Antiviral Agents. II. Substituted Morpholinium Quaternary Salts

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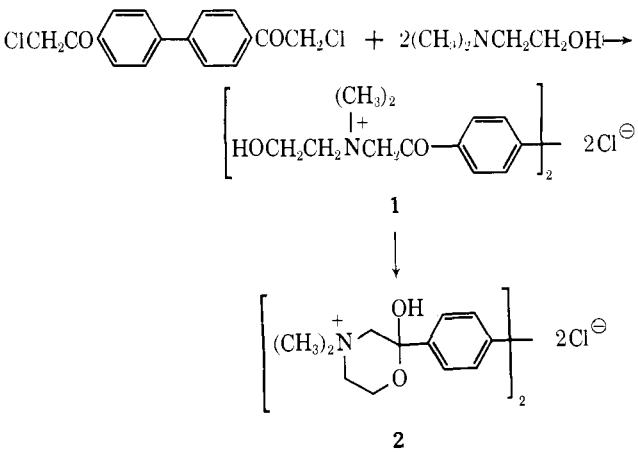
A series of substituted morpholinium quaternary salts was prepared by the reaction of substituted phenacyl halides with various dialkylaminoalkanols. The chemical and antiviral activities of these compounds are discussed.

Numerous workers have reported the formation of heterocyclic compounds from ethanolamine derivatives. For instance, Hill and Powell[1] reported a cyclization and dehydration of N-(3,4-dihydroxyphenacyl)-N-benzoylethanolamine to 2-(3,4-dihydroxyphenyl)-4-benzoyl-5,6-dihydro-1,4-oxazine. Morpholine ring formation from α-(β-hydroxyethylamino)deoxybenzoins was studied[2] and the observation was made that the monoethanolamino compounds either were open chain or involved a relatively labile ring–chain tautomerism. Additional studies in keto–hemiacetal tautomerism[3] of N-phenacyl-N-substituted ethanolamines established that compounds of this type exist largely in the cyclic hemiacetal forms. When N-ethylethanolamine was treated with an excess of phenacyl bromide, the quaternary derivative, 4-ethyl-2-hydroxy-4-phenacyl-2-phenylmorpholinium bromide, was obtained in high yield.

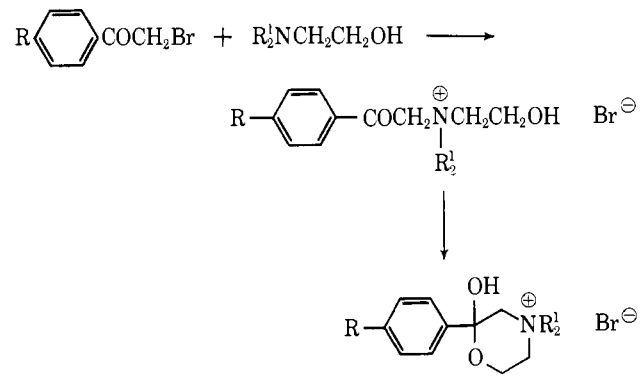
In the course of a program of testing compounds for anticholinesterase activity, Long and Schueler[4] prepared a series of bisphenacylammonium compounds. The reaction of α,α′-dichloro-4,4′-bisacetophenone with dimethylaminoethanol was formulated to give **1**.



Subsequent work[5] established that this product existed as the hemiacetal (**2**) rather than as the open-chain compound previously suggested. Although additional structures of this type were prepared,[6] the open-chain formula was consistently employed.

Since spontaneous ring closure of quaternary ammonium salts to morpholine derivatives does not appear to be well defined, we undertook a series of experiments to study the scope of this reaction more completely. It was found that ring formation followed the general scheme proposed by Cromwell and Tsou[3b] (Scheme I).

SCHEME I



Examination of the infrared spectra of the products provided a convenient method for establishing the structure, since the carbonyl absorption bonds disappeared and strong ether bands appeared in the 9–10-μ region. Additional confirmation was afforded by the ultraviolet absorption spectra. The maximum shifted from the 300–310-mμ range for the open-chain compounds to a characteristic maximum at 252 mμ for the hemiacetals. The compounds thus prepared are listed in Table I.

