753. *Interaction between Carbonyl Groups and Biologically Essential Substituents. Part V.*[1] *The Effect of Ketones on Further Series of Optically Active Amino-derivatives.*

By F. Bergel and Margaret A. Peutherer.

2-Amino-alcohols containing asymmetric centres mutarotate in ketones in a direction opposite to that established for α-amino-esters [2] due to formation of the corresponding optically active oxazolidines. The behaviour of di-peptide esters and α-aminoacyl-2-amino-alcohols is complicated by the possible formation of imidazolidinones and by the difficulty in preparing pure α-amino-acylamino-esters.

As a result of work [2,3] on the rotatory behaviour of optically active amino-derivatives in ketonic solvents under mild conditions, a "rule" for optical configuration based on the direction of mutarotation of L- and D-enantiomorphs of α- and β-amino-esters in such solvents was obtained: for L- and D-α-compounds the change in specific rotation was

[1] Part IV, Bergel, Harrap, and Scott, *J.*, 1962, 1101.
[2] Part I, Bergel, Lewis, Orr, and Butler, *J.*, 1959, 1431.
[3] Part II, Bergel and Butler, *J.*, 1961, 4047.

towards higher negative and positive values, respectively. From results obtained with one L-β-amino-ester, shifts in the opposite sense can be assumed for β-amino-derivatives. A study of the rotatory properties in ketonic solvents of 2-amino-alcohols, α-aminoacylamino-esters (dipeptide esters) and aminoacylamino-alcohols was undertaken, with reference to this " rule."

2-Amino-alcohols.—These compounds were prepared by reduction of the corresponding L- and D-α-amino-esters with lithium aluminium hydride following the method of Karrer *et al.*[4] L-Serine ethyl ester was used for comparison purposes: it was obtained from the hydrochloride[5] by treatment with triethylamine in chloroform. The mutarotation results are shown in Table 1.

TABLE 1.

Maximal $[\alpha]_D$ at 19—22° (*c* 0·8—2·25).

Amino-derivative	EtOH	$COMe_2$	C_5H_8O [a]	$C_6H_{10}O$ [b]	$COBu^iMe$
L(+)-Leucinol	+6·9°	+49·5°	+38·0°	+40·0°	+32·2°
D(−)-Leucinol	−7·0	−59·1	−31·5	—	—
L(−)-Phenylalaninol	−26·5	+21·7	−12·9	+15·0	−26·1
D(+)-Phenylalaninol	+26·1	−26·5	+9·0	—	—
D(−)-Valinol	−17·1	−58·1	−45·6	−47·4	—
L-Serine ethyl ester	+0·6	−55·0	−74·0	—	—

[a] Cyclopentanone. [b] Cyclohexanone.

The time taken for maximal rotation to be reached was 20—180 min., with the exception of cyclohexanone which required only 3—10 min. The rotatory behaviour of 2-amino-alcohols, which might have been expected to correspond to that of their related α-amino-esters, was opposite to that of the latter compounds: for L- and D-2-amino-alcohols respectively, the change in specific rotation was towards higher positive and negative values. L-Serine ethyl ester, still followed the " rule," but the magnitude of mutarotation was less than that observed for other α-amino-esters. In acetone, for example, the molar rotational shift was 74·1° compared with 155·5° for L-alanine ethyl ester. The order of decreasing magnitude of mutarotation for 2-amino-alcohols in ketones was acetone, cyclohexanone, cyclopentanone, isobutylmethylketone; for α-amino-esters it was cyclopentanone, cyclohexanone, acetone. These results are discussed later.

Aminoacylamino-esters and Aminoacylamino-alcohols.—Polarimetric measurements (Table 2) under the same conditions were made with a number of dipeptide esters and with two dipeptide alcohols. The observations with the former compounds must be of a preliminary nature since the preparation of the free dipeptide esters and their purification presented great problems. Solid dipeptide ester hydrochlorides were prepared and from these the free esters were liberated. The usual methods[2,3] employed for α-amino-esters gave unsatisfactory yields. Consequently, the hydrochloride was dissolved in ethanol and treated with Amberlite IR 45, a weakly basic polystyrene ion-exchange resin, until no more chloride could be detected in the ethanol. The resin was filtered off, the solvent was removed at low temperature, and the colourless oil which remained was dissolved immediately in the ketone. The oils, nos. 1, 2, 3, and 10 in Table 2, were subjected to chromatography (Table 7) and were shown to contain only one ninhydrin positive substance in each case. None of the free dipeptide esters has been reported in the literature. Such compounds are unstable and are liable to dioxopiperazine formation: Anderson and Callahan[6] report production of 73% dioxopiperazine from glycyl-DL-phenylalanine ethyl ester after 5 hr. at room temperature. The mutarotation results obtained with freshly liberated dipeptide esters (Table 2) are, however, still of interest.

