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THE THERMOLYSIN-CATALYZED CONDENSATION REACTIONS OF N-SUBSTITUTED ASPARTIC AND GLUTAMIC ACIDS WITH PHENYLALANINE ALKYL ESTERS[1]

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Abstract: The title reactions occur with concurrent peptide synthesis and optical resolutions of racemic substrates. With this method, Z-L-Asp-L-Phe-OMe, the precursor to the synthetic sweetner, could be obtained in high yield.

In recent years, the synthesis of peptides by the reverse reaction of proteinase-catalyzed hydrolysis of a peptide bond has been drawing increasing attention as a practical preparative method.[2] In order to exploit the potential usefulness of the enzymatic method further, we investigated the thermolysin[3]-catalyzed reactions of the N-protected but side chain unprotected acidic amino acids with phenylalanine alkyl esters.

The reactions were carried out in a manner as described below. The mixture of 2-4 mmoles each of substrates in about 10 ml H_2O at pH 6-8 was stirred at 40°C for 3-5 hr in the presence of about 10 mg of thermolysin. At the end of the reaction, the resulting deposit was collected and recrystallized. We investigated the reactions shown in the table, and it was found that all of the condensation products were obtained as the salts with the amine substrates. It was also found that, when racemic substrates were used, only L-isomers were involved in the condensation reactions to give L-L dipeptides, and that the amine parts

Substrates	Yield	Product	$[\alpha]_D^{25}$ a)
(1) Z-L-Asp + L-Phe-OMe	96%	Z-L-Asp-L-Phe-OMe·L-Phe-OMe	-14.7
(2) PMZ-L-Asp + L-Phe-OMe	83%	PMZ-L-Asp-L-Phe-OMe·L-Phe-OMe	-11.5
(3) Z-L-Asp + L-Phe-OEt	83%	Z-L-Asp-L-Phe-OEt·L-Phe-OEt	-14.3
(4) Z-L-Glu + L-Phe-OMe	45%	Z-L-Glu-L-Phe-OMe·L-Phe-OMe	-12.0
(5) PMZ-L-Asp + L-Phe-OEt	90%	PMZ-L-Asp-L-Phe-OEt·L-Phe-OEt	-15.0
(6) Z-L-Asp + DL-Phe-OMe	92%	Z-L-Asp-L-Phe-OMe·D-Phe-OMe	-14.7
(7) Z-DL-Asp + L-Phe-OMe	86%	Z-L-Asp-L-Phe-OMe·L-Phe-OMe	-15.0
(8) Z-DL-Asp + DL-Phe-OMe	95%	Z-L-Asp-L-Phe-OMe·D-Phe-OMe	-14.8
(9) PMZ-L-Asp + DL-Phe-OMe	87%	PMZ-L-Asp-L-Phe-OMe·D-Phe-OMe	-12.1

a) portion of dipeptide. All of the optical rotations were measured in methanol (c=1). $[\alpha]_D^{25}$ of separated Phe-OMe·HCl from its salts were; (6)-15.7; (8)-14.8; (9)-15.9. The value of authentic D-Phe-OMe·HCl was -15.7.

of the salts were almost exclusively D-isomers, as indicated by the optical rotations (see the table). Therefore it is possible to attain the peptide synthesis and optical resolutions of both substrates simultaneously, since the unreacted D-isomer of carboxylic substrate remains in the solution, whereas the salt, which can be easily separated into L-L dipeptide and D-isomer of amine substrate by aqueous hydrochloric acid, is deposited in almost quantitative yield under suitable reaction conditions.

As see above, these condensation reactions occur exclusively at the α-carboxylate, thus the protection of side chain carboxyl group is unnecessary. This is advantageous in peptide synthesis, since in the chemical synthesis the side chain carboxylate is usually masked as the benzyl ester to avoid the undesirable side reactions. Moreover the selective esterification at the side chain carboxylate is rather troublesome and usually in low yields.[4] It is also known that side chain ester groups have the tendency of undergoing ring closure reactions, one of the most disturbing side reactions in peptide synthesis.[5]

Finally, the most significant outcome of the present work is that it provides the novel and elegant method for the preparation of L-Asp-L-Phe-OMe (APM), the synthetic sweetner which is about 200 times as sweet as sucrose.[6] This sweetner is known to be produced via the reactions of Z-L-Asp(β-Bzl),[6] or anhydrides of L-Asp,[7] Z-L-Asp,[8] and HCO-L-Asp,[9] with L-Phe-OMe. However all of these chemical methods have some problems; the first method involves the problems relating to the side chain benzyl ester as mentioned above, and the latter three methods inevitably produce the mixture of α and β APM, and thus require the separation. With this enzymatic method, Z-L-Asp-L-Phe-OMe, which is easily converted to APM by the usual catalytic hydrogenolysis, can be obtained in the simple manner and in high yield, and moreover, unexpensive racemic raw materials and the crude enzyme preparation can be used. Therefore this seems to be a very useful method for the large scale production of the sweetner, and hence the study is now under way toward this end.

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