To obtain IIb, the residue dissolved in 2 ml of benzene was chromatographed on silica gel. The elution with hexane–ether (7:1 v/v) afforded a first fraction containing a mixture of p-chloronitrobenzene and IIb. The second fraction was evaporated under reduced pressure. The residue, after crystallization from hexane chilled in a dry ice–acetone bath to avoid losses, gave 150 mg of 1-(4-chlorophenyl)-3-*tert*-butylperoxymethyl-3-methyltriazene (IIb), mp 44.5°; UV (ethanol): 218, 222, and 282 nm; NMR: δ 1.23 (s, 9H), 3.30 (s, 3H), 5.52 (s, 2H), and 7.35–7.43 (m, 4H).

Anal.—Calc. for $C_{12}H_{18}ClN_3O_2$: C, 53.04; H, 6.68; N, 15.46. Found: C, 53.03; H, 6.64; N, 15.50.

Compounds Ic and IIc—The residue dissolved in 2 ml of benzene was chromatographed on silica gel. Three fractions resulted from elution with 5% hexane in benzene (v/v). The first fraction was p-bromonitrobenzene. The second fraction, after evaporation of the solvent under reduced pressure, gave 200 mg of 1-(4-bromophenyl)-3-*tert*-butylperoxymethyl-3-methyltriazene (IIc), mp 54°; UV: 218, 222, and 281 nm.

Anal.—Calc. for $C_{12}H_{18}BrN_3O_2$: C, 45.58; H, 5.74; N, 13.29. Found: C, 45.80; H, 5.84; N, 13.10.

The third fraction gave 200 mg of 1-(4-bromophenyl)-3-formyl-3-methyltriazene (Ic), mp 102–104°, after crystallization from methanol and from hexane; UV: 224 and 284 nm; IR: 1733 (C=O) cm^{-1}; NMR: δ 3.37 (s, 3H), 7.55 (m, 4H), and 9.24 (s, 1H).

Anal.—Calc. for $C_8H_8BrN_3O$: C, 39.69; H, 3.33; N, 17.36. Found: C, 39.63; H, 3.30; N, 17.30.

Compounds I*d* and II*d*—The residue was suspended in 2 ml of methanol and filtered. The filtrate was chromatographed on silica gel, and elution with 5% hexane in benzene gave three fractions. The first fraction, which contained p-iodonitrobenzene, was discarded. The second fraction, after evaporation of the solvent, gave 250 mg of 1-(4-iodophenyl)-3-*tert*-butylperoxymethyl-3-methyltriazene (II*d*), mp 56°; UV: 224 and 284 nm.

Anal.—Calc. for $C_{12}H_{18}IN_3O_2$: C, 39.68; H, 4.99; N, 11.57. Found: C, 39.80; H, 4.93; N, 11.30.

The third fraction gave 50 mg of 1-(4-iodophenyl)-3-formyl-3-methyltriazene (I*d*), mp 123–124°, after crystallization from methanol; UV: 230 and 293 nm; IR: 1700 (C=O) cm^{-1}; NMR: δ 3.38 (s, 3H), 7.25–7.95 (m, 4H), and 9.30 (s, 1H).

Anal.—Calc. for $C_8H_8IN_3O$: C, 33.24; H, 2.79; N, 14.54. Found: C, 33.23; H, 2.69; N, 14.45.

REFERENCES

(1) C. Malaveille, G. F. Kolar, and H. Bartsch, *Mutation Res.*, **36**, 1 (1976).

(2) R. Preussmann, S. Ivankovic, C. Landschutz, J. Gimmy, E. Flohr, and U. Griesbach, *Z. Krebforsch.*, **81**, 285 (1974).

(3) T. A. Connors, P. M. Goddard, K. Merai, W. C. J. Ross, and D. E. V. Wilman, *Biochem. Pharmacol.*, **25**, 241 (1976).

(4) G. F. Kolar and R. Preussmann, *Z. Naturforsch.*, **26**, 950 (1971).

(5) P. Kleihues, G. F. Kolar, and G. F. Margison, *Cancer Res.*, **36**, 2189 (1976).

(6) G. F. Kolar, "Advances in Antimicrobial and Antineoplastic Chemotherapy," Proceedings of the 7th International Congress of Chemotherapy, Prague, 1971, vol. II, Urban and Schwarzenberg, Munich, West Germany, 1972, p. 121.

