The metabolites of *Trichoderma longibrachiatum*. Part 1. Isolation of the metabolites and the structure of trichodimerol[1]

ROMANO ANDRADE, WILLIAM A. AYER,[2] AND PAUL P. MEBE
Department of Chemistry, University of Alberta, Edmonton, Alta., Canada T6G 2G2

Received March 31, 1992

ROMANO ANDRADE, WILLIAM A. AYER, and PAUL P. MEBE. Can. J. Chem. **70**, 2526 (1992).

Trichoderma longibrachiatum Rifai aggr. is a fungus reported to be antagonistic to the fungus *Mycena citricolor*, the causative agent of the American leaf spot disease of coffee. We have investigated the metabolites produced when *T. longibrachiatum* is grown in liquid culture and have isolated the known compounds sorbicillin (**2**), bisvertinol (**3**), and bisvertinolone (**4**) as well as several new compounds including trichodimerol (**1**), an interesting new natural product possessing a proper axis of symmetry. The structure of trichodimerol (**1**) was determined by a combination of spectroscopic techniques, including ^{1}H and ^{13}C nuclear magnetic resonance, infrared, ultraviolet, circular dichroism, optical rotatory dispersion, and mass spectrometry, and by transformation to octahydrotrichodimerol (**6**) and symmetrical and unsymmetrical derivatives thereof. The relative stereochemistry of bisvertinol (**3**) and bisvertinolone (**4**) has been revised. The metabolites described are not biologically active against *Mycena citricolor*.

ROMANO ANDRADE, WILLIAM A. AYER et PAUL P. MEBE. Can. J. Chem. **70**, 2526 (1992).

Le *Trichoderma longibrachiatum* Rifai aggr. est un champignon qui serait antagoniste du champignon *Mycena citricolor*, l'agent causant des taches sur les feuilles de café américain. On a examiné les métabolites produits par culture liquide du *T. longibrachiatum* et on a identifié la sorbicilline (**2**), le bisvertinol (**3**) et la bisvertinolone (**4**), des composés connus, ainsi que plusieurs nouveaux composés, dont le trichodiméral (**1**), un nouveau produit naturel intéressant possédant un axe de symétrie propre. On a déterminé la structure du trichodiméral (**1**) par une combinaison de techniques spectroscopiques, dont la résonance magnétique nucléaire du ^{1}H et du ^{13}C, l'infrarouge, l'ultraviolet, le dichroïsme circulaire, la dispersion rotatoire optique et la spectrométrie de masse ainsi que par la transformation de **1** en octahydrotrichodiméral (**6**) et en des dérivés symétriques et asymétriques de ce dernier. On a révisé la stéréochimie relative du bisvertinol (**3**) et de la bisvertinolone (**4**). Les métabolites décrits ne sont pas biologiquement actifs contre la *Mycena citricolor*.

[Traduit par la rédaction]

Introduction

Fungi of the genus *Trichoderma* Persoon are widespread and their taxonomical classification is difficult. This has led to the development of a *species aggregate* system of classification (1), which groups together several "species". It has been noted, for example, that under the name *Trichoderma hamatum* (Bon.) Bain aggr. there may be two or three or more different but morphologically very similar species, and "that isolates considered to belong to this species aggregate may behave quite differently under different conditions" (1). It is not surprising, then, that there is no clear pattern among the wide variety of secondary metabolites produced by *Trichoderma* strains (2, 3). In recent years considerable interest has been shown in the use of *Trichoderma* species as biological control agents (4). In spite of the importance of the genus, there are no studies reported of the metabolites of strains of *Trichoderma longibrachiatum* Rifai aggr., or of other "yellow" *Trichoderma* strains, although Rifai (1) has documented the existence of yellow pigments in *Trichoderma* strains of the species aggregates *T. aureoviride* Rifai aggr., *T. longibrachiatum*, and *T. harzianum*.

Our interest in the chemistry of the metabolites produced by *T. longibrachiatum* was stimulated by its potential use as a biocontrol agent for the American leaf spot disease of coffee (ojo de gallo), caused by the fungus *Mycena citricolor* (5). When grown in agar plate co-cultures, *T. longibrachiatum* caused lysis of the hyphae of *M. citricolor*.[3]

Unfortunately the antagonistic activity sometimes observed with *T. longibrachiatum* against *M. citricolor* was not observed with the extracts obtained when *T. longibrachiatum* was grown on liquid still or shake culture. However, the isolation of some colorful and apparently structurally unique compounds from these extracts prompted us to examine the compounds in more detail.

We describe herein the isolation of several new natural products, trichodimerol (**1**), trichodermolide, sorbiquinol, bislongiquinolide, and 5-hydroxyvertinolide, along with the known compounds sorbicillin (**2**), bisvertinol (**3**), and bisvertinolone (**4**). The details for the determination of the structure of trichodimerol, a unique natural product, are described and evidence for the revision of the structures of bisvertinol and bisvertinolone is presented.

Results and discussion

Trichoderma longibrachiatum was grown in both liquid shake and liquid still culture on a medium, developed by our collaborators in Costa Rica, containing yeast extract, malt extract, glucose (or lactose), peptone, and trace elements. Extraction of either the mycelium ($CHCl_3$:CH_2Cl_2) or the broth ($CHCl_3$) provided a similar mixture of metabolites in approximately equal amounts. Attempted separation by

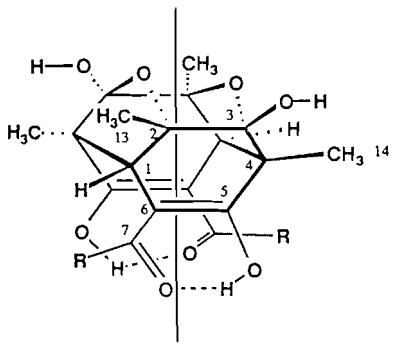
[1]This paper is dedicated to the memory of Prof. José Calzada, our collaborator and friend at the Universidad de Costa Rica, who first interested us in this problem.

[2]Author to whom correspondence may be addressed.

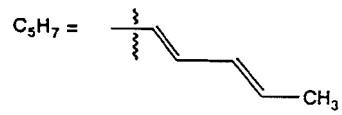
[3]Prof. E. Vargas, Facultad de Agronomia, Universidad de Costa Rica, personal communication.

chromatography over silica gel led to large losses of material and some metabolites were denatured as indicated by comparison of the ^{1}H nmr spectra of the fractions obtained using polar eluants with the ^{1}H nmr spectra of the initial mixtures. Sephadex LH20 chromatography proved to be a very useful tool in handling these silica-sensitive compounds. Sephadex LH20 chromatography of the extracts, followed by silica gel chromatography when possible, led to the isolation of the three known compounds, sorbicillin (**2**), bisvertinol (**3**), and bisvertinolone (**4**), and the five new polyketides trichodimerol (**1**), trichodermolide, sorbiquinol, bislongiquinolide, and 5-hydroxyvertinolide.

Sorbicillin (**2**), an orange crystalline compound, was first isolated as an impurity in penicillin by Cram and Tishler in 1948 (6) and chemical methods were used to determine its structure (7). It has also been isolated from *Verticillium intertextum* (8, 9) and has been synthesized (10). Compound **2** was converted by catalytic hydrogenation to tetrahydrosorbicillin, similar in all respects to the product described by Dreiding and co-workers (9).

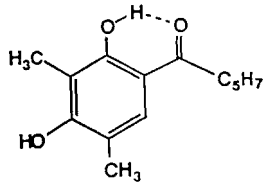


C2

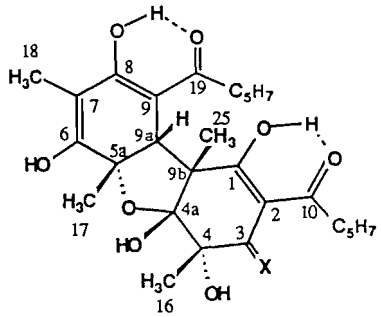


1 R = C_5H_7

6 R = *n*-C_5H_{11}



2



3 X = H_2

4 X = O

Trichodimerol (**1**) is a pale yellow, optically active, crystalline compound. Its molecular formula, $C_{28}H_{32}O_8$, was obtained from the high-resolution electron impact mass spectrum (hreims) and the molecular ion was confirmed by fast atom bombardment mass spectrometry (fabms: *m/z* 497, $M^+ + 1$, 35%). Its ^{13}C nmr spectrum shows 14 signals, indicating an element of symmetry in trichodimerol.[4] The compound gives a positive ferric chloride test and its uv maximum (λ_{max} 362 nm) shows a bathochromic shift upon addition of base (λ_{max} 378 nm), showing the acidic character of the chromophore. The presence of an enolized β-diketone is suggested by the ir (hydroxyl 3420 cm^{-1} and strongly chelated carbonyl 1613 cm^{-1}) and ^{1}H nmr spectra (enolized β-diketone hydrogen at δ 16.33 ppm).

