acid. It seems plausible that this substance not only yields valine but is the direct precursor of penicillamine, β,β'-dimethylcysteine. Such a transformation would be entirely analogous to that of serine to cysteine or of homoserine to homocysteine.

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Syntheses of β-*dl*-Aspartyl Peptides with Maleic Anhydride

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β-*dl*-Aspartyl peptides have been synthesized, starting with maleic anhydride. This was opened by the appropriate free amino acid esters to yield maleamic acids, to the double bond of which one mole of benzylamine was added. By hydrolysis of the resulting N-benzyl-β-*dl*-aspartyl dipeptide esters and subsequent hydrogenolysis, the free dipeptides were produced.

The preparation of aspartyl peptides by means of fumaric acid derivatives was first attempted by Emil Fischer.[1] He described the syntheses of aspartylmonoglycine and of aspartyldialanine, obtained by reaction of fumaryl chloride with the free amino acid ester and subsequent heating with aqueous ammonia of the intermediate fumaryldiglycine (fumaryldialanine) in a closed tube. He was, however, unable to establish whether the aspartylglycine in question represented an α- or a β-peptide,[2] a problem which has now been solved by us. Moreover, only in this case did he actually obtain a peptide in which only one carboxyl group of aspartic acid was involved, whereas in the instance of alanine only aspartyldialanine was isolated.

We had found that β-aspartyl amides (*e.g.*, β-asparagine) may be synthesized from maleic anhydride[3] by opening it in the cold with the appropriate amine and adding one mole of benzylamine to the double bond of the resulting maleamic acid. By subsequent hydrogenolysis of the N-benzyl derivative thus formed, the β-amide is finally obtained in good over-all yield.

On using free amino acid esters instead of primary amines, we expected the maleamic acid to react with benzylamine preferably through its double bond with no or merely little formation of amides by aminolysis. In fact, we have never isolated such amides from the reaction mixture, whereas N-benzyl-β-*dl*-aspartyl dipeptide esters could be obtained generally in good yields. Formation of α-peptides was not observed, except in the case of glycine, where a small amount was isolated (about 10%) which could be separated quantitatively from the β-isomer, as we have shown earlier.[4]

The following dipeptides have been prepared by this scheme: β-*dl*-aspartylglycine, β-*dl*-aspartyl-*dl*-alanine, β-*dl*-aspartyl-β-alanine, β-*dl*-aspartyl-*dl*-α-amino-*n*-butyric acid, β-*dl*-aspartyl-*dl*-phenylalanine and β-*dl*-aspartyl-*dl*-valine.

It is interesting to note that the reactivity of the maleamic acids, as regards the addition of one mole of benzylamine to their double bond, is decreasing in the order given in Table II. This may be due to steric hindrance by the alkyl groups of various size attached to the α-carbon atom of the amino acids. Also, neither hydrolysis nor hydrogenolysis of N-benzyl-β-*dl*-aspartyl-*l*-leucine ethyl ester could be carried out, even on prolonged treatment, and only the starting materials were recovered. Leucine, of course, has a structure for which the hindrance effect would be most pronounced.

As to the intermediate free N-benzyl dipeptides, only in the case of N-benzyl-β-*dl*-aspartylphenylalanine could this type of compound be isolated in crystalline form, on saponification of the corresponding peptide ester. By direct hydrogenolysis of the N-benzyl-β-*dl*-aspartyl dipeptide esters, the free β-*dl*-aspartyl dipeptide esters were obtained.

All β-dipeptides as well as their esters gave negative biuret[4] but positive ninhydrin reactions. Ascending paper-partition chromatography (phenol–water as mobile phase) produced bluish spots.[5]

Emil Fischer's *dl*-aspartylglycine[1] has been found to be identical with β-*dl*-aspartylglycine. It gave no biuret reaction and chromatography produced a blue spot. The substance crystallizes with one molecule of water in whetstone-like crystals ("Wetzsteine" according to Fischer), but the melting point reported by him (165° cor.) seems to be too high. A sample prepared in our laboratory by Fischer's route, as well as our dipeptide melted at 156°, even after repeated recrystallizations, as determined with a Fisher–Johns apparatus.

Experimental

Micro-combustion analyses were made by Drs. Weiler and Strauss. Melting points were determined in a Fisher–Johns apparatus and the ascending method of paper-partition chromatography was used.

Procedure for one typical example of each reaction step is given and the remainder summarized in Tables I–IV.

