In I, a weak positive Cotton effect thus appears to be completely hidden by an exceptionally large negative background. The reason for this anomaly is not apparent; the similar behavior of I, IX, and X shows that it cannot be ascribed to any simple conformational distortion of the double bond by, *e.g.*, formation of an additional ring through covalent (as in IX) or hydrogen bonds (as in I). In addition to this anomaly of their rotatory dispersions, I and its relatives are also anomalous in showing composite curves of circular dichroism (see Fig. 4), while those of the other acids investigated are simple. These facts indicate that the case of I is a complex one and that attempts at a more detailed interpretation should be postponed until more experimental material is available. It is remarkable, however, that the empirical rule of Bose and Chatterjee[11] correctly predicts that I should have a more negative $[\alpha]_D$ value than II.

The conjugated chromophore of I thus appears to behave entirely normally, but this fact could be detected only by study of the circular dichroism, not by that of optical rotatory dispersion, where the effect of this chromophore is completely obscured by background influences.[12]

The occurrence of Cotton effects in this area is not restricted to the free acids; their salts and esters seem to show the same behavior. Thus the sodium salt of V in aqueous solution gives a rotatory dispersion curve very similar to that of the free acid but shifted to greater negative rotations; though at 273 mμ, $[\Phi]$ −1900°. Similarly, several α,β-unsaturated lactones have been found to exhibit extrema in this area; *e.g.*, digitoxigenin, $[\Phi]_{256}$ +4600°, α-levantenolide,[13] $[\Phi]_{266}$ = +5930°, and β-levantenolide,[13] $[\Phi]_{266}$ = −5940°. The investigation is being extended to other compounds of this type.

It remains to be seen, when additional experimental material becomes available, whether the observation of such rotatory anomalies may be of value for the study of stereochemical problems, and what correlation exists between the direction of these Cotton effects and the configuration of the molecule.

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Preparation of *t*-Butyl Esters of Free Amino Acids[1]

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Most amino acids dissolve in dioxane–sulfuric acid mixtures and react with isobutene to form the *t*-butyl esters in 60–75% yield. The monobenzyl esters of aspartic and glutamic acid form benzyl-*t*-butyl esters, which can be hydrogenated to the mono *t*-butyl esters. β-*t*-Butyl L-aspartate and γ-*t*-butyl L-glutamate gave the N-carboxyanhydrides when treated with phosgene.

The *t*-butyl esters of amino acids are useful carboxyl-protecting groups in peptide synthesis because they are cleaved readily by acids. The esters have been prepared from N-acylated amino acids by reaction with isobutene,[2] or *t*-butyl acetate[3] and by conversion of the α-chloro-*t*-butyl esters to the amino esters *via* the azide,[4] a process which yields racemic esters. Some amino esters have been prepared from the free amino acids by reaction with *t*-butyl acetate and perchloric acid.[5] In a preliminary communication[6] we described a procedure for converting free amino acids to their *t*-butyl esters by reaction with isobutene in a mixture of dioxane and sulfuric acid. The yields reported at that time were around 45%. By using a more dilute reaction mixture, we have been able to raise the yields to 60–75% in many cases. The yield depends on the solubility of the amino acid in dioxane–sulfuric acid; L-phenylalanine is quite soluble and is converted rapidly to its *t*-butyl ester in 75% yield, while glycine is only slightly soluble and forms little ester. Diethylene glycol–sulfuric acid is also a satisfactory solvent for the reaction.

The four benzyl-*t*-butyl diesters of L-aspartic and L-glutamic acid were prepared from the monobenzyl esters. Hydrogenation of these provided the four

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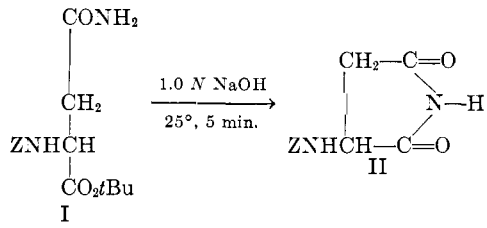
mono-*t*-butyl esters. β-*t*-Butyl L-aspartate reacted with carbobenzoxy chloride to form an oily product which formed a crystalline salt with dicyclohexylamine, identical to that obtained by alkaline hydrolysis of carbobenzoxy-β-*t*-butyl α-benzyl L-aspartate.[7]

β-*t*-Butyl L-aspartate and γ-*t*-butyl L-glutamate reacted readily with phosgene to form the N-carboxyanhydrides. These ought to be useful intermediates in the preparation of poly-L-aspartic and poly-L-glutamic acid.

