CHAPTER I: INTRODUCTION

- *Trichothecenes:*

1. Origin

"Trichothecene contamination of human food and animal feed is a continuing worldwide problem".[1] Multiyear surveys in the US and Canada indicate that maize and wheat are often contaminated with trichothecenes but at levels that are generally below the recommended tolerance level of 2 ppm.

Trichothecenes are widely occurring fungal metabolites and they have been linked to outbreaks of alimentary toxic aleukia that occurred in the former Soviet Union in the 1940's (1942-1947) and outbreaks of a similar disease called akakabi-byo or red mold disease in Japan.[2] Trichothecenes are responsible for moldy corn toxicosis in the USA, and stachybotryotoxicosis in central Europe. Dendrochiotoxicosis of horses in Europe is also presumed to be induced by these secondary metabolites, since the responsible fungus, *Dendrodochium toxicum,* is synonymous with *Myrothecium rodium,* which is able to produce a macrocyclic trichothecene.[3] The most controversial aspect of human exposure to trichothecene toxins has been the charge that they were used as chemical warfare agents in South Asia in the early 1980's, as part as the agent known as yellow rain.[1] Reports

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[1] Desjardins, A. E.; Hohn, T. M.; McCormick, S. P. *Microb. Rev.* **1993**, *57*, 595.
[2] Joffe, A. Z. *Fusarium Species - Their Biology and Toxicology*; John Wiley & sons: New York, **1986**.

denouncing the use of "yellow rain" in Laos were obtained in 1975-76. Similar reports and evidence were received from Kampuchia (1978) and Afghanistan (1979). Scientists believed that the principal active compound of yellow rain was T2-toxin.[2] This hypothesis was later disputed[4] because it takes six to eight weeks of ingestion of food intoxicated with T2-toxin to kill, therefore this trichothecene alone may not be the agent responsible for the sudden death toxic syndrome associated with yellow rain.

The trichothecenes are a group of closely related sesquiterpenoid mycotoxins[5] produced by various species of fungus imperfecti.[6] The first compounds of this class were discovered at Imperial Chemical Industries in 1946 during an extensive search for new antibiotics. Historically, glutinosin was the first trichothecene isolated, but it was later shown to be a mixture of verrucarins A and B. The name trichothecene is derived from the first isolated and purified member of the class, trichothecin, from the fungus *Trichotecium roseum,* in 1948. Since that date, over 80 trichothecenes have been identified from nine genera of fungi: *Fusarium, Myrothecium, Trichothecium, Trichoderma, Cephalosporium, Cyclindrocarpen, Stachybotrys, Verticimonosporium* and *Calonectria.*[7]

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[3] Ueno, Y. *Pure Appl. Chem.* **1977**, *49*, 1737.

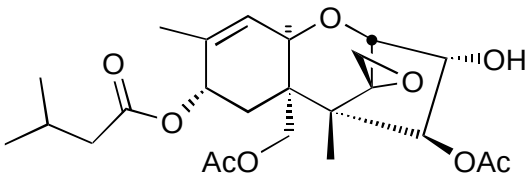
[4] From a letter to *Science* by Rosen, J. D. *Science* **1983**, 222 (4622), p 698:"I firmly believe that is not possible to isolate *Fusarium* species of the sporotrichiella section from tropical regions (Laos and Cambodia) or Subtropical areas with warm climate (India or Israel) but only from cold temperature climate regions. Therefore I cannot agree with the notion that the trichothecenes isolated from yellow rain samples are associated with natural occurrences in Southeast Asia"

[5] Mycotoxins means: fungal toxins that affect animals.

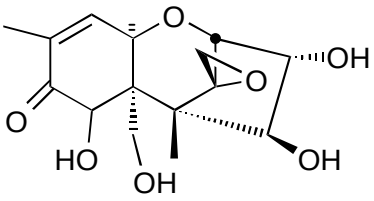
[6] McDougal, P. G.; Schmuff, N. R. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer - Verlag: New York, **1985**; Vol. 47, p 153.

[7] Corley, D. G.; Rottinghaus, G. E.; Tracy, J. K.; Tempesta, M. S. *Tetrahedron Lett.* **1986**, *27*, 4133.

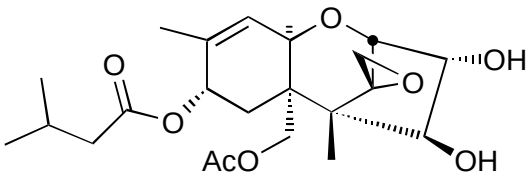
The *Fusarium* genus, which are isolated only from temperate regions, produces the most toxic trichothecene metabolites. The most toxic trichothecenes isolated to date are T2-toxin, HT2-toxin, neosolaniol, diacetoxy scirpenol, nivalenol, fusarion-x and deoxynivalenol.[2]



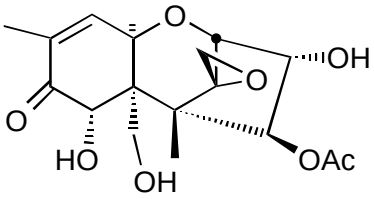
T2-toxin



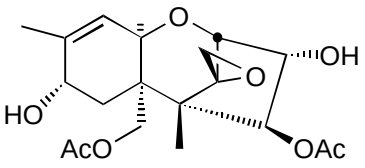
Nivalenol



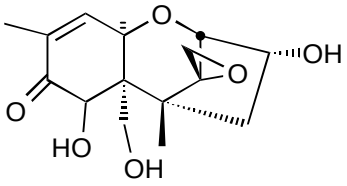
HT2-toxin



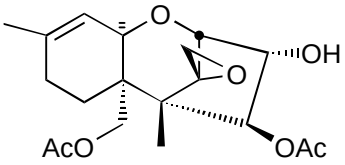
Fusarion-x



Neosolaniol



Deoxynivalenol



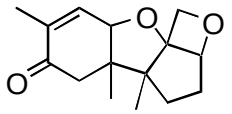
Diacetoxy scirpenol

The only known occurrence of these metabolites in higher plants is from an extract of the Brazilian shrub *Baccharis megapotamica,*[8] which exhibited *in vivo* antileukemic activity and led to the isolation of new macrocyclic trichothecenes, the "baccharins". These shrubs are resistant to trichothecenes and actually accumulate them on their seed

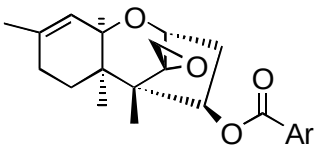
coats. It was originally suggested[9] that the baccharins are fungal metabolites absorbed from the soil and subsequently biotransformed by the plants. This hypothesis was refuted after further investigation as the baccharins were confirmed to originate from the plant.

2. Structure

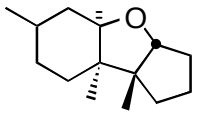
The original structure proposed for the skeleton of the trichothecene molecules was derived from chemical reactivity studies in the laboratories of Freeman,[10] Tamm[11] and Fishman.[12] This structure was later revised[13] based on the x-ray analysis of a single crystal of the p-bromobenzoate derivative of trichodermol. The originally proposed structure was more closely related to the apotrichothecane skeleton obtained from the rearrangement of the trichothecenes.



Proposed Structure



p-Bromobenzoate derivative of trichodermol (X-ray structure)



Apotrichothecane skeleton

[8] Jarvis, B. B.; Stahly, G. P.; Pavanasasivam, G.; Midiwo, J. O.; DeSilva, T.; Holmlund, C. E.; Mazolla, E. P.; Geoghegan Jr, R. F. *J. Org. Chem.* **1982**, *47*, 1117.

[9] Jarvis, B. B.; Stahly, G. P.; Pavanasasivam, G.; Mazzola, E. P. *J. Med. Chem.* **1980**, *23*, 1054.

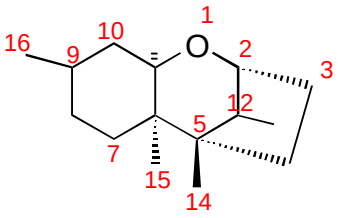
[10] Freeman, G. G.; Gill, J. E.; Waring, W. S. *J. Chem. Soc.* **1959**, 1105.

[11] Harri, E.; Loeffler, W.; Sigg, H. P.; Stahelin, H.; Stoll, C.; Tamm, C. H.; Wiesinger, D. *Helv. Chim. Acta* **1962**, *45*, 839.

[12] Fishman, J.; Jones, E. R. H.; Lowe, G.; Whiting, M. C. *J. Chem. Soc.* **1960**, 3948.

