ether (3 × 50 mL). The ether layers were combined, washed with water, and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the crude residue was chromatographed on a silica gel column with a 5% pentane–ether mixture as the eluant to give pure aryl halide.[22]

(22) All the aryl halides obtained were identical in every detail with authentic samples.

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Registry No. 1a, 13056-98-9; **1b**, 36719-51-4; **1c**, 52416-22-5; **1d**, 79664-67-8; **3a**, 591-50-4; **3b**, 624-31-7; **3c**, 13329-40-3; **3d**, 3058-39-7; **3e**, 108-86-1; **3f**, 106-38-7; **3g**, 99-90-1; **3h**, 623-00-7; trimethylsilyl iodide, 16029-98-4; trimethylsilyl bromide, 2857-97-8.

Communications

Synthesis of an Aspartame Precursor by Immobilized Thermolysin in an Organic Solvent

Summary: The synthesis of *N*-(benzyloxycarbonyl)-L-aspartyl-L-phenylalanine methyl ester, the precursor of the synthetic sweetener aspartame, from *N*-(benzyloxycarbonyl)-L-aspartic acid and L-phenylalanine methyl ester was carried out in an apparent single phase of the organic solvent by using thermolysin immobilized with various methods.

Sir: Proteinase-catalyzed syntheses of peptides have been drawing increasing attention,[1] and in many cases the synthesis is based on the deposition of a product,[2] which causes the shift of the equilibrium toward a less favorable product.[3] From the practical point of view the use of an immobilized enzyme is of great value,[4] but it is rather impractical in the enzymatic peptide syntheses because of the problem of the separation of an immobilized enzyme from a deposition product. Recently Kuhl et al. have shown that an immobilized enzyme could be applied to the peptide synthesis by employing biphasic water–organic systems.[5] However, this approach is still unsatisfactory, since we found that the column operation, one big attraction with an immobilized enzyme system, is difficult due to the channeling of the two layers in a packed bed of an immobilized enzyme. We extended our research[6] on the enzymatic synthesis of the dipeptide sweetner aspartame[7] and report the new approach to the synthesis of *N*-(benzyloxycarbonyl)-L-aspartyl-L-phenylalanine methyl ester, the precursor of the sweetener, by using immobilized thermolysin in an apparent single phase of an organic solvent.

Table I. Reaction of *N*-(Benzyloxycarbonyl)-L-aspartic Acid with L-Phenylalanine Methyl Ester by Immobilized Thermolysin in Ethyl Acetate[a]

supporting matl for immobilization[b]	reaction time, h	% yield
Amberlite XAD-7	10	85
Amberlite XAD-8	10	93
Amberlite IRA-94	24	61
Toyopearl EAGA	24	83
glass beads CPG-10	24	55
glass beads CPG-10[c]	24	48

[a] Reaction conditions are given in footnote 10. [b] The preparations of these materials are given in footnotes 8 and 9. [c] The second run used immobilized thermolysin recovered from the first run.

Immobilized enzymes were prepared by using crude thermolysin as follows: (1) physical adsorption to Amberlyte XAD-7 and XAD-8;[8] (2) ionic bonding to the anionic ion-exchange resin Amberlite IRA-94;[8] (3) the enzyme was supported by porous glass beads as an aqueous solution without any special interaction between the enzyme and the supporting material;[8] (4) covalent bonding to the ethylenediamine-derivatized hydrophilic polymer gel Toyopearl through glutaraldehyde (this gel is designated as Toyopearl EAGA in Table I).[9] The aqueous suspension of the immobilized enzyme obtained as above was filtered, washed with 0.5% aqueous calcium acetate solution (pH 7.5), and used as the water-wet material for the synthesis of the dipeptide. The reaction between *N*-(benzyloxycarbonyl)-L-aspartic acid with L-phenylalanine methyl ester was carried out by incubating the mixture of the substrates and the water-wet immobilized enzyme in ethyl acetate at 40 °C.[10]

(1) For recent examples, see: Inoue, K.; Watanabe, K.; Morihara, K.; Tochino, Y.; Emura, T.; Sakakibra, S. *J. Am. Chem. Soc.* **1979**, *101*, 751. Kullmann, W. *J. Biol. Chem.* **1980**, *255*, 8234. For a recent review, see: Oyama, K.; Kihara, K. *Jpn. Chem. Rev.*, in press, and references therein.

