Notes

Structure-Activity Relationships of Phenytoin-like Anticonvulsant Drugs

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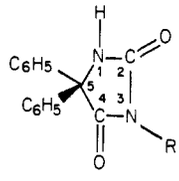
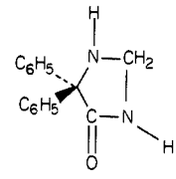
A limited series of compounds structurally related to phenytoin has been tested in the maximal electroshock seizure (MES) test in order to define the impact on their antiepileptic activity of reducing the ability to form a hydrogen bond. A net stepwise decrease of the anticonvulsant activity was observed when the hydantoin ring structure was altered into succinimide and pyrrolidinone and when these rings were N-methylated. The pharmacological data analyzed in terms of structure–activity relationships (SAR) indicate the importance of the capability of forming hydrogen bonds. Further insight into the molecular mode of action of phenytoin was gained by a ^{13}C NMR study of three phenytoin analogues deuterated in one of the phenyl groups, i.e., *o*-, *m*-, *p*-deuteriophenytoin. This approach allowed the unambiguous assignment of the chemical shifts of the ortho, meta, para, and ipso carbons. Measurement of the T_1 relaxation times provided a value of 0.2 s for the para carbon and 0.8 s for the meta and ortho carbons of the phenytoin phenyl rings. These data are consistent with the view that the two phenyl groups possess a certain degree of rotational freedom along the para–ipso axis. More generally, the present results, as well as some literature data, support the concept that the ability to form hydrogen bonds as well as a certain degree of motional freedom of the phenyl groups are important SAR features in antiepileptic phenytoin-like drugs.

Phenytoin (5,5-diphenylhydantoin, **1**) was introduced



1, R = H
4, R = CH_3



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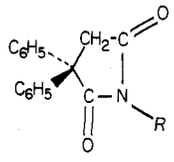
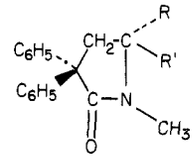


3, R = H
5, R = CH_3



(*R*)-**6**, R = H; R' = CH_3
(*S*)-**6**, R = CH_3; R' = H

in 1938 as an antiepileptic agent[1] and is still regarded as the drug of choice for the treatment of generalized tonic clonic seizures ("grand mal") or elementary partial (focal motor) seizures.[2] In 1978, the National Institutes of Health evaluated 16 commercially available antiepileptic drugs according to well-standardized testing methods; **1** was ranked first in the maximal electroshock seizure (MES) test[3] but, in contrast, was found to be inactive in the subcutaneous metrazol threshold (scMET) test. In spite of numerous studies dealing with the mode of action of antiepileptic drugs structurally related to **1**, limited information concerning the structure–activity relationships (SAR) is avaible;[5-8] a general discussion of the topic has been presented recently by Jones and Woodbury.[9] In this context, an interesting contribution was made by the X-ray crystallography studies of Camerman and Camerman, who pointed out that many anticonvulsant drugs (phenytoin, diazepam,[10] procyclidine,[11] trihexylphenidyl,[12] ethylphenacemide,[13] and diphenylsilanediol[14]) have closely related three-dimensional conformations in the solid state. This was apparent from the fact that all these compounds possess two hydrophobic regions and two electron-rich centers interconnected with similar relative orientations. On this basis, Camerman and Camerman proposed that these stereochemical properties represent a major molecular requirement for antiepileptic activity.[15] Regarding the molecular mode of action of phenytoin, Smythies suggested that **1** forms an array of molecules stacked on a β-turn segment of the proteic part of a putative receptor site through the formation of hydrogen bonds with the carbonyl in position 2 and the NH in position 3.[16] However, the simple fact that doxenitoin (**2**) is a potent antielectroshock drug[17] (and is not metabolized to phenytoin[18]) suggests that other modes of hydrogen bond formation may also take place. In this connection, **1** shows the remarkable feature of having two alternate C(=O)NH edges and two prochiral phenyl groups connected to the quaternary carbon in position 5: this arrangement makes

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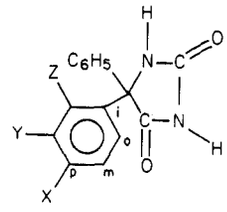
1 particularly receptive toward various hydrogen bond donors or receptors. In view of this, a limited series of compounds was synthesized, keeping the sequence (C_6H_5)C(C=O)N constant on one edge and decreasing the ability to form hydrogen bonds on the other edge, and tested in the MES test to define the role of hydrogen bonds in the antielectroshock activity of these drugs.

