sodium solution. The data had an accuracy of 100.2% with a standard deviation of 0.47% for the 12 assays (Table III). The experiment was conducted over 18 days. A precision study was conducted on a composite sample obtained by grinding 40 commercial tablets. The mean from the 16 assays was 99.8% over 2 months with a standard deviation of 1.07%. The data demonstrated that the method gives precise and accurate data.

Common tablet and capsule excipients such as starch, lactose, cellulose, and stearate lubricants were taken through the assay and did not interfere.

The assay was used to follow the stability of aged tablets and capsules containing up to an equivalent of 400 mg of tolmetin (490.1 mg of tolmetin sodium). Tablets were assayed for up to 5 years at room temperature and at various temperatures up to 80° for 3 months. The stability data demonstrated that the tablets and capsules were very stable. TLC was conducted on the aged samples using the two solvent mixtures. The data showed that tolmetin sodium remained intact in the dosage forms even after aging for 5 years at room temperature and accelerated aging at temperatures up to 80°. Less than 2% degradation was noted under the accelerated conditions.

Two solvent mixtures of different polarity were chosen for TLC to minimize the possibility of unresolved degradation products that could not be predicted. The compounds chosen to demonstrate the specificity of the TLC methods were, with one exception, previously postulated to be theoretical impurities in tolmetin sodium (5). The data (Table I) demonstrated that the solvent systems are suitable for separating tolmetin sodium from possible degradation products as well as theoretical impurities. Further confirmation was made on an aged tablet sample that was chromatographed and isolated from the TLC plate. The material was found to be identical to tolmetin sodium by UV, IR, and mass spectrometry.

The assay was used additionally to follow the stability of tolmetin sodium solutions buffered from pH 4.5 to 10.0 at 80°[6]. The data supported the reflux experiments, which showed that tolmetin sodium is relatively stable at neutral and alkaline pH values but unstable at acidic pH.

The data demonstrated that the method is capable of monitoring the stability of tolmetin sodium in solid dosage forms. The method is precise and accurate and utilizes common laboratory equipment. The hydrolysis experiments illustrated that tolmetin is stable under neutral and alkaline conditions but not under acidic conditions. The TLC methods can monitor qualitatively tolmetin sodium stability in aged dosage forms.

REFERENCES

(1) J. R. Carson, D. N. McKinstry, and S. Wong, *J. Med. Chem.*, **14**, 646 (1971).

(2) M. L. Selley, J. Thomas, and E. J. Triggs, *J. Chromatogr.*, **94**, 143 (1974).

(3) W. A. Cressman, B. Lopez, and D. Sumner, *J. Pharm. Sci.*, **64**, 1965 (1975).

(4) K. T. Ng, *J. Chromatogr.*, **166**, 527 (1979).

(5) R. K. Gilpin and C. A. Janicki, *ibid.*, **147**, 501 (1978).

(6) J. W. Ayres, E. Sakmar, M. R. Hallmark, and J. G. Wagner, *Res. Commun. Chem. Pathol. Pharmacol.*, **16**, 475 (1977).

(7) G. F. Smith, in "Advances in Heterocyclic Chemistry," vol. 2, A. R. Katritzky, Ed., Academic, New York, N.Y., 1963, pp. 287–309.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Mrs. R. Rocco, Dr. J. S. Ranweiler, Dr. C. Y. Ko, Mrs. C. M. Moral, Mr. L. T. Olszewski, and Dr. R. H. Erlich.

[6] W. A. Cressman, McNeil Laboratories, Fort Washington, PA 19034, personal communication.

Preparation and Biological Actions of *N*-2,2,2-Trifluoroethyl-2-(3,4-dihydroxyphenyl)ethylamine

JOHN P. O'DONNELL *x, A. J. AZZARO ‡, and P. R. URQUILLA ‡

Received April 9, 1979, from the **School of Pharmacy and the ‡Departments of Neurology and Pharmacology, School of Medicine, West Virginia University Medical Center, Morgantown, WV 26506.* Accepted for publication August 22, 1979.

