## REFERENCES

(1) I. Nabih and M. El-Wassimi, *J. Pharm. Sci.*, **57**, 1202(1968).
(2) I. Nabih, M. El-Wassimi, and J. Metri, *ibid.*, **57**, 1609(1968).
(3) M. Nolan and E. Mann, *Amer. J. Trop. Med. Hyg.*, **2**, 716 (1953).
(4) A. Lehninger, C. Cooper, T. Devlin, and J. Gamble, Jr., *Science*, **128**, 450(1958).
(5) A. Lehninger, *Ann. Rev. Biochem.*, **31**, 47(1962).
(6) I. Nabih and J. Metri, *J. Pharm. Sci.*, **60**, 1242(1971).
(7) F. Bell and J. Gibson, *J. Chem. Soc.*, **1955**, 24.
(8) A. Green and F. Rowe, *ibid.*, **113**, 955(1918).
(9) H. Hodgson and H. Walker, *ibid.*, **1933**, 1205.
(10) G. Schroeter, *Ann.*, **426**, 104(1922).
(11) A. Zayed, M.S. thesis, Faculty of Science, Cairo University, Cairo, Egypt, 1969.



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# Anticonvulsant Properties of Mannich Base Derivatives of 2-Phenylsuccinimide III


**EDWARD O. MAGARIAN▲, GERALD W. BECKER, and LOUIS DIAMOND**

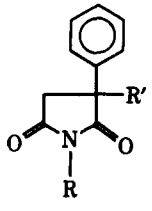


**Abstract** □ Several Mannich base derivatives of 2-phenylsuccinimide were synthesized and screened for anticonvulsant activity. All products were evaluated by maximal electroshock seizure and pentylenetetrazol seizure threshold tests.

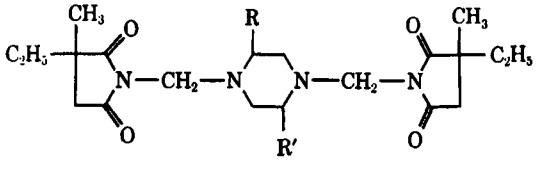


**Keyphrases** □ 2-Phenylsuccinimide, Mannich base derivatives—synthesis, screened for anticonvulsant properties □ Anticonvulsants, potential—synthesis and screening of Mannich base derivatives of 2-phenylsuccinimide


The preceding papers (1, 2) of this series reported the preparation and anticonvulsant properties of several derivatives of phensuximide[1], *N*-methyl-2-phenylsuccinimide (I). One series of compounds (II), consisting of C-Mannich base derivatives of I, proved interesting since several members were more effective than the parent molecule in eliminating the tonic extensor component of electroshock seizures.



I: R = $CH_3$, R′ = H
II: R = $CH_3$, R′ = *tert*-aminomethyl
III: R = *tert*-aminomethyl, R′ = H



IV: R = $CH_3$, R′ = H
V: R = $CH_3$, R′ = $CH_3$

Some researchers (3, 4) reported the preparation of mono- and bis-Mannich bases of succinimides (III–V). These compounds were shown to possess significant anticonvulsant properties.

In view of the foregoing, it was of interest to prepare for evaluation the *N*,2-bis-Mannich bases of 2-phenylsuccinimide (Compounds 1–6 in Table I). The corresponding mono bases (Compounds 7–12 in Table I) were also prepared for comparison purposes. All products were subjected to preliminary pharmacological screening, and the results are presented in Tables II and III.

## EXPERIMENTAL[2]

**2-Phenylsuccinimide**—The title compound was prepared from 2-phenylsuccinic acid and concentrated ammonium hydroxide according to Miller and Long's (5) procedure. It was obtained as a white solid in a yield of 78%, m.p. 79–81° [lit. (6) m.p. 90°].

**Preparation of Bis-Mannich Bases (Table I)**—To 0.05 mole of the appropriate amine, 5.0 ml. of 40% formalin (2.0 g., 0.067 mole formaldehyde) was added slowly with cooling. This mixture was added immediately to 4.4 g. (0.025 mole) of 2-phenylsuccinimide in 50 ml. of 95% ethanol, after which it was heated at reflux for various periods of time (Table I). The solvent was removed *in vacuo*, leaving either a solid or an oil, which usually solidified upon standing. Occasionally, extraction of the oil with petroleum ether and evaporation of the solvent *in vacuo* yielded solid material. If this procedure failed, an ethereal solution of the oil was washed with 10% NaOH and then with water until the washings were neutral to litmus. The solution was dried (magnesium sulfate), and the ether was removed *in vacuo* to give a solid product.