(7) A. Gescher, J. A. Hickman, R. J. Simmonds, M. F. G. Stevens, and K. Vaughan, *Tetrahedron Lett.*, **50**, 5041 (1978).

(8) G. F. Kolar and R. Carubelli, *Cancer Lett.*, **7**, 209 (1979).

(9) V. Zvěřina, J. Diviš, J. Marhold, and M. Matrka, *Collect. Czech. Chem. Commun.*, **36**, 1598 (1971).

(10) W. C. Cornelius, K. Terada, and B. E. Bryant, *Inorg. Synth.*, **6**, 147 (1960).

ACKNOWLEDGMENTS

The authors thank Dr. A. Bigotto, Istituto di Chimica Fisica, Università di Trieste, for advice in the examination of IR spectra and Mr. Paolo D'Errico for elemental analyses and technical assistance.

Stability of Solid Drugs: Degradation of Ergocalciferol (Vitamin D_2) and Cholecalciferol (Vitamin D_3) at High Humidities and Elevated Temperatures

L. T. GRADY and K. D. THAKKER ˣ

Received November 1, 1979, from the *United States Pharmacopeia, Rockville, MD 20852.* Accepted for publication March 17, 1980.

Abstract □ Ergocalciferol and cholecalciferol powders were studied at 25 and 40° and at different humidities. Ergocalciferol decomposed rapidly at 25 and 40° when stored in dry air. Decomposition of ergocalciferol led to the formation of products of higher polarity. Cholecalciferol was not as labile under dry conditions, but decomposed rapidly at high temperature.

Keyphrases □ Vitamin D—ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), effects of temperature and humidity on decomposition □ Stability—ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), effects of humidity and temperature □ Ergocalciferol—stability, effects of temperature and humidity □ Cholecalciferol—stability, effects of temperature and humidity

Ergocalciferol and cholecalciferol are fat-soluble vitamins essential for their antirachitic activity. The adverse effects of light, air, and temperature on solutions of these 9,10-secosterols are well documented (1–4).

Previous studies on the solid-state decomposition of ergocalciferol (initially a white, crystalline powder) showed the formation of a yellow powder having a lowered melting point (5). These observations also were made in this laboratory. GLC analysis of ergocalciferol and cholecalciferol resulted in the decomposition of the parent product, with the formation of two peaks (6). These two peaks were identified as pyro- and isopyro vitamin D[1], formed as a result of thermal cyclization of vitamin D (6). The decomposition of ergocalciferol in powder preparations was reported to depend directly on the surface acidity of the excipients and their ability to adsorb moisture (7).

These studies on the decomposition of vitamin D are valuable but do not predict its stability under normal storage conditions. A labile reference material such as vitamin D must be stored at optimum conditions. Therefore, to determine these conditions, the effects of temperature and humidity on the stability of ergocalciferol and cholecalciferol in the absence of light were investigated.

[1] Vitamin D refers to vitamins D_2 and D_3.

0022-3549/80/0900-1099$01.00/0

© 1980, American Pharmaceutical Association

Table I—Degradation of Ergocalciferol and Cholecalciferol at 25°

Days	Ergocalciferol Remaining[a,b], %			Cholecalciferol Remaining[a,b], %		
	In a Desiccator	85% RH	Anaerobic	In a Desiccator	85% RH	Anaerobic
1	101, 106	101, 101	100			99
7	99, 97	99, 100		99, 96	99, 96	
15	88, 89, 88, 87	97, 96	100, 98	100, 100	99, 100	101, 103
25	71, 72	100, 100	98	100, 101, 100	91, 90	99, 100
56	3, 5, 4, 2	79, 77	101, 100	99, 99	86, 89	101, 103

[a] Samples always were stored in the dark. [b] Individual values are individual assays of separate weighings, and each is based on an average of at least two injections. When more than two values appear in a single block, they were obtained at 6-month intervals as a check of reproducibility.

