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Keyphrases

Local anesthesia–guinea pig cornea
Procaine–interaction with corneal surface
Density, ionic–corneal amphoteric colloid
pH effect–corneal ionic density
Anesthesia, corneal–relation to charge density

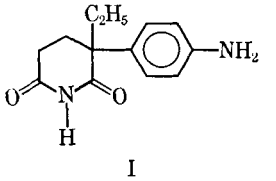
Synthesis of Derivatives of *N*-Methyl-2-phenylsuccinimide Involving a Lithium Salt Condensation and a Novel Application of the Mannich Reaction

Potential Anticonvulsant Agents

By HARRY CARL CLEMSON*, EDWARD O. MAGARIAN†, GEORGE C. FULLER, and RONALD O. LANGNER

The preparation of several Mannich bases of *N*-methyl-2-phenylsuccinimide was accomplished under a variety of conditions. There is no report in the literature of the application of the Mannich reaction to *N*-substituted-2-arylimides. The next higher homologs of these Mannich bases were prepared by a base displacement reaction in toluene between the lithium salt of *N*-methyl-2-phenylsuccinimide and the appropriate β-chloroethylamine hydrochlorides. Preliminary pharmacological data are reported.

GLUTETHIMIDE, a well-known sedative and hypnotic agent, has been reported as having an unreliable anticonvulsant effect at normal hypnotic doses (1). Introduction of an amino group into the *para* position of the benzene ring produces amino-glutethimide (I) which was used, until recently, in the clinical control of epileptic seizures. In their investigation of the effect of the presence of an amino group on the anticonvulsant properties of mephobarbital, Craig and Shideman (2) demonstrated that II has a higher



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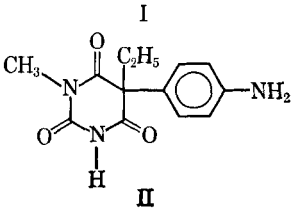

II

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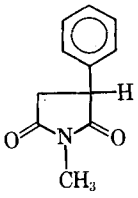
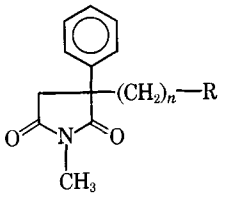
protective index (median toxic dose/median effective dose) than mephobarbital against electroshock seizures in mice. Mainly as a result of this apparent beneficial effect of an amino group on the anticonvulsant properties of these compounds, the authors were prompted to investigate the effect of more basic amino moieties on the anticonvulsant properties of phensuximide (III),



III

a known antiepileptic agent. Specifically, the interest was in introducing aminoalkyl groups into the 2-position of III. The resulting compounds would have the general structure IV, in which R may be either dialkylamino or heterocyclic amino groups and $n = 1$ or 2.



IV

Prior to the beginning of this project, substituted succinimides were prepared by the cyclization of appropriately substituted succinic acid intermediates with ammonia or a primary amine (3–13). The intent was to substitute aminoalkyl groups directly into the 2-position of III. Direct substitution into the ring appeared only recently in the literature (14), but this was not until several products had already been prepared.

DISCUSSION

Mannich Bases—It was envisioned that the synthesis of the alkylaminomethyl derivatives of *N*-methyl-2-phenylsuccinimide (IV, $n = 1$) may be accomplished by subjecting the parent compound (III) to Mannich conditions. A careful search of the literature revealed that there is no report of the successful application of the Mannich reaction to either *N*-substituted-2-phenylsuccinimides or *N,N*-disubstituted-2-phenylamides. Therefore, the authors became interested in establishing if, indeed, *N*-methyl-2-phenylsuccinimide (III) would undergo the Mannich reaction.

The most common solvent used in the Mannich reaction is ethanol. This solvent was initially employed, along with paraformaldehyde and dimethylamine hydrochloride under acidic conditions, in an attempt to prepare 2-dimethylaminomethyl-*N*-methyl-2-phenylsuccinimide hydrochloride (compound *1*, Table I). Several attempts under these conditions with various reaction times proved unsuccessful. Only starting material could be isolated. It was then decided to try higher boiling solvents which differ widely in their polarities. Four solvents were investigated: 2-butanol, glacial acetic acid, cyclohexanol, and toluene.