The time taken for the final values to be reached varied from 22—197 hr. For the L-L- and D-D-dipeptide esters nos. 1—6, the direction of mutarotation in ketonic solvents

[4] Karrer, Portmann, and Suter, *Helv. Chim. Acta*, 1949, **32**, 1156.
[5] Stammer, Wilson, Spencer, Bachelor, Holly, and Folkers, *J. Amer. Chem. Soc.*, 1957, **79**, 3236.
[6] Anderson and Callahan, *J. Amer. Chem. Soc.*, 1960, **82**, 3359.

TABLE 2.

$[\alpha]_D$ at 19—24° (*c*, 0·7—1·9).

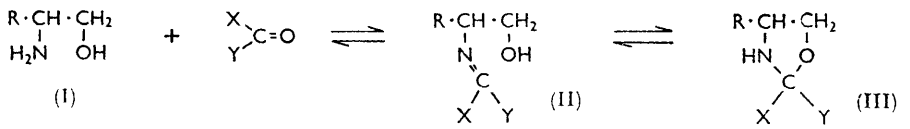
	Dipeptide Et ester	EtOH	$COMe_2$	C_5H_8O [a] $[C_6H_{10}O]$ [b]
1	L-Ala-L-phe-	+8·5°	+39·6° ⟶ −65·9°	+51·2° ⟶ +15·6°
2	D-Ala-D-phe-	−22·4	−55·6 ⟶ +82·2	−58·3 ⟶ −12·2
3	L-Ala-L-tyr-	+10·0	+44·4 ⟶ −56·4	+46·7 ⟶ −15·0
				[+44·4] ⟶ [−65·5]
4	L-Val-L-phe-	+6·3	−45·8 ⟶ −87·5	—
5	L-Leu-L-tyr-	+14·0	+3·3 ⟶ −66·6	+7·8 ⟶ −38·9
				[+7·8] ⟶ [−91·1]
6	L-Phe-L-leu-	−20·4	−20·0 ⟶ −61·1	−14·5 ⟶ −41·7
				[−0·6] ⟶ [−75·0]
7	L-Ala-D-phe- [c]	−18·6	+16·7 ⟶ +72·5	+8·8 ⟶ +17·6
8	D-Ala-L-phe- [c]	−2·6	−34·7 ⟶ −65·4	—
9	D-Ala-L-tyr-	+9·6	−16·7 ⟶ −70·8	−26·7 ⟶ −38·5
10	L-Val-D-phe-	+4·4	−15·8 ⟶ +7·9	−16·9 ⟶ −2·1
11	L-Ala-L-phe- [d]	−20·0	+13·0 ⟶ +4·0	+13·3 ⟶ +5·6
12	D-Ala-D-phe- [d]	+20·4	−20·0 ⟶ +1·0	−12·5 ⟶ −10·0

[a] Cyclopentanone. [b] Cyclohexanone. [c] Hydrochlorides were hygroscopic resins and were not analysed. [d] Dipeptide alcohol.

was the same as that for L- and D-amino-esters: for L-L- and D-D-dipeptide esters the specific rotation changed to higher negative and positive values, respectively. Initially, for dipeptide esters nos. 1—3, a change to higher positive and negative rotations as compared with ethanol solutions for L-L- and D-D-compounds respectively was noted. These three dipeptide esters contained alanine as the *N*-terminal amino-acid. No similar initial rotational shift was shown for the dipeptide esters nos. 4—6, which did not contain alanine. The dipeptide esters nos. 7—9 displayed a change towards higher positive and negative values of specific rotation for the compounds of L-D- and D-L-configuration, respectively. The dipeptide ester no. 10, of L-D-configuration, showed mutarotation towards higher positive values of specific rotation after an initial negative shift of rotation on addition of ketone. The extent of mutarotation of the two aminoacylamino-alcohols nos. 11 and 12 was small but the direction was towards less positive and negative values of specific rotation for L-L- and D-D-compounds, respectively. However, immediately on addition of ketone, opposite shifts were observed, like those mentioned above with alanylamino-esters.