Table II—Degradation of Ergocalciferol and Cholecalciferol at 40°

Days	Ergocalciferol Remaining[a,b], %		Cholecalciferol Remaining, %	
	85% RH	45% RH	85% RH	45% RH
0.66	100, 97, 98	99, 99	80, 79	85, 82
1	99, 99, 98	96, 97, 97	65, 63	75, 79
3	91, 94	91, 91	49, 47	69, 68
7	89, 86, 85	20, 19	20, 15, 16	62, 69
10	78, 78, 78, 79	—[c]	7, 10, 9	
14	51, 52			
21	35, 33, 31	—[c]	3, 3	4, 3

[a,b] Same as in Table I. [c] Not detectable.

EXPERIMENTAL

Reagents—Ergocalciferol and cholecalciferol USP[2] were used as received. Hexane[3] and 1-pentanol[4] were high-pressure liquid chromatographic (HPLC) grade.

Controlled-Temperature and Humidity Microenvironments—Commercial constant-humidity ovens[5] were used to achieve 40° with 85% relative humidity (RH) and 40° with 45% RH. For all of the other controlled-temperature–humidity microenvironments, saturated salt solution systems (8) were used to achieve constant humidity in a thermostatically regulated room (25 ± 1°).

A glove box[6] was used to achieve dry, anaerobic conditions as follows. It first was allowed to equilibrate to very low humidity by placing flat dishes of silica gel in it for 2 days. The dry air then was evacuated, and dry nitrogen was flushed throughout the glove box; this evacuation–flushing cycle was repeated twice. While the glove box was used, dry nitrogen was bled into it continuously at a low rate to ensure an anaerobic environment above the samples.

Sample Assay—The assay to determine the percentage of vitamin D remaining in the preweighed samples after storage at specified time intervals was based on the method described by deVries *et al.* (9). The gradient elution high-pressure liquid chromatograph[7] was equipped with a variable-wavelength UV detector[8], a recorder[9], and an integrator[10]. A commercial liquid chromatographic column[11] packed with microporous silica was the stationary phase. A mixture of 1-pentanol and dry hexane (9) was the mobile phase. The concentration of 1-pentanol in hexane was varied (0.05–0.1% v/v) when necessary to meet the system suitability test.

System Suitability Test—A mixture of cholecalciferol and its known isomers, *e.g.*, precholecalciferol lumisterol, isotachysterol, tachysterol, and 7-dehydrocholesterol, was available from samples left from a previous collaborative study (9). This mixture was injected onto the HPLC column. The resolution, R, between cholecalciferol and tachysterol and the resolution between precholecalciferol and *trans*-cholecalciferol were determined by:

$$R = 2D/B + C \quad \text{(Eq. 1)}$$

where D is the distance between the peak maxima and B and C are the peak widths of the two peaks.

The system conditions were adjusted to obtain $R > 1.0$ for cholecalciferol–tachysterol peaks and $R > 0.8$ for precholecalciferol–*trans*-cholecalciferol peaks. Under these conditions, separation of all isomers listed was possible; their elution order remained fixed so that the peaks could be identified by comparison with reported chromatograms (9).

Procedure—About 1–2 mg of ergocalciferol or cholecalciferol was weighed on an electronic balance[12] as quickly as possible and transferred to a disposable, aluminum weighing boat. Weighings were done in subdued light only. The samples were introduced immediately into the various controlled-temperature–humidity microenvironments. At the designated time intervals, the samples were removed from the microenvironments, dissolved promptly in the mobile phase, and transferred to a 10-ml volumetric flask. The sample dish was washed successively with small volumes of the mobile phase to ensure quantitative transfer. After the volume was adjusted to 10 ml, ~10 μl was injected onto the chromatographic column with a loop injector[13].

Standard Solution—About 1–2 mg of ergocalciferol USP or cholecalciferol USP, accurately weighed, was transferred to a 10-ml volumetric flask and dissolved in the mobile phase. After the volume was adjusted to 10.0 ml, 10 μl of the resulting solution was injected into the HPLC column[11] with a loop injector[13]. The standard solution was prepared fresh daily and was injected at least three times for each assay.

Calculations—The integrator counts for the peak due to ergocalciferol or cholecalciferol (duplicate injections) were compared with the area counts obtained for the peak due to ergocalciferol USP or cholecalciferol USP in the standard solution, and the percentage of ergocalciferol or cholecalciferol remaining in the samples was calculated with a correction applied for the weights of the powders.

Other Tests—Reversed-phase HPLC separation of ergocalciferol and its decomposition products also was performed. The same liquid chromatograph[7] was used as in the assay of decomposed samples. The stationary phase consisted of octadecylsilane chemically bonded to microporous silica[14], and the mobile phase was methanol–acetonitrile (50:50 v/v).