The hydroxyl group of tyrosine is not etherified extensively under our conditions; the reaction yields tyrosine-*t*-butyl ester in 45% yield. The ether–ester has been prepared from carbobenzoxy-L-tyrosine.[8]

Carbobenzoxy-*t*-butyl L-asparaginate (I) reacted readily with cold sodium hydroxide to form carbobenzoxy-α-amino-L-succinimide (II). This observation is surprising in view of the known resistance of t-butyl esters to alkaline hydrolysis and indicates that a β-*t*-butyl aspartate residue in a peptide chain will probably undergo cyclization to the succinimide derivative in base as the methyl ester does.



As an example of the use of *t*-butyl esters in peptide synthesis, L-cystinylbis-L-valine was prepared by coupling dicarbobenzoxy-L-cystinyl dichloride and *t*-butyl L-valinate. The four protecting groups were removed with hydrogen bromide in acetic acid, the solution of the dihydrobromide in water was adjusted to pH 4.9 with lithium hydroxide, and the free peptide was precipitated with ethanol.

All the *t*-butyl ester hydrochlorides have an infrared absorption band of medium strength in the 830 to 845-cm.$^{-1}$ region. Strong absorption in the 800–920-cm.$^{-1}$ region has been observed for the *t*-butoxy group in peroxides[9] and attributed[10] to skeletal vibration of the *t*-butoxy group.

TABLE I

AMINO ACID *t*-BUTYL ESTER HYDROCHLORIDES, R·O-*t*-Bu·HCl[a]

R	Yield, %	M.p., °C,	$[\alpha]^{25}D$ c = 2. EtOH	—Calcd.— C	H	—Found— C	H
α-Bz L-Asp[b]	60	110–112	−2.6°	57.05	7.02	57.29	7.10
β-Bz L-Asp	73	115–117	+23.3°	[c]			
α-Bz L-Glu	67	124–126	+13.8°	[c]			
γ-Bz L-Glu	62	107–108	+16.4°	58.26	7.33	58.32	7.31
ε-Z-L-Lys[d]	65	147–149	+13.6°	57.97	7.84	57.93	7.94
L-Pro	27	110–112	−30.5°	52.04	8.73	52.29	8.79
L-Ileu	60	158–160	+30.9°	[c]			
L-Leu	62	166–167	+12.4°	[c]			
ε-Tos-L-Lys[e]	72	136–138	+14.8°	[c]			
L-Phe	75	[f]	+44.2°	[c]			
L-Tyr	45	143–145[g]	+24.4°	[c]			
L-Val	65	147–149	+20.5°	[c]			

[a] See example 1 for procedure used. [b] Bz is benzyl. [c] Analysis reported in ref. 6. [d] Z is benzyloxycarbonyl. [e] Tos is *p*-toluenesulfonyl. [f] Decomposes without melting. [g] The free amino ester.

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Experimental[11]

Dioxane was distilled from calcium hydride or from sodium before use.

1. **β-Benzyl α-*t*-Butyl L-Aspartate Hydrochloride.**—Twenty-five milliliters of liquid isobutene was added to a solution of 3.0 g. (0.013 mole) of β-benzyl L-aspartate in a mixture of 25 ml. of dioxane and 2.5 ml. of concentrated sulfuric acid in a 500-ml. pressure bottle, and the mixture was shaken mechanically at room temperature for 4 hr.[12] The solution was poured immediately into a cold mixture of 200 ml. of ether and 125 ml. of 1 *N* sodium hydroxide, and the aqueous phase was washed well with ether. The ether solution was dried over sodium sulfate and evaporated under vacuum to about 5 ml. This was diluted with 25 ml. of ether. Addition of dry hydrogen chloride gave the crystalline hydrochloride.[13] After recrystallization from ethyl acetate, it weighed 3.0 g. (73%) and had m.p. 115–117°.

All of the esters listed in Table I except those of L-tyrosine and ε-tosyl L-lysine were prepared by this method. Acetone–ether and ethanol–ether were also used for recrystallization of the hydrochlorides.

***t*-Butyl L-Tyrosinate.**—Three grams of L-tyrosine was dissolved in a mixture of 25 ml. of dioxane and 6.0 g. of *p*-toluenesulfonic acid monohydrate or 2.5 ml. of concentrated sulfuric acid. Twenty-five milliliters of liquid isobutene was added slowly and the reaction mixture was shaken for 20 hr. The solution was added to a cold mixture of 100 ml. of ethyl acetate, 100 ml. of water and 5 ml. of 5 *N* sodium hydroxide, the pH was adjusted to 9.1, and the product extracted twice with ethyl acetate. Evaporation of the solvent left a crystalline residue of 1.8 g. (45%), m.p. 140–143°. The ester was recrystallized for analysis from ethyl acetate–petroleum ether, m.p. 143–145°. The pK_a' values in 66% dimethylformamide were 7.45 and 12.7.

***t*-Butyl ε-Tosyl-L-lysinate.**—This was prepared as in example 1 except that the aqueous phase was adjusted to pH 9.5 before extracting the product.

