oxygen atoms are taken as the electron-donating atoms, the O9–O12 separation of 3.17 Å fits within the active range; however, the phenyl ring to O9 distance is long (5.96 Å), and the phenyl rings of DPH and **6a** do not superimpose in an orientation that places O9 and O12 over the carbonyl oxygen atoms of DPH.

Plots of the superpositions of the structures of cyhepatmide and DPH, as well as phenacyloxindole and DPH, show some striking differences in the shapes of the molecules. As shown in Figure 3a,b, the anticonvulsants studied in this work overlap nicely with DPH in the amide region and in the region of one hydrophobic group; however, overall it is evident that DPH has a different shape from the other molecules, because to superimpose both of the two hydrophobic portions of cyheptamide and diphenylhydantoin the planes of the amide portions of the two would have to be perpendicular to each other. Otherwise, when the amide groups are superimposed, the diphenylhydantoin has both phenyl rings in the general hydrophobic region occupied by one phenyl ring of cyheptamide and the phenacyl ring of the phenacyloxindole compound. The other hydrophobic region, which is occupied by a phenyl ring in cyheptamide and the indole in **6a**, is partially filled by the non-amide part of the hydantoin ring. A comparison of cyheptamide and phenacyloxindole (Figure 3c) shows that they occupy the same volume in space with one exception: the indole ring in **6a** is approximately perpendicular to the comparable phenyl ring in cyheptamide. The conclusions obtained from the comparisons in Figure 3 are enhanced by a superposition of the structures of carbamazepine and cyheptamide, which are nearly identical; the largest deviation of two similar atoms in the least-squares fit of the two structures is 0.6 Å. Thus, three structures, carbamazepine, cyheptamide, and phenacyloxindole, fit nearly the same three-dimensional molecular envelope for the amide group and one aromatic group and overlap partially for the other aromatic group.

A model that would accommodate *all four drugs* with anti-MES induced seizure activity would require an amide group with delocalized electrons and a planar nitrogen atom and would have only one binding site for an aromatic moiety. If both hydrophobic groups are required for binding, then it is probable that the stereochemical requirements for these compounds are imprecise because only a rather large hydrophobic pocket could accommodate both groups in all of the anti-MES anticonvulsants studied. Comparison of diphenylhydantoin to the other compounds indicates that the spatial requirement is probably for one hydrophobic group. It is possible that the main function of the hydrophobic groups is to assure bioavailability. Structure–activity studies[5,14] on anticonvulsants indicate that brain concentrations of the drugs are quite different from those in the blood; these differences may account for much of the data on activity in the literature and for the apparent need for two hydrophobic groups.

Clearly, further evaluation of the π-electron delocalization trends, as well as structural studies on a large number of similar anticonvulsants, are necessary for the development of a predictive model for anticonvulsant activity. Semiempirical calculations, as well as X-ray structural analyses, on several anticonvulsants are in progress.

Acknowledgment. This work was supported by the Alberta Heritage Foundation for Medical Research and the Medical Research Council of Canada (Grant MA 8087). The authors thank Dr. Gary L. Jones for suggesting the study of anticonvulsants and providing a sample of cyheptamide. We also thank F. D. Popp for supplying the phenoxyindole sample, M. Sax for making the carbamazepine results available before publication, and K. A. Kerr for use of the diffractometer.

Registry No. **2**, 57-41-0; **4**, 298-46-4; **5**, 7199-29-3; **6a**, 88730-73-8.

Supplementary Material Available: Positional and anisotropic thermal parameters for all non-hydrogen atoms, positional and isotropic thermal parameters and bond distances for hydrogen atoms, and lists of observed and calculated structure factor amplitudes (35 pages). Ordering information is given on any current masthead page.

Phosphorus Analogues of γ-Aminobutyric Acid, a New Class of Anticonvulsants

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A series of phosphorus compounds, designed as analogues of γ-aminobutyric acid (GABA) in that they possess a P=O moiety separated by three atoms from an amino or acetamido group, was synthesized and tested by using in vitro $GABA_A$ and $GABA_B$ receptor binding, GABA uptake assays, and was examined for anticonvulsant activity. Weak $GABA_B$ receptor affinity was noted for one agent, whereas six compounds displayed moderate to high potencies as inhibitors of electroshock- and pentylenetetrazol-induced seizures. The best anticonvulsant effect was found with the (*m*-aminophenyl)phosphinic acid compounds, with members of this class selected for further study.