Discussion.—For optically active amino-esters, the mutarotation in ketones is associated solely with azomethine formation.[2] * The reason for the difference in behaviour between amino-esters, 2-amino-alcohols, aminoacylamino-esters and aminoacylamino-alcohols must be found in the structures of the potential intermediate and/or end products of condensation with ketones.

With carbonyl compounds, 2-amino-alcohols (I) and L-serine esters can form the azomethine (II), the oxazolidine (III) or both.



Over sixty years ago Knorr and Matthes [7] condensed aldehydes with ethanolamines and, ignoring the possibility of Schiff's base formation, considered the products to be oxazolidines by virtue of their low boiling points and ease of hydrolysis. In 1942, Cope and Hancock [8] prepared a large number of compounds from ethanolamine and carbonyl compounds by azeotropic distillation in benzene, and assigned structures to them by comparison of the

* *Added, December 9th*, 1963.—See also Bonnett, David, Hamlin, and Smith, *Chem. and Ind.*, 1963, 1836.

[7] Knorr and Matthes, *Ber.*, 1901, **54**, 3484.
[8] Cope and Hancock, *J. Amer. Chem. Soc.*, 1942, **64**, 1503.

observed and calculated molecular refractions for the azomethines and the oxazolidines. The products were considered to be azomethines for all ketones studied with the exception of cyclohexanone, methylpropyl ketone and diisobutyl ketone. From these ketones the products were, respectively, the oxazolidine, an equilibrium mixture of azomethine and oxazolidine, and the azomethine with a small amount of oxazolidine. From infrared spectra,[9,10] in which the azomethine (C=N) shows a band between 1620 and 1680 cm.$^{-1}$ and the oxazolidine ring a triplet between 1080 and 1200 cm.$^{-1}$ from dipole moments,[11] and molecular refraction measurements Bergmann [12] concluded: (*a*) aliphatic ketones gave mixtures of the azomethine and oxazolidine; (*b*) isobutyl groups favoured the formation of open forms; (*c*) cyclohexanone and cyclopentanone gave the oxazolidine and azomethine, respectively.

We condensed both D(−)-valinol and L(−)-leucinol with cyclohexanone by azeotropic distillation of the components in benzene. The products were distilled and the infrared absorption spectra of the oils obtained were measured. The presence of triplets at 1080, 1140, and 1160 cm.$^{-1}$ for the product from D(−)-valinol and at 1080, 1140, and 1170 cm.$^{-1}$ for that from L(+)-leucinol, coupled with the absence of C=N absorption showed that both products were oxazolidines. The experimental molecular refraction, 54·1, of the D-valinol–cyclohexanone product was closer to that calculated for the cyclic structure, 53·3 (+0·8), than that of the Schiff's base, 55·3. A comparison between the molecular rotation of these oxazolidines and the maximal molecular rotations of D(−)-valinol and L(+)-leucinol in cyclohexanone is shown in Table 3.

TABLE 3.

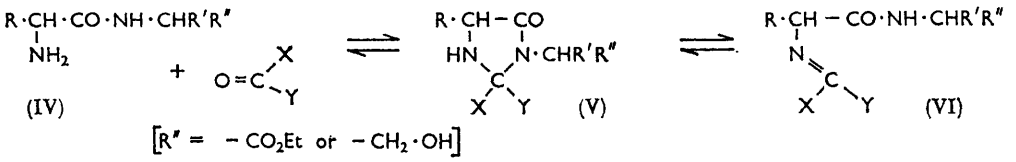
	$[M]_D$	
	EtOH	Cyclohexanone
D(−)-Valinol	−17·5°	−48·9°
D-Cyclohexanespiro-2′-(4′-isopropyloxazolidine)	−59·6	
L(+)-Leucinol	+8·2	+46·9
L-Cyclohexanespiro-2′-(4′-isobutyloxazolidine)	+67·0	

