resolution: More than 800 injections were completed on a second column with no change in the separation.

Analysis of Additional Over-the-Counter Pharmaceutical Formulations—The six additional preparations, shown in Table I, were examined to determine whether the silica adsorbent was appropriate for other basic drugs. Chromatograms were obtained using the same mobile phase and detector wavelength as for quantitation of syrup A, and no attempt was made to optimize the separations. For example, Fig. 3 illustrates that other basic drugs are also eluted in this mobile phase system.

CONCLUSIONS

The separation of lipophilic bases on unbonded silica stationary phases in the reverse-phase mode is rapid and convenient. No amine modifiers or gradients are necessary for the separation; only simple buffers are required for pH control. Separations of several samples indicated that a variety of compounds may be separated with this system. Assay of a single sample indicated that linearity, reproducibility, and length of column life are excellent.

REFERENCES

(1) B. A. Bidlingmeyer, *J. Chromatogr. Sci.*, **18**, 525 (1980).

(2) B. A. Bidlingmeyer, J. K. Del Rios, and J. Korpi, *Anal. Chem.*, **54**, 442 (1982).

Hypolipidemic Activity of Phthalimide Derivatives V: Reduced and Hydrolytic Products of Simple Cyclic Imides

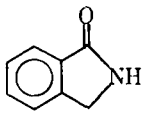
JAMES M. CHAPMAN, JR., STEVEN D. WYRICK, P. JOSÉE VOORSTAD, JAMES H. MAGUIRE, GEORGE H. COCOLAS, and IRIS H. HALL ˣ

Received February 24, 1983 from the *School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina* 27514. Accepted for publication September 8, 1983.

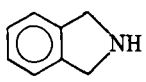
Abstract □ A series of cyclic imides and related compounds have previously been shown to possess hypolipidemic activity at the low dose level of 20 mg/kg/d. Hydrolytic and reduced products of the cyclic imides were synthesized and examined to discern if possible metabolic products were the active chemical species of these hypolipidemic agents. Phthalimide proved to be the most active cyclic imide tested. Unfortunately, the new products did not, in general, improve hypolipidemic activity in rodents. The exceptions were piperidine which demonstrated improved hypotriglyceridemic activity, and 3,4,5,6-dibenzohomopiperidin-2-one, which demonstrated improved hypocholesterolemic activity compared to phthalimide.

Keyphrases □ Cyclic imides—phthalimide derivatives, reduced and hydrolytic products, hypolipidemic activity □ Phthalimide derivatives—cyclic imides, hypolipidemic activity, reduced and hydrolytic products □ Hypolipidemic activity—phthalimide derivatives, cyclic imides, reduced and hydrolytic products

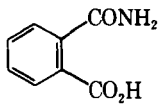
A series of cyclic imides including phthalimide (I), succinimide, 1,8-naphthalimide, and saccharin have been shown to be potent hypolipidemic agents in rodents (1). After conducting dose response studies on these compounds, the optimum dose appears to be 20 mg/kg/d ip when tested in mice. Thus, we have selected that dose for this structure-activity relationship study. The mode of action of these derivatives is different from standard therapeutic agents on the market in that they do not inhibit the regulating enzyme of cholesterol synthesis, HMG CoA reductase; rather, they regulate mitochondrial citrate exchange and the availability of acetyl CoA, the key intermediate required in the synthesis of fatty acids and cholesterol (1). These agents also decreased cholesterol absorption in the intestine and accelerated cholesterol excretion by the biliary route. The agents had no effect on appetite, organ weights, or body weight, and there was no evidence of organ toxicity or deleterious systemic effects (1). Since these are potentially hypolipidemic agents, we expanded the types of imide rings and examined a series of their reduced and hydrolytic products, which may be potential metabolic products of the parent imide yet retain pharmacological activity.