Derivatives of γ-aminobutyric acid (GABA), an inhibitory central neurotransmitter, were synthesized and tested for in vitro and in vivo biological activities associated with convulsive disorders. Anticonvulsant drugs have been developed that mimic GABA at its brain receptor or elevate endogenous levels of the amino acid by inhibiting catabolic enzymes.[1] The success of these approaches led to the design and study of diverse chemical types of phosphorus compounds structurally related to GABA and potentially capable of producing similar therapeutic effects. Most of these agents are of the following general formula, which involves a P=O or P=S moiety in lieu of the carbonyl group present in GABA and a separation of these by three atoms from an amino or acetamido substituent:

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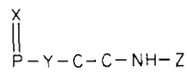
(1) Saelens, J. K.; Vinick, F. J. *Annu. Rep. Med. Chem.* **1978**, *13*, 31.

0022-2623/84/1827-0654$01.50/0

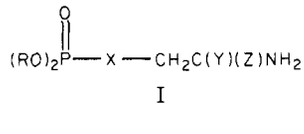


X = O, S; Y = O, NH, CH_2; Z = H, $COCH_3$

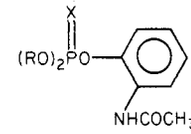
As defined by Johnston et al.,[2] all but five final products herein reported are strict GABA analogues, those with three atoms separating an amino and carboxyl function or their equivalents.

Prior to this study only two phosphorus compounds, *tert*-butyl bicyclophosphate and 4-[(5′-phosphopyridoxyl)amino]butyric acid, were reported in this field of study as having GABA antagonistic and γ-aminobutyric acid–ketogluturate transaminase (GABA-T) inhibitory effects, respectively.[1]

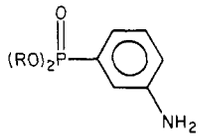
Members of six chemical types of organophosphorus compounds I–VI and some miscellaneous agents were



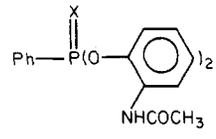
I



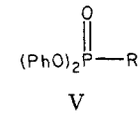
II



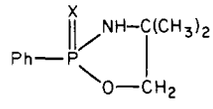
III



IV



V



VI

studied after a rationale was devised for each. The design of GABA analogues took into account the concepts of bioisosterism and a range of log *P* values encompassing that of ca. 2.0 ± 0.7, which has been established as optimum for organic CNS-acting drugs to overcome the blood–brain barrier.[3,4] Thus, compounds **3** and **4** of type I differ in having either a CH_2 or NH situated adjacent to the P=O group. It was originally intended that the oxygen analogue would be included in this bioisosteric study. However, the attempted synthesis of *o*-amino alcohol esters from nitro esters by catalytic reduction led to the formation of salts.[5] In this type, as well as in II and III, different esters were investigated on the basis of their varying solubility properties and interference with zwitterion formation. Of the amino acids studied (**1**, **2**, **5**, and **10**), the phosphonic acid **5** is the closest phosphorus analogue of GABA to be studied up to the present.[6] Lipophilicity was increased in each of the first three types, for example, by ca. 2.3 π units by substituting two ethyl groups with two phenyl groups. Type III compounds, (*m*-aminophenyl)phosphonic acid and its esters, have structures related to gabaculline, a dihydro-*m*-aminobenzoic acid, which is a potent inhibitor of GABA-T[1]. The *o*-acetanilido derivatives of types II and IV retain the three-atom separation of P=O and P=S groups and the nitrogen atom. Compounds **7** and **8** and **15** and **16**, as well as **20** and **21** of type VI, constitute three pairs of derivatives in which a sulfur atom replaces an oxygen atom of a P=O group and this constitutes another type of bioisosteric replacement. The effect of this substitution is an increase in log *P* values of 1.53 units. This figure is the average of differences in log *P* values between 11 pairs of phosphates and thiophosphates (Table I). Trivalent **23–25** were included as analogues lacking a P=O or P=S sulfur or oxygen atom. Compounds **17** and **18** were designed with branched alkyl side chains similar to that found in the anticonvulsant valproic acid, while type VI agents are 1,3,2-oxazaphospholidines, which are structurally related to oxazolidinedione anticonvulsants. Known compound **22** can be considered as a congener of succinimide, whose derivatives are also useful in convulsive disorders.

