Table II—Analysis of Spiked Serum Samples of Carbinoxamine and Hydrocodone at Low Concentration

Concentration, ng/ml	Peak Area Ratios[a]	
	Carbinoxamine/IS	Hydrocodone/IS
0.2	0.0065 ± .0018 (28.2%)[b]	0.0088 ± .002 (25.9%)
0.5	0.0206 ± .0042 (20.3%)	0.0423 ± .0061 (14.4%)
1.0	0.0736 ± .0075 (10.2%)	0.091 ± .0079 (8.7%)

[a] Mean ± SD; n = 4. [b] Coefficient of variation (%).

Calibration curves of peak area ratios *versus* concentration in serum were linear up to at least 20 ng/ml. Assay sensitivity or the minimum quantifiable concentration was evaluated by determining the assay precision in the 0.2–1.0-ng/ml range. By defining the sensitivity as that concentration of drug that has a ±25% CV, the sensitivity for both carbinoxamine and hydrocodone is ~0.2 ng/ml using a 2-ml sample volume (*Table II*).

To assess the validity of this analytical procedure, serum samples from a human volunteer were assayed. The serum carbinoxamine and hydrocodone concentrations from a human volunteer orally administered, under fasted conditions, a solution containing 4 mg of carbinoxamine maleate and 5 mg of hydrocodone bitartrate are shown in Table III. Typical chromatograms from a human volunteer are also shown in Fig. 1. This assay provides the precision and sensitivity to conduct pharmacokinetic and bioavailability studies in humans receiving a single oral dose of carbinoxamine maleate and hydrocodone bitartrate.

Table III—Serum Carbinoxamine and Hydrocodone Concentrations after Oral Administration of 4 mg of Carbinoxamine Maleate and 5 mg of Hydrocodone Bitartrate to a Human Volunteer

Time Postdose, hr	Serum Concentration, ng/ml	
	Carbinoxamine	Hydrocodone
0	0	0
0.5	1.1	4.6
1	3.8	9.1
1.5	4.6	10.7
2	8.0	10.4
3	7.5	7.5
4.5	6.3	4.3
6	5.5	4.2
9	3.2	1.7
12	1.7	0.6
24	1.1	0
36	0.7	0

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Hypolipidemic Activity of Phthalimide Derivatives IV: Further Chemical Modification and Investigation of the Hypolipidemic Activity of *N*-Substituted Imides

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Received June 14, 1982, from the *Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27154.* Accepted for publication September 24, 1982.

Abstract □ A further investigation of *N*-substituted derivatives of phthalimide for hypolipidemic activity has revealed that the chain length, as well as the type of substitution on the *N*-alkyl chain of phthalimide is critical for biological activity. In these studies the hypolipidemic activity was not improved by extending the chain length beyond five carbon atoms in the alkyl and alkanoic acid series. Imido nitrogen substituents, other than alkanoic acids, methyl ketones, and alkyl groups, caused a reduction in hypolipidemic activity, *e.g.*, hydroxy, amino, hydroxymethyl, or carbethoxy. Reduction of the keto group in the side chain to an alcohol, as well as forming derivatives of the keto group, did not improve the hypolipidemic activity with the exception of 1-*N*-phthalimidobutan-3-one semicarbazone. This compound demonstrated improved hypocholesterolemic activity over phthalimide and 1-*N*-phthalimidobutan-3-one. Substitution of the 3-position of the aromatic moiety of phthalimide with an amino or nitro group, as well as substituting a pyridine or cyclohexyl ring for the phenyl ring, led to the loss of hypolipidemic activity.