Abstract □ *N*-2,2,2-Trifluoroethyl-2-(3,4-dihydroxyphenyl)ethylamine was synthesized and compared to *N*-ethyl-2-(3,4-dihydroxyphenyl)-ethylamine and dopamine for activity on adenylate cyclase in the rat striatum. Both dopamine and *N*-ethyl-2-(3,4-dihydroxyphenyl)ethylamine stimulated adenylate cyclase activity in a dose-dependent fashion. The *N*-trifluoroethyldopamine analog at 1×10^{-4} *M* induced a weak effect. The compounds were evaluated further by studying their relaxant effects in isolated rabbit renal and ear arteries. Both the *N*-ethyl- and *N*-trifluoroethyldopamine analogs produced a relaxant effect but demonstrated no selectivity for dopamine receptors.

Keyphrases □ Adenylate cyclase activity—dopamine, *N*- and *N,N*-substituted dopamine analogs, peripheral and central dopamine receptors, isolated striata and blood vessels □ Dopamine—antiparkinson drug, adenylate cyclase activity, dopamine analogs □ *N*-Ethyl-2-(3,4-dihydroxyphenyl)ethylamine—dopamine analog, adenylate cyclase activity □ *N*-2,2,2-Trifluoroethyl-2-(3,4-dihydroxyphenyl)ethylamine—dopamine analog, adenylate cyclase activity

The treatment of parkinsonism with levodopa has beneficial effects; however, in some patients with severely impaired dopaminergic neurons, the use of levodopa may be ineffective because the tissue cannot perform the necessary enzymatic decarboxylation to form dopamine (1, 2). An effort to generate new dopaminergic drugs for parkinsonism led to the synthesis of *N*- and *N,N*-substituted dopamine analogs (3–9). The binding of simple dopaminergic ligands to the receptor appears to be independent of substitution on the nitrogen (5). Unlike dopamine, the *N*- and *N,N*-substituted dopamine analogs cross the blood–brain barrier (3, 4). In addition, these agents are resistant to deamination by monoamine oxidase since they are secondary and tertiary amines. Moreover, these *N*- and *N,N*-substituted dopamine analogs act in the central nervous system as direct acting dopamine receptor agonists and, therefore, are independent of prejunctional dopaminergic mechanisms (4, 8).

To improve absorption and distribution of the *N*-alkyl-substituted dopamine analogs, *N*-2,2,2-trifluoroethyl-2-(3,4-dihydroxyphenyl)ethylamine (I) was prepared. The 2,2,2-trifluoroethyl group is well suited to replace the ethyl group because of similar steric properties and because the fluorine atom increases both lipid solubility and membrane penetration.

The agonistic properties of I, *N*-ethyl-2-(3,4-dihy-

0022-3549/80/0200-0149$01.00/0

© 1980, American Pharmaceutical Association

droxyphenyl)ethylamine, and dopamine at central and peripheral dopamine receptors were analyzed pharmacologically. These studies measured the effects of each agent on the activity of dopamine-sensitive adenylate cyclase in rat neostriatum and evaluated their relaxant effects on isolated rabbit renal and ear arteries.

EXPERIMENTAL

Chemistry[1]—*N-Trifluoroacetyl -2- (3,4-dimethoxyphenyl)ethylamine (III)*—Trifluoroacetic anhydride (20 ml, 0.129 mole) was added very slowly, with stirring, to 20 g (0.115 mole) of 2-(3,4-dimethoxyphenyl)ethylamine. The resulting glassy solid was allowed to set overnight. Trituration of the solid mass with water gave a white suspension, which was filtered and washed with water. The crude amide was recrystallized from benzene–hexane to afford 27.4 g (86%), mp 84°; IR (neat): 1715 cm^{-1} (C=O); NMR ($CDCl_3$): δ 2.60–3.70 (m, 4H, CH_2), 3.84 (s, 6H, OCH_3), and 6.60–6.80 (m, 3H, 3 aromatic H).

