Experimental

Reaction of Dibenzylphosphoryl Chloride with Amino Acid Esters. (a).—Amino acid ester hydrochloride (1 mole) was suspended in dry chloroform and dry ammonia was then passed into the solution under cooling (ice–salt-bath at 0°) till saturation (about 20 minutes). Ammonium chloride was filtered off and washed with fresh chloroform. The excess ammonia was removed by passing dry nitrogen into the cold solution. To the ammonia-free chloroform solution at 0° was added triethylamine (1 mole) followed by slow addition, with stirring, of DBPCl (1 mole) (freshly prepared from dibenzyl hydrogen phosphite and sulfuryl chloride according to Atherton, Howard and Todd.[16] All the apparatus and reagents were protected from moisture. After the addition had been completed (about 20 minutes), the reaction mixture was taken out of the cooling bath and stirring continued for 30 minutes, at which time the white precipitate separated out. Next day the precipitate was filtered off and the filtrate was washed successively with water, N-hydrochloric acid, 10% sodium hydrogen carbonate and water, then dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was dissolved in benzene and the benzene evaporated. After cooling in the ice-box at −18° the residue crystallized in needles and was recrystallized from benzene and petroleum ether. The results are presented in Table I.

(b).—To a suspension of 1 mole of amino acid ester hydrochloride in chloroform was added at 0°, slowly with stirring, DBPCl (1 mole) in the presence of 2 moles of triethylamine. The compound was isolated as in (a).

Preparation of N-Phosphoryl Amino Acid Esters.—One gram of dibenzylphosphoryl amino acid ester was subjected to hydrogenolysis in dry methanol in the presence of about 0.04 g. of palladium oxide. After the reaction had been completed (in 1 hour), the catalyst was filtered off and the solvent removed under reduced pressure in a water-bath of about 50°. The residue crystallized on cooling and was recrystallized from methanol and ether. The results are presented in Table I.

Preparation of DL-Phenylalanine Methyl Ester Phosphate.—One gram of DL-phenylalanine methyl ester hydrochloride dissolved in dry methanol was neutralized under cooling with freshly prepared sodium methoxide using phenolphthalein as indicator. The precipitate of sodium chloride was filtered off. To the filtrate was added about 0.4 ml. (in excess) of phosphoric acid drop by drop, whereby a white precipitate separated out. Ether was added to obtain more crystals. The crude product was recrystallized from methanol and ether in needles; m.p. 184–185° (decomposed with gas evolution).

Anal. Calcd. for $C_{10}H_{16}O_6NP$ (277.2): N, 5.05; P, 11.2. Found: N, 4.91; P, 11.3.

Acknowledgment.—The author is deeply grateful to Dr. Robert E. Eakin for his encouragement and very helpful interest in this work. Thanks are also due to Dr. Roger J. Williams and Dr. Lorene L. Rogers for their valuable advice.

AUSTIN 12, TEXAS

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTH TEXAS STATE COLLEGE]

Antitubercular Studies. V. 4-Aminobenzamides and 4-Aminobenzenesulfonamides

BY PRICE TRUITT, GEORGE SAMMONS[1] AND DAVID ZACHRY[1]

RECEIVED JULY 14, 1952

Series of 1-(4-nitrobenzoyl)- and 1-(4-aminobenzoyl)-4-alkylpiperidines, 1-(4-nitrobenzenesulfonyl)-1-(4-acetylaminobenzenesulfonyl-1-(4-aminobenzenesulfonyl)-4-alkylpiperidines and 1,2,3,4-tetrahydroquinolines are described. A partial evaluation of the antituberculous activity of these amides is given. One derivative, 1-(4-aminobenzoyl)-4-(1-octyl)-piperidine, is rather active against the tubercle bacilli.

Recent reports from this Laboratory[2,3] have indicated that certain substances which contain the piperidine or 4-alkylpiperidine moiety may possess limited antitubercular activity. These compounds include 1-phenacylpiperidines and 1-diphenylmethylpiperidines.

1-(4-Aminophenyl)-piperidine[4] is reported to be active against the tubercle bacilli. Also, 4-nitrobenzoylpiperidine[5] has been found to possess a slight antistreptococcal and antipneumococcal activity. 1-(4-Aminobenzoyl)-piperidine[6] has been prepared and tested for local anesthetic activity. The 4-nitro- and 4-aminobenzoyl-4-alkylpiperidines are not described.

Thus, it seemed desirable to prepare a number of the 1-(4-nitrobenzoyl)-4-alkylpiperidines and 1-(4-aminobenzoyl)-4-alkylpiperidines in which there was some range in size of the alkyl group. Of particular interest were the preparations where this alkyl group contained from five to nine carbons.