Keyphrases □ Phthalimide—*N*-substituted derivatives, synthesis, hypolipidemic activity, mice, structure-activity relationships □ Hypolipidemic agents—potential, *N*-substituted derivatives of phthalimide, structure–activity relationships, mice □ Structure–activity relationships—*N*-substituted phthalimide derivatives, hypolipidemic activity, mice

Previously, it was shown that phthalimide (I) is a potent hypolipidemic agent in rodents. Serum cholesterol levels were reduced 43% in mice after administration for 16 days at 20 mg/kg/day. Serum triglyceride levels were also reduced 56% after 14 days of administration in mice at the same dose. The phthalimide derivatives were more potent

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Table I—Hypolipidemic Activity of Miscellaneous *N*-Substituted Cyclic Imides in Male Mice at 20 mg/kg/day ip

	Compound	Percent of Control[a] Day 14 Serum Triglyceride	Day 9 Serum Cholesterol	Day 16 Serum Cholesterol
I	Phthalimide	44 ± 8[b]	63 ± 8[b]	57 ± 7[b]
II	*N*-Hydroxyphthalimide	66 ± 5[b]	100 ± 11	70 ± 6[b]
III	*N*-Hydroxymethylphthalimide	54 ± 7[b]	79 ± 9[b]	75 ± 9[b]
IV	*N*-Aminophthalimide	81 ± 6[b]	94 ± 10	89 ± 9
V	1-*N*-Phthalimidopropan-2-one	48 ± 10	80 ± 16[c]	67 ± 12[b]
VI	1-*N*-Phthalimidopropan-2-ol	55 ± 5[b]	69 ± 8[b]	62 ± 4[b]
VII	1-*N*-Phthalimidobutan-2-one	69 ± 12[b]	64 ± 7[b]	65 ± 5[b]
VIII	1-*N*-Phthalimidobutan-2-ol	64 ± 4	88 ± 4	72 ± 7
IX	1-*N*-Phthalimidobutan-3-one	58 ± 7[b]	67 ± 11[b]	63 ± 7[b]
X	1-*N*-Phthalimidobutan-3-ol	98 ± 12	96 ± 5	88 ± 7
XI	1-*N*-Phthalimidobutan-3-ketoxime	84 ± 9[b]	89 ± 6[e]	73 ± 5[b]
XII	1-*N*-Phthalimidobutan-3-ketoxime acetate	91 ± 11	81 ± 7[b]	96 ± 5
XIII	1-*N*-Phthalimidobutan-3-semicarbazone	75 ± 6[b]	57 ± 6[b]	43 ± 4[b]
XIV	*N*-n-Butylphthalimide	82 ± 16[c]	72 ± 10[b]	54 ± 6[b]
XV	*N*-*n*-Butyl-3-aminophthalimide	72 ± 10	85 ± 7[b]	82 ± 6[b]
XVI	*N*-n-Butyl-3-nitrophthalimide	88 ± 9[c]	100 ± 9	81 ± 4[b]
XVII	*N*-*n*-Butyl-pyridyl-2,3-dicarboximide	86 ± 5[b]	99 ± 6	75 ± 5[b]
XVIII	*N*-*n*-Butyl-*cis*-1,2-cyclohexyldicarboximide	81 ± 8[b]	98 ± 7	83 ± 8[b]
XIX	8-*N*-Phthalimidooctanoic acid	87 ± 4[b]	78 ± 8[b]	65 ± 2[b]
XX	12-*N*-Phthalimidodecanoic acid	89 ± 4[b]	95 ± 9	75 ± 7[b]
XXI	1-*N*-Phthalimido-3-phenyl-2-propene	77 ± 7[b]	89 ± 9[c]	74 ± 3[b]
XXII	α-(*N*-Phthalimido)ethylphenyl ketone	98 ± 5	84 ± 5[b]	73 ± 6[b]
XXIII	*N*-Carbethoxyphthalimide	113 ± 9	80 ± 6[b]	80 ± 6[b]
XXIV	*N*-*n*-Octadecylphthalimide	92 ± 6	96 ± 12	91 ± 9
XXV	*N*-(Phthalimidomethyl)acetamide	59 ± 6[b]	67 ± 3[b]	70 ± 7[b]
XXVI	Isatin	92 ± 7	87 ± 8[c]	77 ± 7[b]
	1% Carboxymethylcellulose	100 ± 6	100 ± 5[d]	100 ± 8[e]

[a] Mean ± *SD*; $n = 6$. [b] $p \le 0.001$. [c] $p \le 0.010$. [d] Control value 132 ± 7 mg%. [e] Control value 128 dl/liter.

than clofibrate, and preliminary experiments suggested they were nontoxic. Further investigation of SAR requirements for hypolipidemic activity of phthalimides was undertaken by substituting the nitrogen of the imide ring with a series of alkyl, methyl ketone, carboxylic acid, and acetate ester substituents of varying chain lengths (1).

