Table I—*In Vitro* Inhibition of Choline Acetyltransferase Activity from Two Sources by α-Phenyl-β-(3,4-dimethoxy)phenethylamine Derivatives

Compound[a]	Inhibition Constants *Torpedo* I_{50}, M	K_i, μM	*Spodoptera* I_{50}, M	K_i, μM
III	7.0×10^{-5}	60	2.7×10^{-5}	40
VIII	5.8×10^{-5}	45	2.5×10^{-5}	32
IX	4.0×10^{-5}	35	1.8×10^{-5}	25
X	1.3×10^{-5}	10	9.0×10^{-6}	6

[a] The I_{50} values were compared with those determined for diisopropylfluorophosphonate ($I_{50} = 5 \times 10^{-4}$ M from both sources). Lit. (10) data reported for diisopropylfluorophosphonate using choline acetyltransferase (house fly or mouse): $I_{50} = 1 \times 10^{-4}$ M.

acetyltransferase from both sources. From the I_{50} and K_i values obtained, the *Spodoptera* larval enzymes were shown to be more sensitive toward the synthesized compounds (III and VIII–X) than the *Torpedo* electric organ enzymes. However, the best *in vitro* choline acetyltransferase inhibitor was the charged compound (X), whereas the uncharged compounds (III, VIII, and IX) were relatively poor inhibitors (Table I).

Several choline analogues ($SCNCH_2CH_2\overset{+}{N}R^1R^2R^3X^-$) were previously tested (22) on rats as possible *in vivo* choline acetyltransferase inhibitors; the data revealed poor correlation between the K_i value and the insecticidal activity of such compounds when tested against several insect species. The most likely explanation was that since the molecule is charged, it would not readily penetrate the nerve cord and reach the cells in which the enzyme is located. The present work was thus directed toward *in vitro* inhibition studies using some uncharged phenethylamines (III, VIII, and IX) to avoid the problems of compound penetration to the target. The quaternary analogue (X) was also investigated.

REFERENCES

(1) H. B. Murphree, in "Drill's Pharmacology in Medicine," J. R. Di Palma, Ed., McGraw-Hill, New York, N.Y., 1965, p. 251.

(2) K. K. Chen and C. F. Schmidt, *J. Pharmacol. Exp. Ther.*, **24**, 339 (1925).

(3) D. J. Triggle, in "Medicinal Chemistry," Part II, A. Burger, Ed., Wiley, New York, N.Y., 1970, p. 1239.

(4) L. S. Goodman and A. Gilman, Eds., "The Pharmacological Basis of Therapeutics," 4th ed., Macmillan, London, England, 1970, pp. 753 and 754.

(5) R. M. Shafik, R. Soliman, and A. M. M. Hassan, *J. Pharm. Sci.*, **67**, 991 (1978).

(6) F. G. Canepa, P. Pauling, and H. Sorum, *Nature (London)*, **210**, 907 (1966).

(7) M. S. Steinberg, N. S. Cohen, and W. W. Weber, *Biochem. Biophys. Acta*, **235**, 89 (1971).

(8) R. Roskoski, Jr., *J. Biol. Chem.*, **249**, 2156 (1974).

(9) R. D. O'Brien, "Insecticides: Action and Metabolism" Academic, New York, N.Y., 1967.

(10) C. C. Yu and G. M. Booth, *Life Sci.*, **10**, 337 (1971).

(11) D. R. Haubrich and P. F. L. Wang, *Biochem. Pharmacol.*, **25**, 669 (1976).

(12) G. I. Henderson and B. V. Rama Sastry, *Biochem. Pharmacol.*, **27**, 1331 (1978).

(13) A. K. Chaturvedi, P. P. Rowell, and B. V. Rama Sastry, *J. Pharm. Sci.*, **67**, 657 (1978).

(14) P. P. Rowell, A. K. Chaturvedi, and B. V. Rama Sastry, *J. Pharmacol. Exp. Ther.*, **205**, 624 (1978).

(15) S. Owari, *Pharm. Bull.* (*Jpn.*), **1**, 174 (1953).

(16) J. Sam, R. M. Shafik, and K. Aparajithan, *J. Pharm. Sci.*, **59**, 59 (1970).

