[CONTRIBUTION FROM THE WILLIAM H. NICHOLS LABORATORY, NEW YORK UNIVERSITY]

THE EFFECT OF ALKALI AND ACID UPON THE ROTATORY POWER OF CERTAIN DIPEPTIDES[1]

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It is well known that the salt of an optically-active substance may exhibit a rotation different from that of the parent substance. The alkali salts of optically-active α-bromo acids, for instance, show different rotations from those of the free acids, frequently with reversal of sign (1). Similarly the salts of optically-active amines show large changes in rotation as compared with the bases. The curves for the changes in rotation of optically-active acids and bases with the basicity or acidity of the medium bear a close relationship to the titration curves of the respective compounds in that they reflect the proportion of undissociated acid or base and ion in the solution. Attempts have been made to use such curves for the calculation of acidic and basic dissociation constants (2). Usually the curves are of relatively simple form; however, amino acids and proteins, which include both acidic and basic functional groups as structural elements, would be expected to exhibit a more complex behavior. About eighteen years ago Lutz and Jirgensons (3) determined the effect of different degrees of acidity and basicity upon the rotation of a number of amino acids. They were the first to plot as a continuous curve the changes in rotation accompanying the stepwise shift from strongly acidic to strongly basic solutions. Earlier work has been reviewed in a previous paper (4). They observed that all the naturally-occurring amino acids had curves of the same general shape and exhibited a minimum positive or maximum negative rotation in the isoelectric region which led them to conclude that the amino acids commonly found in proteins were configurationally related. Since several of the simpler natural amino acids had been shown to be configurationally related to L-lactic acid, it was proposed that all the natural amino acids belonged to the L-series. Although the natural occurrence of enantiomorphs of several amino acids has been recognized, no exceptions to the rule that members of the L-series have a minimum rotation in the isoelectric region have been encountered.

More recently attempts have been made to extend the relationship between configuration and rotational changes evoked by acids or bases to compounds having two or more asymmetric carbon atoms in their structure. Akasi (5) attempted to establish the configuration of octopine in this manner. Attention has been called to certain errors in his results and the conclusions have been critically reviewed (6). The complexity of the rotation curves of octopine and

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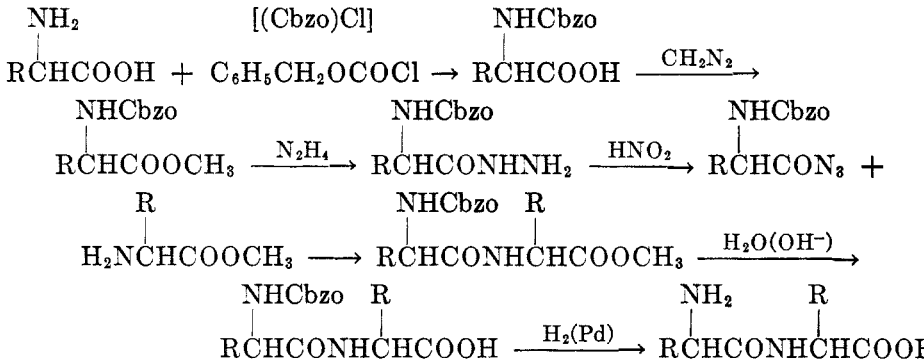
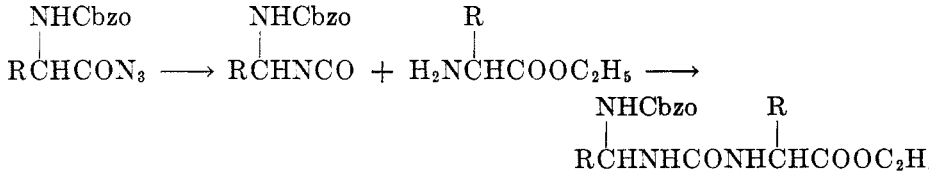
some closely related analogs emphasized the need for a study of the rotatory properties of simpler compounds in which the results would not be complicated by a multipicity of functional groups. For this purpose the rotation curves of dipeptides seemed well suited since such factors as the number of asymmetric carbon atoms, the number and variety of functional groups, and the effects of chemical changes such as acylation could be studied systematically. With this in mind a number of simple dipeptides having one or two asymmetric carbon atoms and only a single free amino and carboxyl group in their structure have been prepared and their rotation curves determined.

