sulting in an increase in disintegration time, the effect increasing as the additive content of the tablet increases. For example, conventional tablets (Table III) typically disintegrate within ~10 s, but tablets with additives present at 1% of the tablet weight require 20–30 s for all additives except povidone. Tablets formulated with 2% of either additive V or additive VIII require ~100 s for disintegration. These times refer to tablets aged 2 years at 25°C. While 100 s is near the UPS limit for 2 min, a reduction in tablet density will significantly reduce the disintegration time. The data quoted above refer to a fixed tablet density (1.16 g/mL). Thus, it may be possible to reduce the tablet density by using less lactose, thereby reducing the disintegration time to a more acceptable level.

With the exception of tablets containing additive I (povidone), the disintegration times for fresh tablets are essentially the same as for aged tablets. Fresh tablets containing 1% povidone typically disintegrate within 20 s, but after prolonged aging (more than 2 years at 25°C) they may require 1–2 min for disintegration. Evidently the povidone cross-links to some extent which retards disintegration. When these tablets are left undisturbed in water for some time, a "ghost" of insoluble material in the shape of the tablet remains. Thus, with the exception of severely aged povidone-containing tablets, excessive disintegration time is not a problem for the tablet systems studied (Table III), at least for ≤1% additive.

CONCLUSIONS

A number of nitroglycerin-soluble additives sufficiently lower the vapor pressure of nitroglycerin to stabilize the content uniformity of molded nitroglycerin tablets. In general, an additive–nitroglycerin weight ratio, R, of 1 is adequate for this purpose. Tablet potency losses in open-dish evaporation tests are roughly proportional to the vapor pressure of nitroglycerin in the tablet, and higher levels of additive (R = 2) are normally needed to achieve a significant improvement in open-dish stability. However, with few exceptions (VII and XV) high additive levels lead to reduced chemical stability, particularly at high temperature, and a compromise must be made between chemical stability and open-dish stability. For packaging in sealed glass bottles, open-dish potency loss is of little importance, and a compromise of chemical stability is not necessary. For example, additives VII and XV stabilize content uniformity with no measurable loss of chemical stability and several other additives (V in particular) do not seriously affect chemical stability when used at R = 1[10].

REFERENCES

(1) R. R. Shangraw and A. M. Contractor, *J. Am. Pharm. Assoc.*, **N512,** 633 (1972).

(2) S. Fusari, *J. Pharm. Sci.*, **62,** 122 (1973).

(3) S. Fusari, *J. Pharm. Sci.*, **62,** 2012 (1973).

(4) M. J. Pikal, A. L. Lukes, and L. F. Ellis, *J. Pharm. Sci.*, **65,** 1278 (1976).

(5) F. W. Goodhart, H. Gucluyildiz, R. E. Daly, L. Chafetz, and F. C. Ninger, *J. Pharm. Sci.*, **65,** 1466 (1976).

(6) H. Gucluyildiz, F. W. Goodhart, and F. C. Ninger, *J. Pharm. Sci.*, **66,** 265 (1977).

(7) M. J. Pikal, D. A. Bibler, and B. Rutherford, *J. Pharm. Sci.*, **66,** 1293 (1977).

(8) D. Stephenson and J. F. Humphreys-Jones, *J. Pharm. Pharmacol.*, **3,** 767 (1951).

(9) W. Kross and H. Roth, *Pharm. Ztg.*, **120,** 1490 (1975); *Chem. Abstr.*, **84,** 22035b (1976).

(10) P. Suphajettra, J. Lim, and J. Strohl, Abstracts of 124th APhA Annual Meeting, APhA Academy of Pharmaceutical Sciences, May 1977.

(11) D. P. Page, N. A. Carson, C. A. Buhr, P. E. Flinn, C. E. Wells, and M. T. Randall, *J. Pharm. Sci.*, **64,** 141 (1975).

(12) M. J. Pikal and A. L. Lukes, *J. Pharm. Sci.*, **65,** 1269 (1976).

(13) G. N. Lewis and M. Randall, "Thermodynamics," 2nd ed., revised by K. S. Pitzer and L. Brewer, McGraw Hill Co., New York, N.Y., 1961, p. 294.

(14) "Handbook of Chemistry and Physics," 38th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1956.

(15) V. V Gorbunov, *Teor. Vzryvchatykh Veshchestv*, **1963,** 219; *Chem. Abstr.*, **59,** 9728b (1963).

(16) R. C. Farmer, *J. Chem. Soc.*, **117,** 806 (1920).

(17) M. C. Crew and F. J. Dicarlo, *J. Chromatogr.*, **35,** 506 (1968).

[10] Only two stabilized nitroglycerin tablet products are available in the U.S. market. Parke-Davis' formulation uses polyethylene glycol 4000 as the additive while the Lilly formulation currently uses povidone.

