overnight. Purification was effected by recrystallization from benzene using charcoal.

3-Methyl-1-[2-(4-pyridyl)ethyl]-2-pyrazolin-5-one (20).—The condensation of 1 mole of ethyl acetoacetate with 1 mole of 2-(4-pyridyl)ethylhydrazine was carried out by the general procedure above. The recrystallization solvent was ethanol–benzene (1:10).

1-Acetyl-2-ethylidene-1-[2-(4-pyridyl)ethyl]hydrazine (21).—To 68.5 g (0.5 mole) of 2-(4-pyridyl)ethylhydrazine was added slowly 40 ml (0.7 mole) of acetaldehyde while cooling in an ice bath. Water was removed from the resulting mixture by adding 200 ml of benzene and refluxing over a Dean–Stark trap. The dried benzene solution was treated with 130 ml (1.38 moles) of acetic anhydride and refluxed for 45 min. The volatile materials were removed on the steam bath at reduced pressure and the product was obtained by vacuum distillation of the residue.

6-Methyl-2-[2-(4-pyridyl)ethyl]-3-pyridazinone (16) and 6-Methyl-2-(4-pyridylmethyl)-3-pyridazinone (19).—To a boiling solution of 0.5 mole of the corresponding dihydropyridazinone (1 or **6**) in 1 l. of glacial acetic acid was added dropwise during 1.5 hr 25.5 ml (0.5 mole) of bromine. The solution was then allowed to cool to about 85–90° and treated with a solution of 98 g (1 mole) of anhydrous potassium acetate in 650 ml of glacial acetic acid. The mixture was refluxed for 1 hr, then cooled in ice. The crystallized KBr was removed by filtration, and the filtrate was stripped of acetic acid by distillation under reduced pressure. The residue was extracted and crystallized from the solvent indicated in Table II.

Levulinic Acid 2-(4-Piperidyl)ethylhydrazone (22).—To 22.1 g (0.15 mole) of 2-(4-piperidyl)ethylhydrazine was added slowly while cooling in ice, 18.0 g (0.15 mole) of levulinic acid. The resulting solid was triturated with alcohol, filtered, and washed with ethanol and ether. The crude product was recrystallized by boiling in ethanol and adding water slowly to complete solution.

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3-Aminopiperidones.[1a] II. 2-(N,N-Diethylamino)-2-phenylglutarimide[1b,c]

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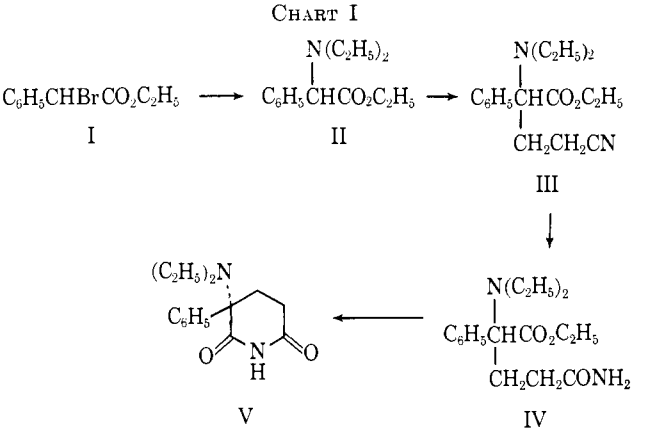
Of the very large number of compounds investigated[2] for antiepileptic activity relatively few carry polar substituents.[3] This fact, coupled with the success of aminoglutethimide, prompted the synthesis of the model compound, 2-(N,N-diethylamino)-2-phenylglutarimide. Steps to the final synthesis of this compound were accomplished after extensive studies of several possible routes. The difficulties experienced in attempts to synthesize certain intermediates along some of these routes and mechanistic studies of problems associated with them have been explored.[1a,4] The most successful route is presented herein.

Ethyl α-bromophenylacetate (I) was prepared by a modification of the procedure of Anschütz,[5] or using the thionyl chloride catalyzed method of Schwenk and Papa.[6] In the subsequent displacement reaction (I → II) using diethylamine as the nucleophile, some aminolysis of the ester (I) was anticipated since it is known that α-halo esters react at elevated temperature with amines to give both α- and β-aminoamides.[7] Since aminolysis reactions are equilibrium controlled, whereas displacement reactions are in general kinetically controlled, a short-time, high-temperature method was chosen (see Experimental Section).