[1] Milontin, Parke, Davis and Co., Detroit, MI 48232

[2] All melting points were determined on a Thomas-Hoover melting-point apparatus and are corrected. IR spectra were obtained on a Beckman IR-8 spectrophotometer using KBr pellets. The two carbonyl stretching bands, which are characteristic of imides, were present in all products. NMR spectra were obtained on a Varian Associates A-60A spectrometer and are consistent with the assigned structures. Elemental analyses were performed by Micro-Analysis, Inc., Wilmington, Del. Intermediates used in this work are available commercially unless specified otherwise.

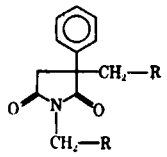
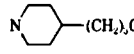
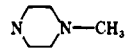
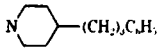
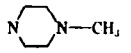


Table I—Mannich Bases of 2-Phenylsuccinimide

| Compound Number | R | Recrystallization Solvent | Melting Point | Formula | Analysis, % Calc. | Analysis, % Found | Yield, % | Reflux Time, hr. |
|---|---|---|---|---|---|---|---|---|
| 1 |  | Ethanol (95%) | 104.5–105.5° | $C_{40}H_{51}N_3O_2$ | C 79.30<br>H 8.48<br>N 6.94 | 79.27<br>8.24<br>6.98 | 46 | 19 |
| 2 |  | Hexane | 112.5–115.5° | $C_{22}H_{31}N_5O_2$ | C 66.14<br>H 8.32<br>N 17.53 | 66.27<br>8.39<br>17.44 | 24 | 93 |
| 3 |  | Isopropanol | 83.5– 84.5° | $C_{22}H_{27}N_3O_2$ | C 72.30<br>H 7.45<br>N 11.50 | 72.47<br>7.51<br>11.43 | 48 | 21 |
| 4 |  | Isopropanol–chloroform (1:1) | 178.5–181° | $C_{28}H_{39}N_3O_2$ | C 74.80<br>H 8.74<br>N 9.35 | 74.50<br>8.89<br>9.60 | 64 | 21 |
| 5 |  | Isopropanol | 118–119° | $C_{20}H_{27}N_3O_4$ | C 64.32<br>H 7.29<br>N 11.25 | 64.36<br>7.42<br>11.10 | 18 | 65 |
| 6 |  | Isopropanol | 97–98° | $C_{22}H_{31}N_3O_2$ | C 71.52<br>H 8.46<br>N 11.37 | 71.26<br>8.36<br>11.18 | 24 | 21 |
| 7 |  | Ethanol (95%) | 121–123° | $C_{26}H_{30}N_2O_2$ | C 76.89<br>H 7.74<br>N 7.17 | 76.99<br>7.54<br>7.27 | 86 | |
| 8 |  | Isopropanol | 127–129° | $C_{16}H_{21}N_3O_2$ | C 66.88<br>H 7.37<br>N 14.62 | 66.95<br>7.45<br>14.52 | 67 | |
| 9 |  | Isopropanol | 100.5–105° | $C_{16}H_{18}N_2O_2$ | C 71.09<br>H 6.71<br>N 10.36 | 71.17<br>6.84<br>10.31 | 84 | |
| 10 |  | Isopropanol | 105–106.5° | $C_{19}H_{24}N_2O_2$ | C 73.05<br>H 7.74<br>N 8.97 | 72.96<br>7.47<br>8.93 | 94 | |
| 11 |  | Isopropanol | 113–114.5°[a] | $C_{15}H_{18}N_2O_2$ | — | — | 86 | |
| 12 |  | Ethanol (95%) | 110–111°[b] | $C_{16}H_{20}N_2O_2$ | — | — | 69 | |

[a] Lit. (3) m.p. = 111–112°. [b] Lit. (3) m.p. = 107–108°.

**Preparation of Mono-Mannich Bases (Table I)**—To 0.05 mole of the appropriate amine, 5.0 ml. of 40% formalin (2.0 g., 0.067 mole formaldehyde) was added slowly with cooling. This mixture was added immediately to a solution of 8.8 g. (0.05 mole) of 2-phenylsuccinimide in 20 ml. of 95% ethanol. The reaction mixture was stirred at room temperature for 15–30 min. and cooled in an ice bath, and the product was removed by filtration.