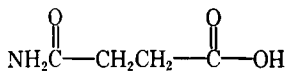
Phthalimidine (II)



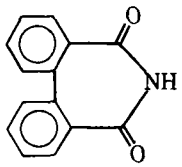
Isoindolin (III)



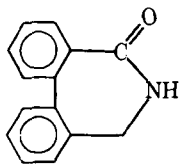
Phthalamic Acid (IV)



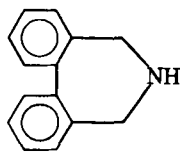
Succinamic Acid (IX)



Diphenimide (XVI)



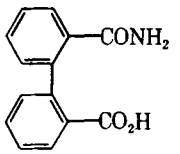
3,4,5,6-Dibenzohomo piperidin-2-one (XVII)



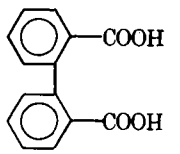
3,4,5,6-Dibenzohomo piperidine (XVIII)

$HO_2C(CH_2)_3CONH_2$

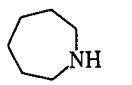
Glutaramic Acid (XIV)



Diphenamic Acid (XIX)



Diphenic Acid (XX)



Homopiperidine (XXIII)

$HO_2C(CH_2)_3CONH_2$

Adipamic Acid (XXIV)

0022-3549/84/1000-1482$01.00/0

Table I—Hypolipidemic Activity of Reduced and Hydrolytic Products of Cyclic Imides in CF_1 Male Mice at 20 mg/kg/d ip [a]

Compound	Serum Cholesterol, Day 9	Serum Cholesterol, Day 16	Serum Triglyceride, Day 14
I Phthalimide	63 ±8[b]	57 ± 7[b]	44 ± 8[b]
II Phthalimidine	89 ± 6	87 ± 14	78 ± 7[b]
III Isoindoline	86 ± 8	74 ± 5[b]	69 ± 6[b]
IV Phthalamic acid	85 ± 10	52 ± 9[b]	70 ± 4[b]
V Phthalic acid	91 ± 7	78 ± 7[b]	86 ± 4[c]
VI Succinimide	78 ± 9[b]	73 ± 8[b]	68 ± 7[b]
VII 2-Pyrrolidinone	86 ± 7	82 ± 5[b]	80 ± 8[c]
VIII Pyrrolidine	82 ± 7[b]	69 ± 5[b]	93 ± 6
IX Succinamic acid	93 ± 3	87 ± 8	93 ± 7
X Succinic acid	85 ± 6	79 ± 9[c]	94 ± 12
XI Glutarimide	80 ± 4[b]	78 ± 5[b]	77 ± 6[b]
XII Valerolactam	102 ± 4	74 ± 5[b]	94 ± 7
XIII Piperidine	81 ± 6[b]	73 ± 7[b]	34 ± 4[b]
XIV Glutaramic acid	90 ± 7	81 ± 6[b]	58 ± 6[b]
XV Glutaric acid	92 ± 4	74 ± 8[b]	80 ± 4[b]
XVI Diphenimide	81 ± 7[b]	82 ± 4[b]	81 ± 7
XVII 3,4,5,6-Dibenzohomopiperidin-2-one	84 ± 6[b]	49 ± 4[b]	73 ± 5[b]
XVIII 3,4,5,6-Dibenzohomopiperidine	70 ± 6[b]	51 ± 3[b]	52 ± 4[b]
XIX Diphenamic acid	80 ± 7[b]	78 ± 6[b]	56 ± 5[b]
XX Diphenic acid	81 ± 7	82 ± 10	58 ± 2[b]
XXI Adipimide	100 ± 7	76 ± 7[b]	89 ± 5
XXII ϵ-Caprolactam	104 ± 8	75 ± 5[b]	84 ± 7
XXIII Homopiperidine	72 ± 6[b]	71 ± 6[b]	80 ± 8
XXIV Adipamic acid	89 ± 7	79 ± 4[b]	85 ± 6
XXV Adipic acid	73 ± 6[b]	71 ± 7[b]	68 ± 6[b]
1% Carboxymethylcellulose	100 ± 5[b]	100 ± 6[e]	100 ± 6[f]

[a] Expressed as percentages of control (mean ± *SD*). [b] $p < 0.001$. [c] $p < 0.010$. [d] 125 mg%. [e] 122 mg%. [f] 137 mg/dL.

EXPERIMENTAL SECTION

All chemicals used as synthetic intermediates were purchased and used as obtained from the manufacturers[1]. Melting points were obtained on a capillary melting point apparatus[2] and are uncorrected. Column chromatography was performed on silica gel 60 (70–230 mesh)[2]. TLC was performed using precoated silica gel plates with fluorescent indicators[3]. ^{1}H-NMR spectra were obtained on a 60-mHz Fourier transform spectrometer[4]. Elemental analyses for prepared compounds are correct within ±0.4% of theoretical values.