Anal.—Calc. for $C_{12}H_{14}F_3NO_3$: C, 51.99; H, 5.08; N, 5.05. Found: C, 51.89; H, 5.11; N, 5.04.

N-(2,2,2-Trifluoroethyl) -2- (3,4-dimethoxyphenyl)ethylamine (IV)—To an ice-chilled mixture of tetrahydrofuran (100 ml) and 27.4 g of III (0.999 mole) was added slowly, with stirring, 233 ml (0.23 mole) of 1 *M* diborane in tetrahydrofuran[2] under nitrogen. The resulting mixture was stirred at 0° for 1 hr and then refluxed for 12 hr. The reaction mixture again was cooled to 0°, 50 ml of water was added, and the volatiles were removed from the reaction mixture. The residue was taken up in water and extracted three times with chloroform. The combined chloroform extracts were dried over magnesium sulfate and filtered, and the filtrate was treated with anhydrous hydrochloric acid. The volatiles were again removed, and the residue was recrystallized from ether to afford 27.8 g (90%) of crystals, mp 208°; NMR (free base) ($CDCl_3$): δ 2.50–3.45 (m, 6H, CH_2), 3.85 (s, 6H, OCH_3), and 6.68 (s, 3H, aromatic H).

Anal.—Calc. for $C_{12}H_{17}ClF_3NO_2$: C, 48.25; H, 5.39; N, 4.69. Found: C, 48.27; H, 5.48; N, 4.63.

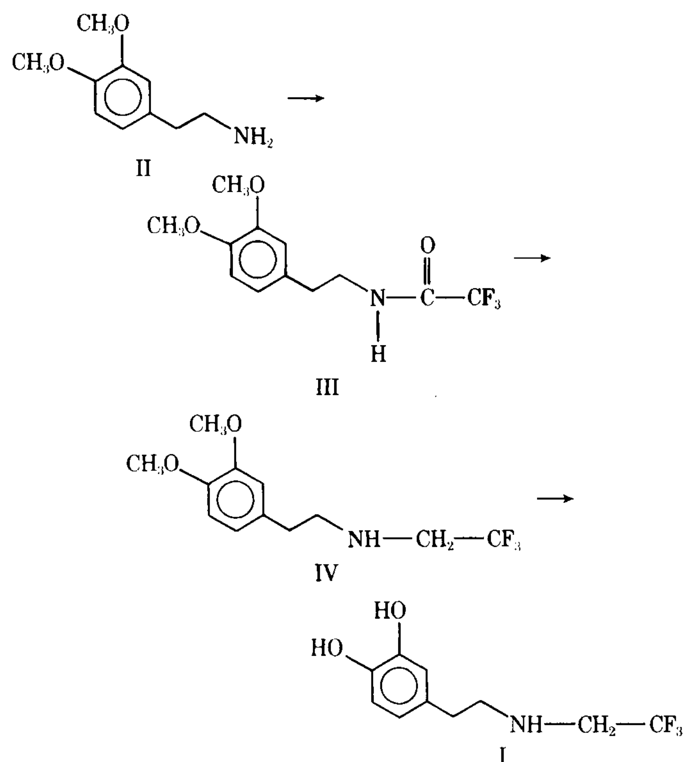
N-(2,2,2-Trifluoroethyl)-2-(3,4-dihydroxyphenyl)ethylamine (I)—Compound IV (3.0 g, 0.01 mole) was dissolved in 48% HBr (20 ml). The system was flushed with nitrogen for 0.25 hr and then heated to 150° for 2 hr. Excess acid was removed under reduced pressure to give a residue. Two recrystallizations from isopropyl alcohol–ether afforded 2.12 g (70%) of product, mp 179–180°. The pKa of I was determined by titration (10); IR (KBr): 3250 cm^{-1} (broad, OH); NMR (D_2O): δ 2.60–4.20 (m, 6H, CH_2) and 6.62–6.80 (m, aromatic H).