[4]The element of symmetry present in trichodimerol must be a proper axis of symmetry, not a plane or point of symmetry, since the compound is optically active.

The ^{1}H nmr spectrum of trichodimerol (Table 1) also displays methyl singlets (δ 1.43 and 1.46 ppm), a methine singlet (δ 3.00 ppm), a hydroxyl hydrogen (δ 3.20 ppm), and signals characteristic of a sorbyl chain (δ 1.89 (d, 6.5 Hz, 3H), 6.11–6.34 (m, 3H), 7.32 (dd, 10, 15 Hz, 1H)). Further evidence of the presence of the sorbyl chain is given by the hreims, which shows a base peak at *m/z* 95 (C_6H_7O, 100%). The stereochemistry of this unsaturated chain was assigned as *E,E* on the basis of the magnitude of the ^{1}H nmr coupling constants measured in benzene solution (J = 15 Hz between δ 5.55 and 5.88 and between δ 6.25 and 7.38).

The ^{13}C nmr spectrum (Table 2) of trichodimerol shows 14 signals: carbons of the sorbyl chain, the methyls, a methine (δ 57.6), and a series of quaternary carbons (δ 58.9, 78.9, 102.8, 104.1, and 176.0). The quaternary carbon signals are assigned as follows: δ 102.8 and δ 176.0 to oxygenated carbons of the enolized β-diketone, δ 58.9 to an

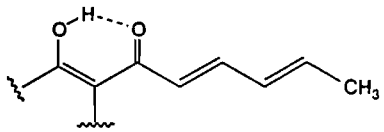
TABLE 1. The ^{1}H nmr data[a] of trichodimerol (**1**), octahydrotrichodimerol (**6**), monoacetyl derivative (**7**), monoacetyloctahydrotrichodimerol (**8**), diacetyloctahydrotrichodimerol (**9**), triacetyloctahydrotrichodimerol (**10**), and tetraacetyloctahydrotrichodimerol (**11**) ($CDCl_3$, 360 MHz)

	Chemical shifts in δ (ppm)[b], multiplicity, *J* in Hz						
H	**1**	**6**	**7**	**8**	**9**	**10**	**11**[d]
1	3.00, s	3.00, s	2.98, s[c]	3.00, s[c]	3.03, s	3.11, s	3.09, s
1′	—	—	3.19, s[c]	2.96, s[c]	—	3.02, s	—
3 (OH)	3.20, s	3.28, s	6.12, s	2.98, s	—	—	—
5 (OH)	16.33, s	16.66, s	16.23, s[d]	16.40, s	16.41, s	—	—
5′ (OH)	—	—	15.94, s[d]	16.69, s	—	16.51, s	—
8	6.11–6.34, m	2.44–2.52 m	2.25–2.40 m	2.25–2.40, m	2.25–2.40, m	3.40, ddd[c]	3.11, m, 2.40, m
8′	—	—	2.25–2.40, m	2.25–2.40, m	—	2.25–2.40, m	—
9	7.32, dd, 15.5, 10	1.6, m	1.6, m	1.6, m	1.6, m	1.6, m	1.6, m
10	6.11–6.34, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m
11	6.11–6.34, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m	1.3, m
12 and 12′	1.89, d, 6.5	0.92, t, 7	0.85, m	0.85, t, 7	0.88, t, 7	0.85, m	0.86, t, 7
13	1.43, s[a]	1.46, s	1.44, s[a]	1.43, s[a]	1.49, s[a]	1.46, s[a]	1.45, s[a]
13′	—	—	1.47, s[b]	1.48, s[b]	—	1.48, s[b]	—
14	1.46, s[a]	1.46, s	1.47, s[b]	1.36, s[b]	1.33, s[a]	1.314, s[b]	1.31, s[a]
14′	—	—	1.51, s[a]	1.41, s[a]	—	1.35, s[a]	—
$COCH_3$	—	—	2.03, s	2.11, s	2.12, s	2.12, s	2.11, s
$COCH_{3'}$	—	—	—	—	—	2.12, s	—
$COCH_{3''}$	—	—	—	—	—	2.24, s	2.23, s

[a]Signals with the same superscript in the same column are interchangeable.
[b]Relative to the residual solvent signal (7.262 for chloroform).
[c]ddd, *J* = 13.5, 8.5, 5 Hz. The other H-8 signal is in the multiplet at 2.40 ppm.
[d]Preparation to be described in Part 2.

aliphatic carbon, δ 78.9 to a monooxygenated aliphatic carbon, and δ 104.1 to a dioxygenated carbon. Since there are only two oxygen-bearing sp^2 carbons, we may conclude that the carbonyl of the sorbyl chain is part of the enolized β-diketone system. These data are consistent with the presence of fragment **5**, an enol-sorbyl system, in trichodimerol.

The molecular formula of trichodimerol indicates it has 13 unsaturations. Since trichodimerol is dimeric, eight unsaturations may be attributed to two enol-sorbyl systems (**5**), leaving five unassigned unsaturations and indicating that trichodimerol is pentacyclic. There are eight oxygen atoms present in trichodimerol, four forming part of the enol-sorbyl systems. Since the ^{13}C nmr spectrum of trichodimerol displays two other signals attributed to oxygen-bearing carbons (the monooxygenated and dioxygenated carbons at δ 78.9 and 104.1), the remaining four oxygens must be present as two hemiacetals.



5

To obtain information about the relative position of the groups further ^{1}H nmr experiments were carried out. Nuclear Overhauser effect (nOe) difference experiments (Table 3) show that there is a large nOe enhancement between the δ 3.00 methine, the sorbyl hydrogen at δ 6.12, and the methyl groups at δ 1.43 and 1.46, which suggests that the methine is in close proximity to these functionalities. In solvent studies with pyridine, the ^{1}H nmr spectrum of trichodimerol shows that the methyls, the methine, and the hydroxyl group are significantly shifted (by 0.44, 0.57, 0.64, and 1.87 ppm, respectively). These shifts are considerably larger than those observed when benzene is used as solvent, suggesting that the methyl groups are vicinal, 1,3-, or in some other way close to the hemiacetal hydroxyl (11).

Of the various "dimeric" structures that can account for the structural features outlined above, only one, structure **1**, has a proper axis of symmetry and can account for the observed optical activity of trichodimerol. The absolute stereochemistry of trichodimerol was determined to be as depicted in **1** by use of the exciton chirality method (12). The cd spectrum of trichodimerol shows a split Cotton effect (amplitude) (A) = $\Delta\varepsilon_1 - \delta\varepsilon_2 = -25$) with a negative first Cotton effect, corresponding to a negative chirality.

Trichodimerol is unstable, decomposing to an insoluble brittle solid upon standing. It may be transformed to a stable derivative, octahydrotrichodimerol (**6**), by hydrogenation over palladium on carbon. The molecular formula of compound **6**, $C_{28}H_{40}O_8$, was confirmed by hreims. Its uv spectrum, λ_{max} 340 (ε = 390) and 294 (14 000) nm, is consistent with the presence of a enolized β-diketone chromophore (13). In addition to the signals expected for a five-carbon saturated chain, the ^{1}H nmr spectrum of **6** (Table 1) shows signals for a methine hydrogen at δ 3.00 (s), a hydroxyl hydrogen at δ 3.28 (br s), a six-hydrogen singlet at δ 1.46 (which may be resolved into two singlets when either pyridine or benzene is used as solvent), an enolized β-diketone hydrogen at δ 16.66, and no olefinic absorptions. The product retained its symmetry as shown by its ^{13}C nmr spectrum (Table 2, only 14 signals).

