method of testing to the author's attention.

Synthesis and Biological Activity of 7-Bromo-8-methyl-10-(1-D-ribityl)isoalloxazine, an Analog of Riboflavin[1,2]

R. D. FAULKNER AND J. P. LAMBOOY

Department of Biochemistry, University of Nebraska College of Medicine, and the Eugene C. Eppley Institute for Research, Omaha, Nebraska

Received October 13, 1965

7-Bromo-8-methyl-10-(1-D-ribityl)isoalloxazine has been synthesized by the condensation of 2-bromo-4-nitro-5-chlorotoluene with D-ribamine to produce 4-bromo-5-methyl-2-nitro-N-D-ribitylaniline. The latter was reduced and the obtained *o*-phenylenediamine was condensed with alloxan monohydrate. This new flavin stimulates the growth of the riboflavin-deficient rat at all quantities used but, in spite of this activity, the higher quantities are lethal for the animal. Since riboflavin protects the animal against the toxicity of this flavin, the analog is a reversible antagonist of riboflavin in the rat. The flavin is also an antagonist of riboflavin in *Lactobacillus casei*, but it stimulates growth of the organism in the presence of small quantities of the vitamin and under specific conditions.

Weygand, Löwenfeld, and Möller[3] synthesized 7,8-dibromo-10-(1-D-ribityl)isoalloxazine (7,8-dibromoflavin) and compared its ability to inhibit the growth of *Streptobacterium plantarum* P32 with the activity of the previously known 7,8-dichloro-10-(1-D-ribityl)isoalloxazine (7,8-dichloroflavin) against this organism. The 7,8-dichloroflavin possessed an inhibition index (*II*)[4] of 398 while the 7,8-dibromoflavin, with an inhibition index of 1750, was far less potent against *S. plantarum* P32. We have prepared 7,8-dichloroflavin also, and our improved procedure yielded a product of unquestioned purity.[5]

The bacterium, *S. plantarum*, does not require an exogenous source of riboflavin, and we considered it important to test 7,8-dichloroflavin on two test systems which do require exogenous riboflavin. We found that 7,8-dichloroflavin was not an antagonist of riboflavin in *Lactobacillus casei*[6] (ATCC 7469),[7] and thus confirmed an earlier report by Snell, *et al.*,[8] and further demonstrated that this material was also completely devoid of biological activity in the rat.[6] We have synthesized 7-chloro-8-methyl-10-(1-D-ribityl)isoalloxazine[9] (7-chloro-8-methylflavin) and studied its biological activity in both the rat and *L. casei*.[10] It is a potent inhibitor of *L. casei* with an *II* of 76 (in this test system, it is exceeded in potency by only 7-methyl-8-chloro-10-(1-D-ribityl)-isoalloxazine).[11] The 7-chloro-8-methylflavin is the most potent antagonist of riboflavin in the rat to be described to date. This antagonism is expressed in terms of lethality because, paradoxically, the compound is an excellent stimulant for the growth (but not survival) of the riboflavin-deficient rat.[10]

Weygand, *et al.*,[3] found 7,8-dibromoflavin to be far less active (*II* = 1750) than 7,8-dichloroflavin (*II* = 440) as an antagonist of riboflavin for *S. plantarum*. We have found 7-chloro-8-methylflavin to be a potent inhibitor (*II* = 76),[10] while 7,8-dichloroflavin was inert[6] as an antagonist of riboflavin in *L. casei*. It was of interest to us to learn if the incorporation of one bromine atom would produce an analog whose activity was related to that of 7,8-dibromoflavin in a way which resembled the relationship we had discovered between the analogs containing one and two chlorine atoms.

Chemistry.—2-Bromo-5-chloro-4-nitrotoluene was condensed with D-ribamine to produce 4-bromo-5-methyl-2-nitro-N-D-ribitylaniline. This compound was reduced to 2-amino-4-bromo-5-methyl-N-D-ribitylaniline which was immediately condensed with alloxan monohydrate in the presence of boric acid to produce 7-bromo-8-methyl-10-(1-D-ribityl)isoalloxazine.

Biological Activity.—7-Bromo-8-methyl-10-(1-D-ribityl)isoalloxazine is a strong antagonist of riboflavin in *L. casei;* the *II* was found to be 137. It appears to be a slightly better stimulant for the growth of the riboflavin-deficient rat and, also, it appears to be less toxic than the previously studied 7-chloro-8-methyl-10-(1-D-ribityl)isoalloxazine. The antagonism of the an-

(1) This work was supported in part by Grant CY-2940 from the National Cancer Institute, U. S. Public Health Service.

(2) The numbering system used for the flavins named in this article conforms to that of *Chemical Abstracts*. All papers constituting the literature citations have made use of the older ring-numbering system. The older numbering system will conform to the *Chemical Abstracts* system by increasing each position number by one.

(3) F. Weygand, R. Löwenfeld, and E. Möller, *Chem. Ber.*, **84**, 106 (1951).

(4) Inhibition index (*II*) = micrograms of analog at half maximum growth/0.3 μg of riboflavin times the molecular weight of riboflavin/molecular weight of analog.

(5) The melting point of our material remained 288–290° dec through repeated recrystallizations; R. Kuhn, R. Weygand, and E. F. Möller, *Ber.*, **76**, 1044 (1943), reported mp 273–275° dec.

(6) J. P. Lambooy, R. A. Scala, and E. E. Haley, *J. Nutr.*, **74**, 466 (1961).

(7) American Type Culture Collection, Rockville, Md. 20852.

(8) E. E. Snell, O. A. Klatt, H. W. Bruins, and W. W. Gravens, *Proc. Soc. Exptl. Biol. Med.*, **82**, 853 (1953).

(9) E. E. Haley and J. P. Lambooy, *J. Am. Chem. Soc.*, **76**, 5093 (1954).

(10) E. E. Haley and J. P. Lambooy, *J. Nutr.*, **72** 169 (1960).

(11) Synthesis, ref 9; biological activity, ref 6.