2-(Phthalimidomethyl)-4-benzylidene-2-oxazolin-5-one (19). To a solution of 1.03 g (2.91 mmol) of phthaloylglycylphenylalanine (**18**) in 5 ml of acetic anhydride containing 1 drop of pyridine was added 651 mg (2.65 mmol) of *o*-chloranil, and after 2 h 0.5 ml of pyridine was added. After standing for 1 day, the reaction mixture was filtered, and the solid precipitate was dried in vacuo giving 851 mg (96%) of crude **19**, mp 215–220 °C. Crystallization from ethyl acetate–hexane gave 445 mg (50%) of an analytical sample of **19**: mp 232–233 °C; UV (95% ethanol) λ_{max} 333 nm (ϵ 22 500); IR (Nujol) 1810 and 1780 (C=O), 1665 cm^{-1} (C=N); NMR (Me_2SO-d_6), 90 MHz) δ 8.23–8.02 (m, 2 H, ortho H's of C_6H_5CH=), 7.94 (s, 4 H, phthaloyl H's), 7.48–7.36 (m, 3 H, meta and para H's of C_6H_5CH=), 7.33 (s, 1 H, C_6H_5CH=), and 4.90 ppm (s, 2 H, CH_2); ^{13}C (Me_2SO-d_6) 166.8, 166.3, and 163.3 (amide C=O, oxazolin C=O, C=N), 134.8, 132.7, 132.0, 131.4, 128.8, and 123.4 (aromatic and vinyl), 35.5 ppm (CH_2N); mass spectrum *m/e* (rel intensity) 332 (41), 188 (22), 161 (42), 160 (45), 133 (19), 116 (18), 104 (42), 89 (28), 77 (50), 76 (50), 63 (21), 51 (39), 50 (39), 39 (13).

Anal. Calcd for $C_{19}H_{12}N_2O_4$: C, 68.67; H, 3.64; N, 8.43. Found: C, 68.75; H, 3.64; N, 8.43.

***N*-Phthaloylglycinedehydrophenylalanine Ethyl Ester (20).** To a solution of 1 ml of 0.5 N sodium ethoxide in 25 ml of absolute ethanol, 200 mg (0.69 mmol) of **19** was added. After stirring for 30 min at room temperature the reaction mixture was poured into a flask containing 4 ml (7.0 mequiv) of Amberlite IR-120H resin in 4 ml of ethanol. After 30 min, the resin was removed by filtration and the solvent was evaporated in vacuo. The crude residue was crystallized from 2:1 ethanol–watr, giving 200 mg (88%) of **20**: mp 200–202 °C (lit.[6] 200–201 °C); IR (KBr) 3270, 1775, and 1730 (C=O), 1690 and 1650 cm^{-1} (amide C=O).

Acknowledgment. We gratefully acknowledge the financial support of the Vice President for Research, University of Georgia, for part of this work.

Registry No.—**1a,** 60422-60-8; **1b,** 60422-61-9; **1c,** 60422-62-0; **1d,** 60422-63-1; **1e,** 60422-64-2; **1f,** 60422-65-3; **1g,** 60676-46-2; **2a,** 60422-66-4; **2b,** 60422-67-5; **2d,** 56538-58-0; **2e,** 60442-68-6; **3a,** 60422-70-0; **3b,** 60676-45-1; **4,** 60422-77-7; **5a,** 60676-48-4; **5b,** 60422-81-3; **5c,** 60422-82-4; **5d,** 60676-49-5; **6,** 60676-50-8; **7a,** 60422-83-5; **7c,** 60422-84-6; **9,** 7554-10-1; **12,** 60422-80-2; **16a,** 60676-51-9; **16b,** 26924-22-1; **16c,** 60676-52-0; **16d,** 27573-05-3; **16e,** 60676-53-1; **18,** 60676-54-2; **19,** 60676-55-3; **20,** 55424-41-4; $HO_2CCH(R)NHCOR'$ (R = $PhCH_2$; R' = Ph), 2901-76-0; $HO_2CCH(R)NHCOR'$ (R = $(CH_3)_2CH$; R' = Ph), 2901-80-6; $HO_2CCH(R)NHCOR'$ (R = CH_3; R' = Ph), 1205-02-3; $HO_2CCH(R)$-$NHCOR'$ (R = H; R' = Ph), 495-69-2; $HO_2CCH(R)NHCOR'$ (R = Ph; R' = Ph), 29670-63-1; $HO_2CCH(R)NHCOR'$ (R = $(CH_3)_2CHCH_2$; R = CH_3), 17966-67-5; *o*-chloranil, 2435-53-2; DL-phenylalanine, 150-30-1; aniline, 62-53-3; benzyl mercaptan, 100-53-8.

