mixture was heated for 2.5 hr. at 60–70°, cooled to room temperature, diluted with ether, and filtered. The product was washed with ether and dried, leaving 1.32 g. (91%) of colorless plates which were analyzed directly.

Trialkylsulfonium Perchlorates. (A) **Without Removing Water.**—A solution of 5 mmoles of dialkyl sulfide in 5 ml. of the corresponding alcohol was mixed with 0.72 g. (5 mmoles) of 70% perchloric acid and heated under reflux for 24 hr. The mixture was cooled to room temperature, diluted with 50 ml. of ether, and filtered to collect any sulfonium salt formed. Under these conditions the following yields were obtained: *n*-butyl, 46% (45% after 48 hr. reflux); *n*-propyl, 24%; ethyl, < 1%. The yield of triethylsulfonium perchlorate was raised to 16% if the reaction was carried out in ethanol solution in a sealed vessel at 120° for 24 hr.

(B) **With Removal of Water.**—A solution of 0.73 g. (5 mmoles) of di-*n*-butyl sulfide in 15 ml. of *n*-butyl alcohol was mixed with 0.72 g. (5 mmoles) of 70% perchloric acid and refluxed under a condenser with provision for periodic removal of solvent by distillation. A total of 6 ml. of distillate was collected over a 24-hr. period. Near the end of the reflux period an insoluble oil appeared in the reaction flask. The mixture was cooled and poured into 50 ml. of ether, precipitating 1.27 g. (84%) of tri-*n*-butylsulfonium perchlorate.

(C) **Benzyldi(*n*-butyl)sulfonium Perchlorate.**—A mixture of di-*n*-butyl sulfide (0.53 g., 3.59 mmoles), 70% perchloric acid (0.52 g., 3.59 mmoles) and 5 ml. of benzyl alcohol was heated at 70–80° for 4 hr., cooled and diluted with ether. The white solid product (1.02 g., 83.5%) had m.p. 70–72°. Recrystallization from acetone/ether in a Dry Ice–acetone bath left the melting point (71–73°) essentially unchanged.

Anal. Calcd. for $C_{15}H_{25}ClO_4S$: C, 53.48; H, 7.48. Found for crude product: C, 54.05; H, 7.48. Found after purification: C, 53.25; H, 7.21.

Our product was identical with a sample synthesized by the reaction of benzyl bromide and dibutyl sulfide in ether at 25° followed by conversion to the perchlorate with perchloric acid; m.p. 70–72°, mixture m.p. 72.5–73.5°; found on elemental analysis C, 53.44; H, 7.36. Our directly synthesized product thus is a single compound of unambiguous structure.

The formation of the sulfonium salts under the conditions of synthesis was shown not to be a reversible process by refluxing 3.02 g. (10 mmoles) of tri-*n*-butylsulfonium perchlorate, 10.0 ml. of dry butanol, and 0.43 g. of water for 24 hr. These conditions duplicate the solvent composition in the synthetic reaction (46% yield), but 2.78 g. (92%) of the sulfonium salt was recoverable unchanged by dilution with ether.

A 0.5 *N* solution of potassium *n*-butoxide was prepared by dissolving cautiously 9.775 g. (0.25 g.-atom) of potassium metal in 500 ml. of *n*-butyl alcohol, previously distilled from sodium. This reagent, prepared and stored under nitrogen, was used to titrate the perchloric acid content of butanol solutions[7] after refluxing for 24 hr. in the absence of dialkyl sulfide. The reagent was standardized by titration against benzoic acid in butanol. It was shown that a solution of 14.4 g. (0.1 mole) of 70% perchloric acid in 100 ml. of butanol retained 101% of the original acid concentration after 24 hr. reflux. Unfortunately, azeotropic removal of water led to formation of a black polymer in the butanol mixture.

Acknowledgment.—The authors wish to thank Professor D. H. R. Barton for a helpful discussion of this work.

(7) This is a modification of a standard method for the quantitative determination of potassium; see F. P. Treadwell and W. T. Hall, "Analytical Chemistry," Vol. II, 9th ed., John Wiley and Sons, Inc., New York, N. Y., 1955, p. 272.

O-Acylation of Tyrosine during Peptide Synthesis

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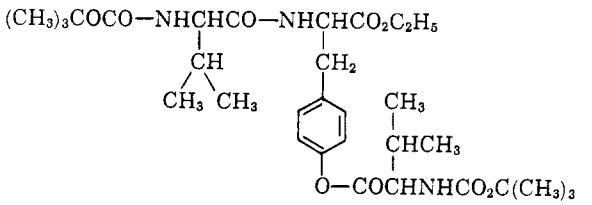
In the synthesis of angiotensin II amide-1 a great deal of difficulty was encountered in forming the valyl-tyrosine bond.[1,2] Only after many attempts were we able to obtain a 50% yield[2] (subsequently raised to 67%) of ethyl *t*-butyloxycarbonyl-L-valyl-L-tyrosinate (I) from N,N′-carbonyldiimidazole,[3,4] ethyl L-tyrosinate[5] and *t*-butyloxycarbonyl-L-valine.[6] The *N,N′*-dicyclohexylcarbodiimide,[7] tetraethylpyrophosphite,[8] and *p*-nitrophenyl ester[9] methods gave only intractable oils. The mixed anhydride method[10] gave a 65% yield of I as the two isomorphic forms (m.p. 139.5–141° and m.p. 151–152°) described earlier.[2] Dimorphism also occurred with N,N′-carbonyldiimidazole preparations but in an erratic fashion.