TLC also was used to separate ergocalciferol and the decomposition products. The following four systems were used on silica gel[3] plates: 1, cyclohexane–ether (1:1 v/v); 2, chloroform; 3, chloroform–ether–methanol (4:4:2 v/v/v); and 4, toluene–hexane–ether (2:2:1 v/v/v). All solvents were chromatographic grade[4].

RESULTS

Table I shows the stability of ergocalciferol and cholecalciferol at room temperature for 56 days. In general, ergocalciferol was more labile at room temperature in the presence of a desiccant. After 21 days, samples stored in dry air (in a desiccator) had only ~66% remaining; those stored under very moist air (85% RH) or anaerobic (dry) conditions (dry nitrogen box) had >95% remaining. On the other hand, cholecalciferol was more stable

[2] Provided by Philips-Duphar, Weesp, The Netherlands.
[3] E. Merck, Darmstadt, West Germany.
[4] Fisher Scientific Co., Fair Lawn, N.J.
[5] Blue-M Electric Co., Blue Island, Ill.
[6] Lab-Con-Co., Kansas City, Mo.
[7] Varian Associates, Palo Alto, Calif.
[8] Gilson Medical Electronics, Middleton, Wis.
[9] Linear Instruments, Irvine, Calif.
[10] Spectra-Physics, Santa Clara, Calif.
[11] Zorbaxsil, Dupont Instruments, Wilmington, Del.
[12] Mettler Instruments Corp., Princeton, N.J.
[13] Valco Instruments, Houston, Tex.
[14] Micropak MCH-10, Varian Associates, Palo Alto, Calif.

Table III—Degradation of Ergocalciferol and Cholecalciferol on Different Container Surfaces

Container	Isomer Remaining after 7 Days at 40° and 45% RH, %	
	Ergocalciferol	Cholecalciferol
Aluminum weighing boats	16, 17	69, 62
Glass weighing dishes	19, 20	69, 69
Polystyrene weighing boats (disposable)	38, 35	66, 72
High-density polyethylene bottles	26, 30	73, 73
Polypropylene bottles (multiple-unit containers)	45	76

at room temperature. After 21 days, cholecalciferol samples stored under all three conditions had >99% remaining, indicating no degradation.

After 15 days, ergocalciferol samples that were stored in a desiccator acquired a yellow tinge, which deepened as time progressed. On thin-layer chromatograms, separation of ergocalciferol and the decomposition products was obtained in System 1, but the new spot due to the decomposition product remained at the origin. There were no extra peaks on the chromatograms of ergocalciferol (obtained during adsorption chromatography), although the samples turned distinctly yellow. However, separation of ergocalciferol and the degradation products was obtained on reversed-phase liquid chromatograms. The decomposition products from a yellowed sample of ergocalciferol eluted with the solvent front (Fig. 1).

Table II shows the stability of ergocalciferol and cholecalciferol at 40°. Both compounds were less stable at elevated temperatures. After 7 days, samples of ergocalciferol stored at 40° and 85% RH had 87% remaining; those stored at 40° and 45% RH had only 20% remaining. Both samples had turned yellow. Cholecalciferol showed the reverse trend. After 7 days, samples stored at 40° and 85% RH had 15% of the cholecalciferol remaining; those stored at 40° and 45% RH had about 65% of the cholecalciferol remaining.

Table III shows the stability of ergocalciferol and cholecalciferol (at 40° and 45% RH for 7 days) on different types of containers (surfaces) used as sample dishes. Both ergocalciferol and cholecalciferol were more stable when stored on plastic containers than when stored on glass or aluminum weighing dishes. In all cases, cholecalciferol was more stable than ergocalciferol.

