A comparison of the data in this study for aspirin solutions to previous data for aspirin tablets (10) showed that plasma SAL levels are 6.5 times higher at 10 minutes, 4.3 times at 20 minutes, and 3.3 times at 30 minutes. The ratios are much less for buffered aspirin solutions—2.7 at 10 minutes, 1.9 at 20 minutes, and 1.6 at 30 minutes. The essential difference between these two solutions is one of acidity. Aspirin in solution at this concentration gives a pH of about 3.0; whereas, when combined with magnesium carbonate and dihydroxyaluminum glycinate, the pH is 4.6. It was this difference in absorption of two aspirin solutions differing mainly in pH that prompted the last portion of the investigation with buffer adjustment of intragastric acidity.

The pKa of salicylic acid is 3.0 and that of acetylsalicylic acid is 3.5. According to a pH partition hypothesis for drug absorption (21), the rate of salicylate absorption from the solute state into the systemic circulation is dependent upon the concentration of unionized, lipid soluble molecules which increase at lower pH values. Schanker (22) showed that absorption of salicylates was more rapid from 0.1 *N* HCl than from bicarbonate solution in the rat, using ligation to assure gastric absorption. The tests of this hypothesis in dogs and in humans in this study generally agree with the idea that absorption is greater at lower pH values. However, in the human study and in some of the dog patterns, a second peak of absorption appears near neutrality. The optimum pH for absorption in this experiment was about pH 3.5 to 5.0 for dogs and 3.0 to 4.0 for humans. Owing to difficulties of dissolving aspirin at low pH values it cannot be said that the lower pH values represent the true lower limit of maximal absorption rates. Rubin, *et al.* (23), claimed that the amounts of antacid buffers included in buffered aspirin tablets were unable to alter gastric pH significantly. These results suggest that the environmental pH conferred upon aspirin particles by the presence in the same tablet of magnesium carbonate and dihydroxyaluminum glycinate with a buffer action at about pH 4.5 (3) do not markedly decrease aspirin absorption as shown in the dissolved aspirin test and by buffer adjustment of intragastric pH. Indeed, this pH of 4.5 may represent an optimum between high pH values which increase the rate of dissolution and low pH values which increase the rate of gastric absorption.

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Pharmacology of Alkylamino Ethanol Esters of *p*-Ethoxybenzoic Acid

By A. E. WADE and F. FORD MILLIKAN

A series of eight alkylaminoethanol esters of *p*-ethoxybenzoic acid were examined for local anesthetic activity on the rabbit cornea and frog sciatic nerve, for acute toxicity in mice, for irritancy on the rabbit cornea and by the trypan blue test in rabbits, and for their spasmolytic activity on the isolated rabbit ileum. In general, sciatic nerve block was hastened, irritancy was increased, acute toxicity increased, and duration of topical anesthesia decreased as the alkylamino portion of the molecule was enlarged.

THE SYNTHESIS and pharmacology of methylbenzyl monoethanolamine paraethoxybenzoate hydrochloride was previously reported by Millikan and Feurt (1). Although this compound appeared to have adequate spasmolytic and local anesthetic activities, its irritant properties were too pronounced for its acceptance as an injectable drug. The present work constitutes a study of

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structure-activity relationships of a series of compounds of this general type (2).

EXPERIMENTAL

Acute Toxicity.—Aqueous unbuffered solutions of each compound were administered intraperitoneally into randomly selected adult female albino Webster strain Swiss mice weighing 22 ± 4 Gm. The doses were spaced at 0.04 logarithmic intervals, and the volume of solution was maintained between 0.16 and 1.3 ml. Four to seven groups of ten mice were used for each LD_{50} determination. The LD_{50} and its standard error were calculated from the percentage of deaths occurring within 24 hours by the method of Miller and Tainter (3).

Topical Anesthesia.—The surface anesthetic activity was determined by the ability of each compound to cause loss of wink reflex when applied to the cornea of albino rabbits using a method similar to that described by Luduena and Hoppe (4). Three to five concentrations of each of the aqueous unbuffered solutions spaced at 0.3 logarithmic intervals were applied to the cornea of five to 11 rabbits each. The concentration of anesthetic necessary to produce corneal anesthesia for 10 minutes was estimated from dose-response curves according to the method of Luduena and Hoppe (5) and reported as threshold anesthetic $concentration_{10}$ (TAC_{10}).

