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

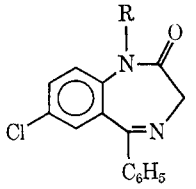
We have used adult Rockland's male mice (20–25 g). The drugs assayed were suspended in 2% carboxymethyl cellulose and given all by oral route (gastric sound). In the determinations of the lethal effect, the drugs were dissolved in DMA and injected ip (4.0 ml/kg); 8

(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).

TABLE I



No.	R	Yield, %[a]	Recrystn solvent	Mp, °C[b]	Formula[c]
I	$CH_3CH_2OCH_2CH_2$	56	Cyclohexane	101–102	$C_{19}H_{19}ClN_2O_2$
II	$CH_3(CH_2)_3OCH_2CH_2$	15	PhH–cyclohexane	155–156	$C_{21}H_{23}ClN_2O_2$
III	$C_6H_5OCH_2CH_2$	50	PhH	166–167	$C_{23}H_{19}ClN_2O_2$
IV	$C_6H_5O(CH_2)_3$	43	EtOH–PhH	163–164	$C_{24}H_{21}ClN_2O_2$
V	p-$CH_3C_6H_4OCH_2CH_2$	16	EtOH–PhH	121–122	$C_{24}H_{21}ClN_2O_2$
VI	p-$NH_2C_6H_4OCH_2CH_2$	75	PhH	148–149	$C_{23}H_{20}ClN_3O_2$
VII	p-$ClC_6H_4OCH_2CH_2$	79	EtOH–PhH	165–166	$C_{23}H_{18}Cl_2N_2O_2$
VIII	p-$NO_2C_6H_4OCH_2CH_2$	81	EtOH–PhH	170–171	$C_{23}H_{18}ClN_3O_4$
IX	$C_6H_5SCH_2CH_2$	12	Cyclohexane	97–98	$C_{23}H_{19}ClN_2OS$
X	$C_6H_5SO_2CH_2CH_2$	67	EtOH–PhH	178–179	$C_{23}H_{19}ClN_2O_3S$
XI	β-$C_{10}H_7OCH_2CH_2$	44	Cyclohexane	104–105	$C_{27}H_{21}ClN_2O_2$

[a] Values obtained are from a single experiment; no attempts have been made to obtain optimal yields. [b] Melting points are uncorrected. [c] All compounds were analyzed for C, H, and N. Values are within ±0.4% of theoretical.

TABLE II

	Psychopharmacologic and neuromuscular activity (mice) ED50, mg/kg p.o.		Anticonvulsant activity (mice) ED50, mg/kg p.o.			Acute toxicity (mice)
No.	Tube test	Mobility	Antypentylenetetrazole	Antimax shock	Antimin shock	LD50, mg/kg i.p.
Diazepam[a]	6	3	2	15	10	300
I	18	5	4	10	18	340
II	7	4	4	12	15	550
III	27	48	40	400	>500	>600
IV	40	34	85	>500	>500	>600
V	25	15	30	65	76	>500
VI	19	10	15	75	39	>500
VII	73	105	57	>500	>400	>600
VIII	200	120	92	160	500	>600
IX	16	14	15	80	43	>500
X	29	19	18	70	47	>500
XI	35	150	25	500	350	>500

[a] Reference compound.

mice and 3 dose levels were used for each determination. Each drug was tested after 2–3 hr The dose of drug (mg/kg) required to produce the desired endpoint in 50% of animals (ED_{50}), in every test was calculated by interpolation on the dose–effect curve relationship.

Psychopharmacologic and Neuromuscular Action.—This was carried out according to the method of Boissier, Tardy, and Diverres[4] and was considered the dose that inhibited the climbing backward up a tube held vertically, in 50% of mice.

Motility.—This was determined by the number of passages in a cylindric grey box (170-mm diameter) with 2 photocells. We only considered the mice that had an average of passages of between 100 and 180 during the first 15 min (control period). The ED_{50} was considered as the dose that decreased the motility 50% in relation to the control period.

Anticonvulsant Activity.—The antipentylenetetrazole test was carried out by the Everett and Richards method.[5] The ED_{50} was calculated as the dose which prevented convulsions in half of mice after administration of 125 mg/kg sc of pentylenetetrazole. The minimal electroshock convulsant threshold was measured by the method of Swinyard, *et al.*[6] The dose level at which half of the mice were protected against a minimal seizure was recorded as the ED_{50}.

Lethal Effects.—The LD_{50} was calculated according to Trevan's method.[7]

Experimental Section

1-Substituted-7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-ones (Table I).—A mixture of 0.5 g (0.01 mol) of NaOMe, 2.5 g (0.01 mol) of 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (mp 210–212°), and 40 ml of *i*-PrOH was refluxed 15 min with stirring and the Na salt crystallized; 0.015 mol of the apropiate bromoalkyl ether was then added and the mixture was refluxed 4 hr and filtered; compounds III, IV, V, VII, and X crystallized on cooling; in the other cases the solvent was evaporated *in vacuo* and if the residue resulted in a paste, it was chromatographed through alumina and later recrystallized from the proper solvent.