The requisite phenacyl halides were generally prepared by bromination of the corresponding ketones in carbon tetrachloride. In some instances, it was more convenient to prepare them by acylation of the aromatic compound with chloroacetyl chloride under normal Friedel–Crafts reaction conditions.[7]

The quaternizations were carried out by refluxing equimolar quantities of reactants in chloroform or acetone for 4 or 5 hr. The products usually precipitated as solids which could be separated and purified by recrystallization from isopropyl alcohol or an alcohol–ether mixture. Occasionally it was necessary to concentrate the solution until an oil or a solid separated. Decantation and trituration with alcohol or acetone hastened crystallization.

Formation of the morpholine ring system appeared to be quite general with the variety of dialkylamino

(1) R. Hill and G. Powell, *J. Am. Chem. Soc.*, **67**, 1462 (1945).
(2) R. E. Lutz, J. A. Freek, and R. S. Murphey, *ibid.*, **70**, 2015 (1948).
(3) (a) R. E. Lutz and R. H. Jordan, *ibid.*, **71**, 996 (1949); (b) N. H. Cromwell and K. C. Tsou, *ibid.*, **71**, 993 (1949).
(4) J. P. Long and F. W. Schueler, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 79 (1954).
(5) F. W. Schueler, *J. Pharmacol Exptl. Therap.*, **115**, 127 (1955).
(6) F. N. Marshall and J. P. Long, *ibid.*, **127**, 236 (1959).
(7) (a) C. C. Price, *Org. Reactions*, **3**, 1 (1946); (b) R. Simonoff and W. H. Hartung, *J. Am. Pharm. Assoc., Sci. Ed.*, **35**, 306 (1946).

TABLE I

No.	R¹	R²	R³	R⁴	R⁵	Mp, °C	Yield, %	Formula	C, % Calcd	C, % Found	H, % Calcd	H, % Found	N, % Calcd	N, % Found
3	Biphenyl	H	H	CH_3	CH_3	187–189	69	$C_{18}H_{22}BrNO_2$	59.35	59.28	6.09	6.37	3.85	3.49
4	Biphenyl	H	H	C_2H_5	C_2H_5	179–181	72	$C_{20}H_{26}BrNO_2$	61.23	61.02	6.68	6.81	3.57	3.65
5	Biphenyl	H	H	CH_3	CH_2CH_2OH	169–171	65	$C_{19}H_{24}BrNO_3$	57.87	57.84	6.11	6.45	3.55	3.27
6	Biphenyl	H	CH_3	CH_3	CH_3	159–162	87	$C_{19}H_{24}BrNO_2$	60.32	60.11	6.39	6.42	3.70	3.82
7	Biphenyl	CH_3	H	CH_3	CH_3	230–231	31	$C_{19}H_{24}BrNO_2$	60.32	60.41	6.39	6.54	3.70	3.71
8	Biphenyl	H	CH_2OH		$-(CH_2)_5-$	145–146	61	$C_{22}H_{28}BrNO_3$[a]	60.21	60.19	6.55	6.61	3.19	3.18
9	Biphenyl	H	H		$-(CH_2)_2O(CH_2)_2-$	224–226	27	$C_{20}H_{24}BrNO_3$	59.12	59.09	5.95	5.98	3.45	3.23
10	Phenyl	H	H	CH_3	CH_3	188–190	57	$C_{12}H_{18}BrNO_2$	50.01	49.76	6.30	6.25	4.86	4.92
11	Phenyl	CH_3	H	CH_3	CH_3	216–218	42	$C_{13}H_{20}BrNO_2$	51.66	51.43	6.67	6.67	4.63	4.49
12	4-Nitrophenyl	H	H	CH_3	CH_3	213–214	71	$C_{12}H_{17}BrN_2O_4$	43.26	43.45	5.14	5.20	8.41	8.18
13	4-Chlorophenyl	H	H	CH_3	CH_3	215–216	85	$C_{12}H_{17}BrClNO_2$	44.67	44.72	5.31	5.39	4.34	4.26
14	4-Hydroxy-3,5-dimethylphenyl	H	H	CH_3	CH_3	145–146	26	$C_{14}H_{22}ClNO_3$[a,b]	57.53	57.78	7.76	7.75	4.78	4.73

[a] Analyzed as the quarter hydrate. [b] Analyzed as the chloride salt instead of bromide.

alcohols and phenacyl halides which we used. The preparation of morpholine compounds with substituents in the 3-position was readily effected by using substituted α-methylphenacyl bromides which were prepared by bromination of the appropriate propiophenone. A methyl group could be introduced into the 6-position (**6**) by using 1-dimethylamino-2-propanol, and a methylol group (**8**) similarly by using piperidino-2,3-propanediol. Substituents on nitrogen could be varied merely by selection of the dialkylamino alcohol, or, by using morpholine or piperidino alcohols, the spiro compounds (**8** and **9**) could be obtained.