(2) Borsook, H. *Adv. Protein Chem.* **1953**, *8*, 127.

(3) Carpenter, F. H. *J. Am. Chem. Soc.* **1960**, *82*, 1111.

(4) There are many books and reviews on immobilized enzymes; see for example: Chibata I. "Immobilized Enzymes"; Kodansha Scientific: Tokyo, 1975.

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(6) Isowa, Y.; Ohmori, M.; Ichikawa, T.; Mori, K.; Nonaka, Y.; Kihara, K.; Oyama, K.; Satoh, H.; Nishimura, S. *Tetrahedron Lett.* **1979**, 2611. Oyama, K.; Kihara, K.; Nonaka, Y. *J. Chem. Soc., Perkin Trans. 2* **1981**, 356.

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(8) Immobilization of the enzyme by physical adsorption onto Amberlite XAD-7 and XAD-8, by ionic bonding to Amberlite IRA-94 (all are from Rohm & Haas Co., Ltd.), and by being supported on glass beads (CPG-10, from Electro-Nucleonics, Inc.) were carried out by stirring 10 g of each of the above water-wet supporting materials in 50 mL of 0.5% aqueous calcium acetate solution (pH 7.5) containing 3 g of crude thermolysin (Thermoase, purchased from Daiwa Kasei Co., Ltd., Osaka, Japan, activity 1.6 × 10^6 PU/g) for 3 h. The immobilized enzyme was collected by filtration and washed with 20 mL of the calcium buffer.

(9) The covalent bonding to the hydrophilic polymer gel was performed as follows. The ethylenediamine-derivatized hydrophilic polymer gel (10 g, Toyopearl, commercially available as the column packing material for gel-permeation chromatography from Toyo Soda Manufacturing Co., Ltd.) was stirred in 20 mL of distilled water containing 3 mL of 25% glutaraldehyde at room temperature for 2 h. The activated gel was collected by filtration, washed with water, and then reacted with the enzyme in 50 mL of the calcium buffer containing 3 g of crude thermolysin.

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The representative results are summarized in Table I, which shows that immobilized enzyme can be used for the synthesis of the peptide in an apparent single phase of the organic solvent. From the results one may see that the enzyme is staying in the inner sphere of the pore of the supporting material as the aqueous solution, and hence the enzyme is protected from denaturation by the organic solvent. On the other hand, substrates move from the organic layer to the aqueous layer in the support, where the reaction takes place, and then the product diffuses back to the organic layer effectively, thus shifting the equilibrium toward the less favorable synthesis side. The reaction rate is rather slow as compared with that in aqueous solution.[6] This may be explained by the slow diffusion of the substrates and the product through the layers and the decrease in some of the enzyme activity by ethyl acetate dissolved in the aqueous layer.[11] Among the immobilization methods studied, the physical adsorption to Amberlite XAD-7 and XAD-8 gave excellent yields which are due to their high adsorptive tendency toward proteins.[12]

One point to be noted in the present study is that the enzyme "immobilized" on such a support as glass beads can be used although they have no special interaction with the enzyme. This fact is of special interest, since such "immobilization" is impossible in an aqueous solution or in biphasic systems, because we found that the enzyme leaks rapidly from the supporting material, but possible in the apparent single-phase system as shown here. A further advantage is that when the activity of the "immobilized" enzyme is decreased, the regeneration can be done by simply washing out the enzyme with water and immersing it in the aqueous fresh enzyme solution.