Conduction Anesthesia.—The ability of these compounds to suppress nervous conduction through the frog sciatic nerve was determined on the grass frog *Rana pipiens* by a modification of the method of Rider (6). To avoid excessive trauma due to removing the viscera and to avoid the possibility of dilution of the anesthetic solution by blood and tissue fluids, the sciatic nerves were exposed by carefully dissecting the caudal portions of the urostyle. This bone needed only to be lifted to expose the sciatic nerves, its section from the upper vertebral column being unnecessary. The local anesthetic solution was then applied to pledgets of cotton carefully placed beneath both nerves. Another pledget saturated with the anesthetic solution was then placed on top of the nerves in juxtaposition to the first pledget to prevent the nerves from contacting cut muscle surfaces and to insure contact of the anesthetic on their circumference. Four to eight observations at each of four concentrations spaced at 0.3-logarithmic intervals were made for each compound. The potency of the anesthetics was determined from time of onset of anesthesia/log concentration of anesthetic curves and was reported as the concentration of anesthetic necessary to suppress reflex withdrawal in response to a pinch stimulus applied to the foot in 10 minutes (AC_{10}).

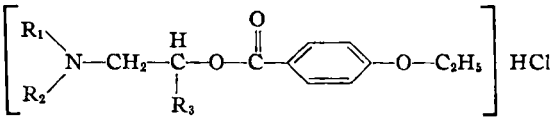
Irritancy.—The local tissue irritant properties of these compounds were estimated following the intradermal injection into the clipped abdominal skin of rabbits. The solutions were made isoosmotic with sodium chloride. A minimum of three concentrations of each compound spaced at 0.3-logarithmic intervals were used and each concentration was injected into six rabbits. The relative degree of irritation was estimated from the amount of local extravasation of intravenously administered trypan blue (7) and reported as the concentration resulting in an irritant score of four (threshold $irritant\ concentration_4$) (4).

Antispasmodic Activity.—The *in vitro* antispasmodic activity was determined by the ability of the drugs to overcome the spasmogenic action of acetylcholine on the isolated rabbit ileum (8). Each segment of ileum was used for no more than six tests. A minimum of three concentrations (spaced at intervals of 0.3 log) were used for each compound; five to eight tests on different ilea were used for each concentration.

Molar Procaine Ratios.—Molar procaine ratios of the results obtained in all of the above tests were calculated by dividing the mean response to the experimental compound into the mean response to procaine, thus giving the ratio of activity of the compound to that of procaine (9). All molar concentrations and procaine ratios are calculated on the basis of the hydrochloride of the compounds.

Correlation Coefficients.—*r* Values were obtained on standard I.B.M. electronic data processing equip-

TABLE I.—CHEMICAL STRUCTURE AND LOCAL ANESTHETIC ACTIVITY OF ALKYLAMINO ETHANOL ESTERS OF PARAETHOXYBENZOATE HCl



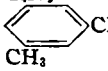
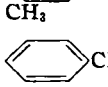
Compd. No.	Substituent Group R1	R2	R3	Local Anesthetic Activity: Rabbit Cornea, Threshold Anesthetic $Concn._{10}$ m*M*	Molar Procaine Ratio	Frog Sciatic Nerve, Anesthetic $Concn._{10}$ m*M*	Molar Procaine Ratio
I	CH_3	H	H	13.87	1.3	34.32	0.6
II	C_2H_5	H	H	17.18	1.1	26.57	0.8
III	iso C_3H_7	H	H	18.78	1.0	21.45	1.0
IV	iso C_4H_9	H	H	8.95	2.0	24.04	0.9
V	C_4H_9	H	H	25.86	0.7	7.96	2.7
VI		H	H	36.65	0.5	9.65	2.2
VII	CH_3	CH_3	CH_3	49.02	0.4	34.77	0.6
VIII		CH_3	H	43.18	0.4	49.76	0.4
Procaine HCl				17.96	1.0	21.19	1.0