The sign and magnitude of the rotation of these compounds prepared under more vigorous conditions and identified as oxazolidines, are compatible with the idea that the direction of mutarotation, when 2-amino-alcohols react with ketones under mild conditions, is due to formation of oxazolidine side by side with varying amounts of the corresponding azomethine. The latter was detected by infrared absorption at 1670 cm.$^{-1}$ in reaction mixtures of L(+)-leucinol with acetone or cyclopentanone after maximal rotations had been reached (Table 8). Similarly, absorption at 1680, 1660, and 1675 cm.$^{-1}$ showed the presence of at least 5% azomethine in reaction mixtures of L(−)-phenylalaninol and acetone, isobutyl methyl ketone and cyclopentanone, respectively. No azomethine absorption was detected in the reaction with cyclohexanone, a fact which is an agreement with Bergmann's [12] conclusion concerning this ketone. Detection of oxazolidine in such reaction mixtures was difficult since both the alcohols and the ketones absorb in the 1080—1200 cm.$^{-1}$ region. The position of the equilibrium between azomethine and oxazolidine under mild conditions is not known: it depends on the nature of both the 2-amino-alcohol and the ketone. Despite the production of some azomethine when 2-amino-alcohols react with ketones other than cyclohexanone, the net rotational shift of all such alcohols studied in ketonic solvents was towards that of the corresponding oxazolidine and thus these compounds carry their own " rule " of optical rotation shifts.

With the dipeptide esters and aminoacylamino-alcohols (IV), there is the possibility of formation of an imidazolidinone (V) as well as the azomethine (VI).
Riebsomer [13] prepared a large number of imidazolidines by the azeotropic distillation of

[9] Bergmann, Zimkin, and Pinchas, *Rec. Trav. chim.*, 1952, **71**, 168.
[10] Kahovec, *Acta Phys. Austriaca*, 1948, **1**, 307.
[11] Bergmann, Fischer, Zimkin, and Pinchas, *Rec. Trav. chim.*, 1952, **71**, 213.
[12] Bergmann, *Chem. Rev.*, 1953, **53**, 309.
[13] Riebsomer, *J. Org. Chem.*, 1950, **15**, 237.

1,2-diamines in the presence of aldehydes. Similarly, Epstein [14] reacted 1,2-diamines with piperonal and found that, when both amino-groups were primary, a di-Schiff's base was formed, but when there was one primary and one secondary amino-group, the product was



an imidazolidine. Bergmann, Herman, and Zimkin [15] reported the formation of hexahydropyrimidines from the condensation of 2,4-diamino-4-methylpentane with isobutyl methyl ketone, cyclohexanone and acetophenone, and the imidazolidine from ethylenediamine and cyclohexanone. The reaction between benzyloxycarbonylamino-acid amides and carbonyl compounds was studied by Zehavi and Ben-Ishai [16] and the product from cyclohexanone was found to be the imidazolidinone.

In order to clarify the behaviour of optically active compounds, L-alanine methylamide, $CH_3{\cdot}CH(NH_2){\cdot}CO{\cdot}NH{\cdot}CH_3$, which can form imidazolidinones and azomethines, was taken as a model for both the aminoacylamino-esters and aminoacylamino-alcohols, and its mutarotation in acetone and cyclopentanone was studied (Table 4).

TABLE 4.

Maximal $[\alpha]_D$ at 24° (*c* 1·4).

	EtOH	$COMe_2$	Cyclopentanone
L-Alanine methylamide	+7·7°	+150·4°	+168·6°

The maximal reading was reached after 2 hours. A shift towards higher positive values of specific rotation was observed, representing an opposite change to that given by the corresponding L-α-amino-ester in ketones. L-Tyrosine amide in acetone had been reported [2] to mutarotate towards higher negative values of specific rotation associated with Schiff's base formation. The rotational behaviour in ketonic solvents of L-alanine methylamide, dipeptide esters and aminoacylamino-alcohols may be explained, tentatively, by the establishment of an equilibrium between imidazolidinone and azomethine, the position of this equilibrium depending on the nature of the amino-compound and the ketone. Using this explanation, for dipeptide esters and dipeptide alcohols of the L-L- and D-D-configuration, it is suggested that the direction of mutarotation is associated with production of an azomethine after initial formation of imidazolidinone in the case of the dipeptide esters nos. 1—3 (Table 2) with alanine as *N*-terminal amino-acid, and in the case of the aminoacylamino-alcohols. With the dipeptide esters of L-D- and D-L-configuration, one could speculate that the direction of mutarotation was due to imidazolidinone formation, except no. 10, L-valyl-D-phenylalanine ethyl ester, where Schiff's base may have been present initially.

