# STEREOCHEMISTRY OF THE RORIDINS

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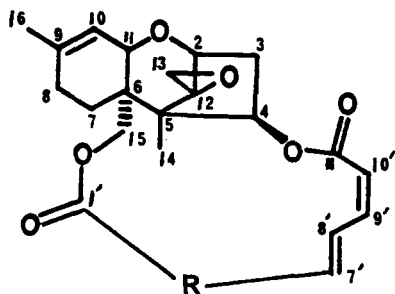
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ABSTRACT.—The configuration of the macrocyclic trichothecene roridin A has been established by an X-ray diffraction analysis. Roridin A was shown to be epimeric at C13' with a new trichothecene, isororidin A. Roridins were oxidatively cleaved to the corresponding verrucarins in high yield by treatment with pyridinium dichromate in DMF.
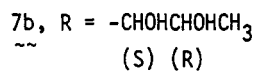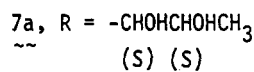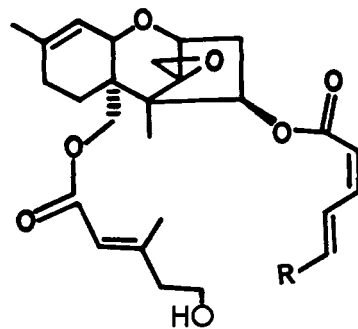
The trichothecene complex of antibiotics has attracted a great deal of interest mainly due to the potent biological activity of these sesquiterpenes (1–3). Our interests have centered on the more complex and largest membered subclass of these mycotoxins, the macrocyclic trichothecenes. Within this group, the roridins (e.g. 4–6) appear to be the central members in that further oxidative bio-transformations lead to the verrucarins (e.g. 1–3) (4–6), satratoxins (7, 8), vertisporin (9), 7β,8β-epoxyroridins (10, 11), and the baccharinoids (12). The roridins themselves appear to arise from ring-closure of the trichoverrins (7) (13), whose absolute structures have been established (13, 14). The stereochemistry at C6' in any of the roridins (4–6) has never been established, although this center is known to be (R) in the closely related baccharinoids (12). We have recently presented evidence that the baccharinoids, the only trichothecenes ever to be isolated from a higher plant, are the result of a plant absorbing, altering and chemically modifying roridins, presumably produced by a soil fungus in close association with the plant (15). The fact that the baccharinoids appear to be derived from the roridins strongly suggests that the roridins also will have the (R) configuration at C6'. Furthermore, the baccharinoids as found in *Baccharis megapotamica* (12) exist as sets of diastereomers, epimeric at C13'. Of the roridins, only roridin E (6) has been reported as having a naturally occurring diastereomer, isororidin E (6a) (10, 11), which must differ in configuration from roridin E at C6' and/or C13'. Herein, we report the determination of the configurations at C6' and C13' in roridin A (4), as established by X-ray diffraction, and the isolation of isororidin A (4a), which differs in configuration from roridin A at C13'.

## RESULTS AND DISCUSSION

A large scale fermentation with *Myrothecium verrucaria* (ATCC 24571) has produced a number of new trichothecenes as well as most of the previously characterized roridins and verrucarins (16). The principal metabolites isolated from this fermentation were verrucarins A (1) and J (3), roridin A (4), and a mixture of roridin E (6)-isororidin E (6a). A careful examination of chromatography fractions associated with roridin A yielded a new macrocyclic trichothecene, isororidin A (4a), which from ¹H and ¹³C nmr spectroscopy is clearly closely related to roridin A. In particular, the chemical shifts of the carbon atoms in 4 and 4a differ by 0.4 ppm or less except for C6', where for roridin A this resonance occurs at $\delta$ 83.7 ppm, and for isororidin A this carbon atom resonates at $\delta$ 82.6 ppm. The ¹H nmr spectra of 4 and 4a are nearly indistinguishable. Especially notable for both 4 and 4a is the doublet of quartets ($J = 3$ and 6 Hz) for the C2' proton at $\delta$ 4.1 ppm, which is the result of coupling to both H3' and the hydroxyl proton. This signal

1,  R = -CHOHCHCH₃CH₂CH₂OC-
    (S) (R)

$1,\ R = -\underset{(\underline{S})\ (\underline{R})}{CHOHCHCH_3CH_2CH_2O\overset{O}{\underset{6'}{C}}-}$

