Ethyl ω-Phthalimidoocetoacetate Ethylene Thioketal.—Ester II (5 g, 0.018 mol) and 2.7 g (0.028 mol) of ethanedithiol were dissolved in $CHCl_3$ and 5.8 ml of BF_3-etherate was added. The solution was stirred 3 hr at 25°, then was washed with $NaHCO_3$ solution, dried, and concentrated. After long standing it partly crystallized from *i*-Pr_2O, 3.1 g, mp 71–72°. *Anal.* ($C_{16}H_{17}NO_4S_2$) C, H, N, S.

Pyrrolidine-2,4-dione 4-Ethylene Ketal (XIV).—NaOMe (0.32 g, 0.006 mol) was added to a solution of 2 g of XI (0.006 mol) and 0.23 g (0.007 mol) of NH_2OH in MeOH. The solution turned pale yellow, then bright yellow, orange and, after 15 min, red. Shortly afterwards a solid precipitated. After 16 hr, the solid Na salt of *N*-hydroxyphthalimide (0.6 g) was filtered, the filtrate was diluted with a large volume of *i*-Pr_2O and more Na salt (0.25 g) was removed. The filtrate was concentrated giving 1.5 g of crude XIV, as an oil. This material slowly recrystallized and was then sublimed *in vacuo* and recrystallized from *i*-Pr_2O, to give 0.7 g of XIV, mp 98–101°. The compound shows a typical lactam absorption in the ir at 5.92 μ. *Anal.* ($C_6H_9NO_3$) C, H, N.

Acknowledgment.—We are indebted to Dr. Timothy Jen for the synthesis of *N*-phthaloylmuscimol, to Dr. Herbert Winicov for the muscimol, and to Mr. Edward Macko and Dr. Richard McLean for the biological test results.

Benzimidazole-5(6)-alanine and Related Compounds. 1. Synthesis of Amino Acids as Inhibitors of Norepinephrine Biosynthesis[1a]

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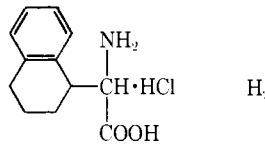
Received October 27, 1969

The potential usefulness of inhibitors of norepinephrine biosynthesis at the level of tyrosine hydroxylase is widely recognized. Inhibitors of phenylalanine hydroxylase offer a potential model for phenylketonuria.

1,2,3,4-Tetrahydronaphthyl-1-glycine hydrochloride (**3**) was prepared by standard reactions as described in the Experimental Section. 3-Nitro-4-aminophenylalanine (**6**) was prepared by standard reactions from *p*-nitrophenylalanine. Benzimidazole-5(6)-alanine dihydrochloride (**7**) was prepared by reduction of **6** and cyclization of the resulting *o*-phenylenediamine by the Phillips method.[2] The unstable intermediate *o*-phenylenediamine analog was not isolated. 3,4-Ethylenedioxyphenylalanine hydrochloride was obtained by the hydrogenation of the oxime formed from the reaction of isopropyl nitrite with the condensation product of 3,4-ethylenedioxybenzyl chloride and sodium diethyl malonate.



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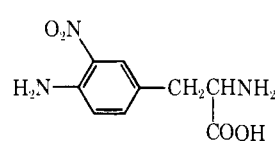

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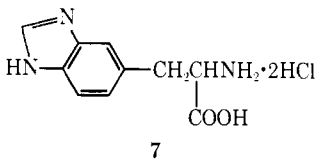

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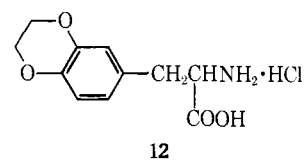

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Biological Activity.—Bovine tyrosine hydroxylase was prepared and purified by slightly modifying previously described methods.[3,4] Tyrosine hydroxylase inhibition was determined[5] using a concentration of 5×10^{-5} *M* L-tyrosine (see Table I).

