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%.

(11) Nakajima, H.; Suzuki, K.; Imahori, K. *Nippon Nogei Kagaku Kaishi* **1975**, *49*, 317.

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

(13) A part of our comprehensive study on the synthesis of aspartame by immobilized enzyme will appear in: Hagi, N.; Nishimura, S.; Oyama, K. *Toyo Soda Kenkyu Hokoku.*

(14) A similar approach, but not for the peptide synthesis, has been reported for the esterification of *N*-acetyl-L-tryptophan: Klibanov, A. M.; Samokhin, G. P.; Martinek, K.; Berezine, I. B. *Biotechnol. Bioeng.* **1977**, *19*, 1351.

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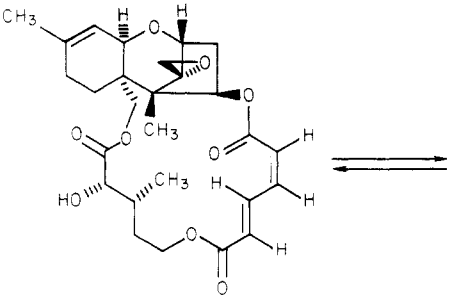
Synthesis of Verrucarin A

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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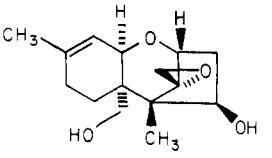

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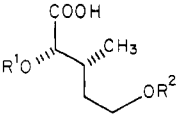

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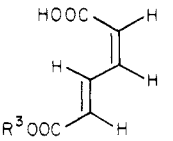


3

(1) Review: Ch. Tamm, *Fortschr. Chem. Org. Naturst.*, **31**, 63 (1974); Ch. Tamm in "Mycotoxins in Human and Animal Health", J. V. Rodricks, C. V. Hesseltine, and M. A. Mehlman, Eds., Pathatox Publishers, Park Forest South, IL, 1977, p 209.

(2) Review: J. R. Bamburg and F. M. Strong in "Microbial Toxins", Vol. 7, Academic Press, New York, 1971, p 207.

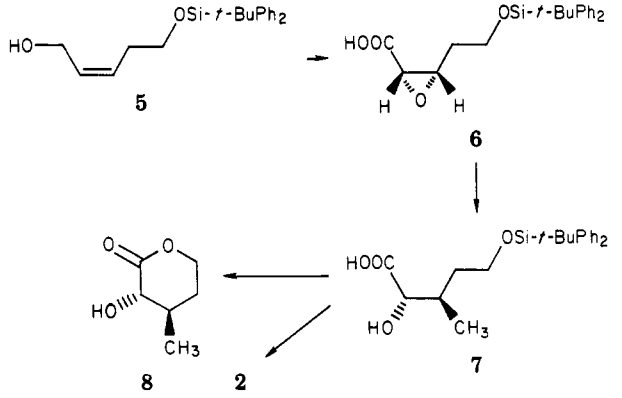
(3) E. Härri, W. Loeffler, H. P. Sigg, H. Stähelin, Ch. Tamm, and D. Wiesinger, *Helv. Chim. Acta*, *45*, 839 (1962).

(4) W. Breitenstein and Ch. Tamm, *Helv. Chim. Acta*, **61**, 1975 (1978). See also: E. A. Noregen, M. Tori, and Ch. Tamm, *ibid.*, **64**, 316 (1981).

0022-3263/81/1946-5242$01.25/0

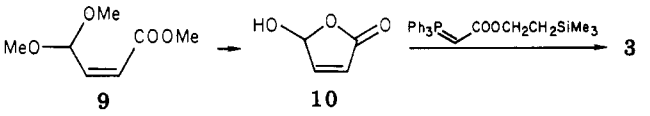
The general scheme of construction involves the cyclization of appropriate derivatives of verrucarin A's saponification products, i.e., verrucarinic acid (**2**),[6] (*E,Z*)-muconic acid (**3**), and verrucarol (**4**).[7] These materials are prepared in turn from propargyl alcohol, furfural, and anguidine with R^1 = Ac, R^2 = Si-*t*-BuPh$_2$, and R^3 = $CH_2CH_2SiMe_3$.