TABLE II.—TOXICITY, IRRITANCY, AND ANTISPASMODIC ACTIVITY OF ALKYLAMINO ETHANOL ESTERS OF PARAETHOXYBENZOIC ACID

	Irritancy (Trypan Blue Test)		Intraperitoneal Toxicity in Mice			Antispasmodic Activity	
Compd. No.	Threshold Irritant Concn. mM	Molar Procaine Ratio	LD_{50} ± S.E. mg./Kg.	LD_{50} mM/Kg.	Molar Procaine Ratio	EC_{50} mM	Molar Procaine Ratio
I	38.5	4.95	319 ± 20	1.229	0.60	0.20	0.54
II	35.1	5.43	270 ± 10	0.987	0.75	0.39	0.28
III	31.6	6.03	229 ± 7	0.796	0.93	0.26	0.42
IV	29.5	6.46	285 ± 6	0.945	0.78	0.24	0.46
V	16.6	11.48	239 ± 10	0.792	0.93	0.14	0.76
VI	[a]	...	272 ± 11	0.810	0.91	0.14	0.79
VII	46.9	4.06	354 ± 8	1.231	0.60	0.25	0.44
VIII	17.27	11.04	627 ± 27	1.793	0.41	[a]	...
Procaine	190.6	1.0	202 ± 5	0.740	1.0	0.11	1.0

[a] Compounds too insoluble to acquire data for these tests.

ment using the statistic $r = Sx_1x_2/\sqrt{(Sx_1^2)(Sx_2^2)}$ with levels of significance determined using $n-2$ degrees of freedom (10).

RESULTS AND DISCUSSION

The chemical structures and physical properties of the compounds included in this series were reported by Millikan and Wade (2). The pharmacological activities are summarized in Tables I and II. The following structural-activity relationships appear to hold for the secondary amines of this series in which R_1 was varied. These compounds will subsequently be referred to as the homologous series.

The degree of irritancy as measured by the trypan blue test increased as the weight of the alkylamino substitution increased ($r = 0.8921$; $p < 5\%$). These data strongly suggest that there exists an inverse relationship between duration of corneal anesthesia and irritancy, since those compounds producing longer anesthetic activity on the cornea were least irritant ($r = 0.8966$; $p < 5\%$). Since irritation of the cornea increases lacrimation, it is possible that the decreased duration of action on the cornea is merely a reflection of the speed at which the anesthetic was washed out of the cornea. It appears also that the irritating qualities of these compounds were directly proportional to the speed of onset of action in the frog sciatic nerve ($r = 0.9263$; $p < 5\%$), and to their surface activity ($r = 0.9394$; $p < 5\%$). This would indicate that anesthetic compounds having the ability to lower surface tension would have greater penetrability through the nerve sheath. Luduena, *et al.* (11), found a highly significant positive correlation between surface activity and irritancy and postulated that the irritancy as measured by the trypan blue method might be due to a mechanism related to surface activity or to molecular interaction with protein receptors. Menkin (12) states, however, that tissue injury resulting from irritation causes leukotaxine to be liberated which results in increased capillary permeability and, in this test, increased extravasation of trypan blue.

Although secondary amines are generally considered more toxic and irritating than tertiary amines (13), it appears from this work that even tertiary nitrogen compounds having large alkyl substitutions on the nitrogen are highly irritant. Although the compound showing the least degree of irritancy by this test is a tertiary nitrogen compound, it does not approach procaine in this desireable quality.

As the lipophilic alkylamino portion of the secondary amines of the homologous series increased, the resulting compounds became more potent in inducing sciatic nerve blockade ($r = 0.8463$; $p < 5\%$) but tended to produce corneal anesthesia of a shorter duration ($r = 0.7555$). This finding indicates that lipoid solubility is relatively more important in the production of conduction block of intact sheathed nerves than it is in inducing blockade of the free nerve endings of the cornea. Luduena, Hoppe, and others (4, 5, 9, 14) in testing homologous series of local anesthetic compounds have repeatedly shown the positive correlation that exists between local anesthetic activity, toxicity, and irritancy with the length of the lipophilic side chain. Shaumann (15) concluded that there was a close relationship between conduction anesthesia and systemic toxicity but not between the latter and topical anesthesia.

