considerable production experience, and sufficient in-process controls exist, low dosage variation in individual compressed tablets may be achieved. Modern quality control concepts emphasize that quality should be built into the product rather than tested in. The results of this study indicate what can be achieved by following this concept.

It should be noted that the tablets studied were round, discoid shape, containing more than 20% active ingredient, and the assay procedures involved have a high degree of reliability. Further studies should be performed with tablets of irregular shapes and lower percentage of active ingredient. It would also be interesting to examine the effects of assay procedures of greater variability on this type of study.

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Notes

Synthesis of Selected Amides of Mono- and Bis(carboxypiperidino)alkanes

By RONALD P. QUINTANA and WAYNE A. SHRADER

The synthesis of selected amides of mono- and bis(carboxypiperidino)alkanes is reported.

THE SYNTHESIS of selected amides of mono- and bis(carboxypiperidino)alkanes has been undertaken to expand significantly those series of compounds previously reported (1–4). The compounds described in this communication were chosen on the basis of enzymodynamic studies, wherein particularly interesting variations in biochemical response relative to modifications in chemical constitution were noted and parallel effects upon surface and interfacial tension were observed. The fact that in some instances, among the monomethyl-, dimethyl-, monoethyl-, and diethylcarboxamido derivatives, the latter was the only one effecting appreciable or even significant inhibition in isolated human plasma "pseudo"-cholinesterase systems (5), prompted us to explore variations in the amide group in terms of steric factors and the electrophilic character of the carbonyl carbon. Since the mono- and bis[3-(N,N-diethylcarboxamido)piperidino]-ethanes and decanes reflected perhaps the most interesting relationships between molecular constitution, cholinesterase inhibition (5), and surface and interfacial tension (6), these four analogs were selected as model molecules for this study.

In general, the synthetic procedures employed in this investigation were those utilized by Lassio and co-workers (1, 2, 4). The compounds 1-ethyl-3-(N,N-diethylcarboxamido)-1,2,5,6-tetrahydropyridine hydrochloride (XVIII) and 1-decyl-3-(N,N-diethylcarboxamido)-1,2,5,6-tetrahydropyridine hydrochloride (XIX) were synthesized by sodium borohydride reduction of the appropriate pyridinium salts, a method employed by Lyle and co-workers (7) in the preparation of arecoline (methyl 1-methyl-1,2,5,6-tetrahydronicotinate) and methyl 1-methyl-1,2 5,6-tetrahydroisonicotinate.

The position of the double bond in compounds XVIII and XIX was confirmed by a comparison of the ultraviolet and infrared spectra of these compounds with the corresponding spectra of 1-methyl-3-(N,N-diethylcarboxamido)-1,2,5,6-tetrahydropyridine hydrochloride (XX) (1), prepared from arecoline (see Table I). Lyle's recent interpretation (8) of the mechanism involved in the sodium borohydride reduction of pyridinium ions provides further substantiation in this regard.

EXPERIMENTAL

4-(N,N-Diethylcarboxamido)pyridine (I).—This

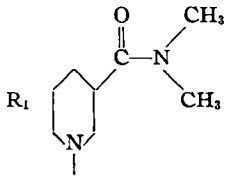
TABLE I.—ULTRAVIOLET AND INFRARED SPECTRA

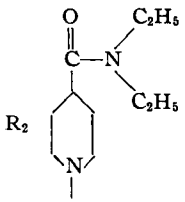
Compd. No.	Infrared Spectra, μ[a]	Ultraviolet Spectra[b] $\lambda_{max.}$ mμ	ϵ
XVIII	5.99 (m),[c] 6.20 (s)[d,e]	206	7800
XIX	5.99 (m),[c] 6.20 (s)[d,e]	206	8100
XX[f]	5.99 (m),[c] 6.20 (s)[d,e]	206	7880

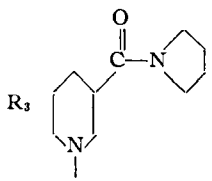
[a] Infrared spectra were run in chloroform. [b] Ultraviolet spectra were run in ethanol by Huffman Microanalytical Laboratories, Wheatridge, Colo. [c] Attributed to the double bond. [d] Attributed to the conjugated amide carbonyl function. [e] Band is broad. [f] Lasslo, *et al.* (1).