Substitution of 2-butanol for ethanol was also unrewarding. However, initial results were obtained when glacial acetic acid was used as the solvent; five Mannich bases were prepared: 2-dimethylaminomethyl - *N* - methyl - 2 - phenylsuccinimide hydrochloride (compound *1*, Table I), 2 - diethylaminomethyl - *N* - methyl - 2 - phenylsuccinimide hydrochloride (compound *2*, Table I), *N*-methyl-2-phenyl-2-(1-pyrrolidinomethyl) succinimide hydrochloride (compound *3*, Table I), *N*-methyl - 2 - (4 - morpholinomethyl) - 2 - phenylsuccinimide hydrochloride (compound *5*, Table I), and *N*-methyl-2-(4-methyl-1-piperazinomethyl)-2-phenylsuccinimide dihydrochloride (compound *7*, Table I). The yields, however, were generally poor.

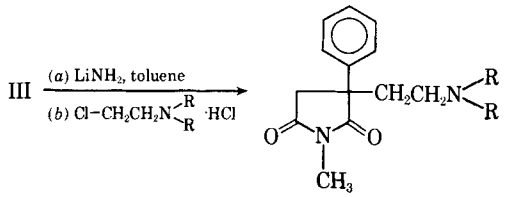
Several attempts to synthesize *N*-methyl-2-phenyl - 2 - (1 - piperidinomethyl)succinimide hydrochloride (compound *4*, Table I) using the glacial acetic acid method failed, but this compound was finally obtained in cyclohexanol.

N - Methyl - 2 - phenyl - 2 - (1 - pyrrolidinomethyl)succinimide hydrochloride (compound *3*, Table I), which was prepared previously in glacial acetic acid, was obtained in a significantly higher yield in cyclohexanol. The synthesis of 2-(3-azabicyclo[3.2.2]non - 3 - ylmethyl) - *N* - methyl - 2-phenylsuccinimide hydrochloride (compound *8*, Table I) was also accomplished in cyclohexanol. The use of 3-azabicyclo[3.2.2]nonane as the amine in the Mannich reaction has been reported only in recent years (15).

Four Mannich bases (compounds *2*, *4*, *6*, and *8*, Table I) were prepared in toluene. In every case but one, the yields were substantially increased over those obtained in glacial acetic acid or cyclohexanol.

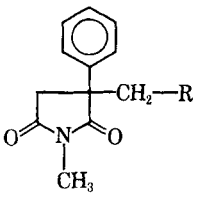
In reviewing the data, it was observed that in every case in which ethanol was used as the solvent, paraformaldehyde was the source of formaldehyde. Therefore, in order to ascertain if the source of formaldehyde might have made a difference, the synthesis of compound *2*, Table I, was again attempted in ethanol, but 36% formalin was substituted for paraformaldehyde. The product was obtained in a 49% yield which is significantly lower than the 67% yield obtained in toluene.

New Higher Homologs—The next higher homologs of the Mannich bases (IV, $n = 2$) were prepared according to the reaction shown in Scheme I.