γ-Aminobutyric Acid Uptake Inhibition and Anticonvulsant Activity of Nipecotic Acid Esters

A. MICHAEL CRIDER *§x, J. D. WOOD ‡, KATHRYN D. TSCHAPPAT *, CHRISTINE N. HINKO *, and KAREN SEIBERT *

Received May 16, 1983, from the **College of Pharmacy, The University of Toledo, Toledo, OH 43606* and the ‡*Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.* Accepted for publication October 5, 1983. Present address: § School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209.

Abstract □ *n*-Alkyl esters of nipecotic acid were prepared by Fischer esterification, and the esters were evaluated against bicuculline-induced seizures in mice. Evaluation of the alkyl esters for inhibition of γ-aminobutyric acid uptake into mouse whole brain mini-slices revealed that the order of potency was proportional to chain length. The octyl ester inhibited γ-aminobutyric acid and β-alanine uptakes by apparently nonspecific mechanisms. A variety of phenyl esters of nipecotic acid were also synthesized utilizing either dicyclohexylcarbodiimide or 1,1′-carbonyldiimidazole as the condensing agent. Most of the phenyl esters were potent inhibitors of γ-aminobutyric acid uptake. The uptake inhibition appeared to involve specific and nonspecific (detergent-like) mechanisms. The *m*-nitrophenyl and *p*-nitrophenyl esters were particularly potent against bicuculline-induced seizures in mice.

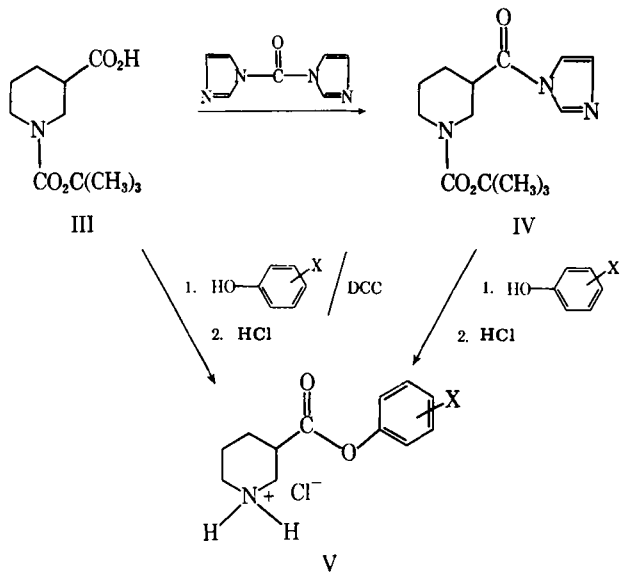
Keyphrases □ Nipecotic acid esters—synthesis, anticonvulsant activity □ Anticonvulsant agents—potential, nipecotic acid esters, synthesis

γ-Aminobutyric acid appears to act as a major inhibitory neurotransmitter in the central nervous system (1–3). Impairment in γ-aminobutyric acid neurotransmission may contribute to the symptoms of Huntington's disease, Parkinsonism, and epilepsy (4). Thus, compounds that potentiate γ-aminobutyric acid neurotransmission have considerable therapeutic potential.

(±)-Nipecotic acid (I) has been found to be a potent inhibitor of γ-aminobutyric acid uptake into rat cerebral cortex (5) and mouse whole brain mini-slices (6). The *R*-(−) enantiomer of nipecotic acid (I) has approximately five times greater affinity for the γ-aminobutyric acid uptake carrier than the *S*-(+) enantiomer (7).

As with several other compounds (8) having the potential of interacting with the γ-aminobutyric acid system, (±)-nipecotic acid (I) does not readily penetrate the blood–brain barrier (9). However, prodrug esters of nipecotic acid (I) that pass into the central nervous system have been evaluated for anticonvulsant activity (10–13) and γ-aminobutyric acid uptake inhibition (13, 14).

0022-3549/84/1100-1612$01.00/0



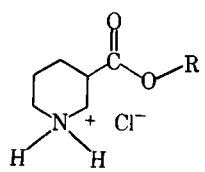
Scheme I

In a previous report, the *p*-nitrophenyl ester Vc was found to exhibit potent anticonvulsant activity and γ-aminobutyric acid uptake inhibition. The octyl ester IIc was also a strong inhibitor of γ-aminobutyric acid uptake (13). The results of this preliminary study indicated that Vc inhibited γ-aminobutyric acid uptake into mouse whole brain mini-slices by a combination of specific and nonspecific (detergent-like) effects. The specific effect is due to the hydrolysis product, nipecotic acid (I), while the nonspecific effect is caused by the intact ester. The octyl ester IIc apparently is not hydrolyzed under the assay conditions of the γ-aminobutyric acid uptake and the inhibition is entirely due to the intact ester.

Based on the activity of Vc, it was of interest to examine the structure-activity relationships of substituted-phenyl esters of I. Specifically, the lipophilicity and the electronic character of the phenyl substituent were varied in order to determine the effect on anticonvulsant activity and γ-aminobutyric acid uptake inhibition. An additional objective of this study was to evaluate the γ-aminobutyric acid uptake inhibition of the heptyl IIb and nonyl IId esters to determine if their action was nonspecific, as was the case with IIc.