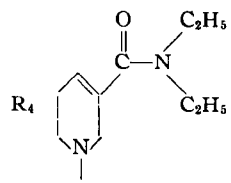
Received January 31, 1963, from the Department of Pharmaceutical and Medicinal Chemistry, College of Pharmacy, University of Tennessee, Memphis.
Accepted for publication April 10, 1963.
The authors acknowledge valuable discussions with Dr. Andrew Lasslo, and the technical assistance of Mr. Thomas H. Bratten, Jr., NSF Undergraduate Research Fellow.
This investigation was supported by Grant MY-2072/MH-04379 from the National Institute of Mental Health, U. S. Public Health Service, Bethesda, Md., and a grant from the Geschickter Fund for Medical Research, Inc.

TABLE II.—COMPOUNDS PREPARED BY PROCEDURES DESCRIBED









Compd. No.	Alkane	Substitution	Prepn., Method	Yield, %[b]	Salt	Recrystn. Solvent[c]	M.p., °C.[d]	Analyses[a] C, % Calcd.	C, % Found	H, % Calcd.	H, % Found	Br, % Calcd.	Br, % Found	Cl, % Calcd.	Cl, % Found	N, % Calcd.	N, % Found
III	Ethane	1-(R_1)	A	71.6	HBr	E-EA	218.0–218.5	45.29	45.47	7.98	7.81	30.13	30.13	...	...	10.56	10.58
V	Decane	1-(R_1)	A	66.4	HBr	E-EA	167.0–167.3	57.28	57.20	9.88	10.09	21.17	21.00	...	...	7.42	7.56
VI	Ethane	1,2-Bis(R_1)	B	50.2	2HBr	M-EA	268.6–268.9[e]	43.21	43.33	7.25	7.33	31.94	32.06	...	...	11.20	11.40
VII	Decane	1,10-Bis(R_1)	B	73.7	2HBr	E-EA	196.0–199.0	50.98	50.78	8.56	8.49	26.09	26.22	...	...	9.15	8.90
VIII	Ethane	1-(R_2)	A	60.8	HBr[f]	E-EA	220.8–221.0	49.15	49.15	8.59	8.31	27.25	27.20	...	...	9.55	9.51
IX	Decane	1-(R_2)	A	68.7	HBr	E-EA	200.0–200.8[e]	59.24	59.39	10.19	10.12	19.71	19.65	...	...	6.91	6.86
X	Ethane	1,2-Bis(R_2)	B	48.9	2HBr	M	300.0–300.5[e]	47.49	47.44	7.97	8.02	28.72	28.84	...	...	10.07	9.88
XI	Decane	1,10-Bis(R_2)	B	65.8	2HBr	E-EA	253.0–253.2[e]	53.89	53.68	9.05	8.80	23.90	23.77	...	...	8.38	8.33
XII	Ethane	1-(R_3)	A	74.2	HBr	E-EA	163.0–163.7	49.49	49.38	7.96	8.09	27.44	27.20	...	...	9.62	9.51
XIII	Decane	1-(R_3)	A	87.4	HBr	E-EA	161.8–162.4	59.54	59.70	9.74	9.49	19.81	19.55	...	...	6.94	6.90
XIV	Ethane	1,2-Bis(R_3)	B	15.0	2HBr	E-EA	268.7–269.3[e]	47.83	47.60	7.30	7.50	28.93	28.74	...	...	10.14	10.42
XV	Decane	1,10-Bis(R_3)	B	39.2	2HBr	E-EA	232.0–234.0[e]	54.21	54.19	8.49	8.66	24.05	24.15	...	...	8.43	8.62
XVIII	Ethane[g]	1-(R_4)	C	29.5	HCl[h]	E-EA	116.5–117.0	58.40	58.51	9.39	9.45	...	...	14.37	14.34	11.35	11.20
XIX	Decane[g]	1-(R_4)	C	15.8	HCl	EA-EE	132.7–133.3	66.91	66.72	10.95	11.02	...	...	9.88	10.06	7.80	7.85

[a] Analyses by Drs. G. Weiler and F. B. Strauss, Oxford, England. [b] Crude yield. [c] E, Ethanol; EA, ethyl acetate; EE, ethyl ether; M, methanol. [d] Melting points uncorrected. [e] Decomposition. [f] Lit. (11) m.p. for HI 124–126°. [g] The synthesis of the corresponding bis-substituted derivative is in progress. [h] Free base, b.p. 102–104°/0.27 mm. Hg.

compound was prepared from isonicotinic acid. The product distilled at 94°/0.15 mm. Hg, uncorrected (lit. (9) b.p. 123 to 123.5°/3 mm. Hg, corrected; lit. (10) b.p. 109°/0.5 mm. Hg, uncorrected).