In order to synthesize dipeptides whose configurational relationship to the constituent amino acids is known unequivocally, the elegant techniques devised by Bergmann and his co-workers (7) involving the N-carbobenzoxy derivatives of the amino acids were the procedures of choice. An attempt was made to prepare L-valyl-L-valine[4] from N-carbobenzoxy-L-valine but difficulties were encountered in the preparation of the acid chloride. To circumvent the preparation of the acid chloride the Curtius technique (8) which had been adapted to their procedures by Bergmann, Zervas, and Greenstein (9) was employed.



R = $(CH_3)_2CH$—, $(CH_3)_2CHCH_2$—

No difficulty was encountered in the preparation of carbobenzoxy-L-valylhydrazide and this appeared to be converted into the azide by interaction with nitrous acid. However, attempts to couple the azide with D- or L-valine ethyl ester did not proceed smoothly. Although a very small amount of the desired carbobenzoxyvalylvaline was isolated in each case, the main product of the reaction was invariably the substituted urea formed by interaction of the Curtius rearrangement product of the azide and the amino acid ester.



[4] The compounds designated as *d*- and *l*-valine by Fischer (14) are now assigned the L and D configurations, respectively. The convention of indicating configurational relationship by the symbols L and D as suggested by Vickery (21) has been used throughout this discussion.

Since the valylvalines could not be prepared readily in sufficiently large amounts for rotation studies, attention was turned to the preparation of leucine peptides. Adequate amounts of both L-leucyl-L-leucine and L-leucyl-D-leucine were prepared in the manner indicated above. The L-leucyl-L-leucine could be obtained in anhydrous form by drying at 78° over phosphorus pentoxide in a vacuum but was so hygroscopic in this form that it was impractical to weigh the material. On the other hand, when placed in a moist atmosphere, it attained a

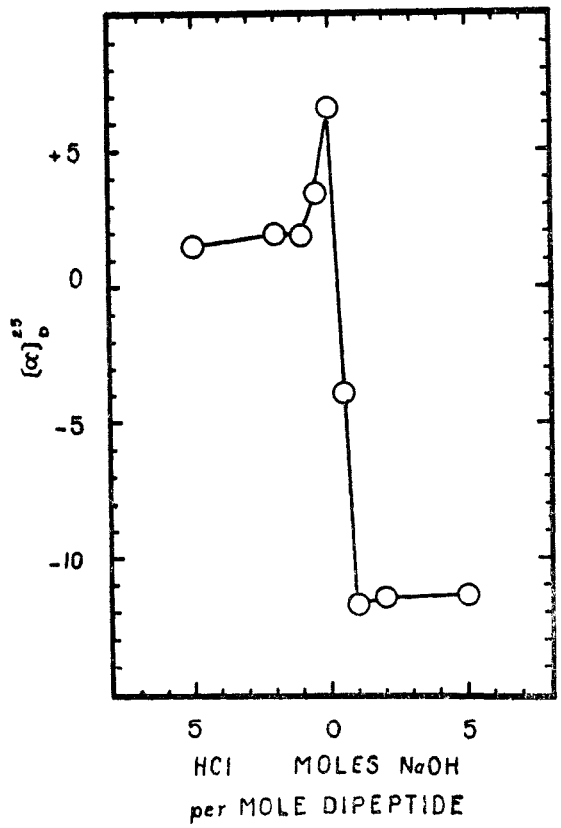


FIGURE 1. The effect of hydrochloric acid and sodium hydroxide on the specific rotation of L-leucyl-L-leucine.

constant weight after about two and a quarter moles of water had been absorbed and in this form it could be handled without difficulty. By application of the same technique to carbobenzoxy-β-alanine it was possible to prepare adequate quantities of β-alanyl-L-leucine. Carbobenzoxy-L-leucylglycine was prepared similarly, but the L-leucylglycine formed on hydrogenolysis of the former could not be obtained in solid form even by means of the expedients suggested by Fischer (10). Glycyl-L-leucine was prepared easily by the interaction of carbobenzoxyglycyl chloride and L-leucine ethyl ester followed by hydrolysis of the ester linkage and hydrogenolysis of the carbobenzoxy group.

