Macrocyclic and Other Novel Trichothecenes: Their Structure, Synthesis, and Biological Significance

BRUCE B. JARVIS*

Department of Chemistry, University of Maryland, College Park, Maryland 20742

EUGENE P. MAZZOLA

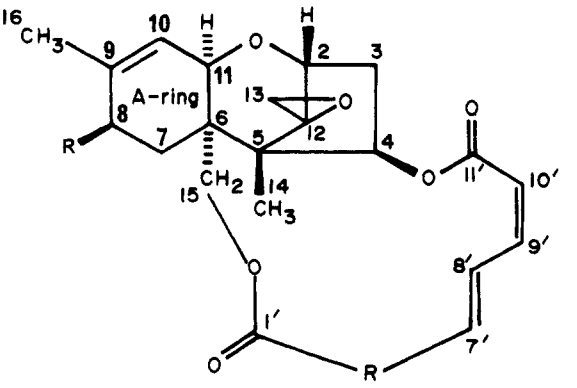
Division of Chemistry and Physics, Food and Drug Administration, Washington, D.C. 20204

Received December 28, 1981 (Revised Manuscript Received July 20, 1982)

The burst of energy in the past 10 years in organic synthesis has been fueled in part by the isolation of a vast array of natural products of unusual structure and bioactivity. The ability of chemists to isolate these highly biologically active compounds, usually present in natural sources in only small concentrations, is due principally to tremendous advancements in separation techniques and to the use of bioassays to monitor fractionation procedures. One of the most intensive programs in this area is that initiated by the National Cancer Institute (NCI) over 25 years ago to screen higher plants, cultures of microorganisms, and, as of late, marine animals for anticancer activity.[1] The rationale for the screening of natural products for anticancer activity is well delineated in a paper by the late Professor S. Morris Kupchan;[2] he pointed out that, in such a search, one is bound to find structures of varied and unusual type in nature that the chemist might use as prototypes for drug development.

One of the last plants studied by the Kupchan group was the Brazilian shrub *Baccharis megapotamica* Spreng (Asteraceae),[3] which was shown to contain baccharin (10) (see Figure 2, ref 5), a compound highly active in vivo against P388 mouse leukemia.[6] Later work showed that 10 was only one of several closely related macrocyclic trichothecenes (Charts I and II) called baccharinoids present to the extent of ca. 0.02% by dry weight in *B. megapotamica*.[7] For several years our work has been centered on the chemistry and biology of these interesting sesquiterpenes, which are members of the well-known group of potent antibiotics called trichothecenes.

Chart I



compound	R	R¹
verrucarin A (1)	-CHOHCHCH₃CH₂CH₂OC- (S R; 2' 3' 12' 4' 5' 6'; O)	H
verrucarin J (2)	-CH=CCH₃CH₂CH₂OC- (E; O)	H
roridin A (3)	-CHOHCHCH₃CH₂CH₂OCHCHOHCH₃ (S R R R; 2' 3' 12' 4' 5' 6' 13' 14')	H
8β-hydroxyverrucarin A (4)[a]	-CHOHCHCH₃CH₂CH₂OC- (S R; O)	OH
8β-hydroxyroridin A (5)[a]	-CHOHCHCH₃CH₂CH₂OCHCHOHCH₃ (S R R R)	OH
baccharinols B3 (6) and B7 (7)	-CHOHCHCH₃CH₂CH₂OCHCHOHCH₃ (R R R; *)	OH
baccharinols B4 (8) and B6 (9)	-CH(O)CCH₃CHOHCH₂OCHCHOHCH₃ (S R S R; *)	OH

[a] Semisynthetic compounds. *Denotes Center of Epimerization.

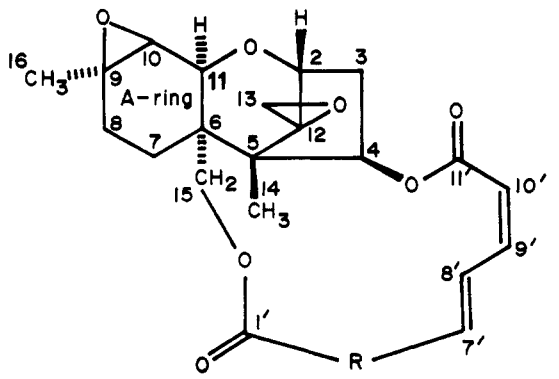
Bruce B. Jarvis was born in Van Wert, OH, in 1942. He graduated from Ohio Wesleyan University, took his Ph.D. degree (with Stan Cristol) from the University of Colorado, spent a year at Northwestern University with Fred Bordwell working on reaction mechanisms in organo sulfur chemistry, and joined the faculty at the University of Maryland in 1967. For several years his research concerned mainly studies of reaction mechanisms involving radicals, carbanions, carbonium ions, and small ring compounds. In 1975-1976, he was Visiting Scholar in Residence at the University of Virginia working in the laboratories of Professor Morris Kupchan on the isolation and identification of anticancer compounds from higher plants. Although he continues to work in the area of organic reaction mechanisms, he has become increasingly involved in natural products work in recent years.

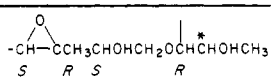
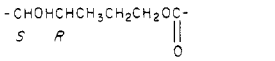
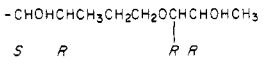
Eugene P. Mazzola is in charge of the Bureau of Foods' NMR Spectroscopy Laboratory of the Food and Drug Administration as well as Adjunct Professor of Chemistry at the University of Maryland. He was born in Montclair, NJ, in 1942 and received an A.B. degree from Franklin and Marshall College and Ph.D. degree from the University of Pittsburgh (with C. E. Griffin). He was a postdoctoral fellow at the California State University, Los Angeles (with H. Goldwhite), before joining FDA. His research interests include application of NMR spectroscopy to structural elucidation problems and theory and applications of the nuclear Overhauser effect.

(1) (a) Douros, J.; Suffness, M. *Cancer Chemother. Pharmacol.* **1978**, *1*, 91. (b) Suffness, M.; Douros, J. *Methods Cancer Res.* **1979**, *14*, 73.
(2) Kupchan, S. M. *Recent Adv. Phytochem.* **1974**, *9*, 167.
(3) *B. megapotamica* is one of ca. 400 species of *Baccharis*, none of which have any reputed medicinal or agricultural use. These plants grow mainly in Central and South America, and none has any reported economic significance to man.[4]
(4) Herz, W. In "The Biology and Chemistry of the Compositae"; Heywood, V. H., Harborne, J. B., Turner, B. L., Eds.; Academic Press: New York, 1977; pp 567-576.
(5) Kupchan, S. M.; Jarvis, B. B.; Dailey, R. G., Jr.; Bright, W.; Bryan, R. F.; Shizuri, Y. *J. Am. Chem. Soc.* **1976**, *98*, 7092.