Duration of anesthesia of secondary amino compounds is generally longer than the corresponding tertiary amines (13). These data confirm this since the concentration of anesthetic required to maintain corneal anesthesia 10 minutes in the rabbit was highest with the two tertiary amines of this series. The branched propanol derivative appears to be relatively less active in anesthetic potency than the ethanol derivatives. Suter (13) also refers to this relationship in local anesthetic compounds.

From the data acquired in these tests it appears that tertiary nitrogen compounds of this type are acutely less toxic than secondary amines (Table I). Although not statistically significant, there was a strong positive correlation between the systemic toxicity of the homologous secondary amines and the length of their alkylamino substituent ($r = 0.7567$). This finding agrees with previous reports (4, 5, 13).

No significant correlation appeared between molecular weight or other findings with the *in vitro* antispasmodic activities of these compounds. No compound of this series was of equal potency to procaine in this spasmolytic effect.

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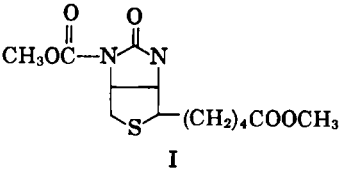
Chemical Reactivity of Models Related to a Proposed CO_2~Biotin Enzyme Complex II

By HOWARD J. SCHAEFFER and PARMATMA S. BHARGAVA

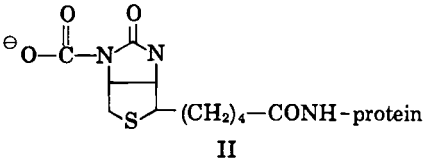
In order to gain more information about the mechanism by which biotin may function in biochemical carboxylations, a study of model compounds which are related to a proposed CO_2~biotin-enzyme complex was undertaken. The model compounds which were employed were the N-arylcarbamyl-2-imidazolidones and the N-alkyloxycarbonyl-2-imidazolidones. It has been shown that the carbonyl group of the model compounds is transferred to an attacking nucleophilic reagent. The significance of these reactions is discussed.

BIOTIN HAS BEEN the subject of numerous studies in which attempts were made to determine the biochemical functions of this vitamin; it has been observed that one of the actions of biotin is in biochemical carboxylation reactions. In the biosynthesis of fatty acids, it has been shown that a biotin-containing enzyme is involved in the carboxylation of acetyl CoA to malonyl CoA (1). Studies of fatty acid synthesis in certain cell-free extracts have demonstrated that carbon dioxide is activated by a biotin-containing enzyme and that the activated CO_2~biotin enzyme complex acts as the carboxylating reagent (2). In addition, biotin is necessary for the biosynthesis of purines since it has been observed (3) that biotin is required in the carboxylation of 5-aminoimidazole ribotide to 5-aminoimidazole-4-carboxylic acid ribotide, which after several further reactions is converted into inosinic acid.

A theory on the mechanism of biotin action has been proposed by Lynen and his co-workers from work on β-methylcrotonyl CoA carboxylase (2). These investigators found that this enzyme could not only carboxylate β-methylcrotonyl CoA but in addition could utilize free D-biotin as a substrate to give an unstable carboxybiotin. The unstable product was not isolated but after treatment with diazomethane gave the methyl ester of N-carbomethoxybiotin (I) (4). This result has led to the suggestion that the chemical structure of the CO_2~biotin enzyme complex may be represented by II.



I



II

Recently, Wakil and Waite have presented evidence that for acetyl CoA carboxylase the uriedo carbonyl group of the enzyme-bound biotin is the active carbon and that it is involved in the carboxylation reactions (5, 6). For example, it was found that in the presence of adenosine triphosphate and Mn^{2+}, acetyl CoA carboxylase incorporated C^{14}-bicarbonate to form a $C^{14}O_2$~biotin enzyme complex. Hydrolysis of this complex gave free C^{14}-biotin in which the carbon-14 was located in the ureido carbon atom (5, 6). In addition, it was also shown that growth of *Lactobacillus arabinosus* on limiting amounts of ureido-C^{14}-biotin resulted in the loss of most of the radioactivity from the biotin. Although this data is in conflict with that of Melville, Pierce, and Partridge (7), it was shown that the previous investigators had employed

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