The next step in the reaction sequence, the addition of a 3-carbon chain to ethyl α-N,N-diethylaminophenylacetate (II), proved to be difficult. Electronic and steric effects, operating at close proximity, decrease considerably the acidity of the α-hydrogen and interfere with the approach of the attacking nucleophile.

Efforts toward effecting a Michael condensation of the ester (II) with acrylamide, acrylonitrile, and methyl acrylate failed to achieve the desired addition, and so were attempted alkylations of II with halopropionitriles following several published procedures.[8] However, employing the general procedure recently developed by Zaugg, *et al.*,[9] it was possible to alkylate II with β-bromopropionitrile in fair yields. The treatment of III with polyphosphoric acid[10] gave a 91% yield of the amido ester (IV) (see Chart I). A clean product was obtained, in comparable yield, by heating the carbethoxynitrile in a 1:1 mixture of concentrated sulfuric and glacial acetic acids at steam-bath temperature for 30 min.

CHART I



(1) (a) Part I: C. F. Martin and V. L. Narayanan, *J. Org. Chem.*, **26**, 2127 (1961). (b) This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171. (c) This paper was presented before the Division of Medicinal Chemistry at the 144th National Meeting of the American Chemical Society, Los Angeles, Calif., April 1963. (d) Communications regarding this paper should be addressed to the Department of Organic Chemistry, Squibb Institute for Medical Research, New Brunswick, N. J. 08903. (e) This paper was abstracted in part from the Ph.D. Dissertation, Washington State University, August 1962.

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(10) H. R. Snyder and C. T. Elston, *ibid.*, **76**, 3039 (1954).

The cyclization of IV to the desired imide (V) met with partial success. Upon treating IV by the procedure of the alkylation step (II → III), it was observed that hydrogen abstraction (as evidenced by the cessation of the evolution of gas) was essentially complete after 2 hr of heating at 50°. However, if the reaction were stopped at this point, the starting material was completely recovered. It was determined that continued heating of the reaction mixture at a higher bath temperature (100–105°) after the initial hydrogen abstraction was completed did effect the desired cyclization to V. The imide portion of V is relatively flat, and since both the phenyl and diethylamino groups are large, they tend to assume positions that are symmetrical with respect to the adjacent carbonyl and are neither axial nor equatorial.[11]

The pharmacology[12] of 2-(N,N-diethylamino)-2-phenylglutarimide (V) has been compared with those of four glutarimides and phenobarbital. With two techniques that measured neurotoxicity and pentylenetetrazol seizure threshold, the onset, peak, and duration of activity of the compounds under study were plotted. Compound V exhibited a rapid onset of action with an ED_{50} of 80 mg/kg, and produced the least increase in seizure threshold. However, V was devoid of any sedative effect, indicating perhaps a different mechanism of antiepileptic action.

Experimental Section[13]

Ethyl α-Bromophenylacetate (I). A.—A modification of the procedure described by Auschütz[5] was used in this instance. To a stirred mixture of 68.1 g (0.5 mole) of phenylacetic acid, and 3 g of red phosphorus, 160 g (1.0 mole) of bromine was added at 60–70° over a period of 3 hr. The mixture was stirred for an additional 3 hr at 60–70°. After cooling, 23.0 g (0.5 mole) of absolute ethanol was added slowly with stirring, and the reaction mixture was heated at 60–70° for 10 hr. At the end of the period, the mixture was poured into ice water, and the ester layer was separated, washed successively with 10% sodium bisulfite solution and water, and dried (Na_2SO_4). After filtering, the oil was distilled to yield 73.5 g (60%) of a liquid, bp 116–120° (2.5 mm). Redistillation gave a central cut of product I bp 99–101° (0.4 mm); $n^{20}D$ 1.5368 [lit.[14] bp 102–104° (0.4 mm), $n^{22}D$ 1.5380]; infrared (CCl_4), 5.72 μ (ester C=O).

B.—This procedure was patterned after the method of Schwenk and Papa[6] for the α bromination of dicarboxylic acids. A mixture of 68.1 g (0.5 mole) of phenylacetic acid and 100 ml of $SOCl_2$ was heated under reflux for 2 hr. After slight cooling, 48.0 g (0.55 mole) of bromine was added during a period of 3 hr maintaining gentle refluxing during the addition. After standing overnight, the excess $SOCl_2$ was removed under reduced pressure, and the residual solution was added portionwise, with stirring, to 100 ml of absolute ethanol. The mixture was stirred for 3 hr at room temperature. Fractional distillation yielded 109.5 g (90%) of the product, bp 117–119° (2.4 mm). The infrared spectra of the ethyl α-bromophenylacetates obtained by methods A and B are superimposable.

