Synthesis of Carbamoylpiperidine-Type Cholinesterase Inhibitors

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A series of selected alkynyl, alkenyl, and aralkyl substituted carbamoylpiperidines was prepared for the evaluation of their effect upon isolated cholinesterase systems.

As an extension of previous synthetic work (1–4) on and biochemical (4, 5) evaluation of carbamoylpiperidine derivatives, a series of carbamoylpiperidines substituted with alkynyl, alkenyl, and aralkyl functions has been prepared.

Since the series of mono- and bis[3-(*N,N*-diethylcarbamoyl)piperidino]alkanes exhibited rather interesting inhibitory properties in human plasma cholinesterase systems (5), it was felt that a comparison of their biochemical response and molecular constitution with the activity and more rigid molecular configuration of the corresponding unsaturated derivatives would facilitate a more effective interpretation of relationships in terms of theoretical chemistry.

The alkynyl and alkenyl derivatives described in this paper (Fig. 1) were prepared utilizing methods previously reported (6–10). The structures of the geometric isomers (Fig. 1, III and V) were confirmed by infrared analysis. While *trans*-1,4-bis[3-(*N,N*-diethylcarbamoyl)piperidino]-2-butene (III) exhibited significant absorption in the 10.2–10.4 μ region, the corresponding *cis*-derivative (V) showed only weak absorption in this region. This observation is consistent with that reported by others (7, 11) and with our observations on the infrared spectra of *trans*- and *cis*-1,4-dichloro-2-butene.

Aralkyl derivatives (Fig. 1, X, XI, XIII) were prepared utilizing the method employed by Sakal (12) for some corresponding derivatives of 3-(*N,N*-dimethylcarbamoyloxy)pyridine, followed by partial hydrogenation (13) of the resulting bis-quaternary compounds.

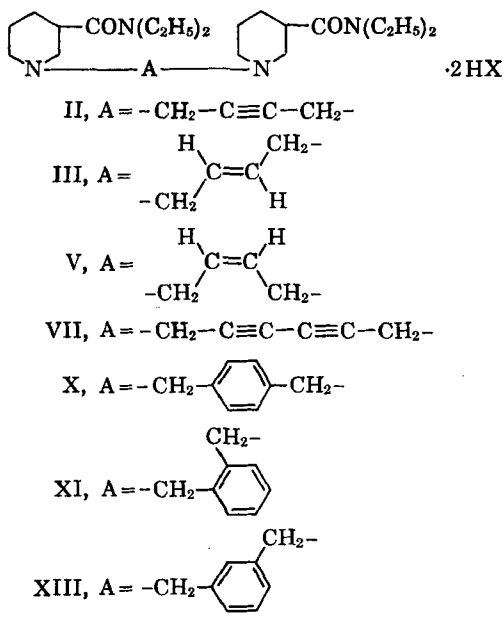

Fig. 1.—Carbamoylpiperidine derivatives.

EXPERIMENTAL[1]

N,N-Diethylnipecotamide (I).—This compound was prepared by hydrogenation of *N,N*-diethylnicotinamide according to the method of Lasslo and co-workers (1). The product distilled at 94°/0.15 mm., in accordance with the literature (1).

1,4-Bis[3-(N,N-diethylcarbamoyl)piperidino]-2-butyne Dihydrochloride (II).—*Method A.*—To a cold solution of *N,N*-diethylnipecotamide (I) (74.8 Gm., 0.406 mole) in 250 ml. of anhydrous benzene, 1,4-dichloro-2-butyne (Aldrich Chemical Co.) (12.5 Gm., 0.101 mole) was added slowly with stirring. The reaction mixture was stirred in the cold for 15 min., at room temperature for 3 hr., then refluxed for an additional 3 hr. It was cooled and treated with a cold concentrated potassium hydroxide solution. The benzene layer was separated and the aqueous layer extracted with benzene. The combined benzene extracts were dried over anhydrous magnesium sulfate, filtered, and the benzene removed by distillation under reduced pressure. The resulting oil was subjected to distillation *in vacuo*. After excess *N,N*-diethylnipecotamide was removed, the product (15.5 Gm., 36.6%) isolated by distillation under reduced pressure was a viscous yellow oil. The dihydrochloride salt was prepared by treating a solution of the oil in dry ether with a solution of dry HCl in dry ether. The product, recrystallized from absolute ethanol–anhydrous ether, melted at 235.8–236.0° dec.

Anal.—Calcd. for $C_{24}H_{44}Cl_2N_4O_2$: C, 58.64; H, 9.02; Cl, 14.43; N, 11.40. Found: C, 58.51; H, 8.95; Cl, 14.60; N, 11.40.

trans-1,4-Bis[3-(N,N-diethylcarbamoyl)piperidino]-2-butene Dihydrochloride (III).—This compound was prepared from *trans*-1,4-dichloro-2-butene (Eastman) by *Method A*, except that the reaction mixture, after being stirred at room temperature for 20 min., was refluxed for 5 hr. The yellow oily product (13.5 Gm., 31.8%), isolated by distillation under reduced pressure, was converted to the dihydrochloride; after recrystallization from absolute ethanol–anhydrous ether, it melted at 281.0–282.0° dec.