Table I. Contribution of the Thionophosphorus Sulfur Atom to Log *P*

R_1	X	R_2	log *P*, obsd[a]	Δ log *P*
CH_3	O	2-Cl, 4-NO_2Ph	1.83	1.61
CH_3	S	2-Cl, 4-NO_2Ph	3.44	
CH_3	O	3-Cl, 4-NO_2Ph	1.83	1.62
CH_3	S	3-Cl, 4-NO_2Ph	3.45	
CH_3	O	3-CH_3, 4-NO_2Ph	1.69	1.61
CH_3	S	3-CH_3, 4-NO_2Ph	3.30	
C_2H_5	O	4-NO_2Ph	1.64	1.34
C_2H_5	S	4-NO_2Ph	2.98	
C_2H_5	O	Ph	1.64	1.82
C_2H_5	S	Ph	3.46	
C_2H_5	O	3-Cl, 4-CH_3SPh	3.21	1.85
C_2H_5	S	3-Cl, 4-CH_3SPh	5.06	
C_2H_5	O	2-Cl, 4-CH_3SPh	3.05	1.35
C_2H_5	S	2-Cl, 4-CH_3SPh	4.40	
CH_3	O	3-$CH(CH_3)_2$, 4-NO_2Ph	2.57	1.48
CH_3	S	3-$CH(CH_3)_2$, 4-NO_2Ph	4.05	
CH_3	O	3,5-$(CH_3)_2$, 4-NO_2Ph	2.57	1.32
CH_3	S	3,5-$(CH_3)_2$, 4-NO_2Ph	3.89	
CH_3	O	3-Et, 4-NO_2Ph	2.19	1.55
CH_3	S	3-Et, 4-NO_2Ph	3.84	
CH_3	O	4-NO_2Ph	1.27	1.24
CH_3	S	4-NO_2Ph	2.51	
				1.53 ± 0.20

[a] Experimental values (octanol/water system) with averages used when more than one value for the un-ionized species is given. Taken from data maintained by the Pomona College Medicinal Chemistry Project, Claremont, CA.

Results and Discussion

Biological Activities. In Vitro. The potencies of 20 of the derivatives to inhibit $GABA_A$ and $GABA_B$ receptor binding are shown in Table III. Although most of the compounds tested showed some potency at the various GABA receptor sites, they are rather weak in comparison to GABA itself. The fact that these compounds were virtually inactive in potentiating diazepam binding is consistent with the notion that they are probably not $GABA_A$ receptor agonists. On the other hand, it would appear that compound **5** may have some affinity for $GABA_B$ binding sites, inhibiting 50% of the specifically bound GABA at a concentration of 6 μM (IC_{50}). Nevertheless, this is still substantially less potent than GABA or baclofen at this site, compounds having IC_{50} values of

(2) Johnston, G. A.; Allan, R. D.; Kennedy, S. M. E.; Twitchin, B. In "GABA-Neurotransmitters"; Krogsgaard-Larsen, P.; Scheel-Krüger, J.; Kofod, H., Eds.; Academic Press: New York, 1979; pp 149–164.
(3) Lien, E. J.; Tong, G. L.; Chou, J. T.; Lien, L. L. *J. Pharm. Sci.* **1973**, *62*, 246.
(4) Lien, E. J.; Liao, R. C. H.; Shinouda, H. G. *J. Pharm. Sci.* **1979**, *68*, 463.
(5) Cates, L. A.; Li, V.-S., unpublished results.
(6) Phosphinic acid derivatives, which possess the HP(O)OH, as compared to the $P(O)(OH)_2$ moiety of phosphonic acid, are considered closer analogues of carboxylic acids. Some of these are currently being prepared in our laboratory.

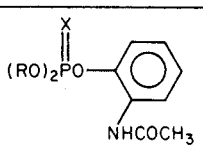
Table II. Physical Properties of Compounds

$R_1O(R_2)P(O)XCH_2CH_2NH_2$

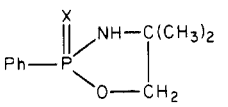
no.	R_1	R_2	X	mp or bp (mm), °C	formula
1	H	PhO	O	245-246 dec[a]	$C_8H_{12}NO_4P$
2	H	Ph	O	265-267 dec	$C_8H_{12}NO_3P$
3	Et	EtO	CH_2	92-95 (0.01)[b]	$C_7H_{18}NO_3P$
4	Et	EtO	NH	108-109 (0.15)[c]	$C_6H_{17}N_2O_3P$
5	H	HO	CH_2	274	$C_3H_{10}NO_3P$

$(PhO)_2P(O)-R$

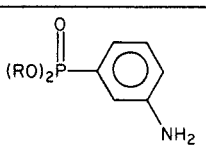
no.	R	mp or bp, °C	formula
17	$OCH_2CH(CH_2CH_3)_2$	78-79	$C_{18}H_{29}O_4P$
18	$NHC(CH_3)_2CH_2C(CH_3)_3$	172-175/0.3	$C_{20}H_{28}NO_3P$
19	$NHCH_2CH(CH_3)NHP(O)(OPh)_2$	118-119	$C_{27}H_{28}N_2O_6P_2$



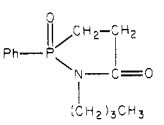
no.	R	X	mp or bp (mm), °C	formula
6	Ph	O	77-78	$C_{20}H_{18}NO_5P$
7	Et	O	156-157 (0.1)	$C_{12}H_{18}NO_5P$
8	Et	S	47-49	$C_{12}H_{18}NO_4PS$
9	Me	S	48-50	$C_{10}H_{14}NO_4PS$

