Synthesis and Pharmacological Evaluation of Spiro-Analogues of 5-Benzyl-5-ethyl Barbituric Acid

S. Bruce King,[1,3] Eugene S. Stratford,[1,6] Charles R. Craig,[2] and E. Kim Fifer,[1,4,5]

Received December 12, 1994; accepted March 17, 1995
KEY WORDS: anticonvulsant; barbiturate; spirobarbiturate; 5-benzyl-5-ethylbarbituric acid.

INTRODUCTION

5,5-Disubstituted barbituric acid derivatives, such as phenobarbital (**1a**), generally exhibit CNS depressant activity (1). However, slight structural modifications in many depressant barbiturates produce compounds with excitatory or convulsant activity, e.g., 5-benzyl-5-ethylbarbituric acid (**1b**) (1–3) a homologue of phenobarbital.

The structure and conformation of the C-5 side chains of the barbiturates have been suggested to be determinants of the different biological activities (2–7). Many 5,5-disubstituted barbituric acid derivatives have been synthesized in an effort to develop a structure-activity relationship for these compounds (1–6). However, syntheses and pharmacological evaluations of conformationally restricted analogues utilizing the 5,5-spirobarbiturate ring system have not been as common (8–10 and references therein). Thus, in order to examine the relationship between the conformation of the C-5 side chains of the barbiturates and their anticonvulsant activity, we designed a series of conformationally restricted spiro-analogues (**2** and **3a-b**) of **1b**. The spiro ring effectively restricts the aromatic ring to different conformations without a large increase in carbon number or steric bulk. Restriction of the aromatic rings to different areas relative to the barbiturate ring may produce compounds with different pharmacological activity. This paper describes the synthesis and preliminary pharmacological testing of **2** and **3a-b**.

[1] Department of Basic Pharmaceutical Sciences, School of Pharmacy, West Virginia University, Morgantown, West Virginia 26505.
[2] Department of Pharmacology and Toxicology, School of Medicine, West Virginia University, Morgantown, West Virginia 26505.
[3] Present address: The Scripps Research Institute, La Jolla, California 92037.
[4] Present address: Department of Biopharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205.
[5] To whom correspondence should be addressed at College of Pharmacy, Slot 522, University of Arkansas for Medical Sciences, 4301 West Markham Street, Little Rock, Arkansas 72205.
[6] Deceased.

MATERIALS AND METHODS

Synthesis

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained with a Perkin-Elmer Model 1320 infrared spectrophotometer. Unless otherwise indicated, ^{1}H NMR spectra were recorded on Varian Model T-60 or EM360 spectrometers. High field NMR spectra were obtained on a JEOL Model JNM-GX 270 FT NMR spectrometer. Mass spectra were recorded at an electron energy of 70 eV on a Finnigan Model 4500MS/DS. Elemental analyses were performed by Atlantic Microlab Inc., Atlanta, GA.

3,4-Dihydro-2,2(1H)-naphthalenedicarboxylic acid (**6**). A solution of 3.65 g (0.016 mol) of **5** in 100 ml of EtOH was reduced over 0.20 g of 10% Pd-C at 30 psi H_2. Filtration and concentration gave 2.3 g (67%) of **6** as a white solid which was recrystallized from ethyl acetate/heptane (1:1): mp 175–178° C [lit (13) mp 176°C].

Dimethyl 3,4-dihydro-2,2(1H)-naphthalenedicarboxylate (**7a**). A solution of 5.95 g (0.027 mol) of **6** and 1 ml of concentrated sulfuric acid in 50 ml of MeOH was refluxed for 24 hours. The reaction volume was reduced by one-half. Ether and water were added, and the aqueous layer was extracted with Et_2O. The combined Et_2O layers were washed with saturated $NaHCO_3$, dried over $MgSO_4$, and concentrated to yield 4.2 g (64%) of **7a**: IR (neat) 2960, 1735 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.3 (t, 2H, $ArCH_2CH_2$), 2.9 (t, 2H, $ArCH_2CH_2$), 3.3 (s, 2H, $ArCH_2$), 3.8 (s, 6H, CO_2CH_3), 7.1 (s, 4H, ArH); m/e 248.2 (m^+), 129.2 (base); Anal. Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.64; H, 6.53.

