(2) H. Pracejus, *Tetrahedron*, **21**, 2257 (1965).
(3) R. M. Sweet and L. F. Dahl, *J. Am. Chem. Soc.*, **92**, 5489 (1970).
(4) R. W. Holley, *Science*, 117, 23 (1953).
(5) R. H. Earle, D. T. Hurst, and M. Viney, *J. Chem. Soc. C*, 2093 (1969).
(6) G. M. Blackburn and J. D. Plackett, *J. Chem. Soc., Perkin Trans.* 2, 1386 (1972).
(7) L. H. Sternbach, *Angew. Chem.*, **10**, 34 (1971).
(8) L. H. Sternbach, L. O. Randall, R. Banziger, and H. Lehr in "Drugs Affecting the Central Nervous System", A. Berger, Ed., Vol. 2, Marcel Dekker, New York, N.Y., 1968, p 237.
(9) A. Camerman and N. Camerman, *J. Am. Chem. Soc.*, **94**, 268 (1972).
(10) S. Garattini, E. Mussini, and L. O. Randall, "The Benzodiazepines", Raven Press, New York, N.Y., 1973.
(11) L. H. Sternbach and L. O. Randall in "CNS Drugs", G. S. Sidhu, Ed., Council of Scientific and Industrial Research, New Delhi, 1966, p 53.
(12) L. H. Sternbach, L. O. Randall, and S. R. Gustafson in "Psychopharmacological Agents", M. Gordon, Ed., Academic Press, New York, N.Y., 1964, p 137.
(13) G. Zbinden and L. O. Randall, *Adv. Pharmacol.*, **5**, 213 (1967).
(14) L. H. Sternbach, D. D. Sancilio, and J. F. Blount, *J. Med. Chem.*, **17**, 374 (1974).
(15) N. R. Draper and H. Smith, "Applied Regression Analysis", Wiley, New York, N.Y., 1968, p 18.
(16) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).
(17) R. E. Tedeschi, D. H. Tedeschi, A. Mucha, L. Cook, P. A. Mattis, and E. J. Fellows, *J. Pharmacol. Exp. Ther.*, **125**, 28 (1959).
(18) G. M. Everett and R. K. Richards, *J. Pharmacol. Exp. Ther.*, 81, 402 (1944).
(19) L. O. Randall, W. Schallek, G. A. Heise, E. F. Keith, and R. E. Bagdon, *J. Pharmacol. Exp. Ther.*, **129**, 163 (1960).
(20) T. Blair and G. A. Webb, unpublished results.

Potential Long-Acting Anticonvulsants. 2. Synthesis and Activity of Succinimides Containing an Alkylating Group on Nitrogen or at the 3 Position

Milton J. Kornet,* A. Michael Crider,

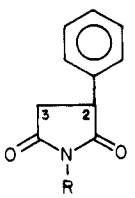
College of Pharmacy, University of Kentucky, Lexington, Kentucky 40506

and Edward O. Magarian

College of Pharmacy, North Dakota State University, Fargo, North Dakota 58102. *Received January 24, 1977*

The synthesis of succinimide derivatives in which alkylating groups have been attached to the imide nitrogen or to the 3 position of the ring is described. The synthesis of one bis-alkylating derivative **19** is also described. The alkylating groups used were (a) α-haloacetyl, (b) α-haloacetamido, (c) maleamyl, and (d) maleimido. These compounds were prepared as potential long-acting anticonvulsants. None of the compounds showed activity against maximal electroshock or metrazole-induced seizures.