2. **β-*t*-Butyl L-Aspartate.**—A suspension of 5.93 g. (0.0188 mole) of α-benzyl β-*t*-butyl L-aspartate hydrochloride in 200 ml. of ether was treated with 20 ml. of 25% potassium carbonate solution and the liberated ester was immediately extracted into the ether and the aqueous solution washed again with 50 ml. of ether. The ether was dried over sodium sulfate and evaporated under vacuum. The oily residue was dissolved in a mixture of 125 ml. of 95% ethanol and 75 ml. of water, 0.2 g. of 5% palladium on charcoal was added, and the solution was shaken under 3 atm. of hydrogen for 1 hr. The catalyst was removed by filtration and the solution was evaporated under vacuum to 50 ml. When 400 ml. of acetone was added, a gel formed, which changed to a crystalline precipitate when the mixture was stirred. The yield of material of m.p. 194–195° dec. was 2.69 g. (76%).

The other mono *t*-butyl esters of aspartic and glutamic acid listed in Table II were prepared in a similar way.

Carbobenzoxy-β-*t*-butyl L-Aspartate-dicyclohexylamine.—A solution of 0.63 g. (0.0033 mole) of β-*t*-butyl L-aspartate in 3.3 ml. of 1.0 *N* sodium hydroxide was treated with two portions each of 0.28 ml. of carbobenzoxy chloride and 1.8 ml. of 1 *N* sodium hydroxide over a 10-min. period. The solution was stirred vigorously during this time and for another 45 min. at room temperature. It was washed with ether, adjusted to pH 3.2 with hydrochloric acid, and extracted with ether to yield 1.00 g. (93%) of the oily carbobenzoxy-β-*t*-butyl-L-aspartate. The dicyclohexylamine salt was prepared as described by Schwyzer[7]; m.p. 126–128°.

3. **β-*t*-Butyl L-Aspartate-N-carboxyanhydride.**—Phosgene gas was passed into a stirred suspension of 0.60 g. (0.0032 mole) of β-*t*-butyl L-aspartate in 25 ml. of dioxane at room temperature until the solid dissolved (about 10 min.). Stirring was continued for 2 hr. A stream of dry nitrogen was passed through the solution for 2 hr. and the solution was evaporated to dryness under vacuum. The white solid residue was stored *in vacuo* over potassium hydroxide overnight, then dissolved in 15 ml. of hot ethyl acetate, and filtered to remove a small amount of polymerized material. Petroleum ether was added until the solution

(11) Melting points are corrected. The analyses were done by Midwest Microlaboratories, Indianapolis, Ind.
(12) A Parr hydrogenation apparatus is satisfactory. About 15-p.s.i. pressure is developed during the reaction; the container is cooled to reduce the pressure before opening.
(13) Some of the hydrochlorides required addition of petroleum ether.

TABLE II

MONO *t*-BUTYL ESTERS OF L-ASPARTIC AND L-GLUTAMIC ACID, R·O-*t*-Bu[a]

R	Yield,[b] %	M.p., °C.	$[\alpha]^{25}D$	R_f[c]	Calcd. C	Calcd. H	Found C	Found H
β-L-Asp	76	198–199 dec.	+8.5° *c*, 1.3, H_2O	0.63	50.78	7.99	50.77	8.49
α-L-Asp	71	178–179 dec.	+25.4° *c*, 1.1, H_2O	.66	50.78	7.99	50.79	7.83
γ-L-Glu	86	190–191 dec.	+17.3° *c*, 1.1, H_2O	.69	53.18	8.43	52.92	8.32
α-L-Glu	75	143–144	+16.0° *c*, 1.0, H_2O	.71	53.18	8.43	53.10	8.43

[a] See example 2 for procedure used. [b] From the benzyl *t*-butyl ester hydrochloride. [c] In *n*-butyl alcohol–acetic acid–water 4:1:1.

was turbid and the product crystallized; 0.48 g. (70%). It melts with decomposition at 138–140°, if placed in the bath at 135°. $[\alpha]^{25}D$ −35.6° (*c*, 2.0, EtOAc).

Anal. Calcd. for $C_9H_{13}O_5N$: C, 50.23; H, 6.09. Found: C 50.41; H, 6.02.

γ-*t*-Butyl L-Glutamate-*N*-Carboxyanhydride.—This was prepared from 0.602 g. (0.00296 mole) of γ-*t*-butyl L-glutamate as in example 3. It was recrystallized from ethyl acetate–petroleum ether; yield 0.54 g. (87%); m.p. 95–96°. $[\alpha]^{25}D$–19.0° (*c*, 2.0, EtOAc).

Anal. Calcd. for $C_{10}H_{15}O_5N$: C, 52.39; H, 6.60. Found: C, 52.89; H, 7.16.