Pharmacological Studies. Progressive reduction of the ability to form hydrogen bonds via the replacement of a carbonyl group or an NH by a methylene group produces compounds with less anti-MES activity than the parent compound. This is illustrated by the ED_{50} of doxenitoin[17] (**2**, ED_{50} = 134 μmol/kg) and 3,3-diphenylsuccinimide[19] (**3**, ED_{50} = 179 μmol/kg). N-Methylation also results in a diminished ability to form hydrogen bonds and, correspondingly, also decreases the anti-MES activity as illustrated by the ED_{50} value of 3-methyl-5,5-diphenylhydantoin (**4**, ED_{50} = 218 μmole/kg[6]). A comparison of various ED_{50} values obtained from different sources is often a hazardous process. Therefore, in an effort to confirm the validity of our hypothesis, we have determined the ED_{50} of compounds **1–5** in a single series of experiments in the MES test. The results are shown in the Table I. The pharmacological data fully agree with the above model in that a net stepwise decrease of the anticonvulsant activity was observed when the hydantoin structure was modified (methylated) into succinimide and pyrolidinone structures. Additional evidence for the poor antiepileptic activity of (*RS*)-, (*R*)- and (*S*)-**6**[20] was obtained from the phamacological data provided by the Anticonvulsant Screening Project of the NIH (Bethesda, MD), which did not demonstrate any activity in the MES and MET tests.

^{13}C NMR Studies. At this point, there remains the question concerning the origin of the superiority of the twin phenyl substitution on the C(5) carbon over any other type of C(5) substitution. A potential reason for this situation could be that the two phenyls directly interact with the receptor site. The work of Tamir et al.[21] compares the comformational behavior of cannabidiol and phenytoin by theorical calculations (PCILO method) and ^{1}H and ^{13}C NMR studies. These authors conclude that both compounds showed "rather similar spatial relationship in the crystal and in solution". More specifically, the ^{13}C NMR results indicated the presence of a single conformer of phenytoin in solution by virtue of a single resonance "at 110 ppm in the aromatic range". Obviously, these results were rather questionable, and, as a consequence, we have undertaken a ^{13}C NMR study of phenytoin and some deuterio analogues [specifically deuterated in the para (**7**),[22]



7, X = ^{2}H; Y = Z = H
8, Y = ^{2}H; X = Z = H
9, Z = ^{2}H; X = Y = H

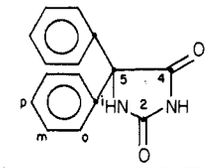
meta (**8**), and ortho position (**9**)[23] of one of the phenyls]

Table I. Antiepileptic Potencies of Phenytoin-like Compounds in the MES Test

no.	ED_{50},[a] μmol/kg
1	28 (24-32)
2	129 (71-244)
3	107 (73-158)
4	149 (120-185)
5	357 (267-481)
6	3834 (1253-11 744)

[a] Numbers in parentheses are 95% confidence intervals.

Table II. ^{13}C NMR Parameters of Phenytoin



C[a]	δ (ppm)	NOE	T_1, s
2	156.0	1.25	4.6
4	174.8	1.40	2.8
5	70.3	1.75	6.2
i	139.8	1.40	6.5
o	126.57	2.0	0.8
m	128.35	1.85	0.8
p	127.93	1.90	0.2

[a] i = ipso; o = ortho; m = meta; p = para.

in solution in Me_2SO-d_6. The 174.8- and 156.0-ppm peaks were assigned to the C(4) and C(2) carbonyls, respectively, by comparison with the spectrum of **2**, which shows a single peak at 176.4 ppm. The aromatic region shows four peaks, and this is consistent with the presence of four carbon types, i.e., ipso, ortho, meta, and para carbons. The ipso carbon was located at 139.8 ppm by comparison with other monosubstituted phenyl compounds[24] and an off-resonance experiment. The resonances of the meta, para, and ortho carbons were assigned by comparing the broad-band proton decoupled and undecoupled (NOE experiment) spectra of **7–9**. Indeed, carbons bonded to deuterium generally display no NOE and produce broadened lines. As a consequence, they are of weak intensity and are often overlapped by neighboring resonances of protium carbons. Fortunately, the deuterium also induces a shielding effect of 0.1 ppm on the adjacent β-carbon.[24] Accordingly, the presence of a deuterium in the para position (**7**) produces a second line at 128.25 ppm in addition to the preexisting line at 128.35 ppm (as in **1**), which is therefore attributed to the protium on the -meta carbon. Similarly the presence of a deuterium in the meta position (**8**) results in twin signals at 127.93 and 127.82 ppm (para carbon) and at 126.57 and 126.48 ppm (ortho carbons). In compounds **9**, a shoulder at 128.20 ppm was observed in the 128.34-ppm resonance of the meta carbons. The different carbon chemical shifts (δ) and associated NOE and T_1 value are reported in Table II. The fact that the meta and ortho carbons have significantly longer T_1 values than the para carbons is consistent with the concept of internal rotation along the ipso–para carbon axis.[24]

Discussion and Conclusion

As mentioned earlier, Tamir et al.[21] computed the energy changes due to the rotation of the two phenyls of pheny-