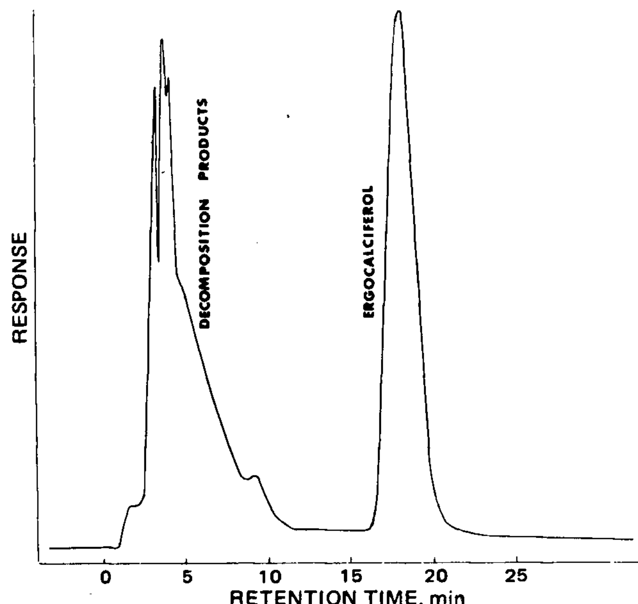


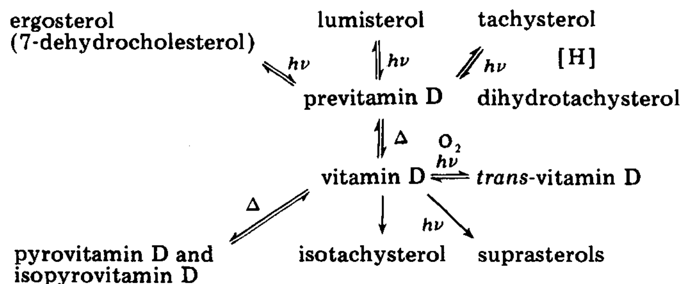
Figure 1—*Chromatogram of a partially decomposed, yellowed sample of ergocalciferol on Micropak MCH-10. The mobile phase was methanol–acetonitrile (50:50 v/v).*

DISCUSSION

Solutions of ergocalciferol and cholecalciferol undergo thermal, photochemical, and oxidative reactions that lead to a host of degradation products (1–4). Scheme I shows the known pathways of decomposition for these compounds.

Powder preparations of ergocalciferol were reported to undergo isomerization, which resulted in the formation of 5,6-*trans*-vitamin D_2 (7). Neither this isomer nor any known ergocalciferol isomer (Scheme I) was present in any decomposed sample. Since Scheme I lists reactions involving the sterol nucleus, which require considerable freedom of molecular motion, the absence of these isomers in decomposed ergocalciferol samples indicates that such freedom of molecular motion is not available to molecules of ergocalciferol for solid-state decomposition.

The effect of moisture on reactions of ergocalciferol is apparent. Moisture adsorbed on the surface of the molecules seems to inhibit the decomposition of ergocalciferol. Such inhibition by moisture adsorbed by the excipients was reported for some powder preparations of ergocalciferol (7). Furthermore, the decomposition is faster in the presence of air since oxygen is required. In short, it can be inferred that the solid-state decomposition reactions of ergocalciferol in the absence of light is of a dry, oxidative type.



Scheme I—Some known reactions of vitamin D and related compounds

The decomposition reactions of cholecalciferol apparently differ from these of ergocalciferol (Tables I–III). Cholecalciferol was more stable than ergocalciferol under all but one condition. The sharp decomposition of ergocalciferol in a dry aerobic atmosphere was not seen with cholecalciferol. Decomposition of cholecalciferol was a little faster at the more humid of the two conditions at 40°. This finding was the exact opposite of the behavior seen for ergocalciferol.

Since the only structural difference between the two molecules is in the side chain, the solid-state decomposition reactions of both compounds must involve the side chain only. This observation also is consistent with the observation that no known isomers involving reactions of the sterol nucleus are found. If the sterol nucleus were to take part in the solid-state decomposition of ergocalciferol and cholecalciferol, such differences as were obtained on decomposition of these two compounds (Table II) would not have been obtained. A double bond in the side chain of ergocalciferol must make it a substrate for attack by oxygen. Since the solid-state reactions must occur with little freedom of movement, the decomposition reactions of ergocalciferol (involving the double bond of the side chain and oxygen) may occur *via* a free radical pathway. Free radical reactions can be accomplished in the solid state and may be effected more readily by the presence of molecules with high dipole moment. Cholecalciferol is stable at room temperature but is quite labile at 40°; thus, the decomposition reaction of cholecalciferol seems to have a low activation energy.