Anal.—Calc. for $C_{10}H_{13}BrF_3NO_2$: C, 37.99; H, 4.11; N, 4.28. Found: C, 37.95; H, 4.22; N, 4.41.

Adenylate Cyclase Activity—Adult male Sprague-Dawley rats[3], 200–250 g, were decapitated, and their brains were removed rapidly and placed in ice-cold saline. Each corpus striatum was dissected in the cold, as described by Glowinski and Iverson (11), and placed in ice-cold pH 7.4 Krebs–Henseleit bicarbonate buffer. A cell-free striatal homogenate was prepared in 25 volumes (w/v) of 2 m*M* tris(hydroxymethyl)aminomethane maleate buffer (pH 7.4) containing 2 m*M* edetic acid (ethylenediaminetetraacetic acid) using a polytef-glass tissue homogenizer[4]. Aliquots (50 μl) of this cell-free homogenate were assayed for adenylate cyclase activity using the procedure of Kebabian *et al.* (12). 3-Isobutyl-1-methylxanthine[5] (1 m*M*) was used to inhibit phosphodiesterase activity. A 25-μl aliquot of each sample was assayed for cyclic AMP content by the method of Gilman (13) as modified by Tovey *et al.* (14).

A wide concentration range (*i.e.*, 10^{-7}–10^{-3} *M*) of dopamine and of each experimental agent was tested for striatal adenylate cyclase activity. Haloperidol[6] was dissolved in propylene glycol–3 *N* HCl. The haloperidol solution was adjusted to pH 7.4 and brought to 50 ml with normal saline. In experiments where haloperidol was employed, an equal volume of the vehicle used to dissolve haloperidol was added to samples without haloperidol.

[1] Melting points were determined in open glass capillaries using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. IR absorption spectra were recorded on a Beckman model 18A spectrophotometer on liquid films or as potassium bromide pellets. NMR spectra were recorded on a Varian T-60 spectrometer with tetramethylsilane as the internal standard. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.
[2] Aldrich Chemical Co., Milwaukee, Wis.
[3] ARS-Sprague-Dawley, Madison, Wis.
[4] Kontes, Vineland, N.J.
[5] Sigma Chemical Co., St. Louis, Mo.
[6] McNeil Laboratories, Fort Washington, Pa.



Scheme I

Statistical analysis to determine differences between sample means was performed using the Student *t* test or the Student *t* test for paired sample comparisons (15).

Isolated Rabbit Renal and Ear Arteries—Albino rabbits, 3–4 kg, were killed by air embolism, and the left renal artery and the central ear arteries were removed carefully. Two cylindrical segments (5 mm × 0.5 mm o.d.) were taken from each artery. The cylinders were set up for isometric recording of tension changes in the manner described for cerebral vessels (16, 17). The recording system included a polygraph[7] and force-displacement transducer[8]. A resting tension of 0.5 g was applied to the tissue and readjusted every 15 min during the initial 1-hr equilibration. The organ bath (pH 7.3–7.4) contained 10 ml of Krebs–Henseleit buffer continuously bubbled with 95% O_2–5% CO_2 and maintained at 37°. Edetic acid (30 m*M*) was added to minimize oxidation of dopamine and its analogs. Solutions of dopamine and its analogs were prepared in saline containing 0.01% (w/v) ascorbic acid.

To avoid possible concomitant activation of α- and β-adrenergic receptors by dopamine and the test compounds, the experiments were carried out in tissues pretreated with 29 m*M* phenoxybenzamine for 0.5 hr and in the presence of propranolol (3.4×10^{-7} *M*). Since phenoxybenzamine also has been reported (18) to inhibit neuronal uptake of catecholamines, its use in this study might have the additional advantage of preventing dopamine diversion from the receptor region toward the adrenergic nerve endings.