Octahydrotrichodimerol (**6**) is much more stable than trichodimerol (**1**) and remains unchanged after long periods of time. As in trichodimerol the methyl groups and the methine hydrogen of octahydrotrichodimerol (**6**) display large

TABLE 2. The ^{13}C nmr data of trichodimerol (**1**), octahydrotrichodimerol (**6**), monoacetyl derivative (**7**), monoacetyloctahydrotrichodimerol (**8**), diacetyloctahydrotrichodimerol (**9**), triacetyloctahydrotrichodimerol (**10**), and tetraacetyloctahydrotrichodimerol (**11**) ($CDCl_3$, 75.5 MHz)

	Chemical shift in δ (ppm)[a,b]						
C	**1**[c,d]	**6**[c,d]	**7**[c]	**8**[d]	**9**[c,d]	**10**[c,d]	**11**[e]
1	57.6	57.3	53.3[a]	58.0[a]	57.7	56.7	56.7
1′	—	—	56.8[a]	57.5[a]	—	57.5	—
2	78.9	78.8	89.0	78.9[e]	78.9	79.1[d]	79.1
2′	—	—	78.4	78.6[e]	—	78.7[d]	—
3	104.1	103.9	104.4	109.5	109.2	108.7	108.9
3′	—	—	202.2	103.5	—	109.1	—
4	58.9	58.0	60.8[b]	58.6	58.6	60.8	57.8
4′	—	—	63.8[b]	58.0	—	58.5	—
5	197.9	193.4	196.5[c]	187.3	187.5	167.4[e]	167.5
5′	—	—	195.1[c]	193.5[b]	—	188.6	—
6	102.8	103.3	106.0[g]	102.0[c]	101.4	117.5	117.2
6′	—	—	106.4[g]	102.7[c]	—	101.0	—
7	176.0	191.9	188.4[c]	196.5[b]	196.3	194.5[a]	193.6
7′	—	—	188.4[c]	191.8[b]	—	194.7[a]	—
8	118.6	34.7	33.6	33.3	33.4	33.7	33.3
8′	—	—	33.7	34.7	—	32.9	—
9	143.6	25.2	25.0[d]	25.8	25.8	26.8	26.6
9′	—	—	25.9[d]	25.1	—	25.3	—
10	131.0	31.5	31.6	31.6	31.6	31.4	31.8
10′	—	—	31.7	31.5	—	31.6	—
11	140.3	22.4	22.4[e]	22.4	22.4	22.4	22.4
11′	—	—	23.8[e]	22.4	—	22.4	—
12	18.7	13.9	13.9	13.9	13.9	13.9	13.9
12′	—	—	13.9	13.9	—	13.9	—
13	21.3	21.2	23.8[f]	21.5[d]	21.4[a]	21.3[b]	21.3[a]
13′	—	—	22.4[f]	21.1[d]	—	21.4[b]	—
14	18.9	18.8	21.4[f]	20.3[d]	20.2[a]	20.4[b]	20.2[a]
14′	—	—	20.0[f]	18.6[d]	—	20.2[b]	—
CO	—	—	173.3	169.0	169.0	169.2	169.1
Me	—	—	20.3[f]	?	20.7[a]	20.6[b]	20.7[a]
CO	—	—	—	—	—	167.2[e]	167.1[b]
Me	—	—	—	—	—	21.0[b]	20.9[a]
CO	—	—	—	—	—	167.4[e]	—
Me	—	—	—	—	—	21.1[b]	—

[a]Signals with the same superscript in the same column are interchangeable.
[b]Relative to the carbon of the solvent (77.0 for chloroform).
[c]APT obtained.
[d]Also obtained from [1-^{13}C]acetate incorporated trichodimerol.
[e]Only obtained from [1-^{13}C]acetate incorporated trichodimerol (see Part 2).

shifts in the ^1H nmr spectrum when pyridine is added. The methyl groups and the methine gave mutual nOe[5] enhancements, which are summarized in Table 3, together with the other observed enhancements. Because of its stability, compound **6** was used for additional spectroscopic studies and for the preparation of several derivatives.

Further evidence supporting the proposed structure of trichodimerol (**1**) was obtained from a $^{13}C[^1H]$ nOe enhancement experiment and a natural abundance ^{13}C INADEQUATE experiment on octahydrotrichodimerol (**6**). The location of the methine observed in the ^1H nmr spectrum relative to the non-hydrogen bearing carbons was investigated by the use of a $^{13}C[^1H]$ nOe difference experiment. Selective irradiation of H-1 of octahydrotrichodimerol gave enhancements to C-6, C-2, C-4, C-8, and a negative enhancement to C-3. The negative nOe must arise from an homonuclear nOe between H-1 and the two adjacent methyls (Me-13 and Me-14) followed by a heteronuclear nOe onto the carbons adjacent to the methyls. As well, the homonuclear nOe between the methine and the methyls is observed in the $^1H[^1H]$ nOe experiments. Indirect nOe enhancements of this type have been observed previously (14).

The abundant sample of octahydrotrichodimerol (**6**) justified a natural abundance ^{13}C two-dimensional INADEQUATE-type experiment (15). An INADSYM (16) spectrum was obtained and showed connectivities between C-3 and C-4, C-1 and C-6 and C-2, and between C-2 and C-3. These connectivities confirm that the structure of trichodimerol is as shown in **1**.

The formation of the different acetyl derivatives of octahydrotrichodimerol (**6**) demonstrates its interesting symmetry properties and confirms the presence of two

[5]Verified in benzene-d_6 where the two methyls give separate signals.

TABLE 3. The ^{1}H nmr nOe experiments with trichodimerol (**1**), octahydrotrichodimerol (**6**), monoacetyl derivative (**7**), monoacetyloctahydrotrichodimerol (**8**), diacetyloctahydrotrichodimerol (**9**), and triacetyloctahydrotrichodimerol (**10**) ($CDCl_3$, 360 MHz)

Signal saturated	Signal enhanced (%nOe)[a]	Signal saturated	Signal enhanced (%nOe)[a]
1		**8**	
1.43 (H-13)	3.00 (H-1) (2.8)	1.36 (H-14)	2.96 (H-1′) (4)
1.46 (H-14)	3.00 (H-1) (3.2)	1.41 (H-14′)	3.00 (H-1) (6.2)
	6.12 (H-8) (1.0)	1.43 (H-13)	3.00 (H-1) (3.4)
3.00 (H-1)	1.43 (H-13) (5)	1.48 (H-13′)	2.96 (H-1′) (2.5)
	1.46 (H-14) (7.8)		
	6.12 (H-8) (16)	**9**	
		1.33 (H-14)	3.03 (H-1) (9.6)
6		1.49 (H-13)	3.03 (H-1) (11)
1.46 (H′s-13, 14)	2.44–2.52 (H-8′s) (0.6)	3.03 (H-1)	1.33 (H-14) (5.3)
	3.00 (H-1) (3.9)		1.49 (H-13) (3.3)
	3.28 (OH-3) (1.6)		2.35 (H-8) (27)
2.44 (H-8a)	3.00 (H-1) (5.1)		
2.52 (H-8b)	3.00 (H-1) (5.7)		
3.00 (H-1)	2.44–2.52 (H-8′s) (12)	**10**	
	1.46 (H-13 and 14) (10)	1.32 (H-14)	3.02 (H-1′) (1.2)
3.28 (OH-3)	1.46 (H-13 and 14) (7.4)		2.30 (H-8) (2)
		1.35 (H-14′)	3.11 (H-1) (2.2)
7		1.46 (H-13)	3.11 (H-1) (5.7)
1.44 (H-13)	2.98 (H-1) (3.7)	1.48 (H-13′)	3.02 (H-1′) (3.7)
	6.12 (OH-3) (12)	3.02 (H-1′)	1.32 (H-14) (6)
1.47 (H-14, H-13′)	3.19 (H-1′) (4.5)		1.48 (H-13′) (5)
	6.12 (OH-3) (1.3)		2.30 (H-8) (17)
1.51 (H-14′)	2.38 (H-8′s) (0.9)	3.11 (H-1)	1.35 (H-14′) (7.6)
	2.98 (H-1) (5.2)		1.46 (H-13) (4)
2.98 (H-1)	1.44 (H-13) (6.4)		2.30 (3)
	1.51 (H-14′) (5.3)	3.40 (H-8a)	2.30 (H-8b) (24)
	2.38 (H-8′s) (10.6)		
	1.469 and 1.474 (H-13′		
3.19 (H-1′)	and H-14) (11.1)		
	2.31 (H-8′) (11.6)		
6.12 (OH-3)	1.44 (H-13) (9.3)		

[a]Ratio of the enhanced area over the irradiated signal's area in the difference spectra.