(17) A. P. Black and F. H. Babers, in "Organic Syntheses," Coll. Vol. II, A. H. Blatt, Ed., Wiley, London, England, 1947, p. 512.

(18) H. Kauffman, *Chem. Ber.*, **25B**, 1422 (1919).

(19) W. D. McPhee and E. S. Eriskson, Jr., *J. Chem. Soc.*, **68**, 624 (1946).

(20) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(21) F. Fonnum, *J. Neurochem.*, **20**, 1351 (1975).

(22) C. A. Baillie, J. R. Corbett, J. R. Dowsett, D. B. Sattelle, and J. J. Callec, *Pestic. Sci.*, **6**, 645 (1975).

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Excitatory Amino Acid Receptor Interactions of a Novel α-Phosphinic Acid Analogue of α-Methylaspartic Acid

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Received October 14, 1983, from the *Department of Medicinal Chemistry, College of Pharmacy, University of Houston, Houston, TX 77004* and the ‡*Departments of Neuroscience, Pharmacology, and Psychiatry, Division of Child Psychiatry, Johns-Hopkins University, School of Medicine, Baltimore, MD 21205.* Accepted for publication November 30, 1983.

Abstract □ An α-phosphino analogue of α-methylaspartate has been synthesized. The compound may not interact with excitatory amino acid receptors directly, as assessed by direct *in vitro* radioreceptor binding methods; however, it possesses weak anticonvulsant activity and exhibits an excitant action *in vitro* that is apparently not mediated by a *N*-methyl-D-aspartate receptor.

Keyphrases □ Excitatory amino acid receptors—*N*-methyl-D-aspartate, glutamate, kainate □ Anticonvulsants—*in vitro*, rats, excitatory amino acids □ Phosphinic acids—excitatory amino acid receptors, anticonvulsant activity

Phosphorus analogues of amino acids have been investigated previously; however, most studies have involved phosphonic acid derivatives such as aminomethanephosphonic acid (I). Only in recent years have aminophosphinic acids with unsubstituted amino groups been prepared. The first, glycine analogue II, was synthesized in 1964 by a procedure which provides only limited types of products (1).

Phosphinic acids possess a hydrogen atom in lieu of one of the two hydroxyl groups found in phosphonic acids and, therefore, are monobasic with less bulk on the phosphorus atom. Phosphinic acids are the closest phosphorus analogues of carboxylic acids and this similarity has led to a limited number of investigations of aminophosphinic acids for biological activity. In 1961, Linfield *et al.* prepared phosphinic acids bearing substituted α-anilino and phenyl groups (III) by the addition of hypophosphorus acid to the appropriate

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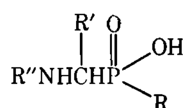
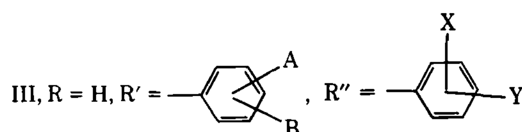
Schiff bases and reported antibacterial properties associated with some of these derivatives (2). Except for II, the only unsubstituted α-aminophosphinic acids reported are those by Khomutov and Osipova (3) and Bayliss *et al.* (4), which were synthesized by the addition of hypophosphorus acid to oximes and diphenylmethylimines, respectively. The amino acid analogues resulting from the latter process were investigated for antibacterial properties with alanine (IV), valine (V), and methionine (VI) derivatives displaying significant activity. In addition, the effect of V was reversed by valine, an indication that a false substrate mechanism was operative.