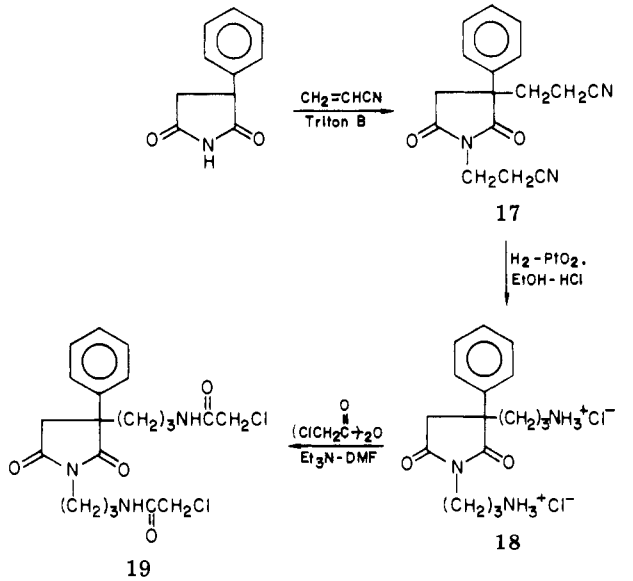
In a previous report[1] we described the synthesis and biological activity of potential long-acting succinimide anticonvulsants. Alkylating groups were attached at the 2 position of the ring and at the para position of the 2-phenyl substituent of phensuximide (**1**, R = CH_3). We now report the synthesis and biological activity of compounds in which the alkylating group is attached predominantly to the imide nitrogen. In addition, one compound containing an alkylating group at the 3 position and an *N*,-2-bis-alkylating derivative are described. The alkylating groups used include (a) α-haloacetyl, (b) α-haloacetamido, (c) maleamyl, and (d) maleimido. These groups were attached at varying distances from the imide nitrogen in an effort to search the area at or adjacent to the active site for an appropriate nucleophile. Proper positioning of the alkylating group to a nucleophile species (SH, NH_2, COO^-, or OH), at or near the active site, can result in a rapid neighboring group reaction with covalent bond formation. Attachment of the succinimide derivative to its active site via covalent bond formation should result in an unusually long-acting anticonvulsant.



1

Chemistry. The 2-phenylsuccinimides containing an alkylating group attached to the nitrogen atom and their precursors were obtained for the most part by conventional procedures.[1] Methods for others are described in the Experimental Section. The physical properties of the compounds are given in Table I.

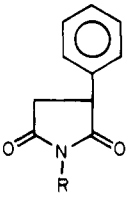
Scheme I

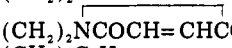
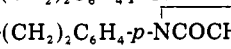


In an attempt to produce an ultra long-acting anticonvulsant, our efforts were turned toward the preparation of the bis-alkylating compound **19**. The synthetic pathway is shown in Scheme I.

Introduction of an alkylating group into the 3 position of the succinimide ring was accomplished by the route shown in Scheme II. The NMR spectrum of **23** showed

Table I. N-Substituted 2-Phenylsuccinimides



Compd	R	Mp, °C	Recrystn solvent	Yield, %	Formula[a] (analyses)
2	$-COCH_2Cl$	112-113	EtOH-Et_2O	45	$C_{12}H_{10}ClNO_3$ (C, H, N)
3	$-CH_2CN$	122-123	EtOH	54	$C_{12}H_{10}N_2O_2$ (C, H, N)
4	$-(CH_2)_2NH_3^+Cl^-$	212-213.5	EtOH	76	$C_{12}H_{15}ClN_2O_2$ (C, H, N, Cl)
5	$-(CH_2)_2NHCOCH_2Cl$	91-92.5	C_6H_6-n-C_6H_{14}	43	$C_{14}H_{15}ClN_2O_3$ (C, H, N)
6	$-(CH_2)_2NHCOCH{=}CHCOOH$ (cis)	142.5-144	EtOH	40	$C_{16}H_{16}N_2O_5$ (C, H, N)
7	$-(CH_2)_2$ 	*b*		29	$C_{16}H_{14}N_2O_4$ (C, H, N)
8	$-(CH_2)_2C_6H_5$	75-76.5	EtOH	64	$C_{18}H_{17}NO_2$ (C, H, N)
9	$-(CH_2)_2C_6H_4$-*p*-NHAc	136.5-139.5	C_6H_6	53	$C_{20}H_{20}N_2O_3$ (C, H, N)
10	$-(CH_2)_2C_6H_4$-*p*-NH_2	149.5-151	EtOH	82	$C_{18}H_{18}N_2O_2$ (C, H, N)
11	$-(CH_2)_2C_6H_4$-*p*-$NHCOCH_2Cl$	133-134.5	EtOH	60	$C_{20}H_{19}ClN_2O_3$ (C, H, N)
12	$-(CH_2)_2C_6H_4$-*p*-$NHCOCH{=}CHCOOH$ (cis)	168-169.5	EtOH	75	$C_{22}H_{20}N_2O_5$ (C, H, N)
13	$-(CH_2)_2C_6H_4$-*p*- 	121-123	EtOH	31	$C_{22}H_{18}N_2O_4$ (C, H, N)