$2,\ R = -\underset{(\underline{S})(\underline{R})}{\underset{O}{\underset{\diagdown/}{CH-CCH_3CH_2CH_2O\overset{O}{C}-}}}$

$3,\ R = -\underset{(\underline{E})}{CH=CCH_3CH_2CHO\overset{O}{C}-}$

$4,\ R = -\underset{(\underline{S})\ (\underline{R})}{CHOHCHCH_3CH_2CH_2O}\underset{\underset{T}{(\underline{R})(\underline{R})}}{CHCHOHCH_3}$

$4a,\ R = -\underset{(\underline{S})\ (\underline{R})}{CHOHCHCH_3}\underset{(\underline{R})(\underline{S})}{CH_2CH_2OCHCHOHCH_3}$

$5,\ R = -\underset{(\underline{S})(\underline{R})}{\underset{O}{\underset{\diagdown/}{CH-CCH_3CH_2CH_2O\overset{|}{C}HCHOHCH_3}}}$

$6,\ R = -\underset{(\underline{E})}{CH=CCH_3CH_2CH_2O\overset{|}{C}HCHOHCH_3}$



7a,  R = -CHOHCHOHCH₃
     (S) (S)

7b,  R = -CHOHCHOHCH₃
     (S) (R)

collapses to a doublet ($J = 3$ Hz) upon exchange with deuterium oxide.   These data suggest that the expected normally rapid exchange of the C2' hydroxyl proton is significantly reduced by intramolecular hydrogen bonding with the adjacent ester carbonyl group.   Also, these data show that C2' has the same (S) configuration in isororidin A as is found for roridin A (6).

To establish whether roridin A and isororidin A differ in configuration only at C13', as in the baccharinoids, we sought a method to oxidize the hydroxyethyl group at C6' to the corresponding methyl ketone.   Although earlier workers (17) had oxidized 4 to a methyl ketone, this compound was isolated only in low yield, and the identity of the compound was not clearly established.[1]   Treatment of roridin A with Jones' reagent or pyridinium chlorochromate (PCC) gave a complex mixture of products; Collins' reagent also gave a mixture of products but one appeared to be predominant.   However, pyridinium dichromate (PDC) (18) re-

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[1]In particular, the oxidation product isolated (*ca.* 25% yield) (17) showed a ketone carbonyl absorption in the ir spectrum at $\nu = 1660$ cm⁻¹ which clearly cannot be assigned to a nonconjugated carbonyl group.

acted with both **4** and **4a** in either methylene chloride or DMF (the latter solvent is preferable since the reaction goes faster) to give the same single product in high yield. We were somewhat surprised to find that the product of this reaction is not a methyl ketone but rather verrucarin A (**1**). Precedent for this reaction is found in the oxidative cleavage by chromium trioxide of tertiary alcohols to yield ketones (19), although our oxidation conditions are much milder. In addition, benzylic ethers are readily oxidized by some chromium (VI) reagents to give benzoate esters, although PDC appears unreactive (20). Based on the probable reactive intermediates involved in these chromium (VI) oxidizations (21, 22), the oxidative cleavage of the C6'–C13' bond can be rationalized. We have used this reaction to establish unambiguously the absolute structure of roridin D (**5**), since **5** upon treatment with PDC gives verrucarin B (**2**), whose structure has been established by X-ray crystallography (23). Thus, roridin D has the 2'$S$,3'$R$ configuration as is found in verrucarin B. Roridins E and iso-E yield verrucarin J (**3**) in this reaction, which confirms the ($E$) assignment given the C2', C3' double bond in **3** (24).



Roridin A

(4)