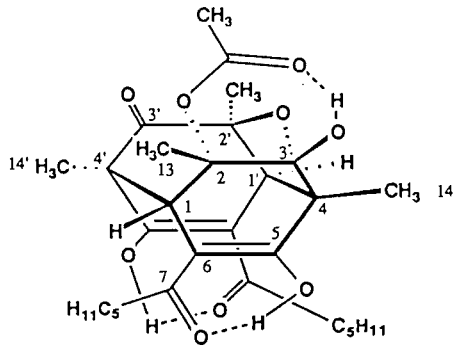
hemiacetals in trichodimerol. Treatment of octahydrotrichodimerol (**6**) with acetic anhydride in refluxing toluene or with acetic anhydride under reflux produced very complex product mixtures with at least 12 components as judged by tlc. When acetic anhydride, pyridine, and 4-*N*,*N*-dimethylaminopyridine (DMAP) in dichloromethane were used for acetylation of **6**, five acetylated products were isolated from the complex mixture formed.

Two different monoacetyl derivatives were obtained. The ir spectrum of monoacetate **7** shows the presence of a hydroxyl group (3400 cm^{-1}), a strongly chelated carbonyl (1605 cm^{-1}), and an acetoxyl carbonyl group (1728 cm^{-1}). The uv spectrum of monoacetate **7** is similar to that observed for octahydrotrichodimerol (**6**), indicating that the enolized β-diketone observed in **6** is unchanged in compound **7**.

The ^{1}H nmr spectrum of monoacetate **7** (Table 1) displays four methyl groups, enolized β-diketone hydrogens (δ 15.94 and 16.23), a hydroxyl hydrogen (δ 6.12 ppm), and methine hydrogens (δ 2.98 and 3.19), all as sharp singlets, indicating that compound **7** is not symmetrical. Accordingly, the ^{13}C nmr spectrum of **7** displays 29 signals, corresponding to 30 carbons. Five carbonyl-like absorptions are observed in the ^{13}C nmr spectrum (Table 2). It is apparent, however, from the integration of the signals that the signal at δ 188.4 corresponds to two carbons. One of the carbonyls can be assigned to the newly introduced acetyl group (δ 173.3), another four arise from the two enolized β-diketone systems (δ 188.4 × 2, 195.1, and 196.5), and the sixth may be assigned to a ketone (δ 202.2). The presence of only three signals in the region δ 100–110 indicates that compound **7** has a single hemiacetal group. Nuclear Overhauser experiments showed that there is a large nOe enhancement (12%) between the methyl hydrogens at δ 1.44 and the hydroxyl hydrogen at δ 6.12. Also, the chemical shift of the hydroxyl hydrogen occurs at unusually low field, suggesting it is chelated. The low-frequency absorption of the acetate carbonyl observed in the ir spectrum of **7** is consistent with this internal hydrogen bonding. These observations, together with the isolation of another monoacetate (**8**) (described below), lead us to conclude that one of the hemiacetals of octahydrotrichodimerol opens to the hydroxy ketone. The tertiary alcohol thus released is then acetylated to produce **7**. The acetyl carbonyl is conveniently located to allow hydrogen bonding with the hydroxyl group. This hydrogen bond explains the acetoxyl carbonyl absorption in the ir spectrum and the large nOe enhancement mentioned above.

The ^{13}C nmr chemical shifts of monoacetate **7** were compared with those of octahydrotrichodimerol (Table 2). A large

downfield shift for C-2 from δ 78.8 to 89.0 (10.2 ppm) is observed due to the acetylation of the tertiary alcohol. Other shifts observed are a downfield shift for C-4′ from δ 58.0 to 63.8 (5.8 ppm), and an upfield shift of C-1 and C-1′ from δ 57.3 to 53.3 and 56.8 (4.0 and 0.5 ppm, respectively) when **7** is compared with **6**. These downfield and upfield shifts are as expected for the change of a hemiacetal (in **6**) into a ketone (in **7**) (17). Furthermore, the nOe experiments (Table 3) allow the assignment of the methines and all the methyl hydrogen signals present in the ^{1}H nmr spectrum of monoacetate **7**.

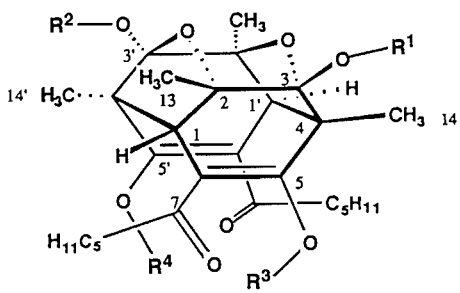


7

A second product obtained from the acetylation of octahydrotrichodimerol is monoacetyloctahydrotrichodimerol (**8**), $C_{30}H_{42}O_9$, which shows absorptions for a strongly chelated carbonyl (1600 cm^{-1}), hydroxyl (3440 cm^{-1}), and acetoxyl carbonyl (1752 cm^{-1}) in its ir spectrum. The ^{1}H nmr spectrum of **8** (Table 1) displays four methyl signals, (δ 1.36, 1.41, 1.43, and 1.48), methines (δ 2.96 and 3.00), acetyl methyl (δ 2.11), enolized β-diketone hydrogens (δ 16.40 and 16.69), and a hydroxyl hydrogen (δ 2.97). These signals indicate that compound **8** is not a symmetrical molecule.

Twenty-nine carbon signals were observed in the ^{13}C nmr spectrum of **8** (Table 2). There are five carbonyl-like carbon absorptions. One is attributed to the newly introduced acetyl carbonyl (δ 169.0) and four to the enolized β-diketone systems (δ 187.3, 191.8, 193.5, and 196.5). Furthermore, there are four carbon signals in the region 100–110 ppm, suggesting that compound **8** has two hemiacetals. Comparison of the ^{13}C nmr spectrum of **8** with that of **6** reveals that in **8** a hemiacetal carbon shifts from δ 103.9 to 109.5 (5.6 ppm). This shift is best explained if one hemiacetal hydroxyl of **6** has been acetylated to give the unsymmetrical acetate **8**.

A diacetate **9**, $C_{32}H_{44}O_{10}$, was also obtained upon acetylation of octahydrotrichodimerol. Its ir spectrum shows acetoxyl carbonyl (1753 cm^{-1}), a strongly chelated carbonyl (1599 cm^{-1}), but no free OH stretching absorption. The ^{1}H nmr spectrum of **9** (Table 1) displays the signals for hydrogens of methyls (δ 1.33 and 1.49), an enolized β-diketone hydrogen (δ 16.41), an acetyl methyl (δ 2.12), and a methine (δ 3.03), all as singlets. The ^{13}C nmr spectrum displays 16 signals, corroborating that compound **9** has an element of symmetry. Three carbon absorptions are carbonyl-like (Table 2): one is assigned to the acetate carbonyl (δ 169.0) and the other two to the enolized β-diketone system (δ 187.5 and 196.3). The hemiacetal carbon (δ 109.2) of **12** is shifted with respect to that observed in the ^{13}C nmr spectrum of octahydrotrichodimerol (**6**). From these data, the structure of the diacetate is assigned as the symmetrical hemiacetal diacetate **9**.



8 R^1 = Ac, R^2 = R^3 = R^4 = H
9 R^1 = R^2 = Ac, R^3 = R^4 = H
10 R^1 = R^2 = R^3 = Ac, R^4 = H
11 R^1 = R^2 = R^3 = R^4 = Ac

A fourth product isolated from the acetylation mixture is triacetate **10**, $C_{34}H_{46}O_{11}$, whose ir spectrum shows absorptions for acetoxyl carbonyl (1752 cm^{-1}), a conjugated ketone carbonyl (1699 cm^{-1}), a strongly chelated carbonyl (1612 cm^{-1}), but no free OH stretching. The uv spectrum of **10** differs from the uv spectrum of octahydrotrichodimerol (**6**) and the other acetates (**7**, **8**, **9**), suggesting that one of the enolized β-diketone systems of **6** has been acetylated.

The ^{1}H nmr spectrum of **10** (Table 1) shows signals for the acetate methyls (δ 2.11, 2.12, and 2.24), the quaternary methyls (δ 1.32, 1.35, 1.46, and 1.48), the methines (δ 3.02 and 3.11), and an enolized β-diketone hydrogen (δ 16.51) as singlets, as well as a one-hydrogen doublet of doublet of doublets at δ 3.40 (*J* 13, 8.5, 5 Hz) (Table 1) attributed to one of the side-chain H-8 hydrogens. Interestingly, in the ^{1}H nmr spectra of acetates **7**, **8**, and **9** the signal for the C-8 methylene hydrogens appears as a multiplet of two diastereotopic hydrogens. Although the chemical shift of the C-8 hydrogens is similar in the ^{1}H nmr spectra of compounds **7**, **8**, and **9**, one of the C-8 hydrogens has shifted more than 1 ppm in the ^{1}H nmr spectrum of **10**. This shift demonstrates that an enol system has been acetylated and suggests that the acetylated enol is endocyclic. If compound **10** had an exocyclic enol acetate, the side chain would retain its rigidity around the C-6, C-7 bond, and no significant change in any of the ^{1}H nmr signals of the C-8 hydrogens would be expected. The ^{13}C nmr spectrum of **10** (Table 2) is in agreement with the structure assigned.