Ethyl α-N,N-Diethylaminophenylacetate (II).—A well-stirred solution of 97.2 g (0.4 mole) of freshly distilled ethyl α-bromophenylacetate and 117 g (1.6 moles) of diethylamine in 100 ml of dry CCl_4 was heated gently under reflux for 12 hr. The mixture of precipitated hydrobromides was filtered and dissolved in water, and the solution was adjusted to pH 9.5 (cooling) and extracted with ether. The CCl_4 solution was concentrated to *ca.* 150 ml and extracted with 10% HCl. Evaporation of the CCl_4 layer yielded 6.0 g of crude ethyl α-bromophenylacetate. The acidic extract was adjusted to pH 9.5 (cooling) and also extracted with ether. The ethereal extracts were combined and dried (Na_2SO_4). The solvent was flash evaporated and the product distilled to yield 83.7 g (89%) of a pale yellow mobile liquid, bp 94–98° (1.0 mm). Other boiling points observed for this compound at different times were 106–109° (2.0 mm) and 85–87° (0.2 mm). Redistillation gave a center cut: bp 92° (0.8 mm); $n^{22}D$ 1.4946; infrared (CCl_4), 5.73 μ (ester C=O).

Anal. Calcd for $C_{14}H_{21}NO_2$: C, 71.45; H, 8.99; N, 5.95. Found: C, 71.11; H, 9.03; N, 6.12.

4-Carbethoxy-4-(N,N-diethylamino)-4-phenylbutyronitrile (III).—All of the glassware used in this reaction was flame dried. All of the reactants were freshly distilled. Reagent grade dimethylformamide (DMF) was dried by heating under reflux with 3% of its weight of NaH and distilled at atmospheric pressure. The entire reaction was run under an atmosphere of dry N_2.

To a well-stirred suspension of 8.2 g (0.34 mole) of NaH in 150 ml of dry DMF, 70.6 g (0.3 mole) of freshly distilled ethyl α-N,N-diethylaminophenylacetate (II) was added at such a rate as to maintain an internal temperature of 40–45° (addition time, 3 hr). The resulting thick red liquid was cooled in ice (0–5°) and 44.2 g (0.33 mole) of freshly distilled β-bromopropionitrile was added dropwise, with continuous stirring, over a period of 4 hr (nitrogen atmosphere). The temperature was not allowed to exceed 5° during the addition. The mixture, containing the precipitated NaBr was stirred overnight at room temperature under dry N_2. The residue left after the removal of the solvent was taken up in 200 ml of ice water and extracted with ether. The ether extract was washed with water and then dried ($MgSO_4$). After removal of ether, the residual product was fractionally distilled to give 45.5 g [52% (corrected to 72% based upon the recovery of the starting material)] of III as a pale, brownish yellow, viscous liquid, bp 140–150° (0.15–0.2 mm). Two further distillations yielded an analytical sample: bp 145° (0.2 mm); $n^{20}D$ 1.5056; infrared (CCl_4), 4.44 (C≡N) and 5.81 μ (ester C=O).

Anal. Calcd for $C_{17}H_{24}N_2O_2$: C, 70.80; H, 8.39; N, 9.71. Found: C, 70.71; H, 8.22; N, 9.94.

4-Carbethoxy-4-(N,N-diethylamino)-4-phenylbutyramide (IV). A.—A mixture of 14.4 g (50 mmoles) of 4-carbethoxy-4-(N,N-diethylamino)-4-phenylbutyronitrile (III) and 150 g of polyphosphoric acid was stirred at a bath temperature of 115–120° for 2 hr. The resulting thick brown mass was cooled to 80° and poured into stirred ice water and neutralized with solid KOH (cooling bath). A thick brownish oil separated and soon solidified. The products were taken up in chloroform, washed with water, dried, and concentrated. Triturating the thick liquid obtained with hexane resulted in the precipitation of 14.1 g (91%) of a brownish white solid. It was crystallized from chloroform–hexane (Norit) to give small white crystals, mp 91°. Two additional recrystallizations from DMF–water netted an analytical sample: mp 92.5°; infrared ($CHCl_3$), 2.85, 2.94, and 3.15 (NH_2), 5.82 (ester C=O), and 5.94 and 6.26 μ (amide C=O).