To further examine the influence of the 1-(4-alkylpiperidyl) radical on antituberculous activity, a number of 1-(4-aminobenzenesulfonyl)-4-alkylpiperidines were prepared to test for antitubercular activity. Sargent and Small[7] synthesized 1-(4-acetylaminobenzenesulfonyl) - 1,2,3,4 - tetrahydroquinoline and certain methoxy substituted tetrahydroquinolines. These compounds were not tested for antitubercular activity. For this reason several methyl substituted tetrahydroquinoline derivatives were included in this study.

The condensation of 4-nitrobenzoyl chloride with the various 4-alkylpiperidines proceeded with ease and the subsequent reduction with iron gave good yields of the expected amines.

The preparation of 4-aminobenzenesulfonyl derivatives was achieved by two routes. 4-Nitrobenzenesulfonyl chloride was treated with the secondary amines and the resultant amide reduced with iron and acetic acid. The yields with catalytic reduction were not as satisfactory as with iron reduction. The second procedure involved the hydrolysis of the 1-(4-acetylaminobenzenesulfonyl) derivative of the 4-alkylpiperidines and tetrahydroquinolines. The latter route gave much better yields but the purifications were somewhat more tedious.

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TABLE I

SULFONAMIDES R—C$_6$H$_4$—SO$_2$—R′

	R	R′	M.p., °C.	Yield, %	Method	Solvent[b]	Empirical formula	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found
1	NO_2	(1-Amyl) P[a]	210–212	83	I	A	$C_{16}H_{24}N_2O_4S$	8.23	8.11	9.41	9.58
2	NO_2	(1-Hexyl) P	133–134	75	II	B	$C_{17}H_{26}N_2O_4S$	7.90	7.80	9.04	
3	NO_2	(1-Octyl) P	123–124	72	I	B	$C_{19}H_{30}N_2O_4S$	7.33	7.39	8.37	
4	NO_2	(1-Nonyl) P	129–130	88	I	C	$C_{20}H_{32}N_2O_4S$	7.07	7.08	8.07	8.32
5	AcNH	(Methyl) P	142–143	89	II	A	$C_{14}H_{20}N_2O_3S$	9.45	9.66	10.71	10.99
6	AcNH	(1-Amyl) P	157–158	94	II	C	$C_{18}H_{28}N_2O_3S$	7.95	8.20	9.08	
7	AcNH	(1-Hexyl) P	149–150	87	I	A	$C_{19}H_{30}N_2O_3S$	7.65	7.90	8.74	8.93
8	AcNH	(1-Octyl) P	150–152	91	I	A–B	$C_{21}H_{34}N_2O_3S$	7.12	7.38	8.12	
9	AcNH	(1-Nonyl) P	150–152	93	I	C	$C_{22}H_{36}N_2O_3S$	6.86	7.23	7.83	
10	$^-NH_2$	(Methyl) P	107–109	70	III	B–D	$C_{12}H_{18}N_2O_2S$	11.02	11.12	12.59	
11	$^-NH_2$	(1-Amyl) P	149–150	87	III, IV	B	$C_{16}H_{26}N_2O_2S$	9.03	9.27	10.32	10.47
12	NH_2	(1-Hexyl) P	123–124	82	III, IV	B	$C_{17}H_{28}N_2O_2S$	8.64	8.91	9.87	10.06
13	NH_2	(1-Octyl) P	112–114	90	III, IV	B	$C_{19}H_{32}N_2O_2S$	7.95	8.23	9.08	
14	NH_2	(1-Nonyl) P	122–125	89	IV	C	$C_{20}H_{34}N_2O_2S$	7.65	7.88	8.72	
15	NO_2	6-Methyl Q[c]	135–136	30	I	B–C	$C_{16}H_{16}N_2O_4S$	8.43	8.39	9.62	9.75
16	NO_2	7-Methyl Q	130–131	41	II	C	$C_{16}H_{16}N_2O_4S$	8.43	8.52	9.62	
17	NO_2	8-Methyl Q	145–147	26	II	C	$C_{16}H_{16}N_2O_4S$	8.43	8.67	9.62	
18	AcNH	6-Methyl Q	192–193	28	I	A–C	$C_{18}H_{20}N_2O_3S$	8.13	8.34	9.29	9.53
19	AcNH	7-Methyl Q	143–144	42	II	A–E	$C_{16}H_{20}N_2O_3S$	8.13	8.11	9.29	
20	AcNH	8-Methyl Q	97–99	32	I	A–E	$C_{18}H_{20}N_2O_3S$	8.13	8.36	9.29	9.34
21	$^-NH_2$	6-Methyl Q	176–177	82	I	B–D	$C_{16}H_{18}N_2O_2S$	9.25	9.53	10.60	10.81
22	NH_2	7-Methyl Q	110–111	41	I	B	$C_{16}H_{18}N_2O_2S$	9.25	9.39	10.60	
23	NH_2	8-Methyl Q	114–115	32	II	B–D	$C_{16}H_{18}N_2O_2S$	9.25	9.42	10.60	

[a] P is to represent 1-piperidyl residue with the alkyl group attached at the 4-position. [b] Solvents for recrystallization: A, dioxane; B, water; C, acetone; D, alcohol; E, ether; F, methanol. [c] Q represents 1-(1,2,3,4-tetrahydroquinolyl) residue.