0001-4842/82/0115-0388$01.25/0

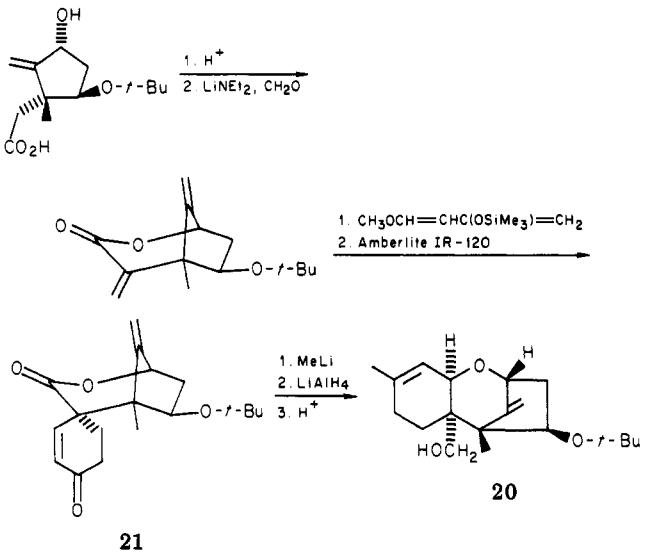
Chart II



compound	R
baccharins B5 (10) and B8 (11)	
9β,19β-epoxyverrucarin A (12)[a]	
9β,10β-epoxyroridin A (13)[a]	

[a] Semisynthetic compound. *Denotes center of epimerization.

Scheme I



21 20

Origin of the Baccharinoids

A curious feature about the isolation of these trichothecene baccharinoids from *B. megapotamica* is that, up to and since that time, trichothecenes have been isolated only from cultures of various genera of fungi (vide infra). In fact trichothecenes are very potent phytotoxins.[8] Through feeding studies, we have established that intact *B. megapotamica* plants can absorb thousands of parts per million of roridin A (3), a compound which is toxic to common agricultural plants such as corn, wheat, beans, and tobacco at concentrations of a few parts per million.[9]

Another interesting fact is that the pattern of A-ring substitution in the baccharinoids [e.g., 8β-hydroxyl derivatives (major series) and 9β,10β-epoxy derivatives (minor series)] is unique; no other fungal-produced trichothecene possesses these particular substitution patterns, although a number of simple trichothecenes[11] and a recently isolated macrocyclic trichothecene [8α-hydroxyverrucarin J (verrucarin L)][12] possess hydroxyl or ester groups at the 8α position. These data suggest that perhaps *B. megapotamica* is acquiring roridins from a fungal source and then oxygenating them to the baccharinoids.

We have recently obtained evidence that this is just what is occurring with *B. megapotamica* in its native habitat. *B. megapotamica* grown in a U.S. Department of Agriculture greenhouse in Beltsville, MD, over a 2-year period is devoid of baccharinoids. Furthermore, we have fed seedlings of *B. megapotamica* roridin A (3) and verrucarin A (1) and have shown that not only are these mycotoxins rapidly absorbed by the roots and translocated to the upper plant, but they are transformed into the 8β-hydroxyl derivatives.[13] In the case of roridin A, the resulting 8β-hydroxyroridin A (5) in the plant undergoes epimerization at C-2′ to baccharinoid 7. Thus, the sources of baccharinoids 7 and 6 in

roridin A (3) —*B. megapotamica*→ 8β-hydroxyroridin A (5) → baccharinol B7 (7)

nature appear to be roridin A and isororidin A,[14] respectively. When this same experiment was repeated with seedlings of tomatoes, peppers, and artichokes, these plants quickly succumbed.[13] One is left to wonder about the ecological and biochemical implications of this finding.[15] What is certain is that this is a unique

(6) The current *in vivo* anticancer screen used by the NCI is against P388 mouse leukemia (PS).[1a] The activity is expressed as T/C values, which are the number of days the test animals live divided by the number of days the control animals live multiplied by 100. Compounds with $T/C \geq 120$ are considered active; those with $T/C > 180$ are considered very active.

(7) Kupchan, S. M.; Streelman, D. R.; Jarvis, B. B.; Dailey, R. G., Jr.; Sneden, A. T. *J. Org. Chem.* **1977**, *42*, 4221.

(8) We have assayed[9] a number of macrocyclic trichothecenes including baccharinoids 8 and 10 in the wheat coleoptile bioassay[10] and find that these compounds are the most toxic ever assayed in this test. Verrucarin A (1) is 100-fold more toxic than abscissic acid and 10 times more toxic than is chaetoglobosin K.[10]

(9) Jarvis, B. B.; Cutler, H. G.; Mandava, N. B., unpublished results.

(10) Cutler, H. G.; Crumley, F. G.; Cox, R. H.; Cole, R. J.; Vormer, J. W.; Springer, J. P.; Latterell, F. M.; Thean, J. E.; Rossi, A. E.; *J. Agric. Food Chem.* **1980**, *28*, 139. Also see: Cutler, H. G.; LeFiles, J. H. *Plant Cell Physiol.* **1978**, *19*, 177.

(11) (a) Bamburg, J. R.; Strong, F. M. In "Microbial Toxins"; Kadis, S., Ciegler, A., Ajl, C. J., Eds.; Academic Press: New York, 1971; Vol. 7, p 207. (b) Bamburg, J. R. In "Mycotoxins and Other Fungal Related Food Problems"; Rodricks, J. V., Ed.; American Chemical Society: Washington, DC, 1976; Adv. Chem. Ser., No. 149, p 144. (c) Doyle, T. W.; Bradner, W. T. In "Anticancer Agents Based on Natural Product Models"; Cassidy, J. M., Douros, J. D., Eds.; Academic Press: New York, 1980; p 43. (d) Ueno, Y. *Adv. Nutr. Sci.* **1980**, *3*, 301. For reviews dealing with the macrocyclic trichothecenes, see: (e) Tamm, Ch. *Fortschr. Chem. Org. Naturst.* **1974**, *31*, 63. (f) Tamm, Ch. In "Mycotoxins in Human and Animal Health"; Rodricks, J. V., Hesseltine, C. W., Mehlman, M. A., Eds.; Pathotox Publishers: Park Forest South, IL, 1977; p 209. (g) Tamm, Ch.; Breitenstein, W. In "The Biosynthesis of Mycotoxins, A Study in Secondary Metabolism"; Steyn, P. S., Ed.; Academic Press: New York, 1980; p 69. (h) Ong, C. W. *Heterocycles* **1982**, *19*, 1685.

(12) Jarvis, B. B.; Midiwo, J. O.; DeSilva, T.; Mazzola, E. P. *J. Antibiot.* **1981**, *34*, 120.

(13) Jarvis, B. B.; Midiwo, J. O., Tuthill, D.; Bean, G. A. *Science (Washington, D.C.)* **1981**, *214*, 460.