Phthalimidine (II)—Preparation of this compound has been reported previously (1). The yield is 47%; mp 150–152°C.

Isoindoline (III)—The procedure of Bornstein and Shields (2) was used to prepare this compound in 43% yield, bp 98°C/10 mm Hg [lit. (bp) 120°C/30 mmHg]; ^{1}H-NMR ($CDCl_3$): δ 7.2 [s, 4, ArH_4] and 4.19 ppm [s, 4, $(CH_2)_2$].

Phthalamic Acid (IV)—Utilizing the process of Chapman and Stephen (3) 8.0 g (0.0054 mol) of phthalic anhydride was added to 40 mL of concentrated ammonium hydroxide, and the reaction mixture was stirred for 1 h at room temperature. The water was removed under reduced pressure, and the colorless solid residue was dissolved in 80 mL of water. Concentrated hydrochloric acid was added and the mixture was cooled on ice to afford a precipitate, which was removed by filtration to give 1.2 g (13%) of a colorless solid, mp 149–152°C; IR (KBr): 3180 (N—H), 1675, and 1645 (C=O) cm^{-1}.

Anal.—Calc. for $C_8H_7NO_3$ for C, 72.17; H, 5.30. Found: C, 72.38, H, 5.28.

Glutaramic Acid (XIV)—The synthesis of this compound has been reported previously (4).

Diphenimide (XVI)—Utilizing the procedure of Underwood and Kochmann (5), diphenamic acid (25.0 g, 0.10 mol) was added to a solution containing 30 mL of glacial acetic acid and 37 mL of acetic anhydride; the mixture was then stirred under reflux for 7.5 h. On cooling, the crude product which precipitated was removed by filtration and recrystallized from ethanol to afford 10.0 g (43%) of a colorless solid, mp 217–219°C.

Anal.—Calc. for $C_{14}H_9NO_2$: C, 75.33; H, 4.06; N, 6.27. Found: 75.53; H, 4.18; N, 6.28.

3,4,5,6-Dibenzohomopiperidin-2-one (XVIII)—The procedure of Chapman, *et al.* (6) was used to reduce XVI to the lactam. Powdered zinc (5.5 g, 0.085 mol) was stirred for 5 min in a solution of 450 mg of mercuric chloride in 1.5 mL of concentrated hydrochloric acid and 13 mL of water. The supernatant was decanted, and the zinc amalgam was washed with 20 mL of water. Diphenimide (XVI) (1.9 g, 0.0085 mol) was added, followed by 10 mL of water and 15 mL of concentrated hydrochloric acid. The mixture was stirred under reflux for 6 h, and was then filtered hot. The cooled filtrate was extracted with dichloromethane. The organic phase was dried (sodium sulfate) and the solvent evaporated under reduced pressure to afford 2.0 g of crude solid, which was purified by column chromatography[5] to afford 250 mg (13%) of a yellow solid, mp 167–169°C: ^{1}H-NMR ($CDCl_3$): δ 7.04–8.18 [m, 8, $(ArH_4)_2$] and 4.12 ppm (m, 2, CH_2).

Anal.—Calc. for: $C_{14}H_{11}NO$: C, 79.68; H, 4.89; N, 6.22. Found: C, 79.35; H, 5.26; N, 6.49.

3,4,5,6-Dibenzohomopiperidine (XVIII)—The procedure of Uffer and Schletterl (7) for reduction of imides to amines was used. To a 766-mg (0.02-mol) suspension of lithium aluminum hydride in 25 mL of ether was added, in a dropwise manner, a solution of 0.5 g (0.0067 mol) of XVI in 30 mL of dry tetrahydrofuran. The mixture was stirred under reflux for 16 h and the excess hydride was decomposed by dropwise addition of water. The resulting suspension was filtered and the filtrate was extracted with 1.0 M HCl. The aqueous extracts were combined and extracted with ether, made alkaline with sodium hydroxide, and extracted again with ether. The organic extracts were dried (sodium sulfate) and evaporated under reduced pressure to afford 400 mg of a viscous oil which was purified by column chromatography[6] to afford 375 mg (29%) of XVIII as a viscous oil. ^{1}H-NMR ($CDCl_3$): δ 7.42 [m, 8, $(ArH_4)_2$] and 3.64 ppm [s, 4, $(CH_2)_2$].