B.—A mixture of 14.40 g (50 mmoles) of III and 15 ml of glacial acetic acid was stirred at room temperature for 15 min. Concentrated H_2SO_4 (20 ml) was added and the mixture heated on a steam bath with stirring for a period of 30 min. The brownish liquid obtained was poured into stirred ice water, and 30% aqueous NaOH was added until the mixture was alkaline (pH 8.5). The product was extracted with chloroform, washed with water, dried, and concentrated. The resulting thick oil was rubbed with hexane which resulted in the precipitation of 14.0 g (91%) of white solid. One recrystallization from chloroform–hexane gave IV as white crystals, mp 92°. The infrared spectra of the products prepared by methods A and B were identical.

2-(N,N-Diethylamino)-2-phenylglutarimide (V).—Reagent grade dried DMF was used. All glassware used was flame dried. A solution of 7.70 g (25 mmoles) of dry 4-carbethoxy-4-(N,N-diethylamino)-4-phenylbutyramide (IV) in 25 ml of dry DMF was added dropwise to a stirred suspension of 0.67 g (28 mmoles) of NaH in 50 ml of dry DMF under an atmosphere of dry N_2. The mixture was stirred at 50° for 2 hr by which time the formation of anion was complete as evidenced by the cessation of hydrogen evolution. The pot temperature was raised to 100

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105° and maintained at this point for 15 hr. The residue, left after distilling the solvent under reduced pressure, was stirred well with ice water and the unreacted starting materials (1.9 g) were filtered off rapidly. The filtrate was adjusted to pH 8.5 (cooling) and the product was extracted with chloroform, washed with water, and dried (Na_2SO_4). Filtration and removal of the chloroform left a thick oil which solidified on rubbing with petroleum ether (bp 30–60°). Recrystallizations from chloroform–hexane (Norit) yielded 1.99 g (29%) of V as white crystals, mp 106–108°. Two additional recrystallizations from chloroform–petroleum ether (bp 30–60°) gave an analytical sample of V as white, needle-shaped crystals: mp 107–109°; infrared ($CHCl_3$), 2.96 and 3.10 (NH), and 5.83 μ (C=O); ultraviolet maximum (95% ethanol), 2.04 mμ (log ϵ 4.43), showing a bathochromic shift (10^{-3} N alcoholic KOH) to 2.37 mμ (log ϵ 3.28).

Anal. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76; mol wt, 260.3. Found: C, 69.06; H, 7.82; N, 10.69; mol wt, 261.8.

Antiviral Compounds. XIII. Aminoacethydrazones of Aromatic α-Ketoaldehydes

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In a previous paper we described the synthesis of water-soluble derivatives obtained by condensing α-ketoaldehydes and Girard reagent.[1] Since several aromatic α-ketoaldehydes have been shown to exhibit antiviral activity,[2-4] we have prepared a series of new phenylglyoxal N,N-disubstituted aminoacethydrazones in order to study their antiviral activity (Table I).

All compounds were tested on embryonated eggs infected with vaccinia virus and A-PR8 virus. They were found inactive against vaccinia virus; the phenylglyoxal derivatives were also inactive against A-PR8 virus. Some derivatives of biphenylglyoxal (**4** and **5**) of *p*-phenylthiophenylglyoxal (**9**), and all derivatives of *p*-phenoxyphenylglyoxal exhibited virucidal activity against A-PR8 virus.

No activity was observed up to a concentration of 50 μg/ml when the compounds were tested for bacteriostatic activity on the following microorganisms: *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes, Mycobacterium tuberculosis, Trychophyton*, and *Candida*.

The compounds were also screened for acute toxicity in mice, for smooth muscle relaxing activity, for effects on blood pressure and respiration, for coronary vasodilatation, and for antiarrhythmic, antitussive, anticonvulsant, and antiinflammatory activity. A low anticonvulsant activity was shown by **3**–**6**; compounds **4**, 8–**10**, and **13** exhibited antiinflammatory activity on formalin edema, but were ineffective as analgesics. The data on acute toxicity, antiviral, anticonvulsant, and antiinflammatory activities are summarized in Table II.

Experimental Section[5]

The α-ketoaldehydes were prepared from the corresponding α,α-dichloroacetophenones.[6]

N-Pyrrolidinoacethydrazide.—A mixture of ethyl N-pyrrolidinoacetate (15.7 g, 0.1 mole), hydrazine hydrate (5 g, 0.1 mole), and 20 ml of ethanol was refluxed for 4 hr. The solvent was evaporated and the crude oil was distilled at 105–110° (0.2 mm), yield 11 g (77%).