Owing to the possibility of end-products other than azomethines, the rule for configuration based on mutarotation in ketonic solvents as previously described for L- and D-α- and -β-amino-esters must be modified for 2-amino-alcohols, and in a preliminary fashion for aminoacylamino-esters and aminoacylamino-alcohols. A 2-amino-alcohol is of the L-configuration if its specific rotation in ketones changes to higher positive values, and of the D-configuration if the opposite change is found. A complete interpretation of the results with dipeptide esters and aminoacylamino-alcohols cannot yet be put forward.

[14] Epstein, *J. Org. Chem.*, 1959, **24**, 68.
[15] Bergmann, Herman, and Zimkin, *J. Org. Chem.*, 1948, **13**, 353.
[16] Zehavi and Ben-Ishai, *J. Org. Chem.*, 1961, **26**, 1097.

Experimental

Solvents were dried and optical rotations were measured as described previously.[2]

Infrared absorption measurements (Table 8) were carried out with a Perkin-Elmer Infracord spectrophotometer. The samples were measured as thin films.

D(−)-*Valinol.*—D(−)-Valine ethyl ester hydrochloride (8·3 g.) was suspended in chloroform (10 ml.) and triethylamine (6·4 ml.) was added, with stirring. Ether (100 ml.) was added and the precipitated triethylamine hydrochloride removed. The filtrate was evaporated to dryness to give the free ester (6·1 g., 92%). The ester, in ether previously dried with lithium aluminium hydride, was added dropwise with stirring and cooling (5°) to a suspension of lithium aluminium hydride (2 g.) in ether (100 ml.). The mixture was refluxed for 1 hr., and the complex which formed was destroyed by the careful addition of water (2 ml.). The mixture was filtered, and the filtrate was dried ($MgSO_4$) and evaporated to dryness to give crystals of D(−)-*valinol* (3·2 g., 74%), m. p. 33°, $[\alpha]_D^{23}$ −17·1° (*c* 1·1 in EtOH) (lit.,[17] gives no physical characteristics, or analysis). D(−)-Valinol was converted into the *oxalate*, by the addition of an ethanolic solution of the amino-alcohol to one of oxalic acid. The crystals which formed were recrystallised from ethanol–ether, and had m. p. 184—185°, $[\alpha]_D^{23}$ −12·7° (*c* 1·1 in EtOH) (Found: C, 43·8; H, 7·8; N, 7·1. $C_5H_{13}NO,C_2H_2O_4$ requires C, 43·5; H, 7·8; N, 7·3%).

D(+)-*Phenylalaninol*, prepared as above, had m. p. 95°, $[\alpha]_D^{22}$ +26·1° (*c* 1·2 in EtOH) (Found: C, 71·6; H, 8·7; N, 9·4. $C_9H_{13}NO$ requires C, 71·5; H, 8·7; N, 9·3%) (lit.,[18] m. p. 91·5°, but no rotation or analysis).

L- and D-leucinol,[19] and L-phenylalaninol [20] had physical characteristics as described in the literature.

*Benzyloxycarbonyl-*D*-alanyl-*D*-phenylalanine Ethyl Ester.*—Isobutyl chloroformate (1·97 ml.) was added to a cooled (5°), stirred solution of benzyloxycarbonyl-D-alanine (3·35 g.) in tetrahydrofuran (10 ml.) containing triethylamine (2·1 ml.), and after 20 min. a solution of D-phenylalanine ethyl ester hydrochloride (3·44 g.) in chloroform containing triethylamine (2·1 ml.) was added. The mixture was left at room temperature overnight, evaporated to dryness and extracted with ethyl acetate (25 ml.). The extract was washed with 2N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate and water, and dried (Na_2SO_4).