Since we were particularly interested in N,N′-carbonyldiimidazole, the formation of I was examined in more detail. A sample of a crude reaction mixture of I was separated on a silica gel column and ethyl N,O-bis(*t*-butyloxycarbonyl-L-valyl)-L-tyrosinate (II) was detected in a ratio of one part of II to eight parts of I. The structure of II was proved by synthesis



II

from I and *t*-butyloxycarbonyl L-valine using N,N′-carbonyldiimidazole as the condensing agent. It was further confirmed by ultraviolet spectra studies.

Of the methods studied the mixed anhydride method gave the most easily purified dipeptide I. Replacing the *t*-butyloxycarbonyl group by a carbobenzoxy group, as in ethyl carbobenzoxy-L-valyl-L-tyrosinate, gave similar findings. Table I shows that the purest product again resulted from mixed anhydride coupling. The melting point was used as the criterion of purity.

TABLE I

PREPARATION OF Z·Val-Tyr·OEt

Method	Yield, %	M.p. °C.
Mixed anhydride[a,b]	64	155–157°
p-Nitrophenyl ester[a,c]	67	145–147°
N,N′-Carbonyldiimidazole[d]	22	152–154°

[a] These literature results have been repeated and confirmed. [b] L. T. Skeggs, Jr., K. E. Lentz, J. R. Kahn, and N. P. Shumway, *J. Exptl. Med.*, **108**, 283 (1958). [c] R. Schwyzer, B. Iselin, H. Kappeler, B. Riniker, W. Rittel, and H. Zuber, *Helv. Chim. Acta*, **41**, 1273 (1958). [d] Worked up by the procedure cited in footnote *b*.

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TABLE II

ULTRAVIOLET STUDIES[a]

Compound	Neutral media maxima		Basic media maxima[b]	
H·Tyr·OH[c] (L)	λ 223 (ε 8440)	λ 275 (ε 1410)	λ 240 (ε 11,600)	λ 294 (ε 2480)
H·Tyr·OEt (L)	λ 226 (ε 9180)	λ 278 (ε 1710)	λ 245 (ε 12,700)	λ 295 (ε 2550)
H·Tyr(OBz)·OH[d] (L)	λ 225 (ε 1210)	λ 275 (ε 107)	Obscured	λ 275 (ε 128)
B·Val-Tyr·OEt (2L)[e]	λ 225 (ε 9700)	λ 277 (ε 1640)	λ 245 (ε 13,450)	λ 295 (ε 2460)
N,O-(B·Val)$_2$-Tyr·OEt (3L)	λ 220 (ε 9010)	λ 265 (ε 322)	λ 245 (ε 13,150)	λ 295 (ε 2450)
N,O-(Ac)$_2$-Tyr·OH[c] (DL)		λ 264 (ε 321)		λ 295 (ε 2180)

[a] Read on a Beckman DU spectrophotometer or a Cary II recording spectrophotometer. [b] Two drops of 2 *N* sodium hydroxide were added to 3 ml. of neutral solution in a cuvette. The peaks were read 5 min. later. [c] Mann Research Laboratories. [d] Cyclo Chemical Corp. This compound is very insoluble in water and the extinction coefficient is probably low. The relative values from neutral to basic solution are accurate, however. [e] B = *t*-Butyloxycarbonyl.

Experimental

Isolation of By-products.—To a solution of 2.18 g. (10.0 mmoles) of *t*-butyloxycarbonyl-L-valine[6] in 10 ml. of tetrahydrofuran was added 1.95 g. (10.0 mmoles, 83% purity) of N,N′-carbonyldiimidazole.[3] After 30 min., 2.09 g. (10.0 mmoles) of ethyl L-tyrosinate[5] was added and the solution was left standing over the weekend. It was then concentrated under vacuum. The residue was taken up in 50 ml. of ether, washed with 40 ml. of *N* sulfuric acid, 20 ml. of saturated aqueous sodium bicarbonate and 40 ml. of water. The ethereal layer was dried over anhydrous sodium sulfate and evaporated to dryness. Scratching solidified the residue, giving 3.73 g. (91%), m.p. 127.5–150°. One gram of this solid was dissolved in chloroform and placed on 30 g. of silica gel in a column, 3.3 cm. × 8.5 cm. The column was eluted with seven 100-ml. portions of 20% ethyl acetate–80% chloroform, then six 100-ml. portions with progressively higher percentages of ethyl acetate. Each fraction was examined by thin layer chromatography on silica gel using 30% ethyl acetate in chloroform. On development with chlorine gas and *o*-tolidine–potassium iodide reagent,[11] fraction 3 gave one spot R_f 0.62 and fraction 4 showed two spots R_f 0.38 and 0.61. Fractions 5–9 gave only one spot at 0.38 as did a pure sample of I. The other fractions did not contain any material. Fraction 4 was rechromatographed and the new fractions combined with the corresponding old. Fractions 5–9 were recrystallized from ethyl acetate-petroleum ether to give 0.55 g. (50%) of I, m.p. 140–140.5°. Fraction 3 was recrystallized from 3 ml. of isopropyl alcohol to give 0.046 g. (4.2%) of a compound, m.p. 125–126.5°, presumed to be ethyl N,O-bis(*t*-butyloxycarbonyl-L-valyl)-L-tyrosinate (II). The ratio of II to I was 1 to 8. A mixed melting point of II with an authentic sample (see below) gave no depression. Both had identical R_f's on silica gel thin layer chromatography in 30% ethyl acetate in chloroform. The ultraviolet spectra was identical to that of an authentic sample of II.