Carbobenzoxy *t*-Butyl L-Asparaginate (I).—A solution of 3.0 g. of N-carbobenzoxy-L-asparagine, 25 ml. of dioxane and 2.5 ml. of concentrated sulfuric acid was treated with 25 ml. of liquid isobutene and the mixture was shaken for 4 hr. The solution was poured into a mixture of 100 ml. of ether and 250 ml. of 5% sodium bicarbonate, and extracted with ether (2 × 50 ml.). The ether solution was washed with 5% sodium bicarbonate, dried over sodium sulfate, and evaporated *in vacuo*. When the solid residue was recrystallized from ethyl acetate–petroleum ether, there was obtained 2.0 g. (55%) of material which melted at 105–106°. $[\alpha]^{25}D$-14.9° (*c*, 2.0, ethanol).

Anal. Calcd. for $C_{16}H_{22}O_5N_2$: C, 59.61; H, 6.88. Found: C, 59.77; H, 6.86.

Reaction of I with Sodium Hydroxide.—A solution of 1.61 g. (0.005 mole) of carbobenzoxy *t*-butyl L-asparaginate in 10 ml. of methanol and 5 ml. of water was treated with 5.0 ml. of 1.0 *N* sodium hydroxide, stirred for 3 min., acidified with 6 *N* hydrochloric acid, and evaporated to dryness under vacuum. The residue was dissolved in 25 ml. of ethyl acetate and 5 ml. of pH 7 buffer. The ethyl acetate was washed twice with pH 7 buffer, dried over sodium sulfate, and evaporated. The residue was recrystallized from ethyl acetate–petroleum ether to give II, 0.50 g. (40%), m.p. 78–82°. $[\alpha]^{26}D$ −43° (*c*, 2.0, 95% ethanol). Electrometric titration indicated solvent of crystallization. A sample was dried at 60° for 24 hr. before analysis.

Anal. Calcd. for $C_{12}H_{12}O_4N_2$: C, 58.06; H, 4.87. Found: C, 58.35; H, 5.00.[14]

Di-*t*-butyl Dicarbobenzoxy-L-cystinylbis Valinate (III).—A mixture of 4.19 g. (0.02 mole) of *t*-butyl L-valinate hydrochloride in 75 ml. of chloroform and 25 ml. of aq. 25% potassium carbonate at 10° was stirred while the solid diacid chloride obtained from 5.0 g. (0.01 mole) of dicarbobenzoxycystine[15] was added. After the mixture was stirred for 30 min., the chloroform layer was washed with 5% sodium bicarbonate (3 × 100 ml.), 1 *N* hydrochloric acid (2 × 100 ml.), and 20 ml. of saturated sodium chloride solution, and dried over sodium sulfate. After evaporation of the solvent under vacuum, the solid residue was recrystallized from ethyl acetate–petroleum ether; yield 6.7 g. (82%), m.p. 172–174°. A sample recrystallized for analysis had m.p. 173–175°. $[\alpha]^{25}D$ −80° (*c*, 1.0, dimethylformamide).

Anal. Calcd. for $C_{40}H_{58}O_{10}N_4S_2$: C, 58.70; H, 7.13; N, 6.83. Found: C, 58.27; H, 6.97; N, 6.88.

L-Cystinylbis-L-valine (IV).—A solution of 5.73 g. (0.007 mole) of the protected peptide III in 30 ml. of 2 *M* hydrogen bromide in acetic acid was allowed to stand at 25° for 1 hr., then heated at 100° for 1 min. The hydrobromide was precipitated by the addition of 200 ml. of ether, filtered, washed with ether, and allowed to stand *in vacuo* over potassium hydroxide overnight. It was dissolved in 50 ml. of water and washed with ether to remove benzyl bromide. The pH of the solution was adjusted to 4.9 by adding 13.5 ml. of 1 *N* lithium hydroxide and 400 ml. of ethanol was added. The precipitated peptide was filtered, washed well with ethanol, and dried. The yield was 2.8 g. (90%). $[\alpha]^{27}D$ −22.7° (*c*, 0.81, 5 *N* HCl).

Anal. Calcd. for $C_{16}H_{30}O_6N_4S_2 \cdot H_2O$: C, 42.04; H, 7.07. Found: C, 41.99; H, 7.31.

The product gave one ninhydrin-positive spot on a paper chromatogram in *n*-butyl alcohol–acetic acid–water (4:1:5), R_f 0.29.

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(14) The physical properties agree with those found by E. Sondheimer and R. Holley, *J. Am. Chem. Soc.*, **76**, 2467 (1954).

(15) Prepared according to the procedure of V. du Vigneaud and G. Miller, *Biochem. Prep.*, **2**, 75 (1952). and used immediately.