al of the solvent in vacuo the residual oil solidified on scratching to give the ω-nitroacetophenone.

Hydrolysis Products. All ω-nitroacetophenones were hydrolyzed with dilute aqueous NaOH at room temperature, a procedure which could equally well be applied to the hydrolysis of **3**.

Method. A mixture of the ester (0.10 mol) and 5% aqueous NaOH (400 ml) was stirred at room temperature for 1 hr, acidified, and allowed to stand in ice for a further 30 min. Those compounds having no alkyl or aryl substituent in the side chain (**2c,n,r**) crystallized at this stage and after filtration were purified by recrystallization. Compounds bearing these substituents in the side chain, however (**2g–i,p**), separated as oils. Isolation in this case was by $CHCl_3$ extraction, drying ($MgSO_4$), and solvent removal in vacuo. After trituration of the oily residue with ligroine the products crystallized and were purified by recrystallization.

Rat PCA Test. The rat PCA test and the evaluation of the results was carried out as previously described.[1]

Compounds **2j**, **2k**, and **2l** were injected subcutaneously as a suspension in isotonic saline buffered to pH 7 with phosphate buffer, PBS (Bacto Hemagglutination buffer, Difco Laboratories), and containing 0.5% methylcellulose, PBS/MC. Compound **2n** was given subcutaneously as a solution in PBS neutralized to pH 7 with sodium bicarbonate. The compounds were free of nitroindandiones and these did not form under the condition in which the compounds were dissolved or suspended prior to administration.

Bioconversion of the Methyl Ester of 2-Carboxy-4,5-dimethyl-ω-nitroacetophenone (2j) to 5,6-Dimethyl-2-nitroindandione (9). In Vivo. Groups of three male Wistar rats of 250–350 g were bled by cardiac puncture under halothane anaesthesia 1 hr after receiving a subcutaneous dose of compound **2j** of 50 mg/kg as a suspension in PBS/MC. The blood was allowed to clot over a period of 5 min and the serum was separated by centrifuging. The serum was assayed for the presence of the nitroindandione **9** and compound **2j**.

a. **Uv Spectroscopy.** 5,6-Dimethyl-2-nitroindandione (**9**) exhibits a pronounced uv absorbance at 353 nm (ϵ^{H_2O} 23,200) and a smaller absorbance at 316 nm (ϵ^{H_2O} 16,300) whereas the acetophenone **2j** shows only a small absorption in this range at 343 nm (ϵ^{EtOH} 2946). By direct comparison of the intensity of the 353 nm peak with that at 316 nm therefore, a qualitative evaluation of the dimethylindandione **9** could be made in the presence of **2j**. The evaluation was facilitated by initial deproteination of the serum with trifluoroacetic acid followed by extraction into butanol. Using this procedure cyclized **9** was detected in the serum taken from rats that had been dosed subcutaneously 60 min previously.

b. **High-Pressure Liquid Chromatography (HPLC).** A better assessment of 5,6-dimethyl-2-nitroindandione (**9**) in the presence of **2j** could be carried out using HPLC since each compound could be individually measured and quantitated. Under our conditions using a Bondapack phenyl corasil column the cyclic material **9** had a retention time of 7.5 min, compared with 9.0 min for **2j**. Moreover, the need to deproteinate the serum was no longer important since identification of each component could be achieved by scanning the eluent with light at 353 nm. Evidence for up to 16 μg/ml of the cyclic material **9** at 60 min after subcutaneous injection of **2j** to rats was found. Residual **2j** up to levels of 32 μg/ml was also found.

In Vitro. Fresh rat livers, of about 15 g, were washed with 0.1 *M* Tris–HCl buffer, pH 7.9, containing 10% sucrose (Tris–sucrose), and then homogenized at 4° in 10 ml of Tris–sucrose for 2 min using an electric homogenizer (Ultra-turrax). The suspension was then centrifuged for 15 min at 4000 rpm and the layer between the solid debris and the fat was separated to give undiluted liver extract. A diluted liver extract was prepared from this by diluting to four times its own volume with Tris–sucrose, centrifuging for 10 min at 4000 rpm, and collecting the supernatant. Equal volumes of solutions of **2j** or **9** in PBS neutralized with sodium bicarbonate were added to Tris–sucrose, fresh rat serum, diluted liver extracts, and undiluted liver extracts to give a final concentration of **2j** or **9** in solution of 100 mg/ml. The solutions were incubated at 37° or stood at room temperature and then assayed for the presence of **2j** or **9** using HPLC calibrated with standard solutions of **2j** and **9**.

When **2j** was stood in undiluted rat liver extract at room temperature for 2 hr there was a 92% conversion to **9** and a 67% conversion after 1 hr and 13 min. When **2j** was incubated at 37° in the diluted rat liver extracts there was a progressive increase in conversion of **2j** to **9** with time so that there was a 78% conversion after 3 hr. Incubation of **2j** in fresh rat serum or Tris–sucrose for 3 hr at 37° gave no conversion to **9**. Compound **9** was stable to liver extracts.

Acknowledgment. The authors express their thanks to Mr. W. Neville for the HPLC work.

References and Notes

(1) D. R. Buckle, N. J. Morgan, J. W. Ross, H. Smith, and B. A. Spicer, *J. Med. Chem.*, **16**, 1334 (1973).
(2) L. P. Zalukaev, *J. Gen. Chem. USSR* (*Engl. Transl.*), **26**, 1039 (1956).
(3) E. H. Rodd, "Chemistry of Carbon Compounds", Vol. III, Elsevier, New York, N.Y., 1956, p 833.
(4) D. R. Buckle and H. Smith, paper presented at the 167th National Meeting of the American Chemical Society, Los Angeles, Calif., 1974.
(5) J. Freimanis and G. Vanags, *Zh. Obshch. Khim.*, 31, 1945 (1961); *Chem. Abstr.*, 55, 27229 (1961).
(6) G. Vanags, J. Freimanis, and G. Zakis, *Zh. Obshch. Khim.*, 27, 2509 (1957); *Chem. Abstr.*, **52**, 7247e (1958).