TABLE II

BENZAMIDES R—C$_6$H$_4$—CO—N⟨S⟩—R′

No.	R	R′	M.p., °C.	Yield, %	Method	Solvent[a]	Empirical formula	Analyses, % Nitrogen Calcd.	Analyses, % Nitrogen Found
1	NO_2	CH_3	97	75	V	D	$C_{13}H_{16}N_2O_3$	11.30	11.42
2	NO_2	1-C_5H_{11}	45–46	89	V	B–F	$C_{17}H_{24}N_2O_3$	9.22	9.23
3	NO_2	1-C_6H_{13}	91	92	V	B	$C_{18}H_{26}N_2O_3$	8.85	9.04
4	NO_2	1-C_8H_{17}	60	85	V	D	$C_{20}H_{30}N_2O_3$	8.08	8.05
5	NO_2	1-C_9H_{19}	59.5–60	81	V	D	$C_{21}H_{32}N_2O_3$	7.78	7.93
6	NH_2	CH_3	89	75	IV	B–F	$C_{13}H_{18}N_2O$	12.91	12.93
7	NH_2	1-C_5H_{11}	129	76	IV	C–E	$C_{17}H_{26}N_2O$	10.22	10.39
8	NH_2	1-C_6H_{13}	100	80	IV	C–E	$C_{18}H_{28}N_2O$	9.77	9.97
9	NH_2	1-C_8H_{17}	75–75.5	82	IV	F	$C_{20}H_{32}N_2O$	8.86	8.93
10	NH_2	1-C_9H_{19}	60–61	85	IV	B–F	$C_{21}H_{34}N_2O$	8.49	8.47

[a] See Table I for solvent designations.

Physiological Results

Although most of the compounds reported in this research were inactive in the antitubercular test in the presence of bovine serum, compound number 9 (Table II) was active at 0.078 mg. % and was also active in the presence of serum. This compound, 1-(4-aminobenzoyl)-4-(1-octyl)-piperidine, was amebistatic at 1:500 dilution and showed moderate activity against *Neisseria catarrhalis*, *Streptococcus hemolyticus* and *Brucella suis*.

The sulfones were inactive in the presence of serum and were inactive in the amebiasis test.

Experimental

Method I. 1-(4-Acetylaminobenzenesulfonyl)-4-methylpiperidine.—A mixture of 6.9 g. (0.03 mole) of 4-acetylaminobenzenesulfonyl chloride, 3.0 g. (0.03 mole) of 4-methylpiperidine, 75 ml. of dioxane and 1.2 g. (0.03 mole) of sodium hydroxide (in 4 ml. of water) was refluxed for several hours. The hot reaction mixture was filtered, the filtrate treated with carbon black and clarified. The filtrate was made turbid with water at 70° and cooled overnight in the ice-box. The product was recrystallized.

Method II.—Same as method I except sodium bicarbonate replaced the sodium hydroxide for neutralization of the hydrogen chloride as it was released.

Method III. Hydrolysis of Acetylamino Derivatives.—A mixture of 3.5 g. (0.01 mole) of 1-(4-acetylaminobenzenesulfonyl)-4-(1-amyl)-piperidine and 100 ml. of 10% hydrochloric acid in 50% dioxane–water solution was refluxed for four hours. The hot mixture was filtered and the filtrate cooled. The precipitate was recrystallized from a water–dioxane mixture.

Method IV. Reduction of the Nitro Derivatives.—A mixture of 5 g. of nitro compound, 15 g. of iron powder and 100 ml. of 50% aqueous dioxane was refluxed for eight hours. The reaction mixture was filtered hot and the filtrate concentrated to 60 ml. Traces of nitro compound were removed by extraction of the amine with hydrochloric acid and subsequent neutralization of the salt.

Method V. 1-(4-Nitrobenzoyl)-4-(1-octyl)-piperidine.—A solution of 9.4 g. (0.05 mole) of 4-nitrobenzoyl chloride

and 20 g. (0.102 mole of 4-1-octyl)-piperidine in 200 ml. of ether was warmed to reflux for two hours. The amine hydrochloride was removed by filtration and the ether evaporated from the filtrate. White crystals were recovered from the filtrate and recrystallized.