Benzyl 2-bromopropionate (**9**). Ethyl 2-bromopropionate (50 g, 0.28 mol), 35.7 g (0.33 mol) of benzyl alcohol, and 1.7 g of *p*-toluenesulfonic acid were dissolved in 70 ml of benzene and refluxed for 24 hours with the removal of ethanol via a Dean-Stark trap. The mixture was washed with saturated $NaHCO_3$, and the organic portion was dried over Na_2SO_4, and concentrated. Elution through a silicic acid column (120 mm × 70 mm) with chloroform afforded 57.8 g (86%) of **9** as a pale yellow liquid: IR 2980, 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.7 (d, 3H, CH_3), 4.3 (q, 1H, CH), 5.1 (s, 2H, CH_2), 7.3 (s, 5H, ArH).

3-Benzyl 2,2-dimethyl 1-phenyl-2,2,3-butanetricarboxylate (**10**). Sodium hydride (4.8 g, 0.10 mol) was stirred in 50 ml of dimethylformamide under nitrogen while 17.1 g (0.08 mol) of **8** in 20 ml of dimethylformamide was added at a rate which kept the temperature at 40–50°C. After evolution of gas ceased, 24.2 g (0.01 mol) of **9** in 20 ml of dimethylformamide was slowly added. The mixture was then heated at 60° overnight. Water (100 ml), saturated $NaHCO_3$ (100 ml), and Et_2O (100 ml) were added. The ethereal layer was washed with saturated NaCl, dried over $MgSO_4$, and concentrated to yield 26.4 g (88%) of **10** as an oil: IR (neat) 2960, 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.3 (d, 3H, CH_3), 3.2 (s, 2H, $ArCH_2$), 3.4 (m, 1H, CH), 3.6 (d, 6H, CO_2CH_3), 5.1 (s, 2H, CO_2CH_2Ar), 7.1–7.6 (m, 10H, ArH); m/e 384 (m^+), 91 (base); Anal. Calcd for $C_{22}H_{24}O_6$: C, 68.74; H, 6.29. Found: C, 68.66; H, 6.32.

2,2-Dimethyl 1-phenyl-2,2,3-butanetricarboxylic acid (**11**). A solution of 9.0 g (0.023 mol) of **10** in 100 ml of MeOH

0724-8741/95/0800-1240$07.50/0 © 1995 Plenum Publishing Corporation

was reduced over 0.80 g of 10% Pd-C at 60 psi H_2. Filtration and concentration gave a residue which was dissolved in Et_2O and extracted with saturated $NaHCO_3$. The combined aqueous layers were acidified, extracted with Et_2O, dried over Na_2SO_4, and concentrated to give 3.47 g (51%). Recrystallization from ethyl acetate/hexane afforded 1.40 g of **11**: mp 98–99°C; IR (KBr) 3400–2500, 1710 cm^{-1}; ^1H NMR (acetone-d_6) δ 1.2 (d, 3H, CH_3), 3.0 (m, 1H, CH), 3.2 (s, 2H, $ArCH_2$), 3.6 (s, 6H, CO_2CH_3), 7.1 (s, 5H, ArH), 7.9 (s, 1H, CO_2H); m/e 294 (m^+), 189 (base); Anal. Calcd for $C_{15}H_{18}O_6$: C, 61.22; H, 6.16. Found: C, 61.28; H, 6.18.

Dimethyl 3,4-dihydro-3-methyl-4-oxo-2,2(1H)-naphthalenedicarboxylate (**12**). A solution of 7.0 g (0.024 mol) of **11** in 25 ml of concentrated sulfuric acid was heated at 45°C for 4 hours. The mixture was poured onto ice and the precipitate was extracted into Et_2O, dried over Na_2SO_4, and concentrated to leave 4.9 g (75%) of a white solid. Recrystallization from ethyl acetate/hexane afforded 3.8 g of **12**: mp 111–113°C; IR (KBr) 2970, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2 (d, 3H, CH_3), 3.1 (m, 1H, CH), 3.4 (s, 2H, $ArCH_2$), 3.8 (d, 6H, CO_2CH_3), 7.1–7.9 (m, 4H, ArH); m/e 276 (m^+), 217 (base); Anal. Calcd for $C_{15}H_{16}O_5$: C, 65.21; H, 5.83. Found: C, 65.27; H, 5.86.