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Structure of Satratoxin H, a Metabolite of *Stachybotrys atra*. Application of Proton and Carbon-13 Nuclear Magnetic Resonance

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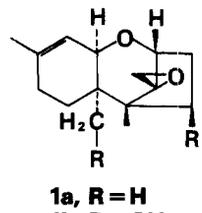
The structure of satratoxin H, a toxic metabolite of *Stachybotrys atra*, has been shown to be **4a** by spectroscopic studies. Satratoxin H is a macrocyclic dilactone derivative of the sesquiterpene 12,13-epoxytrichothec-9-ene and is structurally similar to roridin E, a known metabolite of *Myrothecium verrucaria*. It is most probably one of the causative agents of stachybotryotoxicosis, a food-borne disease which has affected both livestock and humans and which presents a potentially serious public health hazard.

Stachybotryotoxicosis is a food-borne disease which has affected both livestock and humans and which presents a potentially serious public health hazard.[1] The disease results from eating foods contaminated with toxic metabolites of the fungus *Stachybotrys atra*. Recent work in our laboratories has demonstrated that several derivatives of 12,13-epoxytrichothec-9-ene (**1a**) are produced by this mold and are most probably the cause of this disease.[2] Two of these metabolites, initially designated satratoxins C[3] and D,[2] were found by thin layer chromatography and ^1H NMR and mass spectral examination to be, in fact, the trichothecenes verrucarin J (**2**)[4] and roridin E (**3**),[5] respectively, reported by Tamm and co-

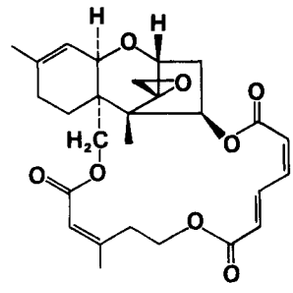
Table I. ^{1}H NMR Data[a] of Roridin E (3), Satratoxin H (4a), and Satratoxin H Diacetate (4b)

Position	3[b,c]	4a[d]	4b[c]
2	3.8 m	3.9 m	3.8 m
3	2.5 m	2.45 dd (7.5, 15)	2.5 m
3	2 m	2.20 dt (4, 5, 15)	2.2 m
4	6.22 dd (4, 8)	5.9 m	5.9 m
7	2 m	1.9 m	2 m
8	2 m	2.1 m	2 m
10	5.5 d (5)	5.46 d (5)	5.5 d (5)
11	3.7 m	3.62 d (5)	3.65 d (5)
13	3.0[e] AX (4)	2.81 d (4)	2.85 d (4)
		3.12 d (4)	3.15 d (4)
14	0.82 s	0.83 s	0.85 s
15	4.15[e] AX (12)	3.88 d (12)	3.86 d (12)
		4.56 d (12)	4.54 d (12)
16	1.75 s	1.74 s	1.75 s
2′	5.98 s	5.85 d (2)	5.85 s
4′A	2.4–2.7[f] m	3.74 dt (3, 10)	3.8 m
4′B		2.6 m	2.6 m
5′	3.5–4.0 m	3.9 m	3.8 m
7′	5.7–6.0 m	6.09 d (17.5)	6.05 d (17.5)
8′	7.53 dd (11, 15)	7.36 dd (10.5, 17.5)	7.4 dd (10.5, 17.5)
9′	6.58 t (11)	6.63 t (10.5)	6.65 t (10.5)
10′	5.75 d (11)	5.91 d (10.5)	5.9 d (10.5)
12	2.30 d (1.5)	3.97 s	5.3 s
13′	3.7 q (6)	4.38 q (7)	5.6 q (7)
14′	1.22 d (6)	1.16 d (7)	1.1 d (7)

[a] In $CDCl_3$, parts per million from Me_4Si, J values in hertz in parentheses. [b] Reference 4. [c] Spectra recorded at 100 MHz. [d] Spectra recorded at 300 MHz. [e] Center of AX system. [f] Both protons reported in this region.