A fifth acetate derivative was isolated from the acetylation of [1-^{13}C]acetate labelled octahydrotrichodimerol prepared during biosynthetic studies.[6] The ^{1}H and ^{13}C nmr

[6]Details of the biosynthetic studies will be presented in a subsequent paper.

spectra indicate it possesses structure **11**. The ir spectrum shows absorption for acetoxyl carbonyl (1751 cm^{-1}), conjugated carbonyl (1700 cm^{-1}), and double bond (1611 cm^{-1}) consistent with an enol acetate – ketone system.

The ^1H nmr spectrum of **11** (Table 1) shows absorptions corresponding to acetyl methyl hydrogens (δ 2.11 and 2.23), methyl hydrogens (δ 1.30 and 1.45), a methine hydrogen (δ 3.09), and a hexanoyl chain (δ 3.10 (m, 1H), 2.50 (m, 1H), 1.45 (m, 2H), 1.28 (m, 4H), and 0.88 (m, 3H). These characteristics indicate that tetraacetate **11** is symmetrical and that the enol acetates are endocyclic since, as indicated for triacetyloctahydrotrichodimerol (**10**), the chemical shift of the H-8 hydrogens changes to δ 3.11 and δ 2.50 in the ^1H nmr spectrum of tetraacetyloctahydrotrichodimerol (**11**). The ^{13}C nmr spectrum of **11** (Table 2) shows 18 signals and the assignment of these signals is in agreement with the proposed structure.

Octahydrotrichodimerol (**6**) is symmetrical and the progressive introduction of acetyl groups upon acetylation gives the following results. Introduction of one acetyl group, as in monoacetyloctahydrotrichodimerol (**7**) and monoacetyloctahydrotrichodimerol (**8**), destroys the symmetry present in octahydrotrichodimerol (**6**). Introduction of two acetyl groups, as in diacetyloctahydrotrichodimerol (**9**) yields a symmetrical product, while introduction of three acetyl groups, as in triacetyloctahydrotrichodimerol (**10**), destroys the symmetry again. The introduction of four acetyl groups restores the symmetry and gives tetraacetyloctahydrotrichodimerol (**11**).

As mentioned above, bisvertinol (**3**) and bisvertinolone (**4**), previously isolated by Dreiding and co-workers from *Verticillium intertextum* (18), were also obtained from *T. longibrachiatum*.[7] The previous workers favored a *trans* C-5a, C-9a ring junction and did not make a stereochemical assignment at the other ring junction (C-4a, C-9b).

We have investigated the stereochemistry of bisvertinol by nOe experiments with the following results: H-9a (δ 3.63) gave nOe enhancement to Me-17 and Me-25 (δ 1.26 (4%) and 1.42 (4%)), and to H-20 (δ 6.49 (18%)). H-3 (δ 2.44) gave an nOe enhancement to H-11 (δ 6.39 (7%)), to H-3′ (δ 2.71 (12%)), and to Me-16 (δ 1.19 (2%)). Furthermore, Me-16 gave enhancement to H-3 (1.4%) and Me-25 to H-20 (1.4%). Me-18 (δ 1.40 ppm) did not give rise to any nOe enhancement. These results allow us to assign the ^1H nmr signals of the quaternary methyls of bisvertinol.

The results of our nOe experiments and the cd spectrum (extrema of opposite sign at 410 and 353 nm (A = 75)) of bisvertinol indicate that the relative stereochemistry of Me-17, H-9a, and Me-25 in bisvertinol should be revised to an all-*cis* stereochemistry. The stereochemistry of the C-4a hydroxyl should then be *cis* on the basis of the enhanced stability of a *cis* 5–6 ring junction over a *trans* 5–6 ring junction, while the stereochemistry at C-4 should remain the same as proposed on biogenetic arguments (18). The stereochemistry of bisvertinol is thus as depicted in **3**. Application of the exciton chirality theory to the cd spectrum of bisvertinol indicates that its absolute stereochemistry is as shown in **3**.

Nuclear Overhauser enhancement experiments with bisvertinolone (**4**) showed similar results to the ones obtained for bisvertinol (**3**) and therefore the stereochemistry of bisvertinolone should also be revised from that proposed by Dreiding and co-workers (18).

The structures of the other new metabolites isolated from *T. longibrachiatum* will be described in Part 2 of this series.

[7]Identity was established by comparison of ^1H and ^{13}H nmr spectra with published values (9).

Experimental

General

High-resolution electron impact mass spectra (hreims) were recorded on an AEI MS-50 mass spectrometer coupled to a DS-50 computer. The hreims data are reported as m/z (relative intensity) except for the molecular ion, which is reported as m/z found (m/z calculated, relative intensity). Unless diagnostically significant, peaks with intensity less than 20% of the base peak are omitted. Chemical ionization mass spectra (cims) were recorded on an AEI MS-12 mass spectrometer with ammonia as the reagent gas. The data were processed using DS-55 software and a Nova-4 computer. The cims data are reported as m/z (assignment, relative intensity). Fourier transform infrared (ftir) spectra were recorded (as a cast from $CHCl_3$ solution unless otherwise noted) on a Nicolet FTIR 7199 interferometer. Ultraviolet (uv) spectra were recorded on a Hewlett Packard 8450A diode array spectrophotometer. Optical rotations were recorded on a Perkin Elmer 141 polarimeter. ^1H and ^{13}C nuclear magnetic resonance (nmr) spectra were measured (in $CDCl_3$ unless noted) on Brucker WM-360 and WH-300 (operating at 75.5 MHz for ^{13}C nmr) spectrometers, coupled to Aspect 2000 and Aspect 3000 computer systems, respectively. The multiplicity of the ^{13}C nmr signals was verified by APT experiments when possible. Chemical shifts are reported in parts per million (d value from tetramethylsilane (TMS)). The residual solvent signal was used as the internal standard, $CDCl_3$: ^1H: δ 7.262 ppm; ^{13}C: δ 77.00 ppm; acetone-d_6: ^1H: δ 2.04 ppm, ^{13}C: δ 29.0 ppm; MeOH-d_4: ^1H: δ 3.30 (methyl) ppm, ^{13}C: δ 49.00 ppm; C_6D_6: δ ^1H: 7.15 ppm, ^{13}C: δ 128.00 ppm relative to TMS.

Growth of Trichoderma longibrachiatum and isolation of the metabolites

Trichoderma longibrachiatum (UAMH 5068) was obtained from Professor Edgar Vargas of the University of Costa Rica and is deposited at the University of Alberta Microfungus Herbarium (UAMH 5068).

Trichoderma longibrachiatum (strain UAMH 4159) was obtained from the University of Alberta Mold Herbarium. It was originally deposited as *Trichoderma reesei* Simmons, mutant of ATCC 24449 (QM 9123). This strain (UAMH 4159) was previously deposited at the American Type Culture Collection (ATCC) as ATCC 26921, at the Commonwealth Mycological Institute (CMI) as CMI 192656, and at the Quatermaster Research and Development Center (QM) as QM 9414. *Trichoderma reesei* ATCC 24449 is a mutant of *T. reesei* ATCC 13631 (*T. viride* QM 6a) (CMI 45548), which was isolated from a "cotton duck shelter" by D. I. Fennell (19).

Slant cultures (PDA) of the fungi were stored in a refrigerator at 4°C. A small piece of mycelium and agar was removed from the slant culture and was used to inoculate an agar plate (PDA), and the plate culture was allowed to grow for 1 week. A portion of the plate culture was used to inoculate a liquid culture (potato dextrose media, 250 mL), which was allowed to grow with constant shaking (160 rpm) for 1 week. Inocula (20 mL) from the small shake culture were used to inoculate still cultures grown in Fernbach flasks (1 L, media composition: *vide infra*), while inocula (25 mL) were used to inoculate large shake cultures (500 mL of media).