Dimethyl 3,4-dihydro-3-methyl-2,2(1H)-naphthalenedicarboxylate (**7b**). A solution of 9.7 g (0.035 mol) of **12**, 10 ml of perchloric acid and 400 ml of MeOH was reduced over 0.70 g of 10% Pd-C at 60 psi H_2. The mixture was filtered and concentrated to about one-third volume. Saturated NaCl was added, and the mixture was extracted with Et_2O, dried over $MgSO_4$, and concentrated to leave a yellow oil. This oil was dissolved in 250 ml of MeOH along with 10 ml of concentrated H_2SO_4, then refluxed for 24 hours. Approximately three-fourths of the MeOH was removed under reduced pressure. Water was added, and the mixture was extracted with Et_2O, dried over $MgSO_4$, and concentrated to give 7.7 g (84%) of **7b**: IR (neat) 2970, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.1 (d, 3H, CH_3), 2.8–3.0 (m, 3H, $ArCH_2CH$ and CH), 3.2 (s, 2H, $ArCH_2$), 3.8 (s, 6H, CO_2CH_3), 7.1 (s, 4H, ArH); m/e 262 (m^+), 143 (base); Anal. Calcd for $C_{15}H_{18}O_4$: C, 68.69; H, 6.92. Found: C, 68.58; H, 6.94.

7-Phenyl-2,4-diazospiro[5.5]undecane-1,3,5-trione (**2**). Urea (1.17 g, 0.019 mol) in 6 ml of dimethyl sulfoxide was slowly added to a suspension of 1.02 g (0.04 mol) of sodium hydride in 9 ml of dimethyl sulfoxide under nitrogen. When evolution of H_2 gas had ceased, 4.7 g (0.016 mol) of **4** in 6 ml of dimethyl sulfoxide was slowly added. After stirring for 1 hour at room temperature and 2.5 hours at 80°C, the mixture was cooled in an ice bath, acidified, and diluted with water. The precipitate was stored overnight at 4°C, filtered, and dried under reduced pressure to afford 2.46 g (58%) of **2** which was recrystallized from 95% EtOH (39% recovery): mp 215–217°C; IR 3300, 2950, 1720 cm^{-1}; ^1H NMR (270 MHz, acetone-d_6) δ 1.54–1.69 (m, 4H, $CCH_2CH_2CH_2$), 2.58 (ddd, 1H, CCH_2, axial), 3.30 (dd, 1H, CH), 7.12–7.27 (m, 5H, ArH), 9.93 (s, 2H, NH); m/e 272 (m^+), 91 (base). Anal. Calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 66.27; H, 5.95; N, 10.23.

3,4-Dihydrospiro[naphthalene-2(1H),5'(2'H)-pyrimidine]-2',4',6'(1'H,3'H)-trione (**3a**). The synthetic procedure used to prepare **2** was used to convert **7a** to **3a** (95% yield): mp 231–233°C; IR (KBr) 3100–3000, 1740, 1450 cm^{-1}; ^1H NMR (270 MHz, acetone-d_6) δ 2.25 (t, 2H, $ArCH_2CH_2$), 2.83 (t, 2H, $ArCH_2CH_2$), 3.31 (s, 2H, $ArCH_2C$), 7.09–7.13 (m, 4H, ArH), 10.11 (s, 2H, NH); m/e 244 (m^+, base); Anal. Calcd for $C_{13}H_{12}N_2O_3 \cdot 1/2H_2O$: C, 61.65; H, 5.17; N, 11.06. Found: C, 61.19; H, 5.12; N, 11.17.