Synthesis of an Active Hydroxylated Glutethimide Metabolite and Some Related Analogs with Sedative–Hypnotic and Anticonvulsant Properties

Hassan Y. Aboul-Enein,* Charles W. Schauberger, Alfred R. Hansen, and Lawrence J. Fischer

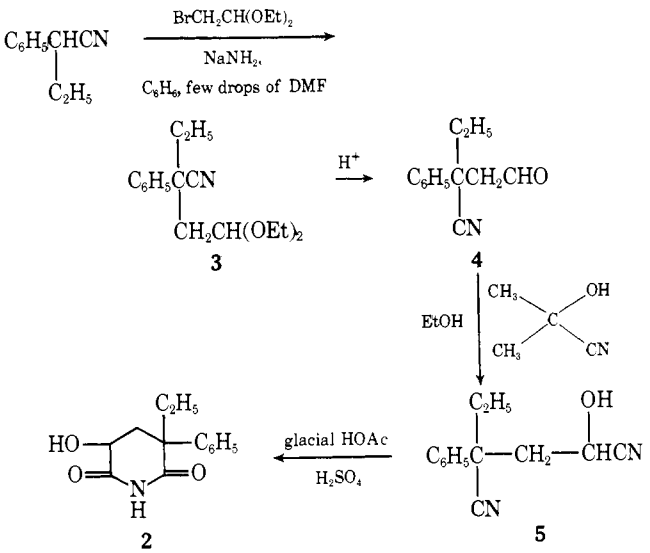
The Toxicology Center, Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa 52242. *Received January 6, 1975*

Two synthetic pathways are described for the preparation of 4-hydroxy-2-ethyl-2-phenylglutarimide (**2**), an active hydroxylated metabolite of glutethimide (**1**). Fourteen other glutethimide analogs were also synthesized and tested for biological activity. Most of the analogs exhibited sedative–hypnotic properties and compound **2** possessed the greatest activity compared to the parent drug. 4-Amino-2-ethyl-2-phenylglutarimide and 4-hydroxy-2-ethyl-2-phenylglutaconimide (**13**) exhibited the greatest potential as anticonvulsant agents. The structure–activity relationships of the series are discussed.

Glutethimide [(±)-2-ethyl-2-phenylglutarimide, **1**] is a nonbarbiturate sedative–hypnotic agent. The metabolism of **1** has been studied in the rat, dog, guinea pig, and man and has been shown to involve hydroxylation at several sites in the molecule.[1–4] Injection of the glucuronide conjugates of hydroxylated metabolites of **1** into mice produced no sedative effect.[5] In a more recent study, Ambre and Fischer[6] showed that a hydroxylated metabolite of **1** accumulates in the plasma of humans intoxicated by glutethimide overdose. This metabolite was subsequently isolated[7] from the urine of dogs given large doses of **1** and was chemically identified as 4-hydroxy-2-ethyl-2-phenylglutarimide

*Address correspondence to this author at USV Pharmaceutical Corporation, Tuckahoe, N.Y. 10707.

Scheme I



(2). This isolated metabolite was found to possess twice the sedative–hypnotic activity of 1,[7] which led to the conclusion that its accumulation plays an important role in acute glutethimide poisoning.[8]

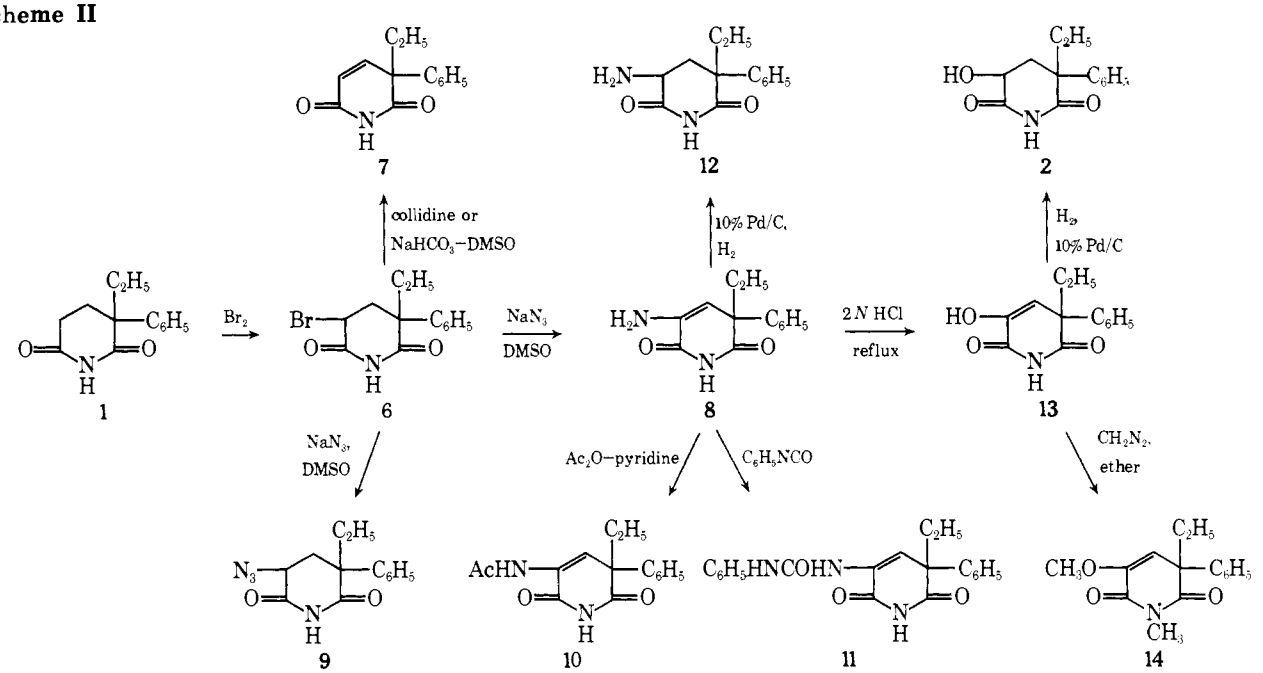
Some glutethimide analogs have been synthesized,[9-13] but none has exhibited sedative–hypnotic activity equal to or greater than 1 itself. Since an isolated metabolite of 1 was found to be more active than the parent drug, we have investigated the possibility that other metabolites and analogs of 1 may possess significant biological activity. We wish to report details of the synthesis of 2 and some other glutethimide metabolites and analogs and to give the results of the pharmacologic tests of these compounds.