Trichoderma longibrachiatum strain UAMH 5068 was grown on still and shake culture using the following media: glucose 2%, yeast extract 0.5%, malt extract 0.5%, K_2HPO_4 0.25%, $MgSO_4$ 0.2%,

$(NH_4)_2SO_4$ 0.1%, bactopeptone 0.5%. The still culture was harvested after 26 days and the shake culture after 11 days of growth.

Trichoderma longibrachiatum strain UAMH 4159 was grown on still culture using the same media, but with lactose instead of glucose as the main carbon source. This growth was harvested after 27 days.

The mycelium was separated from the broth by gravity filtration through cheese cloth. The mycelial mass was dried for 24 h in a fume hood and then extracted for 24 h with $CHCl_3$:CH_2Cl_2 (1:1) in a percolator. The solvent was removed under reduced pressure to give the crude mycelial extract.

The broth was concentrated under reduced pressure to approximately one quarter of its original volume. Butanol was added to avoid excessive foaming during the evaporation. The concentrated broth was then extracted under stirring with chloroform (approx. 4 × 1/2 volume). The chloroform extract was concentrated under reduced pressure to give the crude broth extract as a gum.

Isolation of the metabolites of Trichoderma longibrachiatum

The crude mycelium extract (9.5 g) from a still culture of the *T. longibrachiatum* (UAMH 5068, 10 L) was separated in the following way. A Sephadex LH20 column (70 g, 36 × 2.8 cm i.d.) was packed in methanol, and increasing amounts of chloroform and petroleum ether were used to precondition the column and the column was rinsed with 250 mL of the least-polar solvent mixture to be used. Elution of the metabolites from the crude extract was accomplished using a stepwise increase in solvent polarity. The solvents used were petroleum ether:$CHCl_3$ 2:3 (500 mL), 1:2 (750 mL); petroleum ether:$CHCl_3$:acetone 1:2:1 (250 mL), 1:2:2 (250 mL); $CHCl_3$:acetone 1:1 (250 mL); and methanol. The first fraction (75 mL) was discarded, subsequent fractions (15–20 mL) collected, and like fractions (tlc) combined.

Trichodimerol (**1**) eluted in fractions 20–33 and 56–73 (0.86 g). Sorbicillin (**2**) eluted in fractions 33–44 as a 1:1 mixture with trichodimerol (0.26 g). Sorbiquinol coeluted with trichodimerol in fractions 45–55 (0.27 g). Crude bisvertinol (**3**) eluted in fractions 74–82 (3.5 g). Bisvertinol was purified further by column chromatography (Sephadex LH20, petroleum ether:$CHCl_3$:acetone 1:1:0.4) to give 470 mg of bisvertinol. An analytical sample, prepared by precipitation (twice) from petroleum ether:Et_2O gave bisvertinol, mp 151.5–152.5°C (82 mg). Column chromatography (silica gel; 1% EtOH:$CHCl_3$) of the fraction containing sorbicillin (60 mg) gave pure sorbicillin (20 mg). Column chromatography (silica gel; 2% EtOH:$CHCl_3$) of the fraction containing sorbiquinol (80 mg) afforded sorbiquinol, $C_{28}H_{32}O_7$ (19.4 mg), as a yellow solid, and, after increasing the polarity (6% EtOH:$CHCl_3$), trichodimerol (19.4 mg).

The broth extract (7.5 g) of the still culture of *T. longibrachiatum* (UAMH 5068) was separated as described above. Fractions 15–30 contained impure trichodimerol (0.76 g). Fractions 31–35 gave sorbicillin and trichodimerol (87 mg), 36–57 impure trichodimerol (0.6 g), and 58–69 impure bisvertinol (1.70 g). Sorbiquinol was not obtained. After repeating the Sephadex column with fractions 4–15 (3.25 g), 370 mg of bislongiquinolide, $C_{28}H_{32}O_8$, was obtained in fractions 79–85 as a yellow gum, and 100 mg of bisvertinolone (**4**) in fractions 87–90.

The crude broth extract (3.7 g) from the shake culture of *T. longibrachiatum* (UAMH 5068) was separated as before by Sephadex LH20 chromatography. Impure bisvertinolone (**4**) was obtained in fractions 56–65. This was precipitated from petroleum ether:Et_2O to give 350 mg of bisvertinolone as an amorphous powder, mp 153–155°C. The mycelial extract of the shake culture provided only trichodimerol.

The mycelium extract of *T. longibrachiatum* (UAMH 4159) (9.3 g) was separated by Sephadex LH20 chromatography as described above. Trichodermolide was found in fractions 1–3 and was purified by column chromatography (silica gel; Bz:Et_2O 10:1 to 5:1) to give 235 mg of pure trichodermolide, $C_{24}H_{28}O_4$, as a colorless wax. Fractions 11–18 contained almost pure trichodimerol (1.5 g), fractions 19–38 gave mainly a mixture (2.4 g) of trichodimerol, sorbicillin, and sorbiquinol, while fractions 58–66 contained impure bisvertinol (1.5 g).

The broth extract of *T. longibrachiatum* (UAMH 4159) (8 g) was separated by Sephadex chromatography as described above. Fractions 1–5 contained impure trichodermolide, which after column chromatography (silica gel; Bz:Et_2O 3:1) gave 71 mg of pure trichodermolide. Fractions 32–50 (1.2 g) were combined and subjected to further Sephadex LH20 chromatography using petroleum ether:$CHCl_3$:acetone (2:3:0.4) as eluant. The first 150 mL was discarded. 5-Hydroxyvertinolide, $C_{14}H_{18}O_5$, eluted in fractions 34–48, with fractions 39–40 containing pure 5-hydroxyvertinolide (40 mg).

Trichodimerol (1): $[\alpha]_D$ −376 (*c* 0.26, MeOH); ftir ν_{max} (cm^{-1}): 3420, 2970, 2920, 1613, 1546, 1414, 1288, 1251, 1150, 1125, 992, 935; uv (MeOH) λ_{max} nm (log ε): 362 (4.48), 307 (4.20), 295 (4.17), 240 (4.00); NaOH: 378 nm: HCl regenerated the original spectrum; cd (MeOH) $\lambda_{extremum}$ nm (Δε): 336 (+11), 382 (−14); amplitude (*A*) = −25; ord (MeOH) $\lambda_{extremum}$ nm ([ϕ]): 230 (+10 900), 296 (−22 800), 357 (+52 600), 408 (−20 000); ^{1}H nmr spectrum: see Table 1; ^{1}H nmr spectrum (C_6D_6, 360 MHz) δ: 1.42 (dd, 1.6, 7 Hz, 1H), 1.46 (s, 3H), 1.54 (s, 3H), 3.12 (s, 1H), 3.35 (br s, 1H), 5.55 (dq, 15, 7 Hz, 1H), 5.88 (ddq, 15, 11, 1.5 Hz, 1H), 6.25 (d, 15 Hz, 1H), 7.38 (dd, 11, 14.5 Hz, 1H), 17.14 (s, 1H); ^{13}C nmr spectrum ($CDCl_3$): see Table 2; hreims: 496.2090 (calcd. for $C_{28}H_{32}O_8$: 496.2098, 14%), 401 (3), 248 (22), 232 (7), 205 (30), 137 (33), 95 (C_6H_7O, 100); cims: 514 (M^+ +18, 0.4%), 497 (M^+ +1, 1.2%), 249 (1/2 M^+ +1, 30%); fabms: 497 (M^+ +1, 35%), 249 ($M/2^+$ +1, 30%); tlc: R_f 0.73 (CH_2Cl_2:acetone 3:1).

Sorbicillin (2): ftir ν_{max} (c^{-1}): 3360, 2920, 1640, 1620, 1559, 1480, 1383, 1281, 1154, and 990: uv (MeOH) ν_{max} nm (ε): 318 (27 000); NaOH: 405 (29 000); HCl regenerated the original spectrum; ^{1}H nmr spectrum δ: 1.92 (d, 6 Hz, 3H), 2.16 (s, 3H), 2.24 (s, 3H), 5.26 (br s, 1H), 6.32 (dq, 12, 6 Hz, 1H), 6.38 (dd, 10, 12 Hz, 1H), 6.98 (d, 15 Hz, 1H), 7.48 (dd, 10, 15 Hz, 1H), 7.48 (s, 1H), 13.58 (s, 1H); (C_6D_6, 200 MHz) δ: 1.44 (d, 6.5 Hz, 1H), 1.94 (s, 3H), 1.96 (s, 3H), 4.62 (br s, 1H, exhanges upon addition of D_2O), 5.70 (dq, 15, 6.5 Hz, 1H), 6.00 (ddq, 11, 15, 1.5 Hz, 1H), 6.73 (d, 14.5 Hz, 1H), 7.24 (s, 1H), 7.61 (dd, 10.5, 14.5 Hz, 1H), 14.42 (s, 1H); ^{13}C nmr spectrum δ: 7.0 (q), 15.1 (q), 18.4 (q), 109.9 (s), 113.0 (s), 113.9 (s), 121.3 (d), 128.2 (d), 130.0 (d), 140.6 (d), 144.0 (d), 158.2 (s), 162.0 (s), 192.0 (s); hreims: 232.1096 (calcd. for $C_{14}H_{16}O_3$: 232.1100, 97%), 217 (100), 191 (70), 189 (20), 175 (25), 165 (68), 136 (72), 95 (6); cims: 233 (M^+ +1, 100); tlc: R_f 0.41 (2% EtOH:$CHCl_3$), 0.63 (6%).