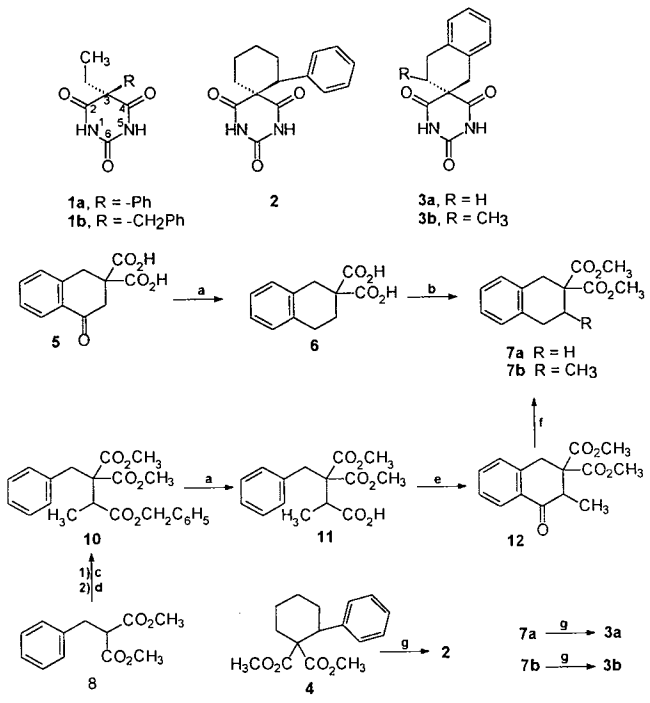
3,4-Dihydro-3-methyl-spiro[naphthalene-2(1H),5'(2'H)-pyrimidine]-2',4',6'(1'H,3'H)-trione (**3b**). The synthetic procedure used to prepare **2** was used to convert **7b** to **3b** (40% yield): mp 207–208°C; IR (KBr) 3000, 1680, 1410 cm^{-1}; ^1H NMR (270 MHz, acetone-d_6) δ 1.08 (d, 3H, CH_3), 2.47 (m, 1H, CH), 2.86 (m, 2H, $ArCH_2CH$), 3.38 (dd, 2H, $ArCH_2C$), 7.09 (s, 4H, ArH), 10.11 (br s, 2H, NH); m/e 258 (m^+, base); Anal. Calcd for $C_{14}H_{14}N_2O_3$: C, 65.12; H, 5.46; N, 10.85. Found: C, 65.01; H, 5.48; N, 10.79.

Pharmacology

Target compounds were submitted to the Antiepileptic Drug Development Program of the National Institute of Neurological and Communicative Disorders and Stroke (14) to be screened for anticonvulsant activity using the maximal electroshock seizure (MES) test and the subcutaneous pentylenetetrazol (scMet) test as originally established by Swinyard, et al. (15). The MES test evaluates the ability of a compound to prevent the spread of a seizure through neural tissue and is performed by administering, via corneal electrodes, a 50 mA (mice) or 150 mA (rats), 60 Hz alternating current for 0.2 sec duration. Protection is defined as the ability of a compound to prevent the hind limb tonic extension component of the electrically induced seizure. The scMet test evaluates the ability of a compound to raise seizure threshold in excitable neural tissue and is performed by administering pentylenetetrazole (85 mg/kg) subcutaneously. Protection is defined as the ability of a compound to prevent a single episode of clonic spasms of five second duration. Toxicity to the central nervous system was evaluated in the rotorod test. A positive toxic response is defined as the animals failure to remain for one minute on a knurled plastic rod rotating at six rpm. Compounds were suspended in a 0.5% aqueous suspension of methylcellulose and were administered intraperitoneally in mice (male Carworth Farm No. 1, 18–25 g) and orally in rats (male Sprague-Dawley, 100–150 g).

RESULTS AND DISCUSSION

Compounds (**2**, **3a–b**) were synthesized by first preparing the properly substituted malonic esters followed by condensation with urea as illustrated in Scheme I. Malonic ester **4**, required for the synthesis of **2**, was prepared by the method of Zimmerman and Cutshall (11). This was condensed with urea to afford **2**. Tetralone **5**, prepared by the method of Hathaway *et al.* (12), was catalytically reduced to the tetralin dicarboxylic acid (**6**), then esterified to give malonic ester **7a**. Condensation of **7a** with urea gave **3a**. Benzyl 2-bromopropionate (**9**), prepared by transesterification of ethyl 2-bromopropionate with benzyl alcohol, was alkylated with **8** to give the triester (**10**). Catalytic hydrogenolysis of the benzyl group of **10** to give **11**, followed by cyclodehydration afforded tetralone **12**. The benzyl ketone of **12** was



Scheme I

catalytically reduced and esterified under Fisher conditions to give **7b**. Condensation with urea gave **3b**.