Active development of tension to 0.99 ± 0.089 g (mean ± *SEM*) was induced with 28–38 m*M* KCl. At the plateau level of the response to potassium chloride, dopamine and its analogs were added in increasing concentrations (*i.e.*, 10^{-7}–10^{-4} *M*) to the tissue segment, while the other segment served as a control to check for a decline of the plateau tension that might have occurred with time. Only one analog was tested in each segment. The plateau tension was stable, and no correction of the changes observed with dopamine was necessary. Dose–response curves for sodium nitrite also were determined in both arteries to assess vascular relaxation independently of the dopaminergic mechanism.

DISCUSSION

N-(2,2,2-Trifluoroethyl)-2-(3,4-dihydroxyphenyl)ethylamine (I) was prepared according to Scheme I. 2-(3,4-Dimethoxyphenyl)ethylamine (II) was reacted with trifluoroacetic anhydride to form the amide III, which was reduced with diborane to give the amide IV. The methyl ethers

[7] Model 7, Grass, Quincy, Mass.
[8] Model FT03, Grass, Quincy, Mass.

Table I—Relaxant Effects of Dopamine, Dopamine Analogs, and Sodium Nitrite in Rabbit Renal and Ear Arteries

Compound	$ED_{40} \times 10^{-6}$ *M* Renal Artery	Ear Artery	Relaxation Ratio, Ear Artery/ Renal Artery	Number of Experiments
Dopamine hydrochloride	14.3 ± 8.4[a]	395.8 ± 7.8	27.6	5
6,7-Dihydroxy-2-aminotetrahydronaphthalene hydrobromide	10.4 ± 5.8	288.5 ± 4.8	27.7	6
Compound V	25.3 ± 6.2	44.3 ± 6.4	1.7	5
Compound I	76.3 ± 6.1	76.4 ± 5.8	1.0	5
Sodium nitrite	45.5 ± 2.1	89.1 ± 1.6	1.9	4

[a] Values are means ± *SEM*.

of IV were cleaved with hydrobromic acid. *N*-Ethyl-2-(3,4-dihydroxyphenyl)ethylamine (V) was prepared in an analogous procedure and also was evaluated for biological activity as a structural comparison to I.

One problem in characterizing dopaminergic activity in isolated blood vessels is the lack of a specific dopamine antagonist. Recent work (19) demonstrated that typical dopamine antagonists cannot be used to characterize peripheral dopamine activity in isolated vessels. In contrast, the induced vasodilator activity in dogs may be attenuated selectively by butyrophenone and phenothiazine neuroleptic agents (20). An alternative to the lack of vascular specificity and a specific dopamine antagonist is the use of two vascular preparations with different sensitivities to dopamine (19). The ED_{40} values and the ratios of rabbit ear arteries to renal arteries for dopamine and analogs are presented in Table I. The ED_{40} was selected because certain dopamine analogs at doses above the ED_{40} demonstrated contractile effects. A comparison of the relaxation ratio in the very responsive renal artery *versus* the less responsive ear artery for dopamine and the specific renal dopamine agonist, 6,7-dihydroxy-2-aminotetrahydronaphthalene (21), gave a similar ratio for dopamine receptor agonist activity. A nonspecific relaxant, sodium nitrite, has a similar potency on both vessels. Since the effect of I and V was comparable to that of sodium nitrite, these compounds demonstrated no specific activity.

The activity of dopamine-sensitive adenylate cyclase in rat neostriatum is a useful biochemical measure of the effectiveness of agents as agonists or antagonists at central dopamine receptors (3, 22–24). Agents that act as dopamine receptor agonists stimulate striatal adenylate cyclase activity (12). In addition, dopamine receptor antagonists attenuate the stimulating effect of dopamine or other dopamine receptor agonists on striatal adenylate cyclase activity.

Dopamine, I, and V were tested for their ability to enhance adenylate cyclase activity in rat corpus striatum (Table II). Dopamine and V stimulated the adenylate cyclase activity in a dose-dependent fashion. In this regard, both agents were similar in potency. Compound I induced only a weak stimulating effect on adenylate cyclase at 1×10^{-4} *M*.