The previous study revealed that alkyl substitutions of four carbon atoms or an oxygen atom substituted for one carbon atom afforded the best hypolipidemic activity in mice. Consequently, a number of other chemical modifications of phthalimides were undertaken. Many of these compounds were chemical probes to investigate the potential hypolipidemic activity and do not represent a full series of compounds for complete SAR analysis.

RESULTS AND DISCUSSION

Substitution of the phthalimido nitrogen with a hydroxyl group (II), a hydroxymethyl group (III), or an amino group (IV) resulted in compounds with decreased hypolipidemic activity compared with the parent molecule, phthalimide, in both the serum cholesterol and triglyceride screens. It may be noted that *N*-hydroxyphthalimide (II) reduced serum triglyceride levels 36%, whereas phthalimide reduced triglyceride levels 56%. Reduction of *N*-phthalimidopropan-2-one (V) and *N*-phthalimidobutan-2-one (VII) to the corresponding alkanols (VI and VIII) resulted in compounds which showed essentially no difference in hypolipidemic activity. However, reduction of 1-*N*-phthalimidobutan-3-one (IX) to 1-*N*-phthalimidobutan-3-ol (X) resulted in loss of hypolipidemic activity. Derivatization of the carbonyl group of the ketone moiety in 1-*N*-phthalimidobutan-3-one (IX) as in the ketoxime (XI), the ketoxime acetate (XII), and the semicarbazone of the ketone produced varied results in hypolipidemic activity. Compounds XI and XII did not significantly lower serum cholesterol and triglyceride levels in mice, but the semicarbazone (XIII) reduced serum cholesterol levels 47% and serum triglyceride levels 25%. This magnitude of reduction of serum cholesterol levels by the semicarbazone demonstrated that it was one of the more potent imide derivatives in the hypocholesterolemic screen in mice.

Substitution of an amino (XV) or nitro group (XVI) at the 3-position of the phenyl ring of *N*-*n*-butylphthalimide or substitution of the aromatic moiety of phthalimide by a pyridine (XVII) or cyclohexyl (XVIII) moiety resulted in no change in hypotriglyceridemic activity; however, there was a marked reduction in biological activity in the cholesterol screen. Elongation of the *N*-alkyl substitution on the imido nitrogen in the *N*-phthalimido alkanoic acid series led to a decrease in hypolipidemic activity. Previously, we have shown that *N*-substituted alkanoic acid series, from acetic to caproic, were potent as hypolipidemic agents in mice. Interestingly, the pharmacological data for these compounds demonstrated that the shorter the side chain, the higher the hypotriglyceridemic activity and the longer the side chain, the higher the hypocholesterolemic activity. However, the two acids tested in this present study were less active in both screens compared with those in the original series.

Random substitution of the side chain, *e.g.*, 1-*N*-phthalimido-3-phenyl-2-propene (XXI) and α-(*N*-phthalimido)ethylphenyl ketone (XXII), resulted in compounds which retained moderate (>25%) hypocholesterolemic activity, but only XXI demonstrated any hypotriglyceridemic activity (~23%). *N*-Carbethoxyphthalimide was essentially inactive in both screens.

We have previously synthesized a homologous series of *N*-*n*-alkylphthalimides, including the methyl through octyl derivatives. The butyl analogue showed the most potent activity with decreasing activity displayed by the pentyl through octyl derivatives. Further chain elongation, *e.g.*, *N*-*n*-octadecylphthalimide (XXIV), afforded a compound essentially devoid of hypolipidemic activity.

Substitution of a nitrogen for a methylene group of 1-*N*-phthalimidobutan-3-one (IX) resulted in XXV, which was active as a hypolipidemic agent lowering serum triglyceride levels 41% and serum cholesterol levels 30%. Isatin (XXVI), an isomer of phthalimide, demonstrated no hypotriglyceridemic activity and only moderate hypocholesterolemic activity (~23%).