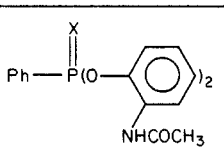


no.	X	mp, °C	formula
20	O	178-179	$C_{10}H_{14}NO_2P$
21	S	57.5-58	$C_{10}H_{14}NOPS$

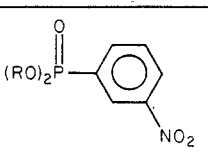


no.	R	mp, °C	formula
10	H	315 dec[d]	$C_6H_8NO_3P$
11	Me	77-78	$C_8H_{12}NO_3P$
12	Ph	70-72	$C_{18}H_{16}NO_3P$
13	Et	51-53[e]	$C_{10}H_{16}NO_3P$
14	*i*-Pr	104-105	$C_{12}H_{20}NO_3P$

no.	structure	mp or bp, (mm), °C	formula
22		150-165 (0.05-0.1)[f]	$C_{13}H_{18}NO_2P$
23	$H_2PCH_2CH_2CH_2NH_2$	75-80 (40)[g]	$C_3H_{10}NP$
24	$(Ph)_2PCH_2CH_2CH_2NH_2$	144-149 (0.4)[h]	$C_{15}H_{18}NP$
25	$PhP(CH_2CH_2CH_2NH_2)_2$		$C_{12}H_{21}N_2P$



no.	X	mp, °C	formula
15	O	157-158	$C_{22}H_{21}N_2O_5P$
16	S	119-121	$C_{22}H_{21}N_2O_4PS$



no.	R	mp or bp (mm), °C	formula
26	Me	120-122 (0.1)	$C_8H_{10}NO_5P$
27	Et	51-53[i]	$C_{10}H_{14}NO_5P$
28	*i*-Pr	104-105	$C_{12}H_{18}NO_5P$
29	Ph	*j*	$C_{18}H_{14}NO_5P$

[a] Literature mp 243-244.[13] [b] Literature bp 110 °C (0.1 mm).[19] [c] Synthesis reported without physical constants.[20] [d] Literature mp 290 °C dec.[21] [e] Literature bp 133-137 °C (0.2).[14] [f] Literature bp 159 °C (0.05 mm).[22] [g] Literature bp 58.5-62 °C (52 mm).[23] [h] Literature bp 150 °C (0.4 mm).[24] [i] Literature mp 47-50 °C.[14] [j] Undistillable oil.

80 and 130 nM, respectively.[7]

In Vivo. Nineteen phosphorus analogues of GABA and three miscellaneous compounds were evaluated for neurotoxicity and abilities to protect against electroshock- and pentylenetetrazol-induced seizures in mice (Table IV). Appreciable anticonvulsant effects were noted in **4**, **7**, and **21** and in three members (**11**, **13**, and **14**) of type III derivatives. Compound **4** provided medium protection and, with the exception of **19**, was the only phosphoramidic acid analogue of GABA studied, and possibly more active anticonvulsants might be found among other derivatives of this chemical class. Moderate effects were also shown by **7**, which is the only N-acetylated derivative of five tested to be classified as active. The high activity of the methyl ester **11**, the first of the type III agents to be tested, prompted the synthesis and screening of the remaining members. Amino acid **10** and the phenyl ester **12** were inactive, while the ethyl ester **13** and isopropyl ester **14** gave moderate anticonvulsant effects. It is likely that **11** possesses superior steric or partitioning properties or is subject to preferential metabolism to an active form. Since the log *P* values of dimethyl (0.95) and diethyl (1.64) phenyl phosphate have been experimentally determined and the π values of appropriate atoms and groups are known,[8,9] those of **11**–**14** can be reliably estimated as –0.28, 3.02, 0.72, and 1.32,[10] respectively, while active derivatives **4**, **7**, and **21** can similarly be calculated as –0.47, 0.67, and

(7) Bowery, N. G. *Trends Pharmacol. Sci.* **1982**, *3*, 400.

(8) Hansch, D.; Leo, A. In "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley: New York, 1979; pp 49–52 and 171–330.

(9) Tute, M. S. *Adv. Drug Res.* **1971**, *6*, 67.

(10) Dimethyl phenylphosphonate (0.95) + NH_2 (–1.23) = 11; 11 + $2CH_2$ (0.5 each) = **13**; **13** + $2CH_2$ + branching (–0.2) = **14**; 11 – $2CH_3$ (0.5 each) + 2Ph (2.15 each) = **12**.