Chemistry. The metabolite 2 was synthesized as shown in Scheme I. Using a modification of a published procedure,[14] α-phenylbutyronitrile was alkylated with bromoacetaldehyde diethylacetal to give 3. Compound 3 was hydrolyzed in good yield to the corresponding aldehyde 4 which was then converted to the cyanohydrin.[15,16] Cyclization of 5 was effected by heating at reflux in glacial acetic acid containing sulfuric acid to give a low yield of 2.[17]

A more efficient procedure was finally developed and most of the glutarimide ring-substituted analogs were obtained through that route (Scheme II). 4-Bromo-2-ethyl-2-phenylglutarimide (6) was prepared according to a modified procedure described by Urech et al.[18] The position of the bromine was established by NMR spectroscopy to be at the C_4 position. 2-Ethyl-2-phenylglutaconimide (7) was obtained by dehydrohalogenation of 6 with collidine.[18] However, compound 7 could be obtained in a higher yield by dehydrohalogenation of 6 with sodium bicarbonate in DMSO.[19] The reaction between sodium azide and 6 in hot aqueous DMSO unexpectedly gave 4-amino-2-ethyl-2-phenylglutaconimide (8),† instead of the anticipated azide 9. The enamine function of 8 could be acetylated to give 10 or reacted with phenyl isocyanate to give the urea derivative 11.

The NMR data from 8 indicated that it existed almost entirely as its enamine tautomer. Catalytic hydrogenation of 8 using 10% Pd/charcoal gave 12. Taking advantage of the ease of hydrolysis of compounds containing an aminovinyl group,[20] we found that when 8 was heated to reflux with 2 *N* HCl in aqueous dioxane 4-hydroxy-2-ethyl-2-phenylglutaconimide (13) was obtained in good yield. Compound 13 gives characteristic enol reactions (e.g., reddish color with $FeCl_3$). When 13 was treated with excess CH_2N_2, 4-methoxy-*N*-methyl-2-ethyl-2-phenylglutaconimide (14) was obtained. Catalytic hydrogenation of 13 gave compound 2 as a diastereoisomeric mixture. The diastereoisomeric pairs of 4-hydroxy-2-ethyl-2-phenylglutarimide (2) were separated using TLC (see Experimental Section).

Scheme II

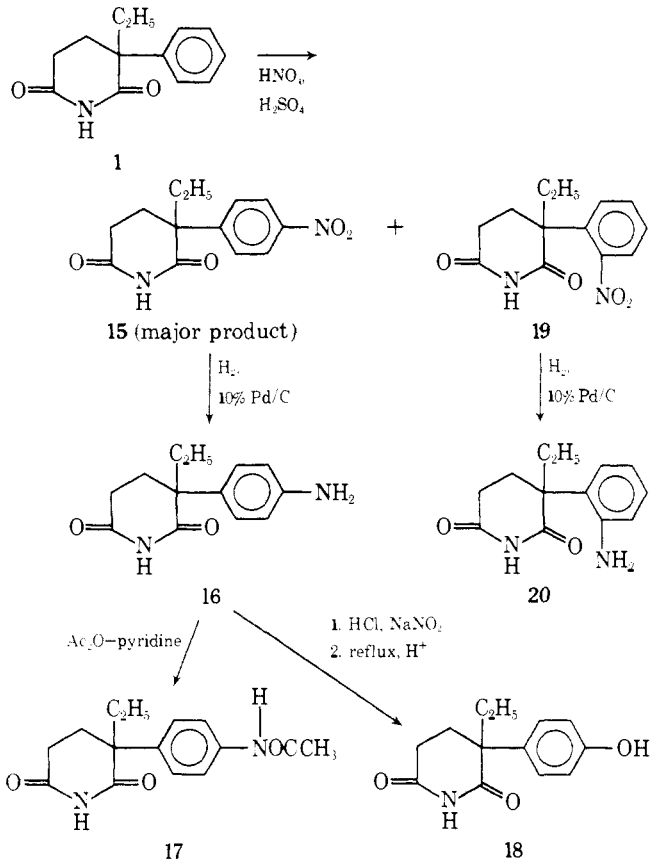


The aromatic-substituted glutethimide analogs were prepared according to previously reported procedures[21] (Scheme III). 2-(*p*-Hydroxyphenyl)-2-ethylglutarimide (18) was prepared by diazotization of 16 followed by reflux in acidic medium.

Pharmacology. The compounds synthesized as previously described were subjected to a screening procedure in which various types of CNS activity and estimates of potency were determined.[24] Sedative–hypnotic activity, as determined by the screening procedure in mice, was usually manifested by positional passivity, impaired righting, and ataxia. All compounds tested showed a sedative–hypnotic

†A full account on the mechanism of this reaction is submitted for publication elsewhere.

Scheme III



profile with the exception of compound 11, which produced no discernible pharmacologic effects (Table I). No CNS effects, other than sedative–hypnotic activity, were observed during the screening procedure. The compounds (except 11) elicited sedative–hypnotic effects at doses of 33–333 mg/kg and the minimum lethal dose ranged from 333 to 1000 mg/kg (Table I). Seven compounds showing relatively high potency as CNS depressants were subjected to further tests which included quantitative determinations of potency as sedative–hypnotics[25] and anticonvulsants[26] along with an estimation of lethality.[27] Data derived from these tests are shown in Table II. All of the analogs except compound 2 had sedative–hypnotic activity but of a lower potency than glutethimide. 4-Hydroxy-2-ethyl-2-phenylglutarimide (2) had an onset of action similar to glutethimide and for hypnotic and anticonvulsant activities this latency was approximately 10 min.

The therapeutic index of 4-hydroxy-2-ethyl-2-phenylglutarimide (2) in mice was similar to that of glutethimide (1) (Table II). Because of differences between laboratory animals and man in the metabolism and elimination of the two compounds, the metabolite 2 may have a greater margin of safety than 1 in man. This is because much of the toxicity seen following glutethimide (1) overdose in man is attributable to the accumulation of 2, a potent, active metabolite.[8b] Since 2 does not accumulate in mice following toxic doses of 1 (unpublished observations), the therapeutic indices determined in this species reflect only the relative toxicities of unchanged 1 and unchanged 2. Compound 2 has a higher ED_{50} (rotarod) and LD_{50} than that reported for the metabolite that was isolated from dog urine.[7] The difference must be due to the fact that compound 2 was tested as a diastereoisomeric mixture with possibly one isomer having less potency.