The diprotected verrucarinic acid segment (**2**, R^1 = Ac, R^2 = Si-*t*-BuPh$_2$) was prepared as outlined below by protection (EtOCH=CH$_2$, *p*-TsOH) and alkylation of the magnesium salt of propargyl alcohol with ethylene oxide (*i*-PrMgCl, THF; CH_2OCH_2; 57% yield). Differential protection [(1) ClSi-*t*-BuPh$_2$, $C_3H_4N_2$, DMF, (2) HOAc, H_2O] and semihydrogenation (H_2, Lindlar catalyst, MeOH) gave the monoprotected *cis*-pentenediol **5** in 92% overall yield. Asymmetric epoxidation (*d*-diethyl tartrate, *t*-BuOOH, Ti(O-*i*-Pr)$_4$, CH_2Cl_2, −20 °C;[8] 87% yield) and oxidation gave the optically active epoxy acid **6**. The oxidation of the epoxy alcohol intermediate to **6** was not a trivial matter. While many oxidants afforded only traces of carboxylic acid, pyridinium dichromate[9] in wet DMF produced a 30–50% yield of the desired epoxy acid and Jones reagent gave up to 60% epoxy acid but only with substantial epimerization to the trans isomer. It was

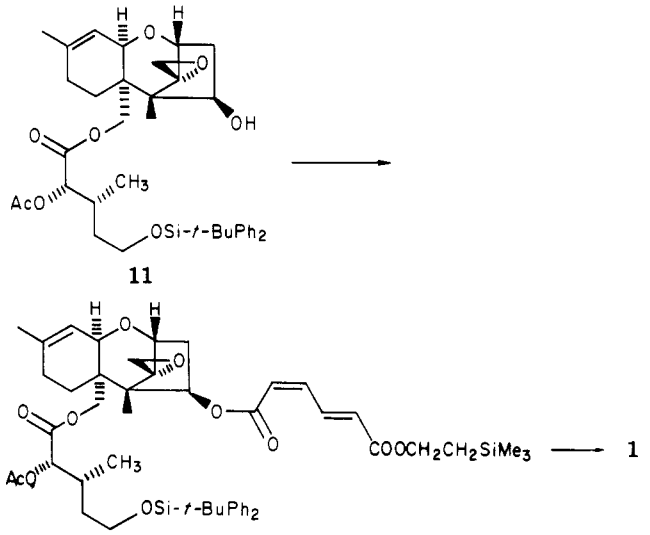


found however that the new ruthenium-catalyzed oxidation of Sharpless and co-workers (2% $RuCl_3$, $NaIO_4/CCl_4$, CH_3CN, H_2O)[10] led cleanly to **6** in 79% yield without detectable epimerization. The only remaining problem in the preparation of **2** was a regiochemical one involving directional control of the epoxide opening of **6** by a methyl anion equivalent. Other work here at Columbia had shown that regioselective β-methylation of epoxy acids and alcohol derivatives may often be effected with trimethylaluminum.[11] In the instance of **6**, almost exclusive β-addition was observed with 3 equiv of trimethylaluminum in petroleum ether (25 °C, 40 h) and led smoothly to **7** in 87% yield. Acetylation (Ac_2O, C_5H_5N) then gave **2** (R^1 = Ac, R^2 = Si-*t*-BuPh$_2$; 90%). The identity of the verrucarinic acid derivative thus produced was confirmed by conversion [(1) CH_2N_2, Et_2O; (2) *p*-TsOH, CH_2Cl_2] of **7** to a well-known verrucarin A degradation product, verrucarinolactone **8** (77% yield, mp 103 °C, $[\alpha]^{23}_D$ −10.4°; lit.[5] mp 103–104 °C, $[\alpha]^{23}_D$ −9°).