Scheme I

TABLE I—MANNICH BASES OF *N*-METHYL-2-PHENYLSUCCINIMIDE



Compd.	R	Method	Yield, %	M.p., °C.	Formula	Anal. Calcd.	Anal. Found	% Protection[k] (Electro-shock)
1	—$N(CH_3)_2$·HCl	*A*	20	210–212 dec.	$C_{14}H_{19}ClN_2O_2$	C, 59.47 H, 6.77 N, 9.91 O, 11.32	C, 59.28 H, 6.63 N, 9.87 O, 11.50	60
2	—$N(C_2H_5)_2$·HCl	*A*[a] *B*[b, c] *D*	10 67 49	181–183 dec.	$C_{16}H_{23}ClN_2O_2$	C, 61.87 H, 7.40 N, 9.02 O, 10.30	C, 62.06 H, 7.34 N, 9.12 O, 10.40	30
3	—N⟨ ⟩·HCl	*A* *C*[d]	14 42	211–213 dec.	$C_{16}H_{21}ClN_2O_2$	C, 62.23 H, 6.85 N, 9.07 O, 10.36	C, 62.41 H, 6.89 N, 9.03 O, 10.39	0
4	—N⟨ ⟩·HCl	*B*[e, f] *C*[g]	56 34	188–189 dec.	$C_{17}H_{23}ClN_2O_2$	C, 63.25 H, 7.13 N, 8.68 O, 9.91	C, 63.38 H, 7.25 N, 8.68 O, 9.85	90
5	—N⟨ ⟩O·HCl	*A*	42	178–182 dec.	$C_{16}H_{21}ClN_2O_3$	C, 59.17 H, 6.52 N, 8.62 O, 14.78	C, 59.35 H, 6.42 N, 8.76 O, 14.87	0
6	—N⟨ ⟩O	*B*[h, f]	79	131–133	$C_{16}H_{20}N_2O_3$	C, 66.65 H, 6.99 N, 9.72 O, 16.64	C, 66.47 H, 6.90 N, 9.62 O, 16.72	...
7	—N⟨ ⟩N—CH_3 ·2HCl	*A*	18	210–235 dec. (sealed capillary)	$C_{17}H_{25}Cl_2N_3O_2$	C, 54.55 H, 6.73 N, 11.23 O, 8.55	C, 54.36 H, 6.58 N, 11.05 O, 9.13	66
8	—N⟨ ⟩·HCl	*B*[i, f] *C*[i]	16 16	183–184 dec.	$C_{20}H_{27}ClN_2O_2$	C, 66.19 H, 7.50 N, 7.72 O, 8.82	C, 66.11 H, 7.29 N, 7.63 O, 9.38	30

[a] The starting amine, diethylamine, was used in the form of its hydrochloride; total reaction time was 4 hr. [b] Recrystallized from 95% ethanol. [c] 36% formalin. [d] Total reflux time 2 hr. [e] Recrystallized from isopropanol. [f] 95% paraformaldehyde. [g] Total reflux time 24 hr. [h] Isolated as free base and recrystallized from 95% ethanol. [i] Recrystallized from isopropanol. [j] Total reflux time 18 hr. [k] Dose level = 200 mg./Kg; see body of paper for experimental protocol.

The addition of III to a suspension of commercial lithium amide in anhydrous toluene generated its lithium salt, which was allowed to react with the appropriately substituted β-chloroethylamine hydrochlorides. Since the starting amines were used in the form of their hydrochlorides, it was necessary to use at least two equivalents of the lithium amide. In subsequent preparations of some of the compounds, it was found that an increase in the number of equivalents of lithium amide used in the reaction produced an increase in the yields of the products. In specific cases the increases were quite significant (Table II).

EXPERIMENTAL

All melting points have been determined on either a Fisher-Johns block or the Kofler micro-hot stage and have been corrected. The infrared spectra were obtained using a Beckman IR-8 spectrophotometer. All imide derivatives displayed the two characteristic carbonyl stretching frequencies between 1775 and 1690 $cm.^{-1}$ (16–18). Also, the "ammonium bands" characteristic of tertiary amine hydrochlorides were present between 2700 and 2250 $cm.^{-1}$ (19).

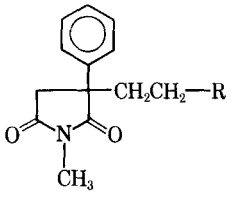
The elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and Micro-Analysis, Inc., Wilmington, Del.

All chemicals were obtained commercially unless specified otherwise.

Intermediates

2-Phenylsuccinic Acid—The preparation of this compound was according to the method of Allen *et al.* (20).