Structure–Activity Relationships. Compound 2 displayed the greatest sedative–hypnotic activity. Replacement of the hydroxyl by an amino group as in 12, a bromo as in 6, or a methoxy as in 14 resulted in decreased activity. Some other glutethimide derivatives having substitution at the 4 position have been reported to exhibit sedative–hypnotic activity.[9] The 2-ethyl-2-phenylglutaconimide derivatives 7, 8, and 13 generally showed lower activity both as sedative hypnotics and as anticonvulsants in comparison with their hydrogenated analogs. Thus, introduction of unsaturation into the glutarimide ring generally decreases activity. However, it is interesting to note that mice given compound 8 (333 mg/kg) slept for as long as 36 hr with full recovery. Usual sleeping time for glutethimide (100 mg/kg) was about 1 hr. Further research on the mechanism of the long sleeping time of this analog is indicated.

Replacement of the aromatic para and ortho hydrogen in 1 by a nitro group as in 15 and 19, respectively, an amino group as in 16 and 20, respectively, or a *p*-hydroxy group as in 18 caused a reduction in sedative hypnotic activity (Table I). It is apparent from the data that ortho substitution in the aromatic ring produced further decreases in the sedative hypnotic activity when compared to the para-substituted analogs.

Some substitutions in the glutarimide ring of the parent compound 1 enhanced anticonvulsant activity relative to sedative–hypnotic activity. The ratio between rotarod ED_{50} and minimum electroshock (MES) ED_{50} serves as a guide to the potential usefulness of a compound as an anticonvulsant. A high ratio may indicate a compound which could be used as an anticonvulsant with a relative lack of undesirable sedative effects. Compounds 12, 13, and 16 exhibited relatively high ratios of sedative–hypnotic/anticonvulsant activity compared to compound 1 as shown in Table II.

Hydroxylation at C_4 in the glutaconimide ring of 7 increased anticonvulsant activity relative to sedative–hypnotic activity but the same substitution in the glutarimide ring of 1 to produce compound 2 failed to produce this effect. Substitution at C_4 did not alter the relatively high sedative–hypnotic activities of compounds 1 or 7.

It is interesting that anticonvulsant activity was retained after introduction of the amino group at C_4 in the glutethimide molecule as in 12. This substitution resulted in a loss of sedative–hypnotic activity compared to glutethimide.

Table I. Compounds Tested with Primary Screen Technique

Compd	Min sedative–hypnotic dose,[a] mg/kg	Min lethal dose, mg/kg
1	33	333
2	33	333
6	100	2000
7	100	1000
8	100	1000
10	100	1000
12	66	1000
13	100	1000
14	100	1000
15	100	1000
16	100	333
17	333	1000
18	100	
19	333	1000
20	333	1000

[a] That dose which produced marked ataxia in all animals tested. Ataxia was usually accompanied by significantly increased positional passivity and a decreased righting reflex.

Table II. Synthesized Analogs of Glutethimide. Sedative and Anticonvulsant Activity

Compd	Rotarod ED_{50}[a] (confidence limits), mg/kg	MES ED_{50}[b] (confidence limits), mg/kg	LD_{50} (confidence limits), mg/kg	Ratio of rotarod ED_{50}/ MES ED_{50}	Therapeutic index LD_{50}/ rotarod ED_{50}	Therapeutic index LD_{50}/ MES ED_{50}
1 (glutethimide)	45.8 (40.3–53.9)	21.2 (13.7–24.8)	400 (326–474)	2.6	8.3	19.5
2	42.2 (38.3–46.9)	16.1 (12.8–18.0)	340 (300–400)	2.6	8.1	21.1
6	247.0 (215.3–298.0)	97.9 (75.9–118.6)	>2000	2.5	>8.1	>20.4
7	137.6 (112.0–162.7)	79.8 (69.8–90.5)	>2000	1.7	>14.5	>25.1
8	94.8 (84.6–106.8)	41.2 (15.0–53.1)	775 (692–857)	2.3	8.2	18.8
12	95.8 (87.8–105.5)	20.5 (16.9–23.4)	600 (375–824)	4.7	6.3	29.3
13	125.9 (115.1–140.4)	31.0 (12.5–39.0)	616.7 (555.4–678.0)	4.1	4.9	19.9
16	75.2 (62.1–81.7)	14.0 (12.9–17.6)	625 (424–826)	5.4	8.3	44.7

[a] Sedative–hypnotic activity. [b] Anticonvulsant activity.

The anticonvulsant activity of **12** compares favorably with that of **16** where the amino group is at the para position of the aromatic ring. *p*-Aminoglutethimide (Elipten) (**16**) has been used as an anticonvulsant[22] and is also known to inhibit steroid biosynthesis.[23,28] Further studies will be conducted on compound **12** and other analogs to determine their effects on steroid metabolism.

In summary, of the chemical modifications introduced into glutethimide (**1**) either by substitution at the 4 position of the glutarimide ring or by ortho and para aromatic substitution, compound **2** showed the most promising activity as a sedative–hypnotic agent. Compounds **12** and **13** showed the most potential as anticonvulsant agents.

Experimental Section

Melting points were determined on a Thomas-Hoover (open capillary) apparatus or on Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 457 spectrophotometer and NMR data with a Varian T-60 spectrometer using tetramethylsilane as an internal standard. The ir and NMR spectra of each compound were consistent with the assigned structure. Elemental analyses were performed by PCR, Inc., Gainesville, Fla., and were within ±0.4% of the theoretical values. Glutethimide was donated by USV Pharmaceutical Corp., Tuckahoe, N.Y.

Mass spectra were obtained on a Finnagan Model 1015SL quadrupole mass spectrometer. Catalytic hydrogenations were carried out in a Parr hydrogenator at a starting pressure of 60 lb/in.^{2} of hydrogen. Gas chromatographic analyses were performed on a Varian aerograph series 1400 to check for purity of the compounds used in pharmacologic tests.