Table III. Affinity for GABA Receptor Binding in Rat Brain[a]

	% inhibn of GABA receptor binding	
no.	$GABA_A$	$GABA_B$
1	13	40
2	15	16
4	b	
5	12	91[c]
6	54	36
7		
8		35
9		ND[d]
10		ND
11	13	13
12		51
15	46	
16		ND
18		65
19		20
20		
21		15
22	17	67

[a] All compounds were tested for their ability to displace (^{3}H)GABA from $GABA_A$ and $GABA_B$ binding sites at a concentration of 100 μM, and the values expressed represent the percent inhibition seen relative to that observed in the presence of 1 mM GABA and 100 μM baclofen for $GABA_A$ and $GABA_B$ binding, respectively. In these experiments, GABA was found to have an IC_{50} of 20 nM for $GABA_A$ binding and 80 nM for $GABA_B$ binding. None of the compounds tested showed any ability to inhibit (^{3}H)GABA uptake or to activate benzodiazepine binding. IC_{50} values reported for compound **5** and for GABA are the means of three experiments performed in triplicate, the standard error being less than 10%. The values in the table are the means of two separate experiments, each of which was performed in triplicate. [b] Denotes less than 10% inhibition of binding. [c] IC_{50} calculated to be 6 μM. [d] ND, not determined.

Table IV. In Vivo Anticonvulsant Activity and Neurotoxicity

	anticonvulsant act.[a]				toxicity, 600 mg/kg[b]	
	MES[c]		SCM[d]			
no.	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
4	+	−	++	−	3/4	0/2
7	++	++	+	−	2/4	2/2
11	+	+	+++	−	0/4	0/2
13	++	+	+	+	4/4	1/2
14	++	+	−	−	4/4	2/2
18	+	−	−	−	0/4	0/2
21	+	−	−	+++	0/4	0/2
Phensuximide	112[e]	(104-131)[f]				

[a] +++, ++, and + indicate activity at 100, 300, and 600 mg/kg, respectively; − signifies no activity observed at 600 mg/kg. Compounds **1-3, 5, 6, 8, 10, 12, 15-17, 19, 20, 23,** and **24** were inactive. [b] Number of animals exhibiting neurotoxicity in the rotorod test/number of animals tested. Compounds **1, 2, 5, 6, 8, 10, 12, 15-17, 19,** and **20** gave 0/4 and 0/2 results after 0.5 and 4 h, respectively. Compounds **3, 23,** and **24** similarly gave results of 1/4, 0/2; 4/4, 0/2; and 4/4, 0/1, respectively. [c] Maximal electroshock seizure test. [d] Subcutaneous pentylenetetrazol (Metrazol) seizure threshold test. [e] ED_{50}. [f] 95% confidence limits.

2.99, respectively.[11] From an examination of the present data there appears to be no relationship between electronic or solubility properties and anticonvulsant activity or toxicity. Compounds **7, 11, 13,** and **14** are presently undergoing more complete pharmacological testing.

Absorption and transport to target sites are important factors in the activity of anticonvulsants and are related to the partitioning properties of the drugs. In order to achieve the proper hydrophilic–lipophilic balance, especially as concerns the penetration of agents across the blood–brain barrier, prodrug forms, such as progabide, which transports and releases the hydrophilic amide of GABA as well as acting as a receptor stimulant per se, have been designed.[12] This method is presently being applied to the more hydrophilic agents reported herein.

Experimental Section

Synthesis. Melting points were determined by the capillary method (oil bath) and are corrected to reference standards. IR spectra (KBr for solids, neat for liquids) were recorded on a Perkin-Elmer 282 spectrophotometer, and mass spectra were taken on a Hewlett-Packard 58930 GC/MS with 5933A data system. ^{1}H NMR spectra were measured with either a Varian Associates T-60 or Varian Associates FT-80A spectrometer with tetramethylsilane as the internal standard and deuteriochloroform as the solvent, except for **1, 2,** and **10** (deuterium oxide). Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, on new compounds **2, 6–9, 11, 12, 14–16, 18–21,** and **29** for C, H, and N and on **17** for C and H, with results being within ±0.4%. NMR and IR spectra were recorded for all compounds except **17** (NMR only) and **23** (IR only), and mass spectra were determined for **2, 3,** and **18–22**. All spectra agreed with assigned structures. Silica gel 60 (70–230 mesh) was used for column chromatography. During the synthesis of **6–9, 15–21, 23,** and **24**, the (di)chloride was added dropwise to the alcohol, phenol, amine, or amino alcohol with stirring and cooling at 0–10 °C. The reaction mixtures were refluxed for 16–18 h and filtered, and the filtrates were evaporated under reduced pressure to yield residues.

2-Aminoethyl Phenylphosphonate (2). This compound was synthesized by a modification of the procedure of Tsizin and Preobrazhenskii,[13] which was used to prepare **1**. Aziridine (6.5 g, 0.15 mol) in anhydrous acetone (100 mL) was added with stirring and cooling (10–18 °C) to phenylphosphonic acid (23.7 g, 0.15 mol) in anhydrous acetone (120 mL). The reaction mixture was heated at 56 °C for 5 h and left at 25 °C under N_2 for 16 h. The solvent was decanted, and the viscous residue was dissolved in hot MeOH (50 mL). The precipitate obtained upon cooling was collected on a sintered glass funnel and washed with cold MeOH until a white solid remained. The residue was recrystallized from MeOH to give the pure product.