We performed the preliminary study on the continuous reaction using a packed column of immobilize thermolysin on Amberlite XAD-7. The catalyst life is dependent on the operation conditions, and we are now carrying out a more detailed study in order to optimize the conditions. The results will be published at a later time.[13]

Usually enzymes are inactive and unstable in an organic solvent, whereas most substrates are insoluble in water but soluble in organic solvents. Therefore, the use of an apparent single-phase system may find wide application in many reactions by immobilized enzymes.[14]

Registry No. *N*-(Benzyloxycarbonyl)-L-aspartyl-L-phenylalanine methyl ester, 33605-72-0; *N*-(benzyloxycarbonyl)-L-aspartic acid, 1152-61-0; L-phenylalanine methyl ester, 2577-90-4.

(10) The enzymatic condensation was carried out as follows. The acid component (12 mmol, 3.2 g), 24 mmol of the amine component (4.3 g), and 10 g of the water-wet immobilized thermolysin prepared as above were mixed in 40 mL of ethyl acetate saturated with water and then incubated at 40 °C. After the reaction, the mixture was filtered to remove the catalyst, and the filtrate was evaporated to dryness on a rotary evaporator. The residue was dissolved in 0.8% aqueous sodium acetate and the yield of *N*-(benzyloxycarbonyl)-L-aspartyl-L-phenylalanine methyl ester was determined by high-performance liquid chromatography as described before.[6] The recovered immobilized enzyme was used for the second reaction, the result of which is included in Table I. In another identical experiment using the enzyme immobilized on Amberlite XAD-8, the ethyl acetate solution after the separation of the catalyst was concentrated on a rotary evaporator. The addition of *n*-hexane to the solution resulted in the deposition of the salt of *N*-(benzyloxycarbonyl)-L-aspartyl-L-phenylalanine methyl ester with L-phenylalanine methyl ester in the yield of 82%.

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(12) Ton, H. Y.; Hughes, R. D.; Silk, D. B. A.; Williams, R. *J. Biomed. Mater. Res.* **1979**, *13*, 407.

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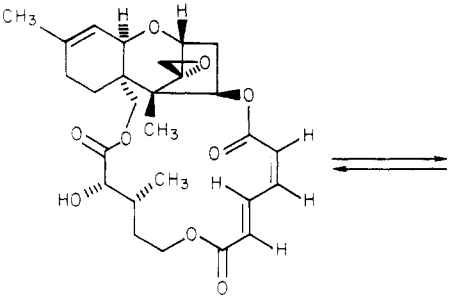
Synthesis of Verrucarin A

Summary: Starting from anguidine, propargyl alcohol, and furfural, the first synthesis of a natural macrocyclic trichothecane ester is described.

Sir: The macrocyclic trichothecane esters are an important class of naturally occurring toxins consisting of the macrotrilactonic verrucarins and the macrodilactonic roridins.[1] These compounds exhibit a variety of significant biological properties including antibiotic, antifungal, antiviral, and antitumor activities and are among the most potent cytostatic materials known.[2] Verrucarin A (1) is one of the most active of these compounds and has been shown to cause 50% inhibition of the growth of mastocytom P-815 tumor cells in mice at a concentration of 6×10^{-4} μg/mL.[3] Although a tetrahydro derivative of verrucarin J has been prepared by Tamm and co-workers,[4] no synthesis of a naturally occurring macrocyclic trichothecane ester has been reported previously. We now report the first such synthesis in the form of a preparation of verrucarin A (1).[5]



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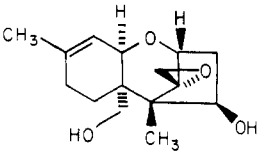

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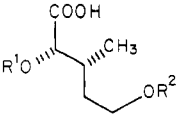

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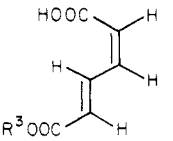

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