Whereas the compounds presented here were generally no more potent than the ones previously reported in this series (1), they do demonstrate chemical modifications that will probably not result in the development of a potent hypolipidemic imide.

EXPERIMENTAL

Chemistry—Melting points were determined using a melting point apparatus[1] and are uncorrected. NMR data were obtained utilizing a 60-MHz spectrometer[2]. Compounds I, II, IV, XXIII, and XXVI were obtained from a commercial source[3] and were tested without further

[1] Mel Temp.
[2] JEOL C 60 HL.
[3] Aldrich Chemical Co., Milwaukee, Wis.

purification. Syntheses of I, III, V, IX, and XIV were reported previously (1).

Compounds VI, XVI–XX, and XXIV—Equimolar amounts (0.006–0.15 mole) of the appropriate anhydride and amine were refluxed overnight in 75–200 ml of toluene, collecting the water of reaction by azeotropic distillation. The toluene was removed *in vacuo*, and the residue was distilled or recrystallized.

1-N-Phthalimidopropan-2-ol (VI)—The amount obtained was 26.7 g (72% yield) (ethanol-ligroine); mp 89–91.5° [lit. (2) mp 88–90°].

N-n-Butyl-3-nitrophthalimide (XVI)—The amount obtained was 19.0 g (62% crude yield) (ethanol); mp 69–70° [lit. (3) mp 71–72°].

Anal.—Calc. for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87. Found: C, 58.21; H, 4.82.

N-n-Butylpyridyl-2,3-dicarboximide (XVII)—The amount obtained was 0.52 g (8% yield) (ethanol–H_2O); mp 84–85°. ^{1}H-NMR ($CDCl_3$): 7.72–9.19 (m, 3, aromatic), 3.81 (t, 2, N—CH_2), and 0.75–2.02 ppm (m, 7, —$CH_2CH_2CH_3$).

Anal.—Calc. for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92. Found: C, 64.59; H, 5.95.

N-n-Butyl-cis-1,2-cyclohexyldicarboximide (XVIII)—The amount obtained was 12.9 g (62% yield); bp 119–123°/0.27–0.68 mm Hg [lit. (4) bp 115–118°/2 mm Hg].

Anal.—Calc. for $C_{12}H_{19}NO_2$ C, 68.87; H, 9.15. Found: C, 68.96; H, 9.32.

8-N-Phthalimidooctanoic Acid (XIX)—The amount obtained was 1.0 g (55% yield) (hexane); mp 89–90°; ^{1}H-NMR ($CDCl_3$): 7.63–8.07 (m, 4, aromatic), 3.77 (t, 2, N—CH_2), 2.45 (t, 2, CH_2CO_2), and 1.15–2.01 ppm [m, 10, $(CH_2)_5CH_2CO_2$].

Anal.—Calc. for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.24; H, 6.42, N, 4.83.

12-N-Phthalimidodecanoic Acid (XX)—The amount obtained was 2.5 g (36% yield) (hexane); mp 87–88°; ^{1}H-NMR ($CDCl_3$): 7.50–7.96 (m, 4, aromatic), 3.68 (t, 2, N—CH_2), 2.20 (t, 2, CH_2CO_2), and 1.10–1.88 ppm [m, 18, $(CH_2)_9CH_2CO_2$].

Anal.—Calc. for $C_{20}H_{27}NO_4$: C, 69.54; H, 7.88; N, 4.06. Found: C, 69.61; H, 8.12; N, 3.94.

N-n-Octadecylphthalimide (XXIV)—The amount obtained was 28.1 g (70% yield) (ethanol); mp 78–79° [lit. (5) mp 80–81°].

Anal.—Calc. for $C_{26}H_{41}NO_2$: C, 78.15; H, 10.34. Found: C, 78.10; H, 10.61.