The monoprotected (*E,Z*)-muconic acid fragment was readily constructued by electrochemical oxidation (Pt electrodes, MeOH, Et_4NClO_4, 1.5 amps, 18 h)[12] of furfural to **9** (74%) and hydrolysis (1 N H_2SO_4, 25 °C, 18 h) to the pseudoacid **10** (79%). Wittig olefination followed in methylene chloride using the (trimethylsilyl)ethyl-protected ylide[13] shown below and yielded **3** (mp 56–58 °C) (R^3 = $CH_2CH_2SiMe_3$) in 72% yield. Only the desired *E,Z* isomer could be detected by 80-MHz ^{1}H NMR [($CDCl_3$) δ8.34 (1 H, ddd, *J* = 17, 12, 1 Hz), 6.73 (1 H, br t, *J* = 12 Hz), 6.12 (1 H, br d, *J* = 17 Hz), 5.95 (1 H, br d, *J* = 12 Hz)].



The selective esterifications of verrucarol (**4**)[14] by the verrucarinic acid and muconic acid intermediates described above were accomplished by the DCC method of Hassner and Alexanian.[15] Under these conditions (1.2 equiv of **2**, DCC, 4-pyrrolidinopyridine, CH_2Cl_2; 25 °C, 5 h), only the primary alcohol was esterified and **11** was isolated in 95% yield. Although the product appeared unreactive toward diesterification with **2**, the more reactive muconic acid derivative **3** added cleanly under the same conditions[16] to afford **12** (86%). Deprotection with tetrabutylammonium



fluoride (THF, 25 °C, 3 h) then led to secoacetoxyverrucarin A. Macrolactonization using the Mitsunobu procedure (Ph_3P, $EtO_2CN{=}NCO_2Et$; 25 °C, 20 h)[17] at a

(5) J. Guzwiller and Ch. Tamm, *Helv. Chim. Acta*, **48**, 157 (1965); *ibid.*, **53**, 2071 (1970); A. T. McPhail and G. A. Sim, *J. Chem. Soc. C*, 1394 (1966).

(6) Cf. R. Achini, U. Meyer, and Ch. Tamm, *Helv. Chim. Acta*, **51**, 1702 (1968); B. M. Trost, M. Ochiai, and P. G. McDougal, *J. Am. Chem. Soc.*, **100**, 7103 (1978).

(7) The first synthesis of verrucarol has recently been completed by Professor R. H. Schlessinger and co-workers at the Univeristy of Rochester.

(8) T. Katsuki and K. B. Sharpless, *J. Am. Chem. Soc.*, **102**, 5974 (1980).

(9) E. J. Corey and G. Schmidt, *Tetrahedron Lett.*, 399 (1979).

(10) P. H. J. Carlsen, T. Katsuki, V. S. Martin, and K. B. Sharpless, *J. Org. Chem.*, **46**, 3936 (1981).

(11) Unpublished results of Kenneth Shaw (our laboratory) and A. Pfaltz (Professor G. Stork's laboratory).

(12) H. Tanaka, Y. Kobayasi, and S. Torii, *J. Org. Chem.*, 41, 3482 (1976).

(13) Prepared in 60% yield as follows: (1) ICH_2COOH, $HOCH_2CH_2SiMe_3$, DCC, 4-pyrrolidinopyridine;[15] (2) Ph_3P; (3) NaH/THF.

(14) Prepared from anguidine by the method of Tulshian and Fraser-Reid [D. B. Tulshian and B. Fraser-Reid, *Tetrahedron Lett.*, 4549 (1980)]: (1) a, NaH, CS_2, THF; b, CH_3I; (2) Bu_3SnH, xylene; (3) $NaOCH_3$, CH_3OH; 65% overall yield. We thank Professor Fraser-Reid for the details of his procedure and Dr. T. Doyle of Bristol Laboratories for the donation of a sample of anguidine.

(15) A. Hassner and V. Alexanian, *Tetrahedron Lett.*, 4475 (1978).

(16) Other esterification methods (e.g., the acylimidazole method used by Breitenstein and Tamm[4]) caused significant isomerization leading to (*E,E*)-muconate derivatives.