***o*-Acetamidophenyl Phosphates and Thiophosphates (6–9).** To *o*-hydroxyacetanilide (7.55 g, 50 mmol) and triethylamine (5.56 g, 55 mmol) in CH_2Cl_2 (200 mL) was added 50 mmol of diphenyl or diethyl phosphorochloridate (for **6** or **7**) of diethyl or dimethyl phosphorothiochloridate (for **8** or **9**) in CH_2Cl_2 (50 mL). The residue was extracted with ether, and the residues from the evaporation of the solutions were subjected to column chromatography with 2% MeOH in CH_2Cl_2 (for **6** and **8**) or 5% MeOH in CH_2Cl_2 (for **7**) as the eluants. Crude **9** was chromatographed with 2% MeOH in CH_2Cl_2 (100 mL) and then with 3% MeOH in CH_2Cl_2 (150 mL) as the eluants. The residue from evaporation of the eluant was distilled at 130–160 °C (0.05 mm), and the distillate was chromatographed with 2% MeOH in CH_2Cl_2 to yield pure **9**.

Esters of (3-Aminophenyl)phosphonic Acid (11–14). To **26–29** (6 g) dissolved in MeOH (50 mL) and placed in a Parr hydrogenation bottle was added 0.6 g of 10% Pd/C under N_2. The mixtures were shaken under 50–60 psi of H_2 until about 50 psi of gas was absorbed and then filtered, and the filtrates were evaporated under reduced pressure. Three of the resulting residues were purified by column chromatography with the following

(11) $O_2P(O)N$ (−2.34)[5] + 2Et (2.0) + ethylamine (−0.13) = 4; diethyl phenyl phosphate (1.64) + arom $NHC(O)CH_3$ (−0.97) = 7; dimethyl phenylphosphonate (0.95) + ring closure (−0.09) + $2CH_3$ (1.0) + 2 branching (−0.4) + 1.53 P=S in lieu of P=O (Table I) and given equivalence of O and N = **21**.

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eluants: 5% MeOH in $CHCl_3$ (for **11**); 2% MeOH in $CHCl_3$ (100 mL), followed by 5% MeOH in $CHCl_3$ (for **12**); and 1, 2, 3, 4, and 5% MeOH in $CHCl_3$ (100 mL each) in sequence (for **13**). Compound **14** was recrystallized from Et_2O–CH_2Cl_2.

Bis(2-acetamidophenyl) Phenylphosphonate and Phenylthiophosphonate (15 and 16). Phenylphosphonic dichloride (4.9 g, 25 mmol) (for **15**) or phenylphosphorothioic dichloride (5.3 g, 25 mmol) (for **16**) in CH_2Cl_2 (50 mL) was reacted with *o*-hydroxyacetanilide (8.3 g, 55 mmol) and triethylamine (5.6 g, 55 mmol) in CH_2Cl_2 (200 mL) under N_2. The residues were purified by column chromatography with the following eluants: CH_2Cl_2 (100 mL), 2% (600 mL) and 3% MeOH in CH_2Cl_2 (200 mL) sequentially (**16**) and 40% EtOAc in CH_2Cl_2 (for **15**). The eluted material obtained was recrystallized from CH_2Cl_2–Et_2O to give pure **15** and **16**.

Diphenyl 2-Ethyl-1-butyl Phosphate (17) and Diphenyl (1,1,3,3-Tetramethylbutyl)phosphoramidate (18). Diphenyl phosphorochloridate (13.8 g, 50 mmol) in Et_2O (100 mL) was added dropwise to 2-ethyl-1-butanol (5.6 g, 60 mmol) (for **17**) or 1,1,3,3-tetramethylbutylamine (7.7 g, 60 mmol) in Et_2O (200 mL). The residues were purified by column chromatography with 5% MeOH in $CHCl_3$ as the eluant. Eluted material was recrystallized from MeOH (**17**) or vacuum distilled (**18**).

Tetraphenyl *N,N'*-Propylenebis[phosphoramidate] (19). Diphenyl phosphorochloridate (26.8 g, 100 mmol) in CH_2Cl_2 (150 mL) was reacted with 1,2-diaminopropane (7.4 g, 100 mmol) in CH_2Cl_2 (75 mL). The oily residue was washed with water and recrystallized from EtOH–H_2O.

2-Phenyl-4,4-dimethyl-1,3,2-oxazaphospholidine 2-Oxide (20) and 2-Sulfide (21). Phenylphosphonic dichloride (19.5 g, 100 mmol) or phenylphosphonothioic dichloride (21.1 g, 100 mmol) in Et_2O (100 mL) was reacted with 2-amino-2-methyl-1-propanol (9.8 g, 110 mmol) and triethylamine (25.3 g, 250 mmol). The residues were purified by recrystallization from acetone (**20**) or by column chromatography with 100 mL each of $CHCl_3$ and 2, 3, 4, and 5% MeOH in $CHCl_3$ sequentially as the eluants (**21**).

