(8Z)-Eicosa-4,5,8-trienoic Acid (7). A mixture of 0.60 g (25 mmol) of magnesium turnings in 15 mL of THF was treated with 1.0 mL (13 mmol) of ethyl bromide and briefly refluxed to initiate the Grignard reaction. The mixture was cooled to room temperature and 0.487 g (1.4 mmol) of bromide **6a** in 3 mL of THF was added. The mixture was refluxed for 15 min, cooled, and poured into a slurry of 100 g of CO_2 (s) and 100 mL of ether. When the CO_2 had evaporated, the reaction mixture was poured into saturated aqueous NH_4Cl and extracted with ether. The organic solution was washed with brine, dried over Na_2SO_4, concentrated, and chromatographed on 40 g of silica gel, eluting with 10% ether/hexane to give 0.083 g (19%) of acid **7**: IR 1960 (C=C=C) and 1710 cm^{-1} (C=O); ^1H NMR (300 MHz) δ 5.4 (m, 2, H-8,9), 5.16 (m, 2, H-4,6), 2.73 (m, 2, H-7), 2.47 (t, 2, J = 7 Hz, H-2), 2.32 (m, 2, H-3), 2.02 (dt, 2, J = 6 and 6 Hz, H-10), 1.3 (m, 18, CH_2), and 0.88 ppm (t, 3, J = 6 Hz, H-20).

Anal. Calcd for $C_{20}H_{34}O_2$: C, 78.38; H, 11.18. Found: C, 78.44;, H, 11.01.

Carboxylic acids **8** and **9** were prepared in the same manner from bromides **6b** and **6c**.

(8Z,11Z)-Eicosa-4,5,8,11-tetraenoic acid (8): 10% yield; IR 1960 and 1710 cm^{-1}; ^1H NMR (300 MHz) δ 5.4 (m, 4, H-8,9,11,12), 5.19 (m, 2, H-4,6), 2.78 (m, 4, H-7,10), 2.47 (t, 2, J = 7 Hz, H-2), 2.32 (m, 2, H-3), 2.05 (dt, 2, J = 6 and 6 Hz, H-13), 1.3 (m, 12, CH_2), and 0.89 ppm (t, 3, J = 6 Hz, H-20).

Anal. Calcd for $C_{20}H_{32}O$: C, 78.90; H, 10.59. Found: C, 78.61; H, 10.38.

(8Z,11Z,14Z)-Eicosa-4,5,8,11,14-pentaenoic acid (9): 35% yield; IR 1960 and 1710 cm^{-1}; ^1H NMR (300 MHz) δ 5.4 (m, 6, H-8,9,11,12,14,15), 5.20 (m, 2, H-4,6), 2.8 (m, 6, H-7,10,13), 2.47 (t, 2, J = 7 Hz, H-2), 2.32 (m, 2, H-3), 2.05 (dt, 2, J = 6 and 6 Hz, H-16), 1.3 (m, 6, CH_2), and 0.90 ppm (t, 3, J = 7 Hz, H-20).

Anal. Calcd for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.58; H, 10.32.

(2E)-Dodec-2-en-1-ol (17). To a mixture of 1 L of NH_3 (liquid) and 0.3 g of ferric nitrate in a 3 L three-necked flask equipped with a dry ice condenser, KOH drying tube, and mechanical stirrer was added 3.1 g of lithium wire in small portions, allowing the blue color to discharge between additions. A solution of 10 mL of propargyl alcohol in 250 mL of THF was added over 30 min, and the reaction mixture was allowed to reflux 1 h. Then 20 mL of 1-bromononane in 200 mL of THF was added over 30 min, and the reaction mixture was allowed to reflux 1 h. At the end of this period 2.1 g of lithium wire was added in portions that resulted in a persistent dark blue color. After 10 min NH_4Cl was added to discharge the blue color and the NH_3 was evaporated with a stream of N_2. The residue was poured onto 1 L of ice, and the resulting mixture was saturated with NaCl and extracted with ether (3 × 400 mL). The extracts were dried over K_2CO_3, concentrated, and distilled to give 15.33 g (89%) of alcohol **17**: bp 95-97 °C (0.8 mm); IR 3400 cm^{-1}; ^1H NMR (90 MHz) δ 5.66 (m, 2, =CH), 4.1 (d, 2, J = 5 Hz, OCH_2), 2.05 (m, 2, =CCH_2), 2.30 (m, 14, CH_2), and 0.9 ppm (t, 3, J = 6 Hz, CH_3).

Anal. Calcd for $C_{12}H_{24}O$: C, 78.20; H, 13.12. Found: C, 78.34; H, 12.90.