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toin about their axes and found two noninterconvertible minima, which, as a matter of fact, represent the very same conformer. These authors claimed that their quantum-mechanical calculations were corroborated by their ^1H and ^{13}C NMR data and concluded that a single conformer existed in solution. The observation of a single resonance in the aromatic region does not necessarily imply the existence of a single conformer in solution. Indeed, the aromatic carbons are enantiotopic, and the rapid internal motion is expected to give rise to single individual resonances for ipso, ortho, meta, and para carbons. Examination of a CPK model of phenytoin shows that the phenyl groups can rotate, but not independently. The T_1 values measured for phenytoin reveal a certain degree of motional freedom of the two phenyl groups, which is mediated through concomitant rotation along the para–ipso carbon axis and libration along the ipso–C(5) bonds. In this connection, it should be noted that minor out-of-plane deformations require little energy: for example, a 5° deviation from planarity of the dihedral angle formed by the 1-, 2-, 3-, and 4-carbons of benzene requires less than 4 kJ/mol, while a 10° deviation requires less than 12 kJ/mol.[25]

The motional freedom of the solvated phenyl groups of phenytoin does not contradict the crystallographic evidence of Camerman and Camerman[14] and is consistent with the view of Smythies[15] in that the strong hydrophobic shield produced by the phenyl rotors protects the hydrogen bonds formed by the imidic part of the hydantoin ring and a β-bend protein segment, from the water extrusion. An additional proof for the correlation between the antiepileptic activity and the capability of forming hydrogen bonds can be found in the equipotency of 3-amino-5,5-diphenylhydantoin and 1 in the MES test.[26] Moreover, it has been shown that cyclized (and therefore immobilized) forms of phenytoin [obtained by bridging an ortho carbon and the N(1) position with two methylene groups] had poor antiepileptic activity.[27] These considerations indicate conclusively that the ability to form hydrogen bonds as well as a certain degree of motional freedom at the level of the phenyl groups are important SAR features in antiepileptic phenytoin-like compounds.

Experimental Section

^{13}C NMR spectra were recorded on Brucker WP-80-SY and WM-250-spectrometers operating in the FT mode at 20.115 and 62.860 MHz respectively. The compounds were dissolved in Me_2SO-d_6 to form 0.5 M solutions. The probe temperature was 38 °C. The central peak of Me_2SO-d_6 was positioned at 39.60 ppm and was used as reference. Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are corrected. Elemental analyses are indicated only by symbols of the elements and are within 0.4% of the theoretical values. Optical rotations were measured with a Perkin-Elmer Model 141 spectrophotometer at room temperature (c 1, C_2H_5OH); IR and mass spectra were consistent with proposed structures. Compound **2** was prepared according to Biltz.[28] Compounds **3** and **5** were synthetized according to Miller and Long.[29] Compound **4** was prepared according to Queen.[30] (*RS*)-**6** was obtained according to Gardner et al.[20] By the same approach, (*R*)-(−)- and (*S*)-(+)-2,2-diphenyl-4-(dimethylamino)pentanenitrile[31,32] were transformed into (*R*)- and (*S*)-(−)-**6**. (*R*)-(−)-**6**: mp 148–150 °C; $[\alpha]_{546}$ −12.0°. Anal. ($C_{18}H_{19}NO$) C, H, N. (*S*)-(−)-**6**: mp 148–150 °C; $[\alpha]_{546}$ +13.0°. Anal. ($C_{18}H_{19}NO$) C, H, N. Compound **8** was prepared by deuteriolysis of 5-(3-bromophenyl)-5-phenylhydantoin[33] over palladium on charcoal, mp 294–296 °C. Anal. ($C_{15}H_{11}DN_2O_2$) C, H, N; ^2H content = 91%.

Antiepileptic Activity. MES Test. Each compound was administered orally to male NMRI mice (20–25 g) as a suspension in a solution of tragacanth (1%). The dose–effect behavior of the six products tested was examined by the administration of five different doses of each compound, treating ten mice at each dose. One hour after administration of the drug, the animals were submitted to the MES test. Maximal electroshock seizures were elicited with a current of 30 mA at 50 Hz delivered for 0.2 s via corneal electrodes. Mice were considered as protected if the tonic extension of hindlimbs was not observed. The ED_{50} and the 95% confidence intervals were computed according to the method of Litchfield and Wilcoxon. (*R*)-, (*S*)-, and (*RS*)-**6** were tested in phase I of the Antiepileptic Drug Development Program (Department of Health an Human Services, National Institutes of Health, Bethesda, MD) and on single animals at 30, 100, 300, and 600 mg/kg in the MES and sc MET test. No protection was observed.

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Registry No. 1, 57-41-0; **2**, 3254-93-1; **3**, 3464-15-1; **4**, 4224-00-4; **5**, 5685-20-1; (*RS*)-**6**, 87185-02-2; (*R*)-**6**, 87246-92-2; (*S*)-**6**, 87246-93-3; **8**, 87185-03-3; (*R*)-(−)-2,2-diphenyl-4-(dimethylamino)pentanenitrile, 7576-16-1; (*S*)-(+)-2,2-diphenyl-4-(dimethylamino)pentanenitrile, 7576-08-1; 5-(3-bromophenyl)-5-phenylhydantoin, 87185-04-4.

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