Esters of 3-Nitrophenyl Phosphate. (3-Nitrophenyl)-phosphonic acid (6.0 g, 30 mmol) and PCl_5 (13 g, 62 mmol) were mixed to yield a liquid.[14] The $POCl_3$, HCl, and excess PCl_5 were removed at 90 °C under reduced pressure. The crude dichloride was distilled through a short path distilling head at 120–125 °C at 0.05-mm pressure to yield a yellow liquid, which solidified on cooling. The purified dichloride (5.3 g, 22 mmol) in CH_2Cl_2 (50 mL) was added to a solution of a large excess of the appropriate alcohol or phenol (4.5 g, 48 mmol) and triethylamine (4.8 g, 48 mmol) in CH_2Cl_2 (100 mL) at 0–5 °C. The reaction mixtures were stirred for 4 h at 25 °C, the solvent was removed under reduced pressure, and the residues were extracted with Et_2O. Compound **26** was distilled, and the others were purified by column chromatography with 5% MeOH in $CHCl_3$ (for **27** and **28**) or 2% MeOH in $CHCl_3$ (for **29**) as the eluants.

In Vitro Biological Testing. Animals. Male Sprague–Dawley rats (150–200 g, Timco Breeding Labs, Houston, TX) were used in all experiments. The animals were killed by decapitation, and the brains were removed rapidly, dissected, and stored at −20 °C until assayed. For GABA uptake studies, the brain tissue was used immediately.

Receptor Binding Assay. $GABA_A$ receptor binding to rat brain homogenates was performed by the method of Enna and Snyder (1977).[15] Briefly, the frozen tissue was homogenized in 100 vol of 50 mM Tris–citrate (pH 7.1 at 4 °C) and centrifuged at 48000*g* for 10 min. The resulting pellet was resuspended in the same volume, incubated with Triton X-100 (0.05% final concentration) for 30 min at 37 °C, and then centrifuged as above. The resultant pellet was resuspended in buffer to yield a final protein concentration of 0.5 to 1.0 mg/mL. One-milliliter portions of this membrane suspension were added to tubes containing 1 mL of buffer plus 8 nM (^{3}H)-γ-aminobutyric acid [(^{3}H)GABA; 57 Ci/mmol] in the presence and absence of various test drugs or 1 mM unlabeled GABA. The mixture was incubated for 5 min at 4 °C, and the reaction was terminated by centrifugation at 48000*g* for 10 min. The resulting pellets were rinsed with 15 mL of buffer, dissolved in 1 mL of Protosol, and analyzed by liquid scintillation spectrometry. Total specific receptor binding was defined as the amount of (^{3}H)GABA displaced by 10^{-3} M unlabeled GABA.

$GABA_B$ receptor binding was analyzed according to the method of Hill and Bowery (1981),[16] with 10 nM (^{3}H)GABA as a ligand and 100 μM isoguvacine used to inhibit attachment to $GABA_A$ receptors. Total specific binding was defined as the amount of (^{3}H)GABA displaced by 10^{-4} M baclofen.

In order to determine whether the test compounds are capable of activating benzodiazepine binding, a measure of $GABA_A$ receptor activity, (^{3}H)diazepam (87.6 Ci/mmol) binding in rat brain membranes was performed by a standard procedure,[17] with the exception that the tissue was not exposed to Triton X-100. Specific benzodiazepine receptor binding was defined as the amount of (^{3}H)diazepam displaced by 10^{-6} M unlabeled diazepam.

GABA Uptake. GABA uptake was measured in rat brain synaptosomes by the general procedure of Peck et al.[18] Synaptosomes were prepared by homogenizing rat brains in 15 vol of 0.32 M sucrose and centrifuging at 1000*g* for 10 min. The supernatant was then centrifuged at 20000*g* for 20 min, and the resulting pellets were resuspended in Krebs–Hepes buffer and assayed. Assay tubes contained 800 μL of buffer, 200 μL of tissue suspension (200–300 μg of protein), and (^{3}H)GABA (0.5 μM, final concentration). The incubation was carried out at 23 °C for 10 min and terminated by filtration over Whatman GF/B glass-fiber filters. Radioactivity was analyzed by liquid scintillation spectrometry. Total specific GABA uptake was defined as the difference between the uptake of (^{3}H)GABA that occurred in the absence or presence of 10^{-2} M nipecotic acid, a GABA uptake inhibitor.