The rotation of each of the solid dipeptides was determined in the presence of varying amounts of acid and base. From the results it appears that both L-leucyl-L-leucine (Figure 1) and L-leucyl-D-leucine (Figure 2) exhibit a maximum value in water in the isoelectric region, the rotation falling to less positive or more negative values upon the addition of either base or acid. After addition of one equivalent the further addition of acid or base caused only slight changes in the rotation such as would result on changing the solvent medium. These

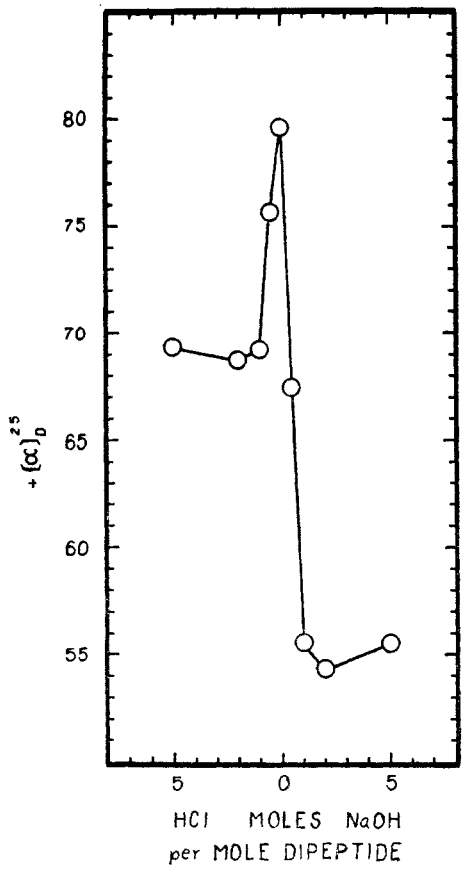


FIGURE 2. The effect of hydrochloric acid and sodium hydroxide on the specific rotation of L-leucyl-D-leucine.

curves are of the same type as those observed by Lutz and Jirgensons for simple monoaminomonocarboxylic acids except that they are the mirror images of the latter exhibiting a maximum rather than a minimum rotation in the isoelectric region. The similarity of the curves for the two leucylleucines suggests that when the two residues are similar the configuration of the amino acid entity carrying the free amino group determines the shape of the curve; however, further evidence would be required to establish this hypothesis.

The curves for glycyl-L-leucine and β-alanyl-L-leucine (Figure 3) appear to

be difficult to interpret at first glance. The latter is of the same general form as those for the leucylleucines and has a maximum in the isoelectric region, while the former is essentially the mirror image and exhibits a minimum rotation in the same region. The discrepancy between the curves for the leucylleucines and glycylleucine is, however, only apparent as becomes clear from an analysis of the contributions of the individual asymmetric carbon atoms according to the van't Hoff principle of optical superposition. For the leucylleucines, if A repre-

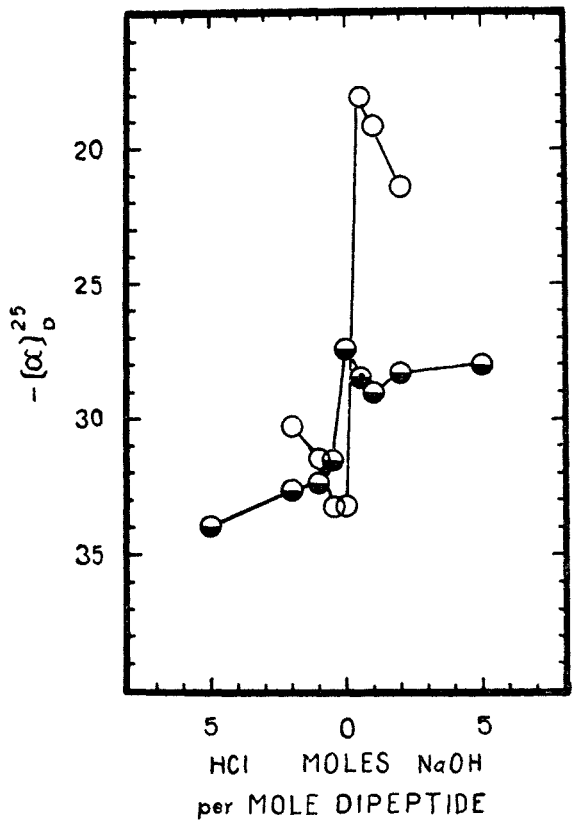


FIGURE 3. The effect of hydrochloric acid and sodium hydroxide on the specific rotations of glycyl-L-leucine (unshaded circles) and β-alanyl-L-leucine (half-shaded circles).

sents the contribution of the leucyl moiety and B represents the contribution of the leucine portion to the molecular rotation of the compound, the molecular rotation of L-leucyl-L-leucine may be expressed as the sum of A and B and that of L-leucyl-D-leucine as the difference between A and B. From the sum of these expressions the value of A may be calculated and from their difference the value of B is obtained. In Table I the values for the contribution of each asymmetric carbon atom for the various combinations of ionic forms are given together with the values for analogous L-leucine portions of glycyl-L-leucine and β-alanyl-L-leucine.