The nature of the decomposition products of ergocalciferol or cholecalciferol is not known, and their chromatographic behavior is puzzling. Normal-phase liquid chromatography and TLC revealed that the decomposition products bound strongly to the silica supports. Reversed-phase liquid chromatography of the same decomposition products revealed that they had a highly polar nature as seen by their fairly high solubility in methanol. These observations cannot be clarified further until more information is gathered to allow proposal of a mechanism for decomposition reactions of ergocalciferol and cholecalciferol.

REFERENCES

(1) T. Kanzawa and S. Kotaku, *J. Pharm. Soc. Jpn.*, **73**, 1357 (1953).

(2) K. S. Keuning, *Pharm. Weekbl.*, **100**, 889 (1965).

(3) L. Velluz, G. Amiard, and A. Petit, *Bull. Soc. Chim. Fr.*, **1949**, 501.

(4) K. H. Hanewald, M. P. Rappoldt, and J. R. Ruborgh, *Recueil*, **80**,

1003 (1961).
(5) S. R. Byrn, *J. Pharm. Sci.*, **65**, 16 (1976).
(6) P. P. Nair and S. deLeon, *Arch. Biochem. Biophys.*, **128**, 663 (1968).
(7) T. Takahashi and R. Yamamoto, *Yakagaku Zasshi*, **89**, 909 (1969).
(8) "Handbook of Chemistry and Physics," 53rd ed., Chemical Rubber Co., Cleveland, Ohio, 1972–1973, p. E-40.
(9) E. J. deVries, J. Zeeman, R. J. E. Esser, B. Borsje, and F. J. Mulder, *J. Assoc. Off. Anal. Chem.*, **62**, 129 (1979).

Synthesis and Antidepressant Activity of 5-(4-Dimethylaminobenzyl)imidazolidine-2,4-dione

F. L. WESSELS ˣ, **T. J. SCHWAN, and S. F. PONG**

Received November 21, 1979, from the *Scientific Affairs Department, Norwich-Eaton Pharmaceuticals, Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication March 24, 1980.

Abstract □ 5-(4-Dimethylaminobenzyl)imidazolidine-2,4-dione was prepared by catalytic hydrogenation of the corresponding benzylidene compound. Antidepressant testing in mice indicated that its ED_{50} values for antagonism of tetrabenazine-induced ptosis and potentiation of levodopa-induced behavioral changes were 42 and 17 mg/kg po, respectively. *In vitro* neurochemical studies demonstrated that this compound did not inhibit the uptake of selected biogenic amines into crude synaptosomes of mouse whole brain, and it did not have significant monoamine oxidase inhibitory activity *in vivo* and *in vitro*. Thus, this compound possesses potential antidepressant activity with a mechanism different from that of the tricyclic antidepressants and monoamine oxidase inhibitors.

Keyphrases □ 5-(4-Dimethylaminobenzyl)imidazolidine-2,4-dione—synthesis and testing for antidepressant activity □ Antidepressant activity—5-(4-dimethylaminobenzyl)imidazolidine-2,4-dione, *in vitro* and *in vivo* testing

The tricyclic antidepressant drugs are the most useful drugs for the treatment of depression. However, these drugs are not effective in all patients and are not without compromising side effects (1, 2). Monoamine oxidase inhibitors also are effective antidepressants; however, because of their propensity to cause hypertensive crisis, they now have limited use in the treatment of depression (3).

As part of a program directed at the discovery and development of novel agents affecting the central nervous system (CNS), 5-(4-dimethylaminobenzyl)imidazolidine-2,4-dione (I) was synthesized as a chemical intermediate and unexpectedly demonstrated antidepressant activity in selected experimental models (4). This paper presents the synthesis and results of pharmacological testing of this compound.

EXPERIMENTAL[1]

Synthesis of 5-(4-Dimethylaminobenzyl)imidazolidine-2,4-dione (I)—A mixture of 69.3 g (0.30 mole) of 5-(4-dimethylaminobenzylidene)imidazolidine-2,4-dione (IV) (5), 600 ml of 1 *N* KOH, and 15 g of wet No. 28 Raney nickel catalyst was shaken with hydrogen at 3–4 atm for 28 hr until the theoretical quantity was consumed. The catalyst was washed with two 100-ml portions of 1 *N* KOH, and the filtrate and combined washings were acidified with acetic acid to give the crude product (60 g). Recrystallization from methanol gave 35 g (50%) of I, mp 180–186°; IR: 3.10, 3.21 (NH), 5.66, 5.90 (C=O), and 6.20 (C=C) μm; NMR (dimethyl sulfoxide-d_6): δ 2.75–2.85 [m, 8, $(CH_3)_2N$ and aryl CH_2], 4.20 (t, 1, CH_2CHNH), 6.60, 7.01 (d, 4, J = 9 Hz, aromatic CH), 7.78 (broad exchangeable s, 1, imidazolidine 1-NH), and 11.0 (broad exchangeable m, 1, imidazolidine 3-NH).