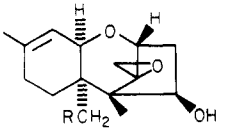
(14) Jarvis, B. B.; Midiwo, J. O.; Mazzola, E. P.; Flippen-Anderson, J. *J. Nat. Prod.* **1982**, *45*, 440.

case of a higher plant acquiring, modifying, and storing large amounts of potent antibiotics with no apparent ill effect.[18]

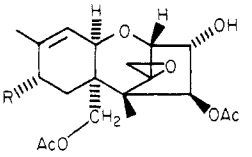
Structures of the Trichothecenes

The trichothecene complex of antibiotics[11] is made up of a series of sesquiterpene polyalcohols and esters produced by various genera of fungi such as *Fusarium*, *Trichothecium*, *Trichoderma*, *Myrothecium*, *Cephalosporium*, *Stachybotrys*, *Verticimonosporium*, and *Cyclindrocarpon*. The biological activities of the trichothecenes span an enormous range, including insecticidal, antifungal, antibacterial and antiviral. They also are phytotoxic and cytotoxic and exhibit a high degree of cytostaticity; the latter property makes them attractive antitumor candidates. Furthermore, trichothecenes are notorious mycotoxins, which have been associated with a wide variety of human and animal intoxications throughout the world.[26] In fact, several of the simple trichothecenes (e.g., T-2 toxin and diacetoxyscirpenol; vide infra) have been reported to be constituents of "yellow rain", a deadly chemical mixture that allegedly has been used in chemical warfare in Afghanistan and Southeast Asia.[27]

The trichothecenes can be divided into two classes: the simple (e.g., **14** and **15**)[11] and the macrocyclic de-



trichodermol (**14a**), R = H
verrucarol (**14b**), R = OH

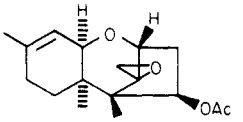


diacetoxyscirpenol (**15a**), R = H
(anguidine)
T-2 Toxin (**15b**), R = $OCOCH_2CH(CH_3)_2$

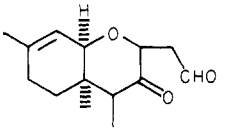
rivatives (Charts I and II).[11e-g] Common to both of these classes is a central tetracyclic ring system that almost always contains a 12,13-spiro epoxide group which is responsible, in the main,[11] for the biological activity of these potent protein synthesis inhibitors.[16]

Synthesis of Macrocyclic Trichothecenes

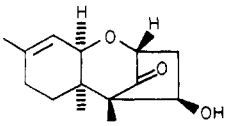
The above-mentioned biological properties of the trichothecenes together with their relative scarcity have made them the object of numerous synthetic efforts. Until recently, there had been no reported synthesis of a macrocyclic trichothecene with the exception of the synthesis of nonnaturally occurring 7′,8′,9′,10′-tetrahydroverrucarin J[28a] and 3′-hydroxy-2′-deoxy-7′,8′,9′,10′-tetrahydroverrucarin A.[28b] The principal stumbling block to the total synthesis of these macrocycles has been the lack of an effective route into verrucarol (**14b**), the central trichothecene from which nearly all of the macrocyclic trichothecenes are derived. Although the synthesis of racemic trichodermin (**16**) was



16



17



18

reported by Colvin, Raphael, and Roberts[29] over 10

(15) The reason for the resistance of *B. megapotamica* to the toxic effects of the macrocyclic trichothecenes is unknown. Clearly, it is not due to the lower toxicity of baccharinoids since these plant-derived trichothecenes are some of the most toxic macrocycylic trichothecenes we have tested in beans, wheat, corn, and tobacco.[9] The trichothecenes are potent inhibitors of eukaryotic protein synthesis and normally bind to the 60S ribosomal subunit, thus inhibiting peptidyltransferase activity.[16] However, trichothecenes do not bind to the 60S ribosomal subunit of *M. verrucaria* and thus do not elicit a toxic effect on this organism.[17] It would be very interesting if this were also the case with *B. megapotamica*.

(16) (a) Ueno, Y.; Hosoya, M.; Morita, Y.; Ueno, I.; Tatsuno, T. J. *Biochemistry (Tokyo)* **1968**, *64*, 479. (b) Cundliffe, E.; Davies, J. E. *Antimicrob. Agents Chemother.* **1977**, *11*, 491. (c) Kaneko, T.; Schmitz, H.; Essery, J. M.; Rose, W.; Howell, H. G.; O'Herron, F. A.; Nachfolger, S.; Huftalen, J.; Bradner, W. T.; Partyka, R. A.; Doyle, T. W.; Davies, J.; Cundliffe, E. *J. Med. Chem.* **1982**, *25*, 579.

(17) Hobden, A. N.; Cundliffe, E. *Biochem. J.* **1980**, *190*, 765.

(18) There are numerous examples of pathogenic fungi,[19] and to a much lesser extent mycorrhizal fungi,[20] which release toxic antibiotics that are absorbed and translocated by the host plant. In some cases, the host can survive by detoxifying the mycotoxins[21] or by releasing phytoalexins,[23] which protect the plant from further attack. A case that is perhaps more closely related to the situation found with *B. megapotamica* is that of maytansine. First isolated from the Ethiopian shrub, *Maytenus serrata*, in only 0.00002% yield, maytansine (and related congeners) was later shown to be present in higher amounts in a variety of higher plants.[1b] A search for a maytansine-producing microorganism in association with the *Maytenus* species gave only negative results.[24] However, several years later, Japanese workers showed that a microorganism (*Nocardia* species) produced maytansinoids.[25] Furthermore, maytansinoids do not appear to be produced by *M. buchananii* in cell culture (Kutney, J. P. *Heterocycles* **1981**, *15*, 1405).

(19) (a) Kuc, J. In "Microbial Toxins"; Ciegler, S., Ajl, S., Eds.; Academic Press: New York, 1972; Vol. 8, p 211. (b) Luke, H. H.; Gracen, V. C. in ref 19a, p 131. (c) Rudolph, K. In "Physiological Plant Pathology"; Heitefuss, R., Williams, P. H., Eds.; Springer-Verlag: New York, 1976; p 270.

(20) (a) Hacskaylo, E. In "Mycorrhizae, First North American Conference on Mycorrhizae, April, 1969", *USDA For. Service Misc. Publ.* **1971**, *No. 1189*, 1981. (b) Watling, R. *McIlvainea* **1977**, *3*, 12.

(21) In most cases of resistant plants, their insensitivity to toxins appears to be the result of a general inertness to the effect of the toxin rather than a chemical modification to yield a nontoxic substance.[22] However, these are examples of host-specific toxins[22] where certain variants or strains of a given species of plant are sensitive to a mycotoxin, whereas most other members of the species remain unaffected. Trichothecenes are nonspecific toxins,[19c] and to our knowledge only *B. megapotamica* of the higher broad leaf plants appears to be unaffected by these potent toxins.

(22) Scheffer, R. P. In ref 19c, p 247.

(23) Grisebach, H.; Ebal, J. *Angew. Chem., Int. Ed. Engl.* **1978**, *197*, 635.

(24) Dr. L. Hanka, Upjohn Co., private communication.

(25) (a) Asai, M.; Mizuta, E.; Izawa, M.; Haibara, K.; Kishi, T. *Tetrahedron Lett.* **1979**, *35*, 1079. (b) Tanida, S.; Hasegawa, T.; Hatamo, K.; Higashide, E.; Yoneda, M., *J. Antibiot.* **1980**, *33*, 192.