*Benzyloxycarbonyl-*D*-alanyl-*D*-phenylalanine ethyl ester* was obtained on evaporation of the ethyl acetate extract as a solid (4·0 g., 68%), [recrystallised from ethyl acetate–light petroleum (b. p. 60—80°)], m. p. 98—99°, $[\alpha]_D^{23}$ +13·5° (*c* 2 in EtOH) (Found: C, 66·2; H, 6·3; N, 7·4. $C_{22}H_{26}N_2O_5$ requires C, 66·3; H, 6·6; N, 7·0%). Other new benzyloxycarbonyl dipeptide esters are shown in Table 5: all were recrystallised from ethyl acetate–light petroleum (b. p. 60—80°).

Table 5.

	Dipeptide Et ester	M. p.	$[\alpha]_D^{23}$ (*c* 2 in EtOH)	Yield (%)	Formula	Found (%) C	Found (%) H	Found (%) N	Required (%) C	Required (%) H	Required (%) N
1	Z-L-Ala-D-phe-	142°	−19·0°	45	$C_{22}H_{26}N_2O_5$	66·4	6·6	7·3	66·3	6·6	7·0
2	Z-D-Ala-L-phe-	136—138	+10·0 *	53	,,	66·4	6·8	7·1	,,	,,	,,
3	Z-L-Val-D-phe-	145	−4·0	51	$C_{24}H_{30}N_2O_5$	67·2	6·8	6·5	67·6	7·1	6·6
4	Z-L-Phe-L-leu-	118—119	−22·5	70	$C_{25}H_{32}N_2O_5$	67·7	7·1	6·3	68·2	7·3	6·4

Z = Benzyloxycarbonyl. * *c* = 1.

Benzyloxycarbonyl ethyl esters of the following dipeptides L-Ala-L-phe- and L-Ala-L-tyr-,[21] D-Ala-L-tyr-,[22] L-leu-L-tyr-,[23] and L-val-L-phe- [24] had physical characteristics as described in the literature.

D-*Alanyl-*D*-phenylalanine Ethyl Ester Hydrochloride.*—The benzyloxycarbonyl-derivative (4·0 g.) was hydrogenated at atmospheric pressure in ethanol (30 ml.) containing ethanolic 2N-hydrogen chloride (2 ml.), acetic acid (2 drops), and 5% palladium–charcoal. Evaporation of

[17] Nakazaki and Arakawa, *Bull. Chem. Soc. Japan*, 1961, **34**, 453.
[18] Karrer and Ehrhardt, *Helv. Chim. Acta*, 1951, **34**, 2202.
[19] Stoll, Peyer, and Hofmann, *Helv. Chim. Acta*, 1943, **26**, 929.
[20] Hunt and McHale, *J.*, 1957, 2073.
[21] Bergmann, M. and Fruton, *J. Biol. Chem.*, 1942, **145**, 247.
[22] Izumiya and Fruton, *J. Biol. Chem.*, 1956, **218**, 59.
[23] Vaughan and Osato, *J. Amer. Chem. Soc.*, 1951, **73**, 5553.
[24] Johnson and Stock, *J.*, 1962, 3806.

the filtered solution and crystallisation from ethanol–ether yielded crystals (2·2 g., 75%) of D-*alanyl*-D-*phenylalanine ethyl ester hydrochloride*, m. p. 145°, $[\alpha]_D^{20}$ −10·0° (*c* 2 in EtOH) (Found: C, 55·7; H, 6·8; Cl, 11·9; N, 9·4. $C_{14}H_{21}ClN_2O_3$ requires C, 55·8; H, 7·0; Cl, 11·8; N, 9·3%).

TABLE 6.

	Dipeptide Et ester hydrochloride	M. p.	$[\alpha]_D$ (*c* 2 in EtOH)	Yield (%)	Formula	Found (%) C	H	Cl	N	Required (%) C	H	Cl	N
1	L-Ala-L-phe-	75—76° (decomp.)	+6·0°	45	$C_{14}H_{21}ClN_2O_3$	55·5	7·0	11·6	9·3	55·8	7·0	11·8	9·3
2	L-Val-D-phe-	225	+32·5	50	$C_{16}H_{25}ClN_2O_3$	58·1	7·6	10·6	8·3	58·4	7·7	10·8	8·5
3	L-Phe-L-leu-	150	−14·5	75	$C_{17}H_{27}ClN_2O_3$	59·2	7·9	10·4	7·9	59·6	7·9	10·4	8·2

Other new dipeptide ester hydrochlorides are shown in Table 6. All were recrystallised from ethanol–ether.

