**(+)-(1*R*,2*S*,3*S*,4*R*)-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrochrysene [(+)-2b].** Direct epoxidation of (-)-**5b** (98 mg) was effected with *m*-chloroperoxybenzoic acid (1 g) in THF (10 mL) for 2 h as described above for the synthesis of (+)-**2a** to give (+)-**2b** as a colorless crystalline powder (80.6 mg, 77.5% yield): mp 233 °C dec.

**(-)-(1*S*,2*R*,3*R*,4*S*)-1,2-Dihydroxy-3,4-epoxy-1,2,3,4-tetrahydrochrysene [(-)-2b].** Direct epoxidation of (+)-**5b** (100 mg) was effected as described above to give (-)-**2b** as a colorless crystalline powder (89 mg, 83.9% yield) mp 233 °C dec.

**Tetrahydrochrysene 3,4-Epoxide. Preparation of Diastereoisomeric MAA Esters of (±)-*trans*-3-Bromo-4-hydroxy-1,2,3,4-tetrahydrochrysene and Their Separation by HPLC.** To a solution of (±)-3-bromo-4-hydroxy-1,2,3,4-tetrahydrochrysene[4c] (642 mg) in dry pyridine (10 mL) was added (-)-menthoxyacetyl chloride (1 g) under cooling at 0 °C within 30 min. The reaction mixture was stirred overnight at room temperature and poured into $CHCl_3$ (200 mL). The $CHCl_3$ solution was washed with water, dilute HCl, and water, dried ($MgSO_4$), and evaporated to give crystals (1.2 g).

Preparative separation (>99.5% diastereomerically pure) of the above diastereomeric mixture (1.2 g) by HPLC was achieved on a 10-μm silica gel column (2.5 cm × 120 cm) elauted with 3% $Et_2O$ in cyclohexane at a flow rate of 44 mL/min ($\alpha$ = 1.34). Evaporation of combined less polar fraction ($k'$ = 2.6) afforded the MAA ester of *trans*-(3*R*,4*R*)-3-bromo-4-hydroxy-1,2,3,4-tetrahydrochrysene (-)-**6** (0.5 g, 39%), as colorless feathers: mp 102-103 °C (petroleum ether); $[\alpha]^{23}_D$ -134° (*c* 1.43, $CHCl_3$); NMR (benzene-$d_6$) $\delta$ 3.90 (s, 2 H, $COCH_2O$), 4.56 (q, 1 H, $H_3$, $J_{3,4}$ = 6, $J_{3,2}$ = $J_{3,2'}$ = 2.2 Hz). Evaporation of combined more polar fractoin ($k'$ = 3.4) afforded the MAA ester of *trans*-(3*S*,4*S*)-3-bromo-4-hydroxy-1,2,3,4-tetrahydrochrysene (+)-**6** (0.5 g, 39%) as colorless feathers: mp 103-104 °C (MeOH) $[\alpha]^{23}_D$ +57.2° (*c* 1.01, $CHCl_3$); NMR (benzene-$d_6$) $\delta$ centered at 3.78 and 3.96 (d, 5, 2 H, $COCH_2O$, $J_{gem}$ = 16 Hz), 4.56 (q, 1 H, $H_3$, $J_{3,4}$ = 6, $J_{3,2}$ = $J_{3,2'}$ = 2.2 Hz.

**(-)-*trans*-(3*R*,4*R*)-3-Bromo-4-hydroxy-1,2,3,4-tetrahydrochrysene [(-)-7].** A mixture of the above less polar MAA ester (-)-**6** (315 mg) and 1 M $B_2H_6$-THF solution (50 mL) was allowed to stand at room termperature for 1 week. After decomposition of excess $B_2H_6$ with ice water, the solvent was evaporated to leave crystals (120 mg), which were washed with cold MeOH. Recrystallization from MeOH afforded colorless needles of (-)-**7**: mp 183-184 °C; $[\alpha]^{23}_D$ -62.5° (*c* 0.59, THF).

**(+)-*trans*-(3*S*,4*S*)-3-Bromo-4-hydroxy-1,2,3,4-tetrahydrochrysene [(+)-7].** The reaction of the more polar diastereoisomer (+)-**6** (300 mg) with 1 M $B_2H_6$-THF solution (50 mL) was effected as described above for its diastereomer to afford (+)-**7** as colorless needles: mp 183-184 °C (MeOH); $[\alpha]^{23}_D$ +61.5° (*c* 0.60).

**(+)-(3*S*,4*R*)-3,4-Epoxy-1,2,3,4-tetrahydrochrysene [(+)-8].** A mixture of (-)-**7** (90 mg), the hydroxide form of dry amberlite IRA-400 (1 g), and THF (5 mL) was stirred under argon gas for 18 h. After filtration to remove the resin, the filtrate was evaporated to leave crystals of (+)-**8** which were recrystallized from $Et_2O$ to give colorless prisms (60 mg): mp 188-189 °C; $[\alpha]^{23}_D$ +89.6° (*c* 0.40, THF); NMR ($CDCl_3$) $\delta$ 3.88 (t, 1 H, $H_3$, $J_{3,4}$ = 4.3, $J_{3,2}$ = $J_{3,2'}$ = 2.5 Hz), 4.75 (d, 1 H, $H_4$, $J_{4,3}$ = 4.3 Hz); mass spectrum (NO-$N_2$, EI), *m/e* 246 ($m^+$).

**(-)-(3*R*,4*S*)-3,4-Epoxy-1,2,3,4-tetrahydrochrysene [(-)-8].** The reaction of (+)-**7** (98 mg) with the hydroxide form of dry Amberlite IRA-400 (1 g) in THF (5 mL) was affected as described above for its enantiomer to afford (-)-**8** as colorless prisms (62 mg): mp 188-189 °C; $[\alpha]^{23}_D$ -89.9° (*c* 0.46, THF); NMR ($CDCl_3$), superimposable with that of (+)-**8**; mass spectrum (NO-$N_2$, EI), *m/e* 246 ($M^+$).

**Registry No.** (-)-(1*S*,2*R*,3*S*,4R)-**1a**, 80433-78-9; (+)-(1*R*,2*S*,3*R*,4*S*)-**1a**, 80433-79-0; (-)-(1*R*,2*S*,3*R*,4*S*)-**1b**, 77123-24-1; (+)-(1*S*,2*R*,3*S*,4*R*)-**1b**, 80433-80-3; (+)-(1*R*,2*S*,3*S*,4*R*)-**2a**, 80446-23-7; (-)-(1*S*,2*R*,3*R*,4*S*)-**2a**, 80433-81-4; (+)-(1*R*,2*S*,3*S*,4*R*)-**2b**, 77123-23-0; (-)-(1*S*,2*R*,3*R*,4*S*)-**2b**, 80433-82-5; (±)-*trans*-**3a**, 80399-21-9; (+)-(3*R*,4*R*)-**3a**, 80433-83-6; (-)-(3*S*,4*S*)-**3a**, 80433-84-7; (-)-(3*R*,4*R*)-**3a** bis(MAA) ester, 80399-22-0; (+)-(3*S*,4*S*)-**3a** bis(MAA) ester, 80433-85-8; (-)-(3*R*,4*R*)-**3a** diacetate, 80433-86-9; (+)-(3*S*,4*S*)-**3a** diacetate, 80433-87-0; (±)-*trans*-**3b**, 67175-75-1; (+)-(1*R*,2*R*)-**3b**, 80433-88-1; (-)-(1*S*,2*S*)-**3b**, 80433-89-2; (+)-(1*R*,2*R*)-**3b** bis(MAA ester, 80399-23-1; (-)-(1*S*,2*S*)-**3h** bis(MAA) ester, 80446-24-8; (-)-(1*R*,2*R*)-**3b** diacetate, 80433-90-5; (+)-(1*S*,2*S*)-**3b** diacetate, 80433-91-6; (-)-(1*S*,2*S*)-**3b** bis[(*p*-dimethylamino)benzoate], 80399-24-2; **4a**, 80399-25-3; **4b**, 80409-34-3; (±)-**5a**, 64501-86-6; (-)-(3*R*,4*R*)-**5a**, 67335-42-6; (+)-(3*S*,4*S*)-**5a**, 67335-43-7; (-)-(3*R*,4*R*)-**5a** diacetate, 80433-92-7; (+)-(3*S*,4*S*)-**5a** diacetate, 80433-93-8; (-)-(3*R*,4*R*)-**5a** bis(MAA) ester, 80399-26-4; (+)-(3*S*,4*S*)-**5a** bis(MAA) ester, 80433-94-9; (-)-(1*R*,2*R*)-**5b**, 77123-18-3; (+)-(1*S*,2*S*)-**5b**, 80433-95-0; (-)-(1*R*,2*R*)-**5b** diacetate, 80433-96-1; (+)-(1*S*,2*S*)-**5b** diacetate, 80433-97-2; (±)-**6**, 80124-65-8; (-)-(3*R*,4*R*)-**6** MAA ester, 80056-88-8; (+)-(3*S*,4*S*)-**6** MAA ester, 80124-35-2; (-)-(3*R*,4*R*)-**7**, 80056-91-3; (+)-(3*S*,4*R*)-**7**, 80433-98-3; (+)-(3*S*,4*R*)-**8**, 80433-99-4; (-)-(3*R*),4*S*)-**8**, 80434-00-0; (*p*-dimethylamino)benzoyl chloride, 4755-50-4; (-)-menthoxyacetyl chloride, 15356-62-4.

# Isolation and Characterization of the Trichoverroids and New Roridins and Verrucarins

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*Myrothecium verrucaria* grown on a large scale (760 L) has yielded a variety of new trichothecenes including the nonmacrocyclic trichoverroids **13-19**, which contain a pendant C4 cis,trans-dienic ester side chain normally common only to the macrocyclic roridins and verrucarins. Several novel macrocyclic trichothecenes (roridins J and K acetate) and verrucarins L and L acetate also were isolated and characterized.