Anal. Calcd for $C_6H_{13}N_3O$: N, 29.35. Found: N, 29.49.

Treatment of an ethanol solution of the free base with HCl gave the hydrochloride salt, which was recrystallized from ethanol, mp 203–205° dec.

Anal. Calcd for $C_6H_{13}N_3O \cdot 2HCl$: C, 33.34; H, 6.99; Cl, 32.82; N, 19.44. Found: C, 33.11; H, 6.96; Cl, 32.34; N, 19.63.

The N-piperidino-, N-morpholino-, and N-diethylaminoacethydrazides were prepared by the same procedure.[7]

Phenylglyoxal N,N-Disubstituted Aminoacethydrazones. General Procedure.—A mixture of the α-ketoaldehyde (0.01 mole) and the corresponding N,N-disubstituted aminoacethydrazide (0.01 mole) in 10 ml of methanol or ethanol was stirred at 20° for 8 hr. After cooling, the products were filtered and recrystallized. The yields, melting points, solvents of crystallization, and analytical data are summarized in Table I.

Biological Testing. Maximal Tolerated Dose (MTD) in the Embryonated Egg.—The compounds were dissolved in saline solution buffered at pH 7.2, containing 500 IU of penicillin G and 0.5 mg of streptomycin/ml. Descending doses of each compound dissolved in 0.1 ml were inoculated into the allantoic sac. Each dose was injected in three embryonated 9-day old eggs. The highest dose which did not provoke mortality within 3 days was defined as the MTD.

Antiviral Methods.—Embryonated 9-day-old leghorn hen eggs and influenza A virus [allantoic fluid containing 10^8–10^9 EID_{50} (median egg-infecting dose) of egg-adapted PR-8 strain] were used. Vaccinia mouse neurotropic virus [(ATCC) CAM (chorioallantoic membrane) homogenized and purified by centrifugation containing 10^6–10^7 ELD_{50} (egg lethal dose) of egg-adapted WR strain] was used.

A. Virucidal Tests.—For each dose, 0.5 MTD dissolved in 10 ml of buffered saline solution was added to 10^2, 10^3, or 10^4 EID_{95} and the three solutions were kept in water baths at 37° for 1 hr. Then the allantoic sacs of 5 eggs (for each dose) were inoculated with 0.1 ml of one of the incubated solutions.

B. Virustatic Tests.—For each dose, the allantoic sacs of five eggs were inoculated with 0.1 ml of allantoic fluid containing either 1, 10, or 100 EID_{95} of virus, and the eggs were stored 1 hr at 37°. Then the allantoic sac was inoculated with 0.1 ml of buffered saline solution containing 0.5 MTD of each compound.

C. Evaluation of the Activity.—For influenza virus, the eggs were stored at 35° for 48 hr, then at 4° for 12 hr, and finally tested for the presence of hemoagglutinin. For vaccinia virus, the eggs were stored at 37° for 7 days and the mortality of chick embryos was recorded.

Antimicrobial and Antifungal Methods.—The compounds were diluted in 1:2 ratios in Difco nutrient agar inoculated with *E. coli* 100, *P. aeruginosa* H2, *P. vulgaris*, *B. subtilis*, *S. aureus* S.G. 511, and in Difco brain heart infusion agar inoculated with *Str. pyogenes humanus* A88, and the results were read after incubation for 18 hr at 35–37°. By the same procedure the compounds were tested in Kirchner–Hermann medium + 10% beef serum inoculated with *M. tuberculosis* 37 Ra, and the results were read after incubation for 10, 17, and 24 days at 35–37°. The compounds were tested also in Sabouraud broth inoculated with *Trichophyton mentagrophytes* 1236 (the results were read after incubation for 4 days at 26°), and with *Candida albicans* 28 (the results were read after incubation for 18 hr at 35–37°).

Pharmacological Methods.—For all tests NMRI albino mice and Wistar albino rats were used. The *acute toxicity* of each compound was determined by administering it intraperitoneally to mice in descending doses. Mortality was recorded over 24 hr and indicative LD_{50} values were estimated.

Smooth muscle relaxing activity was tested *in vitro* by Magnus' method[8] on the small intestine of a guinea pig stimulated by

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