[a] Compounds were analyzed for the elements shown in parentheses and are within ±0.4% of the calculated value. [b] Purification of the crude product by column chromatography using silica gel as the adsorbent and a C_6H_6-$CHCl_3$ mixture as the solvent system resulted in a colorless oil which analyzed correctly.

two singlets at δ 1.91 and 1.72 which integrated for a total of three protons. The presence of individual C–CH_3 signals pointed to the probability that **23** was a mixture of diastereomers. Chromatography using a variety of adsorbents and solvent systems failed to resolve the diastereomeric mixture. Further transformations converted **23** into pure **25**. Because of a lack of biological activity for compound **25**, further efforts at resolving either **24** or **25** were curtailed.

Biological Results. All of the alkylating succinimide derivatives were examined for anticonvulsant activity using methods described in our initial report.[1] None of the compounds showed activity against maximal electroshock or metrazole-induced seizures. The highest dose tested was 300 mg/kg in male Carworth Farms No. 1 mice.

A possible explanation for the lack of activity of these compounds is that they covalently bind in a nonspecific manner to biomolecules and thus are not transported from the site of administration to the receptor site.[2] Alternatively, steric properties may prevent proper binding at the receptor in those instances where prior inactivation does not occur. Such an idea is supported by that fact that **8** which possesses a large nonalkylating N-substituent, in contrast to N-methylated phensuximide, was also inactive.

Preliminary work with the potential alkylating succinimides indicates that anticonvulsant activity is most often retained among compounds where the alkylating group is in the vicinity of the 2 position.[1]

Experimental Section

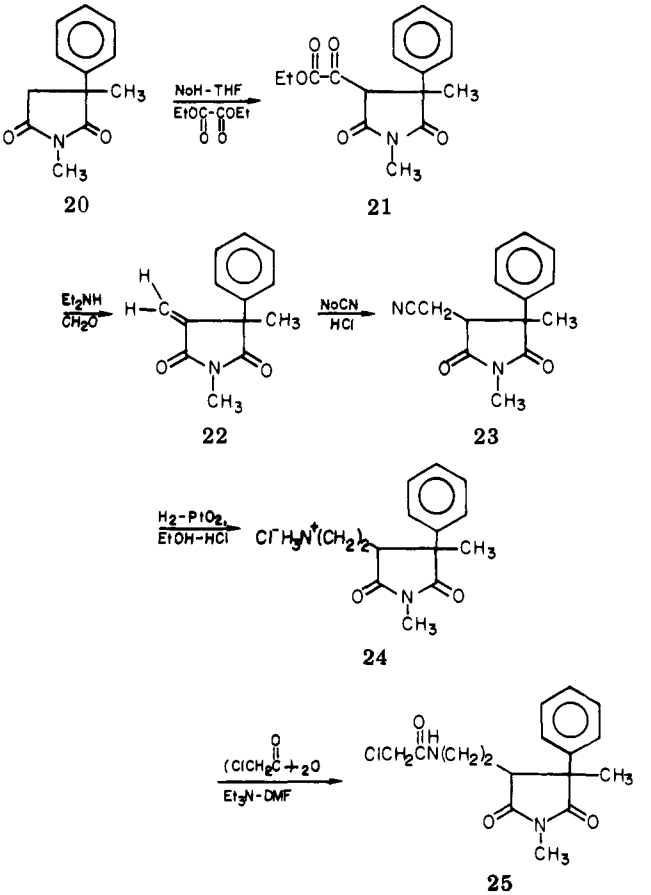
Spectra and Analyses. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The IR spectra were taken on a Beckman IR-8 and a Perkin-Elmer 700 spectrometer as either liquid films or as KBr pellets. The NMR spectra were recorded on a Varian A-60A spectrometer using tetramethylsilane as the internal standard, and the chemical shift data are on file with the editors.