Bisvertinol (3): mp 151.5–152.5°C (power from petroleum ether:Et_2O, lit. (9) mp 139–141°C); $[\alpha]_D$ −1274 (*c* 0.99, MeOH) (lit. (9) $[\alpha]_D$ −1467 ($CHCl_3$)); ftir ν_{max} (cm^{-1}) 3400, 3010, 2975, 2925, 1616, 1556, 1445, 1411, 1379, 1022, 992, 974; uv (MeOH) λ_{max} nm (ε): 227 (9 900), 274 (15 400), 300 (15 000). 313sh, 400 (24 000); NaOH: 203 (74 000), 234 (14 200), 273 (17 300), 298 (14 700), 362 (18 000), 426 (17 000); HCl regenerated the original spectrum; cd (MeOH) $\lambda_{extremum}$ nm (Δε): 410 (−42), 353 (+33); *A* = −75; ord (MeOH) $\lambda_{extremum}$ nm ([ϕ]): 443 (−53 000), 379 (+227 000), 315 (+20 000), 276 (+31 000); ^{1}H nmr spectrum (MeOH-d_4, 360 MHz) δ: 1.19 (s, 3H), 1.26 (s, 3H), 1.40 (s, 3H), 1.42 (s, 3H), 1.85 (d, 7 Hz, 3H), 1.89 (d, 7 Hz, 3H), 2.44 (d, 14 Hz, 1H), 2.71 (d, 14 Hz, 1H), 3.63 (s, 1H), 6.09 (ddq, 10, 15, 1.5 Hz, 1H), 6.25–6.40 (m, 3H), 7.19 (dd, 10, 15, 1H), 7.20 (dd, 10, 15 Hz, 1H); ^{1}H nmr spectrum: 1.30 (s, 3H), 1.38 (s, 3H), 1.50 (s, 3H), 1.58 (s, 3H), 1.92 (d, 6 Hz, 6H), 2.62 (d, 14 Hz, 1H), 2.75 (d, 14 Hz, 1H), 3.68 (s, 1H), 3.98 (br s, 1H), 6.4 (m, 6H), 6.44 (d, 15 Hz, 1H), 7.34 (dd, 15, 11 Hz, 2H), 16.2 (s, 1H), 16.7 (s, 1H); ^{13}C nmr spectrum (MeOH-d_4, 75.5 MHz) δ: 7.1 (q), 18.8 (q), 18.9 (q), 20.0 (q), 22.7 (q), 25.8 (q), 36.5 (t), 54.9 (d), 60.3 (s), 74.2 (s), 80.4 (s), 102.5 (s), 106.0 (s), 107.2 (s), 110.6 (d), 121.7 (d) 2C, 132.2 (d), 137.2 (d), 139.3 (d), 140.5 (d), 143.3 (d),

168.4 (s), 170 (s), 179.7 (s), 193.3 (s), 194 (s) (all carbons within 0.4 ppm of reported (9) values).

Bisvertinolone (4): mp 153–155°C (diisopropyl ether), 124–125°C petroleum ether:Et_2O (lit. (9) mp 156–158°C); ftir ν_{max} (cm^{-1}) 3400, 3020, 2980, 2935, 1760w, 1663s, 1607, 1575, 1412, 1379, and 994; uv (MeOH) λ_{max} nm (ε): 272 (23 000), 294 (sh (23 000), 366 (24 000); NaOH: 276 (32 000), 394 (17 000); HCl regenerated the original spectrum; cd (MeOH) $\lambda_{extremum}$ nm (Δε): 402 (−22), 350 (+20); A = −42; ord (MeOH) $\lambda_{extremum}$ nm ([ϕ]): 437 (−23 500), 370 (+91 000), 325 (+12 000); ^{1}H nmr spectrum δ: 1.35 (s, 3H), 1.42 (s, 3H), 1.43 (s, 3H), 1.46 (s, 3H), 1.86 (d, 6 Hz, 1H), 1.89 (d, 5 Hz, 1H), 3.72 (s, 1H), 4.20 (br s, 1H, exchangeable), 4.54 (br s, 1H, exchangeable), 6.0–6.4 (m, 6H, 1H exchangeable), 7.31 (dd, 11, 15 Hz, 1H), 7.39 (d, 15 Hz, 1H), 7.56 (dd, 16, 10 Hz, 1H), 16.31 (s, 1H), 17.71 (s, 1H): ^{13}C nmr spectrum δ: 6.9 (1), 18.6 (q), 18.7 (q), 19.2 (q), 22.6 (q), 25.6 (q), 54.4 (d), 59.8 (s), 79.0 (s), 79.8 (s), 99.8 (s), 104.0 (s), 107.2 (s), 110.9 (s), 120.0 (d), 121.9 (d), 131.0 (d), 131.2 (d), 137.4 (d), 139.6 (d), 144.0 (d), 148.3 (d), 163.8 (s), 170.2 (s), 185.7 (s), 191.2 (s), 196.4 (s), 199.8 (s), (all carbons within 0.4 ppm of reported (9) values); nOe (C_6D_6): [1.19]: 1.56 (0.8%), OH approx. 6 (1.4%); [1.407]: 4.01 (0.4%), OH approx. 6 ppm (3.5%); [1.56]: 4.01 (0.5%), OH (4.4%), 6.12 (d, 15 Hz) (1%); [1.97]: OH (7.5%); [4.01]: 6.12 approx. (d, 15 Hz) (6.6%), 1.56 (2.6%), 1.407 (3.5%) ppm; nOe ($CDCl_3$): [3.72]: 6.38 (8%), 1.42–1.43 (7%); [1.46]: none; [1.42–1.43]: 3.72 (3.6%); [1.35]: none.

Hydrogenation of trichodimerol (1)

Trichodimerol (**1**) (170 mg) in ethyl acetate was hydrogenated over palladium on carbon at room temperature for 1 h. The reaction mixture was filtered, concentrated, and purified by column chromatography (silica gel; Bz:Et_2O 1:1) to give octahydrotrichodimerol (**6**) (105 mg): mp 147–149°C (EtOAc: petroleum ether), 124–125°C (Bz:Et_2O); $[\alpha]_D$ +184 (*c* 0.70, MeOH); ftir ν_{max} (cm^{-1}): 3420, 2960, 2940, 2910, 1580, 1265, 1129, and 1130; uv (MeOH) λ_{max} nm (ε): 340 (390), 294 (14 100); cd (MeOH) $\lambda_{extremum}$ nm (Δε): 311 (+28), 281 (−4.9); A = +32.9; ord (MeOH) $\lambda_{extremum}$ nm ([ϕ]): 323 (+64 000), 296 (−65 000); ^{1}H nmr spectrum: see Table 1; ^{13}C nmr spectrum: see Table 2; hreims: 504.2727 (calcd. for $C_{28}H_{40}O_8$: 504.2724, 26%), 253 (65), 252 (33), 237 (17), 236 (35), 235 (25), 193 (53), 181 (76), 165 (52), 154 (73), 99 (100), 83 (19), 71 (53); cims: 502 (12%), 501 (32%), 252 (100%), 251 (73%), 233 (19%); tlc: R_f 0.17 (2% EtOH/$CHCl_3$), 0.46 (6%).