The trichothecene complex of antibiotics has generated a great deal of interest during the past 10 years, principally due to the wide spectrum of biological activity exhibited by these sesquiterpene mycotoxins.[1] Our interest in this

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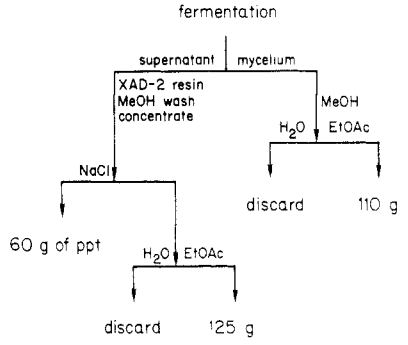
Scheme I. Fermentation Workup of *M. verrucaria*



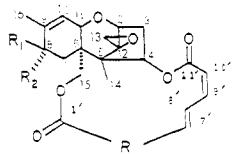

area arose from the report of the isolation of in vivo active antileukemic (P388) macrocyclic trichothecenes from an extract of Brazilian shrub *Baccharis megapotamica*.[2] To date, these compounds, known as baccharinoids, represent the only known examples of trichothecenes isolated from a higher plant; all other members of this series result from cultures of various species of soil fungi.[1] A comparison of the structures of the baccharinoids with the closely related roridins suggests that the known, but in vivo P388 inactive, roridins and verrucarins might be converted readily to derivatives which possess activity resembling that of the activity of the potent in vivo P388 active baccharinoids.[3] To this end, we have conducted a large-scale fermentation with *Myrothecium verrucaria* (ATCC 24571) in order to obtain sufficient quantities of previously reported roridins and verrucarins for further chemical modifications. During the course of this work, we have isolated a number of new trichothecenes whose properties we herein describe in detail.

## Results and Discussion

A strain of *Myrothecium verrucaria* is available from the American Type Culture Collection (ATCC 24571), a culture of which has been reported to yield both verrucarin A (**1**) and roridin A (**5**).[4] Preliminary work indicated that this fungus produced verrucarin A[5] as the major trichothecene metabolite in yields of ca. 15 μg/mL. Our original procedure was modeled after that used by Tamm and his co-workers, who employed Czapek–Dox medium (glucose is the principal carbon source) for the fermentation of the S118 strain of *M. verrucaria*.[6] Our fermentation has been improved by employing a two-stage process where the fungus is allowed to sporulate in Czapek–Dox medium, and this medium then is used to inoculate the production medium (see Experimental Section).

Scheme I outlines the workup of our 760-L fermentation of *M. verrucaria*. The absorption of the trichothecenes from the fermentation beer onto an XAD-2 resin is a substantial improvement over the typical aqueous extraction methods. Concentration of the methanol wash of the resin yielded a substantial amount of a precipitate rich in the more crystalline macrocyclic trichothecenes. The mycelium yielded the less polar of the trichothecenes (e.g., roridin H, **8**).

Chart I



| Compound | -R- | $R_1$ | $R_2$ |
|---|---|---|---|
| Verrucarin A (**1**) | -CHOHCHCH$_3$CH$_2$CH$_2$OC- (S) (R) | H | H |
| Verrucarin B (**2**)[a] | -CH-CCH$_3$CH$_2$CH$_2$OC- (S) (R) | H | H |
| Verrucarin J (**3**) | -CH=CCH$_3$CH$_2$CH$_2$OC- (E) | H | H |
| Verrucarin L (**4**) | -CH=CCH$_3$CH$_2$CH$_2$OC- (E) | H | OH |
| Verrucarin L Acetate (**4a**) | -CH=CCH$_3$CH$_2$CH$_2$OC- (E) | H | OAc |
| Roridin A (**5**) | -CHOHCHCH$_3$CH$_2$CH$_2$OCHCHOHCH$_3$ (S) (R) (R)(R) | H | H |
| Roridin C (**6**) | -CH-CCH$_3$CH$_2$CH$_2$OCHCHOHCH$_3$ (S) (R) | H | H |
| Roridin E (**7**) | -CH=CCH$_3$CH$_2$CH$_2$OCHCHOHCH$_3$ (E) | H | H |
| Isororidin E (**7a**) | -CH=CCH$_3$CH$_2$CH$_2$OCHCHOHCH$_3$ (E) | H | H |
| Roridin H (**8**) | -CH=CCH$_3$CH$_2$CH (E) | H | H |
| Roridin J (**9**) | -CH=CCH$_3$CHOHCH (Z) | H | H |
| Roridin K Acetate (**10**) | -CH=CCH$_3$CH$_2$CH$_2$OCHCHOHCH$_3$ (E) | H | OAc |

[a] Absolute configuration determined by X-ray diffraction analysis: Breitenstein, W.; Tamm, C.; Arnold, E. W.; Clardy, J. *Helv. Chim. Acta* **1979**, *62*, 2699.

Chromatography of the mycelium extract gave a number of fractions containing significant quantities (>3 g) of the known trichothecenes as follows, in the order of elution from the silica gel column: roridin H (**8**),[7] verrucarin J (**3**),[8] verrucarin A (**1**),[9] roridin E[10] (**7**), isororidin E (**7a**),[11] and roridin A (**5**).[12] Smaller amounts (<0.5 g) of the 2′,3′-epoxidized derivatives verrucarin B (**2**)[13] and roridin D (**6**)[14] also were isolated (Chart I).

Careful examination of the chromatography fractions associated with verrucarin A and roridin A led to the isolation of verrucarin L acetate (**4a**) and verrucarin L (**4**), respectively.[15] The major components of the fraction of intermediate polarity to that of verrucarin A (higher $R_f$) and roridin A (lower $R_f$) are roridin E (**7**) and isororidin E (**7a**) (ratio of **7**/**7a** is ca. 1:4). Roridin K acetate (**10**), whose $R_f$ value is slightly lower than that for roridin E/isororidin E, was isolated from this fraction. Compounds

(1) (a) Bamburg, J. R.; Strong, F. In "Microbial Toxins"; Kadis, S., Ciegler, A., Ajl, S. J., Eds.; Academic Press: New York, 1971; Vol. 7, p 207. (b) Bamburg, J. R. In "Mycotoxins and Other Fungal Related Food Problems"; Rodricks, J. V., Ed.; American Chemical Society: Washington, DC, 1976; *Adv. Chem. Ser.* No. 149, p 144. (c) Doyle, T. W.; Bradner, W. T. In "Anticancer Agents Based on Natural Product Models"; Cassidy, J. M., Douros, J. D., Eds.; Academic Press: New York, 1980; p 43.

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

(3) This has been shown to be the case. See: Jarvis, B. B.; Stahly, G. P.; Pavanasasivam, G.; Mazzola, E. P. *J. Med. Chem.* **1980**, *23*, 1054.

(4) Mortimer, P. H.; Campbell, J.; DiMenna, M. E.; White, E. P., *Res. Vet. Sci.* **1971**, *12*, 508.

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

(6) Harri, E.; Loeffler, W.; Sigg, H. P.; Stahelim, H.; Stall, C.; Tamm, C., *Helv. Chim. Acta* **1962**, *45*, 839.

(7) Traxler, P.; Tamm, C. *Helv. Chim. Acta* **1970**, *53*, 1846.

(8) Fetz, E.; Bohner, B.; Tamm, C. *Helv. Chim. Acta* **1965**, *48*, 1664.

(9) Gutzwiller, J.; Tamm, C. *Helv. Chim. Acta* **1965**, *48*, 157.

(10) Traxler, P.; Zurcher, W.; Tamm, C. *Helv. Chim. Acta* **1970**, *53*, 2071.

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

(12) Bohner, B.; Tamm, C. *Helv. Chim. Acta* **1966**, *49*, 2527.

(13) Gutzwiller, J.; Tamm, C. *Helv. Chim. Acta* **1965**, *48*, 177.

(14) Bohner, B.; Tamm, C. *Helv. Chim. Acta* **1966**, *49*, 2547.

(15) Compounds **4** and **4a** were reported in a preliminary communication: Jarvis, B. B.; Midiwo, J. O.; DeSilva, T.; Mazzola, E. P. *J. Antibiot.* **1981**, *34*, 120.

Table I. $^{13}C$ Spectral Data (ppm) of Roridins J and K Acetate and Verrucarins L and L Acetate [a]

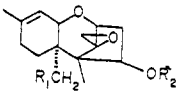
| position | roridin J (9) | roridin K acetate (10) | verrucarin L (4) | verrucarin L acetate (4a) |
|---|---|---|---|---|
| C2 | 79.2 d | 79.1 d | 79.0 d | 79.0 d |
| C3 | 34.7 t | 35.5 t | 35.3 t | 34.9 t |
| C4 | 73.9 d | 73.8 d | 75.1 d | 74.1 d |
| C5 | 49.2 s | 48.6 s | 48.8 s | 49.0 s |
| C6 | 43.3 s | 42.2 s | 42.5 s | 42.2 s |
| C7 | 20.4 t | 27.4 t | 30.1 t | 26.5 t |
| C8 | 27.6 t | 68.8 d | 66.8 d | 68.8 d |
| C9 | 140.4 s | 136.4 s | 139.7 s | 136.5 s |
| C10 | 118.6 d | 124.0 d | 120.9 d | 123.9 d |
| C11 | 67.9 d | 67.0 d | 67.2 d | 67.0 d |
| C12 | 65.6 s | 65.4 s | 65.5 s | 65.3 s |
| C13 | 47.9 t | 48.0 t | 48.1 t | 47.9 t |
| C14 | 7.4 q | 6.8 q | 6.9 q | 7.0 q |
| C15 | 63.4 t | 64.7 t | 65.0 t | 64.5 t |
| C16 | 23.3 q | 21.1 q | 20.6 q | 21.0 q |
| C1′ | 165.9 s | 165.9 s | 165.7 s [b] | 165.5 s [b] |
| C2′ | 119.8 d | 117.6 d | 118.2 d | 117.8 d |
| C3′ | 155.4 s | 159.7 s | 156.6 s | 156.9 s |
| C4′ | 79.8 d | 41.2 t | 40.3 t | 40.2 t |
| C5′ | 103.4 d | 70.3 t | 60.5 t | 60.4 t |
| C6′ | 82.3 d | 84.1 d | 165.5 s [b] | 165.4 s [b] |
| C7′ | 134.5 d | 138.5 d | 127.2 d [c] | 127.8 d [c] |
| C8′ | 126.1 d | 126.5 d | 139.3 d | 138.8 d |
| C9′ | 143.1 d | 143.9 d | 139.3 d | 139.9 d |
| C10′ | 118.9 d | 116.7 d | 125.6 d [c] | 125.2 d [c] |
| C11′ | 166.2 s | 166.2 s | 165.7 s [b] | 165.8 s [b] |
| C12′ | 13.1 q | 20.2 q | 17.3 q | 17.1 q |
| C13′ | 76.5 d | 70.7 d | | |
| C14′ | 16.0 q | 18.3 q | | |
| $COCH_3$ | | 170.8 s | | 170.9 s |
| $COCH_3$ | | 20.7 q | | 20.4 q |

[a] All spectra were taken in deuteriochloroform solvent. The signals were assigned by using $^1H$ single-frequency off-resonance decoupling techniques, by using chemical shift relations, by comparison with literature data (see: Breitenstein, W.; Tamm, C. *Helv. Chim. Acta* **1975**, *58*, 1172 and ref 11), and by comparison of compounds. [b,c] Assignments for those signals of close chemical shift values may be interchanged within each column.