2-Phenylsuccinimide (1a, R = H). This compound was synthesized according to the method of Miller and Long[3] in 51% yield: mp 77–78 °C (lit. mp 88–90 °C).

***N*-Methyl-2-phenylsuccinimide (1b, R = CH_3).** Compound **1b** was prepared according to the conditions previously described.[1]

***N*,2-Dimethyl-2-phenylsuccinimide (20).** Compound **20** was prepared in a similar manner as described for the synthesis of 2-cyanomethyl-*N*-methyl-2-phenylsuccinimide[1] using the quantities 5.05 g (0.12 mol) of a 57% mineral oil dispersion of NaH, 18.9 g (0.10 mol) of **1b**, and 21.3 g (0.15 mol) of CH_3I. Recrystallization of the crude product from cyclohexane yielded 13.2 g (62%) of a white crystalline solid: mp 51.5–53 °C (lit.[3] mp 52 °C).

Scheme II



20 21 22 23 24 25

***N*-Phenethyl-2-phenylsuccinimide (8).** This synthesis is a modification of the method of Miller and Long.[3] A mixture of 10.0 g (0.051 mol) of phenylsuccinic acid and 6.22 g (0.051 mol)

of phenethylamine was heated until the temperature of the mixture reached 210 °C. Water was distilled off during the reaction via a Dean-Stark trap. The viscous mixture was cooled, dissolved in 175 mL of $CHCl_3$, and washed successively with 25-mL portions of saturated $NaHCO_3$ solution, 10% HCl, and H_2O. The $CHCl_3$ phase was dried ($MgSO_4$), filtered, and evaporated. Recrystallization of the residue produced 9.1 g of a white powder.

***N*-(*p*-Acetamidophenethyl)-2-phenylsuccinimide (9).** See synthesis of 8 and Table I.

***N*-Chloroacetyl-2-phenylsuccinimide (2).** A total of 0.25 g (0.011 g-atom) of Na metal under a nitrogen atmosphere was treated with 15 mL of absolute EtOH. After all the Na had reacted, the ethanolic sodium ethoxide solution was cooled to room temperature and treated with 2.0 g (0.011 mol) of 2-phenylsuccinimide (1a, R = H) in 50 mL of anhydrous $C_6H_5CH_3$. A Dean-Stark trap was fitted and the EtOH–$C_6H_5CH_3$ azeotrope distilled. Heating was stopped when the vapor temperature reached 110 °C. After cooling to 10 °C in an ice bath, the thick slurry was treated with 1.24 g (0.011 mol) of $ClCH_2COCl$ in 10 mL of $C_6H_5CH_3$. The reaction mixture was stirred overnight at room temperature and heated at 90 °C for an additional 1 h. After cooling, the mixture was filtered to remove the inorganic precipitate. The filtrate was washed successively with 25-mL portions of H_2O and saturated $NaHCO_3$ solution, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The white residue which remained was washed with Et_2O and suction filtered. The yield of the crystalline solid was 1.05 g. An analytical sample was obtained by recrystallization from an EtOH–Et_2O mixture.