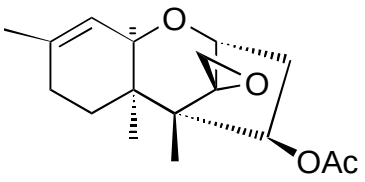
[13] Jarvis, B. B.; Vrudhula, V. M.; Midiwo, J. O.; Mazzola, E. P. *J. Org. Chem.* **1983**, *48*, 2576.

To date, over 80 naturally occurring trichothecenes have been identified and can be classified into three distinct structural groups:[14] simple trichothecenes, macrocycle trichothecenes and the recently discovered trichoverroids. Their chemical structures vary in both the position and the number of hydroxylations as well as in the position and complexity of esterification.

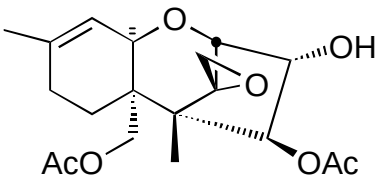


Trichothecane revised skeleton

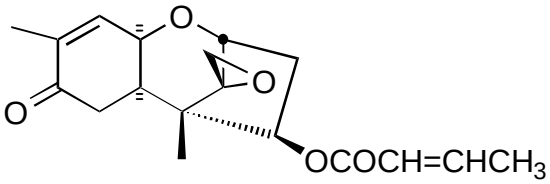
The simple trichothecenes contain the basic mono- or polyhydroxylated sesquiterpene skeleton, with zero, one or more of the hydroxyl groups esterified. Examples include trichodermin, anguidine, T2- toxin, trichothecin and verrucarol.



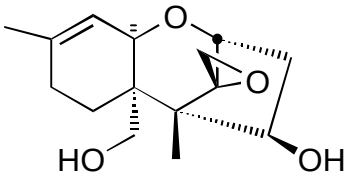
Trichodermin



Anguidine



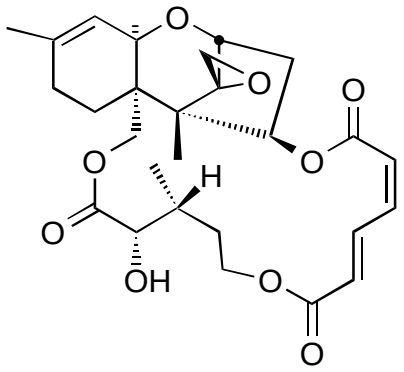
Trichothecin



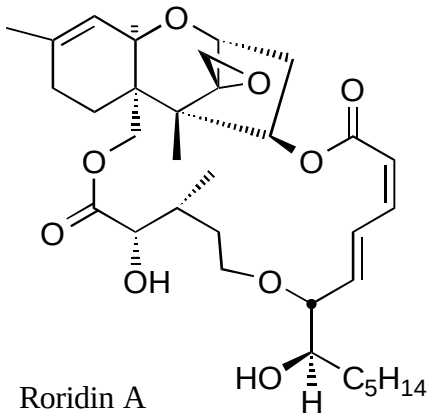
Verrucarol

[14]Corley, D. G.; Rottinghaus, G. E.; Tempesta, M. S. *Tetrahedron Lett.* **1986**, *27*, 427.

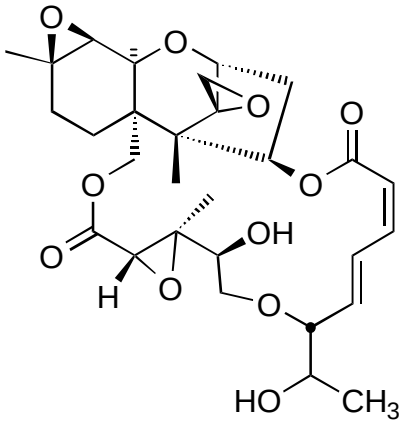
In the second structural group, the macrocyclic trichothecenes, a di- or tri-lactide ribbon bridges the hydroxy groups at C-4 and C-15. Verrucarol is most often the sesquiterpene onto which the macrocycle is attached. Examples in this class are: verrucarin-A, roridin-A, verrucarin K[15] and the baccharins (which were discussed earlier). Verrucarin K was the first trichothecene isolated that lacks the 12,13-epoxide unit.



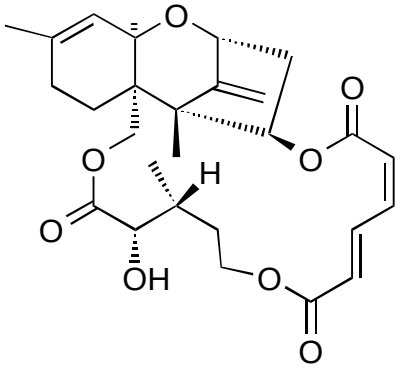
Verrucarin A



Roridin A



Baccharin B5(13'-R)

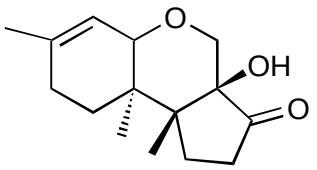


Verrucarin K

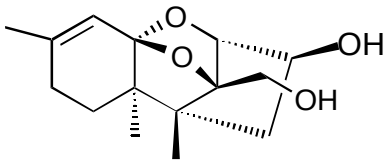
The last group of trichothecenes is the trichoverroids. These *seco* macrocyclic metabolites have either partial or complete carbon chains at C-4 and C-15 characteristic of

[15] Breitenstein, W.; Tamm, C. *Helv. Chim. Acta* **1977**, *60*, 1522.

the macrocyclic compounds but lack the requisite ring forming bonds. Other trichothecenes that cannot be classified into any of the above categories include Sambucoin and Sambucinol.[16]



Sambucoin



Sambucinol

3. Biological Activity

All animal species that have been tested appear to be sensitive to trichothecene toxins. A wide variety of biological activities have been ascribed to the Trichothecene mycotoxins.[17,18,19] These include alimentary toxic aleukia,[20] vomiting, weight loss, skin inflammation, anorexia, inflammation of the gastrointestinal tract, hypo-tension, anemia, diarrhea, ataxia, hematuria, leukopenia, lymphoid necrosis, immunosuppression, hemorrhage, emesis, degeneration of nerve cells in the central nervous system, degeneration and hemorrhaging of cardiac muscle, lymph nodes, testis and thymus, feed

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[16] Mohr, P.; Tamm, C.; Zucher, W.; Zehnder, M. *Helv. Chim. Acta* **1984**, *67*, 406.

[17] Corley, D. G.; Rottinghaus, G. E.; Tempesta, M. S. *J. Org. Chem.* **1987**, *52*, 4405.

[18] Oltz, E. M.; Nakanishi, K.; Yagen, B.; Corley, D. G.; Rottinghaus, G. E.; Tempesta, M. S. *Tetrahedron* **1986**, *42*, 2615.

[19] Cole, R. J.; Cox, R. H. In *Handbook of Toxic Fungal Metabolites*; Academic Press: New York, **1981**; Vol. 5, p 152.

[20] Since the number of white blood cells of the patients decreased markedly, the diagnostic name ATA (alimentary toxic aleukia) was given to the disease.

refusal and death in humans and farm animals from ingestion of infected grains. There is evidence that they are teratogenic but not carcinogenic.[13]

An extensively studied and potentially important property of the trichothecenes is their cytostatic activity. In 1962, Harri[11] and coworkers found that verrucarin-A caused 50% inhibition of mouse tumor cell *P-815* growth at a concentration of 0.6 ng/mL, making it one of the most active cytostatic agents known. This activity is not limited to the macrocyclic trichothecenes, as anguidine has exhibited cytopathogenic effects against baby hamster kidney cells at a concentration of 1.5 ng/mL. In fact, the majority of the trichothecenes have been shown to possess *in vitro* cytotoxic activity. Anguidine has completed Phase II in clinical trials against colon and breast cancer conducted by the National Cancer Institute.

The trichothecene mycotoxins induce cellular damage characterized by karyorrhexis[21] and destruction of the actively dividing cells in the thymus, testis, intestines, spleen and others. These observations led scientists to believe that this type of mycotoxin interferes with the synthesis of biomolecules. Comparative toxicology with various kinds of trichothecenes has revealed that all the tested compounds inhibit protein[22] and DNA synthesis, in whole cell and in cell-free systems.[3, 23] No inhibitory effect was observed on bacterial replication. Inhibition[24] is believed to be caused by the binding of trichothecene

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[21] Karyorrhexis means the destruction of the nucleus.