It is reasonable to assume, as appears from the values in Table I, that the contribution of the L-leucyl portion is the same in both leucylleucines. Similarly the contributions of the L- and D-leucine portions should be numerically the same but of opposite sign. It will be noted that the contribution calculated for the L-leucine moiety exhibits a minimum value in the isoelectric region and that acylation of the amino group has endowed the residue with a much greater *levo* rotation as compared with the free amino acid (3). An approximate value for the rotation of formyl-D-leucine in alkaline aqueous solution given by Fischer and Warburg (11) is included in the table since it supports the sign attributed to the rotation of the leucine portion of the peptide. Amide formation with the

TABLE I

CONTRIBUTION OF INDIVIDUAL ASYMMETRIC CARBON ATOMS TO THE MOLECULAR ROTATION[a] OF LEUCINE PEPTIDES

	$\overset{NH_2}{RCHCO}$ —— $\overset{R}{NHCHCOOH}$				
	L		L		D
Leucylleucines	(NH_3^+)	+86.9	(COOH)	−82	+82
	(NH_3^+)	+104.9	(COO^-)	−89.1	+89.1
	(NH_2)	+53.7	(COO^-)	−82.7	+82.7
Formyl-D-leucine (11)			(COO^-)		+74 appr.
Glycyl-L-leucine	(NH_3^+)	—	(COOH)	−59	
	(NH_3^+)	—	(COO^-)	−62	
	(NH_2)	—	(COO^-)	−35.7	
β-Alanyl-L-leucine	(NH_3^+)	—	(COOH)	−65.2	
	(NH_3^+)	—	(COO^-)	−55.5	
	(NH_2)	—	(COO^-)	−58.6	

[a] Molecular rotation $= [\alpha]_D \times \frac{\text{Mol. Wt.}}{100}$

carboxyl group, as exemplified by the L-leucyl portion of the peptide, has reversed not only the sign of the rotation for this configuration but has also reversed the direction of change of the rotation so that a maximum value is reached in the isoelectric region in going from acidic to basic solutions. Consequently, the rotational changes which the two asymmetric carbon atoms undergo are opposed in the L-leucyl-L-leucine, while in the diastereoisomer they are in the same direction and enhance each other, effects which are reflected in the larger over-all changes in rotation of the L-D-isomer as compared with the L-L-isomer. Another interesting point is the markedly greater effect produced upon the rotation by the ionization of the amino group as compared with the ionization of the carboxyl group.

Considering now the rotation curve of glycyl-L-leucine, it will be noted that the changes in rotation are those due to the L-leucine residue and that these are qualitatively in complete agreement with the results for the comparable portion of the leucylleucines. The greater effect of ionization of the amino group is noteworthy. It is perhaps surprising that β-alanyl-L-leucine should show a maximum

rotation in the isoelectric region since, here too, the asymmetry is in the leucine residue. However, the discrepancy may be due to the shift of the amino group to the *beta* position of the alanyl residue and thus may be related to other differences in properties associated with the β-amino acids. Further investigation of this point is desirable.

EXPERIMENTAL

DL-*Valine.* (a). Valine was prepared from isobutyraldehyde by interaction with a mixture of ammonium chloride and sodium cyanide suspended in methanol, followed by hydrolysis of the aminonitrile with conc'd hydrochloric acid. The yield of recrystallized amino acid was 36%. (b). A second preparation of DL-valine was carried out from isovaleric acid which was brominated according to the procedure of Marvel (12) and then aminated by the method of Cheronis and Spitzmueller (13). Yield, based on isovaleric acid, 47%.

*Formyl-*DL-*valine.* A solution of 100 g. of DL-valine in 500 ml. of 98–100% formic acid was heated to 50° and 300 g. of acetic anhydride was added. The temperature of the reaction mixture first dropped to 45° and then rose to 75° where it was maintained for twenty minutes. The solution was then evaporated under reduced pressure to a syrupy consistency, cooled and treated with 500 ml. of ice-water. The resulting aqueous solution was evaporated under reduced pressure until crystallization began, when, after chilling and filtering, 61.4 g of formyl-DL-valine was obtained. Concentration of the mother liquor gave an additional 36 g. After recrystallization from five times its weight of water, 89 g. of the formyl derivative was isolated as colorless plates, m.p. 142–142.5° after sintering at 136°. Fischer (14) reported a less sharp melting point in the same range.