***N*-Cyanomethyl-2-phenylsuccinimide (3).** A suspension of 4.63 g (0.11 mol) of a 57% mineral oil dispersion of NaH in 20 mL of DMF was treated with 17.5 g (0.10 mol) of 2-phenylsuccinimide (1a, R = H) in 55 mL of DMF under a N_2 atmosphere. During the addition, the temperature of the reaction mixture increased to 30 °C and a considerable amount of precipitate formed. After the exothermic reaction had subsided, the suspension was heated at 80 °C for 2 h and cooled to room temperature. After cooling, 8.31 g (0.11 mol) of $ClCH_2CN$ in 20 mL of DMF was added in a dropwise fashion. During the addition, the temperature rose to 50 °C but decreased after stirring for several minutes. The reaction mixture was heated at 80 °C for 18 h, cooled, and decomposed with a few milliliters of EtOH. The light brown solution was poured into 300 mL of an ice–H_2O mixture. Vigorous stirring produced a light brown solid. Recrystallization from 95% EtOH yielded 11.5 g of white prisms.

***N*-(2-Aminoethyl)-2-phenylsuccinimide Hydrochloride (4).** See Table II. This compound was prepared from 3 according to a described method.[1]

***N*-(2-Chloroacetamidoethyl)-2-phenylsuccinimide (5).** See Table II. The synthesis of compound 5 was accomplished by the reaction of $(ClCH_2CO)_2O$ with 4 following a general procedure.[1]

***N*-(2-Maleamylethyl)-2-phenylsuccinimide (6).** See Table I. Compound 6 was synthesized from 4 by a reported procedure.[1]

***N*-(2-Maleimidoethyl)-2-phenylsuccinimide (7).** See Table I. Compound 7 was prepared from 6 according to conditions previously described.[1]

***N*-(*p*-Aminophenethyl)-2-phenylsuccinimide (10).** Compound 9, 5.6 g (0.0166 mol), was suspended in 50 mL of 6 N HCl, and the suspension was heated at reflux for 0.5 h. The mixture was cooled in an ice bath, and concentrated NH_4OH was added until the solution was strongly basic. Upon cooling, a cream-colored solid formed. The solid was filtered and dried to yield 4.0 g (82%) of 10. Analytically pure 10 was obtained by recrystallization from 95% EtOH.

***N*-[*p*-(Chloroacetamido)phenethyl]-2-phenylsuccinimide (11).** A mixture of 0.70 g (0.0034 mol) of 10, 0.42 g (0.0041 mol) of Et_3N, and 50 mL of dry THF was treated dropwise with a solution of 0.46 g (0.0041 mol) of $ClCH_2COCl$ in 5 mL of dry THF at room temperature. Immediately, a white precipitate formed and the reaction mixture was stirred at room temperature for 17 h. The reaction mixture was filtered to remove the Et_3N·HCl, and the filtrate was concentrated under reduced pressure leaving a white residue. The residue was washed with 100 mL of H_2O and suction filtered. The H_2O-insoluble material was recrystallized from 95% EtOH to yield 0.585 g of 11.

***N*-(*p*-Maleamylphenethyl)-2-phenylsuccinimide (12).** See synthesis of 6 and Table I.

***N*-(*p*-Maleimidophenethyl)-2-phenylsuccinimide (13).** See synthesis of 7 and Table I.

***p*-Aminophenylacetonitrile Hydrochloride (14).** A 200-mL ethanolic mixture of 21.8 g (0.134 mol) of *p*-nitrophenylacetonitrile (Eastman Kodak), 35 mL of concentrated HCl, and 1.8 g of 5% Pd/C was shaken on the Parr hydrogenator for 1 h. The reaction mixture was diluted with 50 mL of H_2O and filtered, and the solvent was removed under reduced pressure. The yellow solid which remained was dried to yield 16.0 g (71%) of yellow crystals: mp 230–235 °C (lit.[4] mp 229–231 °C).

***p*-Acetamidophenylacetonitrile (15).** A total of 16.0 g (0.095 mol) of 14 was dissolved in 250 mL of H_2O and treated with 1 g of Darco. The solution was filtered and heated to 50 °C. To the warm solution was added 10.7 g (0.105 mol) of $(CH_3CO)_2O$ followed by 7.8 g (0.095 mol) of NaOAc in 50 mL of H_2O. The solution was cooled, whereupon a white solid crystallized from solution. Recrystallization from H_2O produced 11.0 g (60%) of white crystals: mp 96–98 °C (lit.[4] mp 96 °C).