RESULTS AND DISCUSSION

The alkyl esters (IIa-d) were synthesized by Fischer esterification of I with an appropriate alcohol in the presence of dry hydrogen chloride (13). Phenyl esters (Va-q) of nipecotic acid were prepared by utilizing either dicyclohexylcarbodiimide or 1,1′-carbonyldiimidazole as the condensing agent (Scheme I); the physical properties of these compounds are given in Table I.



IIa, R = C_6H_{13}
IIb, R = C_7H_{15}
IIc, R = C_8H_{17}
IId, R = C_9H_{19}

Termination of γ-aminobutyric acid with its postsynaptic receptor is brought about by high-affinity uptake systems in nerve terminals and glial cells (3). These carriers exhibit different substrate specificities for γ-aminobutyric acid and its derivatives (15, 16). The results of Breckenridge *et al.* (16) have confirmed that both neuronal and glial uptake processes prefer a folded conformation in the molecule. These same workers found that neuronal γ-aminobutyric acid uptake is markedly influenced by compounds having long alkyl chains. In contrast, inhibition of β-alanine uptake was more strongly affected by compounds having shorter alkyl chains. The speculation was that the glial uptake carrier has a more shallow hydrophobic bonding site.

Based on the work by Breckenridge and co-workers (16), we were interested in evaluating homologues of IIc for γ-aminobutyric acid uptake inhibition. The nonyl ester IId completely inhibited γ-aminobutyric acid uptake, while the heptyl ester IIb and hexyl ester IIa were less potent uptake inhibitors (85 and 53%, respectively). The results of IIc on β-[^{3}H]alanine uptake, a measure of glial uptake inhibition (16), are given in Table II. Since 1 mM nipecotic acid (I) only inhibits β-alanine uptake by 85%, the 100% uptake inhibition by IIc strongly suggests a nonspecific cationic detergent effect. Such a mechanism is further supported by the fact that IIc more potently inhibits γ-aminobutyric acid (100 *versus* 96%) and L-proline (13) (99 *versus* 21%) uptake than the parent amino acid I. Although IIc apparently inhibits both γ-aminobutyric acid and β-alanine by nonspecific mechanisms, it is rather difficult to explain the differences in uptake inhibition of γ-aminobutyric acid and β-alanine on dilution of the inhibitor (16 *versus* 50%) at 0.5 mM, respectively.

All of the phenyl esters, with the exception of Vd, were potent inhibitors of γ-aminobutyric acid uptake (Table III). As was the case with *p*-nitrophenyl ester Vc (13), these derivatives probably inhibit γ-aminobutyric acid uptake by a combination of specific and nonspecific effects. The phenyl substituents vary considerably in terms of lipophilic and electronic character. However, no readily apparent correlation exists between these parameters and either γ-aminobutyric acid uptake inhibition or anticonvulsant activity.

The esters were also evaluated for their ability to protect against bicuculline-induced seizures (Table IV). A single dose (150 mg/kg sc) of each compound was tested, with this dose being administered 60 min prior to bicuculline challenge. The dose and pretreatment time were chosen on the basis of preliminary testing of other compounds in the series (13). A subcutaneous route of injection was used to reduce peripheral hydrolysis. Currently, individual compounds are being evaluated at a variety of doses and pretreatment times in an effort to calculate specific ED_{50} values and times of peak effect.

As indicated in Table IV, all compounds (with the exception of Vd and Vn) demonstrated a significant degree of protection against clonic convulsions, tonic convulsions, or death induced by bicuculline. Although complete protection from clonic spasms was not observed with any of the compounds tested at this dose, IIb, IId, and Vb provided protection from clonic convulsions in seven of eight mice and IIb, Vc, and Ve protected all eight animals in each group from tonic convulsions and death. In addition, IIc, IId, Vb, Vk, Vp, and Vq prevented tonic convulsions and death in at least seven of eight animals. Antagonism of convulsions induced by bicuculline, a putative γ-aminobutyric acid receptor antagonist, suggests that these nipecotic acid esters may be producing their effects by interaction with the γ-aminobutyric acid system.

EXPERIMENTAL SECTION[1]

***n*-Heptyl 3-Piperidinecarboxylate Hydrochloride (IIb)**—This compound was prepared according to conditions previously described (13) in 21% yield, mp 74-75°C; IR (KBr): 2500 (NH_2^+), 1760 (C=O), and 1225 cm^{-1} (C—O); ^{1}H-NMR ($CDCl_3$): δ 0.90 (t, 3, CH_3), 1.07-3.80 (m, 19, aliphatic CH_2, ring CH, and ring CH_2), 4.10 (t, 2, OCH_2), and 9.45 ppm (br s, 2, NH_2^+).