To determine the specificity of dopamine and V on adenylate cyclase activity, both agents were tested in the presence of haloperidol, a dopamine receptor antagonist (12, 25, 26). Haloperidol (10^{-4} *M*) did not alter the control level of adenylate cyclase activity (Fig. 1). However, haloperidol completely attenuated the stimulating action of dopamine and *N*-ethyldopamine on striatal adenylate cyclase. Although not shown here, the weak stimulation of adenylate cyclase activity by I (10^{-4} *M*) was attenuated completely by haloperidol (10^{-4} *M*).

Table II—Adenylate Cyclase Activity in Striatal Homogenates [a]

Agent	Concentration, μ*M*	Cyclic AMP[b], pmoles above control	Percent of Control
Dopamine	10	3.6 ± 2.4	105
	100	28.8 ± 3.9[c]	138
	1000	33.8 ± 6.3[c]	144
Compound V	10	13.0 ± 5.4[c]	117
	100	23.3 ± 4.5[c]	131
	1000	27.6 ± 4.3[c]	137
Compound I	10	7.4 ± 4.8	108
	100	11.7 ± 3.0[c]	113
	1000	−0.8 ± 3.4	99

[a] Cell-free homogenates of rat corpus striatum were assayed for adenylate cyclase activity by measuring the conversion of ATP (0.5 m*M*) to cyclic AMP. [b] Mean ± *SEM* of six to eight experiments. Controls for each agent were as follows: dopamine, 76.7 ± 5.2 pmoles/mg/2.5 min; V, 75.3 ± 8.1 pmoles/mg/2.5 min; and I, 89.7 ± 5.1 pmoles/mg/2.5 min. [c] $p < 0.05$ as compared to control.

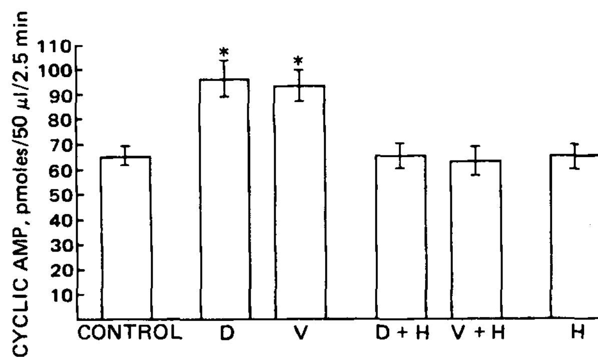


Figure 1—*Effect of haloperidol (H) (10^{-4} M) on the dopamine (D) and Compound V stimulation of adenylate cyclase in striatal homogenates. Cell-free homogenates of rat corpus striata were assayed for adenylate cyclase activity. Each value for cyclic AMP represents the mean ± SEM of five experiments. A concentration of 1×10^{-4} M dopamine or V was used to stimulate adenylate cyclase activity (* = $p < 0.05$ with respect to control).*

These results show that even if I would demonstrate improved absorption and distribution characteristics, it cannot stimulate adenylate cyclase. However, V stimulated adenylate cyclase but to a lesser degree than dopamine. These findings were unexpected due to the close structural similarity of I to V. An explanation for the lack of dopaminergic activity may be attempted by comparing the ionization ratios of the amine groups. The amine groups of dopamine (10) and V have pKa values of 9.74 and 10.61, respectively. Compound I has a pKa value of 5.10. Thus, altering the alkyl substitution by replacement of the protons with the electronegative fluorine atoms changes the ratio at physiological pH from the zwitterion to predominantly the uncharged species, in contrast to dopamine and V at physiological pH, at which the predominant species is the phenolic ammonium salt.

It was proposed recently (10) that the active species at dopamine receptors may be the neutral uncharged form of dopamine. The limited data of this study suggest that the activity of dopaminergic agonists may depend on the ability of the dopamine ligand to undergo readily an equilibrium transformation on the receptor between the zwitterion and the uncharged species.

REFERENCES

(1) A. N. Lieberman, A. L. Goodgold, and M. Goldstein, *Neurology*, **22**, 1205 (1972).