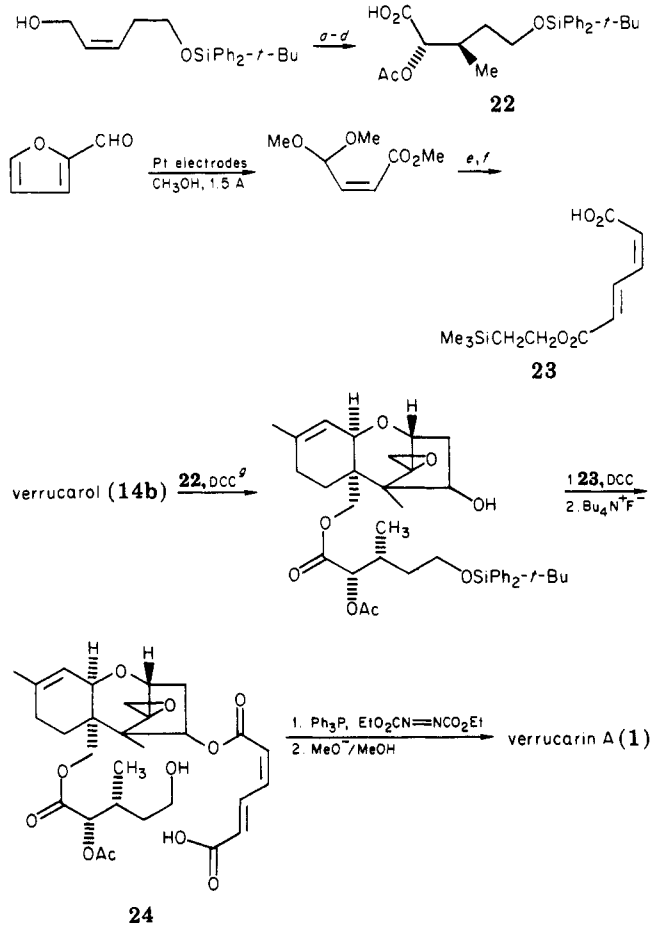
(26) (a) Pathre, S. V.; Mirocha, C. J. In ref 11f, p 299. For references dealing with toxicosis caused by macrocyclic trichothecenes see: (b) Harrach, B.; Mirocha, C. J.; Pathre, S. V.; Palyusik, M. *Appl. Environ. Microbiol.* **1981**, *41*, 1428. (c) Schneider, D. J.; Marasas, W. F. O.; Kuys, J. C. D.; Kriedk, N. P. J.; Van Schalkwyk, G. C., *J. S. Afr. Vet. Assoc.* **1979**, *50*, 73 and references therein.

(27) See *Chem. Eng. News* **1981**, Sept 21, 7; Oct 26, 15; Nov 16, 10; Nov 30, 29; Dec 14, 21; **1982**, July 5, 16. *Science (Washington, D.C.)* **1981**, *218*, 1008; Minocha, C. J.; Powlostzy, R. J.; Chatterjee, K.; Watson, S.; Hayes, W., 96th International Meeting of the Assocation of Official Analytical Chemists, Oct. 28, 1982, Washington, DC.

(28) (a) Breitenstein, W.; Tamm, Ch. *Helv. Chim. Acta* **1978**, *61*, 1975. (b) Notegen, E.-A.; Tori, M.; Tamm, Ch. *Ibid.* **1981**, *64*, 316.

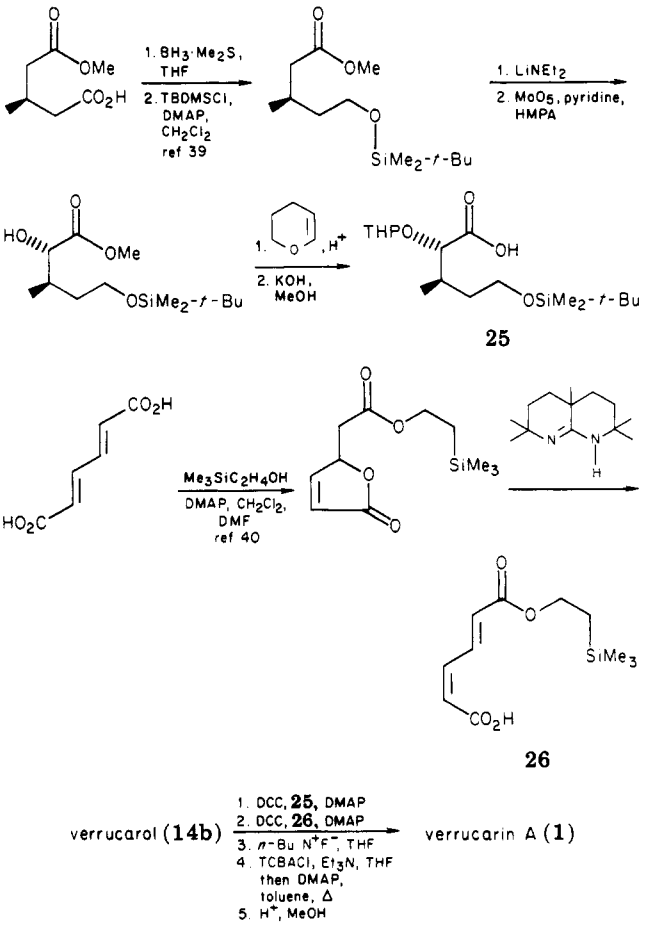
(29) (a) Colvin, E. W.; Raphael, R. A.; Roberts, J. S. *Chem. Commun.* **1971**, 858. (b) Colvin, E. W.; Malchenko, S.; Raphael, R. A.; Roberts, J. S., *J. Chem. Soc., Perkin Trans. 1* **1973**, 1989.

Scheme II



[a] Diethyl *d*-tartrate, *t*-BuOOH, $Ti(OCHMe_2)_4$, CH_2Cl_2, -20 °C. [b] 2% $RuCl_3$; $NaIO_4/CCl_4$, CH_3CN, H_2O. [c] 3 equiv of $Al(CH_3)_3$. [d] Ac_2O, C_5H_5N. [e] 1 N H_2SO_4. [f] $Ph_3P{=}CHCOOCH_2CH_2SiMe_3$. [g] DCC = dicyclohexylcarbodiimide.

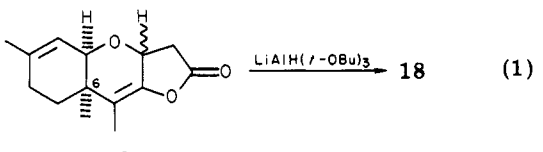
Scheme III[a]



[a] THF = tetrahydrofuran; TBDMSCl = *t*-butyldimethylsilyl chloride; DMAP = 4-(dimethylamino)pyridine; HMPA = $[(CH_3)_2N]_3PO$; THP = tetrahydropyranyl; DMF = dimethylformamide; DCC = dicyclohexylcarbodiimide; TCBACl = 2,4,6-trichlorobenzoyl chloride.

years ago, attempts to extend the reported synthetic methodology to the synthesis of verrucarol failed.[30]

The central problem is the reluctance of keto aldehyde **17** to undergo intramolecular aldol condensation to **18**. This problem was circumvented in the case of trichodermin by the in situ generation of the requisite carbanion through reaction of lactone **19** with lithium hydridotri-*tert*-butoxyaluminate (eq 1). However, the



(1)

reaction goes in only low yield (<10%), and when the quaternary methyl at C-6 was replaced by $CH_2OCH_2OCH_3$, no conversion to the verrucarol ring system was observed.[30,31] Closure of the C-ring by aldol-type condensations, tempting as it appears, does not seem to be a viable route into verrucarol.[32]

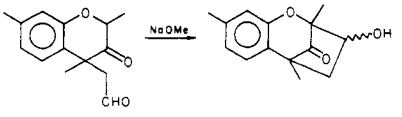
An elegant solution to this longstanding problem of the synthesis of verrucarol (racemic) was recently reported by Schlessinger and Nugent.[34] The key step in their synthesis is the conversion of the spirolactone **20**, via a biomimetic ring-closure reaction,[35] into the verrucarol ring system **21** (Scheme I).