In Vivo Biological Testing. All compounds were tested for anticonvulsant activity and neurotoxicity by contractors of the Antiepileptic Drug Development Program administered by the National Institute for Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD. Tests were performed in Carnworth Farms no. 1 mice with evaluations 0.5 and 4 h after intraperitoneal injections of 30, 100, 300, and 600 mg/kg in one animal per dose (two or four animals per dose in toxicity testing). After one drop of 0.9% saline was instilled in the eye, corneal electrodes were applied, and a 60 cycle alternating current of 50 mA was delivered for 0.2 s to induce maximal electroshock seizures (MES). Abolition of the hind-limb tonic extension component of the seizure was considered as protection. The second test consisted of subcutaneous administration in the posterior midline of 85 mg/kg of pentylenetetrazol as a 0.5% solution. Failure to observe an episode of clonic response of at least 6-s duration was considered protection from pentylenetetrazole (Metrazole) seizure threshold (SMC). Neurotoxicity was evaluated by placing an animal on a 1-in. diameter knurled plastic rod rotating at 6 rpm (rotorod test). Failure of the animal to remain on the rod for 1 min signified neurological toxicity.

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γ-Aminobutyric Acid Esters. 1. Synthesis, Brain Uptake, and Pharmacological Studies of Aliphatic and Steroid Esters of γ-Aminobutyric Acid

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Labeled and unlabeled aliphatic and steroid esters of γ-amino[U-^{14}C]butyric acid (GABA) were synthesized and tested for their capacity to penetrate the blood–brain barrier and for evidence of central neuropharmacological activity in rodents. The uptake of the labeled 9,12,15-octadecatrienyl (linolenyl), 3-cholesteryl, 1-butyl, and the 9-fluoro-11β,17-dihydroxy-16α-methyl-3,20-dioxopregna-1,4-dien-21-yl (dexamethasone) esters of GABA into mouse brain increased 2-, 25-, 74-, and 81-fold over GABA, respectively. The cholesteryl ester of GABA depressed the general motor activity of mice and rats in a dose-dependent manner, whereas the 1-butyl, linolenyl, and dexamethasone esters were inactive by this test. Studies of the rates of hydrolysis, GABA receptor binding capacity, and octanol/water partition coefficients indicated that pharmacological activity of the esters after entry into the central nervous system (CNS) was dependent on their capacity to release GABA by enzymatic hydrolysis and their lipid solubility.

Several investigations report lower than normal levels of γ-aminobutyric acid (GABA) in postmortem tissues of the central nervous system (CNS) of patients with Huntington's disease[1-3] and epilepsy[4-7] and suggest that such deficiencies may contribute to the pathophysiology of these neuropsychiatric disorders. These observations raise the possibility that "replacement" procedures that increase CNS levels of GABA may be useful in the treatment of such neuropsychiatric disorders. GABA, however, crosses the "blood–brain" barrier very poorly,[8] so that methods that alter this property are of interest. Previous investigations have reported that lipophilic esters[9,10] and Schiff's base derivatives[11] can facilitate the uptake of GABA into the brain. With these observations in mind, we have synthesized U-^{14}C-labeled and unlabeled aliphatic and steroid esters of GABA and evaluated the penetration of the labeled compounds through the blood–brain diffusion barrier. In addition, some neuropharmacological properties of the unlabeled products were evaluated as "prodrugs" that release GABA after hydrolysis within the CNS.

Chemistry. The butyl ester of GABA was prepared in good yield by direct esterification with 1-butanol at 100 °C in the presence of anhydrous HCl as the catalyst as shown in Scheme I. The 9,12,15-octadecatrienyl alcohol (linolenol), cholesterol, and dexamethasone esters of GABA were synthesized according to Scheme II. The amino group of GABA was first protected by formation of its *tert*-butoxycarbonyl (*t*-Boc) derivative (**2**) by reaction with *t*-Boc-ON reagent.[12] This product was converted to the symmetrical anhydride (**3**) by using dicyclohexylcarbodiimide[13] (DCC). Each alcohol was esterified by reaction with **3** to yield products **4**, which, after treatment with trifluoroacetic acid to remove the *t*-Boc group, gave the desired compounds **5–7**. Similar methods on a microscale were used to prepare **1** and **5–7** as radioactive derivatives, with all the radioactivity present at ^{14}C in the GABA moiety of the molecules. The IR and NMR spectra and elemental analyses of the compounds were consistent with their proposed structures.

Scheme I

$$\underset{\text{GABA}}{H_2N(CH_2)_3COOH} \xrightarrow[HCl]{CH_3(CH_2)_3OH} \underset{\mathbf{1}}{[H_3N(CH_2)_3CO_2(CH_2)_3CH_3]^+Cl^-}$$

Results and Discussion

Brain Uptake Studies. Initially, a double-labeling method was used to compare the simultaneous uptake of each ^{14}C-labeled GABA ester with [^{3}H]GABA in the brain

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