(2) O. Hornykiewicz, *Fed.Proc.*, **32**, 183 (1973).

(3) J. Ginos, G. C. Cotzias, E. Tolosa, L. C. Tang, and A. LoMonte, *J. Med. Chem.*, **18**, 1194 (1975).

(4) J. G. Cannon, F. L. Hsu, J. P. Long, J. R. Flynn, B. Costall, and R. J. Naylor, *ibid.*, **21**, 248 (1978).

(5) R. Miller, A. Horn, L. Iverson, and R. Pinder, *Nature*, **250**, 238 (1974).

(6) L. I. Goldberg, P. F. Sonneville, and J. L. McNay, *J. Pharmacol. Exp. Ther.*, **163**, 188 (1968).

(7) J. G. Cannon, J. P. O'Donnell, T. Lee, C. R. Hoppin, J. P. Long, M. I. Ilhan, B. Costall, and R. J. Naylor, *J. Med. Chem.*, **18**, 1212 (1975).

(8) J. Z. Ginos and F. C. Brown, *ibid.*, **21**, 155 (1978).

(9) J. Z. Ginos, G. C. Cotzias, and D. Doroski, *ibid.*, **21**, 160 (1978).
(10) J. L. Armstrong and R. B. Barlow, *Br. J. Pharmacol.*, **57**, 501 (1976).
(11) J. Glowinski and L. L. Iverson, *J. Neurochem.*, **13**, 655 (1966).
(12) J. W. Kebabian, G. L. Petzold, and P. Greengard, *Proc. Natl. Acad. Sci. USA*, **69**, 2145 (1972).
(13) A. Gilman, *ibid.*, **67**, 305 (1970).
(14) K. C. Tovey, K. G. Oldham, and J. A. Whelan, *Clin. Chim. Acta*, **56**, 221 (1974).
(15) R. R. Sokal and F. J. Rohlf, "Introduction to Biostatistics," W. H. Freeman, San Francisco, Calif., 1973.
(16) K. C. Nielsen and C. Owman, *Brain Res.*, **27**, 33 (1971).
(17) P. R. Urquilla, E. J. Marco, and S. Luch, *Blood Vessels*, **12**, 53 (1975).
(18) R. F. Furchgott, in "Handbuch der Experimentelles Pharmackologie," vol. 33, H. Blaschko and E. Muschall, Eds., Springer, Berlin, Germany, 1972, p. 283.
(19) L. I. Goldberg and N. Toda, *Circ. Res.* **36**, I-97 (1975).
(20) L. I. Goldberg and B. K. Yeh, *Eur. J. Pharmacol.*, **15**, 36 (1971).
(21) L. I. Goldberg, P. H. Volkman, and J. D. Kohli, *Ann. Rev. Pharmacol. Toxicol.*, **18**, 57 (1978).
(22) R. J. Miller and L. Iverson, *Naunyn-Schmiedebergs Arch. Pharmacol.*, **282**, 213 (1974).
(23) R. J. Miller, A. S. Horn, and L. Iverson, *Mol. Pharmacol.*, **10**, 759 (1974).
(24) R. J. Baldessarini, R. H. Walton, and R. J. Borgman, *Neuropharmacology*, **14**, 725 (1975).
(25) A. Carlsson and M. Lindqvist, *Acta Pharmacol. Toxicol.*, **20**, 140 (1963).
(26) N. E. Anden, H. Corrodi, K. Fuxe, and U. Ungerstedt, *Eur. J. Clin. Pharmacol.*, **15**, 193 (1971).

High-Performance Liquid Chromatographic Analysis of Codeine in Syrups Using Ion-Pair Formation

E. J. KUBIAK and J. W. MUNSON ˣ

Received July 25, 1979, from *Quality Control, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication September 13, 1979.