**4**, **4a**, and **10** upon base hydrolysis yield 4β,8α,15-trihydroxy-12,13-epoxytrichothec-9-ene (**20**), a compound previously characterized in our laboratories.[3] The structure assignments for these new macrocyclic compounds were made firm by a combination of $^1H$ and $^{13}C$ NMR (Table I) and mass spectral data. In particular, the configuration of the substituent at C8 can be assigned with confidence by NMR spectroscopy. Introduction of a hydroxyl group at C8 (either α or β) causes a downfield shift in the C16 vinyl methyl resonance in the $^1H$ NMR spectrum of ca. 0.2 ppm. The C16 carbon resonance in the $^{13}C$ NMR spectra shifts upfield by ca. 2–3 ppm for the C8 α-hydroxyl derivatives and upfield by ca. 4–5 ppm for the C8 β-hydroxyl derivatives.[3] Introduction of a C8 β-hydroxyl substituent has little effect on the chemical shifts of both C15 and H15 in the NMR spectra, whereas introduction of a C8 α-hydroxyl group causes a downfield shift of both H15 (ca. 0.2 ppm) and C15 (ca. 2 ppm).[16] It is interesting that we isolate significantly more of acetate **4a** than the parent alcohol **4**. We obtained roridin K acetate (**10**) only in small amounts; we presume roridin K itself also is present, though we have yet to isolate it. Acetates **4a** and **10** are the first isolated naturally occurring acetates of macrocyclic trichothecenes.

We note that **4**, **4a**, and **10** are the first reported macrocyclic trichothecenes which possess a hydroxyl group or ester function at the α-C8 position, although a number of nonmacrocyclic trichothecenes possess such substituents.[1] The plant-derived macrocyclic baccharinols have a hydroxyl group at C8; however, this group is in the β configuration.[2] We have presented data elsewhere which strongly indicate that the baccharinols are derived from fungal toxins via plant-mediated C8 hydroxylation of roridins.[17]

Chart II



| Compound | $R_1$ | $R_2$ |
|---|---|---|
| Trichodermol (11) | H | H |
| Verrucarol (12) | OH | H |
| Trichodermadiene (13) | H | -OCCH=CHCH=CHCH-CHCH$_3$ (c, t (R), (R)) |
| Trichodermadienediol A (14) | H | -OCCH=CHCH=CHCHOHCHOHCH$_3$ (c, t (S), (S)) |
| Trichodermadienediol B (15) | H | -OCCH=CHCH=CHCHOHCHOHCH$_3$ (c, t (S), (R)) |
| Trichoverrol A (16) | OH | -OCCH=CHCH=CHCHOHCHOHCH$_3$ (c, t (S), (S)) |
| Trichoverrol B (17) | OH | -OCCH=CHCH=CHCHOHCHOHCH$_3$ (c, t (S), (R)) |
| Trichoverrin A (18) | -OCCH=CCH$_3$CH$_2$CH$_2$OH (E) | -OCCH=CHCH=CHCHOHCHOHCH$_3$ (c, t (S), (S)) |
| Trichoverrin B (19) | -OCCH=CCH$_3$CH$_2$CH$_2$OH (E) | -OCCH=CHCH=CHCHOHCHOHCH$_3$ (c, t (S), (R)) |

Chromatography fractions from both the mycelium extract and the supernatant extract rich in verrucarin A also contained minor quantities of roridin J (**9**).[18] Roridin J was separated from **1** by chromatography on alumina[19] and purified by recrystallization. Upon acetylation, **9** gave a monoacetate, and hydrolysis of **9** yielded verrucarol.[12] Inspection of the $^1H$ NMR spectrum of **9** suggests that it is closely related to roridin H. However, the $^{13}C$ NMR spectrum of **9** suggests some definite differences between roridin J and roridin H. In addition to the large downfield shift for C4′ in **9** caused by the introduction of the hydroxyl iso-E, H, and K acetate and verrucarins J, L, and L acetate) possessing a C2′,C3′ double bond. The configuration of this double bond in roridins E, iso-E, and H and the 7β,8β-epoxy derivatives were shown by NOE experiments to be of the *E* configuration.[11] This double bond has also been assigned the *E* configuration in verrucarin J.[20] However, irradiation of the C12′ protons of **9** in an NOE experiment gave a significant (12%) enhancement of the C2′ proton signal, clearly indicating their cis relationship and establishing that in **9** the C2′,C3′ bond has the *Z* configuration. This explains then the difference observed in the chemical shifts of the C12′ carbon atom in **9** when compared with the $^{13}C$ NMR data in the earlier isolated C2′,C3′ unsaturated macrocyclic trichothecenes.

The C2′,C3′ double bond in both vertisporin[21] and satratoxin H[22] is of the same relative configuration as that

(16) Jarvis, B. B.; Mazzola, E. P., unpublished results.

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

(18) Preliminary communication: Jarvis, B. B.; Stahly, G. P.; Pavanasasivam, G.; Mazzola, E. P. *J. Antibiot.* **1980**, *33*, 256.

(19) Compounds **1** and **9** separate on silica gel TLC only after repetitive developments, with **9** having a slightly lower $R_f$ value. However, with the same solvent system (0.5% $CH_3OH$ in $CH_2Cl_2$), **9** exhibits a slightly lower retention time on HPLC (silica column).

(20) Breitenstein, W. Ph.D. Thesis, University of Basel, Basel, Switzerland, 1975.

(21) Minato, H.; Katayama, T.; Tori, K. *Tetrahedron Lett.* **1975**, 2579.

(22) (a) Eppley, R. M.; Mazzola, E. P.; Highet, R. J.; Bailey, W. J. *J. Org. Chem.* **1977**, *42*, 240. (b) Eppley, R. M.; Mazzola, E. P.; Stack, M. E.; Dreifuss, P. A. *Ibid.* **1980**, *45*, 2522.

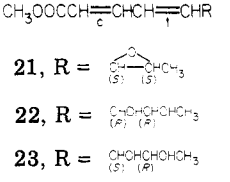
Table II. $^{13}C$ NMR Spectral Data (ppm) of Trichoverroids[a]

| position | trichodermadiene (13) | trichodermadienediol A (14) | trichodermadienediol B (15) | trichoverrol A (16) | trichoverrol B (17) | trichoverrin A (18) | trichoverrin B (19) |
|---|---|---|---|---|---|---|---|
| C2 | 79.2 d | 79.1 d | 79.1 d | 79.0 d | 79.0 d | 79.1 d | 79.2 d |
| C3 | 36.9 t | 37.0 t | 36.9 t | 36.3 t | 36.2 t | 36.9 t | 36.9 t |
| C4 | 75.0 d | 75.0 d | 74.9 d | 75.5 d | 75.5 d | 75.1 d | 75.1 d |
| C5 | 49.2 s | 49.3 s | 49.2 s | 49.1 s | 49.1 s | 48.6 s | 48.6 s |
| C6 | 40.5 s | 40.5 s | 40.5 s | 44.4 s | 44.4 s | 42.9 s | 42.9 s |
| C7 | 24.6 t | 24.5 t | 24.5 t | 21.3 t | 21.2 t | 21.9 t | 21.9 t |
| C8 | 28.1 t | 28.1 t | 28.0 t | 28.2 t | 28.1 t | 27.9 t | 27.9 t |
| C9 | 140.0 s | 140.2 s | 140.3 s | 140.6 s | 140.6 s | 140.5 s | 140.5 s |
| C10 | 118.9 d | 118.7 d | 118.6 d | 118.8 d | 118.8 d | 118.5 d | 118.6 d |
| C11 | 70.6 d | 70.6 d | 70.6 d | 66.9 d | 66.9 d | 66.7 d | 66.7 d |
| C12 | 65.5 s | 66.1 s | 66.1 s | 66.1 s | 66.0 s | 65.8 s | 65.8 s |
| C13 | 47.8 t | 48.1 t | 48.1 t | 48.3 t | 48.3 t | 48.2 t | 48.3 t |
| C14 | 6.0 q | 6.2 q | 6.1 q | 6.8 q | 6.8 q | 6.7 q | 6.7 q |
| C15 | 16.1 q | 16.1 q | 16.0 q | 62.7 t | 62.6 t | 63.5 t | 63.5 t |
| C16 | 23.2 q | 23.2 q | 23.2 q | 23.3 q | 23.2 q | 23.2 q | 23.2 q |
| C1′ | 165.8 s | 166.0 s | 166.0 s | 166.9 s | 167.0 s | 166.0 s | 166.1 s |
| C2′ | 118.4 d | 118.7 d | 118.2 d | 118.2 d | 117.9 d | 118.1 d | 117.9 d |
| C3′ | 143.1 d | 143.4 d | 143.7 d | 144.2 d | 144.3 d | 144.1 d | 144.1 d |
| C4′ | 130.3 d | 127.9 d | 128.0 d | 127.8 d | 128.0 d | 127.5 d | 127.6 d |
| C5′ | 140.4 d | 141.9 d | 141.3 d | 142.5 d | 141.6 d | 142.4 d | 141.4 d |
| C6′ | 58.6 d | 76.6 d | 75.6 d | 76.5 d | 75.5 d | 76.5 d | 75.4 d |
| C7′ | 56.6 d | 70.6 d | 70.3 d | 70.7 d | 70.3 d | 70.5 d | 70.3 d |
| C8′ | 17.5 q | 18.9 q | 17.7 q | 19.0 q | 17.8 q | 18.9 q | 17.9 q |
| C1″ | | | | | | 166.0 s | 166.0 s |
| C2″ | | | | | | 116.9 d | 116.9 d |
| C3″ | | | | | | 157.3 s | 157.2 s |
| C4″ | | | | | | 43.6 t | 43.6 t |
| C5″ | | | | | | 59.7 t | 59.7 t |
| C6″ | | | | | | 19.1 q | 19.2 q |

[a] All spectra were taken in deuteriochloroform solvent. The signals were assigned by using $^1H$ single-frequency off-resonance decoupling techniques, by using chemical shift relations, by comparison of compounds, and by comparison with literature values (Hanson, J. R.; Marten, T.; Siverns, M. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1033).

found in roridin J. In all three of these compounds, the allylic position (C4′ in **9** and C12′ in vertisporin and satratoxin H) has been oxygenated. It may be that in enzymatic oxidation of the position allylic to C2′,C3′, the configuration of the C2′,C3′ double bond is changed.