(17) T. Kurihara, Y. Nakajima, and O. Mitsunobu, *Tetrahedron Lett.*, 2445 (1976).

concentration of 2 mM in benzene proceeded smoothly to give acetoxyverrucarin A in 52% yield from **12**. Interestingly, cyclization of a mixture of secoacetoxyverrucarin A and its (*E,E*)-muconate isomer led only to cyclization of the (*E,Z*)-secoacetoxyverrucarin A component. This circumstance probably results from the strain of the *E,E* macrocycle and could have significant implications for future synthetic efforts in this area. Deacetylation of the acetoxyverrucarin A thus produced with catalytic sodium methoxide (MeOH, 0 °C, 2 h) gave synthetic verrucarin A (70%) which was shown to be identical by all the usual measurements including ^{13}C NMR and 250-MHz ^1H NMR with an authentic sample of natural material kindly provided by Professor B. B. Jarvis.[18]

Registry No. **1**, 3148-09-2; **2** (R^1 = Ac, R^2 = Si-*t*-BuPh$_2$), 79568-65-3; **3** (R^3 = $CH_2CH_2SiMe_3$), 79568-66-4; **4**, 2198-92-7; **5**, 79568-67-5; **6**, 79568-68-6; **7**, 79568-69-7; **8**, 1122-21-0; **9**, 57314-31-5; **10**, 14032-66-7; **11**, 79568-70-0; **12**, 79568-71-1; propargyl alcohol magnesium salt, 65113-89-5; furfural, 98-01-1; (trimethylsilyl)ethylylidene triphenylphosphine, 79414-15-6.

(18) All new compounds were characterized by ^1H NMR, IR, and MS. Yields reported refer to chromatographically pure isolated material. This work was supported by PHS Grant 2R01 CA23094 awarded by the National Cancer Institute, DHHS.

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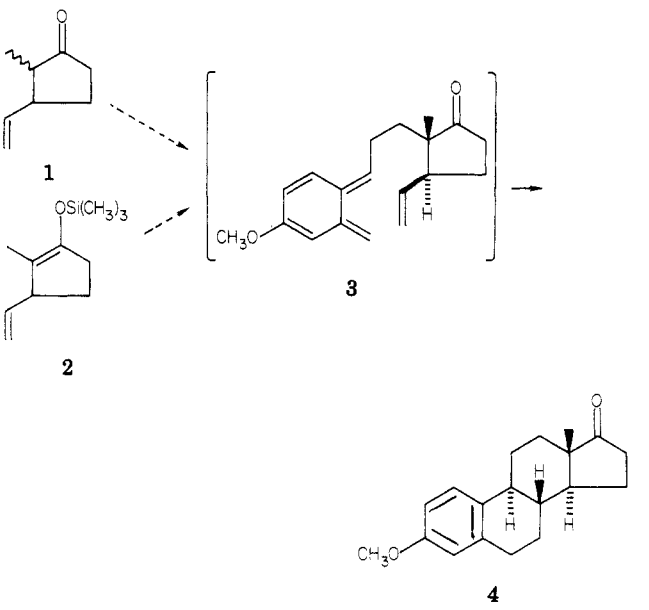
Complete Asymmetric Induction in Synthesis of Enantiomerically Pure Steroid Intermediates of Natural Configuration

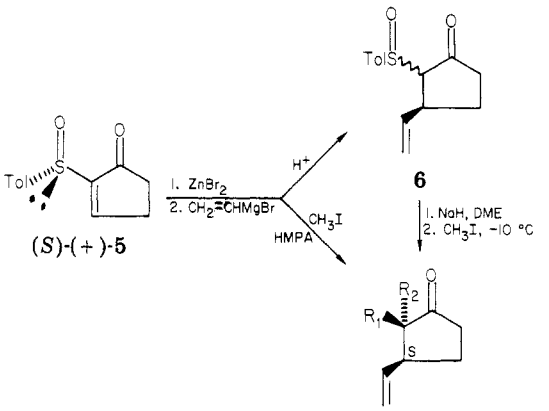
Summary: Enantiomerically pure steroid intermediates (*S*)-**1** and (*S*)-(+)-**2** of natural absolute configuration have been prepared with complete asymmetric induction during zinc dibromide mediated vinylmagnesium bromide conjugate addition to enantiomerically pure, crystalline, stable cyclopentenone sulfoxide (*S*)-(+)-**5**, which has been prepared on a 10-g scale.