(2E)-Dodec-2-enal (18). This aldehyde was prepared from alcohol **18** by PCC oxidation[21] (74%): bp 85 °C (0.4 mm); IR 1685 cm^{-1} (C=O); ^1H NMr (300 MHz) δ 9.50 (d, 1, J = 8 Hz, CHO), 6.84 (dt, 1, J = 17 and 6 Hz, =$CHCH_2$), 6.08 (ddt, 1, J = 17, 8, and 1.5 Hz, =C*H*CHO), 2.33 (m, 2, =CCH_2), 1.25 (m, 14, CH_2), and 0.85 ppm (t, 3, J = 6 Hz, CH_3).

Anal. Calcd for $C_{12}H_{22}O$: C, 79.06; H, 12.16. Found: C, 79.09; H, 11.97.

(7Z,9E)-Nonadeca-3,4,7,9-tetraen-1-yl *tert*-Butyldimethylsilyl Ether (19). The Wittig reaction between aldehyde **18** and the ylide derived from phosphonium salt **1** under the conditions for the preparation of **4a** gave the tetraene **19** in 62% yield: bp 120 °C (0.02 mm); IR 3020 (=CH) and 1960 cm^{-1} (C=C=C); UV 236 nm (E 25,000); ^1H NMR (300 MHz) δ 6.28 (dd, 1, J = 16 and 10 Hz, H-9), 5.99 (dd, 1, J = 10 and 10 Hz, H-8), 5.68 (dt, 1, J = 16 and 7 Hz, H-10), 5.30 (dt, 1, J = 11 and 8 Hz, H-7), 5.11 (m, 2, H-3,5), 3.66 (t, 2, J = 7 Hz, H-1), 2.86 (m, 2, H-6), 2.20 (ddt, 2, J = 7, 7, and 3.5 Hz, H-3), 2.10 (dt, 2, J = 7 and 7 Hz, H-11), 1.4-1.2 (m, 14, CH_2), 0.90 (m, 12, *t*-Bu and H-19), and 0.06 ppm (s, 6, $SiMe_2$); ^{13}C NMR δ 204.75, 135.59, 129.42, 126.73, 125.35, 89.56, 88.37, 63.00, 32.92, 32.65, 31.91, 29.58, 29.53, 29.38, 29.34, 29.28, 27.30, 25.95, 22.69, 18.36, 14.12, and -5.24 ppm.

Anal. Calcd for $C_{25}H_{46}OSi$: C, 76.85; H, 11.87. Found: C, 77.02; H, 11.75.

Registry No. 1, 86118-22-1; **2**, 86118-23-2; **3a**, 112-54-9; **3b**, 68141-15-1; **3c**, 13553-09-8; **4a**, 86118-24-3; **4b**, 86118-25-4; **4c**, 86118-26-5; **5a**, 86118-27-6; **5b**, 86118-28-7; **5c**, 86118-29-8; **6a**, 86118-30-1; **6b**, 86118-31-2; **6c**, 86118-32-3; **7**, 86118-33-4; **8**, 86118-34-5; **9**, 85654-39-3; **10**, 78592-82-2; **11**, 86118-35-6; **12**, 86118-36-7; **13**, 86118-37-8; **14**, 86118-38-9; **15**, 86118-39-0; **16**, 86118-40-3; **17**, 69064-36-4; **18**, 20407-84-5; **19**, 86118-41-4; methanesulfonyl chloride, 124-63-0; arachidonate lipoxygenase, 63551-74-6.

(21) E. J. Corey and J. W. Suggs, *Tetrahedron Lett.*, 2647 (1975).

New Trichoverroids from *Myrothecium verrucaria*: Verrol and 12,13-Deoxytrichodermadiene

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Two new naturally occurring trichothecenes have been isolated as minor metabolites from a culture of *Myrothecium verrucaria*. The metabolites verrol, previously obtained only as a hydrolysis product, and 12,13-deoxytrichodermadiene were characterized by NMR and mass spectral data. The latter compound is only the second trichothecene reported to be lacking the 12,13-epoxy functional group.