Ethyl N-Maleyl-*dl*-α-amino-*n*-butyrate.—To an ice-cooled solution of 4.9 g. (0.05 mole) of maleic anhydride in 80 ml. of ether (in all reactions of this type, ether dried over sodium was used), was added 6.6 g. (0.05 mole) of ethyl *dl*-α-amino-*n*-butyrate in 10 ml. of ether. On addition of petroleum ether, scratching and cooling in a freezing mixture, the substance crystallized; yield 10 g. (87%), m.p. on recrystallization from ethyl acetate–petroleum ether 59°.

Anal. Calcd. for $C_{10}H_{15}O_5N$: C, 52.3; H, 6.5; N, 6.1. Found: C, 51.6; H, 6.4; N, 6.1.

Ethyl N-Benzyl-β-*dl*-aspartyl-*dl*-α-amino-*n*-butyrate.—To ethyl N-maleyl-*dl*-α-amino-*n*-butyrate (7.5 g.) in 25 ml. of dry dioxane was added 4.5 g. of benzylamine and the mixture heated under reflux for 5 hours (oil-bath at 110–120°).

(1) E. Fischer and E. Koenigs, *Ber.*, **37**, 4585 (1904).

(2) J. S. Fruton, "Advances in Protein Chemistry," Vol. V, Academic Press, Inc., New York, N. Y., 1949, p. 41.

(3) Max Frankel, Y. Liwschitz and Y. Amiel, THIS JOURNAL, **75**, 330 (1953).

(4) Y. Liwschitz and A. Zilkha, *ibid.*, **76**, 3698 (1954).

(5) W. J. LeQuesne and G. T. Young, *J. Chem. Soc.*, 24 (1952).

TABLE I

PREPARATION OF N-MALEYLAMINO ACID ESTERS

All substances were recrystallized from ethyl acetate–petroleum ether.

Substance, ethyl N-maleyl-	Yield, %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Glycinate	90	88–89	$C_8H_{11}O_5N$					7.0	7.0
dl-Alaninate	88	80	$C_9H_{13}O_5N$					6.5	6.5
β-Alaninate	93	60	$C_9H_{13}O_5N$	50.1	50.1	6.0	6.0	6.5	6.4
dl-Phenylalaninate	95	103	$C_{15}H_{17}O_5N$	61.8	61.8	5.8	5.5	4.8	4.9
dl-Valinate	98	75–76	$C_{11}H_{17}O_5N$	54.2	54.6	7.0	6.8	5.8	5.8
l-Leucinate	100	94	$C_{12}H_{19}O_5N$	56.0	55.8	7.4	7.2	5.4	5.3

TABLE II

PREPARATION OF N-BENZYL DIPEPTIDE ESTERS

All substances were recrystallized from ethanol

Substance, ethyl N-benzyl-β-*dl*-aspartyl-	Yield, %	M.p., °C.	Reflux time, min.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Glycinate[a,b,c]	74	201	35							
dl-Alaninate[a,b]	69	197	35							
β-Alaninate[b]	66	194–195	55	$C_{16}H_{22}O_5N_2$	59.6	60.0	6.8	6.8	8.7	8.8
dl-Phenylalaninate[b]	51	166	150	$C_{22}H_{26}O_5N_2$	66.2	66.0	6.5	6.5	7.0	6.9
dl-Valinate[d]	34	179–181	150	$C_{18}H_{26}O_5N_2$	61.6	61.6	7.4	7.2	8.0	8.0
l-Leucinate[d]	31	166	180	$C_{19}H_{28}O_5N_2$	62.6	62.2	7.7	7.5	7.7	8.0

[a] Identical with substance prepared by different method (*cf.* ref. 4). [b] The substance crystallized from dioxane during reflux and was filtered off after having stood overnight in an ice-box. [c] On evaporating the dioxane filtrate *in vacuo* and dissolving the residue in hot acetone 11.5% of the α-isomer was recovered (see ref. 4). [d] The substance crystallized from the acetone solution only after having been left in an ice-box for 2–3 days.