[22] Inhibition of protein synthesis in humans was verified in experiments using HeLa cells as well as cell-free systems. The cell-free system is more sensitive than the whole cell, therefore it is suggested that trichothecenes have some effect on cell membrane permeability.

[23] Ueno, Y.; Hosoya, M.; Ishikawa, Y. *J. Biochem. (Tokyo)* **1969**, *66*, 419.

[24] Welch, S. C.; Rao, A. S. C. P.; Gibbs, C. G.; Wong, R. Y. *J. Org. Chem.* **1980**, *45*, 4077.

to polysomes and ribosomes (80 S) from eukaryotic cells,[25] followed by inactivation of protein translation. The trichothecenes affect protein synthesis by interfering with the active site of peptidyl transferase on ribosomes[19] and consequently inhibit the initiation, elongation or termination steps of protein synthesis.

Trichothecenes have been shown to inhibit DNA synthesis in Ehrlich ascited tumor cells[3], but the mechanism of this inhibition is not known. No inhibitory effect on RNA transcription was observed.

The molecular basis of the biological activity for these compounds is still unclear. Ueno has presented evidence that the trichothecenes react with thiol residues at the peptidyl transferase active site in the ribosome. Grove[26] suggested that the 12,13-epoxide in the trichothecane framework might serve as the electrophylic site responsible for this reactivity.[27] Other structure-activity relationship studies demonstrated that the

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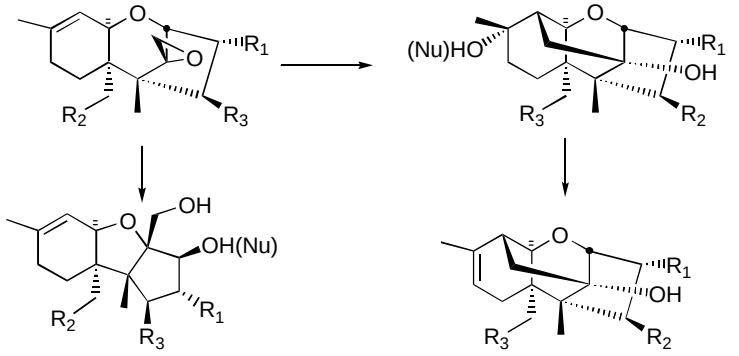
[25] No detectable binding of the trichothecenes with the individual 30S or 50S sub-units of the ribosome was observed.

[26] Grove, J. F. *J. Chem. Soc. Perkin Trans. I* **1985**, 1731.

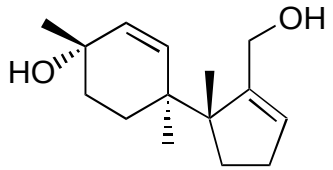
[27] This assumption is not completely supported since it is difficult to reconcile it with the established low reactivity of the 12,13-epoxide unit under non-acidic SN_2 conditions. Normally, the epoxide is protected from rear side nucleophilic attack by ring A and by rigid oxabicyclo[3.2.1]octane system rings B/C so the reactivity of these trichothecenes depends on the ease of generation of ionic centers which can participate *via* an intramolecular attack on the epoxide, this process is frequently accompanied by skeletal rearrangement.



saponification of the esters as well as the hydrogenation of the 9-10 olefin leads to a decrease in toxicological activity.[3]

- *FS-2*

In 1987, Tempesta[17] isolated two new secondary metabolites from fermentation of *Fusarium sporotrichioides* (MC - 72083): FS-2 and trichotriol. FS-2 was isolated in 30 ppb (2 mg) from culture on corn medium at 10 ^{o}C, after incubation in the dark for 21 days. Its structure was elucidated with the aid of high-resolution mass spectroscopy, UV, ^{1}H-NMR (COSY and difference NOE experiments) and ^{13}C-NMR. Its absolute stereochemistry was assigned based on analogy with other trichothecenes.



FS-2

FS-2 was tested for toxicity using the chicken-egg-yolk-sac inoculation bioassay and the results indicated an LD_{50} of 55 ng/egg, similar to T2-toxin embryotoxicity. This suggests that the tetrahydropyran ring (absent in this case) may play only a minor role for toxicity in most trichothecenes.

FS-2, and other closely related compounds recently isolated,[17, 28] represent important clues for the elucidation of the biosynthetic pathway of the trichothecenes and thus presents itself as an interesting target for synthetic organic chemists.

The structure of FS-2 is unusual among the trichothecenes because it lacks the oxygen bridge between C-2 and C-11 (trichothecene numbering, structure section of this chapter) that forms ring B. The absence of ring B increases the flexibility of the molecule and makes the task of controlling the stereochemistry of its substituents during synthesis more difficult, especially for the two adjacent quaternary centers at positions C-5 and C-6 (trichothecene numbering).

[28] Other natural products bearing similar structures include trichodiol, trichotriol and FS-4.

- *Previous synthetic approaches:*

1. General:

Extensive research has been conducted towards the synthesis of the trichothecene family of sesquiterpenes culminating in the total synthesis of trichodermin[29], 12,13-epoxytrichothec-9-ene,[30] verrucarol,[31] trichodermol,[32] calonectrin,[33] trichodiene,[34] anguidine[35] and sporol.[36]

The synthesis of trichothecenes was originally divided into four main synthetic approaches based on the final development of the tricyclic skeleton, according to

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[29] Colvin, E. W.; Malchenko, S.; Raphael, R. A.; Roberts, J. S. *J. Chem. Soc. Perkin Trans. I* **1973**, 1989.

[30] (a) Fujimoto, Y.; Yokura, S.; Nakamura, T.; Morikawa, T.; Tatsuno, T. *Tetrahedron Lett.* **1974**, 2523; (b) Masuoka, N.; Kamikawa, T. *Tetrahedron Lett.* **1976**, 1691; (c) Masuoka, N.; Kanikawa, T.; Kubota, T. *Chem. Lett.* **1974**, 751; (d) Hua, D. H.; Venkataraman, S.; Chan-Yu-King, R.; Paukestelis, J. V . *J. Am. Chem. Soc.* **1988**, *110*, 4741; (e) Hua, D. H.; Venkataraman, S.; Coulter, M. J.; Sinai-Zingde, G. *J. Org. Chem.* **1987**, *52*, 719; (f) Pearson, A. J.; O'Brien, M. K. *J. Org. Chem.* **1989**, *54*, 4663.

[31] (a) Trost, B. M.; Rigby, J. H. *J. Org. Chem.* **1978**, *43*, 2938; (b) White, J. D.; Matsui, T.; Thomas, J. A. *J. Org. Chem.* **1981**, *46*, 3376; (c) Roush, W. R.; D'Ambra, T. E. *J. Am. Chem. Soc.* **1983**, *105*, 1058; (d) Trost, B. M.; McDougal, P. G.; Haller, K. J. *J. Am. Chem. Soc.* **1984**, *106*, 383; (e) Koreeda, M.; Ricca, D. J.; Luengo, J. I. *J. Org. Chem.* **1988**, *53*, 5586; (f) White, J. D.; Kim, N.-S.; Hill, D. E.; Thomas, J. A. *Synthesis - Stuttgard* **1998**, 619; (g) Ishihara, J.; Nonaka, R.; Terasawa, Y.; Shiraki, R.; Yabu, K.; Kataoka, H.; Ochiai, Y.; Tadano, K.-I. *Tetrahedron Lett.* **1998**, *38*, 8311; (h) Ishihara, J.; Nonaka, R.; Terasawa, Y.; Shiraki, R.; Yabu, K.; Kataoka, H.; Ochiai, Y.; Tadano, K. *J. Org. Chem.* **1998**, *63*, 2679.

[32] Still, W. C.; Tsai, M.-Y. *J. Am. Chem. Soc.* **1980**, *102*, 3654.

[33] Kraus, G. A.; Roth, B.; Frazier, K.; Shimagaki, M. *J. Am. Chem. Soc.* **1982**, *104*, 1116.