This sequence was extended to the reaction of γ-dimethylaminopropanol with *p*-phenylphenacyl bromide and, in contrast to the speculation of previous workers,[5] the seven-membered 1,4-oxazepinium compound (**15a**) was obtained in good yield. It was found

15a, R = C_6H_5; X = Br
b, R = OH; X = Cl

that *p*-hydroxyphenacyl chloride reacted with γ-dimethylaminopropanol to give the cyclic **15b** in good yield. The proposed structures were confirmed by the absence of carbonyl absorption and the appearance of ether absorption in the infrared spectra, as well as the characteristic absorption at 252 mμ in the ultraviolet spectra.

Pharmacology.—Quaternary ammonium compounds have a wide range of pharmacologic effects, principally as parasympathetic stimulants.[8] Some compounds of this general class have been shown to have germicidal or antibacterial activity[9] and Drayton[10] reported one of a series of synthetic cationic detergents to be virucidal against *Rous sarcoma* virus, *in vitro*. It was of interest, therefore, to study this series of quaternary salts for antiviral activity. It was shown that some of these compounds exhibited *in vivo* activity against mouse hepatitis infection and herpes simplex encephalitis infection. The most active compound in the series was **3** and the data are shown in Table II. None of the compounds demonstrated significant activity against *Rous sarcoma* virus in chickens using essentially the procedure described by Bryan.[11]

Testing Methods

Virus Preparation. A. Mouse Hepatitis Infection.—The stock virus preparation contained $10^{7.4}$ LD_{50}/ml as determined in 14-g mice. For intraperitoneal infection, approximately 8 LD_{50} values of the virus is employed. Deaths occur beginning at 72 hr with the maximum rate at 96 hr after infection.

B. Herpes Simplex Encephalitis Infection.—A rabbit kidney cell culture preparation of virus is used for testing. Stock virus preparation contained $10^{4.8}$ LD_{50}/ml as determined in 14-g mice. For intracranial infection, approximately 5 LD_{50} values is used. Deaths occur 6 days after infection with the maximum per cent death at 10 days.

(8) A. Burger, "Medicinal Chemistry," Vol. 1, Interscience Publishers, Inc., New York, N. Y., 1951, p 373.

(9) (a) O. Rahn and W. P. Van Eseltine, *Ann. Rev. Microbiol.*, **1**, 173 (1947); (b) M. H. Roberts and O. Rahn, *J. Bacteriol.*, **52**, 639 (1946); (c) E. I. Valko, *Ann. N. Y. Acad. Sci.*, **46**, 451 (1946).

(10) H. A. Drayton, *Brit. J. Cancer*, **15**, 348 (1961).

(11) W. R. Bryan, *J. Natl. Cancer Inst.*, **6**, 225 (1946).

TABLE II
In Vivo ANTIVIRAL ACTIVITY (**3**)

Virus	Virus dose × LD_{50} value	Route	Toxic concn, mg/kg/dose	Concn, mg/kg/dose	% protection
MHV_3[a]	8[b]	Ip	10	5.0	0
				2.5	0
				1.25	20
				0.63	35
				0.32	0
Herpes[c] simplex encephalitis	5[d]	Ic	10	5.0	0
				2.5	0
				1.25	40
				0.63	40
				0.32	25

[a] Average of four experiments. [b] Stock virus contained $10^{7.4}$ LD_{50}/ml for 14-g mice. [c] Average of two experiments. [d] Stock virus contained $10^{4.8}$ LD_{50}/ml for 14-g mice.

Compound Testing.—Swiss, albino mice (14–15 g) are treated subcutaneously with 0.25 ml of each of 5 twofold dilutions of compound, starting at one-half the toxic concentration. Treatments are given 1 hr before and 1 hr after infection and continued twice daily for 4 days thereafter. Ten mice are used for each dose level of compound.

Evaluation of Drug Efficacy.—The compound is considered effective if, over a 14-day observation period, the per cent protection (per cent survival of the drug-treated groups minus per cent survival of untreated controls) is greater than 20% at any given dose level. Duplicate tests are run on compounds found effective in the initial experiment, to confirm the data.