TABLE I

PER CENT INHIBITION OF TYROSINE HYDROXYLASE[a]

Compd	10^{-3} *M*	10^{-4} *M*	10^{-5} *M*
3	32		4
6	29	8	
7	97		22
12	15		6

[a] All values are the average of triplicate runs; iodotyrosine was run as a standard in each run and consistently yielded inhibition of approximately 50% at 10^{-5} *M* and 10% at 5×10^{-7} *M*.

Rat liver phenylalanine hydroxylase was prepared by the method of Kaufman[6] through the first $(NH_4)_2SO_4$ step and stored frozen in 200-μl fractions. Phenylalanine hydroxylase inhibition was carried out as described by Guroff and Abramowitz,[7] the tyrosine produced being measured spectrophotofluorometrically.[8] A K_m of 2.1×10^{-4} *M* for L-phenylalanine was obtained by a Lineweaver–Burke[9] plot of this enzyme preparation.

Compounds **3**, **6**, and **12** were tested at concentrations up to 2×10^{-3} *M* in a system containing 2×10^{-4} *M* L-phenylalanine and no inhibition was noted. The K_i of **7** determined by the method of Lineweaver and Burke, was found to be 2.1×10^{-4} *M* and appeared competitive.

Experimental Section[10]

1,2,3,4-Tetrahydronaphthyl-1-malonic acid (1) was obtained by hydrolysis of ethyl 1,2,3,4-tetrahydronaphthyl-1-malonate;[11]

(1) (a) Supported by Fellowship 4-F1-GM-18,614 and Grant AM-06480 from the National Institutes of Health, U. S. Public Health Service. (b) In partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Maryland, 1967. Present address: Merck, Sharp and Dohme Research Laboratories, Division of Merck & Co., Inc., Rahway, N. J. 07065. (c) Department of Chemistry, University of Northern Illinois, Dekalb, Ill. 60115. (d) To whom inquiries should be addressed.

(2) M. A. Phillips, *J. Chem. Soc.*, 2393 (1928).
(3) T. Nagatsu, M. Levitt, and S. Udenfriend, *J. Biol. Chem.*, **239**, 2910 (1964).
(4) M. Ikeda, L. A. Fahien, and S. Udenfriend, *ibid.*, **241**, 4452 (1966).
(5) S. Udenfriend, P. Zoltzman-Nirenberg, and T. Nagatsu, *Biochem. Pharmacol.*, **14**, 837 (1965).
(6) S. Kaufman, *Methods Enzymol.*, **5**, 809 (1962).
(7) G. Guroff and A. Abramowitz, *Anal. Biochem.*, **19**, 548 (1967).
(8) T. D. Waalkes and S. Udenfriend, *J. Lab. Clin. Med.*, **50**, 733 (1957).
(9) J. L. Webb, "Enzyme and Metabolic Inhibitors," Vol. 1, Academic Press, New York, 1963, p 150.
(10) The melting points, all uncorrected, were determined on a Thomas-Hoover melting point apparatus. Ir spectra were obtained on a Perkin-Elmer Infracord, Model 137 and uv spectra on a Beckman DB spectrophotometer. Where analyses are indicated only by symbols of elements, analytical results obtained for those elements were within 0.4% of the theoretical values.
(11) Synthesized as described by M. Protiva, J. Jilek, F. Vejdelek, and P. Finglova, *Chem. Listy*, **47**, 584 (1953).

recrystallized from PhMe, mp 133–135°. *Anal.* ($C_{13}H_{14}O_4$) C, H.

α-Oximino-1,2,3,4-tetrahydronaphthyl-1-acetic acid (2) was obtained from the reaction of **1** (14.04 g, 0.060 mol) with *i*-PrONO in the presence of dry HCl.[12] The oil obtained was cryst from PhMe to yield 9.0 g (68%), mp 152–154°.