Pharmacology. Methods. Pharmacological testing was conducted using 20–25-g male albino Swiss–Webster mice (Biolabs, Minneapolis, Minn.). The compounds were suspended in 2% carboxymethylcellulose (CMC) and administered intraperitoneally (0.1 ml/10 g of body weight). A single individual carried out all testing to eliminate variation in handling and grading of responses. Potency values were calculated using probit analysis.[27] The compounds tested were chromatographically pure and those substituted in the glutarimide ring were used as the mixture of diastereoisomers.

The primary screening technique utilized was the comprehensive observations assessment described by Irwin.[24] This allows the investigator to distinguish activity associated with certain drug classes and to estimate relative potencies. Compounds were tested using at least three dosage levels with a constant log dose interval. Every screening experiment compared the test compound with a standard drug (glutethimide, 100 mg/kg) and a placebo (vehicle alone). Each experiment was replicated three separate times during a single day to control for possible circadian variations. During the primary screen, onset of drug effect, time of peak effect, and duration of activity were noted.

Sedative–hypnotic activity was investigated using the rotarod test for drug-induced ataxia. A modification[7] of the test described by Sofia[25] was used in this study. At least four dosage levels were tested using ten mice at each level. The animal was tested 10 or 15 min after an ip dose and those times were shown to be optimal in preliminary experiments. An animal exhibited a positive response (ataxia) by falling from the rotating rod twice in a 2-min time interval.

The inhibition of convulsions produced by electroshock (50 mA, 60 Hz, 0.2 sec) was used as a measure of anticonvulsant activity.[26] The blocking of tonic extensor convulsions was considered a positive anticonvulsant response. These experiments were performed immediately after the rotarod experiments.

The LD_{50} values were estimated by the method of Dixon and Mood.[27] The test required that death occur in less than 4 hr after drug administration.

3-Phenyl-3-cyano-*n*-valeraldehyde Diethylacetal (3). A solution of 14.5 g (0.1 mol) of α-phenylbutyronitrile in 20 ml of C_6H_6 was added to a suspension of $NaNH_2$ (freshly prepared from 2.4 g of sodium) in 150 ml of C_6H_6, heated to reflux for 2 hr, and cooled to room temperature, and a solution of 29.5 g (0.15 mol) of bromoacetaldehyde diethylacetal in 50 ml of C_6H_6 was slowly added. The mixture was heated at reflux for 6 hr and cooled. The reaction was quenched by the addition of 50 ml of ice-cooled water and the aqueous layer was extracted with (2 × 150 ml) C_6H_6. The combined C_6H_6 extract was dried ($MgSO_4$) and the solvent was evaporated to yield a brownish liquid, which was distilled at 135–140° (0.15 mm) to yield 18 g (76%) of pale yellow liquid: $n^{26}D$ 1.4843.

3-Phenyl-3-cyano-*n*-valeraldehyde (4). An ethanolic solution of 38.0 g (0.145 mol) of **3** was heated to reflux with 10% H_2SO_4 for 2 hr. The product was extracted with Et_2O (2 × 150 ml), the combined Et_2O extracts were dried ($MgSO_4$), and the solvent was evaporated to yield a pale yellow liquid which was distilled at 125–130° (0.15 mm) to give 20 g (74%) of colorless liquid: $n^{28}D$ 1.5215. Anal. ($C_{12}H_{13}NO$) C, H, N.

3-Cyano-3-phenyl-5-hydroxy-*n*-capronitrile (5). To a solution of 20 g (0.107 mol) of **4** in 100 ml of EtOH, 9.35 g (0.11 mol) of acetone cyanohydrin was added. The mixture was held at reflux for 24 hr. The solvent was evaporated under reduced pressure, water (500 ml) was added, and the oily liquid which separated was extracted with Et_2O (3 × 150 ml). The combined Et_2O extracts were washed several times with water and dried ($MgSO_4$) and evaporated to yield 14.1 g (96%) of the product; ir spectroscopy showed loss of the carbonyl aldehyde at 1730 cm^{-1} and appearance of bands at 3440 (OH) and 2220 cm^{-1} (CN). The crude product

was used without further purification since distillation was found to cause decomposition of the product back to 4.

4-Hydroxy-2-ethyl-2-phenylglutarimide (2). Method A. To a solution of 6 g (28 mmol) of **5** in 50 ml of glacial HOAc, 20 ml of concentrated H_2SO_4 was added in portions at an initial temperature of 70°. During the addition, the temperature rose to 100–110°. The mixture was maintained at this temperature for 30 min, cooled, and poured onto ice and adjusted to pH 8–9 with sodium hydroxide. The alkaline mixture was extracted with CH_2Cl_2 (3 × 50 ml). The combined CH_2Cl_2 extracts were washed with H_2O, dried ($MgSO_4$), and evaporated to yield an oily residue which was recrystallized from Et_2O–petroleum ether (bp 40–60°) to yield 620 mg (10%) of a product identical with the product prepared by method B.

4-Bromo-2-ethyl-2-phenylglutarimide (6). A modification of the procedure of Urech et al.[18] was adopted. A mixture of 6.75 g (31 mmol) of **1** and 7.7 g (48 mmol) of bromine was stirred for 10 min in a round-bottomed Pyrex flask and then heated at 135–140° under a 500-W photo lamp for 2.5 hr. The reddish residue was then dissolved in 50 ml of *i*-PrOH, decolorized with charcoal, and filtered while hot. The filtrate was kept at a room temperature while 5.2 g (57%) of white crystalline solid was obtained: mp 161–164° (lit.[18] 165–167°); ir (KBr) 3200 (NH), 1710 cm^{-1} (C=O imide); NMR ($CDCl_3$) δ 0.9 (t, 3, CH_3CH_2), 1.83 (m, 2, CH_3CH_2), 2.83 (m, 2-CH_2– of glutarimide ring), 4.5 (q, 1, ABX, CH at C_4), 7.33 (s, 5, arom protons), and 8.5 (br s, 1, NH exchanged with D_2O); mass spectrum 296 (M^+), 297 (M + 1), 295 (M^+ – 1). Anal. ($C_{13}H_{14}BrNO_2$) C, H, N, Br.