The chromatography fraction eluting prior to the roridin H fraction contained a new trichothecene less polar than any heretofore reported macrocyclic trichothecene. Although this compound clearly contains the C4 cis,trans-dienic ester side chain typically present in the macrocyclic trichothecenes, proton and carbon magnetic resonance spectroscopy clearly show that this new compound, trichodermadiene (**13**),[23] is nonmacrocyclic. In addition to the $^1H$ NMR signals attributable to the dienic protons at C4′ ($\delta$ 7.83, dd, $J$ = 11, 15.5 Hz) and C3′ ($\delta$ 6.57, dd, $J_{2',3'} = J_{3',4'}$ = 11 Hz), the protons at C13 exhibit the characteristic AB pattern at ca. $\delta$ 3 ($J$ = 4 Hz). However, unlike any known macrocyclic trichothecene, **13** possesses two quaternary methyl groups which resonate at $\delta$ 0.74 (H14) and $\delta$ 1.00 (H15). Furthermore, upon base hydrolysis, **13** gives the known trichothecene trichodermol (**11**). Elemental analysis yielded $C_{23}H_{30}O_5$ as the molecular formula, requiring nine units of unsaturation. Trichodermol and the dienic ester account for eight of these units which leaves the fragment $C_3H_5O$ unaccounted for. Analysis of $^1H$ and $^{13}C$ spectra clearly show this unit is a C6′,C7′ methylepoxy group which from the H6′,H7′ coupling constant ($J$ = 2 Hz) is trans substituted. This structure assignment was made firm by the work of Tulshian and Fraser-Reid,[24] who synthesized the optically active methyl ester **21**; methanolysis of **13** yielded the enantiomer of **21**. Thus, trichodermadiene (**13**) has the *R* configuration at both C6′ and C7′.




In previous reports of the compounds isolated from the cultures of *M. verrucaria*, the most polar of the trichothecenes observed was roridin A. Upon examination of our fractions with $R_f$ values lower than that of roridin A, we observed the presence of a number of compounds. Through a combination of chromatographic methods, six new trichothecenes, **14**–**19**, were isolated.[25] The three sets of diastereomers, trichodermadienediols A (**14**) and B (**15**), trichoverrols A (**16**) and B (**17**), and trichoverrins A (**18**) and B (**19**), could be separated relatively easily, but the separation of the A/B epimers within each set could be accomplished only by HPLC. These new compounds, along with trichodermadiene, constitute a new class of trichothecenes, the trichoverroids, which possess the cis,trans-dienic C4 ester side chain common to the macrocyclic trichothecenes, and yet, the trichoverroids are nonmacrocyclic.

Upon hydrolysis **14** and **15** gave trichodermol (**11**), and **16** and **17** gave verrucarol (**12**). Diols **14** and **15** formed diacetates (acetic anhydride/pyridine) while **16**–**19** gave triacetates. Analysis of the spectral data (Table II) led to

(23) Preliminary communication: Jarvis, B. B.; Midiwo, J. O.; Stahly, G. P.; Pavanasasivam, G.; Mazzola, E. P. *Tetrahedron Lett.* **1980**, 787.
(24) Tulshian, D. B.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1981**, *103*, 474.
(25) Preliminary communication: Jarvis, B. B.; Pavanasasivam, G.; Holmlund, C. E.; DeSilva, T.; Stahly, G. P.; Mazzola, E. P. *J. Am. Chem. Soc.* **1981**, *103*, 472.

the structure assignments.[25] Compounds **14–19** have in common the pendant C4 cis,trans-dienediolic side chain which from $^1$H NMR data appeared to be consistently of the same relative configuration within a series; i.e., the A series of epimers all have the same configuration and the B series a different configuration. The absolute configurations of compounds **14–19** were established by relating their structures to those of methyl esters **22** and **23**, synthesized stereospecificially by Tushian and Fraser-Reid.[24] Upon methanolysis, the B series of epimers (**15**, **17**, and **19**) gave ester **23**, whereas the A series of epimers (**14**, **16**, and **18**) gave the enantiomer of ester **22**. Thus, the A series of compounds is L-threo and the B series is D-erythro, making these compounds epimeric at C7′ and of the *S* configuration at C6′. We also have observed consistent differences in the NMR spectra of the A series vs. the B series. Carbons 6′ and 8′ in the $^{13}$C spectra resonate ca. 1 ppm upfield in the B series relative to the resonances for these carbon atoms observed for the A series. In the acetates, the 7′-H in the L-threo series (A) occurs at ca. $\delta$ 5 as an overlapping doublet of quartets (five lines, $J_{7',8'} \approx J_{6',7'} \approx 6$ Hz) whereas this proton for the D-erythro isomers (B) is a doublet of quartets appearing as an eight-line signal ($J_{7',8'} = 6$ Hz, $J_{6',7'} = 4$ Hz) at ca. $\delta$ 5.

The $^1$H NMR spectra of trichoverrins **18** and **19** closely resemble the spectra of roridin E and iso-E.[10,11] However, in the $^{13}$C NMR spectra, carbons 5″ and 6′ resonate 8–10 ppm upfield from the corresponding carbon atoms in roridins. The origin of this chemical shift difference between the roridins and the trichoverrins is obscure.[26]

The biogenesis of the macrocyclic trichothecenes has been investigated in detail by Tamm and his co-workers,[28] and the biosynthetic origin of the various components of the roridins and verrucarins has been established through labeling studies. However, the steps leading to the formation of the macrocyclic ring have not been delineated. The trichoverrins are attractive candidates as precursors for the macrocycles since dehydration–ring closure would lead to roridin E and/or isororidin E. Indeed, when in separate experiments trichoverrins A and B were fed to a resting culture of *M. verrucaria*, after 1 week they were shown to have been transformed in high conversion to the macrocycles. Thus, from 100 mg of trichoverrin A and 100 mg trichoverrin B, after 1 week in a shake culture with *M. verrucaria*, each gave ca. 50 mg of recovered, unaltered trichoverrin, ca. 12 mg of verrucarin A, and ca. 5 mg of verrucarin B. In addition, each gave substantial amounts (2–5 mg) of verrucarin J and roridins A and (iso) E. These data strongly suggest that the trichoverrins serve as precursors for the macrocyclic trichothecenes. Recently, we[29] determined that the configuration of C6′ in roridin A is *R*, the same as that found in the closely related baccharinoids.[2] It now seems clear that all of the macrocyclic trichothecenes[30] are going to prove to be *R* at C6′. Since the trichoverrins are *S* at this center, ring closure from the trichoverrins to the macrocycles has occurred with inversion of configuration at C6′.

In general, the macrocycles exhibit the highest toxicity of the trichothecenes.[28] Although the role played by the macrolide ring itself is not understood, it is clear that the presence of this ring imparts substantially enhanced potency to these compounds, since the trichoverrins, which differ from roridins E and iso-E by only a molecule of water, are devoid of P388 in vivo activity (and toxicity) at dose levels of 32 mg/kg and below.[31] Also, the cytotoxicity in vitro against L1210 mouse leukemia of the trichoverrins is about 2 orders of magnitude lower than the toxicity of isororidin E.[27]

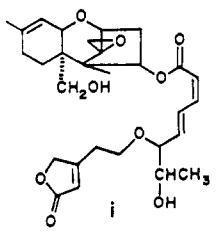
## Experimental Section

**General Methods.** Melting points were determined on a Fisher-Johns hot-stage melting point apparatus and are uncorrected. Ultraviolet spectra were determined on a Cary 15 or a Perkin-Elmer 552 spectrophotometer. Nuclear magnetic resonance spectra were determined in deuteriochloroform, unless otherwise noted, on a Varian XL-100, EM 390, or FT-80 spectrometer with tetramethylsilane as an internal standard. The $^{13}$C NMR signals were assigned by using $^1$H single-frequency off-resonance decoupling techniques, by using chemical shift relations, and by comparison with literature data. Mass spectra were determined on a VG Micromass ZAB-2F and microanalyses were performed by Dr. Franz Kasler of the University of Maryland. Optical rotations were determined on a Perkin-Elmer 241 automatic polarimeter. Thin-layer chromatography (TLC) was carried out on prepared silica gel plates (E. Merck or Analtech), and visualization was effected with short-wavelength UV light or sulfuric acid/ethanol/vanillin (20/3/1) spray. Flash chromatography[32] was carried out on silica gel 60 (230–400 mesh, E. Merck or Whatman LPS-2). Medium-pressure liquid chromatography (MPLC)[33] was carried out on either Licroprep 60 (E. Merck) or Whatman LPS-1 silica gel. High-performance liquid chromatography (HPLC) was performed either with a Waters Model 6000 liquid chromatograph coupled with a Model 660 solvent programmer and an SF 770 Spectroflow monitor or with an Altex Model 332 gradient liquid chromatograph. Separations were carried out on a Whatman Magnum 9 (10/15) semipreparative Partisil column.

Prior to HPLC, the gummy fractions were filtered through TLC silica gel, washing with 10% MeOH/$CH_2Cl_2$. This procedure removes ca. 30% of the weight and much of the yellow coloration. Solvents used for HPLC were obtained from J. T. Baker (HPLC grade) and were filtered before use.