Verrucarin A

(1)
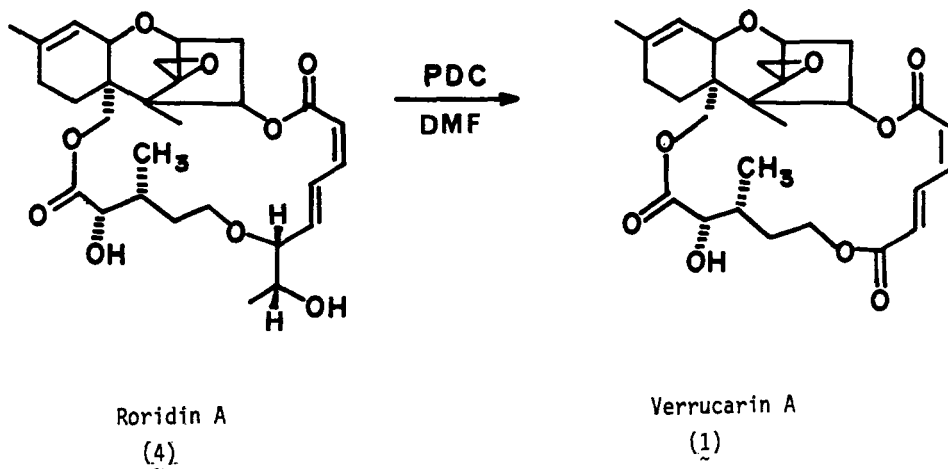
Following the procedure of Kupchan *et al.* (12) and Bohner and Tamm (17), we selectively reduced roridins A and iso-A to their respective tetrahydro derivatives, **8** and **8a**. Oxidation of these compounds with pyridinium chlorochromate gave the identical ketone **9** from both **8** and **8a**. Thus, as in the case of the baccharinoids, roridins A and iso-A are epimeric at C13'.

The configurations at C6' and C13' in **4** were established as C6'$R$, C13'$R$ by single-crystal X-ray diffraction analysis. Crystals of roridin A were grown by slow evaporative crystallization from a solution of **4** in methylene chloride in an atmosphere of hexane, and figure I illustrates the stereoconfiguration of roridin A. This study did not determine the absolute configuration of (**4**); however, the chirality shown in figure 1 was chosen to conform to the absolute configuration of verrucarin A(**1**) as determined by the X-ray study on the *p*-iodobenzenesulfonate derivative of **1** (25). Bond lengths and angles for **4** are listed in table 1. The C(5)–C(6) bond is somewhat long at 1.579(6)Å but is not unexpected in this type of a fused-ring system. The external CCC angles of the epoxide ring [i.e., C(2)–C(12)–C(13) at 125.6(4)° and C(5)–C(12)–C(13) at 126.0(4)°, are significantly larger than the external CCO angles at the epoxide ring, i.e., C(5)–C(12)–O(2) at 117.1(4)° and C(2)–C(12)-O(2) at 115.2(4)°]. These differences have been noted in other compounds having epoxide rings (26). The partially unsaturated terminal