Anal.—Calc. for $C_{14}H_{13}N$: C, 86.16; H, 6.66; N, 7.17. Found: C, 86.03; H, 6.73; N, 6.97.

Diphenamic Acid (XIX)—Utilizing the procedure of Underwood, *et al.* (5), a 93% yield of product was obtained, mp 189–191°C [lit. (5) mp 187.0–187.5°C].

Adipamic Acid (XXIV)—The synthesis of this compound has been reported previously (4).

RESULTS AND DISCUSSION

Examination of the individual imides, I, VI, XI, XVI, and XXI, for hypolipidemic activity showed that the phthalimide moiety was more active than succinimide, glutarimide, diphenimide, and adipimide (Table I). However, all these imides were more active at 20 mg/kg/d than clofibrate, which is inactive at this dose. Clofibrate requires doses of 150–200 mg/kg to observe a 15% reduction of serum cholesterol levels and a 25% reduction of triglyceride levels. Succinimide, for example, reduced serum triglyceride levels 32% and glutarimide reduced the levels 23%.

Examination of derivatives where the carbonyl groups were reduced demonstrated mixed results. In the phthalimide series, reduced compounds were

[1] Compounds I, V, VI, and X were obtained from Eastman Organic Chemicals; VII, VIII, IX, XI, XII, XV, XX, XXI, XXII, XXIII, and XXV from Aldrich Chemical Company; and XIII from Fisher Scientific Company.

[2] Thomas Hoover Company.

[3] E. M. Reagents.

[4] JEOL, deuterochloroform plus tetramethylsilane.

[5] Dichloromethane:methanol (9:1).

[6] Dichloromethane:ethyl acetate (9:1).

Table II—Hypolipidemic Activity of 3,4,5,6-Dibenzohomopiperidine in Male CF_1 Mice [a]

Dose, mg/kg/d	Serum Cholesterol Day 9	Serum Cholesterol Day 16	Serum Triglyceride Day 14
Control (1% Carboxymethylcellulose)	100 ± 5[c]	100 ± 6[d]	100 ± 6[e]
Compound XVIII			
10	61 ± 5[b]	50 ± 5[b]	48 ± 5[b]
20	68 ± 4[b]	48 ± 4[b]	49 ± 5[b]
40	67 ± 6[b]	54 ± 4[b]	44 ± 6[b]
60	69 ± 5[b]	51 ± 4[b]	55 ± 6[b]

[a] Expressed as percentage of control (mean ± *SD*); $n = 6$. [b] $p < 0.001$. [c] 125 mg%. [d] 122 mg%. [e] 137 mg/dL.

less active than I in both the cholesterol and triglyceride screens; interestingly, the completely reduced compound isoindoline (III) was more active than the partially reduced phthalimidine (II) in both screens. In the succinimide series, the reduced compounds VII and VIII demonstrated less hypotriglyceridemic activity; the partially reduced compound 2-pyrolidinone (VII) demonstrated less hypocholesterolemic activity, but the totally reduced compound, pyrrolidine (VIII) was more active than succinimide in the cholesterol screen, causing 31% reduction. In the glutarimide series, the partially reduced compound, valerolactam (XII), demonstrated approximately the same activity in the cholesterol screen as glutarimide, but it demonstrated less hypotriglyceridemic activity than glutarimide. The fully reduced compound, piperidine (XIII) was more effective in both screens, with a marked reduction, 66%, of serum triglyceride levels. The reduction of diphenimide led to improved hypocholesterolemic activity for both derivatives, lowering serum cholesterol levels 51–49% and the fully reduced derivative serum triglyceride levels 48%. In the adipimide series, the reduction of the carbonyl groups led to little change in hypolipidemic effects in either screen.

Examination of the hydrolytic products of the phthalimide series showed that the half amide, phthalamic acid (IV), afforded approximately the same hypocholesterolemic activity as phthalimide; however, IV produced less effect on lowering triglyceride levels than I. The phthalic acid (V) demonstrated less activity in both screens compared to I. The hydrolytic products of the succinimide series (IX and X), the glutarimide series (XIV and XV), and the adipimide series (X, XXIV, and XXV) demonstrated no improvement in hypolipidemic activity over the parent derivatives. In the diphenimide series, the hydrolytic products XIX and XX demonstrated approximately the same hypocholesterolemic activity as XVI.

