Table IV—Anticonvulsant Activity of Thiazole, Thiazine, and Thiadiazine Derivatives of 1-Phthalazine

Compound	Anticonvulsant Activity, % protection[a]	Pentylenetetrazol, % mortality[b]
IId[c]	70	20
IIf	20	50
IIIa	10	90
IIIf	40	50
IVa	30	60
IVb[c]	0	100
IVd	20	70
IVe	30	50
IVf[c]	10	90
Va	50	30

[a] Anticonvulsant activity was determined at doses of 1.0 mmole/kg, equivalent to 200 mg of meprobamate/kg, as described under *Experimental*. Meprobamate (200 mg/kg) and phenobarbital sodium (80 mg/kg) were used as standard anticonvulsants; they exerted 90–100 and 100% protection, respectively, against pentylenetetrazol-induced convulsions in mice under similar conditions. [b] Represents mortality over 24 hr in each group of animals administered pentylenetetrazol at 90 mg/kg. [c] Compounds IId, IVb, and IVf were used in doses equivalent to 50 mg of meprobamate/kg. Their corresponding LD_{50} ($\pm SD$) values were 135 ± 19, 202 ± 7, and 137 ± 12 mg/kg, respectively.

clonic spasm that persisted for a minimum of 5 sec after administration of pentylenetetrazol was considered a threshold convulsion. Transient intermittent jerks and tremors were not counted. Animals devoid of threshold convulsions over 60 min were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of the test compounds was represented as percent protection. The mice then were observed for 24 hr, and the mortality rate in each group was recorded (Table IV). The LD_{50} of some compounds was determined by the graphical method of Miller and Tainter (16).

The data presented in Table IV show that the compounds possess weak to moderate anticonvulsant activity and that the doses required for demonstration of anticonvulsant activity are very close to their corresponding toxic doses.

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Quantitative Determination of Pilocarpine, Isopilocarpine, Pilocarpic Acid, and Isopilocarpic Acid in Clinical Ophthalmic Pilocarpine Formulations by Reversed-Phase Liquid Chromatography

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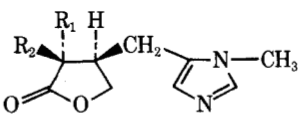
Abstract □ A rapid and convenient reversed-phase high-performance liquid chromatographic procedure for the quantitative determination of pilocarpine and its degradation products was used to analyze 10 clinical ophthalmic pilocarpine formulations.

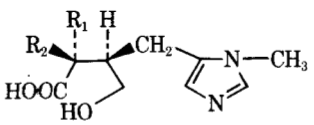
Keyphrases □ Pilocarpine—reversed-phase high-performance liquid chromatographic analysis with isopilocarpine, pilocarpic acid, and isopilocarpic acid in ophthalmic pilocarpine formulations □ High-performance liquid chromatography, reversed phase—analysis, pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid in ophthalmic pilocarpine formulations

(+)-Pilocarpine {(2*S*,3*R*)-2-ethyl-3-[(1-methyl-5-imidazolyl)methyl]-4-butanolide} is an imidazole alkaloid for which a stereoselective synthesis was reported recently (1). It is used frequently in ophthalmology to relieve intraocular pressure, specifically glaucoma. For this purpose, buffered, stabilized, isotonic aqueous solutions of pilocarpine hydrochloride or nitrate usually are used (2).

In an aqueous medium, pilocarpine (I) can hydrolyze to



I: R_1 = H, R_2 = C_2H_5
II: R_1 = C_2H_5, R_2 = H



III: R_1 = H, R_2 = C_2H_5
IV: R_1 = C_2H_5, R_2 = H

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Table I—Composition of Commercially Available Ophthalmic Pilocarpine Formulations (March 1979)

Sample	Expiration Date	Label Claim of Pilocarpine		Pilocarpine Found, %	Relative Composition, %			pH Found	Additives Stated on Label
		Form	Percent		Pilocarpine	Isopilocarpine	Pilocarpic Acid		
1	December 1973	HCl	4	4.3	95.0	1.5	3.5	3.5	—[a]
2	November 1976	HCl	1	1.0	92.7	2.1	5.2	3.7	—[a]
3	November 1976	HCl	1	1.0	93.0	2.0	5.0	3.6	—[a]
4	May 1977	HCl	2	2.1	93.4	3.4	3.2	3.4	—[a]
5	April 1978	HCl	4	4.2	96.6	0.7	2.7	3.2	—[a]
6	February 22, 1979	HCl	2	1.8	82.3	5.7	11.3[b]	5.5	—[c]
7	February 16, 1979	HCl	4	4.3	97.5	0.7	1.8	2.7	None
8	February 16, 1979	HCl	8	8.7	97.9	0.7	1.4	2.4	None
9	—	HNO_3	2	2.1	97.7	1.1	1.2	6.0	—[d,e]
10	—	HNO_3	2	2.2	97.7	0.8	1.5	6.0	—[d,e]