1a, R = H
1b, R = OH



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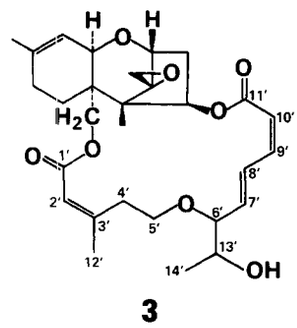

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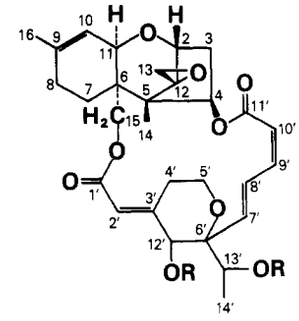


4a, R = H
4b, R = Ac

workers. We now describe spectral studies which show that the structure of another *S. atra* metabolite, designated satratoxin H, is **4a**.

Satratoxin H, $C_{29}H_{36}O_9$, is hydrolyzed in methanolic sodium hydroxide to the dialcohol verrucarol (**1b**).[2] This product accounts for 15 carbon atoms, two ethereal oxygen atoms, and five of the 12 elements of unsaturation. The presence of two alcohol groups in the product and an analogy to the congeners verrucarin J (**2**) and roridin E (**3**) imply that satratoxin H is a cyclic ester of a dicarboxylic acid and the dialcohol (**1b**), a conclusion amply supported by the ^{13}C NMR spectrum (vide infra). Thus, the functional character of a total of six oxygen atoms (two ester and two ether groups) and a total of eight elements of unsaturation are accounted for.

Signals in the 300-MHz ^{1}H NMR spectrum (Table I) arising from the verrucarol nucleus are readily identified.[6] Most notable are the resonances at 0.83 and 1.74 ppm due to the methyl groups at positions 14 and 16, respectively, and the AX pattern centered at 3 ppm, J = 4 Hz, due to the epoxide at position 13. A doublet at 4.56 ppm, J = 12 Hz, was shown by an INDOR experiment (at 100 MHz) to be coupled to a doublet in the complex at 3.8 ppm; the two protons correspond to the C-15 methylene group. An AX system of J = 5 Hz was readily assigned to the olefinic proton, H-10 (5.46 ppm), and the adjacent methine proton, H-11 (3.62 ppm). With the aid of published spectral studies, two broad peaks comprising four protons at 1.9 and 2.1 ppm could be assigned to the methylene groups of C-7 and C-8, respectively. Signals of suitable multiplicities for H-3α (2.45 ppm) and H-3β (2.20 ppm) were visible, but the multiplets of H-2 and H-4 were obscured in complex groups at 3.9 and 5.9 ppm, respectively.

Satratoxin H reacts with acetic anydride in pyridine to form a diacetate of molecular weight 612, identifying two of the remaining three oxygen atoms as hydroxyl groups. Four elements of unsaturation remain to be identified.

The ultraviolet absorption of satratoxin H suggested that

Table II. ^{13}C NMR Data[a] of Satratoxin H (4a) and Roridins A (5a), D (5b), and H (5c)

Position	4a	5a[b]	5b[b]	5c[b]
2	79.1 d	78.8 d	78.8 d	79.0 d
3	34.4 t	34.6 t	34.9 t	34.8 t
4	74.2 d	74.2 d	74.3 d	74.0 d
5	49.0 s	49.1 s	49.0 s	48.9 s
6	43.4 s	43.6 s	43.1 s	43.2 s
7	20.4 t	20.0 t	20.4 t	20.5 t
8	27.6 t	27.5 t	27.4 t	27.6 t
9	140.2 s	140.4 s	140.1 s	139.9 s
10	119.0 d[c]	118.2 d[c]	118.4 d[c]	118.6 d[c]
11	68.2 d	66.9 d	66.9 d	67.6 d
12	65.4 s	64.9 s	65.1 s	65.3 s
13	48.0 t	47.4 t	47.4 t	47.3 t
14	7.6 q	7.2 q	6.8 q	7.0 q
15	64.2 t	64.2 t	64.3 t	63.0 t
16	23.3 q	22.9 q	22.9 q	22.9 q
1′	166.2 s[d]	174.5 s	167.8 s	166.0 s
2′	119.0 d[c]	75.3 d	57.9 d	119.0 d[c]
3′	155.1 s	36.7 d	62.9 s	154.4 s
4′	25.3 dd	33.0 t	39.4 t	47.7 t
5′	60.4 t	69.5 t	67.3 t	100.8 d
6′	81.4 s	83.7 s	85.3 s	81.9 s
7′	134.2 d[e]	139.0 d	138.1 d	134.6 d
8′	132.2 d[e]	126.0 d	126.2 d	126.2 d
9′	143.0 d	143.6 d	142.9 d	142.5 d
10′	120.4 d[c]	117.2 d[c]	117.8 d[c]	118.9 d[c]
11′	167.0 s[d]	166.3 s	166.1 s	166.0 s
12′	73.7 d	14.4 q	17.2 q	18.2 q
13′	69.7 d	70.4 d	70.5 d	76.8 d
14′	15.7 q	18.0 q	17.9 q	16.3 q