Still and Ohmizu[36] have completed the synthesis of verrucarin A (**1**) (Scheme II), which marks the first reported synthesis of a naturally occurring macrocyclic trichothecene; however, they employed verrucarol (**14b**) obtained through the conversion of anguidine (**15a**).[37]

(30) Colvin, E. W.; Malchenko, S.; Raphael, R. A.; Roberts, J. S. *J. Chem. Soc., Perkin Trans. 1* **1978**, 658.

(31) For studies related to verrucarol, see: (a) Trost, B. M.; Rigby, J. N. *J. Org. Chem.* **1978**, *43*, 2938. (b) Roush, W. R.; D'Ambra, T. E. *Ibid.* **1980**, *45*, 3929. (c) White, J. D.; Matsui, T.; Thomas, J. A. *Ibid.* **1981**, *46*, 3376. For a recent synthesis of trichodermol, see: (d) Still, W. C.; Tasi, M.-Y. *J. Am. Chem. Soc.* **1980**, *102*, 3654. (e) Kraus, G. A.; Roth, B.; Frazier, K.; Shimagaki, M. *Ibid.* **1982**, *104*, 1114.

(32) Aldol-type ring closures as illustrated in (i) are known to take



(i)

place in this system.[33]

(33) (a) Fujimoto, Y.; Yokura, S.; Nakamura, T.; Morikawa, T.; Tatsun, T. *Tetrahedron Lett.* **1974**, 2523. (b) Goldsmith, D. J.; John, T. K.; Kwong, C. D.; Painter, G. R., III *J. Org. Chem.* **1980**, *45*, 3989.

(34) Schlessinger, R. H.; Nugent, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1116.

(35) See: ref 31b,c. Masuoka, E.; Kamikawa, T. *Tetrahedron Lett.* **1976**, 1691.

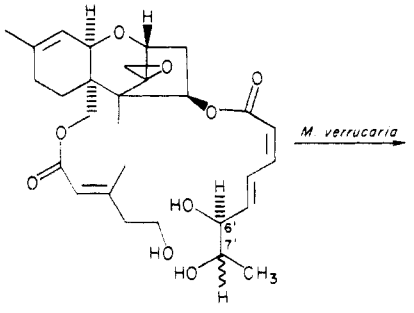
(36) Still, W. C.; Ohmizu, H. *J. Org. Chem.* **1981**, *46*, 5242.

(37) Tulshian, D. B.; Fraser-Reid, B. *Tetrahedron Lett.* **1980**, 4549.

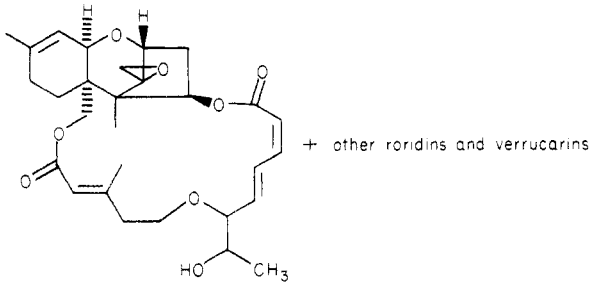
Interestingly, the (*E,E*)-muconate ester isomer of **24** failed to undergo cyclization under the conditions where **24** readily undergoes ring closure.[36]

Tamm's group at Basel[38] recently completed the synthesis of both verrucarin A (**1**) and 3α-hydroxyverrucarin A, a nonnaturally occurring congener. Their synthetic pathways are presented in Scheme III.

Our efforts in this area have been directed mainly at the synthesis and subsequent conversion of the trichoverrins (**27**) to the macrocycles. This line of research was prompted by the observation that the trichoverrins lie along the biosynthetic pathway to the macrocyclic trichothecenes.[11g,41] The ring closure of trichoverrins (**27**) to (iso)roridin E (**28**) by a resting culture of *Myrothecium verrucaria*[41] results in inversion of configuration at C-6′, since this center is *S* in **27** and *R* in most of the roridins.[14]



trichoverrin A (**27a**), 7′ = *S*
trichoverrin B (**27b**), 7′ = *R*



(iso)roridin E (**28a**, **28b**)

The proof of structure for trichoverrins A (**27a**) and B (**27b**) rests, in part, on the correlation of the dienediolic side chain with methyl esters **29** and **30** synthesized stereospecifically by Tulshian and Fraser-Reid.[42] Thus, the methodology for the synthesis of the right-hand side of the trichoverrins was already worked out. The synthesis for the chain on the left side is given in eq 2.[43]

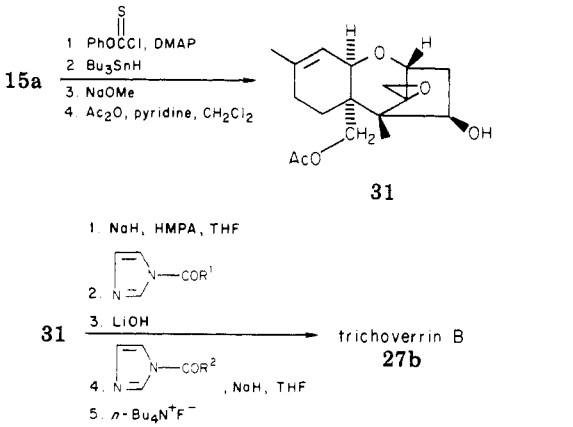
$$\underset{Z\quad E\quad R\quad R}{MeO_2CCH{=}CHCH{=}CHCHOHCHOHCH_3}$$

29

$$\underset{Z\quad E\quad S\quad R}{MeO_2CCH{=}CHCH{=}CHCHOHCHOHCH_3}$$

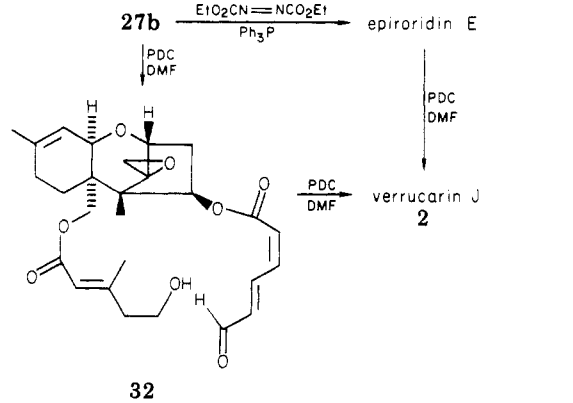
30

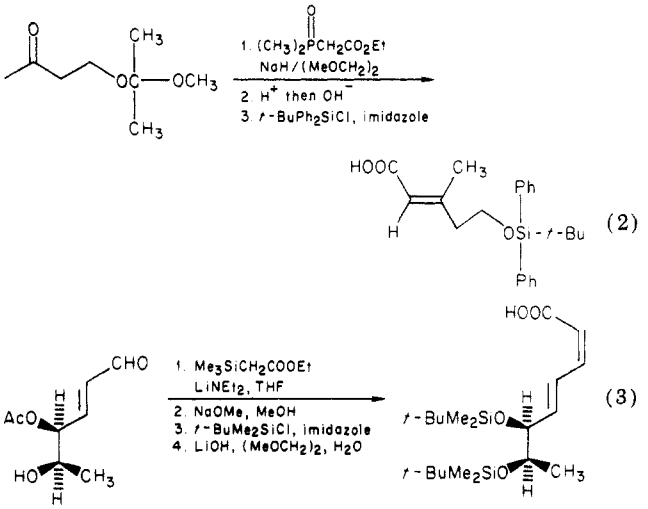
Scheme IV



R^1 = CH = CHCH = CHCH(OSiMe$_2$-*t*-Bu)CHCH$_3$OSiMe$_2$-*t*-Bu
(*Z*) (*E*)