1-(*p*-Aminophenoxyethyl)-7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (VI).—To a boiling mixture of 2.2 g of VIII, 1.7 g of NH_4Cl, 4 ml of H_2O, and 18 ml of methyl cellosolve, 1.2 g of Fe powder was added over 1 hr; the mixture was refluxed 1 hr, filtered, ane evaporated to dryness *in vacuo*, the residue was dissolved in 20% HCl, treated with carbon, and

(4) J. R. Boissier, J. Tardy and J. C. Diverres, *Med. Exp.*, **3**, 81 (1960).
(5) G. M. Everett and R. K. Richards, *J. Pharmacol. Exp. Therap.*, **81**, 402 (1944).
(6) E. A. Swinyard, W. C. Brown, and L. S. Goodman, *J. Pharmacol. Exp. Ther.*, **106**, 319 (1952).
(7) J. W. Trevan, *Proc. Roy. Soc. Ser. B*, **101**, 483 (1927).

filtered. By neutralization with 30% NH_4OH, 1.5 g of crude product VI precipitated (see Table I).

2-Ethoxyethylamino-5-chlorobenzophenone.—A mixture of 2.3 g of I, 15 ml of EtOH, and 7.5 ml of HCl was refluxed 2 hr on a steam bath, cooled, filtered, made alkaline with 20% NaOH and extracted with C_6H_6; the extract was evaporated and the residual oil distilled *in vacuo* to give 1.9 g (93%) of a dense yellow oil, bp 130–135° (4 mm). *Anal.* ($C_{17}H_{18}ClNO_2$) C, H, N.

2-Phenoxyethylamino-5-chlorobenzophenone.—A mixture of 0.5 g of III, 10 ml of EtOH, and 5 ml of HCl was refluxed 2 hr on a steam bath, filtered, and neutralized with NH_4OH; on cooling, 0.3 g (72%) of a yellow solid crystallized; recrystallized from EtOH–H_2O, mp 87–88°. *Anal.* ($C_{21}H_{18}ClNO_2$) C, H, N.

Acknowledgment.—The authors are grateful to Miss B. C. Cieri for the preparation of intermediates and to Miss M. L. Sotil for collaboration in the pharmacological evaluations.

Angiotensin II Analogs. IV.[1] Synthesis and Biological Evaluation of Simplified Angiotensins

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Received February 9, 1970

It was shown earlier[2,3] that the N-terminal portion of the tissue hormone angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) could be greatly simplified and yet retain significant pressor activity. Thus, Gly-Gly-Val-Tyr-Ile-His-Pro-Phe and its polymethylene analog, δAvl-Val-Tyr-Ile-His-Pro-Phe, had pressor activities which were 10[4] and 7%, respectively, of that of angiotensin II. When the terminal amino group was acylated or eliminated, the pressor activity was greatly reduced, thus Ac-Gly-Gly-Val-Tyr-Ile-His-Pro-Phe had 0.4% pressor activity[2] while Ac-Gly-Val-Tyr-Ile-His-Pro-Phe had ~1% pressor activity.[3] From these results it was concluded that a single basic group separated by 5 atoms from the valine N of Val-Tyr-Ile-His-Pro-Phe is sufficient to significantly enhance the pressor activity of the relatively inactive (<1%) hexapeptide.

Because Khosla, *et al.*,[5] have shown that the side-chain of valine in position 3 is not very important (Asp-Arg-Ala-Tyr-Ile-His-Pro-Phe has 68% pressor activity), an attempt was made to simplify angiotensin II further by synthesizing Gly-Gly-Gly-Tyr-Ile-His-Pro-Phe and its polymethylene analog, ηAoc-Tyr-Ile-His-Pro-Phe. These peptides were synthesized as described earlier[3] by the solid phase method of Merrifield[6] and their pressor properties were evaluated in nephrectomized pentolinium-treated male rats anesthetized with pentobarbital by the method of Boucher, *et al.*[7]

Results and Discussion

Gly-Gly-Gly-Tyr-Ile-His-Pro-Phe and ηAoc-Tyr-Ile-His-Pro-Phe both had pressor activities which were only 0.1% of that of angiotensin II. These results were somewhat surprising since both of these peptides should be capable of fitting a receptor as well as Gly-Gly-Val-Tyr-Ile-His-Pro-Phe and δAvl-Val-Tyr-Ile-His-Pro-Phe. The very low activities of these more simplified analogs suggests that the side chain of valine in position 3 plays a more important role in these analogs than would have been expected from the results of Khosla, *et al.*,[5] on peptides containing aspartic acid and arginine. It is not clear whether the function of the valine side chain is steric, hydrophobic, or a combination of both, but in view of these results, it would be very interesting to see the effect of replacing valine by glycine in angiotensin II itself.