Anal.—Calcd. for $C_{24}H_{46}Cl_2N_4O_2$: C, 58.40; H, 9.39; Cl, 14.37; N, 11.35. Found: C, 58.26; H, 9.29; Cl, 14.25; N, 11.31.

cis-1,4-Dichloro-2-butene (IV).—This compound was prepared from *cis*-2-butene-1,4-diol[2] by the

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[1] Analyses by Drs. G. Weiler and F. B. Strauss, Oxford, England. Melting points and boiling points are uncorrected.

[2] This material was provided by Antara Chemicals, Division of General Aniline and Film Corp.

method of Amundsen and co-workers (14). The product distilled at 53–55°/15–16 mm., n_D^{25} 1.4865, in accordance with the literature (14).

cis - 1,4 - Bis[3 - (N,N - diethylcarbamoyl)piperidino]-2-butene Dihydrobromide (V).—This compound was prepared from *cis*-1,4-dichloro-2-butene (IV) by *Method A*, except that the reaction mixture, after being warmed to room temperature, was refluxed for 8 hr. The product (9.4 Gm., 13.5%), a yellow oil isolated by distillation under reduced pressure, was converted to the dihydrobromide, which was recrystallized from absolute ethanol–anhydrous ether. The salt melted at 222.0–223.0° dec.

Anal.—Calcd. for $C_{24}H_{46}Br_2N_4O_2$: C, 49.49; H, 7.96; Br, 27.44; N, 9.62. Found: C, 49.63; H, 8.15; Br, 27.22; N, 9.54.

1- Propargyl - 3 - (N,N - diethylcarbamoyl)-piperidine Hydrochloride (VI).—To a cold solution of *N,N*-diethylnipecotamide (I) (92.1 Gm., 0.500 mole) in 100 ml. of anhydrous ether, propargyl bromide (Aldrich Chemical Co.) (29.7 Gm., 0.250 mole) was added slowly with stirring. The reaction mixture was warmed to room temperature, then refluxed for 12 hr. The viscous liquid product was extracted thoroughly with ether, the combined ether extracts were filtered, and the ether removed by distillation. An oily product (22.2 Gm., 39.9%) was obtained by distillation of the residue, b.p. 114–116°/0.19–0.23 mm., n_D^{25} 1.4954. The hydrochloride salt, recrystallized from absolute ethanol–anhydrous ether, melted at 165.5–165.8°.

Anal.—Calcd. for $C_{13}H_{23}ClN_2O$: C, 60.33; H, 8.96; Cl, 13.70; N, 10.83. Found: C, 60.14; H, 9.09; Cl, 13.61; N, 10.80.

1, 6 - Bis[3 - (N,N - diethylcarbamoyl)piperidino]-2,4-hexadiyne Dihydrochloride (VII).—1-Propargyl-3-(*N,N*-diethylcarbamoyl)piperidine (free base of VI) (28.0 Gm., 0.126 mole) was dissolved in 104 ml. of a solution obtained by mixing 15 Gm. of cupric acetate monohydrate, 70 ml. of dry pyridine, and 70 ml. of methanol, and decanting the supernatent liquid (9). The reaction mixture was allowed to stand at room temperature for 3 days, after which it was treated with 30 ml. of concentrated ammonium hydroxide and 60 ml. of water. When no product came out of solution, the solvent was removed by distillation under reduced pressure. The residue was treated again with a cold mixture of 30 ml. of concentrated ammonium hydroxide and 60 ml. of water, saturated with sodium chloride, and extracted with ether. The combined ether extracts were dried over anhydrous magnesium sulfate, filtered, and treated with a solution of dry HCl in dry ether. The salt (10.9 Gm., 33.5%) which precipitated was recrystallized from absolute ethanol–anhydrous ether. In a melting point determination, the compound darkened progressively and became black at 187° dec.

Anal.—Calcd. for $C_{26}H_{44}Cl_2N_4O_2$: C, 60.57; H, 8.60; Cl, 13.75; N, 10.87. Found: C, 60.52; H, 8.80; Cl, 13.65; N, 10.62.