*Resolution of formyl-*DL-*valine.* The resolution into the optically-active forms followed closely the procedure employed by Fischer (14). From 80 g. of the inactive formylvaline 20 g. of formyl-D-valine, m.p. 148–149°, $[\alpha]_D^{25}$ −13.3° in ethanol, and 19.8 g. of formyl-L-valine, m.p. 148–149°, $[\alpha]_D^{25}$ +13° in ethanol, were obtained.

The free amino acids were obtained on hydrolysis of the formyl derivatives by boiling with 20% hydrochloric acid for two hours. After isolation in the usual manner (14), 9.4 g. of L-valine, $[\alpha]_D^{25}$ +27.2° in 20% hydrochloric acid, was obtained from 14.4 g. of formyl-L-valine and a comparable amount of D-valine, $[\alpha]_D^{25}$ −27.6° in 20% hydrochloric acid, was obtained from formyl-D-valine.

Benzyl chlorocarbonate. This reagent was prepared by the method of Bergmann and Zervas (9) from phosgene and benzyl alcohol in toluene solution.

*Carbobenzoxy-*L-*valine methyl ester.* A solution of 5 g. of L-valine in 40 ml. of 1 *N* sodium hydroxide was treated with 13.6 g. of benzyl chlorocarbonate and 80 ml. of 1 *N* sodium hydroxide was added alternately in small portions during thirty minutes while the mixture was thoroughly shaken and kept at about 0°. Shaking was continued for an additional thirty minutes after addition of the reagents was complete or until the odor of benzyl chlorocarbonate had disappeared. After extraction of the alkaline solution with ether and acidification to Congo Red with hydrochloric acid the carbobenzoxy-L-valine separated as an oil which was taken up in ether and dried over sodium sulfate. Since the product could not be crystallized, it was converted into the methyl ester by treatment with diazomethane in ethereal solution. The methyl ester crystallized on evaporation of the solvent and was isolated in the form of colorless needles, m.p. 55–55.5°, $[\alpha]_D^{30}$ +16.4° in ethanol, yield, 6.6 g. (58%).

Anal. Calc'd for $C_{14}H_{19}NO_4$: N, 5.3. Found: N, 5.3.

*Carbobenzoxy-*L-*valylhydrazide.* A solution of 6.3 g. of carbobenzoxy-L-valine methyl ester in 50 ml. of absolute ethanol was treated with 3.2 g. of anhydrous hydrazine. After three days at room temperature the hydrazide had crystallized; the mixture was diluted with 10 ml. of water and the product filtered. A small amount of insoluble material remained when the hydrazide was dissolved in cold normal hydrochloric acid. After filtration of the acid solution, the hydrazide was precipitated by the addition of sodium acetate and re-

crystallized from ethanol from which it separated as silky needles, m.p. 178°, $[\alpha]_D^{22}$ −22.1° (*c*, 2.3 in 1 *N* HCl), $[\alpha]_D^{27}$ −22.8° (*c*, 2.5 in 1 *N* HCl).

Anal. Calc'd for $C_{13}H_{19}N_3O_4$: N, 15.8. Found: N, 15.9 (microDumas).

L-*Valyl*-D-*valine*. To a solution of 1 g. of carbobenzoxy-L-valylhydrazide in 6 ml. of glacial acetic acid and 30 ml. of dilute hydrochloric acid (1:10) cooled to 0–5° a solution of 0.4 g. of sodium nitrite in 4 ml. of water was added dropwise during five minutes. The oily product which separated was taken up in ether and the ethereal solution washed repeatedly with ice-water before drying over magnesium sulfate at refrigerator temperature for thirty minutes. The azide solution was then added to a solution of 1.57 g. of D-valine ethyl ester in 50 ml. of dry ether. After two days the ethereal reaction mixture was washed successively with dilute hydrochloric acid and 5% potassium carbonate solution, again dried over magnesium sulfate and evaporated to dryness under reduced pressure. Recrystallization of the partially crystalline residue from ether-hexane mixtures gave 0.89 g. of a product which separated as colorless needles, m.p. 176–177°, $[\alpha]_D^{27}$ +8.1° (*c*, 3.58 in ethanol) and appeared to be *N*-*α*-L-*carbobenzoxyaminoisobutyl*-*N'*-*α*-D-*carbethoxyisobutyl urea*, formed by interaction of the Curtius rearrangement product of the azide and the amino acid ester.

Anal. Calc'd for $C_{20}H_{31}N_3O_5$: N, 10.7. Found: N, 10.8.