## PHARMACOLOGY

The anticonvulsant activity of each compound was evaluated by maximal electroshock seizure and pentylenetetrazol seizure threshold tests (7). A group of five male albino mice[3] was used for each test, and the results were compared with vehicle-treated control groups.

For the maximal electroshock seizure tests, animals were stimulated with 60-cycle alternating current for 200 msec. *via* a pair of earclip electrodes. A variable transformer was used to regulate the applied voltage. The desired duration of stimulus was obtained by the inclusion of a relay in the circuit, which was operated by a square-wave stimulator[4]. A cathode ray oscilloscope was used to monitor the voltage drop across a 10-ohm resistor coupled in series with the animal. This enabled the calculation of the corresponding current. The sweep on the cathode ray oscilloscope screen was triggered by the stimulator, so accurate readings could be obtained with each shock. Preliminary studies showed that 12–16 ma. was required to produce a convulsive seizure pattern having a tonic flexor phase, a tonic extensor phase, and a clonic phase.

After adequate control data were obtained, each group of animals received 10–600 mg./kg. i.p. of the drug under study. The maximal electroshock seizure tests were repeated 1, 2, and 3 hr. after drug administration. All drugs were administered as suspensions in 0.5% methylcellulose (4000 cps.).

An animal was considered to be protected if the tonic extensor phase of the seizure pattern was absent.

[3] Swiss-Webster mice obtained from the Laboratory Supply Co., Inc., Indianapolis, IN 46241. Food and water were allowed *ad libitum*.

[4] Grass S-4.

**Table II**—Maximal Electroshock Seizure Tests

| Compound Number | Dose, mg./kg. | Number of Animals Protected[a] 1 hr. | 2 hr. | 3 hr. | Number of Animals Showing Toxicity[a] 1 hr. | 2 hr. | 3 hr. |
|---|---|---|---|---|---|---|---|
| 1 | 600 | 0/5 | 0/4 | 0/2 | 3/5 | 1/4 | 1/2 |
| 2 | 10 | 1/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| | 100 | 3/5 | 2/3 | 0/3 | 0/5 | 0/3 | 0/3 |
| | 600[b] | — | — | — | — | — | — |
| 3 | 100 | 1/5 | 0/4 | 1/4 | 1/5 | 0/4 | 0/4 |
| | 600[b] | — | — | — | — | — | — |
| 4 | 600 | 0/5 | 0/5 | 0/5 | 4/5 | 4/5 | 5/5 |
| 5 | 100 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| | 600 | 5/5 | 5/5 | 3/4 | 5/5 | 5/5 | 3/4 |
| 6 | 100 | 0/5 | 0/3 | 0/2 | 1/5 | 1/3 | 0/2 |
| | 600 | 3/4 | 1/2 | 0/1 | 3/4 | 2/2 | 1/1 |
| 7 | 10 | 0/5 | 0/5 | 0/5 | 0/5 | 1/5 | 1/5 |
| | 100 | 2/5 | 1/5 | 3/5 | 2/5 | 2/5 | 1/5 |
| | 600 | 2/5 | 3/4 | 3/3 | 1/5 | 1/4 | 1/3 |
| 8 | 100 | 1/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| | 600 | 3/3 | 1/1 | 0/0 | 3/3 | 1/1 | 0/0 |
| 9 | 100 | 2/5 | 1/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| | 600 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 | 2/2 |
| 10 | 100 | 0/5 | 0/3 | 0/3 | 0/5 | 0/3 | 0/3 |
| | 600[b] | — | — | — | — | — | — |
| 11[c] | 10 | 1/5 | 1/5 | 0/5 | 1/5 | 1/5 | 1/5 |
| | 100 | 2/5 | 1/5 | 1/5 | 0/5 | 0/5 | 0/5 |
| | 600 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| 12[c] | 100 | 2/5 | 0/5 | 1/5 | 2/5 | 0/5 | 0/5 |
| | 600[b] | — | — | — | — | — | — |
| Diphenylhydantoin | 25 | 5/5 | 5/5 | 5/5 | 0/5 | 0/5 | 0/5 |
| Phenobarbital | 25 | 5/5 | 5/5 | 5/5 | 0/5 | 0/5 | 0/5 |
| Methylcellulose, 0.5% | 0.5 ml. | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |

[a] Animals not surviving are excluded from the ratios. [b] Drug was 100% lethal at this dosage level. [c] See *Reference 3*.