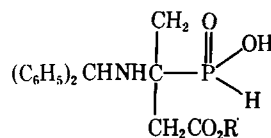


I, R = OH, R′ = R″ = H
II, R = R′ = R″ = H

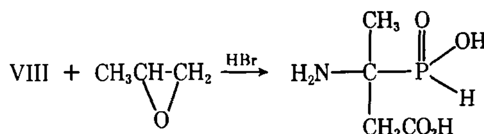


IV, R = R″ = H, R′ = CH_3
V, R = R″ = H, R′ = $—CH(CH_3)_2$
VI, R = R″ = H, R′ = $—CH_2CH_2SCH_3$

In this paper we describe the synthesis (*Scheme I*) and biological testing of DL-α-amino-α-methyl-β-carboxyethanephosphinic acid (IX) and two of its precursors (VII and VIII). Compound IX represents the bioisosteric replacement of one carboxyl group of α-methylaspartic acid with a P(O)H(OH) moiety. α-Methylaspartic acid has been investigated for its ability to reduce aspartic acid utilization (5) and for its effect in many enzyme systems such as inhibition of tyrosine aminotransferase (6) and asparaginase (7). Moreover, IX is related to the antagonists of amino acid receptors including 2-amino-4-phosphonobutyric acid and 2-amino-7-phosphonoheptanoic acid.



VII, R = Et
VIII, R = H



Scheme I

Table I—Effect of VII, VIII, and IX on the Specific Binding of Tritiated Kainic Acid, L-Glutamate, and 2-Amino-7-phosphonoheptanoic Acid to Rat Brain Membranes *In Vitro*[a]

	Specifically Bound Ligand, % Displacement		
Compound	Tritiated L-Glutamate	Tritiated Kainic Acid	Tritiated 2-Amino-7-phosphoheptanoic Acid
		Tris-Citrate	
VII	14	8	0
VIII	0	7	0
IX	0	11	28
		Tris-HCl	
VII	6	—	—
VIII	8	—	—
IX	8	—	—

[a] Compounds (10^{-4} M) were incubated in the presence of the indicated ligand as described. Reference IC_{50} values for L-glutamate, kainic acid, and 2-amino-7-phosphonoheptanoic acid are 1 μM, 5 nM, and 5 μM, respectively.

Three different types of excitatory amino acid receptors have been proposed to exist (8): (a) "glutamate receptors," which may be preferentially activated by L-glutamic acid or quisqualate, and blocked by glutamate diethyl ester or 2-amino-4-phosphonobutyric acid; (b) "kainate receptors," preferentially activated by kainate and closely related structures, for which there are no known receptor antagonists; (c) "*N*-methyl-D-aspartate receptors," preferentially activated by *N*-methyl-D-aspartate and ibotenic acid, and antagonized by 2-amino-5-phosphonopentanoate and 2-amino-7-phosphonoheptanoate. Excitatory amino acids may be the most common neurotransmitters in the brain. Antagonists of excitatory amino acid receptors are sought for application in controlling seizures and suppressing certain neurodegenerative disorders.

RESULTS AND DISCUSSION

Compounds VII-IX were tested for direct interactions with excitatory amino acid receptors in available *in vitro* excitatory amino acid receptor binding assays (9-11). As shown in Table I, none of the compounds possessed inhibitory activity, with the exception of the marginal effects of IX, at the site labeled by tritiated 2-amino-7-phosphonoheptanoic acid.

The major role of inactivation of synaptically released excitatory amino acid neurotransmitters is high-affinity, sodium-dependent reuptake (5). Compound IX inhibited such uptake by slightly more than 50% at a concentration of 5×10^{-4} M (Table II), which is at least 100-fold less potent than L-glutamate or D- or L-aspartate in this respect (12). Thus, IX is a weak inhibitor of high-affinity excitatory amino acid uptake in the rat brain. These data may suggest that IX itself is transported into synaptosomes by the excitatory amino acid carrier, albeit with low affinity. Compounds VII and VIII demonstrated no interaction with the carrier.

In the [^{3}H]acetylcholine release system, which reflects the neuronal excitation induced by excitatory amino acids acting at *N*-methyl-D-aspartate receptors (13), IX caused an increase in spontaneous [^{3}H]acetylcholine release and tended to enhance *N*-methyl-DL-aspartate-evoked [^{3}H]acetylcholine release (Table III). Compound VIII caused an increase in spontaneous [^{3}H]acetylcholine release (Table III).

Compounds VII-IX were tested *in vivo* for ability to protect against electroshock and subcutaneously administered pentylenetetrazol[1]-induced (scMet) seizures. Compounds VII and IX afforded protection against scMet of 25 and 50%, respectively, at doses of 600 mg/kg. No acute disruption of neurological function was detected in the rotorod test for VII-IX at doses of ≥600 mg/kg.