R^2 = CH = CHCH$_3$CH$_2$CH$_2$OSiPh$_2$CMe$_3$
(*E*)





The synthesis of the right-hand side chain was accomplished by a slight modification of the reaction sequence reported earlier[42] in that the ethyl ester rather than the methyl ester of trimethylsilylacetic acid was employed in reaction 3. This gave a 2:1 ratio of the

(38) Mohr, P.; Tori, M.; Grossen, P.; Herold, P.; Tamm, Ch. *Helv. Chim. Acta* **1982**, *64*, 316.
(39) Obtained by the enantioselective hydrolysis of dimethyl 3-methylglutarate by pig liver esterase. See: Huang, F.-C.; Lee, L. F. H.; Mittal, R. S. D.; Ravikumar, P. R.; Chan, J. A.; Sih, C. J. *J. Am. Chem. Soc.* **1975**, *97*, 4144.
(40) Elvidge, J. A.; Linstead, R. P.; Sims, P.; Orkin, B. A. *J. Chem. Soc.* **1950**, 2228, 2235.
(41) Jarvis, B. B.; Pavanasasivam, G.; Holmlund, C. E.; DeSilva, T.; Stahly, G. P.; Mazzola, E. P. *J. Am. Chem. Soc.* **1981**, *103*, 472.
(42) Tulshian, D. B.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1981**, *103*, 474.
(43) Esmond, R.; Fraser-Reid, B.; Jarvis, B. B. *J. Org. Chem.* **1982**, *47*, 3358.

Z,E:*E,E* diastereomers rather than the lower 1:1 ratio observed with the methyl ester.[43]

Verrucarol, prepared from anguidine (**15a**) by a modification of the procedure reported by Tulshian and Fraser-Reid,[37] was selectively monoacetylated and converted to the C-4 dieneic ester, which was converted to trichoverrin B (**27b**) as shown in Scheme IV.[43]

Trichoverrin B can be ring-closed in low yield (ca. 10%) by means of the Mitsunobu procedure (Ph_3P, $EtO_2CN{=}NCO_2Et$, 25°C)[44] to give an epiroridin E,[45] which in turn can be oxidatively cleaved in high yield by pyridinium dichromate (PDC) to give verrucarin J (**2**).[43] This latter reaction is a general one for the conversion of roridins to verrucarins.[14] Interestingly, trichoverrin B is converted to verrucarin J (**2**) via aldehyde **32** with PDC in dimethylformamide (DMF). This reaction presumably occurs via the cyclic hemiacetal.[43]

Due to advances in both separation techniques and synthetic methodology, sufficient quantities of many of the above trichothecenes are now available to permit anticancer studies to be conducted.

Anticancer Studies

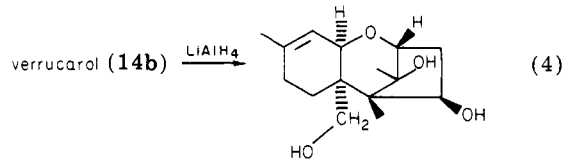
The plant-derived 8β-hydroxy- and 9β,10β-epoxybaccharinoids are distinctive in that they possess the greatest in vivo antileukemic activity of all the known trichothecenes. They stand in marked contrast to the structurally similar roridins and verrucarins, which, at best, show only marginal activity against P388 mouse leukemia.[6,11c] It appears that a slight modification (i.e., A-ring oxygenation) of the roridins or verrucarins would result in compounds possessing comparable in vivo P388 activity to that of the baccharinoids. A survey of the list of microorganisms available from the American Type Culture Collection (ATCC) revealed that there were available several species of *Myrothecium* that appeared to be roridin and verrucarin producers. Submerged shake cultures of *M. verrucaria* (ATCC 24571) produced enough of verrucarin A (**1**) and verrucarin B for us to convert these compounds to their 9β,10β-epoxides with *m*-chloroperbenzoic acid (MCPBA). These compounds proved to be significantly more active in P388 than were the original verrucarins.[46]

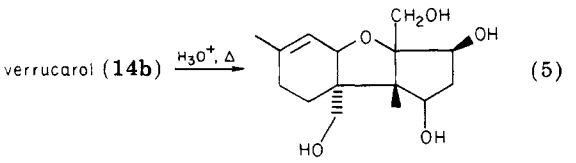
At the outset, it should be noted that, in general, the production and isolation of biologically active natural products from fermentations are usually a much more tractable problem than are the production and isolation of these compounds from higher plants. The problems associated with large-scale collection of plants and the ensuing isolations are often enormous. On the other hand, fermentation techniques are such that production and isolation of antibiotics normally are routine. One usually can feel confident that a small-scale fermentation can be scaled up with little difficulty. Therefore, production of baccharinoid-like compounds by straightforward chemical transformations of the antibiotics produced via fermentation is an attractive alternative to relying on *B. megapotamica* as the source of these compounds.

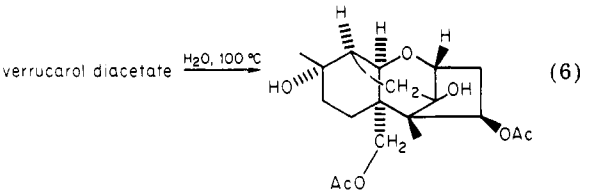
On the basis of our preliminary data for the epoxyverrucarins, NCI agreed to run a large-scale fermentation (760 L) of *M. verrucaria* at the Frederick Cancer Research Facility (FCRF) in order that we might obtain larger amounts of macrocyclic trichothecenes for further chemical modification studies. Besides isolating most of the known roridins (A, D, E, isoE,[47] and H)[11e-g] and verrucarins (A, B, and J)[11e-g] during the course of the workup of this fermentation, we isolated a number of new trichothecenes.[48]

Trichothecenes are potent protein synthesis inhibitors, and there have been extensive studies of the biochemical mechanisms of this process.[16] Interruption of protein synthesis takes place on the ribosomes, and, depending upon the particular trichothecene, cessation occurs at the initiation, elongation, or termination stage of protein synthesis. The stage at which protein synthesis is interrupted by the trichothecenes appears to be related to the size of the substrate. Thus, the larger trichothecenes (e.g., the macrocyclic ones) inhibit protein synthesis at the initiation stage, whereas the smallest ones inhibit at the termination stage. Although the details of how these compounds function at the molecular level are not known precisely, it is clear that they are bioalkylating agents and that the center of activity resides in the 12,13-epoxy group. Any alteration in structure that results in the loss of this group is accompanied by complete loss of biological activity.[11,16]

There are three routes whereby this epoxide group is known to undergo nucleophilic ring opening: (1) attack at C-13 (eq 4); (2) O-1 participation followed by rearrangement to the apotrichothecene ring system (eq 5); and (3) participation of the 9,10-double bond (eq 6).[11] Which of these pathways, if any, is important for







in vivo activity is unknown. However, synthetic analogues having the "wrong" epoxide configuration (e.g., **33**) are inactive, whereas those possessing oxygen atoms anti to the spiro epoxy oxygen atom (e.g., **34**) typically show modest P388 activity.[49] There are a number of

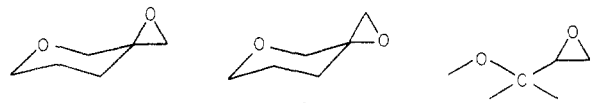
(44) Kurihara, T.; Nakajima, Y.; Mitsunobu, O. *Tetrahedron Lett.* **1976**, 2445.