A similar experimental design was used for conducting the pentylenetetrazol seizure threshold tests. Animals received pentylenetetrazol, 85 mg./kg. s.c., 1 or 2 hr. after the administration of a test compound. Animals that showed no convulsive activity of any kind during a 30-min. observation period were considered protected.

The ability of the selected experimental testing procedures to detect anticonvulsant activity was assessed by including three agents known to provide significant protection against either electrically or chemically induced seizures—*viz.*, diphenylhydantoin, phenobarbital, and phensuximide.

The neurological status of each animal was evaluated prior to each test for anticonvulsant activity. Toxicity was considered present if the animal failed to exhibit a righting reflex or a hind-limb placing reaction or if the animal's equilibrium, gait, or stance deviated from normal.

It appears from the data in Tables II and III that the mono-Mannich bases were generally more active than the corresponding bis-substituted analogs. The products were less effective in preventing seizures with the maximal electroshock seizure test (Table II) than with the pentylenetetrazol seizure threshold test (Table III). As a rule, protection was obtained in the maximal electroshock seizure test only for dosage levels at which toxic effects were observed. In the pentylenetetrazol seizure threshold test, Compound 9 (Table III) displayed an activity pattern equivalent to that of the standard compound phensuximide. Compounds 8 and 11 (Table III) compared favorably with phensuximide in that they also provided full protection at a dosage level of 300 mg./kg.

**Table III**—Pentylenetetrazol Seizure Threshold Tests

| Compound Number | Dose, mg./kg. | Number of Animals Protected[a] 1 hr. | 2 hr. |
|---|---|---|---|
| 1 | 100 | 1/5 | 1/5 |
| | 600 | 0/5 | 2/5 |
| 2 | 100 | 1/5 | 1/5 |
| | 300[b] | — | — |
| 3 | 100 | 0/5 | 1/5 |
| | 600 | 0/2 | 0/0 |
| 4 | 100 | 1/5 | 2/5 |
| | 600 | 1/5 | 2/5 |
| 5 | 100 | 1/5 | 0/5 |
| | 300 | 0/5 | 0/5 |
| 6 | 100 | 1/5 | 0/5 |
| | 400 | 1/5 | 2/5 |
| 7 | 100 | 2/5 | 3/5 |
| | 300 | 0/5 | 1/5 |
| 8 | 100 | 0/5 | 3/5 |
| | 300 | 5/5 | 5/5 |
| 9 | 100 | 4/5 | 1/5 |
| | 300 | 5/5 | 5/5 |
| 10 | 100 | 0/5 | 2/5 |
| 11[c] | 100 | 1/5 | 0/5 |
| | 300 | 5/5 | 5/5 |
| 12[c] | 100 | 3/5 | 0/5 |
| | 600[b] | — | — |
| Phensuximide | 100 | 4/5 | 1/5 |
| | 300 | 5/5 | 5/5 |
| Methylcellulose, 0.5% | 0.5 ml. | 0/5 | 0/5 |

[a] Animals not surviving are excluded from the ratios. [b] Drug was 100% lethal at this dosage level. [c] See *Reference 3*.

## REFERENCES


(1) H. C. Clemson, E. O. Magarian, G. C. Fuller, and R. O. Langner, *J. Pharm. Sci.*, **57**, 384(1968).

(2) H. C. Clemson, E. O. Magarian, and J. T. Reinhard, *ibid.*, **59**, 1137(1970).

(3) M. Eckstein, A. Zejc, and A. Klusek, *Diss. Pharm. Pharmacol.*, **19**, 263(1967).

(4) B. Lucka-Sobstel and A. Zejc, *ibid.*, **23**, 135(1971).

(5) C. A. Miller and L. M. Long, *J. Amer. Chem. Soc.*, **73**, 4895 (1951).

(6) R. Wegscheider and J. Hecht, *Monatsh. Chem.*, **24**, 422 (1903).

(7) E. A. Swinyard, W. C. Brown, and L. S. Goodman, *J. Pharmacol. Exp. Ther.*, **106**, 319(1952).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