Of these results, the most striking is the coincidence of the activity of IX in enhancing spontaneous [^{3}H]acetylcholine release and as an anticonvulsant. Because the response to *N*-methyl-D-L-aspartate was not decreased during exposure to IX, it is unlikely that IX interacts directly with *N*-methyl-D-aspartate receptors. Since IX is only weakly active in inhibiting the specific

[1] Metrazol.

Table II—Effect of VII, VIII, and IX on the Uptake of L-[³H]Glutamate, D-[³H]Aspartate, and L-[³H]Aspartate into Synaptosomes Prepared from Rat Corpus Striatum or Cerebellum [a]

Compound	Specific Uptake, % Inhibition		
	L-[³H]Glutamate	D-[³H]Aspartate	L-[³H]Aspartate
	Corpus Striatum		
VII	0	0	0
VIII	0	7	0
IX	53	56	56
	Cerebellum		
VII	2	0	0
VIII	4	0	5
IX	59	57	59

[a] Synaptosomes were incubated in the presence of various ligands with or without the compounds (at a concentration of 5×10^{-4} M). Reference IC_{50} values for L-glutamate, D-aspartate, and L-aspartate are 1–5 μM (12).

Table III—[³H]Acetylcholine Release Studies [a]

		[³H]Acetylcholine Release, % of Control	
		Spontaneous	Evoked [b]
Control	9	100 ± 3.8	100 ± 9.8
VII, 0.3 mM	4	103 ± 5.0	70 ± 6.1
VII, 0.3 mM	4	116 ± 3.8 [d]	108 ± 4.9
IX, 0.3 mM	3	113 ± 1.3 [c]	87 ± 2.4
IX, 1.0 mM	3	166 ± 5.0 [c]	129 ± 39.3

[a] Test substances were added on interval six. Stimulation, a 2-min application of 50 μM N-methyl-D-aspartate, was applied at intervals three and nine. The effect of test substances on the parameters S_{pII}/S_{pI} and $S_{II} - S_{pII}/S_{I} - S_{pI}$ is expressed as percent of control (13). [b] N-Methyl-DL-aspartate-evoked release. [c] $p < 0.05$, Mann-Whitney two-tailed U test.

binding of the N-methyl-D-aspartate receptor antagonist tritiated 2-amino-7-phosphonoheptanoic acid, interaction of IX with N-methyl-D-aspartate receptors is unlikely to be the mechanism of action of this compound. Further biological investigations are required to determine the mechanism of action by which IX exerts its effects in these various biological systems.

DL-α-Benzhydrylamino-α-methyl-β-carbethoxyethanephosphinic Acid (VII)—This compound was prepared using a modification of previously reported procedures (2, 4). A mixture of 23.65 g (0.129 mol) of 1,1-diphenylmethylamine (14) and 16.78 g (0.129 mol) of ethyl acetoacetate in 80 mL of benzene was refluxed for 2 h and then distilled with azeotropic removal of water using a Dean-Stark trap. To the cooled residue was slowly added 8.96 g (0.129 mol) of 95% hypophosphorous acid, which was obtained by evaporation of a commercial 50% aqueous solution at 30–40°C (2 mm) and the mixture was heated at reflux for 2 h. The resultant solid was removed by filtration, washed with benzene, and dried in a vacuum oven at 50°C to yield 16.2 g (35%) of the product as white granules, mp (ethanol) 172–173°C; IR (KBr): 2600 (P—OH), 2350 (P—H), 1740 (C=O), 1600 (C=C), and 1200 (P=O) cm^{-1}; ¹H-NMR (D_2O-NaOD): δ 1.08 (d, 3, J = 16 Hz, CH_3), 1.19

CH_2O), 5.52 (s, 1, CH), 7.23–7.59 (m, 10, ArH), and 3.52 and 9.94 ppm (1:1 d, 1, J = 510 Hz, P—H).

Anal.—Calc. for $C_{19}H_{24}NO_4P$: C, 63.12; H, 6.69; N, 3.87. Found: C, 63.24; H, 6.72, N, 3.84.