Anal.—Calc. for $C_{13}H_{26}ClNO_2$: C, 59.17; H, 9.95; N, 5.31. Found: C, 59.43; H, 9.87; N, 5.01.

***n*-Nonyl 3-Piperidinecarboxylate Hydrochloride (IId)**—Compound IId was synthesized following the general method (13) in 51% yield, mp 85-86°C; IR (KBr): 2500 (NH_2^+), 1760 (C=O), and 1225 cm^{-1} (C—O); ^{1}H-NMR ($CDCl_3$): δ 0.87 (t, 3, CH_3), 1.03-3.77 (m, 23, aliphatic CH_2, ring CH, and ring CH_2), 4.07 (t, 2, OCH_2), and 9.43 ppm (br s, 2, NH_2^+).

Anal.—Calc. for $C_{15}H_{30}ClNO_2$: C, 61.71; H, 10.38; N, 4.80. Found: C, 61.79; H, 10.27; N, 5.02.

1-(1-*tert*-Butyloxcarbonyl-3-piperidinocarbonyl)imidazole (IV)—A mixture of III (13) (3.00 g, 0.013 mol) and 1,1′-carbonyldiimidazole (2.10 g, 0.013 mol) in 150 mL of tetrahydrofuran was heated at reflux overnight. Removal of the solvent gave a yellow oil, which was dissolved in 50 mL of chloroform

[1] Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. IR spectra were recorded as potassium bromide pellets with a Perkin-Elmer spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian EM 360A spectrometer. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (1%) or, in the case of D_2O (1%), sodium 2,2-dimethyl-2-silapentane-5-sulfonate. Analytical data were obtained from Micro-Analysis Inc., Wilmington, Del.

Table I—Physical Properties of Phenyl 3-Piperidinecarboxylate Hydrochlorides (V)