Evaporation of the mother liquors from the crystallization of the urea derivative left an oily product which was saponified with ethanolic potassium hydroxide solution at room temperature. From the saponification mixture after dilution with water and evaporation of the ethanol under reduced pressure, a solid product separated upon acidification with hydrochloric acid. Recrystallization from ethanol gave 52 mg. of *carbobenzoxy*-L-*valyl*-D-*valine*, needles, m.p. 184–185° with sublimation, $[\alpha]_D^{25}$ −21.3° (*c*, 1.5 in aqueous 1 *N* KOH).

Anal. Calc'd for $C_{18}H_{26}N_2O_5$: N, 8.0. Found: N, 8.1.

Hydrogenation of 33 mg. of the carbobenzoxydipeptide in aqueous ethanol with palladium on charcoal as catalyst permitted the isolation of 11 mg. of L-*valyl*-D-*valine*, needles from aqueous ethanol.

Anal. Calc'd for $C_{10}H_{20}N_2O_3$: N, 13.0. Found: N, 13.0.

The amount of product isolated did not suffice for determination of the rotation.

L-*Valyl*-L-*valine*. From 2 g. of carbobenzoxy-L-valylhydrazide and 1.24 g. of L-valine ethyl ester following closely the procedure used in the preceeding preparation, the following products were isolated in the order given: (a) *N*-*α*-L-*Carbobenzoxyaminoisobutyl*-*N'*-*α*-L-*carbethoxyisobutyl urea* (1.42 g.), needles from ether-hexane mixtures, m.p. 167–168°, $[\alpha]_D^{25}$ +1.6° (*c*, 3.15 in ethanol).

Anal. Calc'd for $C_{20}H_{31}N_3O_5$: N, 10.7. Found: N, 10.8.

(b) *Carbobenzoxy*-L-*valyl*-L-*valine* (0.30 g.), needles from aqueous ethanol, m.p. 139.5–140°, $[\alpha]_D^{20}$ −36.6° (*c*, 3.15 in aqueous 1 *N* KOH).

Anal. Calc'd for $C_{18}H_{26}N_2O_5$: N, 8.0. Found: N, 8.1.

(c) L-*Valyl*-L-*valine* (0.15 g.), needles from aqueous ethanol.

Anal. Calc'd for $C_{10}H_{20}N_2O_3$: N, 13.0. Found: N, 12.9, 13.1.

L-*Leucine*. Commercial L-leucine obtained from the Corn Products Refining Corporation was purified by the method of Dunn (15). The purified product had a specific rotation of +15.2° in 6.15 *N* HCl.

Carbobenzoxy-L-*leucylhydrazide*. This derivative was prepared by essentially the same procedure as that employed by Bergmann and coworkers (16). The melting point, 121°, agreed with that reported; $[\alpha]_D^{25}$ −20.8° (*c*, 4.1 in ethanol).

L-*Leucyl*-L-*leucine*. Carbobenzoxy-L-leucyhydrazide (8.0 g.) was converted into the azide and allowed to react with the free ester from 5.5 g. of L-leucine methyl ester hydrochloride under conditions similar to those employed for the preparation of the valylvalines. Upon evaporation of the solvent from the reaction mixture the carbobenzoxydipeptide ester remained as an oil part of which could be isolated in crystalline form through solution in ethyl acetate-petroleum ether mixtures. After several crystallizations from the same solvent mixture, 2.35 g. of *carbobenzoxy*-L-*leucyl*-L-*leucine methyl ester* was obtained as needles, m.p. 97–98°.

Anal. Calc'd for $C_{21}H_{32}N_2O_5$: N, 7.1. Found: N, 7.1.

Although the analytical result was good, there was some question concerning the ultimate purity of the compound since it could not be freed of an odor reminiscent of the azide solutions.

Saponification of the ester gave an oily carbobenzoxydipeptide which was converted directly into the free dipeptide by hydrogenation in the presence of palladium on charcoal. After two recrystallizations from aqueous ethanol, 750 mg. of L-*leucyl*-L-*leucine* was isolated as very fine crystals, m.p. 266°, $[\alpha]_D^{25}$ +6.5° (*c*, 1.7 in water), −11.3° (*c*, 3.3 in water containing 5 equivalents of sodium hydroxide).

Anal. Calc'd for $C_{12}H_{24}N_2O_3$: total N, 11.5; amino-N, 5.7.
Found: N (Kjeldahl), 11.4; N (van Slyke), 5.8.

Fischer (14) reported m.p. 270°, and $[\alpha]_D^{20}$ +7° in water and −13.36° in 1 *N* sodium hydroxide.