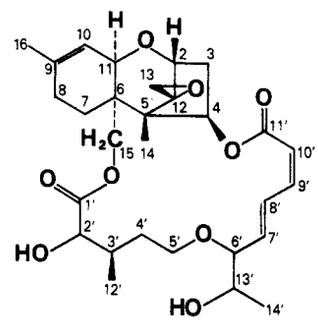
[a] In $CDCl_3$, parts per million from Me_4Si. [b] W. Breitenstein and C. Tamm, *Helv. Chim. Acta*, **58**, 1172 (1975). [c,d,e] These signals could not be more specifically assigned.

this metabolite retained the unsaturated systems common to the roridin and verrucarin groups, in which one ester is conjugated with a double bond and the other with a diene system. The olefinic protons visible in the ^1H NMR spectrum supported the existence of the diene, displaying peaks with couplings suitable to a cis–trans diene attached to a fully substituted carbon atom. A single peak in the olefinic region remained to be assigned, a doublet at 5.85 ppm, $J = 2$ Hz. This chemical shift is suitable for the α proton of an acrylic ester residue; the coupling is that of an allylic proton, and shows the β carbon to be fully substituted.

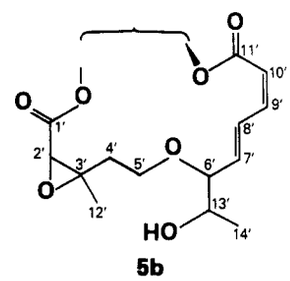
At midfield, a quartet at 4.38 ppm coupled to a methyl group at 1.16 ppm, $J = 7$ Hz, reveals a methyl carbinol system attached to a fully substituted carbon atom; a singlet at 3.97 ppm indicates a secondary alcohol flanked by fully substituted carbon atoms. These inferences are supported by comparison of the spectrum of satratoxin H with that of the diacetate (**4b**), in which both carbinol protons are shifted downfield by approximately 1 ppm.

The ^1H NMR spectra thus support the belief that the verrucarol moiety exists unaltered in satratoxin H and show the presence of an unsaturated diester lactone system closely resembling those of known metabolites in the roridin–verrucarin group.

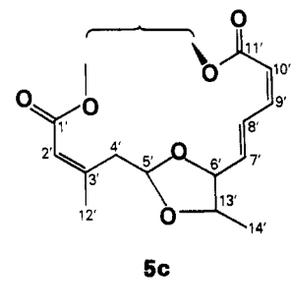
Comparison of the ^{13}C NMR chemical shift data of satratoxin H with those of roridins A, D, and H (**5a–c**) and relevant portions of related compounds[7] (Table II) supports the above conclusions and allows the identification of the 15 signals corresponding to the sesquiterpenoid verrucarol moiety. Those resonances due to the six olefinic and two carbonyl carbons of the macrocyclic dilactone are also readily distinguished. The observed chemical shifts of the carbonyl carbons provide unequivocal evidence (referred to above) for the diester system. In the single-frequency off-resonance decoupled spectra, singlets at 155.1 and 81.4 ppm confirm, moreover, the fully substituted nature of C-3′ and C-6′, while a quartet at 15.7 ppm and a doublet at 69.7 ppm are consistent with the postulated methyl carbinol pendant group.



5a



5b

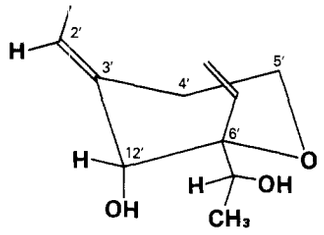


5c

Twenty-six of the 29 carbons are thus accounted for. The remaining three signals appeared in the off-resonance decoupled spectrum as follows: (1) a doublet at 73.7 ppm, (2) a triplet at 60.4 ppm, and (3) a doublet of doublets at 25.3 ppm. The first two signals correspond to methine and methylene carbons, respectively, which are attached to oxygen. The last

resonance is indicative of a methylene carbon which is not attached to oxygen and the protons of which possess widely separated chemical shifts.[8]