TABLE II—NEXT HIGHER HOMOLOGS OF MANNICH BASES



Compd.	R	Yield, % (Equiv. of LiNH2)		M.p., °C.	Formula	Anal. Calcd.	Anal. Found	% Protection[e] (Electroshock)
1	—N(CH3)2·HCl	39[a] (3.4)	...	198–200	$C_{15}H_{21}ClN_2O_2$	C, 60.71 H, 7.12 N, 9.44 O, 10.78	C, 60.69 H, 7.05 N, 9.55 O, 10.90	20
2	—N(C2H5)2·HCl	37[b] (2.5)	54 (3.7)	209–210	$C_{17}H_{25}ClN_2O_2$	C, 62.86 H, 7.76 N, 8.62 O, 9.85	C, 62.90 H, 8.03 N, 8.79 O, 9.90	50
3	—N⟨ ⟩·HCl	32[b] (2.6)	40 (3.7)	184–185 dec.	$C_{17}H_{23}ClN_2O_2$	C, 63.25 H, 7.18 N, 8.68 O, 9.91	C, 63.18 H, 7.23 N, 8.80 O, 9.90	70
4	—N⟨ ⟩·HCl	45[c] (2.4)	72 (5.1)	219–220 dec.	$C_{18}H_{25}ClN_2O_2$	C, 64.18 H, 7.48 N, 8.32 O, 9.50	C, 64.30 H, 7.58 N, 8.53 O, 9.55	44
5	—N⟨ O ⟩·HCl	38[d] (2.2)	41 (3.4)	215–218 dec.	$C_{17}H_{23}ClN_2O_3$	C, 60.26 H, 6.84 N, 8.27 O, 14.17	C, 60.52 H, 6.84 N, 8.29 O, 14.18	44
6	—N⟨ ⟩N—CH3·2HCl	12[a] (3.0)	...	262–266 dec.	$C_{18}H_{27}Cl_2N_3O_2$	C, 55.67 H, 7.01 N, 10.82 O, 8.24	C, 55.55 H, 7.05 N, 10.95 O, 8.36	44
7	—N⟨ ⟩·HCl	39[a] (2.0)	54 (5.4)	240–245 dec.	$C_{21}H_{29}ClN_2O_2$	C, 66.91 H, 7.76 N, 7.43 O, 8.49	C, 67.09 H, 7.81 N, 7.59 O, 8.41	11

[a] After addition of the amine hydrochloride, the reflux time was 24 hr. [b] After addition of the amine hydrochloride, the reflux time was 18 hr. [c] After addition of the amine hydrochloride, the reflux time was 37 hr. [d] After addition of the amine hydrochloride, the reflux time was 42 hr. [e] Dose level = 200 mg./Kg., except compound 4 (100 mg./Kg.); see body of paper for experimental protocol.

N-Methyl-2-phenylsuccinimide (III)—Made by the procedure of Miller and Long (4).

4-Methyl-1-(2-hydroxyethyl)piperazine—The following procedure is a modification of Ratouis, Boissier, and Dumont's method for the preparation of phenoxyalkylpiperazines (21). A mixture of 23.0 Gm. (0.230 mole) of *N*-methylpiperazine, 16.0 Gm. (0.200 mole) of 2-chloroethanol, 31.8 Gm. (0.230 mole) potassium carbonate, and 700 ml. of 2-butanol was heated to 100° for 15 hr. The mixture was allowed to cool to room temperature and filtered to remove the inorganic salts. The 2-butanol was then removed *in vacuo*. The residue, 4-methyl-1-(2-hydroxyethyl)piperazine, was used without further purification in the following procedure.

4-Methyl-1-(2-chloroethyl)piperazine Hydrochloride—The 4-methyl-1-(2-hydroxyethyl)piperazine, prepared by the procedure above, was treated according to the method of Billinghurst (22). That is, a chloroform solution of the alcohol was treated with thionyl chloride and the solid product was recrystallized from methanol to yield 22.4 Gm. (78%, based on 2-chloroethanol) of the title compound.

3-(2-Chloroethyl)-3-azabicyclo[3.2.2]nonane Hydrochloride—The procedure of Eastman Chemical Products, Inc., was modified in the preparation of this compound (23). To 31.2 Gm. (0.250 mole) of 3-azabicyclo[3.2.2]nonane was added, dropwise, 20.1 Gm. (0.250 mole) of 2-chloroethanol over a period of 20 min. After the addition was complete the reaction mixture was warmed on a steam bath. The entire reaction mixture formed a solid mass after 5 min. To this dark solid was added, with stirring, 150 ml. of a 10% solution of sodium hydroxide and 50 ml. of ether. The layers were separated and the basic aqueous solution was washed twice with 25-ml. portions of ether. The combined ethereal washings were dried over anhydrous mag-

nesium sulfate and filtered. The ether was removed *in vacuo* to give 30.1 Gm. (71.2%) of 3-(2-hydroxyethyl)-3-azabicyclo[3.2.2]nonane, which was used without further purification in the next step.