[34] (a) Suda, M. *Tetrahedron Lett.* **1982**, *23*, 427; (b) Schlessinger, R. H.; Schultz, J. A. *J. Org. Chem.* **1983**, *48*, 407; (c) Snowden, R. J.; Sonnay, P. *J. Org. Chem.* **1984**, *49*, 1464; (d) Harding, K. E.; Clement, K. S. *J. Org. Chem.* **1984**, *49*, 3870; (e) Gilbert, J. C.; Wiechman, B. E. *J. Org. Chem.* **1986**, *51*, 258; (f) Gilbert, J. C.; Kelly, T. A. *J. Org. Chem.* **1986**, *51*, 4485; (g) Van Middlesworth, F. L. *J. Org. Chem.* **1986**, *51*, 5019; (h) Kraus, G. A.; Thomas, P. J. *J. Org. Chem.* **1986**, *51*, 503; (i) Harding, K. E.; Clement, K. S.; Tseng, C.-Y. *J. Org. Chem.* **1990**, *55*, 4403; (j) Tanaka, M.; Sakai, K. *Tetrahedron Lett.* **1991**, *32*, 5581; (k) Gilbert, J. C.; Selliah, R. D. *J. Org. Chem.* **1993**, *58*, 6255; (l) Lemieux, R. M.; Meyers, A. I. *J. Am. Chem. Soc.* **1998**, *120*, 5453.

[35] (a) Brooks, D. W.; Grothaus, P. G.; Mazdiyasni, H. *J. Am. Chem. Soc.* **1983**, *105*, 4472; (b) Brooks, D. W.; Grothaus, P. G.; Palmer, J. T. *Tetrahedron Lett.* **1982**, *23*, 4187.

McDougal and Schmuff,[6] as shown in Figure 1.1. Two approaches involved formation of the C ring via an intramolecular aldol reaction (paths a and b). The other two involved the formation of the B ring in a similar manner to the model proposed for the biosynthesis of the trichothecene family (paths c and d) and are referred to as the biomimetic approach.

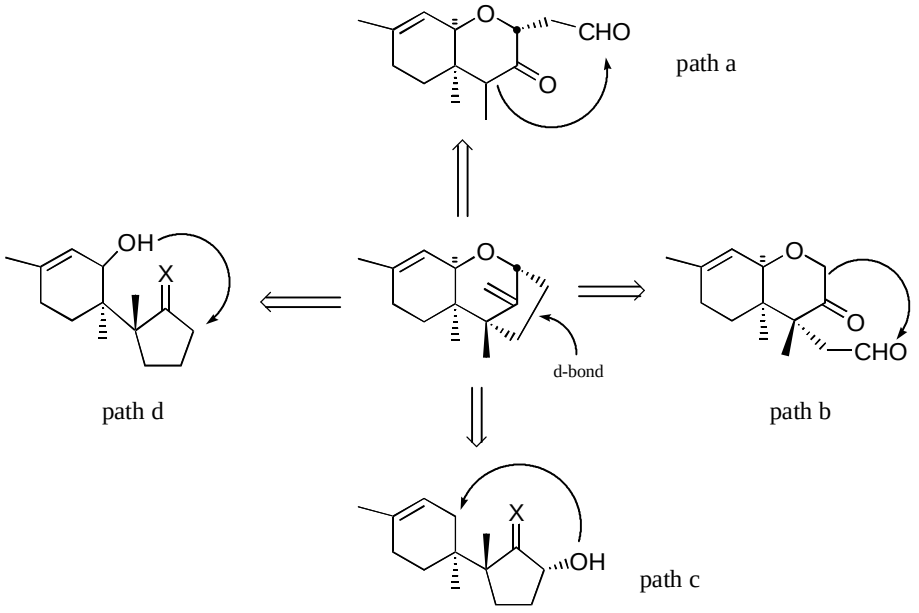

Figure 1.1

2. Aldol approach

The first trichothecene total synthesis was effected by Raphael[29,37] in 1973. He successfully prepared racemic trichodermin following the path-a aldol disconnection (see Figure 1.2).

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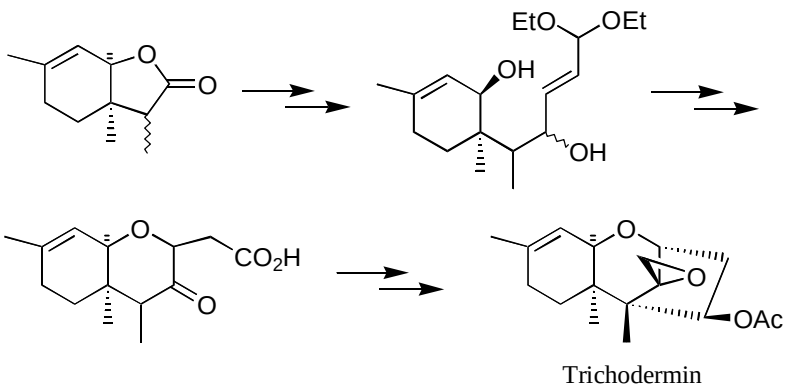
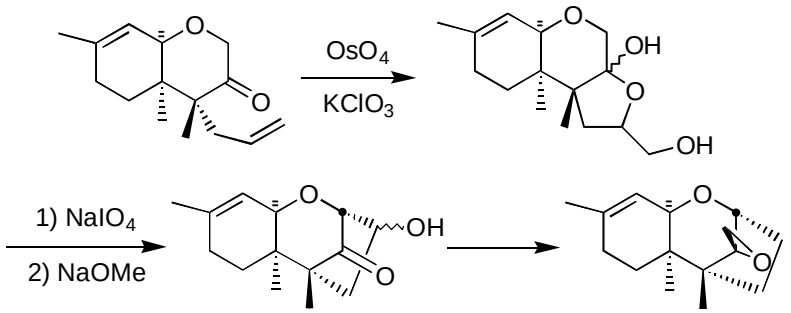
[36] Ziegler, F. E.; Metcalf III, C. A.; Schulte, G. *Tetrahedron Lett.* **1992**, *33*, 3117.



Trichodermin

Figure 1.2

The path-b aldol disconnection was employed successfully in the synthesis of calonectrin by Kraus,[33] T-2 tetraol by Colvin[38] and the preparation of 12,13-epoxytrichothec-9-ene by Fujimoto.[30a] Figure 1.3 illustrates the path-b aldol disconnection for Fujimoto's synthesis of 12,13-epoxytrichothec-9-ene.



12,13-epoxy-
trichothec-9-ene

Figure 1.3

[37] Colvin, E. W.; Malchenko, S.; Raphael, A.; Roberts, J. S. *J. Chem. Soc. Perkin Trans. I* **1978**, 658.
[38] Colvin, E. W.; Egan, M. J.; Kerr, F. W. *J. Chem. Soc. Chem. Comm.* **1990**, 1200.

3. Biomimetic approach:

Many synthetic endeavors have followed the path-c biomimetic strategy, including Masuoka[30b-c] with the synthesis of 12,13-trichothec-9-ene, the application of chiral sulfoxides developed by Hua,[30d-e] the synthesis of enantiomerically pure anguidine by Brooks[35] and the preparation of an advanced intermediate towards the synthesis of anguidine by Ziegler.[39] Still[32] utilized the path-c biomimetic approach in his synthesis of trichodermol as shown in Figure 1.4. The synthesis starts with a Diels-Alder reaction followed by the Favorskii rearrangement and anionic fragmentation to prepare rings A and C and set the relative stereochemistry of the two quaternary centers.

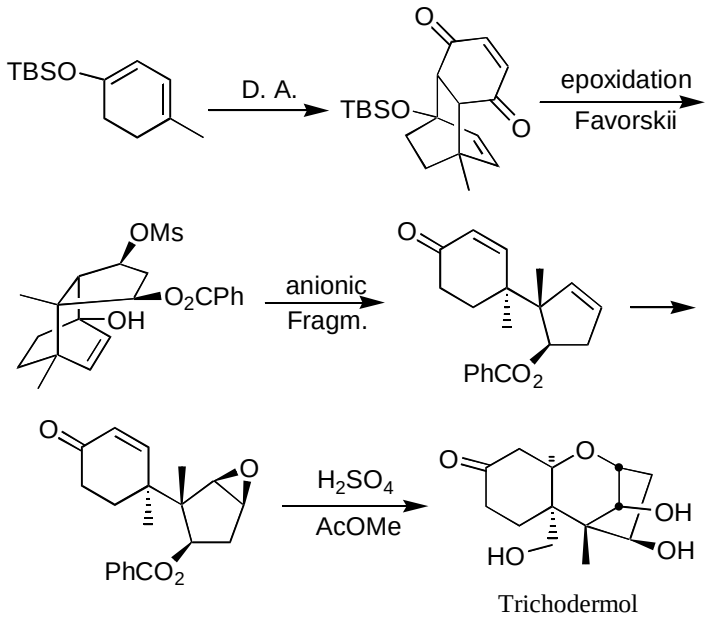


Figure 1.4

[39] Ziegler, F. E.; Sobolov, S. B. *J. Am. Chem. Soc.* **1990**, *112*, 2749.