Compound	X	Method	Melting Point, °C	Yield, %	Formula[a]	IR, cm^{-1}	^1H-NMR[b]
Va	—H	B[c]	161–163	46[k]	$C_{12}H_{16}ClNO_2$	1785 (C=O)	δ 1.97 (m, 4, CH_2CH_2CH), 3.33 (m, 5, $CH_2NH_2^+CH_2CH$), 6.93–7.40 (m, 5, ArH), 9.67 ppm (br s, 2, NH_2^+)
Vb	—*m*-NO_2	A[d]	190–192	44[j]	$C_{12}H_{15}ClN_2O_4$		
Vc	—*p*-NO_2[e]	A[d]	160–162	60[j]	$C_{12}H_{15}ClN_2O_4$	1780 (C=O)	2.0 (m, 4, CH_2CH_2CH), 3.1–3.5 (br m, 5, $CH_2NH_2^+CH_2CH$), 7.3–8.2 ppm (m, 4, ArH)
Vd	—*m*-CN	B[c]	149.5–151.5	14[i]	$C_{13}H_{15}ClN_2O_2$	1750 (C=O)	2.03 (m, 4, CH_2CH_2CH) 2.63–3.80 (m, 5, $CH_2NH_2^+CH_2CH$), 7.60 ppm (m, 4, ArH)
Ve	—*p*-CN	A[c]	148.5–150.0	20[k]	$C_{13}H_{15}ClN_2O_2$	1785 (C=O)	2.13 (m, 4, CH_2CH_2CH), 2.73–4.07 (m, 5, $CH_2NH_2^+CH_2CH$), 7.33 (d, 2, J = 8 Hz, ArH), 7.87 ppm (d, 2, J = 8 Hz, ArH)
Vf	—*o*-CN	B[c]	142–143	36[k]	$C_{13}H_{15}ClN_2O_2$	2270 (C≡N), 1795 (C=O)	2.03 (m, 4, CH_2CH_2CH), 2.60–4.83 (m, 5, $CH_2NH_2^+CH_2CH$), 7.23–8.03 ppm (m, 4, ArH)
Vg	—*m*-CF_3	B[c]	111–113	35[k]	$C_{13}H_{15}ClF_3NO_2$	1810 (C=O)	2.20 (m, 4, CH_2CH_2CH), 3.57 (m, 5, $CH_2NH_2^+CH_2CH$), 7.70 ppm (s, 4, ArH)
Vh	—*p*-CF_3	B[f]	183–184	30[k]	$C_{13}H_{15}ClF_3NO_2$	1800 (C=O)	2.17 (m, 4, CH_2CH_2CH), 2.70–4.07 (m, 5, $CH_2NH_2^+CH_2CH$), 7.43 (d, 2, J = 8 Hz, ArH), 7.83 ppm (d, 2, J = 8 Hz, ArH)
Vi	—*m*-F	B[c]	124–126	48[k]	$C_{12}H_{15}ClFNO_2$		
Vj	—*p*-Cl	B[c]	174.5–175.0	28[k]	$C_{12}H_{15}Cl_2NO_2$	1785 (C=O)	2.07 (m, 4, CH_2CH_2CH), 3.67–3.87 (m, 5, $CH_2NH_2^+CH_2CH$), 7.20 (d, 2, J = 8 Hz, ArH), 7.50 ppm (d, 2, J = 8 Hz, ArH)
Vk	—*p*-Br	B[d]	225.0–226.5	50[l]	$C_{12}H_{15}BrClNO_2$	1750 (C=O)	1.87 (m, 4, CH_2CH_2CH), 2.67–3.57 (m, 5, $CH_2NH_2^+CH_2CH$), 7.17 (d, 2, J = 8 Hz, ArH), 7.67 (d, 2, J = 8 Hz, ArH), 9.50 ppm (br s, 2, NH_2^+)
Vl	—*p*-I	B[g]	255–256	69[k]	$C_{12}H_{15}ClINO_2$	1785 (C=O)	1.90 (m, 4, CH_2CH_2CH), 3.20 (m, 5, $CH_2NH_2^+CH_2CH$), 7.07 (d, 2, J = 8 Hz, ArH), 7.80 (d, 2, J = 8 Hz, ArH), 9.47 ppm (br s, 2, NH_2^+)
Vm	—*p*-$C(CH_3)_3$	B[c]	178–179	31[k]	$C_{16}H_{24}ClNO_2$	1750 (C=O)	1.30 [s, 9, $C(CH_3)_3$], 1.47–3.87 (m, 8, ring CH_2), 4.13 (m, 1, ring CH), 6.97 (d, 2, J = 8 Hz, ArH), 7.36 (d, 2, J = 8 Hz, ArH), 9.70 ppm (br s, 2, NH_2^+)
Vn	—*p*-OCH_3	B[d]	190.0–190.5	57[k]	$C_{13}H_{18}ClNO_3$	1785 (C=O)	2.00 (m, 4, CH_2CH_2CH), 2.67–3.90 (m, 8, including singlet at 3.90, OCH_3), 7.13 ppm (br s, 4, ArH)
Vo	—*p*-$COCH_3$	A[c]	171.5–172.5	30[k]	$C_{14}H_{18}ClNO_3$	1785 (C=O, ester), 1710 (C=O, ketone)	2.03 (m, 4, CH_2CH_2CH), 2.67 (s, 3, $COCH_3$), 2.70–3.83 (m, 5, $CH_2NH_2^+CH_2CH$), 7.20 (d, 2, J = 8 Hz, ArH), 7.93 (d, 2, J = 8 Hz, ArH)
Vp	—*p*-CO_2CH_3	A[h]	186.0–187.5	33[k]	$C_{14}H_{18}ClNO_4$	1800 (C=O, ester), 1755 (CO_2CH_3)	1.40–3.80 (m, 9, ring CH and CH_2), 3.97 (s, 3, $COOCH_3$), 7.27 (d, 2, J = 8 Hz, ArH), 8.07 ppm (d, 2, J = 8 Hz, ArH)
Vq	—*p*-$CO_2C_2H_5$	B[d]	186.0–186.5	46[k]	$C_{15}H_{20}ClNO_4$	1820 (C=O, ester) 1750 ($CO_2C_2H_5$)	1.33 (t, 3, J = 6 Hz, CH_3), 1.90 (m, 4, CH_2CH_2CH), 3.40 (m, 5, $CH_2NH_2^+CH_2CH$), 4.33 (q, 2, J = 6 Hz, CH_2CH_3) 7.40 (d, 2, J = 8 Hz, ArH), 8.07 (d, 2, J = 8 Hz, ArH), 9.60 ppm (br s, 2, NH_2^+)

[a] Compounds Va, Vb, and Vd–q were analyzed for C, H, and N; all values were within ±0.4% of the theoretical value. [b] Compounds Va and Vn were run in Unisol-*d*; Vc–j and Vn–p were run in D_2O; Vd, Vl, and Vq were run in Me_2SO-d_6. [c] Recrystallized from ethanol–ether. [d] Recrystallized from ethanol. [e] Reported in the literature (13). [f] Recrystallized from 95% ethanol–ether. [g] Recrystallized from 95% ethanol. [h] Recrystallized from methanol–ether. [i] Pale-pink crystals. [j] Pale-yellow crystals. [k] White crystals. [l] Brown crystals.

and washed three times with 25-mL portions of water. The organic phase was separated, dried (sodium sulfate), and evaporated to yield an almost colorless oil. Trituration of the oil with hexane gave, after drying, 2.88 g (79%) of a white solid, mp 95–99°C. An analytical sample was prepared by recrystallization from petroleum ether (bp 39–55°C) to yield pure product, mp 105–106°C; IR (KBr): 1735 (C=O, imidazolide) and 1700 cm^{-1} (C=O, carbamate); ^1H-NMR ($CDCl_3$): δ 1.50–4.47 [m, 18, ring CH, ring CH_2, and $C(CH_3)_3$ at 1.50], 7.10 (s, 1, —N—CH=N—CH=CH), 7.50 (s, 1,

Table II—Effect of IIc on the Uptakes of γ-Aminobutyric Acid and β-Alanine [a]

Conc. of Inhibitor, mM	β-Alanine Uptake, % Inhibition		γ-Aminobutyric Acid Uptake, % Inhibition	
	IIc	Nipecotic Acid	IIc	Nipecotic Acid
1.0	100 ± 0	85 ± 1	100 ± 1	96 ± 1
0.2	92 ± 1	71 ± 2	48 ± 3	89 ± 1
0.05	50 ± 2	47 ± 4	16 ± 2	75 ± 1

[a] All values are mean ± *SEM* for six samples.