N,N'-Diacetyl-4-aminophenylalanine (4).—*N*-Acetyl-4-aminophenylalanine[13] (5.2 g, 0.027 mol) was dissolved in warm H_2O and 4.5 ml (0.048 mol) of Ac_2O was added. After standing at room temp for 3 hr, the mixture was refrigerated for 48 hr. The resulting solid was washed with H_2O and dried to yield 4.4 g (70%), recryst from abs EtOH, mp 219–220° (lit.[13] mp 210–211°). *Anal.* ($C_{13}H_{16}N_2O_4$) C, H, N.

3-Nitro-N,N'-diacetyl-4-aminophenylalanine (5).—Compound **4** (1.0 g, 0.0038 mol) was added in small portions to 3 ml of concd H_2SO_4 at room temp. The resulting soln was cooled in an ice bath and 1.0 ml of concd HNO_3 was added dropwise, stirring over a period of 5 to 10 min. The reaction mixture was stirred at ice bath temp for a further 15 min and poured slowly, with stirring, into ice-water. The soln was extracted continuously with $CHCl_3$ overnight. After drying (Na_2SO_4), the $CHCl_3$ was removed under reduced pressure and the residue recryst from EtOAc to produce 0.82 g (70%) of **5**, mp 190–192°. *Anal.* ($C_{13}H_{15}N_3O_6$) C, H, N.

3-Nitro-4-aminophenylalanine (6).—Compound **5** (2.5 g, 0.008 mol) was hydrolyzed by refluxing with 50 ml of 20% v/v HCl for 1 hr. The bright red soln was cooled to room temp, adjusted to pH 7 with NH_4OH, and refrigerated. The resulting ppt was recryst from H_2O to yield 1.5 g (83%) of **6**, mp 235° dec. *Anal.* ($C_9H_{11}N_3O_4 \cdot 0.5H_2O$) C, H, N.

Benzimidazole-5(6)-alanine Dihydrochloride (7).—A soln of 2.25 g (0.01 mol) of **6** in 100 ml of 4 *N* HCl was hydrogenated at 3.2 kg/cm² over 5% Pd–C. The reaction mixture was filtered into a flask containing 5.0 ml of 97–100% formic acid under N_2. The contents were refluxed for 1 hr under N_2 and evaporated to dryness under reduced pressure to yield 2.37 g (86%) of crude **7**. This was recryst by dissolving in a minimum of hot 4 *N* HCl, adding 2–4 vol of EtOH, and then Et_2O dropwise until just cloudy, mp 265° dec. *Anal.* ($C_{10}H_{12}Cl_2N_3O_2 \cdot H_2O$) C, H, N.

3,4-Ethylenedioxybenzyl Chloride (8).—HCl gas was bubbled into a mixture of benzodioxane[14] (74 g, 0.54 mol) and $ZnCl_2$ (74 g, 0.54 mol) in 200 ml of concd HCl for 15 min while the temp was maintained at 10–15°. To this mixture, 100 ml of aq CH_2O (35–40%) was added dropwise with stirring at 10–15°. After the addition, the reaction was stirred for 2 hr at 15–20°. The organic phase was taken up in C_6H_6, washed several times with H_2O, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was distilled to yield 43 g (43%) of **8**, bp 114° (0.55 mm). This compound slowly decomposed on standing giving off HCl and must, therefore, be used within 24 hr.

Ethyl 3,4-Ethylenedioxybenzylmalonate (9).—Na (5.3 g, 0.23 g-atom) was dissolved in dry EtOH (200 ml), protected from atmospheric moisture. To this soln was added diethyl malonate (80.0 g, 0.5 mol), followed by **8** (43.0, 0.23 mol), dropwise, with stirring. The reaction was then refluxed until neutral to litmus (approx 3 hr). EtOH was then removed by distn. The residue was treated with H_2O, the ester separated, and the aq phase extracted twice with Et_2O. The ester and ether phases were combined, washed (H_2O), dried (Na_2SO_4), and the Et_2O was removed then under reduced pressure. Fractionation of the resulting oil yielded 56 g (79%) of **9**, bp 165–170° (0.5 mm) which was characterized as the corresponding acid.