3-(Pyrrolidinoformyl)pyridine (II).—This compound was prepared from nicotinic acid. The product distilled at 114°/0.04 mm. Hg, uncorrected (lit. (10) b.p. 131–133°/0.3 mm. Hg, uncorrected).

The compounds listed in Table II were prepared by the following procedures.

Procedure A: **1-Ethyl-3-(N,N-dimethylcarboxamido)piperidine Hydrobromide** (III).—N,N-Dimethylnicotinamide (25 Gm., 0.167 mole) (IV) and ethyl bromide (48.3 Gm., 0.443 mole) were dissolved in 200–300 ml. anhydrous benzene and refluxed for 48–60 hours. The precipitate formed in the reaction was separated and was dissolved in 150–200 ml. distilled water. The aqueous solution was washed with benzene, treated with charcoal, and filtered. The filtrate was subjected to hydrogenation (aqueous or hydroalcoholic solution) in the presence of 1.0 Gm. platinum oxide (Adams' catalyst) at maximum pressures of 45–50 p.s.i. After absorption of hydrogen ceased, the platinum oxide was filtered off and the solvent was removed by azeotropic distillation under reduced pressure with absolute ethanol and anhydrous benzene. The product was purified by recrystallization.

Procedure B: **1,2-Bis[3-(N,N-dimethylcarboxamido)piperidino]ethane Dihydrobromide** (VI).—N,N-Dimethylnicotinamide (52.1 Gm., 0.347 mole) (IV) and 1,2-dibromoethane (32.6 Gm., 0.174 mole) were dissolved in 200 ml. anhydrous benzene and refluxed for 53–62 hours. The precipitate formed during the reaction was then treated as described under *Procedure A*.

Procedure C: **1-Ethyl-3-(N,N-diethylcarboxamido)-1,2,5,6-tetrahydropyridine Hydrochloride** (XVIII).—1 - Ethyl - 3 - (N,N - diethylcarboxamido)pyridinium bromide (XVII) (2) was prepared by refluxing N,N-diethylnicotinamide (40 Gm., 0.224 mole) (XVI) and ethyl bromide (61 Gm., 0.560 mole) in 250 ml. anhydrous benzene for 48 hours. Excess alkyl halide and solvent were removed by decantation. The residual oil was dissolved in 150 ml. water, the aqueous solution was washed with benzene, and the water was removed under reduced pressure. Last traces of moisture were removed by azeotropic distillation with benzene. The oily product (60 Gm., 0.209 mole) was dissolved in 400 ml. dry methanol and cooled in an ice bath. Sodium borohydride (31.7 Gm., 0.838 mole) was added with stirring to the cold solution over 30 minutes; subsequently the solution was stirred an additional 30 minutes. Methanol was removed by distillation under reduced pressure to give a spongy yellow residue which was dissolved in 230 ml. water and saturated with potassium carbonate. The mixture was extracted with ether, the combined ether extracts were dried over anhydrous potassium carbonate, filtered, and the ether was removed by distillation. The oily residue was purified by conversion to the hydrochloride (anhydrous HCl in anhydrous ether) with or without prior fractionation under reduced pressure. Residual traces of moisture were removed from the oily hydrochloride by azeotropic distillation with benzene. The salt was then purified by recrystallization from ethanol-ethyl acetate.

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Sensitive and Reproducible Assay Method for Chymotrypsin

By B. L. KABACOFF, M. UMHEY, A. WOHLMAN, and S. AVAKIAN

A new assay method for chymotrypsin was developed. This method, based on N-acetyl-L-tyrosine ethyl ester (ATEE), was found to be ten times as sensitive as the proposed N.F. assay method for chymotrypsin. In the new method described, the residual substrate is measured by a single colorimetric determination. The method is convenient and highly reproducible.

THE CLASSICAL assay methods for proteolytic activity such as hemoglobin digestion, casein digestion, and milk clotting, although convenient and simple, lack specificity. N-acetyl-L-tyrosine ethyl ester (ATEE) is a specific substrate for chymotrypsin. The proposed N.F. method for the analysis of chymotrypsin, based on the work of Schwert and Takenaka (1), utilizes ATEE as the enzyme substrate. The rate of hydrolysis of this substrate in pH 7.0 buffer is followed spectrophotometrically at 237 mμ. Readings are taken every 30 seconds for a period of 4 minutes. The temperature must be kept within ±0.1° of 25.0°.

The above method has certain drawbacks which limit its practical application. Elaborate equipment is required to maintain the cell compartment of the spectrophotometer at the exact temperature speci-

Received January 29, 1963, from the Research Department, Wampole Laboratories, Stamford, Conn.
Accepted for publication April 30, 1963.