Sir: During the past four years, several elegant, creative, and convergent total syntheses of racemic A-ring aromatic steroids have been developed (Scheme I). A common feature in all of these approaches is use of an intramolecular Diels–Alder cyclization of an *o*-quinodimethane such as **3** to produce a 19-nor steroid such as **4** having the natural *relative* configuration at all chiral centers.[2] Many of these approaches start with either racemic 2,3-disubstituted cyclopentanone **1** or with racemic cyclopentanone

Scheme I



Scheme II



(*S*)-(+)-**7a**, R_1 = TolSO; R_2 = CH_3
b, R_1 = CH_3; R_2 = TolSO

enol silyl ether **2**.[3] There is, therefore, a well-recognized need for an efficient and reliable source of *enantiomerically pure* steroid intermediates **1** and **2**. Prompted by Quinkert's recent report on partial asymmetric induction in synthesis of 2,3-disubstituted cyclopentanone **1**,[1i] we now report (1) the effect of divalent zinc on the course of vinyl Grignard conjugate addition to enantiomerically pure cyclopentenone sulfoxide (*S*)-(+)-**5**,[4] and (2) virtually *complete asymmetric induction* in the syntheses of optically pure steroid intermediates (*S*)-**1** and (*S*)-(+)-**2** of natural absolute configuration.

Optically pure, crystalline, stable cyclopentenone sulfoxide (*S*)-(+)-**5**,[5] $[\alpha]^{22}_D$ +141.7° (*c* 0.11, $CHCl_3$), prepared reproducibly on 10-g scale, was treated first with zinc dibromide to preform chelate **5a** and then with vinylmagnesium bromide; conjugate addition[6] led to an enolate ion which was protonated to give 2,3-disubstituted cyclopentanone **6** in quantitative yield and which separately was

(1) (a) Oppolzer, W.; Petrzilka, M.; Bättig, K. *Helv. Chim. Acta* **1977**, *60*, 2965. (b) Kametani, T.; Nemoto, H.; and Fukumoto, K. *J. Am. Chem. Soc.* **1977**, *99*, 3461. (c) Funk, R. L.; Vollhardt, K. P. C. *Ibid.* **1977**, *99*, 5483; **1979**, *101*, 215. (d) Oppolzer, W.; Bättig, K.; Petrzilka, M. *Helv. Chim. Acta* **1978**, *61*, 1945. (e) Nicolaou, K. C.; Barnette, W. E.; Ma, P. *J. Org. Chem.* **1980**, *45*, 1463. (f) Djuric, S.; Sarkan, T.; Magnus, P. *J. Am. Chem. Soc.* **1980**, *102*, 6885. (g) Ito, Y.; Nakatsuka, M.; Saegusa, T., *Ibid.* **1981**, *103*, 476. (h) Quinkert, G.; Weber, W.-D.; Schwartz, U.; Dürner, G. *Angew. Chem., Int. Ed. Engl.* **1981**, *19*, 1027. (i) Quinkert, G.; Schwartz, U.; Stark, H.; Weber, W.-D.; Baier, H.; Adam, F.; and Dürner, G. *Ibid.* **1981**, *19*, 1029.

(2) For reviews see: (a) Oppolzer, W. *Synthesis* **1978**, 793; *Heterocycles* **1980**, *14*, 1615. (b) Kametani, T.; Nemoto, H. *Tetrahedron* **1981**, *37*, 3. (c) Funk, R. L.; Vollhardt, K. P. C. *Chem. Soc. Rev.* **1980**, *9*, 41.

(3) A resolved oxocyclopentaneacetic acid has been used in the synthesis of two optically pure estrone derivatives: Oppolzer, W.; Roberts, D. A. *Helv. Chim. Acta* **1980**, *63*, 1703 and ref 1d.

(4) Posner, G. H.; Mallamo, J. P.; Miura, K. *J. Am. Chem. Soc.* **1981**, *103*, 2886.

(5) Prepared as indicated in ref 4.

(6) Posner, G. H. *Org. React.* **1972**, *19*, 1.

0022-3263/81/1946-5244$01.25/0