L-*Leucyl*-D-*leucine*. Carbobenzoxy-L-leucylhydrazide (7 g.) was converted into the azide and allowed to react with the free ester from 4.5 g. of D-leucine methyl ester hydrochloride as previously described. Upon evaporation of the solvent the carbobenzoxy-L-leucyl-D-leucine methyl ester remained as a syrupy residue. Attempts to purify it by means of an adsorption column failed to give a crystalline product. Saponification of the crude ester with ethanolic potassium hydroxide solution gave an oily, acidic material and a small amount of apparently neutral material which was not further investigated. The acidic material had approximately the correct neutralization equivalent and nitrogen content for carbobenzoxy-L-leucyl-D-leucine but was not characterized further. After hydrogenation of the acidic material in 50% aqueous ethanol, L-*leucyl*-D-*leucine* could be isolated as a solid separating from aqueous ethanol as colorless needles in a yield of 0.7 g. Upon drying over phosphorus pentoxide at 78° and 0.01 mm. pressure the dipeptide was anhydrous but exceedingly hygroscopic. On exposure to moisture 701 mg. of the dipeptide reached a constant weight after absorbing 117 mg. of water, corresponding to 14.3% in the hydrated material or 2.27 moles of water per mole of peptide. The hydrated material was used for all analytical procedures, results being corrected for the water content of the product; $[\alpha]_D^{25}$ +69.3° (*c*, 2.76 in water containing 5 equivalents of hydrochloric acid). Fischer and Steingröver (17) reported the specific rotation as +68.95° in 1 *N* hydrochloric acid at 20°.

Anal. Calc'd for $C_{12}H_{24}N_2O_3$: total N, 11.5; amino-N, 5.7.
Found: N (Kjeldahl), 11.5; N (van Slyke), 5.8.

L-*Leucylglycine*. By interaction of the azide prepared from 1 g. of carbobenzoxy-L-leucylhydrazide with glycine ethyl ester in ether solution, essentially as described previously, 0.85 g. of *carbobenzoxy*-L-*leucylglycine ethyl ester* was obtained as colorless needles, m.p. 99°, after several crystallizations from ether-petroleum ether, $[\alpha]_D^{25}$ −26.8° (*c*, 2.6 in ethanol).

Anal. Calc'd for $C_{18}H_{26}N_2O_5$: N, 8.0. Found: N, 8.2.

Since the carbobenzoxydipeptide obtained by saponification of the ester failed to crystallize, it was hydrogenated in the usual manner. The dipeptide was isolated as a syrupy material, readily soluble in ethanol and in water, which could not be induced to crystallize even upon repeated treatment with absolute ethanol as suggested by Fischer (10).

L-*Leucine methyl ester hydrochloride*. Using the Fischer (18) technique 47 g. of L-leucine was esterified with methanolic hydrogen chloride. The methyl ester hydrochloride was isolated in 85% yield after crystallization from methanol and dry ether, m.p. 146–147°. Smith and Brown (19) give m.p. 149–150° for the D-isomer.

Glycyl-L-*leucine*. L-Leucine methyl ester, from 4.5 g. of hydrochloride, and the chloride from 2.5 g. of carbobenzoxyglycine were allowed to react in ether solution (9). Neither the ester nor the carbobenzoxydipeptide formed by saponification thereof could be crystallized, so the *glycyl*-L-*leucine* was liberated by hydrogenation and isolated in a yield of 264 mg. as colorless crystals after several recrystallizations from aqueous ethanol, $[\alpha]_D^{25}$ −33.2° (*c* 2.7 in water).

Anal. Calc'd for $C_8H_{16}N_2O_3$: total N, 14.9; amino-N, 7.4.
Found: N (Kjeldahl), 14.8, 15.0; N (van Slyke), 7.5.

Fischer and Steingröver (17) give the rotation as −35.1° in water at 20°.

Carbobenzoxy-β-alanylhydrazide. The crude ethyl ester hydrochloride prepared from 20 g. of β-alanine by esterification with absolute ethanolic hydrogen chloride, was converted into the carbobenzoxy derivative by treatment with 45 g. of benzyl chlorocarbonate and 12 g. of magnesia suspended in 100 ml. of water and 200 ml. of chloroform. Upon evaporation of the chloroform layer after washing with dilute hydrochloric acid and potassium bicarbonate solution, the carbobenzoxy ester remained as a syrup (40 g.) which was converted into the hydrazide by dissolving in 150 ml. of ethanol and boiling under reflux for two hours with 25 g. of 85% hydrazine hydrate. The hydrazide crystallized upon cooling the reaction mixture. A total of 39 g. of crude hydrazide was obtained after concentration of the mother liquors which gave after recrystallization from water, 35 g. (66%) of pure carbobenzoxy-β-alanylhydrazide, platelets, m.p. 144–145°. Using a different sequence of reactions Sifferd and du Vigneaud (20) obtained the same product, m.p. 143°.