3,4-Ethylenedioxybenzylmalonic Acid (10).—Compound **9** (71 g, 0.23 mol) was hydrolyzed by refluxing in aq KOH, yielding 50.3 g (87%) of **10**, mp 152–155°. *Anal.* ($C_{12}H_{12}O_6$) C, H.

α-Oximino-β-(3,4-ethylenedioxyphenyl)propionic acid (11) was prepared by the same procedure as **7**; yield 71%, mp 149–150.5°. *Anal.* ($C_{11}H_{11}ClNO_5$) C, H, N.

3,4-Ethylenedioxyphenylalanine·HCl (12) was prepared by the same procedure as **3**; yield 72%, mp 208° dec. *Anal.* ($C_{11}H_{14}ClNO_4$) C, H, N.

(12) Oscar Touster, *Org. React.*, **7**, 353 (1953).

(13) Synthesized by the procedure of P. Block, Jr., *J. Org. Chem.*, **21**, 1237 (1956).

(14) Prepared by modification of method of B. N. Ghosh, *J. Chem. Soc.*, **107**, 1588 (1915).

Synthesis and Structure–Activity Relationships of 1-Alkoxyalkyl- and 1-Aryloxyalkyl-1,4-benzodiazepin-2-ones

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Received December 1, 1969

The literature describes only one example of a 1,4-benzodiazepin-2-one, 1-substituted by groups with an ether function[1] (compound I of our series) and its pharmacological properties are not described. We have prepared 11 compounds of this type, listed in Table I, which were screened for sedative, muscle relaxant, and anticonvulsant effects in mice; the LD_{50} was also determined (Table II).

Alkylation of 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one[2] with $NaOCH_3$ and various alkoxy or aryloxyalkyl bromides (chloro derivatives are unreactive) gave 1-substituted benzodiazepin-2-ones. An exception was VI which was obtained by reduction of the nitro group in VIII. The structure of these compounds was confirmed by ir spectra and acid hydrolysis, to give the corresponding N-substituted 2-amino-benzophenones in good yield (two examples are given in the experimental section). We found it to be an excellent method for the preparation of *N*-alkyl derivatives of 2-aminobenzophenones instead of the method of sulfonamide alkylation.[3]

Structure–Activity Relationship.—The replacement of the Me group in diazepam by alkoxyalkyl, aryloxyalkyl, arylmercaptoalkyl group, and its sulfone changes only quantitatively the diazepam activities, producing in general a lower activity. Of the alkoxy derivatives, the butoxy compound II is the most active; the phenyl ethers have lower activity, specially in their anticonvulsant effect. The presence of an electron-releasing substituent in the benzene ring produces a high activity compared with those that have no substituent or have an electron withdrawing group. The thioether and its sulfone have an activity intermediate between the aliphatic and aromatic ether; β-naphthoxyethyl derivative XI combines a minor muscle relaxant effect with its anxiolytic effect. In general the activity has no relation with the weight of the substituent. All compounds show low acute toxicity compared with diazepam.

Pharmacological Methods

We have used adult Rockland's male mice (20–25 g). The drugs assayed were suspended in 2% carboxymethyl cellulose and given all by oral route (gastric sound). In the determinations of the lethal effect, the drugs were dissolved in DMA and injected ip (4.0 ml/kg); 8

(1) Hoffmann-La Roche, Netherlands Patent, 6,510,539; *Chem. Abstr.*, **65**, 732 (1966).

(2) L. H. Sternbach and E. Reeder, *J. Org. Chem.*, **26**, 4936 (1961).

(3) S. C. Bell, T. S. Sulkowski, C. Gochman, and S. J. Childress, *ibid.*, **27**, 562 (1962).