2-Ethyl-2-phenylglutaconimide (7). Method A. According to a modified procedure described by Urech et al.[18] using 6 g (20.28 mmol) of **6** and 6 ml of collidine, after reflux for 3 hr, the mixture was acidified with 3 *N* HCl and extracted with CH_2Cl_2 (3 × 50 ml). The combined CH_2Cl_2 extracts were dried ($MgSO_4$), and the solvent was removed to yield a solid product which was recrystallized from EtOH to yield 2.8 g (64%) of the product: mp 162–163° (lit.[18] mp 163–165°); ir (KBr) 3200 (NH), 1710 (C=O imide), 1630 cm^{-1} (C=C); NMR ($CDCl_3$) δ 0.93 (t, 3, CH_3CH_2), 1.70–2.80 (two sets of quartets, 2, CH_3CH_2, due to the asymmetric center at C_3 and coupling with H_4), 6.37 (d × d, 1 olefinic proton at C_5), 6.8 (d, 1, olefinic proton at C_4, J = 10 Hz), 7.03 (s, 5, aromatic phenyl), and 8.33 (br s, 1, NH, exchangeable with D_2O); mass spectrum, M^+ 215.

Method B. According to the procedure described by Aboul-Enein[19] the product was identical with the one obtained by method A.

4-Amino-2-ethyl-2-phenylglutaconimide (8). A mixture of 6.0 g (20 mmol) of **6**, 4 g (60 mmol) of sodium azide dissolved in 5 ml of water, and 20 ml of DMSO was heated 2 hr at 100°. The reaction mixture was diluted with 200 ml of water and extracted with (3 × 100 ml) CH_2Cl_2. The combined extracts were washed with H_2O, dried ($MgSO_4$), and filtered, and the solvent was evaporated under reduced pressure. A white solid was obtained which was recrystallized from EtOH to yield 3.45 g (75%) of crystalline solid: mp 161–162°; ir (KBr) 3470 and 3380 (NH_2), 3220 (NH imide), 1700 and 1650 (C=O imide), 1600 cm^{-1} (NH bending); uv (λ_{max}^{EtOH}) 302 nm (log ϵ 3.45); NMR ($CDCl_3$) δ 0.9 (t, 3, CH_3CH_2), 1.73–2.73 (q, 2, CH_3CH_2), 3.83 (br s, 2, NH_2 exchanged with D_2O), 5.47 (s, 1, olefinic proton at C_4), 7.3 (s, 5, arom phenyl), 8.37 (br s, 1, NH imide, exchanged with D_2O); mass spectrum, M^+ 230. Anal. ($C_{13}H_{14}N_2O_2$) C, H, N.

4-Acetamido-2-ethyl-2-phenylglutaconimide (10). A solution of 600 mg (2.6 mmol) of **8** in 2 ml of HOAc and 2 ml of Ac_2O was heated at reflux for 1 hr. The solution was cooled and rendered alkaline with 20% $NaHCO_3$, and the oily precipitate was extracted (3 × 75 ml) with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a semisolid which was recrystallized from C_6H_6 to give 420 mg (59%) of the product: mp 162–164°. Anal. ($C_{15}H_{16}N_2O_3$) C, H, N.

4-(*N*-Phenylureido)-2-ethyl-2-phenylglutaconimide (11) was prepared by heating 230 mg (1 mmol) or **8** with 120 mg (1 mmol) of phenyl isocyanate for 10 min on a steam bath. The solid which formed was recrystallized from EtOH to yield 125 mg (35.8%) of white crystals: mp 212–214°. Anal. ($C_{21}H_{19}N_3O_3$) C, H, N.

4-Amino-2-ethyl-2-phenylglutarimide (12). A solution 2.0 g (8.7 mmol) of **8** in 50 ml of EtOH was shaken over 200 mg of 10% Pd/C at 60 psi for 12 hr at room temperature. The catalyst was removed by filtration and the solvent was evaporated under vacuum to yield an oily residue which solidified after a short time. The solid was recrystallized from Et_2O–petroleum ether (bp 40–60°) to yield 1.5 g (75%) of white crystalline solid, mp 117–120°. The hydrochloride salt was prepared with ethereal HCl and recrystallized from EtOH–Et_2O. Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

4-Hydroxy-2-ethyl-2-phenylglutaconimide (13). A mixture of 3.6 g (15.6 mmol) of **8** was heated to reflux overnight with 10 ml of 2 *N* HCl and 6 ml of dioxane. An oily product was formed which, upon dilution with water and scratching with a glass rod, solidified. The solid product was recrystallized from a H_2O–dioxane (3:1) mixture to give 3.3 g (91.6%) of a white crystalline solid: mp 114–117°; gave a deep red color with aqueous $FeCl_3$ solution; uv λ_{max}^{EtOH} 270 nm (log ϵ 3.56); ir (KBr) 3280 (NH, OH), 1690, 1675 (saturated and α,β-unsaturated C=O, respectively), 840 cm^{-1} (C=CH bending); NMR ($CDCl_3$) δ 0.9 (t, 3, CH_3CH_2), 1.75–2.76 (q × q, 2, CH_3CH_2), 5.83 (s, 1, olefinic H at C_3), 7.25 (s, 5, arom phenyl protons), 8.6 (br s, 2, NH and OH protons exchanged with D_2O); mass spectrum, M^+ at *m/e* 231. Anal. ($C_{13}H_{13}NO_3$) C, H, N.

4-Methoxy-*N*-methyl-2-ethyl-2-phenylglutaconimide (14). An ethereal solution of 400 mg (1.7 mmol) of **13** was treated with excess CH_2N_2 in Et_2O and left overnight at room temperature and the progress of the reaction was monitored by GLC. The solvent was evaporated and the residue that solidified after several hours was recrystallized from Et_2O–petroleum ether (bp 40–60°) to yield 250 mg (56.7%) of white solid: mp 95–98°; ir (KBr) no absorbance at 3400–3300 cm^{-1} region (OH absent), 1680, 1650 cm^{-1} (C=O saturated and unsaturated C=O imide, respectively); NMR ($CDCl_3$) δ 0.9 (t, 3, CH_3CH_2), 1.86–2.83 (two sets of q, 2, CH_3CH_2), 3.26 (s, 3, NCH_3), 3.86 (s, 3, OCH_3), 5.56 (d, 1, olefinic H at C_3), and 7.36 (3, 5, arom phenyl). Anal. ($C_{15}H_{17}NO_3$) C, H, N.