DL-α-Benzhydrylamino-α-methyl-β-carboxyethanephosphinic Acid (VIII)—Ten grams of VII in 150 mL of 0.5 M NaOH was heated at 90°C for 4 h. The resultant solution was cooled and treated with concentrated HCl (pH 1). The precipitate thus formed was removed by filtration, washed with water until free of NaCl, and dried in a vacuum oven at 55°C to give 8.4 g (91% yield) of the product as a white powder, mp 190–191°C; IR (KBr): 2500–2700 (P—OH, C—OH), 2300 (P—H), 1700 (C=O), 1600 (C=C), and 1190 (P=O) cm^{-1}; ¹H-NMR (D_2O-NaOD): 1.14 (d, 3, J = 14 Hz, CH_3), 2.46 (d, 2, J = 10 Hz, CH_2), 5.56 (s, 1, CH), 7.25–7.63 (m, 10, ArH), and 3.59 and 10.01 ppm (1:1 d, 1, J = 510 Hz, P—H).

Anal.—Calc. for $C_{17}H_{20}NO_4P$: C, 61.26; H, 6.05; N, 4.20. Found: C, 61.17; H, 6.09; N, 4.20.

DL-α-Amino-α-methyl-β-carboxyethanephosphinic Acid (IX)—Using a modification of the method of Dingwall *et al.* (15), 2.6 g of VIII in 40 mL of 48% HBr was heated at 75°C for 4 h. The benzhydryl bromide was extracted with benzene, and the aqueous layer was treated with propylene oxide at pH 3-4. The resulting solution was concentrated under reduced pressure and the resulting solid was removed by filtration, washed with ethanol and ether, and dried in a vacuum oven at 55°C to yield 0.5 g (38%) of IX, mp 214–215°C; IR (KBr): 3240 (NH_3^+), 2700–2500 (P—OH, C—OH), 2380 (P—H), and 1685 (C=O) cm^{-1}; ¹H-NMR (D_2O): δ 1.47 (d, 3, J = 14 Hz, CH_3), 2.88, 2.76 (dd, 2, J = 9 Hz, CH_2), and 3.53 and 10.32 ppm (1:1 d, 1, J = 540 Hz, P—H).

Anal.—Calc for $C_4H_{10}NO_4P$: C, 28.93; H, 6.07; N, 8.43. Found: C, 28.85, H, 6.03, N, 8.34.

Biological Testing—*Animals*—For *in vitro* neurochemical studies, adult, male, albino rats[3] weighing 150–200 g were employed. *In vivo* testing for anticonvulsant activity and neurological toxicity of compounds was performed using mice[4].

Anticonvulsant Activity and Neurological Toxicity—Anticonvulsant activity of the compounds was evaluated against both maximal electroshock (MES) and pentylenetetrazol-induced seizures (scMet) by contractors of the Antiepileptic Drug Development Program administered by the National Institute for Neurological and Communicative Disorders and Stroke, Bethesda, Md, according to previously described procedures (16).

Neurochemical Assays—*Excitatory Amino Acid Uptake*—Crude synaptosomal fractions of rat cerebellum or corpus striatum were prepared as previously described (17) and resuspended in 0.32 M sucrose. Two hundred-microliter aliquots (100 μg of protein) of the suspension were added to borosilicate tubes containing 800 μL of Krebs HEPES buffer (pH 7.4) and 0.5 μM of D-[³H]aspartate, L-[³H]aspartate, or L-[³H]glutamate with or without the test substance. Following a 3 min incubation at 30°C, the reaction was terminated by rapid filtration over glass fiber filters[5] maintained under reduced pressure. The filters were washed with 1 × 5 mL of ice-cold buffer, dried, and the radioactivity was determined by liquid scintillation counting. Parallel incubations were performed at 2°C to determine nonspecific uptake.