***p*-Acetamidophenethylamine (16).** A mixture of 15.1 g (0.087 mol) of 15, 24.2 mL of concentrated HCl, 0.2 g of PtO_2, and 250 mL of 95% EtOH was shaken on the Parr hydrogenator for 4 h. The reaction mixture was filtered, diluted with 50 mL of H_2O, and evaporated until a total volume of 50 mL remained. The aqueous solution was basified with 2.5 M NaOH and extracted with $CHCl_3$ (4 × 50 mL). The $CHCl_3$ extract was dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The yellow solid which remained was recrystallized from C_6H_6–$CHCl_3$ to give 6.9 g (45%) of a powdery yellow solid: mp 117–120 °C. Anal. ($C_{10}H_{14}N_2O$) C, H, N.

***N*,2-Bis(3-aminopropyl)-2-phenylsuccinimide Dihydrochloride (18).** To a solution of 3.0 g (0.017 mol) of 2-phenylsuccinimide (1a, R = H) in 25 mL of dioxane was added 0.4 mL of a 40% methanolic solution of benzyltrimethylammonium hydroxide (Triton B), and the solution was heated at 60 °C for 1 h. The deep red solution was cooled to room temperature, and 2.2 g (0.041 mol) of CH_2=CHCN in 5 mL of dioxane was added dropwise. The reaction mixture was stirred at room temperature for 0.5 h, followed by heating at 70 °C for 1.5 h. The deep purple solution was cooled and neutralized with 10% HCl. Removal of the solvent under reduced pressure left 4.5 g of a yellow oil. An IR (film) of the oil revealed the presence of a C≡N group at 4.43 μ, and the oil was homogeneous by TLC. The crude nitrile 17 was catalytically hydrogenated without further purification. A mixture of 4.5 g of the crude nitrile 17, 14.4 mL of concentrated HCl, 0.1 g of PtO_2, and 200 mL of 95% EtOH was catalytically hydrogenated to yield 18 following the general procedure[1] for the reduction of nitriles. Recrystallization of crude 18 from EtOH–Et_2O produced 3.5 g (57% based on 2-phenylsuccinimide) of a white powder: mp 240–245 °C. An analytical sample was obtained by recrystallization from EtOH–Et_2O to give analytically pure 18: mp 238.5–240.5 °C. Anal. ($C_{16}H_{25}Cl_2N_3O_2$) C, H, N.

***N*,2-Bis(3-chloroacetamidopropyl)-2-phenylsuccinimide (19).** Compound 18, 1.90 g (0.0053 mol), 1.81 g (0.0106 mol) of $(ClCH_2CO)_2O$, and 1.08 g (0.0106 mol) of Et_3N in 25 mL of dry DMF were allowed to react under conditions previously described.[1] Recrystallization of the crude product from C_6H_6–*n*-C_6H_{14} yielded 0.7 g of white powder, mp 109–111.5 °C. From the mother liquor a second crop of 0.3 g of a white powder was obtained: mp 102–106 °C. The combined yield was 1.0 g (43%): IR (KBr) 6.05 μ (amide C=O); NMR ($CDCl_3$) δ 7.56 (s, 5 H, ArH), 6.99 (br s, 2 H, NH), 4.12 (s, 4 H, $COCH_2Cl$), 3.85–0.72 (m, 14 H, aliphatic ring CH and $CH_2CH_2CH_2N$). Anal. ($C_{20}H_{25}Cl_2N_3O_4$) H; C: calcd, 54.30; found, 55.00; N: calcd, 9.50; found, 8.90.

3-Ethoxalyl-*N*,2-dimethyl-2-phenylsuccinimide (21). Compound 21 was prepared following the procedure of Hauck and Fan[5] in 54% yield: mp 92–95 °C (lit. mp 98–99 °C).