1 - Allyl - 3 - (N,N - diethylcarbamoyl)piperidine Hydrochloride (VIII).—To a cold stirred mixture of *N,N*-diethylnipecotamide (I) (64.3 Gm., 0.349 mole), anhydrous potassium carbonate (27.3 Gm., 0.198 mole), and 300 ml. of anhydrous benzene, allyl bromide (46.5 Gm., 0.385 mole) was added slowly. The reaction mixture was warmed gradually to room temperature, then refluxed for 15 hr. The mixture was cooled, treated with water, the benzene layer separated, and the aqueous layer extracted with benzene. The combined benzene extracts were dried over anhydrous magnesium sulfate, filtered, and the benzene removed by distillation under reduced pressure. The residue was distilled, giving 34.1 Gm. (43.6%) of an oil, b.p. 102–104°/0.32 mm., n_D^{25} 1.4865. The free base was converted to the hydrochloride, which was recrystallized from absolute ethanol–anhydrous ether. It melted at 125.0–125.5°.

Anal.—Calcd. for $C_{13}H_{25}ClN_2O$: C, 59.87; H, 9.66; Cl, 13.59; N, 10.74. Found: C, 59.75; H, 9.54; Cl, 13.42; N, 10.64.

1,4 - Xylylenebis[3 - (N,N - diethylcarbamoyl)-pyridinium Bromide] (IX).—To a solution of *N,N*-diethylnicotinamide (50.0 Gm., 0.281 mole) in 100 ml. of absolute ethanol, there was added α,α'-dibromo-*p*-xylene (29.6 Gm., 0.112 mole) in 200 ml. of acetone. The reaction mixture was refluxed for 6 hr., after which the white solid product (69.2 Gm., 99.6%) was obtained by filtration. After recrystallization from absolute ethanol, it melted at 262.3–262.8° dec.

Anal.—Calcd. for $C_{28}H_{36}Br_2N_4O_2$: C, 54.20; H, 5.85; Br, 25.76; N, 9.03. Found: C, 54.10; H, 6.00; Br, 25.81; N, 8.78.

1,4 - Xylylenebis[3 - (N,N - diethylcarbamoyl)-piperidine Hydrobromide] (X).—*Method B.*—1,4-Xylylenebis[3-(*N,N*-diethylcarbamoyl)pyridinium bromide] (IX) (69.2 Gm., 0.112 mole) was dissolved in 200 ml. of 50% ethanol and hydrogenated, after addition of 1 Gm. of platinum oxide, at a maximum pressure of 43 p.s.i. When absorption of hydrogen ceased, the catalyst was removed by filtration, and the solvent was removed by distillation under reduced pressure. The dry product (57.6 Gm., 81.2%), after recrystallization from absolute ethanol–ethyl acetate, melted at 284.2–284.8° dec.

Anal.—Calcd. for $C_{28}H_{48}Br_2N_4O_2$: C, 53.17; H, 7.65; Br, 25.27; N, 8.86. Found: C, 53.25; H, 7.86; Br, 25.00; N, 8.91.

1,2 - Xylylenebis[3 - (N,N - diethylcarbamoyl)-piperidine Hydrobromide] (XI).—This compound was prepared by *Method B* from 1,2-xylylenebis-[3-(*N,N*-diethylcarbamoyl)pyridinium bromide] (XII), an oil which could not be crystallized. Following hydrogenation and removal of the solvent, residual traces of moisture were removed by azeotropic distillation with benzene. The resulting oil was solidified by repeated trituration with anhydrous ether, followed by drying in a vacuum desiccator. The dry material (34.0 Gm., 48%) was recrystallized from absolute ethanol–anhydrous ether. The analytical sample melted at 214.5–215.0°.

Anal.—Calcd. for $C_{28}H_{48}Br_2N_4O_2$: C, 53.17; H, 7.65; Br, 25.27; N, 8.86. Found: C, 52.94; H, 7.56; Br, 25.30; N, 8.75.

1,3 - Xylylenebis[3-(N,N-diethylcarbamoyl)piperidine Hydrochloride] (XIII).—This compound was prepared by *Method B* from 1,3-xylylenebis[3-(*N,N* - diethylcarbamoyl)pyridinium bromide] (XIV), an oil which could not be crystallized. Following hydrogenation and removal of the solvent, the residual oil was treated with cold 40% potassium hydroxide, and the mixture was extracted with benzene. The benzene extract was dried over

anhydrous magnesium sulfate, filtered, and the benzene removed by distillation under reduced pressure. The oil remaining (48.3 Gm., 91.7%) was converted to the dihydrochloride which, after recrystallization from absolute ethanol–anhydrous ether, melted at 232.5–233.0°.

Anal.—Calcd. for $C_{28}H_{48}Cl_2N_4O_2$: C, 61.86; H, 8.90; Cl, 13.04; N, 10.31. Found: C, 61.60; H, 8.74; Cl, 12.79; N, 10.31.