[a] Hydroxypropyl methylcellulose (4000 cps) at 0.5%, benzalkonium chloride at 1:25,000, phenylmercuric nitrate at 1:75,000, boric acid, sodium citrate, and distilled water. [b] The sample also contained ~0.7% isopilocarpic acid. [c] Borax–boric acid buffer at pH 6.5. [d] Lyophilized eye drops (200 mg of pilocarpine nitrate as solid matter and a vial containing 10 ml of an aqueous liquid). [e] Methylcellulose, boric acid, sodium borate, sodium chloride, thimerosal, and water.

pilocarpic acid (III) and epimerize to isopilocarpine (II), which, in turn, can hydrolyze to isopilocarpic acid (IV). The extent of the degradation depends, among other things, on pH, temperature, and time (3). Since this degradation results in deterioration of the pharmacological effect, ophthalmic aqueous pilocarpine formulations have limited stability.

Several determination methods for pilocarpine are known, including one based on ^{13}C-NMR spectroscopy (4). (Reference 4 summarizes the existing methods and their drawbacks.) However, the application of the ^{13}C-NMR method for the quantitative determination of pilocarpine in ophthalmic formulations is laborious and has other limitations (5, 6).

A quantitative determination was reported recently for pilocarpine and its degradation products by reversed-phase high-performance liquid chromatography (7). This report discusses the successful application of this separation method to 10 commercial, clinical ophthalmic pilocarpine formulations.

EXPERIMENTAL

The quantitative determination is based on the separation of I–IV (7). By the use of UV detection at 215 nm instead of the refractive index, the sensitivity was improved greatly. The detection limit thus was lowered from ~6 to ~0.04 μg. The separation of I–IV was carried out within 30 min on a reversed-phase column with water–methanol (97:3) containing 5% monobasic potassium phosphate. The pH was adjusted to 2.5 with phosphoric acid. No significant difference was found with the chromatogram shown in Ref. 7, although the column contained a slightly different octadecylsilyl packing. Additives such as buffers, antiseptics, and stabilizers, which often are present in clinical formulations, did not disturb the separation.

The formulations studied had been stored unopened. When assayed (March 15, 1979), the preparations, except for Formulations 9 and 10, were past their expiration date (Table I). Starting from the concentration stated on the package, all of the formulations were diluted with water to a concentration of 1%. The diluted solutions were compared with standard solutions of a known concentration of pilocarpine hydrochloride or nitrate. The percentage of pilocarpine was found by measuring the peak height. For the determination of the relative composition, the reversed-phase high-performance liquid chromatograms were compared with those of standard mixtures of pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid dissolved in water.

(+)-Pilocarpine (8) and (+)-isopilocarpine were used as their hydrochlorides. Pilocarpic acid and isopilocarpic acid were obtained by hydrolysis of pilocarpine and isopilocarpine, respectively, in 0.1 *N* aqueous NaOH. A high-performance pump was used[1]; a column (15 × 0.4 cm i.d.) packed with octadecylsilica[2] was employed with a UV detector[3] set at 215 nm. The flow rate was set at 1.5 ml/min.

RESULTS AND DISCUSSION

The method described is superior to existing methods in many respects (accuracy, time of analysis, quantity of sample needed, and ability to determine all four compounds in one run). In addition, the method is cheap and requires little pretreatment of the sample.

The results of the analyses of the 10 pilocarpine formulations are summarized in Table I. All experiments were carried out in duplicate, and deviations were negligible. Beside pilocarpine, these formulations contained, according to their label, buffers, antiseptics, and stabilizers (Table I).

With Samples 1–5 (pH found was ~3.5), the composition was only slightly dependent on age. For Samples 6–8, no information about the additional contents was given on the package. The label of Sample 6 said that it contained borax–boric acid buffer of pH 6.5 (found pH 5.5). The relatively high pH of this sample might account for the large quantity of isopilocarpine and pilocarpic acid found. Furthermore, most, if not all, of the formulations probably initially contained an excess of pilocarpine of at least 10% over the content stated.

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[1] Waters model 6000A pump with a model U6K injector.
[2] Nucleosil C_{18}, Macherey-Nagel & Co., Düren, West Germany.
[3] Pye Unicam LC3.