Knowledge of these remaining atoms may be developed as summarized in the following partial structure:



Only the methine carbon at 73.7 ppm can be the secondary alcohol group, C-12′, with the singlet proton at 3.97 ppm. It is, therefore, attached to the two fully substituted carbons identified as C-3′ and C-6′. However, as the singlet proton shows no allylic coupling, it must be in the plane of the double bond and thus be quite close to H-2′. This situation was demonstrated by a nuclear Overhauser experiment, in which irradiation of H-2′ resulted in a 24% increase in the integrated intensity of the singlet assigned to H-12′ (3.97 ppm). Additionally, decoupling irradiation at 2.6 ppm (H-4′B) caused the collapse of the H-2′ signal to a sharp singlet and altered the appearance of the complex pattern at 3.9 ppm (H-5′). The resonance at 25.3 ppm is assigned to a methylene carbon attached to C-3′, i.e. C-4′. The chemical shift of C-6′ (81.4 ppm) and that of the remaining methylene (60.4 ppm) require that they be attached to oxygen; this situation shows that the last element of unsaturation is the ether ring shown above. The fully substituted character of C-6′ shows that both the diene system and the methyl carbinol are attached here.

The structure of the macrocyclic dilactone moiety is thus complete with a single uncertainty, the stereochemistry at C-6′. There remains a question concerning its attachment to the tetracyclic verrucarol nucleus, i.e., whether C-1′ is connected to C-15 and C-11′ to C-4 or vice versa. Several lines of reasoning argue convincingly in favor of the former mode of attachment. Nine macrocyclic ring-containing trichothecenes have been reported to date: dehydroverrucarin A;[9] verrucarins A,[10] B,[10] and J;[4] roridins A,[11] D,[12] E,[5] and H;[13] and vertisporin.[14] The orientation of the various macrocyclic rings with respect to the verrucarol nucleus is the same for all of those trichothecenes. Because, in part, of similarities in chemical shifts, coupling constants, and general ^{1}H NMR spectral appearance[15] between satratoxin H and these nine trichothecenes, it is likely that the dilactone system of satratoxin H is attached to the sesquiterpenoid verrucarol nucleus in the same manner as the other macrocyclic rings (structure 4).

In addition, as previously stated, two of the metabolites which were isolated with satratoxin H proved to be compounds listed above, viz., verrucarin J and roridin E. From a biogenetic point of view, it is highly unlikely that the mold *S. atra* would produce two metabolites in which a macrocyclic ring is attached to a central nucleus in one manner and a third metabolite in which this mode of attachment is completely reversed.

The ^{13}C chemical shift data presented in Table II also offer compelling evidence for attachment of the dilactone ring of satratoxin H as depicted in structure **4**. Ellison and Kotsonis[7a] and Hanson and co-workers[7b] have demonstrated that subtle structural modifications can affect the chemical shifts of distant carbons in trichothecene systems. Apparently the chemical shifts of those carbons of the verrucarol nucleus close to the macrocyclic ring are sensitive to structural variations in the dilactone ring. The chemical shifts of such close-lying carbons (viz., carbons 4–8 and 15) in satratoxin H and roridins A, D, and H are very nearly identical (Table II), with average differences being of the order of 0.1 ppm. The dilactone system of satratoxin H is, therefore, most probably connected to the sesquiterpenoid moiety in the manner indicated.

The structure of satratoxin H represents an obvious variation on the biogenetic pathway of the roridins,[16] with the addition of a hydroxyl group on C-12′ and a dehydrogenative ring formation between C-6′ and C-12′.

Experimental Section

Satratoxin H was isolated according to the method of Eppley and Bailey.[2]

^{1}H NMR spectra of satratoxin H (no. 1603) were recorded by Dr. T. Suzuki on a Varian SC 300 spectrometer at the Institute of Polymer Science of the University of Akron, Akron, Ohio. Those of the diacetate derivative of satratoxin H were obtained on a Varian XL-100-15 spectrometer.

Proton-decoupled and single-frequency off-resonance decoupled ^{13}C NMR spectra were recorded on a Varian XL-100-15 spectrometer operating at 25.20 MHz.

Registry No.—3, 16891-85-3; **4a,** 53126-64-0; **4b,** 60538-74-1; **5a,** 14729-29-4; **5b,** 14682-29-2; **5c,** 29953-50-2.

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