Spirobarbiturates **2** and **3a–b** were evaluated as anticonvulsants in mice and rats as described above. The results of these screens are presented in Tables I (mice) and II (rats).

In mice at 30 minutes, compound **2** exhibited protection against MES induced seizures only at 300 mg/kg. It also exhibited neurotoxicity at this dose. Its activity in rats at 50 mg/kg was insignificant (1/4 protected against MES and scMet).

Both **3a** and **3b** exhibited activity against MES and scMet in mice with **3b** showing the greatest activity. Similarly, **3b** exhibited neurotoxicity at a lower dose than **3a**. Both compounds were active against MES in rats at 50 mg/kg, with the greatest activity being exhibited at 4 to 6 hours. Both showed weak activity against scMet seizures in rats, and neither exhibited neurotoxicity.

From models it can be seen that the phenyl ring of **2a** is restricted to a radically different area than for **3a** and **3b**. The phenyl ring of **3a** and **3b** is oriented nearly perpendicular to and above the barbiturate ring system, whereas the phenyl ring of **2** is oriented much closer and perpendicular to the barbiturate ring. These data suggest that better activity is seen when the phenyl ring of the C5 side chain is oriented to a position similar to that of **3a** and **3b**.

Table II. Phase I Anticonvulsant Test Results of Spirobarbiturates **2** and **3a-b** in Rats (50 mg/kg)

Compound	Hour	MES[a]	scMet[a]	Tox[b]
2	0.25	0/4	0/4	0/4
	0.5	0/4	0/4	0/4
	1	0/4	0/4	0/4
	2	1/4	0/4	0/4
	4	0/4	1/4	0/4
3a	0.25	1/4	1/4	0/4
	0.5	2/4	—	0/4
	1	2/4	—	0/4
	2	3/4	—	0/4
	4	4/4	—	0/4
	6	4/4	—	—
	24	2/4	—	—
3b	0.25	2/4	1/4	0/4
	0.50	3/4	2/4	0/4
	1	0/4	1/4	0/4
	2	2/4	0/4	0/4
	4	4/4	2/4	0/4
	6	4/4	—	—
	24	2/4	—	—

[a] Number of animals protected/number of animals tested.
[b] Number of animals exhibiting toxicity/number of animals tested.

ACKNOWLEDGMENTS

The authors wish to acknowledge financial support for this project from the West Virginia University School of Pharmacy. The authors would like to thank Mr. Jim Stables for performing the pharmacological analyses through the An-

Table I. Phase I Anticonvulsant Test Results of Spirobarbiturates **2** and **3a-b** in Mice

Compd	Dose (mg/kg)	MES[a] 0.5 hr	MES[a] 4 hr	scMet[a] 0.5 hr	scMet[a] 4 hr	Tox[b] 0.5 hr	Tox[b] 4 hr
2	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	1/1	0/1	0/1	0/1	4/4	0/2
3a	30	0/1	0/1	3/5	0/1	0/8	0/2
	100	1/3	0/3	5/5	2/5	0/12	0/8
	300	1/1	1/1	5/5	4/5	5/8	4/6
3b	30	1/1	0/1	4/5	0/1	0/8	0/2
	100	3/3	1/3	5/5	0/1	4/12	4/4
	300	1/1	1/1	5/5	5/5	8/8	6/6

[a] Number of animals protected/number of animals tested.
[b] Number of animals exhibiting toxicity/number of animals tested.

tiepileptic Drug Development Program, National Institutes of Health.