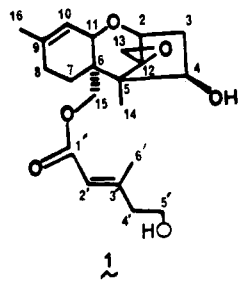
The trichothecene group of antibiotics has attracted a good deal of attention in recent years due principally to the high degree of biological activity associated with these potent mycotoxins.[1] During the course of the workup of

Table I. ^{13}C and ^{1}H NMR Data for Verrol (1), Deoxytrichodermadiene (2), and Verrol Diacetate[a]

position	verrol		verrol diacetate,	deoxytrichodermadiene	
	^{13}C shifts	^{1}H shifts	^{1}H shifts	^{13}C shifts	^{1}H shifts
2	78.8 (d)	3.82 (d, 5.1)	3.83 (d, 5.1)	75.7 (d)	4.47 (d, 5)
3α	39.9 (t)	2.60 (dd, 15.7, 7.5)	2.54 (dd, 7.8, 15.7)		2.62 (dd, 7, 15)
3β		2.00 (m)	1.98 (m)	38.0 (t)	2.00 (m)
4	74.5 (d)	4.50 (br)	5.70 (m)	75.1 (d)	5.70 (m)
5	49.2			52.1	
6	42.8			40.4	
7	21.4 (t)	1.9 (m)	1.9 (m)	23.6 (t)	1.90 (m)
8	28.1 (t)	2.0 (m)	2.0 (m)	28.1 (t)	1.90 (m)
9	140.7			139.8	
10	118.6 (d)	5.42 (d, 5.4)	5.43 (d, 5.6)	119.0 (d)	5.40 (d, 5)
11	66.7 (d)	3.64 (d, 5.4)	3.72 (d, 5.6)	70.6 (d)	3.68 (d, 5)
12	65.6			152.7	
13	47.6 (t)	2.96 (AB, 3.9)	2.99 (AB, 4.0)	104.9 (t)	4.75 (s), 5.16 (s)
14	7.1 (q)	0.87 (s)	0.81 (s)	10.2 (q)	0.98 (s)
15	62.9 (t)	4.05 (AB, 12.4)	4.12 (AB, 12.4)	16.2 (q)	0.98 (s)
16	23.1 (q)	1.70 (br s)	1.71 (br s)	23.3 (q)	1.72 (br s)
1′	166.0			169.4	
2′	117.2 (d)	5.73 (q, 1.2)	5.70 (q, 1.2)	118.3 (d)	5.80 (d, 11)
3′	157.3			143.2 (d)	6.60 (t, 11)
4′	43.9 (t)	2.42 (t, 6.1)	2.48 (t, 6.7)	129.9 (d)	7.75 (dd, 11, 15)
5′	60.3 (t)	3.80 (t, 6.1)	4.22 (t, 6.7)	140.3 (d)	5.70 (dd, 8, 15)
6′	18.8 (q)	2.21 (d, 1.2)	2.20 (d, 1.2)	58.8 (d)	3.21 (dd, 2, 8)
7′				57.0 (d)	3.01 (dq, 2, 5)
8′				17.5 (q)	1.40 (d, 5)
$C(O)CH_3$			2.07		
$C(O)CH_3$			2.05		

[a] Spectra were taken in $CDCl_3$ as solvent with Me_4Si as an internal standard. Chemical shifts are reported in parts per million with Me_4Si at 0.0 ppm. Coupling constants are reported in parentheses along with the multiplicities (s = singlet, d = doublet, etc.).

a large-scale fermentation of *Myrothecium verrucaria* (ATCC 24571),[2] which is a producer of macrocyclic trichothecenes,[3] we have isolated two new minor metabolites which belong to a new class of trichothecenes, the trichoverroids.[2,4] Like many of the other trichoverroids, these compounds, verrol (1) and deoxytrichodermadiene (2),



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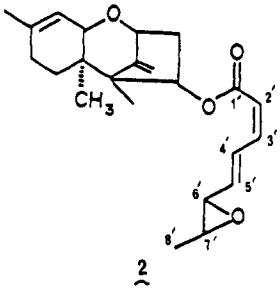

2

possess only a portion of the macrolide ring which spans the C-4, C-15 positions in the macrocyclic trichothecenes (e.g., verrucarins and roridins). Compound 2 is particularly interesting because it is only the second trichothecene isolated from natural sources which lacks the 12,13-epoxy functionality; verrucarin K is the other example.[5]

The crude extract of the large-scale fermentation of *Myrothecium verrucaria* was subjected to a series of partition and adsorption chromatographies.[2] The fraction with an R_f value (SiO_2, 3% methanol in methylene chloride) between those for the trichodermadienediols and trichoverrols[2] was further purified by column chromatography (SiO_2, $MeOH/CH_2Cl_2$) to give verrol (1). The structure assignment was based on spectral data (see Experimental Section). Verrol (1) formed a diacetate, suggesting the presence of two OH groups, and base hydrolysis gave verrucarol (3). Inspection of the ^{1}H NMR spectrum of verrol revealed that it lacks the right-hand dienic portion of the other trichoverroids, roridins and verrucarins, as evidenced by the absence of downfield signals at ca. δ 8 and 6.6. In the ^{1}H NMR spectrum, signals due to the six-carbon acrylic acid ester side chain were evident (Table I). This, along with ^{13}C data, led us to believe that verrol has structure 1. Finally, hydrolysis of verrucarin J gave verrol, as reported earlier by Tamm's group.[6] The spectral data for naturally occurring and synthetically derived verrol were identical.