1-*N*-Phthalimidobutan-2-one (VII) and 1-*N*-Phthalimidobutan-2-ol (VIII)—1-Amino-2-butanol (3.95 g, 0.044 mole), prepared from 1,2-epoxybutane by the procedure of de Montmollin and Achermann (6), was dissolved in 100 ml of toluene, and 6.5 g (0.044 mole) of phthalic anhydride was added. The mixture was refluxed for 2 hr, collecting the water formed in the reaction by azeotropic distillation. The toluene was removed *in vacuo*, and the residue was recrystallized from isopropyl alcohol to afford 4.0 g (41%) of 1-*N*-phthalimidobutan-2-ol (VIII), mp 71–71.5°. ^{1}H-NMR ($CDCl_3$): 7.72–8.10 (m, 4, aromatic), 3.76–4.00 (m, 3, CH_2CH(OH)—), 2.32–2.54 (br s, 1, OH), 1.38–1.88 (m, 2, —CH_2CH_3), and 1.05 (t, 3, CH_3).

Anal. Calc. for $C_{12}H_{13}NO_3$: C, 66.35; H, 5.10. Found: C, 66.58; H, 5.11.

1-*N*-Phthalimidobutan-2-ol (2.02 g, 0.009 mole) in 18 ml of CH_2Cl_2 was added to 2.98 g (0.014 mole) of pyridinium chlorochromate suspended in 20 ml of CH_2Cl_2. The mixture was stirred 2.5 hr, 50 ml of ether was added, and the supernatant was decanted and filtered. Evaporation of the filtrate and repeated recrystallization from water gave 0.27 g (14%) of 1-*N*-phthalimidobutan-2-one (VII), mp 107–108° [lit. (7) mp 107–108°].

Anal.—Calc. for $C_{13}H_{12}NO_3$: C, 66.74; H, 5.98. Found: C, 65.70; H, 5.98.

1-*N*-Phthalimidobutan-3-ol (X)—1-*N*-Phthalimidobutan-3-one (9.78 g, 0.045 mole) was added to a mixture of 32.31 g (0.158 mole) aluminum isopropoxide in 300 ml of isopropyl alcohol. The mixture was refluxed for 6 hr (at this time it gave a negative test for acetone with 2,4-dinitrophenylhydrazine). The isopropyl alcohol was removed *in vacuo*, 450 ml of 0.16 *N* HCl was added, and the mixture extracted with ethyl acetate. Evaporation of the organic phase *in vacuo* yielded a yellow oil which on distillation gave 5.6 g (49%) of 1-*N*-phthalimidobutan-3-ol, mp 58–60° [lit. (8) mp 48°].

Anal.—Calc. for $C_{12}H_{13}NO_8$: C, 65.74; H, 5.98. Found: C, 66.00; H, 5.92.

1-*N*-Phthalimidobutan-3-ketoxime (XI)—1-*N*-Phthalmidobutan-3-one (5.0 g, 0.023 mole) was suspended in 25 ml of ethanol, and 5.0 g of hydroxylamine hydrochloride was added, followed by 25 ml of pyridine. The mixture was refluxed 3 hr, the volatile material removed *in vacuo*, and the residue was recrystallized from ethanol to yield 0.62 g (12%) of 1-*N*-phthalimidobutan-3-ketoxime, mp 179° [lit. (9) mp 178°].

Anal.—Calc. for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.20. Found: C, 62.31; H, 4.94.

1-*N*-Phthalimidobutan-3-ketoxime Acetate (XII)—1-*N*-Phthalimidobutan-3-ketoxime (1.0 g, 0.013 mole) was refluxed for 4 hr in 90 ml of acetic anhydride. The volatile material was removed *in vacuo*, and the residue was recrystallized from water to give 0.22 g (18%) of 1-*N*-phthalimidobutan-3-ketoxime acetate, mp 110–112°; ^{1}H-NMR (acetone-d_6): 7.98 (s, 4, aromatic), 4.02 (t, 2, N—CH_2), 2.73 (t, 2, CH_2C=N), 2.10 (s, 3, CH_3), and 2.02 ppm (s, 3, CH_3).

Anal.—Calc. for $C_{14}H_{14}N_2O_4$: C, 61.31; H, 5.15. Found: C, 61.50; H, 4.98.