**Fermentors.** The fermentors used in this study were 120- and 1200-L geometrically similar vessels. Each vessel was equipped with three turbine impellers having diameters of 12.7 and 27 cm, respectively. Agitation was accomplished through a bottom-entering mechanically scaled shaft. Air sparging was conducted via a sparge ring located below the bottom-most impeller. Fermentation broth samples were withdrawn to determine (1) pH, (2) packed cell volume by using a Delaval Gyro Test centrifuge with 10-cm$^3$ conical tubes, and (3) the concentration of trichothecenes. Dissolved oxygen was determined continuously by using an in-

(26) This difference does not seem to be associated with an effect of the macrocyclic ring since these corresponding carbon atoms in roridin L-2 (i)[27] resonate at chemical shifts close to those of the corresponding carbon atoms in roridin E and iso-E.




i

(27) French, J. Parke-Davis Division of Warner-Lambert, Detroit, MI, private communication.

(28) (a) Tamm, C. *Fortschr. Chem. Org. Naturst.* **1974**, *31*, 61. (b) Tamm, C. In "Mycotoxins in Human and Animal Health"; Rodricks, J. V., Hesseltine, C. W., Mehlman, M. A.; Eds.; Pathotox Publishers: Park Forest South, IL, 1977; p 209. (c) Tamm, C.; Breitenstein, W. In "The Biosynthesis of Mycotoxins, A Study in Secondary Metabolism"; Steyn, P. S., Ed.; Academic Press: New York 1980; p 69.

(29) Jarvis, B. B.; Midiwo, J. O.; Flippen-Anderson, J.; Mazzola, E. P. *J. Nat. Prod.*, in press.

(30) Roridins H and J may prove to be exceptions to this statement, since they may arise by a fundamentally different ring closure process.

(31) Doyle, T. W. Bristol Laboratories, Syracuse, NY, private communication.

(32) Still, W. C.; Kalin, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(33) Meyers, A. I.; Seade, J.; Smith, R. K.; Mihelich, E. D. *J. Org. Chem.* **1979**, *44*, 2247.

place sterilizable galvanic probe.

**Fermentation.** Spores of *Myrothecium verrucaria* grown on N-Z amine agar (80 plates) and suspended in distilled water were transferred to a 120-L fermentor containing 70 L of Czapek–Dox seed media (per liter: 20 g of glucose, 2 g of malt extract, 2 g of yeast extract, 2 g of peptone, 2 g of $KH_2PO_4$, 2 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of $FeSO_4 \cdot 7mH_2O$, and 3 g of $NH_4Cl$) which had been sterilized at 121 °C for 45 min. The fermentor was cultivated for 48 h (aeration 0.25 vvm, 125 rpm at 25 °C and 4 psig). After 2 days, 38 L of the seed media was transferred to a 1200-L fermentor containing 760 L of production media (per liter: 1 g of $NH_4H_2PO_4$, 3 g of $K_2HPO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 5 g of NaCl, 40 g of sucrose, and 10 g of glycerol) which had been sterilized at 121 °C for 45 min. The production fermentation parameters were the same as with the seed fermentation except that the agitation speed was 100 rpm. During the course of 7 days, the pH decreased from 6.83 to 6.00, and the packed cell volume increased to a maximum at 72 h and remained steady. The dissolved oxygen decreased at a constant rate until it was no longer measurable at 96 h. Trichothecene production was observed at 72 h (TLC analysis), plateaued at 144 h, and remained steady through a total of 168 h of production time.

The fermentation was terminated after 7 days by circulating refrigerated water through the fermentor jacket and diverting the sparge gas through the overlay. The broth was delivered to a centrifuge manifold servicing two shapless centrifuges (Model 16) under 19 psig of pressure. Removal of the supernatant left a wet cell paste (ca. 25 lbs) which was stored at 4 °C. The supernatant was passed twice through a column containing 70 L of XAD-2 resin. After each pass, the resin was washed with methanol, yielding a total of ca. 300 L of aqueous methanol. This solution was concentrated in a laboratory-scale wiped-film evaporator to yield ca. 40 L of an aqueous concentrate.

The wet mycelium paste was extracted by being soaked overnight in 5 gal of methanol. This process was repeated twice more, and the methanol was concentrated by rotary evaporation to give ca. 2 L of an aqueous solution. This solution was washed with ethyl acetate (2 × 1 L), and the organic portions were combined, dried ($MgSO_4$), and concentrated to give 110 g of a brown gum.

**Isolation of the Trichoverroids 14–19.** A portion of the aqueous concentrate (6 L) was extracted with ethyl acetate (2 × 3 L). The organic extract was washed with water and saturated salt solution and dried ($MgSO_4$). Removal of the solvent by rotary exaporation yielded 28 g of a dark brown gum. HPLC analysis indicated that the principal trichothecenes present were verrucarin J, verrucarin B, verrucarin A, isororidin E, roridin E, roridin A, trichodermadienediols A and B, trichoverrols A and B, and trichoverrins A and B in the order of increasing retention time on a silica column (ethyl acetate/hexane eluent). Verrucarin A was the major metabolite, and it was present at a concentration of ca. 30 mg/L of media. The 28 g of gum was subjected to flash chromatography (350 g of silica gel) with increasing amounts of methanol in dichloromethane to yield four fractions: I (14.0 g, eluted with 0–1% MeOH in $CH_2Cl_2$), II (4.5 g, eluted with 1–5% MeOH in $CH_2Cl_2$), III (5.1 g, eluted with 5–7.5% MeOH in $CH_2Cl_2$), and IV (4.0 g, eluted with MeOH).

Fraction II was subjected to partition chromatography [sample applied to a column of Celite (100 g) impregnated with 83% aqueous methanol and eluted with 1–5% dichloromethane in petroleum ether] to give 3.0 g of a fraction rich in the trichothecenes. This was chromatographed ($MeOH/CH_2Cl_2$) to yield 0.9 g of an oil, $R_f$ 0.15–0.20 (silica gel, 3% $MeOH/CH_2Cl_2$). This material was subjected to HPLC (1–4% $MeOH/CH_2Cl_2$ gradient, 30 min, followed by HPLC with 70% ethyl acetate in hexane) to yield 60 mg of trichodermadienediol A [**14**, mp 184–185 °C (recrystallized from dichloromethane–hexane)] and 125 mg of trichodermadienediol B (**15**, an oil).

For **14**: $[\alpha]^{28}_D$ +26.3° (*c* 0.39, $CHCl_3$); UV max (MeOH) 260 nm (log $\epsilon$ 4.53); mass spectrum (chemical ionization, methane gas reagent), *m/e* 405.2263 ($M^+$ + H, calcd 405.2277); $^1H$ NMR ($CDCl_3$) $\delta$ 0.76 (3 H, s, 14-H), 0.98 (3 H, s, 15-H), 1.13 (3 H, d, *J* = 6 Hz, 8′-H), 1.74 (3 H, s, 16-H), 2.60 (1 H, dd, *J* = 8 and 15 Hz, 3α-H), 3.03 (2 H, AB, *J* = 4 Hz, 13-H), 3.66 (1 H, d, *J* = 5 Hz, 11-H), 3.87 (1 H, d, *J* = 5 Hz, 2-H), 4.06 (1 H, m, 6′-H), 5.44 (1 H, d, *J* = 5 Hz, 10-H), 5.73 (1 H, d, *J* = 11 Hz, 2′-H), 6.09 (1 H, dd, *J* = 6, 16 Hz, 5′-H), 6.64 (1 H, dd, *J*'s = 11 Hz, 3′-H), 7.65 (1 H, dd, *J* = 11, 16 Hz, 4′-H).

For **15**: $[\alpha]^{28}_D$ -15.8° (*c* 0.80, $CHCl_3$); UV max (MeOH) 260 nm (log $\epsilon$ 4.55); mass spectrum (chemical ionization, methane gas reagent), *m/e* 405.2252 ($M^+$ + H calcd 405.2277); $^1H$ NMR ($CDCl_3$) $\delta$ 0.74 (3 H, s, 14-H), 0.98 (3 H, s, 15-H), 1.16 (3 H, d, *J* = 6 Hz, 8′-H), 1.73 (3 H, s, 16-H), 2.62 (1 H, dd, *J* = 8, 15 Hz, 3α-H), 3.04 (2 H, AB, *J* = 4 Hz, 13-H), 3.66 (1 H, d, *J* = 5 Hz, 11-H), 3.88 (1 H, d, *J* = 5 Hz, 2-H), 4.30 (1 H, m, 6′-H), 5.44 (1 H, d, *J* = 5 Hz, 10-H), 5.72 (1 H, d, *J* = 11 Hz, 2′-H), 6.16 (1 H, dd, *J* = 6, 16 Hz, 5′-H), 6.66 (1 H, dd, *J*'s = 11 Hz, 3′-H), 7.64 (1 H, dd, *J* = 11, 16 Hz, 4′-H).

Diols **14** and **15** (25 mg, each) were acetylated in 0.5 mL each of acetic = 6 to yield, respectively, trichodermadienediol A diacetate: [mass spectrum (chemical ionization, methane gas reagent), *m/e* 489.2455 ($M^+$ + H, calcd 489.2488); $^1H$ NMR ($CDCl_3$) $\delta$ 0.74 (3 H, s, 14-H), 0.98 (3 H, s, 15-H), 1.24 (3 H, d, *J* = 6 Hz, 8′-H), 1.74 (3 H, s, 16-H), 2.08 and 2.14 (3 H each, s, acetates), 2.60 (1 H, dd, *J* = 8, 15 Hz, 3α-H), 3.00 (2 H, AB, *J* = 4 Hz, 13-H), 3.64 (1 H, d, *J* = 5 Hz, 11-H), 3.86 (1 H, d, *J* = 5 Hz, 2-H), 5.10 (1 H, dq, *J*'s 6 Hz, 7′-H), 5.46 (1 H, d, *J* = 5 Hz, 10-H), 6.58 (1 H, dd, *J*'s = 11 Hz, 3′-H), 7.68 (1 H, dd, *J* = 11, 16 Hz, 4′-H)] and trichodermadienediol B diacetate: mass spectrum (chemical ionization, methane gas reagent), *m/e* 489.2436 ($M^+$ + H, calcd 489.2488); $^1H$ NMR ($CDCl_3$) $\delta$ 0.74 (3 H, s, 14-H), 0.98 (3 H, s, 15-H), 1.23 (3 H, d, *J* = 6 Hz, 8′-H), 1.74 (3 H, s, 16-H), 2.06 and 2.12 (3 H each, s, acetates), 2.60 (1 H, dd, *J* = 8, 15 Hz, 3α-H), 3.00 (2 H, AB, *J* = 4 Hz, 13-H), 3.64 (1 H, d, *J* = 5 Hz, 11-H), 3.86 (1 H, d, *J* = 5 Hz, 2-H), 5.16 (1 H, dq, *J* = 4, 6 Hz, 7′-H), 6.60 (1 H, dd, *J*'s = 11 Hz, 3′-H), 7.68 (1 H, dd, *J* = 11, 16 Hz, 4′-H).