Experimental Section[8]

***N*-*t*-Butyloxycarbonylglycylglycylglycine (I).**—To a solution of 1.89 g (10 mmol) of glycylglycylglycine (Fox Chemical Co.) in 20 ml of H_2O were added 0.81 g (20 mmol) of MgO and 2.86 g (20 mmol) of *t*-butyloxycarbonyl azide. The mixture was stirred at 45° for 48 hr then extracted with 50 ml of Et_2O. The aq phase was acidified to pH 3.5 with citric acid then evapd to dryness under high vacuum at 25°. The residue was extracted with $CHCl_3$ (5 × 250 ml). The $CHCl_3$ was removed on a rotary evaporator at 40° leaving 2.2 g of white powder. This powder was extracted with boiling AcOEt (2 × 300 ml). Evaporation of the AcOEt left 1.65 g of powder which was crystallized from AcOEt (240 ml) giving small needles: yield 1.14 g (40%), mp 129–130°. Tlc showed one spot, R_fIII: 0.85, R_fIV: 0.46 (detected by spraying the plate with 6 *N* HCl, heating to 110°, spraying with ninhydrin solution, and heating until spots appeared). *Anal.* ($C_{11}H_{19}N_3O_6$) C, H, N.

***N*-*t*-Butyloxycarbonyl-η-aminooctanoic Acid (II).**—To a solution of 1.59 g (10 mmol) of η-aminooctanoic acid in 20 ml of H_2O were added 0.81 g (20 mmol) of MgO and 2.86 g (20 mmol) of *t*-butyloxycarbonyl azide. The mixture was stirred at 45° for 48 hr, extracted with Et_2O (2 × 20 ml) then acidified to pH 3.5 with citric acid. The oil which separated was extracted into AcOEt (4 × 20 ml). The AcOEt washes were combined, washed with H_2O (4 × 20 ml), then dried ($MgSO_4$). Evaporation of AcOEt at 40° on a rotary evaporator gave a colorless oil which crystallized as small needles when triturated with heptane: yield 1.68 g (65%); mp 37–9°; tlc showed one Cl^9 + spot, R_fI: 0.62, R_fII: 0.82. *Anal.* ($C_{13}H_{25}NO_4$) C, H, N.

Gly-Gly-Gly-Tyr-Ile-His-Pro-Phe (III).—Boc-Tyr(Bzl)-Ile-His(Bzl)-Pro-Phe-polymer[3] (0.9 g, 0.15 mmol), was deprotected

(1) Part III: E. C. Jorgensen, G. C. Windridge, and T. C. Lee, *J. Med. Chem.*, **13**, 352 (1970). This investigation was supported in part by Public Health Service Research Grants AM 08066 and AM 06704 from the National Institute of Arthritis and Metabolic Diseases and by NIH Training Grant No. 5 T01 GM 00728 from the National Institute of General Medical Sciences. The abbreviations used to denote amino acid derivatives and peptides are those recommended in *Biochemistry*, **5**, 2485 (1966). ηAoc stands for η-aminooctanoic acid.

(2) E. C. Jorgensen, G. C. Windridge, W. Patton, and T. C. Lee, *J. Med. Chem.*, **12**, 733 (1969).

(3) E. C. Jorgensen, G. C. Windridge, and T. C. Lee, *ibid.*, **13**, 352 (1970).

(4) Best recent value expressed on a molar basis.

(5) M. C. Khosla, R. R. Smeby, and F. M. Bumpus, *Biochemistry*, **6**, 754 (1967).

(6) (a) R. B. Merrifield, *J. Amer. Chem. Soc.*, **85**, 2149 (1963); (b) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).

(7) R. Boucher, R. Veyrat, J. de Champlain, and J. Genest, *Can. Med. Ass. J.*, **90**, 194 (1964).

(8) Melting points were measured in a Thomas-Hoover Uni-Melt apparatus and are corrected. Amino acid analyses were performed on a Spinco Model 116 amino acid analyzer using the standard 4-hr methodology. Peptides were hydrolyzed under N_2 at 110° in constant boiling HCl containing aspartic acid or alanine as internal standards. Peptide content was calculated in terms of free peptide rather than the hydrated salt. Microanalyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, California. Where analyses are indicated only by symbols of the elements, analytical results obtained were within ±0.4% of the theoretical values. Precoated silica gel G plates (E. Merck) were used for tlc. The following solvent systems were used: I, xylene–pyridine–AcOH (100:15:5); II, *i*-Pr_2O–$CHCl_3$–AcOH (6:3:1); III, MeCOEt–AcOH–H_2O (70:30:25); IV, MeCOEt–AcOH–H_2O (200:30:25); V, *n*-BuOH–pyridine–AcOH–H_2O (15:10:3:12); VI, *n*-BuOH–AcOH–H_2O (3:1:1); VII, *s*-BuOH–3% NH_3 (100:44).

(9) D. E. Nitecki and J. W. Goodman, *Biochemistry*, **5**, 665 (1966).