Receptor Binding Assays—Specific binding of excitatory amino acid analogues to brain membranes was performed, with minor modifications, according to previously published procedures. Briefly, brain membranes were prepared according to the method of Enna and Snyder (18) and stored frozen at −80°C until use. For assay, the membranes were thawed and washed four times by centrifugation, and the final pellet was resuspended in sufficient buffer to yield a protein concentration of 0.15–0.2 mg/mL. Specific binding of [³H]kainic acid (2 nM) was assayed according to the procedure of London and Coyle (9). Specific binding of tritiated 2-amino-7-phosphonoheptanoic acid (50 nM) was assayed as described by Ferkany and Coyle (10). Specific binding of L-[³H]glutamate (100 nM) was assayed by the method of Slevin *et al.* (11), with the exception that the assay was conducted in both Tris-citrate and Tris-HCl buffers (pH 7.1, 37°C, 0.05 M). In all instances, nonspecific binding was defined as that obtained by incubating the membranes in the presence of the indicated ligand and 10^{-3} M L-glutamate. The assays were terminated by centrifugation, and the pellets were rinsed rapidly and superficially with ice-cold buffer and solubilized[6]. Radioactivity was determined by liquid scintillation counting.

[³H]Acetylcholine Release Evoked by N-Methyl-D-aspartate Receptor Stimulation—The release of [³H]acetylcholine from the corpus striatum of the brain, evolved by exposure to excitatory amino acid receptor agonists, was measured as previously described (13). Striatal slices were exposed by [³H]choline, which is selectively accumulated by cholinergic interneurons and synthesized into [³H]acetylcholine. The slices were placed in chambers and superfused with Krebs medium, the superfused phase was collected, and the radioactivity released from the tissue into the superfused phase was measured by liquid scintillation counting. Exposure to agonists of N-methyl-D-aspartate receptors caused an increase in release while addition of magnesium or N-methyl-D-aspartate receptor antagonists, such as 2-amino-5-phosphonopentanoic acid or 2-amino-7-phosphonoheptanoic acid, inhibited release caused by agonists. Agonists or antagonists of other excitatory amino acid receptors (*e.g.*, quisqualate or kainate) have little or no effect in general (13).

The action of test substances in this paradigm were assessed by determining if (*a*) they evoked [³H]acetylcholine release by themselves or (*b*) they inhibited the release of [³H]acetylcholine evoked by N-methyl-DL-aspartate (50 μM).

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REFERENCES

(1) H. D. F. Toy and E. H. Uhing, U.S. Patent No. 3,160,632 (1964).
(2) W. M. Linfield, E. Jungermann, and A. T. Guttmann, *J. Org. Chem.*, **26**, 4088 (1961).
(3) R. H. Khomutov and T. I. Osipova, *Izv. Akad. Nauk SSSR, Ser. Khim.*, **1978**, 1951; *Chem. Abstr.*, **89**, 197655j (1978).
(4) E. K. Bayliss, C. D. Campbell, J. G. Dingwall, and W. Pickles, in "Phosphorus Chemistry- Proceedings of the 1981 International Conference," ACS Symposium Series 171, L. D. Quin and G. Verkade, Eds., American Chemical Society, Washington, D.C., 1981, p. 183.
(5) E. Roberts and P. F. Hunter, *Proc. Soc. Exp. Biol. Med.*, **83**, 720 (1953).
(6) G. Litwack and J. E. Miller, *J. Biol. Chem.*, **246**, 3234 (1971).
(7) M. I. Lerman and I. V. Vereukina, *Biokhimiya*, **27**, 526 (1962).
(8) J. C. Watkins and R. H. Evans, *Annu. Rev. Pharm.*, **21**, 165 (1981).
(9) E. London and J. T. Coyle, *Mol. Pharmacol.*, **15**, 492 (1979).
(10) J. W. Ferkany and J. T. Coyle, *Life Sci.*, **33**, 1295 (1983).
(11) J. Slevin, J. Collins, K. Lindsley, and J. T. Coyle, *Brain Res.*, **249**, 353 (1982).
(12) M. E. Sandoval, P. Harch, and C. Cotman, *Brain Res.*, **142**, 285 (1978).
(13) J. Lehmann and B. Scatton, *Brain Res.*, **252**, 77 (1982).
(14) A. P. Terent'ev and N. I. Gusar, *J. Gen. Chem. USSR*, **35**, 124 (1965).
(15) J. G. Dingwall, W. K. Bayliss, and C. D. Campbell, *Ger. Offen.*, 2,722,162; Chem. Abstr., **88**, 105559j (1978).
(16) DHEW Publication No. (NIH)76-1093, Bethesda, Md., 1976.
(17) D. A. Redburn, D. Bloome, J. W. Ferkany, and S. J. Enna, *Brain Res.*, **152**, 511 (1978).
(18) S. J. Enna and S. Snyder, *Mol. Pharmacol.*, **13**, 442 (1977).