Fraction III (5.1 g) from the original chromatography of the crude extract was subjected to partition chromatography, as described previously for fraction II, and elution with 15% dichloromethane in petroleum ether gave 3.2 g of a gum. Flash chromatography (90% ethyl acetate–petroleum ether) yielded two major fractions, III-A (0.55 g, $R_f$ 0.35 in 90% EtOAc/petroleum ether) and III-B (0.90 g, $R_f$ 0.30 in 90% EtOAc/petroleum ether). After filtration through TLC grade silica gel, each of these fractions was purified by HPLC (1–6% $MeOH/CH_2Cl_2$, gradient, 30 min) to give 70 mg of trichoverrol A (**16**) and 135 mg of trichoverrol B (**17**) from fraction III-A and 100 mg of trichoverrin A (**18**) and 210 mg of trichoverrin B (**19**) from fraction III-B.

For **16**: mp 177–179 °C (EtOAc/petroleum ether); $[\alpha]^{28}_D$ +37.7° (*c* 0.45, $CHCl_3$); UV max (MeOH) 260 nm (log $\epsilon$ 4.56); mass spectrum (chemical ionization, methane gas reagent) *m/e* 421.2226 ($M^+$ + H, calcd 421.2226); $^1H$ NMR ($CDCl_3$) $\delta$ 0.84 (3 H, s, 14-H), 1.19 (3 H, d, *J* = 6 Hz, 8′-H), 1.72 (3 H, s, 16-H), 2.46 (1 H, dd, *J* = 8, 15 Hz, 3α-H), 3.00 (2 H, AB, *J* = 4 Hz, 13-H), 5.49 (1 H, d, *J* = 5 Hz, 10-H), 5.73 (1 H, d, *J* = 11 Hz, 2′-H), 6.66 (1 H, dd, *J*'s = 11 Hz, 3′-H), 7.62 (1 H, dd, *J* = 11, 16 Hz, 4′-H).

For **17**: an oil; $[\alpha]^{28}_D$ -3.3° (*c* 0.39, $CHCl_3$); UV max (MeOH) 260 nm (log $\epsilon$ 4.53); mass spectrum (chemical ionization, methane gas reagent), *m/e* 421.2232 ($M^+$ + H, calcd 421.2226); $^1H$ NMR ($CDCl_3$) $\delta$ 0.84 (3 H, s, 14-H), 1.16 (3 H, d, *J* = 6 Hz, 8′-H), 1.74 (3 H, s, 16-H), 2.47 (1 H, dd, *J* = 8, 15 Hz, 3α-H), 3.07 (2 H, AB, *J* = 4 Hz, 13-H), 4.18 (1 H, m, 6′-H), 5.50 (1 H, d, *J* = 5 Hz, 10-H), 5.74 (1 H, d, *J* = 11 Hz, 2′-H), 6.67 (1 H, dd, *J* = 11, 11 Hz, 3′-H), 7.61 (1 H, dd, *J* = 11, 16 Hz, 4′-H).

For **18**: mp 78–79 °C (EtOAc/petroleum ether); $[\alpha]^{28}_D$ -21.5° (*c* 0.39, $CHCl_3$); UV max (MeOH) 260 nm (log $\epsilon$ 4.60); mass spectrum (chemical ionization, methane gas reagent), *m/e* 533.2714 ($M^+$ + H, calcd 533.2754); $^1H$ NMR ($CDCl_3$) $\delta$ 0.82 (3 H, s, 14-H), 1.21 (3 H, d, *J* = 6 Hz, 8′-H), 1.74 (3 H, s, 16-H), 2.53 (1 H, dd, *J* = 8, 15 Hz, 3α-H), 3.04 (2 H, AB, *J* = 4 Hz, 13-H), 4.18 (2 H, s, 15-H), 5.52 (1 H, d, *J* = 5 Hz, 10-H), 5.75 (1 H, d, *J* = 11 Hz, 2′-H), 5.90 (1 H, s, 2′-H), 6.67 (1 H, dd, *J*'s = 11 Hz, 3′-H), 7.63 (1 H, dd, *J* = 11, 16 Hz, 4′-H).

For **19**: an oil; $[\alpha]^{28}_D$ -32.2° (*c* 0.57, $CHCl_3$); UV max (MeOH) 260 nm (log $\epsilon$ 4.53); mass spectrum (chemical ionization, methane gas reagent) *m/e* 533.2722 ($M^+$ + H, calcd 533.2754); $^1H$ NMR ($CDCl_3$) $\delta$ 0.82 (3 H, s, 14-H), 1.15 (3 H, d, *J* = 6 Hz, 8′-H), 1.70 (3 H, s, 16-H), 2.48 (1 H, dd, *J* = 8, 15 Hz, 3α-H), 3.04 (2 H, AB, *J* = 4 Hz, 13-H), 4.18 (2 H, s, 15-H), 5.51 (1 H, d, *J* = 5 Hz, 10-H), 5.73 (1 H, d, *J* = 11 Hz, 2′-H), 5.93 (1 H, s, 2′-H), 6.68 (1 H, dd, *J*'s = 11 Hz, 3′-H), 7.58 (1 H, dd, *J* = 11, 16 Hz, 4′-H).

The triacetates of these compounds were prepared as described previously.

Trichoverrol A triacetate: mass spectrum (chemical ionization, methane gas reagent), $m/e$ 547.2533 ($M^+ + H$, calcd 547.2543); $^1H$ NMR ($CDCl_3$) $\delta$ 0.82 (3 H, s, 14-H), 1.21 (3 H, d, $J$ = 6 Hz, 8′-H), 1.72 (3 H, s, 16-H), 2.07, 2.09, and 2.15 (3 H each, 3 s, acetates), 2.58 (1 H, dd, $J$ = 8, 15 Hz, 3$\alpha$-H), 2.99 (2 H, AB, $J$ = 4 Hz, 13-H), 4.11 (2 H, AB, $J$ = 12 Hz, 15-H), 5.08 (1 H, dq, $J$'s = 6 Hz, 7′-H), 5.47 (1 H, d, $J$ = 5 Hz, 10-H), 5.75 (1 H, d, $J$ = 11 Hz, 2′-H), 5.92 (1 H, dd, $J$ = 6, 16 Hz, 5′-H), 6.55 (1 H, dd, $J$'s = 11 Hz, 3′-H), 7.64 (1 H, dd, $J$ = 11, 16 Hz, 4′-H).

Trichoverrol B triacetate: mass spectrum (chemical ionization, methane gas reagent) $m/e$ 547.2665 ($M^+ + H$, calcd 547.2543); $^1H$ NMR ($CDCl_3$) $\delta$ 0.82 (3 H, s, 14-H), 1.23 (3 H, d, $J$ = 6 Hz, 8′-H), $\delta$ 1.72 (3 H, s, 16-H), 2.07, 2.11, and 2.13 (3 H each, 3 s, acetates), 2.58 (1 H, dd, $J$ = 8, 15 Hz, 3$\alpha$-H), 2.99 (2 H, AB, $J$ = 4 Hz, 13-H), 4.12 (2 H, AB, $J$ = 12 Hz, 15-H), 5.14 (1 H, dq, $J$ = 4, 6 Hz, 7′-H), 5.47 (1 H, dd, $J$ = 6, 16 Hz, 5′-H), 6.57 (1 H, dd, $J$'s = 11 Hz, 3′-H), 7.64 (1 H, dd, $J$ = 11, 16 Hz, 4′-H).

Trichoverrin A triacetate: mass spectrum (chemical ionization, methane gas reagent), $m/e$ 599.2865 ($M^+ + H$, $CH_3COOH$; calcd for $C_{33}H_{43}O_{10}$ 599.2856); $^1H$ NMR ($CDCl_3$2) $\delta$ 0.82 (3 H, s, 14-H), 1.21 (3 H, d, $J$ = 6 Hz, 8′-H), 1.72 (3 H, s, 16-OH), 2.02, 2.04, and 2.12 (3 H each, 3 s, acetates), 2.20 (3 H, s, 6″-H), 2.99 (2 H, AB, $J$ = 4 Hz, 13-H), 3.74 (1 H, d, $J$ = 5 Hz, 11-H), 3.85 (1 H, d, $J$ = 5 Hz, 2-H), 5.08 (1 H, dq, $J$'s = 6 Hz, 6′-H), 5.46 (1 H, m, 10-H), 5.74 (1 H, d, $J$ = 11, 2′-H), 6.54 (1 H, dd, $J$'s = 11 Hz, 3′-H), 7.64 (1 H, dd, $J$ = 11, 16 Hz, 4′-H).

Trichoverrin B triacetate: mass spectrum (chemical ionization, methane gas reagent), $m/e$ 599.2835 ($M^+ + H$, $CH_3COOH$; calcd for $C_{33}H_{43}O_{10}$ 599.2856); $^1H$ NMR ($CDCl_3$) $\delta$ 0.82 (3 H, s, 14-H), 1.22 (3 H, d, $J$ = 6 Hz, 8′-H), 1.72 (3 H, s, 16-H), 2.04, 2.06, and 2.12 (3 H each, 3 s, acetates), 2.22 (3 H, s, 6″-H), 2.97 (2 H, AB, $J$ = 4 Hz, 13-H), 3.76 (1 H, d, $J$ = 5 Hz, 11-H), 3.88 (1 H, d, $J$ = 5 Hz, 2-H), 5.14 (1 H, d, $J$ = 5 Hz, 11-H), 3.88 (1 H, d, $J$ = 5 Hz, 2-H), 5.14 (1 H, dq, $J$ = 4, 6 Hz, 6′-H), 5.46 (1 H, m, 10-H), 5.76 (1 H, d, $J$ = 11 Hz, 2′-H), 6.56 (1 H, dd, $J$'s = 11 Hz, 3′-H), 7.64 (1 H, dd, $J$ = 11, 16 Hz, 4′-H).