1-*N*-Phthalimidobutan-3-semicarbazone (XIII)—1-*N*-Phthalimidobutan-3-one (4.0 g, 0.018 mole) was added to a solution of 4.0 g (0.035 mole) of semicarbazide hydrochloride and 6.0 g of sodium acetate in 40 ml of ethanol and 22 ml of water. The mixture was warmed until it became milky white and was then allowed to stand for 3 hr; 40 ml of water was added, and the precipitate was collected and recrystallized from methanol to give 1.98 g (40%) of 1-*N*-phthalimidobutan-3-semicarbazone, mp 221–223° [lit. (10) mp 202–203°].

Anal.—Calc. for $C_{13}H_{14}N_4O_3$: C, 56.93; H, 5.15. Found: C, 57.13; H, 5.07.

***N*-*n*-Butyl-3-aminophthalimide (XV)**—*N*-*n*-Butyl-3-nitrophthalimide (XVI) (12.4 g, 0.5 mole) was dissolved in 300 ml of ethanol, added to 4 g of 10% Pd-C, and shaken for 10 min under hydrogen in a low-pressure apparatus[4] (60 psig). The catalyst was removed by filtration, the solvent was evaporated *in vacuo*, and the residue was recrystallized from ethanol–water to give 8.05 g (73%) of *N*-*n*-butyl-3-aminophthalimide, mp 67–70°; ^{1}H-NMR ($CDCl_3$): 6.83–7.62 (m, 3, aromatic), 5.38 (s, 2, NH_2), 3.67 (t, 2, N—CH_2), and 0.75–1.95 ppm (m, 7, $CH_2CH_2CH_3$).

Anal.—Calc. for $C_{12}H_{14}N_2O_2$: C, 66.04; H. 6.47. Found: C, 66.00; H, 6.40.

1-*N*-Phthalimido-3-phenyl-2-propene (XXI)—This compound was prepared by refluxing equimolar amounts of potassium phthalimide and cinnamyl bromide in absolute ethanol for 12 hr. The solvent was removed under reduced pressure, and the residue was washed with water. The residual water-insoluble product was collected by filtration and then recrystallized from toluene to give XXI, in 65% yield, mp 160–161°.

Anal.—Calc. for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.78; H, 5.09; N, 5.39.

α-(*N*-Phthalimido)ethylphenyl Ketone (XXII)—An equimolar amount of potassium phthalimide and α-bromopropiophenone in ethyl alcohol were refluxed for 5 hr. The solvent was removed under reduced pressure, and the residue was washed with water and then recrystallized from toluene–petroleum ether to give XXII in 60% yield, mp 88–89°.

Anal.—Calc. for $C_{17}H_{13}NO_3$: C, 73.11; H, 4.69; N, 5.02. Found: C, 73.27; H, 4.72; N, 5.00.

***N*-(Phthalimidomethyl)acetamide (XXV)**—Utilizing the procedure of Buc (11), 8.85 g (0.05 mole) of *N*-hydroxymethylphthalimide and 3.5 ml (0.066 mole) of acetonitrile were added to 50 ml of concentrated sulfuric acid. The mixture was stirred for 22 hr, 250 ml of ice was added, and the precipitate was collected and recrystallized from ethanol to give 3.5 g (33%) of XXV, mp 224–227° [lit. (11) mp 213–214.5°].

Anal.—Calc. for $C_{11}H_{10}N_2O_3$: C, 60.57; H, 4.59. Found: C, 60.58; H, 4.59.

Biological Studies—All compounds were tested at 20 mg/kg/day and administered intraperitoneally to male mice at 11:00 a.m. On days 9 and 16, the blood was collected via the tail vein. The blood samples were collected between 8:00 and 9:30 a.m. in alkali-free nonheparinized microcapillary tubes, which were centrifuged for 3 min to obtain the serum (12). Duplicate 25-μl samples of nonhemolyzed serum were used to determine the milligram percent serum cholesterol levels by a modification of the Liebermann–Burchard reaction. Using a separate group of mice, which were bled on day 14, serum triglyceride levels (in dl/liter) were measured using duplicate samples of 50 μl[5].