TABLE II

CONCENTRATION, OBSERVED ROTATION, AND *p*H OF DIPEPTIDE SOLUTIONS CONTAINING VARYING AMOUNTS OF ACID AND BASE

EQUIV.	L-Leucyl-L-leucine			L-Leucyl-D-leucine			Glycyl-L-leucine			β-Alanyl-L-leucine		
	MG./CC.	α[b]	*p*H	MG./CC.[a]	α[b]	*p*H	MG./CC.	α[c]	*p*H	MG./CC.	α[b]	*p*H
HCl												
5	33.26	+0.10	0.55	27.57	+3.81	0.39				33.41	−2.27	0.42
2	33.37	+0.13	0.78	27.56	+3.79	0.98	27.03	−0.82	1.00	33.18	−2.17	0.92
1	33.10	+0.127	1.70	27.52	+3.81	2.08	27.03	−0.85	2.02	33.39	−2.16	1.97
¾				27.55	+3.88	2.54						
½	33.41	+0.11	3.30	10.51	+1.59	3.08	27.03	−0.90	3.24	33.45	−2.11	3.08
0	16.74	+0.22	5.65	10.17	+1.62	5.90	27.05	−0.90	5.88	33.22	−1.82	5.20
NaOH												
½	33.06	−0.26	7.90	10.16	+1.37	8.08	27.06	−0.49	8.15	33.37	−1.90	9.20
1	33.41	−0.78	11.48	27.28	+3.03	9.62	27.06	−0.52	9.90	33.41	−1.94	11.25
2	33.25	−0.76	—	27.44	+2.98	—	27.06	−0.58	—	33.43	−1.89	—
5	33.30	−0.76	—	27.56	+3.06	—	—	—	—	33.22	−1.86	—

[a] Calculated as anhydrous dipeptide. [b]Observed rotation in degrees at *t*, 25°, *l*, 2 dcm. [c]Observed rotation in degrees at *t*, 25°, *l*, 1 dcm.

β-Alanyl-L-leucine. By interaction in chloroform solution of the azide prepared from 7.2 g. of carbobenzoxy-β-alanylhydrazide and the ester from 5.0 g. of L-leucine methyl ester hydrochloride followed by saponification of the carbobenzoxydipeptide ester and hydrogenolysis of the carbobenzoxy group, 2.1 g. of pure β-alanyl-L-leucine was obtained as long, matted needles from 80% ethanol, $[\alpha]_D^{25}$ −27.4° (*c*, 3.3 in water). The intermediates were not obtained in crystalline form.

Anal. Calc'd for $C_9H_{18}N_2O_3$: N (total), 13.9; N (amino), 6.9.
Found: N (Kjeldahl), 13.8; N (van Slyke), 7.0.

Rotation curves. Solutions for the determination of rotations were prepared by weighing the samples into calibrated 2.5-ml. flasks. The requisite amounts of standard hydrochloric acid or sodium hydroxide were added and the solutions brought to volume with distilled water. Rotations were determined with a Schmidt and Haensch half-shadow polarimeter using a sodium lamp as the source of monochromatic light. The temperature was maintained at 25° by circulating water from a large thermostat around the polarimeter tube. With the exception of glycyl-L-leucine for which a one-dcm. semimicro tube was employed all determinations were made with the same two-dcm. semimicro tube. The *p*H of each solution was determined with a Beckman *p*H meter, Laboratory Model G. The observed rotation corresponding to each point is the average of a series of at least ten successive readings on the

same solution none of which deviated more than 0.03° from any other reading. In Table II are summarized the concentrations, observed rotations, and *p*H values for all solutions.

SUMMARY

A number of dipeptides of known configuration, including L-leucyl-L-leucine, its diastereoisomer, L-leucyl-D-leucine, glycyl-L-leucine, and β-alanyl-L-leucine were prepared. The changes in optical rotation which these peptides undergo on passing from neutral to alkaline or acid solution were determined. Both leucylleucines exhibited a maximum positive (*dextro*) rotation in the isoelectric region. Calculation of the contribution of each of the asymmetric carbon atoms indicated, when both components of the dipeptide are similar, that the amino acid residue having the free amino group made the greatest contribution to the total rotation of the dipeptide. It also appeared that, in the case of L-leucine, amide formation with the carboxyl group caused the leucyl residue to become more *dextro* rotatory while amide formation with the amino group caused the leucine residue to become more *levo* rotatory. The converse is to be expected with the D-isomer.

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