Deoxytrichodermadiene (2) was isolated from an extract of the mycelium by the use of partition and adsorption chromatographies. The structure assignment was based on spectral data (see Experimental Section). A noteworthy feature of the proton NMR spectrum (Table I) was the absence of the C-13 methylene AB pattern. Instead, two singlets at δ 4.75 and 5.16 were observed for the terminal olefinic protons at position 13. Another curious feature is that the peaks for C-14 and C-15 quaternary methyl groups are coincident with each other at δ 0.98. However, when the spectrum was run in deuteriobenzene,[7] two peaks were observed at δ 0.89 (3 H) and 0.80 (3 H). The ^{13}C chemical shifts for the two carbons, however, were resolved, with C-14 and C-15 occurring at δ 10.2 and 16.2, respec-

(1) (a) Bamburg, J. R.; Strong, F. M. In "Microbial Toxins"; Kadis, S., Ciegler, A., Ajl, S. J., Eds.; Academic Press: New York, 1971; Vol. 7, p 207. (b) Ueno, Y. *Adv. Nutr. Sci.* **1980**, *3*, 301. (c) Doyle, T. W.; Bradner, W. T. In "Anticancer Agents Based on Natural Product Models"; Cassidy, J. M., Duros, J. D., Eds.; Academic Press: New York, 1980; p 43. (d) Ong, C. W. *Heterocycles* **1982**, *9*, 1685.

(2) Jarvis, B. B.; Stahly, G. P.; Pavanasasivam, G.; Midiwo, J. O.; DeSilva, T.; Holmlund, C. E.; Mazzola, E. P.; Geoghegan, R. F. Jr. *J. Org. Chem.* **1982**, *47*, 1117.

(3) (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, 1970; p 69. (d) Jarvis, B. B.; Mazzola, E. P. *Acc. Chem. Res.* **1982**, *15*, 388.

(4) Jarvis, B. B.; Pavanasasivam, G.; Holmlund, C. E.; DeSilva, T.; Stahly, G. P.; Mazzola, E. P. *J. Am. Chem. Soc.* **1981**, *103*, 472.

(5) Breitenstein, W.; Tamm, C. *Helv. Chim. Acta* **1977**, *60*, 1522.

(6) Fetz, E.; Bohner, B.; Tamm, C. *Helv. Chim. Acta* **1965**, *48*, 1669.

(7) Bovey, F. A. "Nuclear Magnetic Resonance Spectroscopy"; Academic Press: New York, 1969; p 44.

tively. The rest of the NMR spectroscopic data, including the pendant epoxide group at C-6′ and C-7′, which show the diagnostic doublet of doublets and doublet of quartets around δ 3.0, match very closely those of trichodermadiene.[2,8] These data are consistent with structure **2** assigned for deoxytrichodermadiene. Table I lists the ^{13}C and ^{1}H NMR chemical shifts of the two new compounds.

Experimental Section

General Methods. Ultraviolet spectra were determined on a Hitachi 100-80A spectrophotometer. Nuclear magnetic resonance spectra were determined in deuteriochloroform, unless otherwise noted, on a Varian XL-200, Varian XL-100, EM-390, or FT-80A 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 by Harvey Chemical Laboratories, Charlottesville, VA, on a VG Micromass 70/70 HS instrument. Thin-layer chromatography was carried out on prepared silica gel plates (E. Merck), and visualization was effected with short-wavelength UV light or sulfuric acid/ethanol/vanillin (20/3/1) spray. Flash chromatography was carried out on silica gel 60 (230–400 mesh, E. Merck). Medium-pressure liquid chromatography (MPLC) was carried out on Licroprep 60 (E. Merck) silica gel. High-performance liquid chromatography (HPLC) was performed with an Altex Model 332 gradient liquid chromatograph. Separations were carried out on a Whatman Magnum 9 (10/15) semipreparative Partisil column.