REFERENCES

1. J. A. Vida and E. H. Gerry. Cyclic Ureides. In J. A. Vida (ed), *Anticonvulsants-Medicinal Chemistry. A series of Monographs*, Vol 15, Academic Press, New York, 1977, pp. 151–173.
2. P. R. Andrews, G. P. Jones, and D. Lodge. Convulsant, anticonvulsant and anaesthetic barbiturates. 5-ethyl-5-(3′-methylbut-2′-enyl)-barbituric acid and related compounds, *Eur. J. Pharmacol.* 55:115–120 (1979).
3. P. R. Andrews, G. P. Jones and D. B. Poulton. Convulsant, anticonvulsant and anaesthetic barbiturates. In vivo activities of oxo- and thiobarbiturates related to pentobarbitone, *Eur. J. Pharmacol.* 79:61–65 (1982).
4. G. P. Jones and P. R. Andrews. Convulsant and anticonvulsant barbiturates. 1. Molecular conformations from classical potential-energy calculations, *J. Med. Chem.* 23:444–448 (1980).
5. P. R. Andrews and G. P. Jones. Convulsant and anticonvulsant barbiturates 2. Molecular orbital calculations, *Eur. J. Med. Chem.-Chimica Therapeutica* 16:139–143 (1981).
6. P. R. Andrews, A. J. Jones, G. P. Jones, A. Marker, and E. A. Owen. Convulsant and anticonvulsant barbiturates 3. Conformational analysis by ^{1}H and ^{13}C NMR, *Eur. J. Med. Chem.-Chimica Therapeutica* 16:145–150 (1981).
7. P. R. Andrews, L. C. Mark, D. A. Winkler, and G. P. Jones. Structure-activity relationships of convulsant and anticonvulsant barbiturates: A computer-graphic-based pattern-recognition analysis, *J. Med. Chem.* 26:1223–1229 (1983).
8. A. W. Dox and L. Yoder. Spiro-pyrimidines. III. Condensation of cyclopropane-1,1-dicarboxylic ester with ureas, *J. Amer. Chem. Soc.* 43:2097–2101 (1921).
9. A. C. Cope, P. Kovacic, M. Burg. Spirobarbituric acids containing a six-membered carbocyclic ring, *J. Amer. Chem. Soc.* 71:3658–3662 (1949).
10. D. B. Reddy, V. Padmavathi, and P. V. Ramana Reddy. Cyclohexanonedicarboxylates as precursors for the synthesis of some spiropyrimidinetetrones and spiropyrazolidinetriones, *Ind. J. Chem.* 31B:774–777 (1992).
11. H. E. Zimmerman and T. W. Cutshall. The stereochemistry of ketonization. VI. Decarboxylation of 2-phenylcyclohexane-1,1-dicarboxylic acid, *J. Amer. Chem. Soc.* 80:2893–2896 (1958).
12. B. A. Hathaway, D. E. Nichols, M. B. Nichols, and G. K. W. Yim. A new, potent, conformationally restricted analogue of amphetamine: 2-amino-1,2-dihydronaphthalene, *J. Med. Chem.* 25:535–538 (1982).
13. J. Braun and F. Zobel. Syntheses in the aliphatic-aromatic series. XIV. Homo-o-xylylene bromide, *Ber.* 56B:2142–2152 (1923).
14. (a) Anticonvulsant Screening Project, Antiepileptic Drug Development Program, National Institutes of Health, DHEW Publ., 1978, NIH 78-1093. (b) R. L. Krall, J. K. Penry, B. G. White, H. J. Kupferburg, and E. A. Swinyard. Antiepileptic drug development. II. Anticonvulsant drug screening, *Epilepsia* 19: 409–428 (1978). (c) E. A. Swinyard, J. H. Woodhead, H. S. White, and M. R. Franklin. General principals. Experimental selection, quantification, and evaluation of anticonvulsants. In R. Levy, R. Mattson, B. Meldrum, J. K. Penry, and F. E. Dreifuss (eds), *Antiepileptic Drugs*, 3rd ed., Raven Press, New York, 1989, pp. 85–102.
15. E. A. Swinyard, W. C. Brown, and L. S. Goodman. Comparative assays of antiepileptic drugs in mice and rats, *J. Pharmacol. Exp. Ther.* 106:319–330 (1952).