Anal.—Calc. for $C_{12}H_{15}N_3O_2$: C, 61.78; H, 6.48; N, 18.02. Found: C, 61.79; H, 6.45; N, 18.02.

Tetrabenazine Antagonism—The method used was similar to that described by Barnett *et al.* (6). Groups of five unfasted male mice (TAC:SW/N fBr), 20–27 g, were pretreated with the test compound at 50 mg/kg po as a 0.5% suspension in 0.5% methylcellulose 4000 cps[2] or methylcellulose alone. Thirty minutes later, each animal received tetrabenazine methanesulfonate[3] (36 mg/kg ip in saline) or saline at a volume of 10 ml/kg. After an additional 30 min, the degree of palpebral narrowing was estimated as a measure of ptosis and compared to the methylcellulose and tetrabenazine control groups. The degree of ptosis was evaluated using a rating scale of 0–4, with a score of 4 representing a normal palpebral opening and scores of 3, 2, 1, and 0 representing slight, moderate, marked, and complete active closure of the palpebral opening, respectively. The percent prevention of ptosis was calculated as described by Barnett *et al.* (6).

Compound I at a dose of 50 mg/kg antagonized the effect of tetrabenazine by 50% or greater and, therefore, was evaluated further for dose-response effect as an ED_{50} value. The method described by Litchfield and Wilcoxon (7) was used for determining the ED_{50} value.

Levodopa Potentiation—The levodopa potentiation model is a reflection of increased dopaminergic activity, and the method used was that described by Everett (8). Groups of unfasted male mice [TAC:(SW)fBr] were administered pargyline[4] at a dose of 40 mg/kg po (0.4% in 0.5% methylcellulose) 4 hr prior to the administration of graded doses of test drugs or methylcellulose only. One hour after administration of the test drugs, levodopa (100 mg/kg ip, 1% in saline) was administered to all mice.

Observations of the levodopa-induced response were recorded 25 min after administration on a scale similar to that described by Everett (8). The degree of potentiation was scored as: 0 = no unusual irritability; 1 = increased alertness and responsiveness to touch; 2 = aggressive response to touch (hyperreactive); and 3 = markedly aggressive response to touch, spontaneous fighting with salivation and piloerection, or near prostrate. The percent of levodopa potentiation was calculated as described by Everett (8). The ED_{50} values were obtained using the method described by Litchfield and Wilcoxon (7).

Tryptamine Potentiation—Tedeschi *et al.* (9) hypothesized that tryptamine increases brain serotonin levels but that convulsions do not occur because the destruction of serotonin by monoamine oxidase keeps pace with its formation. The interruption of the metabolism of serotonin (*i.e.*, by monoamine oxidase inhibitors) and tryptamine will cause convulsions. The method used was similar to that described by Tedeschi *et al.* (9). Groups of 10 unfasted male mice [TAC:(SW)fBr] were administered the test compounds suspended in 0.5% methylcellulose at a dose 10 times their levodopa potentiation ED_{50} values. Four hours later, tryptamine[5] was administered at 50 mg/kg iv. The compounds were considered to potentiate tryptamine if >50% of the animals in the treated group convulsed.

[1] Melting points were determined on a Mel-Temp apparatus and are corrected. The IR spectrum was determined as a mineral oil mull using a Perkin-Elmer 137B spectrophotometer. The NMR spectrum was obtained on a Varian A-60 instrument and was compared with tetramethylsilane as the internal standard.

[2] Methocel, Dow Chemical Co.
[3] Hoffmann-La Roche.
[4] Abbott Laboratories.
[5] Aldrich Chemical Co.

0022-3549/80/0900-1102$01.00/0
© 1980, American Pharmaceutical Association