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.

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

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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 57–9°; 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).

and acylated as previously described[3] with 260 mg (0.90 mmol) of Boc-Gly-Gly-Gly (I) and 186 mg (0.90 mmol) of DCCI in 20 ml of purified DMF[10] since Boc-Gly-Gly-Gly was not soluble in CH_2Cl_2. Complete acylation was achieved using a single 12-hr reaction. The peptide was cleaved from the polymer and hydrogenated as described[3] giving 125 mg. The crude peptide was dissolved in 5 ml of 0.05 *M* NH_4OAc–1 *M* AcOH and applied to a 2.5 × 100 cm column of Sephadex-C25-SE (NH_4^+) packed in the same buffer. The column was eluted at 34 ml/hr with a gradient of NH_4OAc in 1 *M* AcOH using a concentration change of 3.3×10^{-4} *M*/ml starting from 0.05 *M*. The effluent was monitored at 280 mμ and the fractions from the center of the main peak were pooled and lyophilized giving 40 mg. After further purification *via* the picrate salt[2,11] there was 30 mg of white powder, homogeneous on tlc, R_fV: 0.57, R_fVII: 0.33 (detected with Pauly reagent). A 72-hr acid hydrolysate had the following amino acids: Gly, 2.99; Tyr, 0.96; Ile, 1.00; His, 1.00; Pro, 1.00; Phe, 0.99; peptide content, 81%. A 48-hr acid hydrolysate incubated with L-amino acid oxidase (Worthington)[3] had the following amino acids: Gly 2.23, Tyr 0.00, Ile 0.00, His 0.06, Pro 1.00, Phe 0.00.[12]

ηAoc-Tyr-Ile-His-Pro-Phe (IV).—Boc-Tyr(Bzl)-Ile-His(Bzl)-Pro-Phe-polymer[3] (0.9 g, 0.15 mmol), was deprotected and acylated as before with 155 mg (0.60 mmol) of Boc-ηAoc (II) and 125 mg (0.60 mmol) of DCCI in 20 ml of CH_2Cl_2. The peptide was cleaved from the polymer and hydrogenated as previously described[3] giving 140 mg. This crude product was dissolved in 5 ml of 0.1 *M* NH_4OAc–1 *M* AcOH and applied to a 2.5 × 100 cm column of Sephadex-C25-SE (NH_4^+) packed in the same buffer. The column was eluted at 34 ml/hr with a gradient of NH_4OAc in 1 *M* A AcOH using a concentration change of 2.0×10^{-4} *M*/ml starting from 0.1 *M*. The effluent was monitored at 280 mμ and the fractions from the main peak which were homogeneous on tlc in solvents VI and VII were pooled and lyophilized to give 60 mg. After further purification *via* the picrate salt,[2,11] there was 47 mg of chromatographically homogeneous material, R_fVI: 0.70, R_fVII: 0.32 (detected with Pauly reagent). A 72-hr acid hydrolysate had the following amino acids; ηAoc, 1.00; Tyr, 0.97; Ile, 1.02; His, 1.04; Pro, 1.00; Phe, 0.99; peptide content, 96%. ηAoc emerged from the short (5.3 cm) column of the analyzer 20 ml after arginine and had a color value which was 23% that of leucine. A 48-hr acid hydrolysate incubated with L-amino acid oxidase[3] had the following amino acids: ηAoc, 0.80; Tyr, 0.00; Ile, 0.00; His, 0.09; Pro, 1.00; Phe, 0.00.

Acknowledgment.—We are grateful to Dr. D. Nitecki for her helpful discussions during the course of this work.

(10) A. B. Thomas and E. G. Rochow, *J. Amer. Chem. Soc.*, **79**, 1843 (1957).

(11) Both peptides described in this paper gave gummy picrate salts which were decomposed very slowly by the ion exchange resin AG 1 × 2 (acetate) in 1 *M* AcOH. In this case, the procedure described in ref 2 was modified so that the precipitated salt was dissolved in 1 ml of AcOH, H_2O was added until slightly turbid then the AG 1 × 2 (acetate) was added.

(12) These values were corrected for the amount of racemization occurring when a mixture of these amino acids was subjected to the same hydrolytic conditions.