REFERENCES

(1) Lasslo, A., Marine, W. M., and Waller, P. D., *J. Org. Chem.*, **21**, 958(1956).
(2) Lasslo, A., and Waller, P. D., *ibid.*, **22**, 837(1957).
(3) Quintana, R. P., and Shrader, W. A., THIS JOURNAL, **52**, 1186(1963).
(4) Beasley, J. G., Quintana, R. P., and Nelms, G. G., *J. Med. Chem.*, **7**, 698(1964).
(5) Lasslo, A., *et al.*, *ibid.*, **6**, 811(1963).
(6) Biel, J. H., and DiPierro, F., *J. Am. Chem. Soc.*, **80**, 4609(1958).
(7) Neumeyer, J. L., Cannon, J. G., and Buckley, J. P., *J. Med. Pharm. Chem.*, **5**, 784(1962).
(8) Neumeyer, J. L., and Cannon, J. G., THIS JOURNAL, **51**, 804(1962).
(9) Lutz, W. B., Lazarus, S., and Meltzer, R. I., *J. Org. Chem.*, **27**, 1695(1962).
(10) Sperber, N., *et al.*, *J. Am. Chem. Soc.*, **81**, 704(1959).
(11) Burgstahler, A. W., and Aiman, C. E., *J. Org. Chem.*, **25**, 489(1960).
(12) Sakal, E. H., U. S. pat. 2,662,890 (1953); through *Chem. Abstr.*, **49**, 1108g(1955).
(13) Lasslo, A., and Jordan, W. D., *J. Org. Chem.*, **21**, 799(1956).
(14) Amundsen, L. H., *et al.*, *J. Am. Chem. Soc.*, **73**, 2118 (1951).

Antimicrobial Properties of a Propylene Glycol Based Topical Therapeutic Agent

By I. OLITZKY

Propylene glycol is shown to have antimicrobial activity when used as a dermatological vehicle for fluocinolone acetonide.

THE ANTIMICROBIAL activity of propylene glycol (PG) has been studied in great detail with primary interest in air disinfection. Ancillary test tube studies of concentrated PG solutions have revealed significant antimicrobial activity with lower concentrations having virtually no effect. In studies conducted to determine minimal killing or inhibitory concentrations, the concentration–activity relationship was characterized by rather sharp end points. (1–4).

Because of its low toxicity and apparent lack of skin sensitizing properties (5), PG can be used safely in concentrated form as a vehicle for therapeutic agents for topical application. The purpose of the present study was to investigate the *in vitro* antimicrobial activity of a topical steroid preparation in which the active therapeutic ingredient is dissolved in 100% PG.

The microorganisms used were those which might be involved in primary or secondary infections of dermatological lesions. The addition of normal human serum to the test solutions was used to determine the effect of protein material on the antimicrobial activity.

EXPERIMENTAL

The solution[1] tested had the following composition: fluocinolone acetonide, 0.01 Gm.; citric acid, 0.01 Gm.; and propylene glycol, 100% *q.s.* 100.0 ml. [referred to as S-PG (steroid–propylene glycol)].

The microorganisms employed included *Escherichia coli*, *Mycobacterium balnei* (ATTC 11564), *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* (Group A), *Staphylococcus aureus* (phage type 80/81, penicillin resistant), *S. aureus*, (phage type 53, penicillin resistant), *S. aureus*, (phage type 42E, penicillin sensitive), *Candida albicans*, *Microe sporum canis*, *M. audouini*, *Trichophyton mentagrophytes*, *T. rubrum*, and *T. tonsurans*.

Bacteriostatic and Fungistatic Tests.—The bacterial cultures were grown in trypticase soy broth. Various dilutions of S-PG made in the same broth were seeded with 0.1-ml. amounts of 18–24-hr. cultures. The tubes were incubated at 35° and observed for 7 days.

The fungi were grown on Sabourad's agar. Plugs (7-mm. diameter) were cut from the confluent growth and dropped into tubes of Sabourad's broth containing various concentrations of S-PG. Incubation at 30° was continued for 21 days.

Bactericidal and Fungicidal Tests.—Based on the data gathered in preliminary studies, two solutions were selected for further tests. Solution *A* consisted of S-PG (90%) and normal human serum, NHS (10%), v/v. Solution *B* was made up of S-PG

TABLE I.—BACTERIOSTATIC AND FUNGISTATIC ACTIVITY OF A S-PG SOLUTION

Microorganisms	Growth in (% S-PG in v/v)	But Not in Broth,
E. coli	10	20
Ps. aeruginosa	10	20
P. vulgaris	10	20
Streptococcus pyogenes, group *A*	10	15
Staphylococcus aureus, 42E	20	30
S. aureus, 80/81	20	30
S. aureus, 53	20	30
Mycobaclerium balnei	4	5
C. albicans	10	20
Microsporum audouini	5	10
Microsporum canis	5	10
T. mentagrophytes	5	10
T. rubrum	5	10
T. tonsurans	5	10

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[1] Marketed as Synalar Solution (lot 1730133C) by Syntex Laboratories, Inc.