The ethyl ester hydrochlorides of L-Ala-L-tyr- and D-Ala-L-tyr-,[22] L-leu-L-tyr-,[25] and L-val-L-phe-[24] had physical characteristics as described in the literature.

Liberation of Dipeptide Esters.—The hydrochloride was dissolved in dry ethanol, and small amounts of Amberlite 1R 45 resin added until chloride could no longer be detected in the solvent (Beilstein test) and until the polarimeter reading was constant. The resin was removed and the solvent evaporated at low temperature to give the dipeptide ester as an oil.

Chromatography.—The chromatogram (ascending) was developed for 2 hr. in butan-1-ol–ethanol–propionic acid–water (10 : 5 : 2 : 5), dried, and passed through a 0·25% solution of ninhydrin in acetone. R_F values are given in Table 7.

TABLE 7.

Dipeptide Et ester	R_F	Dipeptide Et ester	R_F
L-Ala-L-phe-	0·83	L-Ala-L-tyr-	0·74
D-Ala-D-phe-	0·86	L-Val-D-phe-	0·84

Benzyloxycarbonyl-L-*alanyl*-L-*phenylalaninol.*—Isobutyl chloroformate (0·87 ml.) was added to a cooled (5°), stirred solution of benzyloxycarbonyl-L-alanine (1·48 g.) in tetrahydrofuran (10 ml.) containing triethylamine (0·92 ml.), and after 20 min. a solution of L-phenylalaninol (1·0 g.) in chloroform (5 ml.) was added. After 16 hr. at 20° the mixture was evaporated to dryness and extracted with ethyl acetate. The extract was washed with 2N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water, and dried (Na_2SO_4). *Benzyloxycarbonyl*-L-*alanyl*-L-*phenylalaninol* (2·0 g., 83%) obtained on evaporation was crystallised from ethyl acetate–light petroleum (b. p. 60—80°) and then had m. p. 125°, $[\alpha]_D^{20}$ −45° (*c* 1 in EtOH) (Found: C, 67·3; H, 6·7; N, 8·0. $C_{20}H_{24}N_2O_4$ requires C, 67·4; H, 6·8; N, 7·9%). *Benzyloxycarbonyl*-D-*alanyl*-D-*phenylalaninol* prepared similarly, had m. p. 126°, $[\alpha]_D^{19}$ +44° (*c* 1·3 in EtOH) (Found: C, 67·4; H, 7·0; N, 7·7%).

L-*Alanyl*-L-*phenylalaninol.*—The benzyloxycarbonyl derivative (1·4 g.) was hydrogenated at atmospheric pressure and temperature in ethanol (20 ml.) containing glacial acetic acid (2 drops) and 5% palladium–charcoal. Evaporation of the filtered solution gave a solid which on recrystallisation from ethyl acetate–light petroleum (b. p. 60–80°), yielded prisms of L-*alanyl*-L-*phenylalaninol* (0·7 g., 81%), m. p. 135°, $[\alpha]_D^{20}$ −20° (*c* 1 in EtOH) (Found: C, 64·8; H, 8·1; N, 13·0. $C_{12}H_{18}N_2O_2$ requires C, 64·8; H, 8·2; N, 12·6%). D-*Alanyl*-D-*phenylalaninol*, prepared similarly, had m. p. 142°, $[\alpha]_D^{19}$ +21·4° (*c* 0·7 in EtOH) (Found: C, 64·3; H, 8·2; N, 12·9%).

D-*Cyclohexanespiro*-2′-(4′-*isopropyloxazolidine*).—A mixture of D(−)-valinol (4·9 g.) and cyclohexanone (3·1 g.) in benzene (25 ml.) was refluxed azeotropically until no more water collected in the side arm of a Dean and Stark apparatus. The solvent was removed, and the residue distilled *in vacuo* to give the *oxazolidine* (8·3 g., 95%) as an oil, b. p. 52°/0·01 mm., n_D^{20} 1·4680, $[\alpha]_D^{20}$ −32·0° (*c* 1·3 in EtOH) (Found: C, 72·3; H, 11·4; N, 7·6. $C_{11}H_{21}NO$ requires C, 72·1; H, 11·6; N, 7·6%).