The path-d biomimetic approach involves the attack of ring C by a nucleophilic oxygen substituent in ring A. This approach proved to be effective for Pearson's[30f,40] synthesis of trichodermol and Roush's[31c,41] synthesis of verrucarol. Anderson[42] was studying the entry to the trichothecene ring system while looking at compounds with an aromatic ring A and Nemoto[43] developed the aromatic ring A strategy further by elaborating the tricyclic skeletons into nivalenol-type compounds. Nemoto's approach (see Figure 1.5) was based on the elaboration of a ketone into a cyclobutanone and then an allylic cyclobutene that could undergo Palladium mediated regiocontrolled ring expansion to form ring C. A path-d biomimetic cyclization finalized the assembly of the skeleton.

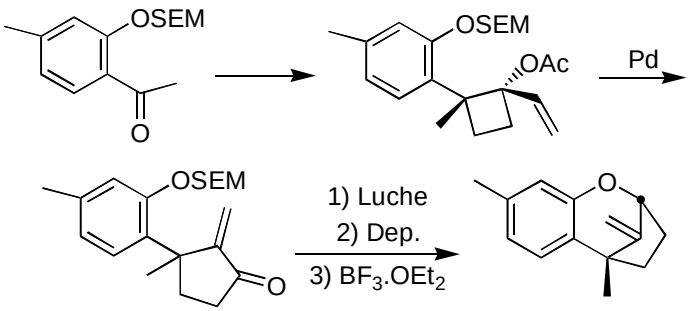


Figure 1.5

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[40] (a) Pearson, A. J.; O'Brien, M. K. *J. Chem. Soc. Chem. Comm.* **1987**, 1445; (b) O'Brien, M. K.; Pearson, A. J.; Pinkerton, A. A.; Schimidt, W.; Willman, K. *J. Am. Chem. Soc.* **1989**, *111*, 1499.

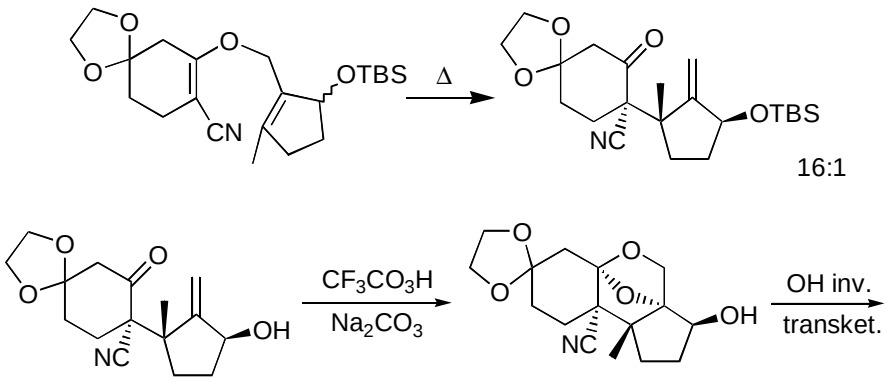
[41] (a) Roush, W. R.; D'Ambra, T. E. *J. Org. Chem.* **1981**, *46*, 5045; (b) Roush, W. R.; D'Ambra, T. E. *J. Org. Chem.* **1980**, *45*, 3927.

[42] Anderson, W. K.; La Voie, E. J.; Lee, G. E. *J. Org. Chem.* **1977**, *42*, 1045.

[43] (a) Nemoto, H.; Miyata, J.; Fukumoto, K. *Heterocycles* **1997**, *44*, 125; (b) Nemoto, H.; Miyata, J.; Fukumoto, K. *Tetrahedron* **1996**, *52*, 10363; (c) Nemoto, H.; Miyata, J.; Fukumoto, K. *Heterocycles* **1996**, *42*, 165.

4. Other approaches:

Some synthetic approaches developed for the assembly of trichothecenes don't fall into any of the above disconnection categories. Fraser-Reid[44] developed a chiral synthetic entry into the tricyclic skeleton starting from D-glucose for preparation of the A/B ring system followed by formation of the C ring via the "d-bond" closure (see Figure 1.1). In the synthesis of neosporol[45] (see Figure 1.6), a Claisen rearrangement set the stereochemistry of the two quaternary centers. The placement of the double bonds in the A and C rings solved previous problems involving mixtures of E and Z olefin geometries that resulted in low selectivity. The methodology developed for the assembly of the trichothecene skeleton involved the epoxidation of the allylic alcohol followed by intramolecular ketal formation and inversion of the alcohol to prompt transketalization. This methodology was applied successfully again for the synthesis of Sporol.[36]



[44] Tsang, R.; Fraser-Reid, B. *J. Org. Chem.* **1985**, *50*, 4659.
[45] Ziegler, F. E.; Nangia, A.; Schulte, G. Tetrahedron *Lett.* **1988**, *29*, 1669.

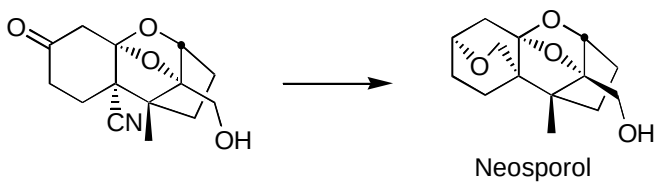


Figure 1.6

Trost[31d,46] accomplished the synthesis of verrucarol shown in Figure 1.7 by use of a tandem Diels-Alder-ene reaction to set the adjacent quaternary centers followed by a retro-ene and a ring expansion/fragmentation reaction to assemble the final skeleton.

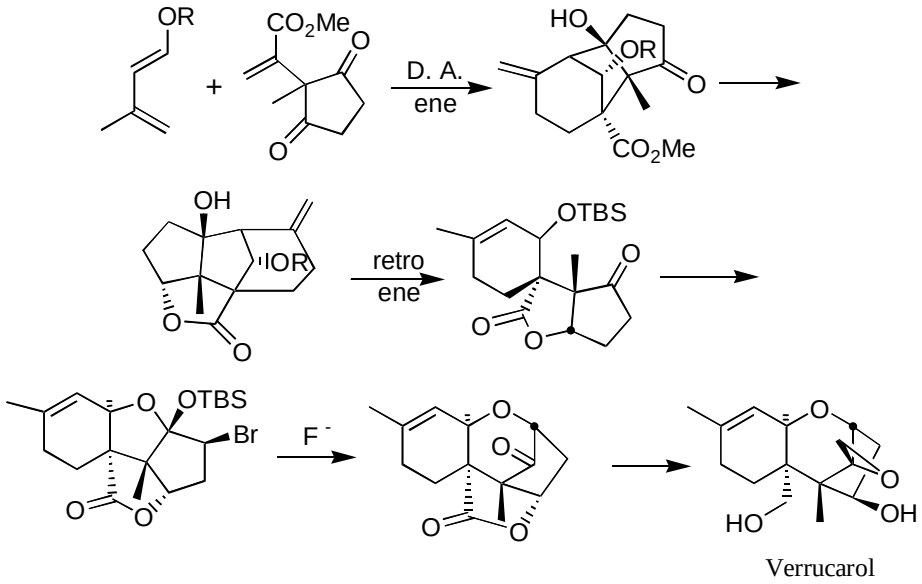


Figure 1.7

Assembly of Ring A via an intramolecular Diels-Alder reaction during flash chromatography in neutral alumina was the final bond connection employed by Koreeda[31e]

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[46] Trost, B. M.; McDougal, P. G. *J. Am. Chem. Soc.* **1982**, *104*, 6110.

on his formal synthesis of verrucarol (see Figure 1.8). This synthesis merges with Trost's lactone advanced intermediate shown in Figure 1.7.

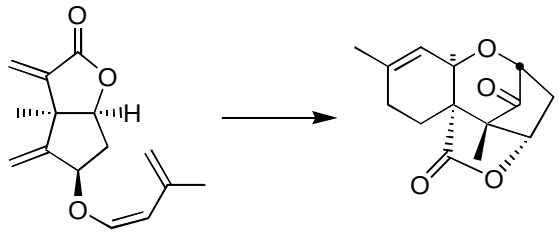
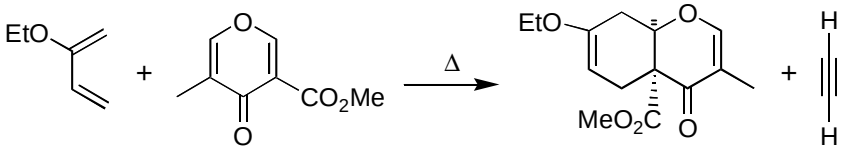


Figure 1.8

White's approach[31b,f,47] for the formal synthesis of Verrucarol took advantage of the Cargill rearrangement (see Figure 1.9). The strategy involved formation of the A/B ring system via a Diels-Alder reaction and introduction of the C ring in the form of a cyclobutene via photochemical reaction with acetylene. Acid catalyzed ring expansion proceeded via cyclobutene fragmentation to give a 6-5-5 tricyclic skeleton. Palladium catalyzed skeleton rearrangement assembled the trichothecene skeleton. The final product is the same lactone previously prepared by Trost[31d] in his synthesis of Verrucarol (Figure 1.7).