The sulfuric acid wash of the crude product from above was examined for ethyl O-(*t*-butyloxycarbonyl-L-valyl)-L-tyrosinate, this type of compound having been described[12] recently. The acidic wash was neutralized with sodium bicarbonate and quickly extracted with ether. The ethereal solution was dried over anhydrous sodium sulfate and then evaporated to dryness. An ultraviolet spectrum was taken on this residue in neutral and in basic media. Normally, compounds with a free phenolic group such as tyrosine, ethyl tyrosinate and I experience a shift in from λ278 to λ295. This is accompanied by an increase in optical density of a factor of 1.5. O-Acylated compounds, as described below, have a shift from λ 264 to λ 295 with a 7.7-fold shift in optical density. The residue had a 2.3-fold shift from λ 277 to λ 295 indicating the presence of an O-acylated phenol. The λ 264 peak being small was obscured in neutral media. Thin layer chromatography of this residue on silica gel showed the presence of an unknown that was neither starting material nor product. No trace of II was detected.

Ethyl N,O-Bis(*t*-butyloxycarbonyl-L-valyl)-L-tyrosinate (II).—After 1.57 g. (7.23 mmoles) of *t*-butyloxycarbonyl-L-valine had been dissolved in 10 ml. of tetrahydrofuran, 1.41 g. (7.23 mmoles, 83% pure) of N,N′-carbonyldiimidazole was added. Thirty minutes later 2.95 g. (7.23 mmoles) of I was added. After 1 hr. the solution was concentrated under vacuum to a clear oil. The oil was worked up 16 hr. later by dilution with 10 ml. of water and 20 ml. ether. The ethereal layer was dried over anhydrous sodium sulfate, evaporated to dryness and the residue dissolved in chloroform. This was placed on a silica gel column and eluted with 20% ethyl acetate–80% chloroform. The ultraviolet absorption of each fraction was taken at λ 265 and λ 277. When a fraction came off whose absorption was greater at λ 277 than at λ 265, it was assumed the N,O-compound (II) was off the column. The purity of the various fractions was checked by thin layer chromatography. Those fractions with the correct ultraviolet absorption and with only one spot, R_f 0.62, were combined and recrystallized from 30 ml. of isopropyl alcohol. The product crystallized and was collected, 2.05 g. (34%), m.p. 124.5–126.5°, $[\alpha]^{25}D$ −35.4° ± 1.2° (*c* 4, ethanol).

Anal. Calcd. for $C_{31}H_{49}N_3O_9$: C, 61.26; H, 8.13; N, 6.91. Found: C, 61.48; H, 8.26; N, 7.09.

Proof of Structure.—A spot of I and one of II on paper were sprayed with ferric chloride in *n*-butyl alcohol and heated to 60° for 48 hr. The N,O-compound (II) gave a negative test indicating the phenolic hydroxyl was not free. The dipeptide (I) gave a positive test.

Ultraviolet spectra are described in Table II. Since phenolic esters are activated, one would expect base to convert II to I. The spectra bear this out. An ultraviolet spectrum of N,O-diacetyl-DL-tyrosine[13] gave detailed structure at the λ 264 peak (shoulder at λ 258, maximum at λ 264 and small peak at λ 272) corresponding exactly to the fine structure of the ethyl N,O bis(*t*-butyloxycarbonyl-L-valyl)-L-tyrosinate (II) peak at λ 265.

Acknowledgment.—We thank Mr. L. Brancone and staff for analysis and Mr. W. Fulmor and staff for optical rotations.

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Preparation and Reactions of Trialkyltinlithium

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Recent reports from this laboratory[1b] have shown a convenient preparation for triphenyltinlithium. This organometallic can be prepared through the reaction between metallic lithium and either triphenyltin chloride or hexaphenylditin in tetrahydrofuran (THF). This paper concerns the extension of the same general synthetic procedure to prepare trialkyltinlithium compounds.

Trialkyltinlithium compounds have been prepared

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