Abstract □ The chromatographic behavior of morphine, codeine, and ethylmorphine was examined using reversed-phase high-performance liquid chromatography and ion-pair formation. Alkyl sulfonates and sulfates significantly increased the retention times for these compounds. The carbon chain length of the pairing ions was linearly related to the log of the capacity factors of these amine drugs. A mechanism for the increased retention based on ion-pair formation in the mobile phase is proposed. The use of dioctyl sodium sulfosuccinate as a pairing ion for codeine is described, and a method utilizing this pairing ion was developed for the quantitation of codeine in syrups. This method was applied successfully to various syrups containing codeine or codeine phosphate.

Keyphrases □ Codeine—analysis, high-performance liquid chromatography, ion-pair formation, effect of carbon chain length of pairing ions, syrups □ Ethylmorphine—analysis, high-performance liquid chromatography, ion-pair formation, effect of carbon chain length of pairing ions □ Morphine—analysis, high-performance liquid chromatography, ion-pair formation, effect of carbon chain length of pairing ions □ High-performance liquid chromatography, ion-pair formation—analysis of codeine, ethylmorphine, and morphine, effect of carbon chain length of pairing ion

The use of ion-pair formation to enhance retention time is becoming popular in reversed-phase high-performance liquid chromatography (HPLC). The counterions used to form ion-pairs generally are detergents such as alkyl sulfates and sulfonates or tetraalkylammonium salts. Anionic surfactants (sulfates and sulfonates) generally are used to form ion-pairs with cationic eluates, and cationic tetraalkylammonium salts are used with anionic eluates. By formation of the ion-pair, the apparent polarity of the eluate is decreased, leading to increased retention times in reversed-phase HPLC.

Since many drugs contain either acidic or basic functional groups, pH conditions can be chosen such that numerous drugs will form ion-pairs with these surfactants. The surfactant molecule often is referred to as the pairing ion, which gives rise to the term ion-pair HPLC. While ion-pair formation can be used in normal-phase HPLC, it is used more widely in reversed-phase HPLC. This technique also has been called soap chromatography (1, 2) because of the surfactants used.

BACKGROUND

The mechanism of retention enhancement has been attributed to two phenomena. The original concept closely follows classical ion-pair extraction techniques in which two oppositely charged molecules form an ion-pair in an aqueous solution and then partition as a complex into a nonpolar organic solvent. In the chromatographic analog of this concept, the analyte forms a complex with a pairing ion added to the mobile phase. The complex then partitions into the stationary phase (nonpolar), thus accounting for the increased analyte retention. An alternative mechanism suggests that the pairing ion first binds to the stationary phase to form an ion-exchanging surface due to the adsorbed charged pairing ions (3). Complex formation at the surface of the stationary phase thus is responsible for the increased analyte retention. Recently, application of solvophobic theory to a reversed-phase ion-pair chromatographic study led to the conclusion that the retention of the analyte involves ion-pair formation in the mobile phase and subsequent partitioning of the neutral complex to the stationary phase (4).

Codeine analysis by HPLC has been of interest. An early study (5) utilized columns of pellicular silica gel coated with polyethylene glycol 300 with an ethanol–heptane (1:10) mobile phase saturated with the stationary phase. In this system, codeine was well resolved from morphine, thebaine, and papaverine. However, quantitative codeine analysis at levels near 3 μg gave a relative error of ~9%. In a study of the liquid chromatographic behavior of alkaloids, the retention times of codeine and morphine on a microparticulate silica column were measured (6). Codeine eluted slightly before morphine with mobile phases of chloroform–methanol (three volume ratios) and ether–methanol (three volume ratios).

The quantification of morphine, codeine, and thebaine was accomplished by isocratic reversed-phase HPLC (7). With a microparticulate (10-μm) octadecylsilane column and a mobile phase of 0.1 *N* monobasic sodium phosphate in 25% acetonitrile–water, codeine in gum opium was measured with a relative standard deviation of 1.3%. Codeine also was separated from other opium alkaloids using a pellicular silica column and

0022-3549/ 80/ 0200-0152$01.00/ 0
© 1980, American Pharmaceutical Association