(45) Isororidin E is *S* at both C6′ and C13′;[14] the configurations of roridin E and epiroridin E at these centers are unknown.

(46) Jarvis, B. B.; Stahly, G. P.; Curtis, C. R. *Cancer Treat. Rep.* **1978**, *62*, 1585.

(47) Matsumoto, M.; Minato, H.; Tori, K.; Ueyama, M. *Tetrahedron Lett.* **1977**, 4093.

(48) (a) Jarvis, B. B.; Eppley, R. M.; Mazzola, E. P. In "Trichothecenes—Chemical, Biological, and Toxicological Aspects", Ueno, Y., Ed.; Kodansha Scientific: Tokyo, in press. (b) Jarvis, B. B.; Stahly, G. P.; Pavanasasivam, G.; Midiwo, J. O.; DeSilva, T.; Holmlund, C. E.; Mazzola, E. P.; Geoghegan, R. F., Jr. *J. Org. Chem.* **1982**, *47*, 1117.

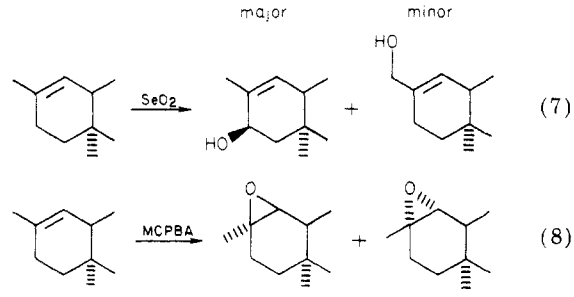


33 34 35

P388 active natural products, besides the trichothecenes, which have the characteristic functionality illustrated in structure **35**.[50]

These data suggest that the biologically important alkylating step in vivo might correpond most closely to that represented in eq 5. However, the 9,10-double bond also plays an important role in the biological activity of trichothecenes since reduction to the 9,10-dihydro derivatives results in a substantial loss of activity.[11c] Whether this is due to an important conformational change or to inability of the resulting reduced trichothecanes to undergo reactions in vivo analogous to eq 6 is unknown.

We have prepared a number of oxygenated derivatives of the roridins and verrucarins by straightforward chemical oxidations (eq 7 and 8).[51] In the case of



verrucarin A (1), we also have prepared the corresponding α-isomers in order to ascertain the importance of the stereochemistry of the A-ring oxygen atom. The 9α,10α-epoxide, 8α-hydroxide, and 8-keto derivatives of verrucarin A are P388 in vivo inactive.[51]

The various synthetic 9β,10β-epoxides exhibit excellent in vivo P388 activity.[51,52] In this regard, the baccharins **10** and **11** clearly serve as useful models for epoxidated roridins and verrucarins. The 8β-hydroxy derivatives of verrucarins A and J and of roridin A are somewhat less active in vivo against P388.[51,53]

We were pleased to find that the 16-hydroxy derivatives of verrucarin A and roridin A (for which there are no known naturally occurring models) exhibit high activity (T/C's $\simeq$ 200–250) at very low dose levels. However, these alcohols are the minor products (<10%) of the selenium dioxide hydroxylation reactions, and we, therefore, sought alternative routes for hydroxylation of the C-16 methyl group.

One approach to the selective hydroxylation of natural products is microbial modification.[54] Virtually nothing has been done in this area with trichothecenes although there are scattered reports concerning other types of modifications in this system.[55] We examined the ability of several microorganisms[56] to oxidize verrucarin A selectively, including *Rhizopus arrhizus* (ATCC 11,145), *Streptomyces roseochromogenus* (ATCC 13,400), *Cephalosporium acremonium* (NRRL 3092), *Wojnovivia graminis* (NRRL 2472), *Hypholom* sp. (NRRL 2471), *Bacillus megaterium* (NRRL 3938), *Hendersonia aciocola* (NRRL 2595), and *Curvularia lunata* (NRRL 2380). Of those studied, *Rhizopus arrihizus* was the only one to yield the desired 16-hydroxyverrucarin A; verrucarin B also is converted to 16-hydroxyverrucarin B in high yield.[57]

Finally, the most active of the compounds that we have prepared[53] have two oxygen functionalities attached to the A ring. The 8β-hydroxy 9β,10β-epoxides of verrucarin A and roridin A exhibit exceptionally high activity against P388; larger amounts of these compounds will be prepared for testing against other tumor systems.[58]

Although the presence of the 9β,10β-epoxide group clearly imparts greatly enhanced in vivo P388 activity to the macrocyclic trichothecenes, the origin of this enhancement is obscure. One of the principal effects of the introduction of this functionality is to lower the toxicity. It is in the dose range of 5–15 mg/kg, where the typical roridins and verrucarins are toxic, that the 9β,10β-epoxides are most active. Interestingly, conversion of anguidine (**15a**) (T/C = 210 at 2.5 mg/kg) to the 9β,10β-epoxide results in the loss of P388 activity at dose levels of 20 mg/kg and below.[16c] One also sees a corresponding drop in acute toxicity.

The role played by the macrolide ring in the macrocyclic trichothecenes with respect to their biological activity is unknown. The macrocyclic ring clearly enhances the potency of these compounds since the trichoverrins, which differ from roridins E and isoE by only a water molecule, are devoid of P388 in vivo activity (and toxicity) at dose levels of 32 mg/kg and below.[59] Also, the cytotoxicity in vitro against L1210 mouse leukemia of the trichoverrins is about 2 orders of magnitude lower than the toxicity of isororidin E.[60]

Future Work

This work has generated a number of new questions concerning the macrocyclic trichothecenes. Among these are why is *B. megapotamica* immune to the toxic effects of the macrocyclic trichothecenes? Is this unique to *B. megapotamica*, or are other *Baccharis* and related species also unaffected by these compounds? What are the toxic effects and metabolic pathways of the simple

(49) (a) Fullerton, D. S.; Chen, C. M.; Hall, I. H. *J. Med. Chem.* **1976**, *19*, 1341. (b) Anderson, W. K.; LaVoie, E. J.; Lee, G. E. *J. Org. Chem.* **1977**, *42*, 1045.

(50) Pettit, G. R. "Biosynthetic Products for Cancer Chemotherapy"; Plenum Press: New York, 1977–1979; Vol. 1–3.