Experimental Section[12]

3,5-Dimethyl-4-hydroxyphenacyl Chloride.—To a cooled, stirred mixture of 183 g (1.5 moles) of 2,6-dimethylphenol and 220 g (1.5 moles) of $AlCl_3$ in 800 ml of tetrachloroethane was added 180 g (1.5 moles) of chloroacetyl chloride at such a rate that a temperature of 0–5° was maintained. When the addition was complete, the reaction mixture was stirred at room temperature for 4 hr and poured into a mixture of 250 ml of HCl and crushed ice. The organic layer was separated, washed with water, and dried. The residue remaining after removal of solvent was recrystallized twice from isopropyl alcohol to give 121 g (41%) of white solid, mp 109–110°.

Anal. Calcd for $C_{10}H_{11}ClO_2$: C, 60.46; H, 5.58. Found: C, 60.39; H, 5.53.

Cyclic Quaternary Salts.—The procedures described are typical for those used to prepare the compounds listed in Table I. The infrared spectra of these products showed no absorption in the carbonyl region and strong ether absorption was observed in the 9–10-μ region. A maximum at 252 mμ was observed in the ultraviolet spectrum.

4,4-Dimethylhexahydro-2-hydroxy-2-(*p*-hydroxyphenyl)-1,4-oxazepinium Chloride (15b).—A solution of 26.0 g (0.153 mole) of *p*-hydroxyphenacyl chloride and 15.7 g (0.153 mole) of N,N-dimethylamino-1-propanol in 200 ml of acetone was stirred at the reflux temperature for 4 hr. The reaction mixture was cooled and the solid was separated by filtration and washed with acetone to give 36 g of off-white material. Two recrystallizations from a mixture of ethanol and ether gave 26.1 g (62%) of white product, mp 189–191°.

Anal. Calcd for $C_{13}H_{20}ClNO_3$: C, 57.03; H, 7.36; N, 5.12. Found: C, 57.25; H, 7.66; N, 5.02.

2-Biphenylyl-4,4-dimethylhexahydro-2-hydroxy-1,4-oxazepinium Bromide (15a).—A solution of 55 g (0.2 mole) of *p*-phenylphenacyl bromide and 21 g (0.2 mole) of N,N-dimethylamino-1-propanol in 300 ml of $CHCl_3$ was stirred at the reflux temperature for 4 hr. The reaction mixture was cooled and the product was collected by filtration, washed ($CHCl_3$), and recrystallized twice from a mixture of isopropyl alcohol and benzene to give 53 g (70%) of white solid, mp 168–170°, after drying *in vacuo* at 70° for 24 hr.

Anal. Calcd for $C_{19}H_{24}BrNO_2$: C, 60.32; H, 6.39; N, 3.70. Found: C, 60.14; H, 6.56; N, 3.76.

2-Hydroxy-2-phenyl-3,4,4-trimethylmorpholinium Bromide (11).—A solution of 115 g (0.38 mole) of α-bromopropiophenone and 40 g (0.38 mole) of N,N-dimethylaminoethanol in 150 ml of $CHCl_3$ was stirred at the reflux temperature for 5 hr. The yellow solid was separated by filtration and recrystallized three times from isopropyl alcohol to give 48 g (42%) of white product, mp 216–218°.

2-Hydroxy-2-(4-hydroxy-3,5-dimethylphenyl)-4,4-dimethylmorpholinium Chloride (14).—A solution of 43 g (0.22 mole) of 4-hydroxy-3,5-dimethylphenacyl chloride and 25 g (0.27 mole) of N,N-dimethylaminoethanol in 150 ml of $CHCl_3$ was stirred at the reflux temperature for 5 hr. The reaction mixture was cooled and an amorphous material was collected by filtration. Three recrystallizations from a mixture of isopropyl alcohol and ether gave 17 g (26%) of white solid, mp 145–146° dec.

2-Biphenyl-2-hydroxy-4,4′-spirobimorpholinium Bromide (9).—A solution of 55 g (0.2 mole) of *p*-phenylphenacyl bromide and 26 g (0.2 mole) of N-β-hydroxyethylmorpholine in 500 ml of $CHCl_3$ was stirred at the reflux temperature for 5 hr. The reaction mixture was cooled, and the white product was separated by filtration and recrystallized twice from methanol to give 22 g (27%), mp 224–226°.

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(12) All melting points were taken on a Thomas–Hoover melting point apparatus. Calibration of the thermometer against standard compounds showed no need for correction.