[47] Fetizon, M.; Khac, D. D.; Tho, N. D. *Tetrahedron Lett.* **1986**, *26*, 1777.

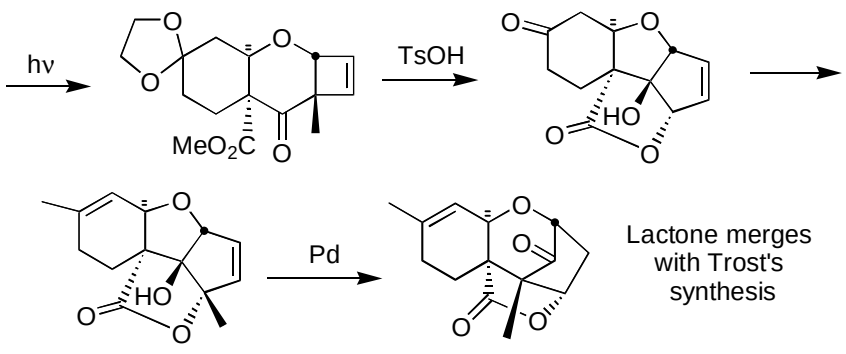
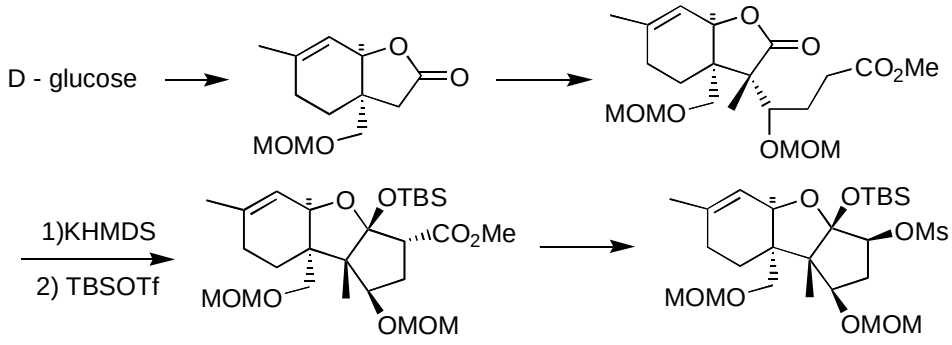
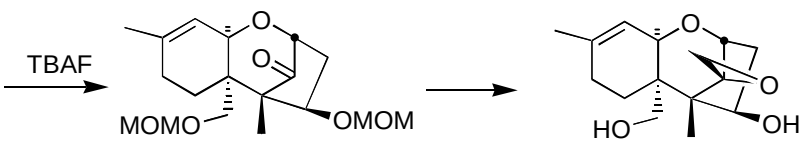


Figure 1.9

Ishihara[31g-h,48] was responsible for the first preparation of Verrucarol in optically active form (Figure 1.10). Starting from D-glucose, elaboration of ring A took advantage of Trost's recognition that the *cis* stereochemistry between rings A and B could be better controlled *via* the formation of a 6-5 ring system. Stereoselective quaternization α to the carbonyl of the lactone took advantage of the stereochemistry bias of the bicyclic molecule. Elaboration of ring C followed via a Dieckmann cyclization, Barton-Crich decarboxylative oxygenation and ring expansion (also developed by Trost, see Figure 1.7).



[48] Ishihara, J.; Nonaka, R.; Terasawa, Y.; Tadano, K.-i.; Ogawa, S. *Tetrahedron Assym.* **1994**, *5*, 2217.



Verrucarol

Figure 1.10

5. Optically active synthesis

Other optically active synthesis of trichothecenes were the synthesis of 12,13-epoxytrichothec-9-ene employing chiral sulfoxides by Hua,[30d-e] an approach towards the synthesis of Anguidine by Enholm[49] starting from the readily available L-(+)-arabinose. Two independent chiral syntheses of trichodiene were accomplished. One by Gilbert,[50] taking advantage of Bakers' yeast reduction of a ketone in 45% ee, and one by Meyers[34l] (see Figure 1.11). Meyers used a chiral auxiliary derived from an amino acid to set the two quaternary centers in trichodiene via a thio-Claisen rearrangement.

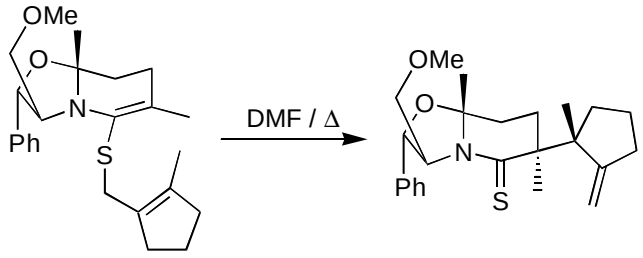


Figure 1.11

[49] Enholm, E. J.; Satici, H.; Trivellas, A. *J. Org. Chem.* **1989**, *54*, 5841.
[50] Gilbert, J. C.; Kelly, T. A. *Tetrahedron Lett.* **1989**, *30*, 4193.

6. Quaternary centers:

A series of syntheses of trichodiene addressed the issue of construction of the two adjacent quaternary centers. These include the use of the Nazarov[34d,i] reaction to introduce the stereochemistry in an electrocyclic fashion. A series of Claisen[51] approaches including the ester enolate Ireland-Claisen[52] adaptation was investigated in detail. The low selectivity of this rearrangement was attributed to the lack of selectivity of E/Z geometry at the double bonds. This problem was solved by incorporating the olefins into rings for the Claisen reaction to achieve selectivity as high as 16:1.[45] Another solution was found by use of chelate controlled Ireland ester enolate Claisen to get as high as 92:8 selectivity, such as Gilbert's synthesis of optically active (-) trichodiene in 1993 (see Figure 1.12).

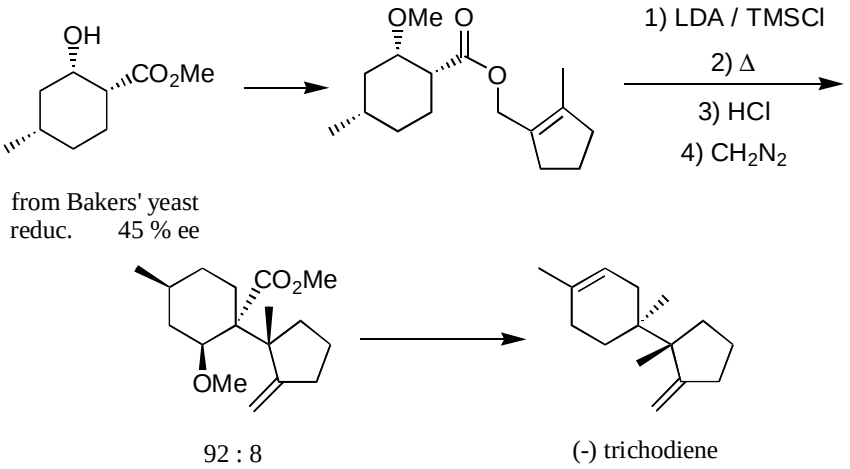


Figure 1.12

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[51] Ziegler, F. E.; Nangia, A.; Schulte, G. *J. Am. Chem. Soc.* **1987**, *109*, 3987; also see references 34a, 34e, 36 and 48.

[52] (a) Gilbert, J. C.; Selliah, R. D. *Tetrahedron Lett.* **1992**, *33*, 6259; (b) Gilbert, J. C.; Selliah, R. D. *Tetrahedron* **1994**, *50*, 1651; also see references 34f,g,h,k, and ref. 55.