Table III—Inhibition of γ-Aminobutyric Acid Uptake by Nipecotic Acid Esters [a]

Inhibitor[b]	Inhibition of Uptake, %[c]
I	96.3 ± 0.2
Va	81.2 ± 0.6
Vb	97.5 ± 0.5
Vc	99.0 ± 0.1
Vd	33.4 ± 2.2
Ve	94.4 ± 0.2
Vf	98.0 ± 0.3
Vg	95.1 ± 0.5
Vh	97.3 ± 0.3
Vi	92.0 ± 0.3
Vj	94.2 ± 0.4
Vk	95.8 ± 0.4
Vl	98.2 ± 0.1
Vm	97.5 ± 0.3
Vn	82.5 ± 0.5
Vo	90.9 ± 0.5
Vp	92.4 ± 0.1
Vq	95.5 ± 0.3
IIa	52.7 ± 1.6
IIb	84.9 ± 1.4
IIc	99.7 ± 0.1
IId	99.8 ± 0.1

[a] Uptake measured into mouse whole brain mini-slices. [b] Inhibitor concentration = 1 mM. [c] All values are mean ± *SEM* for six samples.

$-\overline{N-CH=N-CH=C}H$), and 8.23 ppm (s, 1, $-\overline{NCH=N-CH=CH}$).

Anal.—Calc. for $C_{14}H_{21}N_3O_3$: C, 60.18; H, 7.59; N, 15.04. Found: C, 60.06; H, 7.58; N, 14.87.

Representative Procedures for the Synthesis of the Phenyl 3-Piperidinecarboxylate Hydrochlorides (V)—*Method A*—The synthesis of *m*-nitrophenyl 3-piperidinecarboxylate hydrochloride (Vb) is representative of the general procedure. A solution of III (3.00 g, 0.013 mol) and *m*-nitrophenol (1.82 g, 0.013 mol) in acetonitrile (150 mL) was treated in one portion with dicyclohexylcarbodiimide (2.70 g, 0.013 mol). A white precipitate of dicyclohexylurea formed immediately. Stirring was continued for 48 h, the dicyclohexylurea was removed by filtration, and the filtrate was saturated with hydrogen chloride at 0–5°C. The solvent was evaporated under reduced pressure and the resulting solid was recrystallized from absolute ethanol to yield 1.65 g (44%) of light-yellow crystals, mp 190–192°C; IR (KBr): 1785 cm^{-1} (C=O, ester); ^{1}H-NMR (D_2O): δ 1.65–4.00 (m, 9, ring CH and CH_2) and 7.55–8.45 ppm (m, 4, ArH).

Anal.—Calc. for $C_{12}H_{15}ClN_2O_4$: C, 50.26; H, 5.28; N, 9.77. Found: C, 50.49; H, 5.42; N, 9.63.

Method B—The synthesis of *m*-fluorophenyl 3-piperidinecarboxylate hydrochloride (Vi) is representative of the general method. A solution of IV (2.90 g, 0.010 mol) and *m*-fluorophenol (1.17 g, 0.010 mol) in acetonitrile (200 mL) was heated at reflux for 48 h, and the solvent was removed under reduced pressure. The resulting oil was dissolved in chloroform (200 mL) and washed three times with 25-mL portions of water. The organic phase was dried (sodium sulfate) and saturated with dry hydrogen chloride at 0–5°C. Evaporation of the solvent gave a light-yellow oil which solidified after trituration with ether. Recrystallization from ethanol–ether gave 1.30 g (48%) of analytically pure product, mp 124–126°C; IR (KBr): 1785 cm^{-1} (C=O, ester); ^{1}H-NMR (D_2O): δ 1.67–4.00 (m, 9, ring CH and CH_2) and 6.90–7.80 ppm (m, 4, ArH).

Anal.—Calc. for $C_{12}H_{15}ClFNO_2$: C, 55.49; H, 5.83; N, 5.39. Found: C, 55.76; H, 5.83; N, 5.53.