Acetylation of octahydrotrichodimerol (6)

Octahydrotrichodimerol (129 mg), acetic anhydride (1.5 mL), pyridine (0.5 mL), and dimethylaminopyridine (one crystal) were refluxed in dichloromethane (15 mL) for 7 days. Methanol (5 mL) was added, and the reaction mixture was filtered and evaporated *in vacuo*. Traces of pyridine were removed by azeotropic evaporation with toluene. Purification by column chromatography (silica gel; 12 to 33% ethyl acetate:petroleum ether) afforded the monoacetyl derivative of the keto form (**7**) (3.1 mg), diacetyloctahydrotrichodimerol (**9**) (51.2 mg), triacetyloctahydrotrichodimerol (**10**) (32.3 mg), and impure monoacetyloctahydrotrichodimerol (**8**) (7.7 mg). Compound **8** was purified further by preparative tlc on silica gel (eluant: 4% EtOH:$CHCl_3$) to give pure monoacetyloctahydrotrichodimerol (**8**) (3 mg).

Monoacetyloctahydrotrichodimerol (7): ftir ν_{max} (cm^{-1}): 3400, 2960, 2935, 2895, 2860, 1728, 1605, 1588, 1380, 1262, 1138, 1110, 1041, 948; uv (MeOH) λ_{max} nm (log ε): 295 (4.08); NaOH: 203 (4.53), 307 (4.08); HCl regenerated the original spectrum; ^{1}H nmr spectrum: see Table 1; ^{13}C nmr spectrum: see Table 2; hreims: 546.2834 (calcd. for $C_{30}H_{42}O_9$: 546.2830, 90%), 528 (19), 486 (37), 468 (14), 261 (27), 253 (42), 252 (12), 237 (22), 236 (81), 235 (52), 223 (19), 218 (21), 207 (22), 180 (33), 165 (60), 154 (21), 149 (40), 99 (100), 71 (80); cims: 564 (M^++18, 29%), 547 (M^++1, 14%), 504 (66), 295 (100); tlc: R_f 0.56 (EtOH:$CHCl_3$ 1%), 0.78 (2%), 0.92 (6%).

Monoacetyloctahydrotrichodimerol (8): ftir ν_{max} (cm^{-1}): 3440, 2957, 2932, 2872, 2860, 1752, 1600, 1466, 1462, 1374, 1243, 1076; uv (MeOH) λ_{max} nm (log ε): 292 (4.20); NaOH: 204 (4.69), 308 (4.20); HCl regenerated the original spectrum; ^{1}H nmr spectrum: see Table 1; ^{13}C nmr spectrum: see Table 2; hreims: 546.2822 (calcd. for $C_{30}H_{42}O_9$: 546.2830, 7%), 486 (39), 443 (27), 415 (11), 291 (18), 263 (31), 251 (17), 236 (100), 218 (25), 209 (17), 192 (26), 180 (39), 167 (68), 165 (61), 99 (66), 71 (61); cims: 564 (M^++18, 100%), 547 (M^++1, 6%); tlc 0.65 (EtOH:$CHCl_3$ 4%).

Diacetyloctahydrotrichodimerol (9): ftir ν_{max} (cm^{-1}): 2957, 2885, 1753, 1599, 1580, 1466, 1252, 1231, 1197, 1078, 1064; uv (MeOH) λ_{max} nm (log ε): 290 (4.30); NaOH: 204 (4.74), 299 (4.34); HCl regenerated the original spectrum; ^{1}H nmr spectrum: see Table 1; ^{13}C nmr spectrum: see Table 2; hreims: 588.2931 (calcd for $C_{32}H_{44}O_{10}$: 588.2935, 93%), 447 (26), 370 (34), 317 (37), 279 (20), 278 (32), 272 (30), 263 (21), 251 (48), 237 (41), 236 (95), 235 (43), 233 (20), 223 (24), 218 (27), 192 (31), 180 (42), 167 (47), 165 (86), 99 (100); cims: 606 (M^++18, 100%), 589 (M^++1, 22%); tlc: R_f 0.63 (EtOH:$CHCl_3$ 2%), 0.87 (6%).

Triacetyloctahydrotrichodimerol (10): ftir ν_{max} (cm^{-1}): 2956, 2932, 2880, 2865, 1752, 1699, 1612, 1465, 1373, 1253, 1232, 1193, 1176, 1066; uv (MeOH) λ_{max} nm (log ε): 224 (3.90), 229 (3.90), 255 (4.08), 292 (4.00); NaOH: 205 (4.89), 248 (4.00), 304 (4.26); HCl regenerated the original spectrum; ^{1}H nmr spectrum: see Table 1; ^{13}C nmr spectrum: see Table 2; hreims: 630.3069 (calcd. for $C_{34}H_{46}O_{11}$: 630.3045, 5%), 604 (36), 588 (8), 468 (18), 401 (26), 370 (34), 359 (31), 317 (22), 252 (9), 251 (27), 236 (46), 235 (43), 233 (24), 218 (35), 180 (25), 165 (63), 99 (100), 83 (21), 71 (87); cims: 648 (M^++18, 45%), 631 (M^++1, 22%), 588 (M^+−42, 100%); tlc: R_f 0.32 (EtOH:$CHCl_3$ 2%), 0.73 (6%).

Natural abundance INADSYM

Octahydrotrichodimerol (600 mg) was dissolved in deuterated benzene (1.5 mL). Sixty-four FIDs of 2K points and 640 scans each were obtained with the program INADSYM of Bruker. The delays were optimized for a carbon–carbon coupling constant of 59 Hz. A relaxation delay of 2 s was used between scans. This required a total spectrometer time of 40 h. The spectral window used was of 4545 Hz, corresponding to 50 ppm at 90.56 MHz. A zero filling to 1024 W was done in F_1 and the spectra were Fourier-transformed with a Gaussian multiplication in F_1 (LB_1 = 100, GB_1 = 0.2), and a sinusoidal multiplication in F_2 followed by symmetrization.

Acknowledgements

We acknowledge with thanks the financial support provided by the Natural Sciences and Engineering Research Council of Canada, the International Development Research Centre, and the Alberta Heritage Foundation for Medical Research. We also thank Lynne Sigler, University of Alberta Microfungus Herbarium, for cultures and valuable discussion and Dr. Lois M. Browne for her interest and assistance.

1. M. A. Rifai. Mycol. Pap. Commonw. Mycol. Inst. **116**, 1 (1969).
2. W. B. Turner and D. C. Aldridge. Fungal Metabolites II. Academic Press, New York. 1983. p. 592.
3. R. W. Dunlop, A. Simon, K. Sivasithamparam, and E. L. Ghisalberti. J. Nat. Prod. **52**, 67 (1989), and references therein.
4. G. C. Papavizas. Annu. Rev. Phytopathol. **23**, 23 (1985).
5. W. A. Ayer and C. Dufresne. Bull. Soc. Chim. Belg. **95**, 699 (1986).
6. D. J. Cram. and M. Tishler. J. Am. Chem. Soc. **70**, 4238 (1948).
7. D. J. Cram. J. Am. Chem. Soc. **70**, 4240 (1948).
8. L. Trifonov, A. S. Dreiding, L. Hoesch, and D. M. Rast. Helv. Chim. Acta, **64**, 1843 (1981).
9. L. Trifonov, H. Hilpert, P. Floersheim, A. S. Dreiding, D.

M. Rast, R. Skrivanova, and L. Hoesch. Tetrahedron, **42**, 3157 (1986).
10. J. F. C. McOmie and M. S. Tute. J. Chem. Soc. 3226 (1958).
11. P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari, and E. Wenkert. J. Am. Chem. Soc. **90**, 5480 (1968).
12. N. Harada and K. Nakanishi. Circular dichroic spectroscopy. Exciton coupling in organic stereochemistry. University Science Books, Mill Valley, Calif. 1983. pp. 3–8.
13. A. I. Scott. Interpretation of the ultraviolet spectra of natural products. Pergamon Press, New York. 1964. p. 69.
14. R. N. Henrie II, R. W. Creekmore, and W. Yeager. J. Org. Chem. **53**, 5977 (1988).
15. A. E. Derome. Modern NMR techniques for chemistry research. Pergamon Press, Oxford. 1987. pp. 234–239.
16. R. Freeman and D. L. Turner. J. Magn. Reson. **48**, 158 (1982).
17. R. M. Silverstein, C. G. Bassler, and T. C. Morrill. Spectrometric identification of organic compounds. 4th ed. 1981. p. 289.
18. L. Trifonov, J. H. Bieri, R. Prewo, A. S. Dreiding, D. M. Rast, and L. Hoesch. Tetrahedron, **38**, 397 (1982).
19. S. C. Jong and M. J. Gantt (*Editors*). American type culture collection of fungi/yeasts. 17th ed. American Type Culture Collection, Md. 1987. p. 386.