**Transformations of the Trichoverrins to the Macrocyclic Trichothecenes.** Spores of *M. verrucaria* grown on N-Z amine agar were added to Czapek–Dox media (300 mL) and allowed to grow at 28 °C in a shake culture. After 2 days, the solution was divided into three equal portions and added to three separate Fernback flasks containing 1 L of production media. After 3 days of growth, the mycelium in each flask was separately centrifuged, washed, and resuspended in 1 L of sterile water. Trichoverrin A (100 mg) and trichoverrin B (100 mg) were added separately to flasks one and two; flask three was used as the control. After 7 days, the mycelium was removed, and the supernatants were extracted with ethyl acetate (2 × 500 mL). The ethyl acetate solutions were dried ($MgSO_4$) and concentrated, and the resulting oily material was passed through 10 g of TLC grade silica gel (elution with 100 mL of 5% $CH_3OH$ in $CH_2Cl_2$) to give extract A (100 mg), extract B (115 mg), and extract C (20 mg). HPLC analysis of C showed that it contained only trace amounts of the macrocyclic trichothecenes. Preparative HPLC ($MeOH/CH_2Cl_2$) of A gave 10 mg of verrucarin A, 4 mg of verrucarin B, and 52 mg of recovered unaltered trichoverrin A. Preparative HPLC of B gave 13 mg of verrucarin A, 6 mg of verrucarin B, and 50 mg of recovered unreacted trichoverrin B. Other fractions from the HPLC contained small but significant amounts (ca. 1–2 mg) of verrucarin J, roridins A, and roridin E and/or iso E.

**Roridin J (9).** A 5-L portion of the aqueous concentrate derived from the methanol wash of the XAD-2 resin was treated with 100 g of sodium chloride and allowed to stand for 12 h at 5–10 °C. Filtration gave 7.5 g of a tan solid which was subjected to flash chromatographies (EtOAc/petroleum ether followed by $MeOH/CH_2Cl_2$ eluents). The fractions rich in verrucarin A were combined to give 2.8 g of a solid which was subjected to chromatography on 200 g of neutral alumina (activity grade II) with dichloromethane followed by increasing amounts of methanol in dichloromethane as eluent. Elution with 0–0.5% methanol in dichloromethane afforded fraction B (372 mg). Fraction B was crystallized from dichloromethane–hexane to give 165 mg of roridin J (9): mp 281–285 °C; $[\alpha]^{28}_D$ +21.8° ($c$ 0.65, $CHCl_3$); UV (EtOH) $\lambda_{max}$ 261 nm (log $\epsilon$ 4.28); mass spectrum (electron impact), $m/e$ 528 ($M^+$); $^1H$ NMR ($CDCl_3$) $\delta$ 0.87 (3 H, s, 14-H), 1.36 (3 H, d, $J$ = 6 Hz, 14′-H), 1.74 (3 H, s, 16-H), 2.28 (1 H, d, $J$ = 1.2 Hz, 12′-H), 2.48 (1 H, dd, $J$ = 8, 15 Hz, 3$\alpha$-H), 2.97 (2 H, AB, $J$ = 4 Hz, 13-H), 3.63 (1 H, d, $J$ = 5 Hz, 11-H), 3.85 (1 H, d, $J$ = 5 Hz, 2-H), 3.85 (1 H, d, $J$ = 7 Hz, 4′-H), 4.21 (2 H, AB, $J$ = 12 Hz, 15-H), 5.24 (1 H, d, $J$ = 7 Hz, 5′-H), 5.44 (1 H, d, $J$ = 5 Hz, 10-H), 5.80 (1 H, d, $J$ = 15.5 Hz, 7′-H), 5.90 (1 H, d, $J$ = 11.5 Hz, 10′-H), 6.54 (1 H, dd, $J$'s = 11.5 Hz, 9′-H), 7.70 (1 H, dd, $J$ = 11.5, 15.5 Hz, 8′-H).

Anal. Calcd for $C_{29}H_{36}O_9 \cdot 0.5H_2O$: C, 64.79; H, 6.94. Found: C, 64.84; H, 6.99.

**Roridin J Acetate.** A solution of 55 mg of roridin J in 0.25 mL each of acetic anhydride and pyridine was allowed to stand at room temperature for 24 h. Removal of the solvents in vacuo gave a residue which was subjected to preparative TLC on silica gel (one 0.5 mm plate) with 3% methanol in dichloromethane as the eluent to provide 42 mg (71%) of roridin J acetate: mp 230–235 °C; $[\alpha]^{28}_D$ -40.6° ($c$ 0.55, $CHCl_3$); mass spectrum (chemical ionization, methane gas reagent), $m/e$ 571.2528 ($M^+ + H$ calcd 571.2543); $^1H$ NMR ($CDCl_3$) $\delta$ 0.87 (3 H, s, 14-H), 1.36 (3 H, d, $J$ = 6 Hz, 14′-H), 1.72 (3 H, s, 16-H), 2.10 (3 H, s, acetate), 2.28 (3 H, d, $J$ = 1.2 Hz, 12′-H), 2.47 (1 H, dd, $J$ = 8, 15 Hz, 3$\alpha$-H), 2.97 (2 H, AB, $J$ = 4 Hz, 13-H), 3.63 (1 H, d, $J$ = 5 Hz, 11-H), 3.84 (1 H, d, $J$ = 5 Hz, 2-H), 4.21 (2 H, AB, $J$ = 12 Hz, 15-H), 4.97 (1 H, d, $J$ = 7 Hz, 4′-H), 5.42 (1 H, d, $J$ = 7 Hz, 5′-H), 5.43 (1 H, d, $J$ = 5 Hz, 10-H), 5.81 (1 H, d, $J$ = 15.5 Hz, 7′-H), 5.88 (1 H, d, $J$ = 11.5 Hz, 10′-H), 6.56 (1 H, dd, $J$'s = 11.5 Hz, 9′-H), 7.70 (1 H, dd, $J$ = 11.5, 15.5 Hz, 8′-H).

**Isolation of Compounds from the Mycelium.** A portion (48 g) of the mycelium extract was subjected to partition chromatography on 500 g of Celite impregnated with 250 mL of 18% water in methanol. The column was eluted with petroleum ether followed by increasing amounts of dichloromethane in petroleum ether up to 40% dichloromethane in petroleum ether. Fractions were combined on the basis of TLC analysis to give a total of eight fractions: A(6.0 g), B (3.7 g), C (3.4 g), D (3.0 g), E (4.2 g), F (16.6 g), G (10.0 g), H (1.0 g, methanol wash).

Fraction D on flash chromatography (30–40% ethyl acetate in hexane) gave, after recrystallization, 2.5 g of verrucarin J (3) (from dichloromethane–ether) and 100 mg of verrucarin B (2) (from dichloromethane–hexane). Similarly, fraction C on flash chromatography (20–30% ethyl acetate) gave 2.54 g of roridin H (8) (crystallized from dichloromethane–hexane) and 0.5 g of verrucarin J.

**Trichodermadiene (13).** Flash chromatography of fraction B with 1% methanol in dichloromethane gave 980 mg of 13, followed by 320 mg of roridin H. Trichodermadiene was recrystallized from ether: mp 145–146 °C; $[\alpha]^{27}_D$ +17.7° ($c$ 3.2, $CHCl_3$); UV (EtOH) 264 nm (log $\epsilon$ 4.41); mass spectrum (electron impact), $m/e$ 386 ($M^+$); $^1H$ NMR ($CDCl_3$) $\delta$ 0.74 (3 H, s, 14-H), 1.00 (3 H, s, 15-H), 1.36 (3 H, d, $J$ = 5 Hz, 8′-H), 1.72 (3 H, s, 16-H), 2.66 (1 H, dd, $J$ = 7.5, 15 Hz, 3$\alpha$-H), 2.97 (2 H, AB, $J$ = 4 Hz, 13-H), 2.97 (1 H, dq, $J$ = 2, 5 Hz, 7′-H), 3.21 (1 H, dd, $J$ = 2, 8 Hz, 6′-H), 3.64 (1 H, d, $J$ = 5.5 Hz, 11-H), 3.83 (1 H, d, $J$ = 5 Hz, 2-H), 5.43 (1 H, d, $J$ = 5 Hz, 10-H), 5.7 (1 H, dd, $J$ = 4, 7.5 Hz, 4-H), 5.7 (1 H, d, $J$ = 11 Hz, 2′-H), 5.7 (1 H, dd, $J$ = 8, 15.5 Hz, 5′-H), 6.57 (1 H, dd, $J$'s = 11 Hz, 3′-H), 7.83 (1 H, dd, $J$ = 11, 15.5 Hz, 4′-H).

Anal. Calcd for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.38, H, 8.07.

**Verrucarin L (4).** Flash chromatography of fraction G (3% MeOH in $CH_2Cl_2$) gave a fraction (4.1 g) rich in roridin A, which upon crystallization from dichloromethane–hexane yielded 2.2 g of roridin A. The mother liquor was subjected to HPLC (ca. 30–40 mg/injection, 2% MeOH in $CH_2Cl_2$) to give 15 mg of 4 which elutes just before roridin A. Recrystallization from dichloromethane–ether gave 4: mp 230–235 °C; $[\alpha]^{27}_D$ +15.0° ($c$ 0.92, $CHCl_3$); UV (EtOH) 262 nm (log $\epsilon$ 4.42); mass spectrum (chemical ionization, methane gas reagent), $m/e$ 501.2112 ($M^+ + H$, calcd 501.2124); $^1H$ NMR ($CDCl_3$) $\delta$ 0.86 (3 H, s, 14-H), 1.87 (3 H, s, 16-H), 2.26 (3 H, d, $J$ = 1 Hz, 12′-H), 2.99 (2 H, AB, $J$ = 4 Hz, 13-H), 3.80 (1 H, d, $J$ = 5 Hz, 11-H), 4.45 (2 H, AB, $J$ = 12 Hz, 15-H), 5.2 (1 H, m, 8-H), 5.58 (1 H, d, $J$ = 5 Hz, 10-H), 6.00 (1 H, d, $J$ = 16 Hz, 7′-H), 6.12 (1 H, d, $J$ = 11 Hz, 10′-H), 6.65 (1 H, dd, $J$'s = 11 Hz, 9′-H), 8.10 (1 H, dd, $J$ = 11, 16 Hz, 8′-H).