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Quantitation of Acetaminophen, Chlorpheniramine Maleate, Dextromethorphan Hydrobromide, and Phenylpropanolamine Hydrochloride in Combination Using High-Performance Liquid Chromatography

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Received July 5, 1983, from the **Department of Pharmaceutics, University of Houston, Houston, TX 77030* and *‡Ohm Laboratories, New Brunswick, NJ 08902.* Accepted for publication November 22, 1983.

Abstract □ A high-performance liquid chromatographic (HPLC) method has been developed for the quantitation of acetaminophen, chlorpheniramine maleate, dextromethorphan hydrobromide, and phenylpropanolamine hydrochloride in combination in pharmaceutical dosage forms using a single column and three different mobile phases. The method developed is sensitive for the content uniformity test for tablets. No preliminary extraction procedure is required for liquid preparation and a very simple extraction procedure is required for tablets. The method is accurate and precise with *RSD* (based on five injections) of 1.2, 2.4, 1.9, and 1.6% for acetaminophen, chlorpheniramine, dextromethorphan, and phenylpropanolamine, respectively.

Keyphrases □ Acetaminophen—HPLC, content uniformity □ Chlorpheniramine maleate—HPLC, content uniformity □ Dextromethorphan hydrobromide—HPLC, content uniformity □ Phenylpropanolamine hydrochloride—HPLC, content uniformity

One popular commercial product[1] contains an analgesic (acetaminophen), an antihistamine (chlorpheniramine maleate), an antitussive (dextromethorphan hydrobromide), and a decongestant (phenylpropanolamine hydrochloride). The product is extensively used for the relief of symptoms of coughs and colds. The dosage forms also contain excipients, some of which may interfere with the analysis of the active ingredients. The problem of analysis is further complicated by the presence of 325 mg of acetaminophen *versus* only 1 mg of chlorpheniramine maleate per tablet (or per 15 mL of the liquid). The quantities of dextromethorphan hydrobromide and phenylpropanolamine hydrochloride present are 10 and 12.5 mg per tablet (or 15 mL of the liquid), respectively. Acetaminophen has high absorbance in the useful UV range, but that of phenylpropanolamine hydrochloride is very poor. For example, at 256 nm (the wavelength of maximum absorption of phenylpropanolamine hydrochloride) a 1350-μg/mL aqueous solution of phenylpropanolamine hydrochloride has an absorption similar to a 20-μg/mL solution of acetaminophen. No single method is available to determine the active ingredients quantitatively in this combination.

High-performance liquid chromatographic (HPLC) methods for the quantitation of acetaminophen (1-4), chlorpheniramine maleate (5, 6), dextromethorphan hydrobromide (3, 7, 8), and phenylpropanolamine hydrochloride (6, 9-11) have been reported. None of the reported methods is applicable when ingredients are present in this combination.

This paper reports the quantitation of acetaminophen (I), chlorpheniramine maleate (II), dextromethorphan hydrobromide (III), and phenylpropanolamine hydrochloride (IV) in combination, using HPLC. The method requires three different mobile phases for complete analysis.

EXPERIMENTAL SECTION

Materials—All chemicals and reagents were USP, NF, or ACS quality and used without further purification. The sodium salt of 1-heptanesulfonic acid[2] was used as received.

A liquid chromatograph[3] attached to a multiple-wavelength detector[4] and a recorder[5] was used. The column[6] (30 cm × 4 mm i.d.) was purchased and

[1] Comtrex; Bristol-Myers Products, New York, N.Y.

[2] Eastman Kodak Co., Rochester, N.Y.
[3] Waters ALC202 equipped with a U6K Universal injector; Waters Associates, Milford, Mass.
[4] Spectroflow Monitor 770; Schoeffel Instruments, Ramsey, N.J.
[5] Omniscribe 5213-12; Houston Instruments, Austin, Tex.
[6] μ-Bondapak phenyl; Waters Associates, Milford, Mass.

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