Thyroxine Analogs. XIX.[1] 3,5-Dimethyl-3′-isopropyl-DL-thyronine

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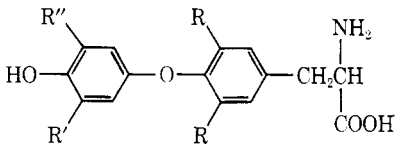
The iodine atoms of the thyroid hormones, L-thyroxine (L-T_4, **1**) and 3,5,3′-triiodo-L-thryonine (L-T_3, **2**),



1, R = R′ = R″ = I
2, R = R′ = I; R″ = H
3, R = Me; R′ = I; R″ = H
4, R = Me; R′ = *i*-Pr; R″ = H
5, R = R′ = R″ = Me

have been replaced, in part, by alkyl or aryl groups, with retention of hormonal activity.[2]

Hormonal activity is also retained by a partial or complete replacement of I by other halogen atoms, particularly Br,[3] or by a combination of Br and alkyl groups, *e.g.*, 3,5-dibromo-3′-isopropyl-L-thyronine.[4] The *i*-Pr group substitution produces an analog more potent than the parent compound when it replaces the 3′-I atom of L-T_3(**2**).[2e,g] *i*-Pr group replacement of the 3,5-I atoms of L-T_3, however, results in loss of hormonal activity.[1,5] The Me group is the only nonhalogen substituent yet reported to be capable of replacing the 3,5-iodines of L-T_3 with retention of thyroxine-like properties. 3,5-Dimethyl-3′-iodo-DL-thyronine (**3**) has been reported to show about 3% the activity of L-T_4 in the rat antigoiter assay.[6] Because of the uniqueness of this finding, it was felt desirable to carry out an independent resynthesis and biological evaluation of **3**.

The establishment or rejection of an essential role for halogen in the hormonal actions of L-T_4 and L-T_3 could aid in a better understanding of the events which initiate the hormonal response. Thus, the heavy atom perturbation theory, whereby I participates in energy transfer mediated by its excitability to the long-lived highly reactive triplet state,[7] could not be valid if a completely alkyl-substituted thyronine proved to be hormonally active. Because of the activities of 3,5-diiodo-3′-isopropyl-L-thyronine (500–1200% L-T_4) and of 3,5-dimethyl-3′-iodo-DL-thyronine (**3**, 3% L-T_4), 3,5-dimethyl-3′-isopropyl-DL-thyronine (**4**) was selected for synthesis as a potentially active halogen-free thyronine derivative. Previous studies indicated that 3,5,3′5′-tetramethyl-DL-thyronine (**5**) was inactive,[8] or possessed questionable weak activity.[6] A reevaluation of **5** at high dose levels in the antigoiter assay was therefore included in the present study.

(1) Paper XVIII: E. C. Jorgensen and J. Wright, *J. Med. Chem.*, **13**, 367 (1970). This research was supported by Research Grant AM-04223 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

(2) (a) E. C. Jorgensen, N. Zenker, and C. Greenberg, *J. Biol. Chem.*, **235**, 1732 (1960); (b) E. C. Jorgensen, P. A. Lehman, C. Greenberg, and N. Zenker, *ibid.*, **237**, 3832 (1962); (c) E. C. Jorgensen and J. A. W. Reid, *J. Med. Chem.*, **8**, 533 (1965); (d) B. Blank, F. R. Pfeiffer, C. M. Greenberg, and J. F. Kerwin, *ibid.*, **6**, 554 (1963); (e) C. M. Greenberg, B. Blank, F. R. Pfeiffer, and J. F. Pauls, *Amer. J. Physiol.*, **205**, 821 (1963); (f) C. S. Pittman, H. Shida, and S. Barker, *Endocrinology*, **68**, 248 (1961); S. B. Barker, M. Shimada, and M. Makiuchi, *ibid.*, **76**, 115 (1965); (g) M. Wool, V. S. Fang, and H. A. Selenkow, *ibid.*, **78**, 29 (1966); (h) C. M. Buess, T. Giudici, N. Kharasch, W. King, D. D. Lawson, and N. N. Saha, *J. Med. Chem.*, **8**, 469 (1965).

(3) M. V. Mussett and R. Pitt-Rivers, *Metab. Clin. Exp.*, **6**, 18 (1957).

(4) (a) E. C. Jorgensen and J. R. Nulu, *J. Pharm. Sci.*, **58**, 1139 (1969); (b) R. E. Taylor, Jr., T. Tu, S. B. Barker, and E. C. Jorgensen, *Endocrinology*, **80**, 1143 (1967).

(5) T. Matsuura, T. Nagamichi, K. Matsuo, and A. Nishinaga, *J. Med. Chem.*, **11**, 899 (1968).

(6) E. C. Jorgensen and R. A. Wiley, *ibid.*, **5**, 1307 (1962).

(7) (a) G. Cilento and M. Berenholc, *Biochim. Biophys. Acta*, **94**, 271 (1965); (b) A. Szent-Györgyi, "Bioenergetics," Academic, New York, N. Y., 1957, pp 24, 27.

(8) (a) C. S. Pittman, H. Shida, and S. B. Barker, *Endocrinology*, **68**, 248 (1961); (b) H. J. Bielig and G. Lützel, *Justus Liebigs Ann. Chem.*, **608**, 140 (1957).