In conclusion, there did not appear to be a trend among all of the reduced products of imide analogues with respect to improvement or loss of activity in the hypolipidemic screens in mice. In general, the new compounds examined in this study did not extensively improve the hypocholesterolemic and hypotriglyceridemic activity of the lead compound, phthalimide (I). Piperidine produced improved hypotriglyceridemic activity compared to I, and XVII demonstrated improved hypocholesterolemic activity compared to I. The compound which demonstrated the best activity in both screens, other than I, was XVIII, which was slightly more potent than I in the cholesterolemic screen (6%) and slightly less active in the triglyceridemic screen (8%) in mice (Table II). These differences are probably not significant. Thus, I and XVIII are very similar in hypolipidemic activity. A dose response study with XVIII from 10–60 mg/kg showed that a 20 mg/kg/d dose was optimum for reducing serum cholesterol levels, and a 40 mg/kg/d dose lowered serum triglyceride levels to the level observed for I at 20 mg/kg. Further investigation of these derivatives is warranted since they are more active than commercially available agents, *e.g.*, clofibrate.

REFERENCES

(1) I. H. Hall, P. J. Voorstad, J. M. Chapman, Jr. and G. H. Cocolas, *J. Pharm. Sci.* **72**, 845 (1983).

(2) J. Bornstein and J. E. Shields, *Org. Synth.*, **Coll. Vol. V**, 1064 (1973).

(3) E. Chapman and H. Stephen, *J. Chem. Soc.*, **127**, 1791 (1925).

(4) J. H. Maguire and K. H. Dudley, *Anal. Chem.*, **49**, 292 (1977).

(5) H. W. Underwood and E. L. Kochmann, *J. Am. Chem. Soc.*, **46**, 2072 (1924).

(6) J. M. Chapman, Jr., G. H. Cocolas and I. H. Hall, *J. Med. Chem.* **28**, 243 (1983).

(7) A. Uffer and E. Schlettler, *Helv. Chim. Acta*, **31**, 1397 (1948).

ACKNOWLEDGMENTS

This research was financially supported by NIH Grant 1-R01-HL25680. We also thank Greg Webb and Walter Spivey for technical assistance.

Liquid Crystals as a Potential Ointment Vehicle

SVEN WAHLGREN *, ANNA L. LINDSTROM ‡, and STIG E. FRIBERG §x

Received May 2, 1983, from **The National Corporation of Swedish Pharmacies, Stockholm, Sweden*, ‡*Aco Company, Solna, Sweden*, and the §*Chemistry Department, University of Missouri-Rolla, Rolla, MO 65401.* Accepted for publication October 28, 1983.

Abstract □ A lecithin–water lyotropic liquid crystal was used as an ointment vehicle for a hydrocortisone formulation. The hydrocortisone was soluble in the liquid crystalline phase up to 5% by weight. The diffusion coefficient determined for the hydrocortisone in the liquid crystalline phase was 5.5×10^{-9} $cm \cdot s^{-1}$, which is four magnitudes higher than the corresponding value for skin.

Keyphrases □ Ointments—vehicles, liquid crystals, lecithin–water □ Lecithin—liquid crystals as potential ointment vehicle □ Hydrocortisone—lecithin–water liquid crystals as potential ointment vehicle

The total therapeutic effect of percutaneous preparations depends not only on the action of the drug itself, but also on other factors related to the structure of the vehicle (1, 2). These latter factors may be divided into two main groups.

The first group contains vehicle–barrier interactions, mostly involving changes in the structure of the stratum corneum caused by the vehicle. These interactions may be evaluated *in toto* by use of standardized tests such as the blanching test (3, 4). Stratum corneum structural changes may facilitate or retard the diffusion of the active substance through this layer, as found for the absorption of solvents through skin (5). It has been claimed that some substances enhance penetration of pharmacological agents when topically applied (6).

The second group includes vehicle–drug interactions. Of these, the capacity of the structure to dissolve (solubilize) the active substance, the related chemical potential difference of the drug in the vehicle and in the stratum corneum, and the diffusion rate of the drug through the vehicle are the most important (7). The vehicle is usually a liquid that is immobilized by the presence of polymers or solid particles (1). The factors mentioned above are, therefore, related to the properties of the liquid phase; the immobilization of the bulk has little importance for the drug diffusion.

The possibilities of using colloidal structures as ointment

0022-3549/84/1000-1484$01.00/0