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High-Performance Liquid Chromatographic Stability-Indicating Assay for Naphazoline and Tetrahydrozoline in Ophthalmic Preparations

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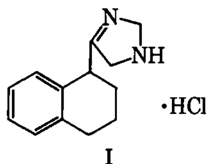
Abstract □ A high-performance liquid chromatographic (HPLC) analysis for tetrahydrozoline and naphazoline in ophthalmic solutions is presented. The analysis allows a more reproducible, direct stability-indicating assay than the colorimetric methods generally employed. The HPLC system is so designed that a variety of ophthalmic solutions containing either naphazoline or tetrahydrozoline can be analyzed concomitantly.

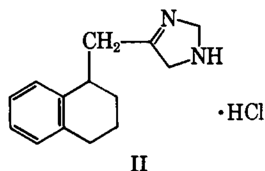
Keyphrases □ High-performance liquid chromatography—stability-indicating assay, ophthalmic preparation, naphazoline, tetrahydrozoline □ Naphazoline—high-performance liquid chromatographic stability-indicating assay, ophthalmic preparation □ Tetrahydrozoline—high-performance liquid chromatographic stability-indicating assay

Tetrahydrozoline (I) and naphazoline (II) are sympathomimetic agents used in the majority of commercially available occular decongestants. The current analytical methodologies are either colorimetric or ultraviolet. Tetrahydrozoline analysis involves color development with either sodium nitroprusside (1, 2) or bromophenol blue (2). Naphazoline is presently assayed by either UV-absorption spectroscopy (3) or colorimetry (1).



I

2-(1,2,3,4-Tetrahydro-1-naphthyl)-2-imidazoline, monohydrochloride



II

2(1-Naphthylmethyl)-2-imidazoline, monohydrochloride

These methods generally involve isolation steps as well as color development times and are subject to interferences. The high-performance liquid chromatographic (HPLC) procedure developed in our laboratory is a direct stability-indicating assay for tetrahydrozoline and naphazoline in ophthalmic solutions.

EXPERIMENTAL

Materials—Methanol[1], citric acid[2], sodium citrate[3], and perchloric acid[4] were used as received. Tetrahydrozoline and naphazoline were USP reference standards. A high-performance liquid chromatograph[5] and a UV visible spectrophotometer[6] were used. A microparticulate octadecylsilane column[7] was used. The temperature was ambient and the flow rate 2.0 ml/min. The analytical wavelength was 265 nm. Injection volume was 20 μl.

Mobile Phase—Six grams of sodium citrate dihydrate and 4 g of anhydrous citric acid were added to 700 ml of water and mixed until dissolved; 7 ml of perchloric acid was added and the pH determined. The pH was adjusted to 2.2 ± 0.2 by further addition of perchloric acid. A 300-ml volume of methanol was added, the solution mixed thoroughly, filtered through a 0.45-μm filter, and deaerated for ~10 min.

Tetrahydrozoline Internal Standard Solution—A solution containing ~150 mg of tetrahydrozoline hydrochloride was prepared by dissolving and diluting to volume with distilled water to 100 ml (~1.5 mg/ml).

Naphazoline Internal Standard Solution—A preparation of ~40 mg of naphazoline in 100 ml of water was prepared and diluted 1/10 with water for use as the internal standard solution (~0.04 mg/ml).

Naphazoline Standard—Approximately 120 mg of naphazoline hydrochloride was weighed accurately into a 100-ml volumetric flask and dissolved and diluted to volume with distilled water. A 5.0-ml volume of this solution was pipetted into a 50-ml volumetric flask and diluted to volume with distilled water (~0.12 mg/ml); 5.0 ml of this solution was pipetted into a 10-ml volumetric flask and diluted to volume with tetrahydrozoline internal standard solution.

[1] Methanol, Burdick and Jackson or equivalent spectrophotometric grade.
[2] Citric acid anhydrous AR Grade.
[3] Sodium citrate dihydrate AR Grade.
[4] Perchloric acid (60%), Fisher Scientific.
[5] Waters Model 6000A Liquid Chromatography Pump; Rheodyne Model 7120 Injector with 20-μl loop; DuPont variable-wavelength detector; Hewlett-Packard 3385A Recording Integrator.
[6] Varian/Cary 219 UV Spectrophotometer.
[7] Waters μBondapak C_{18} liquid chromatographic column.

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