TABLE III

PREPARATION OF FREE DIPEPTIDE ESTERS

Substance, ethyl β-*dl*-aspartyl-	Yield, %	M.p., °C.	Recryst. solvent	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
β-Alaninate	Almost quant.	248	Water	$C_9H_{16}O_5N_2$	46.5	47.1	6.9	6.5	11.9	11.9
dl-Phenylalaninate	Almost quant.	182	Ethanol	$C_{15}H_{20}O_5N_2$	58.4	58.5	6.5	6.6	9.1	9.1
dl-Valinate	84	113	Ethanol	$C_{11}H_{20}O_5N_2 + H_2O$	47.2	47.2	7.9	7.7	10.0	9.6

TABLE IV

PREPARATION OF FREE DIPEPTIDES

Substance, β-*dl*-aspartyl-	Yield, %	M.p., °C.	Recryst. solvent	R_f value	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
β-Alanine	31[a]	225	Water	0.47	$C_7H_{12}O_5N_2$	41.1	41.1	5.9	5.9	13.7	13.6
dl-Phenylalanine	90[b]	204	Water–acetone	.44	$C_{13}H_{16}O_5N_2$	55.7	55.7	5.7	6.2	10.0	10.0
dl-Valine	25[a]	218	Water–acetone	.43	$C_9H_{16}O_5N_2$	46.5	46.6	6.9	6.8	12.1	12.1

[a] Yield is based on N-benzyl dipeptide ester, since the free N-benzyl peptide could not be isolated. [b] Yield is based on N-benzyl-β-*dl*-aspartyl-*dl*-phenylalanine.

The solvent was then removed *in vacuo* and the residue dissolved in 100 ml. of hot acetone. After standing overnight, the meanwhile formed precipitate was filtered off and washed with acetone; yield 5.6 g. (51%). On recrystallization from a little ethanol it melted at 161–162°.

Anal. Calcd. for $C_{17}H_{24}O_5N_2$: C, 60.6; H, 7.1; N, 8.3. Found: C, 61.4; H, 7.1; N, 8.2.

Ethyl β-*dl*-Aspartyl-*dl*-α-amino-*n*-butyrate.—Ethyl N-benzyl-β-*dl*-aspartyl-*dl*-α-amino-*n*-butyrate (2 g.) was dissolved in 40 ml. of glacial acetic acid and 0.2 g. of catalyst added. Hydrogenolysis was carried out for 5 hours (*cf.* ref. 4). After separation of the catalyst, the solvent was removed *in vacuo* and dry ether was added to the residue. On standing overnight in an ice-box white crystals separated. After filtration, the substance was recrystallized from ethanol. Part of it which did not dissolve in hot ethanol was identified as aspartic acid. On addition of a large quantity of ether to the filtrate, 0.9 g. (57%) of the substance was obtained. After recrystallization from a little ethanol (needles joined at center), m.p. 123°.

Anal. Calcd. for $C_{10}H_{18}O_5N_2 + H_2O$: C, 45.4; H, 7.6; N, 10.6. Found: C, 45.8; H, 7.5; N, 10.0.

N-Benzyl-β-*dl*-aspartyl-*dl*-phenylalanine.—Ethyl N-benzyl-β-*dl*-aspartyl-*dl*-phenylalaninate (3 g.) was dissolved in 21 ml. of 1 *N* lithium hydroxide solution and kept at room temperature for 90 minutes. On acidification with hydrochloric acid, the solution was evaporated to dryness *in vacuo*. On trituration of the residue with water, the substance separated in white crystals; yield 1.5 g. (65%), m.p. on recrystallization from methanol–water 206°.

Anal. Calcd. for $C_{20}H_{22}O_5N_2$: N, 7.6. Found: N, 7.5.

β-*dl*-Aspartyl-*dl*-α-amino-*n*-butyric Acid.—Ethyl N-benzyl-β-*dl*-aspartyl-*dl*-α-amino-*n*-butyrate (2 g.) was dissolved in 14 ml. of 1 *N* sodium hydroxide solution and left at room temperature for 90 minutes. After acidification with hydrochloric acid and removal of the solvent *in vacuo*, the residue was redissolved in 30 ml. of glacial acetic acid, the insoluble sodium chloride filtered off and on addition of 0.2 g. of catalyst, the mixture was hydrogenolyzed for 5 hours. The solvent was then removed *in vacuo* and the residue dissolved in water and reprecipitated by addition of acetone. On repeating this procedure twice the free dipeptide was obtained; yield 0.2 g. (15%), m.p. 203°. It gave negative biuret but positive ninhydrin reactions. Chromatography gave a blue spot (R_f value 0.39).

Anal. Calcd. for $C_8H_{14}O_5N_2$: C, 44.0; H, 6.4; N, 12.8. Found: C, 43.6; H, 6.1; N, 12.5.

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