In 200 ml. of chloroform was dissolved 20.1 Gm. (0.178 mole) of the crude 3-(2-hydroxyethyl)-3-azabicyclo[3.2.2]nonane. To this solution was added slowly 30 ml. of thionyl chloride dissolved in 200 ml. of chloroform. The reaction was exothermic. After the addition was complete, the mixture was refluxed for 3 hr. and then allowed to stand at room temperature overnight. The solid was collected by filtration and recrystallized from absolute ethanol to yield 14.3 Gm. (35.8%) of 3-(2-chloroethyl) - 3 - azabicyclo[3.2.2]nonane hydrochloride, m.p. 214–215° dec.; lit. m.p. 198–225°.

Mannich Bases (Table I)

Method A—To 25 ml. of cold glacial acetic acid was added slowly, with stirring, 0.120 mole of the amine as a 40% aqueous solution (for an exception, see compound *2*, Table I). To this solution was added 10 ml. of 36% formalin (0.12 mole formaldehyde), followed by 18.9 Gm. (0.100 mole) of *N*-methyl-2-phenylsuccinimide. The mixture was heated on a steam bath for 1–2 hr.

The reaction mixture was cooled in an ice bath and made basic with 10% sodium hydroxide solution, and then extracted with chloroform. The chloroform extracts were dried over anhydrous magnesium sulfate and filtered, and the solvent was removed *in vacuo*. The residue was dissolved in anhydrous ether and filtered to remove any undissolved material. The ethereal solution was saturated with dry hydrogen chloride and the solid was collected by filtration, washed with anhydrous ether, and recrystallized from absolute ethanol.

Method B—A mixture of 0.10 mole of the amine, 18.9 Gm. (0.100 mole) of *N*-methyl-2-phenylsuccinimide, 60 ml. of toluene, and either 8.0 ml. of 36% formalin (2.9 Gm., 0.10 mole formaldehyde) or 3.4 Gm. (0.10 mole, calculated as formaldehyde) of 95% paraformaldehyde was heated at reflux for 20–24 hr. The volatile liquids were then removed *in vacuo*. An ethereal solution of the residue was dried over anhydrous magnesium sulfate, filtered, and saturated with dry hydrogen chloride. Sometimes a tacky material precipitated, which upon further addition of dry hydrogen chloride, with stirring, usually solidified to a white solid. However, if this treatment failed, then triturating the material with fresh anhydrous ether accomplished the same thing. The hydrochlorides were recrystallized from either 95% ethanol or isopropanol.

Method C—The amine (0.055 mole), 5.0 ml. (1.8 Gm., 0.060 mole formaldehyde) of 36% formalin and 9.4 Gm. (0.050 mole) of *N*-methyl-2-phenylsuccinimide were added to 50 ml. of cyclohexanol. The mixture was heated at reflux for several hours and then allowed to cool to room temperature.

The mixture was then dissolved in ether and the resulting solution was washed four times with 50-ml. portions of 10% hydrochloric acid solution. The combined acidic solutions were made basic with 10% sodium hydroxide solution, which usually precipitated an oil. The basic mixture was then extracted with ether, and the ethereal layer was dried over anhydrous magnesium sulfate, filtered, and saturated with dry hydrogen chloride. The solid was recrystallized from absolute ethanol. As in the previous method, occasionally a tacky material precipitated from the acidified ethereal solution. Treating the material in a manner similar to that in method *B* yielded an amorphous solid which then could be easily recrystallized from absolute ethanol.

Method D—Since only 2-diethylaminomethyl-*N*-methyl-2-phenylsuccinimide hydrochloride (compound *2*, Table I) was prepared by this method, the detailed procedure follows. To 3.7 Gm. (0.050 mole) of diethylamine were added 5.0 ml. (1.8 Gm., 0.060 mole formaldehyde) of 36% formalin, 9.4 Gm. (0.50 mole) of *N*-methyl-2-phenylsuccinimide, and 50 ml. of 95% ethanol. The solution was heated at reflux for 24 hr., and the volatile liquids were then removed *in vacuo*. An ethereal solution of the residue was dried over anhydrous magnesium sulfate, filtered, and saturated with dry hydrogen chloride. The solid was recrystallized from absolute ethanol to yield 2-diethylaminomethyl-*N*-methyl-2-phenylsuccinimide hydrochloride.