(51) Jarvis, B. B.; Stahly, G. P.; Pavanasasivam, G.; Mazzola, E. P. *J. Med. Chem.* **1980**, *23*, 1054.

(52) The baccharins and the 9β,10β-epoxides of verrucarins A, B, and J and roridin A all have T/C's > 200.[6] The baccharinols and the semisynthetic 8β-hydroxy derivatives of verrucarins A and J and roridin A exhibit somewhat lower T/C's (~150–200).

(53) Midiwo, J. O. Ph.D. Thesis, University of Maryland, 1981.

(54) Kieslich, K. "Microbial Transformations of Non-steroid Cyclic Compounds"; Wiley-Thieme Publishers: New York, 1976.

(55) (a) Horvath, I.; Varga, J. M. *Nature (London)* **1961**, *192*, 99. (b) Ellison, R. A.; Kotsonis, F. N. *Appl. Microbiol.* **1974**, *27*, 423. (c) Machida, Y.; Nozoe, S. *Tetrahedron Lett.* **1972**, 1969. (d) Yoshizawa, T.; Morooka, N. *Appl. Microbiol.* **1976**, *29*, 54. (e) Claridge, C. A.; Schmitz, H. *Appl. Environ. Microbiol.* **1978**, *36*, 63.

(56) We also have screened several common soil fungi and find that a number of *Fusarium* species rapidly metabolize both verrucarins and roridins. Preliminary studies[57] suggest that the first reaction to take place is the hydration rearrangement of the 12,13-epoxy group to yield the biologically inactive apotrichothecenes (see eq 5). Apparently, *Fusarium* strains can readily metabolize (detoxify) trichothecenes via apotrichothecenes, thus circumventing the normal toxic effects of the compounds.

(57) Pavanasasivam, G. Ph.D. Thesis, University of Maryland, 1980.

(58) NCI submits certain selected compounds for evaluation in a tumor panel.[1]

(59) Doyle, T. W., unpublished results.

(60) French, J., unpublished results.

trichothecenes with respect to *Baccharis*? What are the details of the biosynthetic conversions of trichoverrins to roridins and verrucarins, and could this knowledge help us devise synthetically useful pathways in constructing macrocyclic trichothecenes from the simple trichothecenes? What are the details, at the molecular level, for biological activity of the trichothecenes, and could this information be useful in devising new anticancer derivatives? These are but a few of the questions that we hope to address in future research.

We are grateful to our collaborators, whose names appear in the references cited. B.B.J. also acknowledges support from the National Cancer Institute, Grant CA 25967.

Asymmetric Synthesis Catalyzed by Transition-Metal Complexes with Functionalized Chiral Ferrocenylphosphine Ligands

TAMIO HAYASHI and MAKOTO KUMADA*

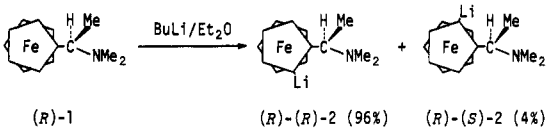
Department of Synthetic Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606, Japan

Received January 28, 1982 (Revised Manuscript Received June 3, 1982)

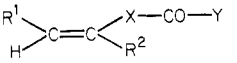
Among various types of asymmetric reactions, reaction with a chiral catalyst is obviously the best choice since catalytic asymmetric reactions can proceed with high stereoselectivity, producing the desired enantiomeric isomer in high yield.[1] Catalytic asymmetric synthesis requires ideally only one molecule of a chiral catalyst in order to produce a large quantity of an optically active substance. Catalytic reactions by homogeneous transition-metal complexes have been rapidly developed recently, and now a wide variety of reactins can be effected by transition-metal catalysts. Since many of the transition-metal complexes used for catalytic reactions have tertiary phosphines as ligands, it is convenient to use optically active phosphine ligands to make the metal complexes function as chiral catalysts. Thus, the most significant point for obtaining high stereoselectivity in catalytic asymmetric reactions is the design and preparation of a ligand that will fit in with a given reaction as efficiently in stereoselectivity as possible.

In 1968, the first asymmetric reaction by homogeneous transition-metal catalysts was reported by Knowles and Horner and their co-workers.[2] They used methylphenylpropylphosphine as a chiral ligand with a rhodium catalyst and got 4–15% optical yields in asymmetric hydrogenation of prochiral olefins. Since that time, over 100 various kinds of ligands have been developed in order to obtain higher optical yield, mostly

Scheme I



in rhodium-catalyzed asymmetric hydrogenations, and some of the phosphine ligands have been found very effective for the hydrogenation of α-(acylamino)acrylic acids, producing α-amino acids of over 90% ee.[1e,g] Recently, it has been shown that the high stereoselectivity attained is due to a characteristic structure of the olefinic substrates as well as chiral phosphine ligands. α-(Acylamino)acrylic acids and analogous functionalized olefins that can be hydrogenated with high stereoselectivity have the structural features shown below,



X = NH, O, CH_2
Y = R, OR

containing the carbonyl oxygen three atoms away from the carbon–carbon double bond, and the carbonyl group can coordinate with the rhodium, forming a chelate in the diastereomeric transition states.[1,3] Thus, attractive

Tamio Hayashi was born in Gifu, Japan, in 1948. He received his Ph.D. from Kyoto University where he studied with Professor M. Kumada and is now Instructor of Chemistry. He spent the year 1976–1977 as a postdoctoral fellow at Colorado State University with Professor L. S. Hegedus.

Makoto Kumada was born in Obama, Fukui, Japan, in 1920. He did his undergraduate work at Kyoto Imperial University and received his doctorate from Osaka University. After 7 years with Tokyo-Shibaura Electric Co., he joined in 1950 the faculty of Osaka City University. In 1962 he moved to Kyoto University, where he is a Professor of Chemistry. His current interests include organosilicon chemistry and organic synthesis catalyzed by transition-metal complexes. He was recipient of the ACS F. S. Kipping Award in Organosilicon Chemistry in 1967.

(1) For reviews: (a) Kagan, H. B.; Fiaud, J. C. *Top. Stereochem.* **1978**, *10*, 175–285. (b) Scott, J. W.; Valentine, Jr., D. *Science (Washington, D.C)* **1974**, *184*, 943. (c) Valentine, Jr., D.; Scott, J. W. *Synthesis* **1978**, 329. (d) Pearce, R. *Catalysis* **1978**, *2*, 176. (e) Caplar, V.; Comisso, G.; Sunjic, V. *Synthesis* **1981**, 85. (f) Bosnich, B.; Fryzuk, M. D. *Top. Stereochem.* **1981**, *12*, 119–154. (g) Merrill, R. E. "Asymmetric Synthesis Using Chiral Phosphine Ligands"; Design Corporation, Inc.: Hillside, NJ, 1979.

(2) (a) Knowles, W. S.; Sabacky, M. J. *Chem. Commun.* **1968**, 1445. (b) Horner, L.; Siegel, H.; Buthe, H. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 942.

(3) (a) Brown, J. M.; Chaloner, P. A. *J. Chem. Soc., Chem. Commun.* **1980**, 344 and their previous papers cited therein. (b) Chan, A. S. C.; Pluth, J. J.; Halpern, J. *J. Am. Chem. Soc.* **1980**, *102*, 5952 and their previous papers cited therein.

0001-4842/82/0115-0395$01.25/0