Table IV—Antagonism of Bicuculline-Induced Convulsions by Esters of Nipecotic Acid

Pretreatment[a]	Number Mice Protected/Total Number Mice[b]		
	Clonic Convulsions	Tonic Convulsions	Death
Saline	0/116	0/116	0/116
IIa	3/8[e]	4/8	5/8
IIb	7/8	8/8	8/8
IIc	6/8	7/8	7/8
IId	7/8	7/8	7/8
Vb	7/8	8/8	7/8
Vc[c]	4/8	8/8	8/8
Vd	0/8[d]	1/8[d]	0/8[d]
Ve	6/8	8/8	8/8
Vf	4/8	6/8	6/8
Vg	3/8[e]	6/8	6/8
Vi	1/7[d]	3/7[e]	2/7[d]
Vj	2/8[d]	4/8	4/8
Vk	5/8	7/8	7/8
Vm	6/8	6/8	6/8
Vn	0/8[d]	0/8[d]	0/8[d]
Vo	1/8[d]	5/8	7/8
Vp	4/8	7/8	7/8
Vq	3/8[e]	7/8	7/8

[a] Compounds (150 mg/kg sc) were administered 60 min prior to bicuculline (3.00 or 3.75 mg/kg sc). Compounds Vh and Vl were also tested but limited solubility and stability of the compounds prevents the reporting of reproducible data. [b] The animal was considered protected from clonic convulsions if it failed to exhibit an episode of clonic spasms for at least 5 s. The animal was considered protected from tonic convulsions if it failed to exhibit hind limb extension. [c] Previously reported (13). [d] Not significantly different from saline controls. [e] Significantly different from saline controls ($p < 0.05$). All other values in the table are significantly different from saline controls ($p < 0.01$).

Biological Testing—Male Sprague-Dawley mice[2] received an injection of 3.0 or 3.75 mg/kg sc of bicuculline in a volume of 0.01 mL/g. The bicuculline was prepared by dissolving the solid in 0.1 M HCl and adjusting to pH 5 with 0.1 M NaOH (13). Each compound was evaluated for its ability to protect against bicuculline-induced seizures at a dose of 150 mg/kg sc injected 1 h prior to bicuculline administration. Groups of eight mice per compound were tested. Groups of four control mice were pretreated with 0.9% NaCl and tested before and after each experimental group to ensure maximal convulsant potency of the bicuculline.

The incidence and onset of clonic and tonic convulsions and fatality induced by bicuculline were recorded for each of the animals. A clonic convulsion was defined as a single episode of clonic spasms of at least a 5-s duration. A tonic seizure was defined as a brief period of hind limb flexion followed by a prolonged period of hind limb extension. The animals were observed for at least 45 min after the injection of bicuculline. The uptakes of γ-[^{3}H]aminobutyric acid and tritiated β-alanine were carried out following reported procedures (6, 17).

REFERENCES

(1) J. K. Saelens and F. J. Vinick, in "Annual Reports in Medicinal Chemistry," Vol. 13, F. H. Clarke, Ed., Academic, New York, N.Y., 1978, p. 31.

(2) D. R. Curtis, in "GABA-Neurotransmitters, Pharmacochemical, Biochemical and Pharmacological Aspects," P. Krogsgaard-Larsen, J. Scheel-Krüger, and H. Kofod, Eds., Academic, New York, N.Y., 1979, p. 18.

(3) A. Schousboe, L. Hertz, O. M. Larsson, and P. Krogsgaard-Larsen, *Brain Res. Bull. Suppl.*, **2**, 403 (1980).

(4) S. J. Enna, *Biochem. Pharmacol.*, **30**, 907 (1981).

(5) P. Krogsgaard-Larsen and G. A. R. Johnston, *J. Neurochem.*, **25**, 797 (1975).

(6) J. D. Wood, D. Tsui, and J. W. Phillis, *Can. J. Physiol. Pharmacol.*, **57**, 581 (1979).

(7) G. A. R. Johnston, P. Krogsgaard-Larsen, A. L. Stephanson, and B. Twitchin, *J. Neurochem.*, **26**, 1029 (1976).

(8) P. S. Callery, L. A. Geelhaar, M. S. B. Nayar, M. Stogniew, and K. G. Rao, *J. Neurochem.*, **38**, 1063 (1982).

(9) D. Lodge, G. A. R. Johnston, D. R. Curtis, and S. J. Brand, *Brain Res.*, **136**, 513 (1977).

(10) R. W. Horton, J. F. Collins, G. M. Anlezark, and B. S. Meldrum, *Eur. J. Pharmacol.*, **59**, 75 (1979).

[2] Harlan Sprague-Dawley, Madison, Wis.

(11) H. H. Frey, C. Popp, and W. Löscher, *Neuropharmacology*, **18**, 581 (1979).
(12) C. G. Wermuth, A. Zinoune, J. J. Bourguignon, and J. P. Chambon, in "The Chemical Regulation of Biological Mechanisms," A. M. Creighton and S. Turner, Eds., The Royal Society of Chemistry, London, 1982. p. 112.
(13) A. M. Crider, T. T. Tita, J. D. Wood, and C. N. Hinko, *J. Pharm. Sci.*, **71**, 1214 (1982).
(14) J. D. Wood, A. Schousboe, and P. Krogsgaard-Larsen, *Neuropharmacology*, **19**, 1149 (1980).
(15) S. L. Earley, E. K. Michaelis, and M. P. Mertes, *Biochem. Pharmacol.*, **30**, 1105 (1981).
(16) R. J. Breckenridge, S. H. Nicolson, A. J. Nicol, C. J. Suckling, B. Leigh, and L. Iversen, *Biochem. Pharmacol.*, **30**, 3045 (1981).
(17) L. L. Iversen and M. J. Neal, *J. Neurochem.*, **15**, 1141 (1968).