Next Higher Homologs (Table II)

To a suspension of lithium amide in 60 ml. of anhydrous toluene, under nitrogen, was added 4.7 Gm. (0.025 mole) of *N*-methyl-2-phenylsuccinimide. The mixture was heated at reflux for 5 hr., and then allowed to cool to room temperature. The amine hydrochloride (0.028 mole) was then added. After having been heated at reflux for several hours, the mixture was cooled in an ice bath and hydrolyzed with 20 ml. of water. The layers were separated and the organic layer was washed with water until the aqueous extracts were neutral. The toluene solution was dried over anhydrous magnesium sulfate, filtered, and the toluene removed *in vacuo*.

An ethereal solution of the residue was saturated with dry hydrogen chloride. If a tacky material precipitated, it was treated according to one of the procedures mentioned under *Method B*. The products were recrystallized from either absolute ethanol or isopropanol.

In some subsequent preparations it was found that if the number of equivalents of lithium amide were increased, the yields could also be increased.

PHARMACOLOGY

Pharmacological tests were conducted to determine if the tertiary amino derivatives of III were effective in abolishing the tonic hind leg extensor seizure of convulsions induced electrically and by pentylenetetrazol. A comparative pharmacological study was conducted on all the experimental compounds using male, albino, Charles River mice weighing 19–26 Gm. The mice were divided into groups of 10 and were fasted for 24 hr. before intraperitoneal injection of the test compound. All the compounds were soluble in water except compound *8* (Table I) which, like III, was suspended in a 7% solution of gum acacia. One hour after the compounds were administered, the mice were challenged with electroshock or pentylenetetrazol.

The electroschock procedure used in this study was a modification of the supramaximal seizure technique of Toman *et al.* (24). A 60-c. alternating current of fixed voltage was delivered for 0.2 sec. to each mouse through two attached ear clips. In order to screen the compounds for antipentylene-

tetrazol activity, 100 mg./Kg. of pentylenetetrazol was injected subcutaneously into each mouse. This dose is considered to be approximately twice the CD_{50} in untreated mice (25). In both tests, an animal was considered protected if the tonic extensor component of the seizure was abolished. The control animals, which were injected with 7% gum acacia or distilled water, showed 0% protection against electroshock and subcutaneous pentylenetetrazol.

All of the test compounds were evaluated at a dose level of 200 mg./Kg. with the exception of compound 4 (Table II, 100 mg./Kg.). However, none of the derivatives of III were shown to be effective in preventing the tonic hind leg extension produced by pentylenetetrazol. It was not possible to test higher doses of the experimental compounds since amounts greater than 200 mg./Kg. produced toxic symptoms.

The results from the electroshock testing demonstrated that several of the analogs were more protective against electroshock than was the parent compound (III). At a dose level of 200 mg./Kg., III protected 20% of the test animals against electroshock. The electroshock data appear in Tables I and II.

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Keyphrases

N-Methyl-2-phenylsuccinimide derivatives–synthesis
Mannich reaction
Lithium amide–higher homolog synthesis
IR spectrophotometry–structure
Anticonvulsive activity against electroshock, pentylenetetrazol

Survival of *Pseudomonas aeruginosa* in Fluorescein Solution

Preservative Action of PMN and EDTA

By MICHAEL R. W. BROWN

Colony counts were made for a year on washed and unwashed cells of *Ps. aeruginosa* inoculated into fluorescein sodium solution, 2 percent in water (pH 8.6), into nutrient broth, into tris buffer (pH 8.6), and into water. Broth exerted a protective effect in all storage liquids (22°) and also supported growth, depending on the amount of broth. Water-washed inocula lost viability in less than a day in fluorescein solution. Viability of broth inocula was maintained for more than a year in all vehicles. Fluorescein solution preserved with PMN-sterilized inocula of about 10^6/ml. *Ps. aeruginosa* cells within 5 hr. EDTA in combination with PMN was less effective.

PSEUDOMONAS AERUGINOSA causes general problems of cross infection (1, 2) and is a particular hazard in ophthalmology (3, 4). Dale *et al.* (5) have reviewed many reports of *Ps. aeruginosa* as a contaminant of fluorescein eye drops.

The B.P.C. recommendation of phenylmercuric nitrate (PMN) as an ophthalmic preservative is supported by many studies, nearly all of which

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