4-Hydroxy-2-ethyl-2-phenylglutarimide (2). Method B. A solution of 600 mg (2.6 mmol) of **13** in EtOH was shaken over 100 mg of 10% Pd/C overnight. The catalyst was removed by filtration and the residue left after evaporation of the solvent was recrystallized from Et_2O–petroleum ether (bp 40–60°) to give 450 mg (74%) of white crystalline solid: mp 135–137°; ir (KBr) 3560, 3450 (OH), 3240, 3100 (NH), 1710 (imide C=O), 1600 cm^{-1} (C=C arom phenyl); NMR ($CDCl_3$) δ 0.87 (t, 3, CH_3CH_2), 1.8–2.93 (m, 4, CH_3CH_2 and –CH_2 of glutarimide ring at C_3), 3.5 (s, 1, OH exchangeable with D_2O), 4.17 (q, 1, CH at C_4), 7.37 (s, 5, arom protons), and 8.7 (br s, 1, NH exchangeable with D_2O). Anal. ($C_{13}H_{15}NO_3$) C, H, N.

The compound exists as a diasteromeric pair which could be separated by TLC on 250-μ silica gel GF (Analtech, Inc., Newark, Del.) to give two spots with a R_f 0.35 and 0.27 (70:30:8 hexane–Et_2O–HOAc), 0.55 and 0.34 (90:10 CH_2Cl_2–HOAc), and 0.33 and 0.22 (90:10 $CHCl_3$–acetone). The more mobile compound corresponds to the isolated biosynthetic metabolite [mp 127–129° (reported 120–121°) and was found to be identical by comparison of ir, NMR, and mass spectrum with the isolated biosynthetic compound]. The other isomer has mp 144–146°.

2-(*p*-Nitrophenyl)-2-ethylglutarimide (15). The procedure described by Hoffmann and Urech was followed.[21] A solution of 4.34 g (0.02 mol) of glutethimide in 16 g of concentrated H_2SO_4 was cooled to −5 to 10° in an ice–salt bath. A nitrating mixture of 2.2 g of 71% HNO_3 and 2.2 g of concentrated H_2SO_4 was added to the above solution slowly with stirring and cooling. The mixture was stirred for an additional 2 hr and poured into an ice–water mixture where a solid precipitated. It was collected by filtration and washed with water until the washings became acid-free. The precipitate was recrystallized from MeOH to give 2.86 g (53%) of the product: mp 135–137° (lit.[21] 137–139°). Anal. ($C_{13}H_{14}N_2O_4$) C, H, N. The ortho isomer **19** was separated from the mother liquor by fractional crystallization from MeOH to yield 620 mg: mp 168–170° (lit.[21] 170–172°).

2-(*p*-Aminophenyl)-2-ethylglutarimide (16). This was prepared by the method described by Hoffmann and Urech[21] using 3.0 g (10.44 mmol) of **15** dissolved in 100 ml of EtOH and reduced catalytically at 60 psi using 500 mg of 10% Pd/C at 60°. After the absorption of hydrogen ceased, the reaction mixture yielded 2.84 g (93%) of the product, mp 148–150° (lit.[21] 149–150°), which was identical with an authentic sample of *p*-aminoglutethimide.

2-(*p*-Acetamidophenyl)-2-ethylglutarimide (17). A solution of 600 mg (2.58 mmol) of **16** in 2 ml of HOAc and 2 ml of Ac_2O was heated at reflux for 1 hr. The solution was cooled, rendered alkaline with 20% $NaHCO_3$, and extracted with CH_2Cl_2 (2 × 50 ml). The combined CH_2Cl_2 extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The resulting oily residue was recrystallized from C_6H_6–petroleum ether to yield 440 mg (62%) of the product: mp 188–189° (lit.[28] 187–188°). Anal. ($C_{15}H_{18}N_2O_3$) C, H, N.

2-(*o*-Aminophenyl)-2-ethylglutarimide (20). This was pre-

pared by the catalytic hydrogenation of a solution of 400 mg (1.53 mmol) of **19** in 50 ml of EtOH and 150 mg of 10% Pd/C at room temperature. After the absorption of hydrogen ceased the reaction mixture yielded 320 mg (90%) of the product, mp 175–178°, which was identical with an authentic sample of o-aminoglutethimide.

2-(*p*-Hydroxyphenyl)-2-ethylglutarimide (18). A solution of 700 mg (3.3 mmol) of **16** in 20 ml of 2 *N* H_2SO_4 was cooled to 5° and diazotized with 480 mg (7 mmol) of $NaNO_2$ dissolved in 2 ml of H_2O at 5°. The solution of diazonium salt was then added dropwise to 10 ml of boiling H_2O and the solution was held at reflux for 1 hr and then allowed to cool. The reaction mixture was saturated with NaCl and extracted with CH_2Cl_2 (3 × 50 ml). The organic layer was dried ($MgSO_4$) and filtered and the solvent evaporated under reduced pressure. The oily residue was recrystallized from Et_2O to yield 470 mg (61%) of a white crystalline solid: mp 143–144°; ir (KBr) 3240 (OH, NH), 1710, 1680 cm^{-1} (C=O imide); NMR (DMSO-d_6) δ 0.86 (t, 3, CH_3CH_2), 1.68–2.33 (m, 6, CH_3CH_2 and 2-methylene glutarimide ring), 6.73 (d, 2, H_3 and H_5 arom protons, J = 8.8 Hz, typical A_2B_2 system of para-substituted benzene), 7.13 (d, 2, H_2 and H_6 arom protons, J = 8.8 Hz), 9.33 (s, 1, OH exchangeable with D_2O), 8.3 (s, 1, NH, exchangeable with D_2O); mass spectrum, M^+ 233. Anal. ($C_{13}H_{15}NO_3$) C, H, N.

Acknowledgments. A preliminary account of this work was presented to the Medicinal Chemistry Section, APHA 121st National Meeting, Chicago, Ill., August 1974. This investigation was supported by U.S. Public Health Service Grant GM 12675. We thank Dr. David E. Nichols for his suggestions and discussions during the preparation of this manuscript. The authors acknowledge Mr. Dennis Charkowski and Mr. Archie Jones for their technical assistance. An authentic sample of *p*-aminoglutethimide and *o*-aminoglutethimide was obtained from Dr. J. J. Clark, Ciba-Geigy Corp., Ardsley, N.Y. 10502.