Acetylation of **4** (10 mg in 0.2 mL each of acetic anhydride and pyridine, 16 h at ambient temperature) after crystallization from ether–hexane gave acetate **4a**, identical in all respects (mixture melting point, $^1$H NMR spectrum, and TLC) with the natural compound isolated below.

**Verrucarin L Acetate (4a).** Fraction E was subjected to MPLC (30–40% ethyl acetate in hexane) to give 100 mg of verrucarin B, 70 mg of verrucarin L acetate (**4a**), and 2.8 g of a mixture of verrucarin A and roridin J. Recrystallization of **4a** from ether–hexane gave crystals: mp 132–135 °C; $[\alpha]^{27}_D$ +29.7° (*c* 0.52, $CHCl_3$), UV (EtOH) 261 nm (log $\epsilon$ 4.28); mass spectrum (chemical ionization, methane gas reagent), *m/e* 543.2217 ($M^+$ + H, calcd 543.2230); $^1$H NMR ($CDCl_3$) $\delta$ 0.86 (3 H, s, 14-H), 1.80 (3 H, s, 16-H), 1.94 (acetate), 2.27 (3 H, d, $J$ = 1 Hz, 12′-H), 2.97 (2 H, AB, $J$ = 4 Hz, 13-H), 3.75 (1 H, d, $J$ = 5 Hz, 11-H), 4.40 (2 H, AB, $J$ = 12 Hz, 15-H), 5.70 (1 H, d, $J$ = 5 Hz, 10-H), 5.96 (1 H, d, $J$ = 16 Hz, 7′-H), 6.07 (1 H, d, $J$ = 11 Hz, 10′-H), 6.61 (1 H, dd, $J$'s = 11 Hz, 9′-H), 8.01 (1 H, dd, $J$ = 11, 16 Hz, 8′-H).

**Roridin K Acetate (10).** Fraction F was subjected to MPLC (30–60% ethyl acetate in hexane) to yield a fraction (6 g) composed principally of a mixture of roridin E and isororidin E in a ratio of ca. 1:4.[34] The following fraction (320 mg) was subjected to HPLC (20% ethyl acetate in hexane, 40 mg/injection) to give 100 mg of roridin D and 65 mg of roridin K acetate, which was recrystallized from dichloromethane–hexane to give **4a**: mp 255–257 °C; $[\alpha]^{25}_D$ +2.1° (*c* 5.6, $CHCl_3$), UV (EtOH) 263 nm (log $\epsilon$ 4.21); mass spectrum (chemical ionization, methane gas reagent), *m/e* 573.2692 ($M^+$ + H, calcd 573.2700); $^1$H NMR ($CDCl_3$) $\delta$ 0.78 (3 H, s, 14-H), 1.19 (3 H, d, $J$ = 6 Hz, 14′-H), 1.76 (3 H, s, 16-H), 1.19 (acetate), 2.30 (3 H, d, $J$ = 1.2 Hz, 12′-H), 2.52 (1 H, dd, $J$ = 7, 15 Hz, 3$\alpha$-H), 2.97 (2 H, AB, $J$ = 4 Hz, 13-H), 3.84 (1 H, d, $J$ = 5 Hz, 2-H), 3.90 (1 H, d, $J$ = 5 Hz, 11-H), 4.31 (2 H, AB, $J$ = 12 Hz, 15-H), 5.75 (1 H, d, $J$ = 11 Hz, 10′-H), 5.78 (1 H, d, $J$ = 16 Hz, 7′-H), 6.10 (1 H, dd, $J$ = 4, 8 Hz, 4-H), 6.57 (1 H, dd, $J$'s = 11 Hz, 9′-H), 7.47 (1 H, dd, $J$ = 11, 16 Hz, 8′-H).

**Hydrolysis of Esters.** All new compounds (ca. 10 mg each) were transesterified at room temperature in 0.3 M sodium methoxide (2–3 h reaction time). The reaction was quenched by passage through a small column packed with Dowex 50W-X4 acidic resin. The solvent was removed, and the crude product was purified by preparative TLC. Trichodermadiene (**13**), **14**, and **15** yielded trichodermol (**11**), mp 116–117 °C (lit.[6] mp 117.5–118 °C). Roridin J (**9**) and **16**–**19** each gave verrucarol; compounds **4**, **4a**, and **10** gave 4$\beta$,8$\alpha$,15-trihydroxy-12,13-epoxytrichothec-9-ene (**20**), mp 177–178 °C (undepressed upon admixture with an authentic sample).[3]

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**Registry No.** **1**, 3148-09-2; **2**, 2290-11-1; **3**, 4643-58-7; **4**, 77101-87-2; **4a**, 77101-88-3; **5**, 14729-29-4; **6**, 14682-29-2; **7**, 16891-85-3; **7a**, 64726-84-7; **8**, 29953-50-2; **9**, 74072-83-6; **9** acetate, 74098-61-6; **10**, 80326-33-6; **11**, 2198-93-8; **12**, 2198-92-7; **13**, 75323-72-7; **14**, 76740-74-4; **14** diacetate, 80326-34-7; **15**, 76685-81-9; **15** diacetate, 80374-38-5; **16**, 76739-71-4; **16** triacetate, 80326-35-8; **17**, 76685-83-1; **17** triacetate, 80374-39-6; **18**, 76739-70-3; **18** triacetate, 80326-36-9; **19**, 76685-82-0; **19** triacetate, 80408-18-0; **20**, 74516-69-1.

(34) These isomers are very difficult to separate. They may be separated with difficulty on a silica column (HPLC) by using 60% ethyl acetate in hexane. Reverse-phase HPLC (C-18 column, MeOH–$H_2O$) works somewhat better.

# *Notes*

## Dehalogenation of $\alpha$-Chloro and $\alpha$-Bromo Ketones. Use of Sodium *O,O*-Diethyl Phosphorotelluroate

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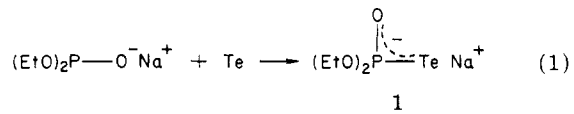
Conversion of $\alpha$-halo ketones into the parent ketones is a reaction that is sometimes used in synthesis,[1] and several new reagents have been introduced recently[2] for this transformation. We have found that sodium *O,O*-diethyl phosphorotelluroate (**1**) is a mild and convenient reagent for such dehalogenations.

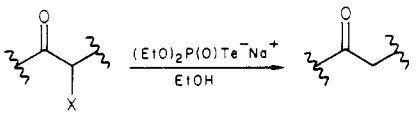
The salt **1** is readily prepared[3] (eq 1) by dissolving



(1)

**1**

metallic tellurium in an ethanol solution of sodium diethyl phosphite. As reported previously,[3] compound **1** converts epoxides into olefins. It also dehalogenates $\alpha$-halo ketones (eq 2), and our results are collected in Table I for a number



(2)

X = Cl, Br

of $\alpha$-chloro and $\alpha$-bromo ketones. In each case the halo ketone, usually in ethanol but sometimes in THF, was injected into a solution containing a stoichiometric amount of the phosphorotelluroate. The reaction was then allowed to proceed at room temperature or at 80 °C with the results indicated.

One of the reaction products is elemental tellurium, and entry 4, parts b and c (Table I) shows that less than 1 equiv of the metal can be used, although, for best results, a

(1) (a) Stork, G.; MacDonald, T. L. *J. Am. Chem. Soc.* **1975**, *97*, 1264. (b) Deprês, J.-P.; Greene, A. E. *J. Org. Chem.* **1980**, *45*, 2036. (c) For conversion of olefins into $\alpha$-halo ketones see: Cardillo, G.; Shimizu, M. *Ibid.* **1977**, *42*, 4268. Sharpless, K. B.; Teranishi, A. Y. *Ibid.* **1973**, *38*, 185.

(2) Olah, G. A.; Arvanaghi, M.; Vankar, Y. D. *J. Org. Chem.* **1980**, *45*, 3531. Alper, H.; Pattee, L. *Ibid.* **1979**, *44*, 2568. Alper, H.; Des Roches, D. *Ibid.* **1976**, *41*, 806. Luh, T.-Y.; Lai, C. H.; Lei, K. L.; Tam, S. W. *Ibid.* **1979**, *44*, 641. Ho, T.-L.; Wong, C. M. *Ibid.* **1974**, *39*, 562. Sauers, R. R.; Hu, C. K. *Ibid.* **1971**, *36*, 1153. Borowitz, I. J.; Kirby, K. C., Jr.; Rusek, P. E.; Lord, E. *Ibid.* **1969**, *34*, 2687. Kuivila, H. G.; Menapace, L. W. *Ibid.* **1963**, *28*, 2165. Denis, J. N.; Krief, A. *Tetrahedron Lett.* **1981**, 1431. Alper, H. *Ibid.* **1975**, 2257. Townsend, J. M.; Spencer, T. A. *Ibid.* **1971**, 137. Olah, G. A.; Vankar, Y. D.; Fung, A. P. *Synthesis* **1979**, 59. Ho, T.-L.; Olah, G. A. *Ibid.* **1976**, 807. Ho, T.-L. *Synth. Commun.* **1979**, *9*, 241. Ho, T.-L.; Wong, C. M. *Ibid.* **1973**, *3*, 237.

(3) Clive, D. L. J.; Menchen, S. M. *J. Org. Chem.* **1980**, *45*, 2347.

0022-3263/82/1947-1124$01.25/0 