ACKNOWLEDGMENTS

A portion of this work was presented at the 14th Central Regional Meeting of The American Chemical Society, Midland, Michigan, June 1982. This investigation was funded by research grants from the Epilepsy Foundation of America, The Upjohn Company, the University of Toledo Faculty Research Awards and Fellowships Program, and the Medical Research Council of Canada. The authors thank David L. Miskell and Eugen Kurylo for their technical assistance.

Accurate, Wide-Range, Automated, High-Performance Liquid Chromatographic Method for the Estimation of Octanol/Water Partition Coefficients I: Effect of Chromatographic Conditions and Procedure Variables on Accuracy and Reproducibility of the Method

J. E. GARST ˣ and W. C. WILSON

Received March 7, 1983, from the *Department of Animal Science, University of Illinois, 126 Animal Sciences Laboratory, 1207 West Gregory Drive, Urbana, IL 61801.* Accepted for publication November 29, 1983.

Abstract □ A high-performance liquid-chromatographic (HPLC) procedure is reported for estimation of the logarithm of the octanol/water partition coefficient, log P(o/w). This automated log P(o/w) measurement (ALPM) circumvents many inherent difficulties with the shake-flask method, yet gives high reproducibility and excellent overall correlation with shake-flask results. Partition coefficients for numerous structurally diverse chemicals, ranging from ~0 to ~8 log P(o/w) units, can be determined; however, values for zwitterionic compounds cannot be obtained. Additional advantages of ALPM include lower cost and greater safety when compared with other HPLC or shake-flask procedures. Chromatographic conditions (*i.e.*, flow rate and temperature) and variables (*i.e*, column length and solvent composition) affecting this method are discussed in detail. ALPM may also find application in quality control of HPLC columns, qualitative-quantitative analysis, and in computer-controlled method development and analysis.

Keyphrases ◻ HPLC-octanol/water partition coefficients □ Partition coefficients-octanol/water, HPLC

The logarithm of the octanol/water partition coefficient of a compound, log P(o/w), often parallels the biological effects of that substance (1, 2). Although log P(o/w) correlations are commonly used to optimize a specific biological response, they can also be a valuable predictor of adverse effects from chemical agents (3, 4). Consideration of this parameter in structure-toxicity as well as structure-activity studies might substantially reduce drug development costs (1). Furthermore, the increasing cost of animals and animal care, combined with growing public discontent over the use of animals in scientific research, will inevitably make prediction of toxicological responses much more important (5, 6).

Ideally, determination of the partition coefficient, P(o/w), requires measurement of the equilibrium ratio of the concentrations of a single component, X, dissolved in nonpolar and polar layers using simple separatory funnel shake-flask procedures (2):

$$P(o/w) = [X]_{octanol}/[X]_{water} \qquad \text{(Eq. 1)}$$

Although the polar phase is nearly always water, the choice of nonpolar phase is arbitrary; chloroform, hexane, and other solvents have been used, but octanol is the most common choice (2). There is, however, nothing unique about biological correlations using octanol/water as opposed to another solvent/water system. In 1954, Collander showed that partition data can be converted between solvent/water systems by least-squares regression (7), but Leo has described limitations in the use of this relationship (8).

Many economic and scientific problems occur with shake-flask log P(o/w) measurements. Since analytical procedures differ with each compound, the traditional method is time consuming and expensive (3). Log P(o/w) measurements may have only limited reproducibility among laboratories. For example, the seven reported shake-flask log P(o/w) values for naphthalene range from 3.01 to 3.59 (2). Analysis error is always a concern, but microemulsions can also alter distribution of the compound between the two phases (9). Formation of other components in the shake-flask could also alter phase equilibrium. The physical difficulties of measurement may be minimized by improved analytical techniques and sample centrifugation, but chemical interactions (changing the number of components) could affect the accuracy of even reproducibly obtained partition coefficients.

While measurement is always preferred, approximate values can be obtained by calculations based on the concept that the overall log P(o/w) reflects the summation of hydrophobic contributions from each constitutive group (π-approach) or fragment (f-approach) (2, 10, 11). Although calculated values often afford good estimates of log P(o/w), additivity of such constants may not always be observed (3). Problems of reproducibly and accurately measuring the shake-flask log P(o/w), knowledge of the increment for a particular molecular unit, and limitations in the additivity of such groups often re-

0022-3549/84/1100-1616$01.00/0