3-Methylidene-*N*,2-dimethyl-2-phenylsuccinimide (22). The synthesis of 22 was accomplished by modification of the method of Seidel and Cook.[6] To a suspension of 11.6 g (0.0038 mol) of 21 in 65 mL of distilled H_2O was added 12.7 mL of 40% aqueous CH_2O and 12.7 mL of 48% aqueous Et_2NH. After stirring for 12 h at room temperature, a dark orange oil had separated. The entire reaction mixture was extracted with $CHCl_3$ (3 × 50 mL), the combined $CHCl_3$ extract was washed with 25 mL of 10% HCl, dried ($MgSO_4$), and filtered, and the solvent was removed under reduced pressure. The dark brown oil which remained was

chromatographed on 50 g of silica gel using a C_6H_6–EtOAc solvent system. The chromatography produced 7.3 g (88%) of a light yellow oil. Anal. ($C_{13}H_{13}NO_2$) C, H, N.

3-Cyanomethyl-*N*,2-dimethyl-2-phenylsuccinimide (23). Compound **23** was prepared following the procedure of Seidel and Cook.[6] A suspension of 35 g (0.71 mol) of NaCN in 70 mL of DMF was cooled in an ice bath and 35 mL of concentrated HCl was added dropwise. After the addition had been completed, 6.1 g (0.0028 mol) of **22** in 70 mL of DMF was added to the suspension. After stirring for 18 h, the dark brown suspension was diluted with 200 mL of H_2O and extracted with $CHCl_3$ (4 × 5 mL). The combined $CHCl_3$ extracts were washed with H_2O (2 × 100 mL) and evaporated until only a few mL of solution remained. The dark red solution was poured into 150 mL of an ice–H_2O mixture and a red oil separated. The oil was dissolved in 100 mL of $CHCl_3$, washed with H_2O (2 × 5 mL), dried ($MgSO_4$), and filtered. Removal of the solvent left 5.0 g (74%) of a red oil: IR (film) 4.42 μ (C≡N); NMR ($CDCl_3$) δ 7.50–7.06 (m, 5 H, ArH), 3.50–2.37 (m, 6 H, aliphatic ring CH, CH_2CN, and NCH_3 at 3.17 and 3.12), 1.91 and 1.72 (s, 3 H, CCH_3). The NMR spectrum indicated the product was a mixture of diastereomers and attempts to separate the diastereomers were unsuccessful. The product was not further purified but used directly in the preparation of **24**.

3-(2-Aminoethyl)-*N*,2-dimethyl-2-phenylsuccinimide Hydrochloride (24). Compound **24** was prepared from 5.0 g (0.021 mol) of crude **23**, 8 mL of concentrated HCl, and 0.100 g of PtO_2 in 150 mL of 95% EtOH following a general procedure.[1] The crude hydrochloride was dissolved in absolute EtOH, treated with Darco G-60, and filtered, and Et_2O was added to the point of cloudiness. Upon cooling a white solid crystallized from solution. After drying in vacuo over P_2O_5 the yield was 1.25 g (21%) of a white powder, mp 190–200 °C. A sample for elemental analysis was obtained by recrystallization from an EtOH–Et_2O mixture to yield a powdery solid: mp 185–200 °C; IR (KBr) 3.00–3.20 μ (br NH_3^+); NMR (D_2O) δ 7.40 (s, 5 H, ArH), 3.31–2.92 (m, 6 H, aliphatic ring CH, CH_2, and NCH_3 at 3.07 and 3.01), 2.35–1.53 (m, 5 H, aliphatic CH_2 and CCH_3 at 1.72 and 1.57). Anal. ($C_{14}H_{19}ClN_2O_2$) H, N; C: calcd, 59.46; found, 55.29.

3-(2-Chloroacetamidoethyl)-*N*,2-dimethyl-2-phenylsuccinimide (25). Compound **25** was prepared from 1.0 g (0.0035 mol) of the hydrochloride **24**, 0.599 g (0.0035 mol) of $(ClCH_2CO)_2O$, and 0.354 g (0.0035 mol) of Et_3N in the manner described for the synthesis of **5**. An almost colorless oil was obtained which was purified by column chromatography using 50 g of silica gel and a C_6H_6–EtOAc solvent system. The chromatography yielded 0.200 g (18%) of a white powder: mp 76–79.5 °C. Anal. ($C_{16}H_{19}ClN_2O_3$) C, H, N.