An organometallic reaction involving tin enolates coupling to cyclohexadienyl iron complexes[40] posed as a potential convergent entry to the system as was shown by Pearson in his syntheses of trichodiene[40b] (see Figure 1.13) and trichodermol.[40c]

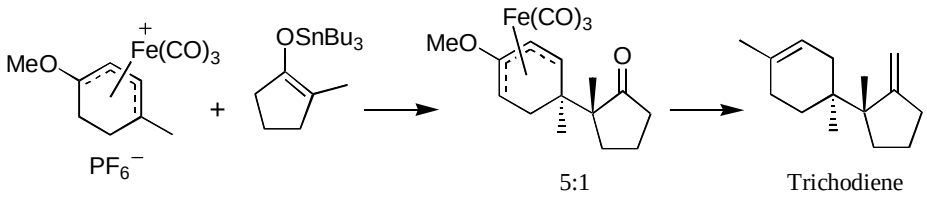

Figure 1.13

The application of a Prins reaction[39] to establish the relative stereochemistry between the two adjacent quaternary centers was showcased in an attempt to prepare anguidine (see Figure 1.14).

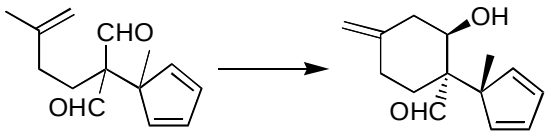

Figure 1.14

A [2+2] intermolecular photoaddition[53] was attempted without much success resulting in a mixture of regioisomers at about 1:1 ratio. A thio-Claisen rearrangement,[34l] aided by a chiral auxiliary, prepared the optically active bond connection. A novel ring transformation involving aldol acetylation and Grob fragmentation was developed by

Tanaka[34js] in a synthesis of trichodiene starting from a prostaglandin type intermediate and is illustrated in Figure 1.15.

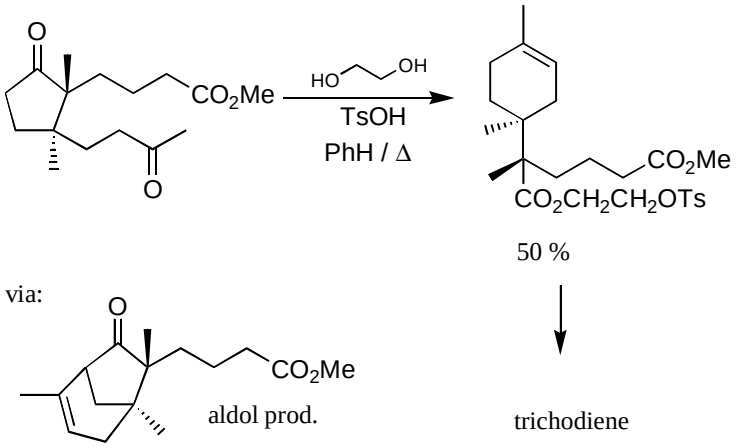

Figure 1.15

For other isolated solutions, an extensive review in the subject has been written by Martin[54] and should be consulted.

7. Interconversion of trichothecenes:

Some attention has been focused on developing the more complicated trichothecenes from readily available intermediates deriving from natural products, such as anguidine and verrucarol. Anguidine can be obtained in 250 to 300 mg/mL from aerated

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[53] (a) Yamakawa, K.; Sakaguchi, R.; Nakamura, T.; Watanabe, K. *Chem. Lett.* **1976**, 991; (b) Yamakawa, K.; Kurita, J.; Sakaguchi, R. *Tetrahedron Lett.* **1973**, 3877.

[54] Martin, S. F. *Tetrahedron* **1980**, *36*, 419.

culture of *fusarium* fungi in pure crystalline form.[55] It has been used as the starting material for the interconversion of trichothecenes because C-15 and C-4 hydroxyl groups are protected as acetate groups while the C-3 is a free hydroxyl. This approach has been the focus of the research of Tamm,[56] Colvin[57] and Wani[58] and has resulted in the synthesis of Verrucarin A, Calonectrin, HT-2 toxin, Neosolaniol, T-2 toxin, Sporotrichiol, Deoxynivalenol (Vomitoxin), Verrucinol and Verrucene. One asymmetrical synthesis of the dilactide ribbon involved in the macrocyclic trichothecenes was reported by Tamm[59] and it involved a Sharpless epoxidation as the source of asymmetry.

- *Retrosynthetic analysis:*

The synthesis of FS-2 was envisioned to start by construction of a rigid structure with well-defined concave and convex faces followed by introduction of the necessary functionality *via* face selective reactions. The desired trichothecene skeleton would be unraveled at the end, with the application of a radical fragmentation methodology under investigation in our laboratory. This methodology offered the possibility of control of the regiochemistry of the fragmentation of a six-membered cyclic thionocarbonate ring fused to a five-membered ring by manipulation of the relative stereochemistry at the ring

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[55] Brian, P. W.; Dawkwns, A. W.; Grove, J. F.; Hemming, A. G.; Lowe, D. *J. Exp. Bot.* **1961**, *12*, 1.

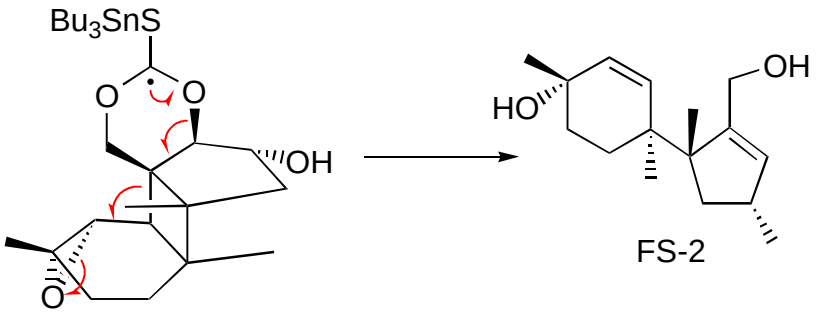
[56] (a) Mohr, P.; Tori, M.; Grossen, P.; Herold, P.; Tamm, C. *Helv. Chim. Acta* **1982**, *65*, 1412; (b) Jeker, N.; Mohr, P.; Tamm, C. *Tetrahedron Lett.* **1984**, *25*, 5637; (c) Jeker, N.; Tamm, C. *Helv. Chim. Acta* **1988**, *71*, 1904; (d) Jerker, N.; Tamm, C. *Helv. Chim. Acta* **1988**, *71*, 1895.

[57] (a) Colvin, E. W.; Cameron, S. *J. Chem. Soc. Chem. Comm.* **1986**, 1642; (b) Colvin, E. W.; Cameron, S. *Tetrahedron Lett.* **1988**, *29*, 493; (c) Cameron, S.; Colvin, E. W. *J. Chem. Soc. Perkin Trans. I* **1989**, 887.

[58] Wani, M. C.; Rector, H.; Cook, C. E. *J. Org. Chem.* **1987**, *52*, 3468.

junction. The radical methodology tolerates a wide array of functionality in the molecule at the time of the fragmentation and offers an entry to the synthesis of trichothecenes of the FS-2 type skeleton and to the normal trichothecene skeleton, making this a general approach to the family.

The radical fragmentation *per se* will be discussed in more detail in chapter three, along with the discussion of results obtained during the study of the fragmentation of some model systems. The transformation proposed is depicted in the scheme below.



The radical fragmentation methodology required the preparation of cyclic thionocarbonate **1** (see Figure 1.16). Preparation of this intermediate could be derived from the epoxidation of the parent olefin and selective cyclization of the 1,3 diol. This strategy assumes that reaction with the primary alcohol would be faster than reaction with the secondary alcohol and that formation of the six-membered ring would be favored over the seven-membered ring alternative. Intermediate **2** was the logical precursor and could be prepared from ketone **3** by stereoselective introduction of a hydroxyl at C-3 (trichothecene numbering) followed by reduction of the ketone and the allylic ether.

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[59] Herold, P.; Mohr, P.; Tamm, C. *Helv. Chim. Acta* **1983**, *66*, 744.