Isolation of Verrol (1). The details of the fermentation and initial chromatographic separations leading to a fraction containing 1, i.e., fraction III, are described elsewhere.[2] The crude fraction (29 g) was subjected to flash chromatography (SiO_2, 0–8% methanol in methylene chloride) to yield three major fractions rich in roridin A (1.2 g), verrol (461 mg), and trichoverrins (195 mg), respectively. The verrol-containing fraction was subjected to flash chromatography again under the conditions described above to yield 230 mg of a fraction which was mostly verrol and trichoverrols. This was further purified by column chromatography on silica (10–25% 2-propanol in hexane) to yield 120 mg of verrol[9] as an oil ($R_f \approx 0.2$ in both 5% MeOH/CH_2Cl_2 and 25% 2-propanol in hexane): mass spectrum (chemical ionization, methane gas reagent), m/e 379.2112 ($M^+ + H$ calcd 379.2120).

Acetylation of Verrol. A mixture of 10 mg of verrol in 25 μL each of acetic anhydride and pyridine was allowed to stand overnight. Removal of solvents in vacuo followed by preparative TLC on silica gel (50% EtOAc in hexane) provided verrol diacetate whose NMR spectral data are presented in Table I.

Isolation of Deoxytrichodermadiene. The workup of mycelium leading to the fraction containing this compound is described elsewhere.[2] Fraction I (6 g) was passed through a silica gel flash column and eluted with dichloromethane to yield several fractions. The first fraction (0.50 g) was subjected to preparative TLC (20% hexane in CH_2Cl_2) on 2-mm silica gel plates to yield 25 mg of an oil. Further purification was done on a Magnum-9 column (Whatman, Inc., 9 mm i.d., SiO_2) under gradient conditions: 90–100% methylene chloride in hexane, 30 min. The procedure gave 12 mg of **2**: oil; $[\alpha]^{25}_D - 5.6°$ (c 0.95, $CHCl_3$); UV max (MeOH) 265 nm (log ε 3.42); mass spectrum (chemical ionization, methane gas reagent), m/e 371.2216 ($M^+ + H$ calcd 371.2222). See Table I for NMR data.

Acknowledgment. This investigation was supported by Grant No. CA-25967 awarded by the National Cancer Institute, DHHS.

Registry No. 1, 84412-91-9; 1 diacetate, 85994-28-1; 2, 85957-00-2.

(8) Jarvis, B. B.; Midiwo, J. O.; Stahly, G. P.; Pavanasasivam, G.; Mazzola, E. P. *Tetrahedron Lett.* **1980**, 787.

(9) From another strain of *M. verrucaria* we found that reverse-phase chromatography on a C-18 column employing 40–60% methanol in water as the solvent can be used to separate and isolate verrol from trichoverrols and trichoverrins, the order of elution being trichoverrol B, trichoverrol A, verrol, trichoverrin B, and trichoverrin A.

Photocyclization of 2,6-Dichlorocinnamic Acid Derivatives to 5-Chlorocoumarin

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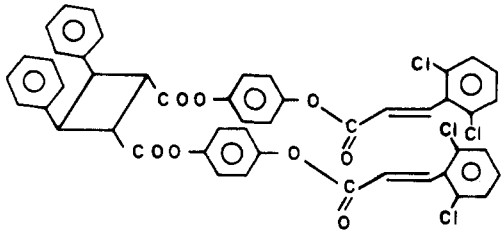
On UV irradation, 2,6-dichlorocinnamic acid and its esters undergo photocyclization with elimination of the elements of HCl or RCl (R = alkyl or aryl) to yield 5-chlorocoumarin (**2**); amide derivatives yield the corresponding imino analogues. Low-temperature irradiation monitored by infrared and optical spectrophotometry allowed the identification of o-quinomethyl ketene as one of the intermediates of this reaction and suggested a mechanism for the photocycloelimination.

While the solution photobehavior of the cyclobutane derivative **1**[1] was being studied (as a possible route to a tricyclic derivative containing two cyclobutane rings), it was found that instead of the anticipated product, a totally



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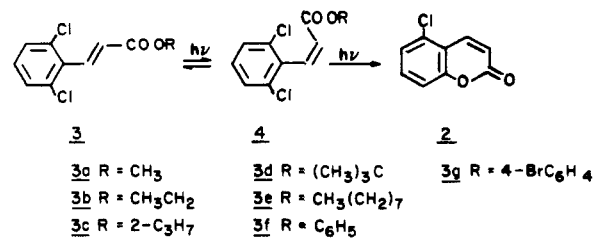
Scheme I



3 **4** **2**

3a R = CH_3
3b R = CH_3CH_2
3c R = 2-C_3H_7
3d R = $(CH_3)_3C$
3e R = $CH_3(CH_2)_7$
3f R = C_6H_5
3g R = 4-BrC_6H_4

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(1) R. Arad-Yellin, Ph.D. Thesis, Weizmann Institute of Science, Rehovot, Israel, 1977.

0022-3263/83/1948-2578$01.50/0