Acknowledgment. One of us (A.M.C.) wishes to thank the American Foundation for Pharmaceutical Education for partial support in the form of a fellowship. The authors are indebted to Dr. Ronald Krall, ADD Program, National Institutes of Health, for providing the anticonvulsant activity data.

References and Notes

(1) M. J. Kornet, A. M. Crider, and E. O. Magarian, *J. Med. Chem.*, **20**, 405 (1977).
(2) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors", Wiley, New York, N.Y., 1967, p 4.
(3) C. A. Miller and L. M. Long, *J. Am. Chem. Soc.*, **73**, 4895 (1951).
(4) J. R. A. Pollock and R. Stevens, Ed., "Dictionary of Organic Compounds", Vol. 1, 4th ed, Oxford University Press, New York, N.Y., 1965, p 196.
(5) F. P. Hauck, Jr., and J. T. Fan, *J. Org. Chem.*, **34**, 1703 (1969).
(6) M. C. Seidel and R. S. Cook, *J. Heterocycl. Chem.*, **3**, 311 (1966).

Inhibitors of Folate Biosynthesis. 1. Inhibition of Dihydroneopterin Aldolase by Pteridine Derivatives

Morris Zimmerman, Richard L. Tolman,* Harriet Morman, Donald W. Graham, and Edward F. Rogers

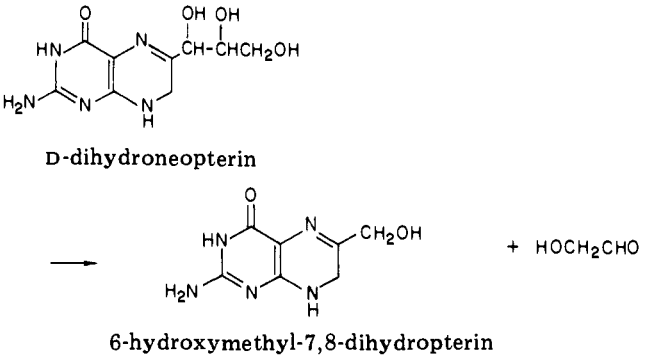
Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065. *Received January 13, 1977*

2-Amino-6-carboxamido-7,8-dihydropteridin-4-one and 2-amino-6-hydroxymethyl-7,7-dimethyl-7,8-dihydropteridin-4-one have been shown to be good inhibitors of *Escherichia coli* dihydroneopterin aldolase, an early enzyme of de novo folate biosynthesis.

Many bacteria and some parasites unlike their mammalian hosts have difficulty concentrating certain of the water-soluble vitamins such as folic acid and riboflavin and must, therefore, synthesize them de novo. A favorite approach to the chemotherapy of bacterial and parasitic diseases has been the interference with these biosynthetic sequences. Both of the vitamins cited above are biosynthesized from a guanosine triphosphate precursor[1,2] in separate multistep procedures.[3] The final steps of tetrahydrofolate biosynthesis, those catalyzed by dihydropteroate (or dihydrofolate) synthetase and dihydrofolate reductase, are common chemotherapeutic targets[4] for antibacterial and antiparasitic agents (e.g., methotrexate, pyrimethamine, ethopabate, and sulfas), but inhibitors of the early steps of folate biosynthesis have not been studied in detail.

Dihydroneopterin aldolase[5] cleaves a two-carbon segment from D-dihydroneopterin in an early step of pteroate synthesis to form 6-hydroxymethyl-7,8-dihydropterin

Scheme I



(Scheme I). The study of chemical inhibitory requirements for this enzyme was particularly attractive since the substrate is not a phosphorylated derivative obviating the conversion of potential inhibitors to the corresponding