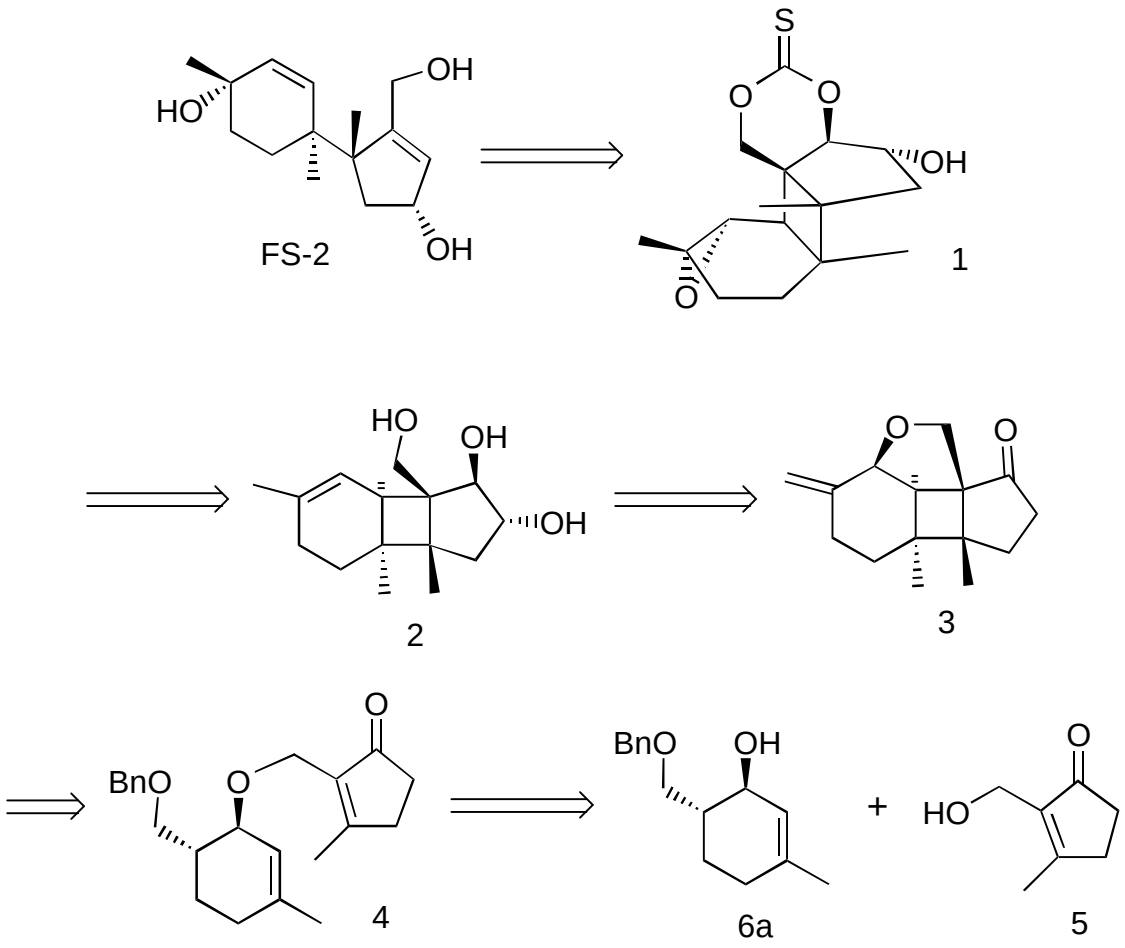
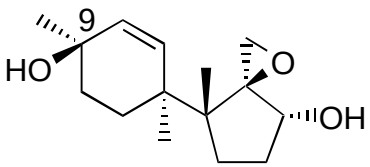


Figure 1.16 : Retrosynthetic Analysis

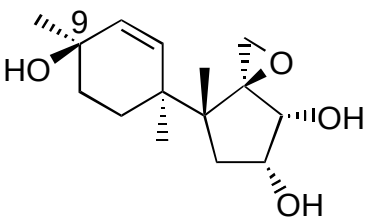
Preparation of the cyclobutane and control of the stereochemistry of the two emerging quaternary centers in ketone **3** could be accomplished through the photoaddition of substrate **4** or an analog. Synthesis of the requisite five- and six-membered ring precursors could be achieved from commercially available material.

- *Revised Structure:*

In 1993, Gilbert[60] published a proposed revision for the structure of FS-2 in which the hydroxyl at C-9 was reassigned as 9-β hydroxy (R) contrary to the original configuration assignment of 9-α (S).[17] This change was a result of research conducted on two molecules, trichodiol and trichotriol, which are structurally related to FS-2.



Trichodiol



Trichotriol

Gilbert's assignments for the stereochemistry of trichodiol and its triol partner were confirmed by through-bond NMR, ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY. The reassignment of the stereochemistry of FS-2 and other naturally occurring products is based solely on the comparative analysis of Gilbert's ^{13}C NMR of trichodiol and trichotriol, which were recorded in $CDCl_3$, and the previously published values[17] of the chemical shifts for FS-2, which were recorded in acetone-d_6. In Gilbert's examples, C-9 bearing an α-OH is consistently at 69 ppm, and the β-OH counterpart appears between 65 and 66 ppm. It should be noted that the reassignment of FS-2 is based on this comparison of the 3-4 ppm difference in the ^{13}C NMR chemical shift data for C-9, collected in two different solvents, in different laboratories.

Computational conformation studies, using Monte Carlo simulations,[61] of both the originally reported configuration (Tempesta) and the reassigned structure (Gilbert) were carried out in order to clarify the structure of FS-2 by comparing the low energy structures for both molecules against the available NMR data. The details of the experiment and the explicit results are presented in Addendum III. The Tempesta configuration can adopt six distinct conformations within 4 Kcal/mol and the Gilbert configuration offers the possibility for a total of nine different conformations within 4 Kcal/mol. The large number of possible conformations within a narrow range of energy for each configuration makes it difficult to choose one conformation based on thermodynamics alone.

The fifteen conformations generated by the Monte Carlo simulations were analyzed against the difference NOE and COSY results reported by Tempesta.[17] The magnitude of the NOEs were not reported in the original paper, which suggested that only strong NOEs (within 3Å) were considered. Because the different conformations are very close in energy, one possibility was that the NOE and coupling constants observed actually represent a fast exchange time averaged structure in solution. That possibility is not likely since the coupling constants reported reflect specific angles encountered in some of the conformations and not an intermediate value.

Analysis of the differential NOE results reported against the calculated structures for the Tempesta and the Gilbert series suggests that conformation #3 of the Tempesta

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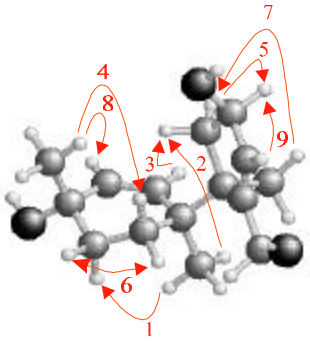
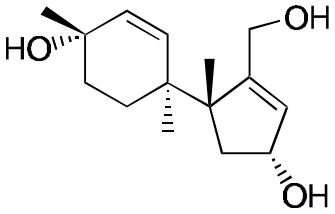
[60] Hesketh, A. R.; Gledhill, L.; Bycroft, B. W.; Dewick, P. M.; Gilbert, J. *Phytochemistry* **1993**, *32*, 93 - 104.

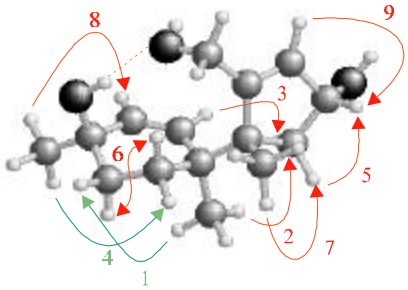
[61] See Addendum III for details on the Monte Carlo simulations.

series is the one that best fits the data, presenting no NOE violations and satisfying the available coupling constant data. Shown below is a 3-dimensional view of Tempesta's conformation #3 with the NOEs reported in Tempesta's paper marked in red. Following that, conformation #1 of the Gilbert configuration is also shown with the same NOEs drawn for comparison.

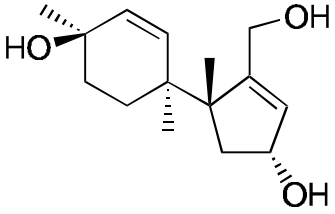


FS-2
Tempesta





FS-2
Gilbert



NOE #	Tempesta (Å)	Gilbert (Å)
1	2.142	3.755
2	2.220	2.239
3	2.246	2.174
4	2.375	4.842
5	2.475	2.461
6	2.481	3.072
7	2.577	2.556
8	2.691	2.562
9	2.744	2.733

The NMR data, along with the Monte Carlo simulations results support the structure assignment published by Tempesta, but this is still not conclusive data to assign the relative stereochemistry of FS-2. The decision to proceed with the original plan of synthesizing the α-hydroxy FS-2 (9-S configuration, Tempesta's original proposal) now had the added intention of establishing the stereochemistry of the hydroxyl at position C-9 for the natural product.