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[CONTRIBUTION NO. 1666 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

The Enzyme-Inhibitor Dissociation Constants of α-Chymotrypsin and Several Series of Bifunctional Competitive Inhibitors[1]

BY H. T. HUANG AND CARL NIEMANN[2]

RECEIVED MARCH 28, 1952

The enzyme-inhibitor dissociation constants of α-chymotrypsin and several series of competitive inhibitors, *i.e.*, benzoic acid, phenylacetic acid, β-phenylpropionic acid, γ-phenylbutyric acid; the corresponding amides; β-indoleacetic acid, β-(β-indole)-propionic acid, γ-(β-indole)-butyric acid; β-(β-indole)-propionamide; and acetanilide, have been determined in aqueous media at 25° and *p*H 7.9 in the presence of a 0.02 *M* tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer. A comparison of the results obtained in this study with those reported previously has shown that the affinity of α-chymotrypsin for certain negatively charged competitive inhibitors may be determined in part by the nature of the buffer present in the reaction system. The relation between the structure of certain of the above competitive inhibitors has also been considered and attention has been called to the possibility that each of the above inhibitors may combine with the active site of the enzyme in more than one way.

α-Amino acids or simple functional derivatives of these compounds that are specific substrates or competitive inhibitors of α-chymotrypsin may be described by the general formula $R_1CHR_2R_3$, where R_1, R_2 and R_3 are the three prominent structural features of these molecules, *i.e.*, the α-amino or acylamino group, the α-amino acid side chain and the carboxyl group or a functional derivative thereof.[3,4] It has been suggested[4] that the above specific substrates and competitive inhibitors may combine with the enzyme *via* an interaction involving the three groups R_1, R_2 and R_3, and a set of centers, ρ_1, ρ_2 and ρ_3 which are assumed to be a characteristic feature of the catalytically active site of the enzyme and which present a complementary aspect to molecules containing a particular set of R groups disposed in either one of the two possible configurations resulting from the asymmetry of the α-carbon atom present in these molecules. The extent to which any given compound of the general formula $R_1CHR_2R_3$ will be bonded to the active site of the enzyme will primarily depend upon the degree of complementariness originally obtaining between the molecule and the asymmetric catalytic surface, and secondarily, by the ability of both the combining molecule and the active site to alter their respective aspects to improve the closeness of fit during the course of the combining process.

The results of studies conducted in these laboratories on the behavior of specific substrates and competitive inhibitors of α-chymotrypsin have, so far, been consistent with the above view.[4-8] It may therefore be inferred that variations in the nature of disposition of the R groups will be reflected by changes in the corresponding enzyme-inhibitor or enzyme-substrate dissociation constants even in the case of bifunctional compounds where one of the R groups is replaced by a sterically unimportant hydrogen atom. Thus with bifunctional competitive inhibitors of the general formula $R_1(CH_2)_nR_2$, $R_1(CH_2)_nR_3$ and $R_2(CH_2)_nR_3$ it should be possible not only to determine the optimum value of n for the best fit to the complementary surface at the active site of the enzyme but also to make a rational estimate of the relative importance of various R groups in the over-all binding process.

In this study we have chosen for examination a group of bifunctional competitive inhibitors $R_2(CH_2)_nR_3$ where R_2 = benzyl or β-indolylmethyl and R_3 = carboxylate or carbamido. When this work was initiated, one member of this group of compounds, *i.e.*, β-phenylpropionic acid, had already been studied[9] although the data were inadequate to establish the nature of the inhibition. The present experiments were all performed at 25° and *p*H 7.9 in aqueous solutions 0.02 *M* with respect to the amine component of a tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer. In all cases the *p*H of the medium remained within the desired range, *i.e.*, 7.9 ± 0.2 even when the inhibitors containing a carboxylate group were introduced in the form of their sodium salts. Except where noted nicotinyl-L-tryptophanamide was used as the specific substrate with an enzyme concentration corresponding to 0.208 mg. of protein nitrogen per ml. Since in every instance the hydrolytic reaction was limited to within 30% hydrolysis all of the systems investigated can be formulated in terms of equations (1) and (2) where $K_S = (k_2 + k_3)/k_1$ and $K_I = k_5/k_4$.[10] The

$$E_f + S_f \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E_f + P_{1f} + P_{2f} \qquad (1)$$

$$E_f + I_f \underset{k_5}{\overset{k_4}{\rightleftharpoons}} EI \qquad (2)$$

(1) Supported in part by a grant from Eli Lilly and Company.
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