References and Notes

(1) H. Keberle, K. Hoffmann, and K. Bernhard, *Experientia*, 18, 105 (1962).
(2) E. Bütikofer, P. Cottier, P. Imhof, H. Keberle, W. Reiss, and K. Schmid, *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol.*, 244, 97 (1962).
(3) D. Post and H. Schutz, *Beitr. Gerichtl. Med.*, **26**, 227 (1969).
(4) W. G. Stillwell, M. Stafford, and M. G. Horning, *Res. Commun. Chem. Pathol. Pharmacol.*, **6**, 579 (1973).
(5) J. Keberle, K. Schmid, K. Hoffmann, J. Vuilleumier, and K. Bernhard, *Helv. Chim. Acta*, **42**, 417 (1959).
(6) J. J. Ambre and L. J. Fischer, *Res. Commun. Chem. Pathol. Pharmacol.*, 4, 307 (1972).
(7) J. J. Ambre and L. J. Fischer, *Drug Metab. Dispos.*, **2**, 151 (1974).
(8) (a) A. R. Hansen and L. J. Fischer, *Clin. Chem.*, **20**, 236 (1974); (b) A. R. Hansen, K. A. Kennedy, J. J. Ambre, and L. J. Fischer, *N. Engl. J. Med.*, **292**, 250 (1975).
(9) R. Brachini, C. Caradonna, G. Casini, and M. L. Stein, *Farmaco, Ed. Sci.*, **16**, 387 (1961); *Chem. Abstr.*, **56**, 5828c (1962).
(10) G. Casini, M. Ferappi, S. Gulinelli, and P. Mazzeo, *Ann. Chim. (Rome)*, **52**, 1254 (1952); *Chem. Abstr.*, **59**, 620c (1963).
(11) G. Casini, O. Cicchetti, and M. Ferappi, *Ric. Sci., Suppl.*, **30**, 2535 (1960); *Chem. Abstr.*, **60**, 7985 (1964).
(12) E. Tagmann, E. Sury, and K. Hoffmann, *Helv. Chim. Acta*, **35**, 1541 (1952).
(13) F. Gross, K. Hoffmann, J. Keberle, and J. Tripod, *Verh. Naturforsch. Ges. Basel*, **67**, 479 (1956).
(14) F. Salmon-Legagneur and Y. Oliver, *Bull. Soc. Chim. Fr.*, 640 (1965).
(15) A. Ercoli and P. DeRuggieri, *J. Am. Chem. Soc.*, **75**, 650 (1953).
(16) B. E. Betts and W. Davey, *J. Chem. Soc.*, 4193 (1958).
(17) K. Hoffmann, B. Tagmann, and E. Tagmann, U.S. Patent 2,673,205 (March 23, 1954).
(18) E. Urech, E. Tagmann, E. Sury, and K. Hoffmann, *Helv. Chim. Acta*, **36**, 1809 (1953).
(19) H. Y. Aboul-Enein, *Org. Prep. Proced. Int.*, in press.
(20) A. S. Tomcufcik and L. N. Starker, "Pyridine and Its Derivatives", Part III, E. Klingsberg, Ed., Interscience, New York, N.Y., 1962, p 81.
(21) K. Hoffmann and E. Urech, U.S. Patent 2,848,455 (1958); *Chem. Abstr.*, **53**, 7103*h* (1959).
(22) E. G. C. Clarke, "Isolation and Identification of Drugs", The Pharmaceutical Press, London, 1969, p 187.
(23) (a) F. W. Kahnt and Neher, *Helv. Chim. Acta*, **49**, 725 (1966); (b) A. E. Daniels-Severs and J. Vernikos-Danellis, *Pharmacology*, **10**, 111 (1973), and references cited therein.
(24) S. Irwin, *Psychopharmacologia*, **13**, 222 (1968).
(25) R. D. Sofia, *J. Pharm. Sci.*, **58**, 900 (1969).
(26) L. S. Goodman, M. Singh Grewal, W. C. Brown, and E. A. Swinyard, *J. Pharmacol. Exp. Ther.*, **108**, 168 (1953).
(27) W. J. Dixon and A. M. Mood, *J. Am. Stat. Assoc.*, **43**, 109 (1948).
(28) R. Paul, R. P. Williams, and E. Cohen, *J. Med. Chem.*, **17**, 539 (1974).

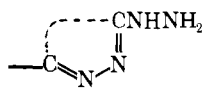
Synthesis and Antihypertensive Properties of New 3-Hydrazinopyridazine Derivatives

Giorgio Pifferi,* Francesco Parravicini, Carlo Carpi, and Luciano Dorigotti

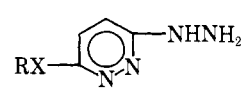
ISF, Italseber Research Laboratories, Trezzano S/N, Milan, Italy 20090. *Received September 25, 1974*

3-Hydrazinopyridazines substituted in position 6 with a primary amine, secondary amine, or an alkoxy group were synthesized and screened for antihypertensive activity. In general, the 6-dialkylamino derivatives are the most active; the (2-hydroxypropyl)methylamino chain provides the best combination of high antihypertensive activity and low toxicity.

The potent and long-lasting antihypertensive activity of hydrazinophthalazines,[1] exemplified by hydralazine and dihydralazine, encouraged the investigation of modified heterocyclic analogs[2] and particularly of six-membered rings containing the essential moiety A.[3] In the case of pyridazine series B, various 6-substituted derivatives with alkyl,[4] aryl,[5] methoxy,[6] phenoxy,[6] carbamyl,[7] and hydrazino[8] groups have been synthesized, but only few of them retained interesting hypotensive properties. Compounds of type B in which X is NR′, NH, or O had received little attention until a remarkable improvement in activity and decrease in toxicity was recently obtained by replacing RX with a secondary amino residue.[9,10] These results prompted us to undertake a more detailed study designed to define structure–activity relationships and to optimize activity in this class of 6-substituted 3-hydrazinopyridazines.



A



B (X = NR′, NH, O)