[25] Izumiya and Yamashita, *J. Biochem.* (*Japan*), 1959, **46**, 337.

Molecular refraction. (*a*) Experimental. Calculated from the formula $[R] = [(n^2 - 1)/(n^2 + 2)]M/\rho$, where the density ($\rho$) was determined five times by weighing known volumes of oxazolidine delivered from a microsyringe and a mean value taken, $[R] = 54{\cdot}1$. (*b*) Calculated, from atomic contributions: [26] oxazolidine; 11 carbon, 26·598, + 21 hydrogen, 23·1, + secondary NH, 2·50, + oxygen in ether, 1·643, — depression for ring, 0·5 = 53·34. Schiff's base: 11 carbon, 26·598, + 21 hydrogen, 23·1, + azomethine N, 4·10, + oxygen in OH, 1·525 = 55·323.

L-*Cyclohexanespiro-2′-(4′-isobutyloxazolidine).*—L(+)-Leucinol (2·5 g.) and cyclohexanone (1·44 g.) in benzene (25 ml.) were refluxed as above. The oxazolidine (3·5 g., 80%) had b. p. 62°/0·02 mm., n_D^{20} 1·4640, $[\alpha]_D^{20}$ +34·0° (*c* 3·8 in EtOH) (Found: C, 73·1; H, 12·0; N, 7·1. $C_{12}H_{23}NO$ requires C, 73·0; H, 11·8; N, 7·1%).

TABLE 8.

Substance	Time at room temp. (hr.)	ν (cm.⁻¹) C=O	C=N	1200—1040
L(+)-Leucinol	—	—	—	1060
Cyclopentanone (C_5H_8O)	—	1720	—	1150
Cyclohexanone ($C_6H_{10}O$)	—	1710	—	1120
L(+)-Leucinol + $COMe_2$	3	—	1670	1150, 1100, 1050
L(+)-Leucinol + $COMe_2$	72	1710	1670	1150, 1100, 1050
L(+)-Leucinol + C_5H_8O	0·75	—	1670	1180, 1080, 1060, 1040
L(+)-Leucinol + C_5H_8O	72	1720	1670	1180, 1080, 1060, 1040
L(−)-Phenylalaninol	—	—	—	1120, 1085, 1070
$COBu^iMe$	—	1720	—	1185
L(−)-Phenylalaninol + $COMe_2$ ...	1	1710	1680	1150, 1080, 1050
L(−)-Phenylalaninol + $COBu^iMe$	12	1720	1660	1175, 1120, 1050
L(−)-Phenylalaninol + C_5H_8O ...	2	1740	1675	1150, 1080, 1050
L(−)-Phenylalaninol + $C_6H_{10}O$ ...	0·5	1710	—	1160, 1150, 1050

L-*Alanine Methylamide.*—L-Alanine ethyl ester (5·0 g.) was dissolved in a saturated methanolic solution of methylamine (10 ml.) and left at room temperature for 50 hr. The excess of reagent was removed and the residue distilled to give L-*alanine methylamide* (3·2 g., 73%) as an oil, b. p. 98°/0·01 mm., n_D^{24} 1·4670, $[\alpha]_D^{24}$ +7·7° (*c* 0·7 in EtOH). The *hydrochloride*, m. p. 230°, was prepared by treatment of the methylamide with 2N-ethanolic hydrogen chloride (Found: C, 34·6; H, 8·1; Cl, 25·6; N, 20·4. $C_4H_{11}ClN_2O$ requires C, 34·7; H, 8·0; Cl, 25·6; N, 20·4%).

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[26] Eisenlohr, *Z. Phys. Chem.*, 1912, **79**, 129, for carbon, hydrogen, and secondary NH; von Auwers, *ibid.*, 1930, **147**, 436, for azomethine N; Vogel, Cresswell, Jeffery, and Leicester, *Chem. and Ind.*, 1950, 358, for oxygen in OH and ether; Cope and Hancock, *J. Amer. Chem. Soc.*, 1942, **64**, 1503, for depression by ring.