**8,**    R = CHOHCH$_3$
         (R)

**9**

**8a,**   R = CHOHCH$_3$
         (S)
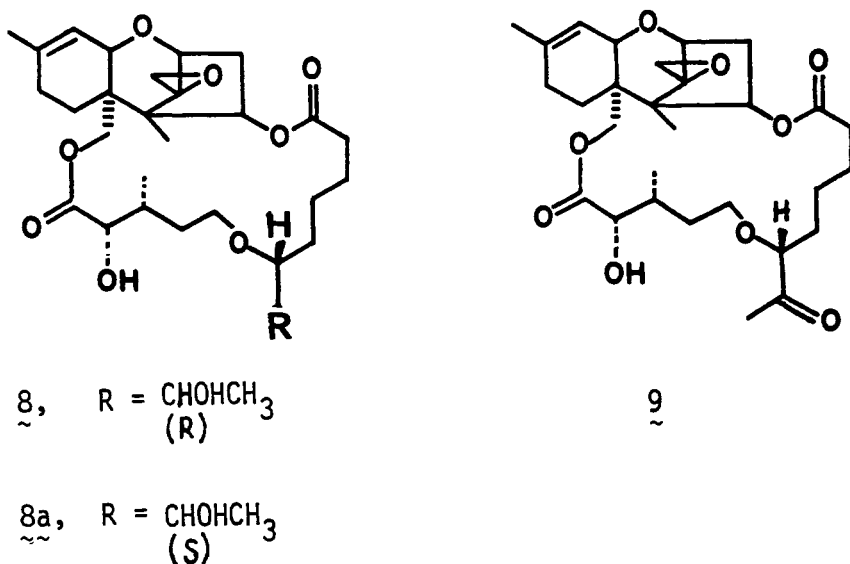
6-membered ring of the trichothecene moiety is in a somewhat flattened half-chair conformation.  The saturated central 6-membered ring has a slightly distorted full-chair conformation [the C(5)–C(6)–C(11)–O(1) torsion angle is only −41.5° as opposed to the expected value of ±60°].  The five-membered ring is in an envelope conformation with C(12) being out of the plane formed by C(2), C(3), C(4) and C(5).  The packing of the molecules in the unit cell is influenced by the presence of two intermolecular hydrogen bonds.  The hydrogen on O(7) is the donor to the carbonyl oxygen O(9) with an O . . . O distance of 2.83 Å and an O–H . . . O angle of 135.7°.  The hydrogen on O(8) is the donor to the ether oxygen O(2) with an O . . . O distance of 2.88 Å and an O–H . . . O angle of 164.4°.
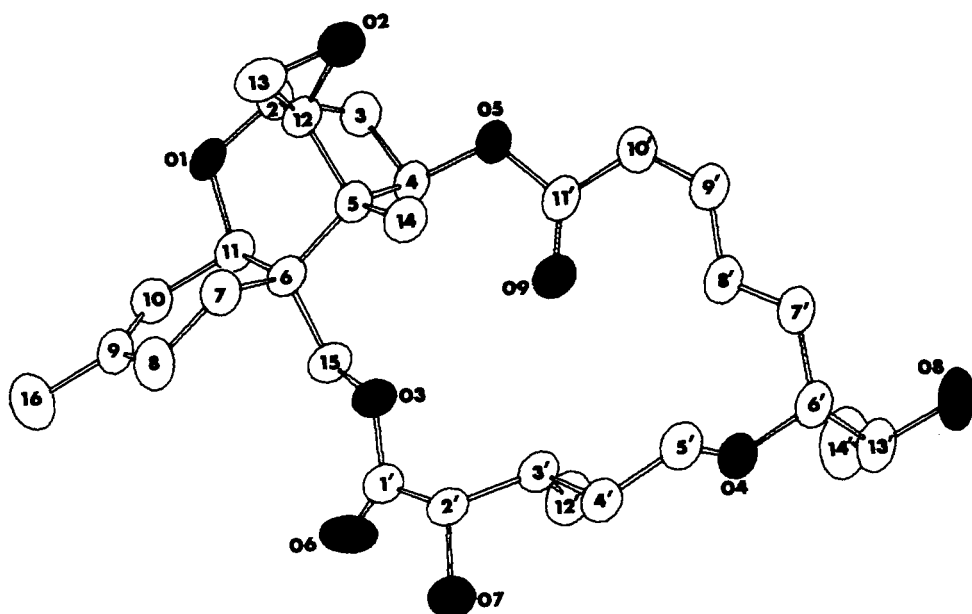


FIGURE 1.  The diagram, drawn by computer using PROGRAM ORTEP (27), is based on the final refined coordinates and thermal parameters at the 50% probability level. Oxygen atoms have been darkened.

TABLE 1. Bond Lengths and Angles for Roridin A[a].

| Atom | Atom | Distance (A) |
|------|------|--------------|
| O(1) | C(2) | 1.431 |
| O(1) | C(11) | 1.459 |
| C(2) | C(3) | 1.535 |
| C(2) | C(12) | 1.488 |
| C(3) | C(4) | 1.560 |
| C(4) | C(5) | 1.559 |
| C(4) | O(5) | 1.457 |
| C(5) | C(6) | 1.579 |
| C(5) | C(12) | 1.529 |
| C(5) | C(14) | 1.527 |
| C(6) | C(7) | 1.545 |
| C(6) | C(11) | 1.555 |
| C(6) | C(15) | 1.541 |
| C(7) | C(8) | 1.536 |
| C(8) | C(9) | 1.482 |
| C(9) | C(10) | 1.333 |
| C(9) | C(16) | 1.515 |
| C(10) | C(11) | 1.502 |
| C(12) | O(2) | 1.452 |
| C(12) | C(13) | 1.467 |
| C(13) | O(2) | 1.449 |
| C(15) | O(3) | 1.452 |
| | | |
| C(1)' | O(3) | 1.328 |
| C(1)' | O(6) | 1.196 |
| C(1)' | C(2)' | 1.507 |
| C(2)' | C(3)' | 1.531 |
| C(2)' | O(7) | 1.406 |
| C(3)' | C(4)' | 1.535 |
| C(3)' | C(12)' | 1.544 |
| C(4)' | C(5)' | 1.509 |
| C(5)' | C(4) | 1.441 |
| C(6)' | O(4) | 1.421 |
| | | |
| C(6)' | C(7)' | 1.504 |
| C(6)' | C(13)' | 1.543 |
| C(7)' | C(8)' | 1.339 |
| C(8)' | C(9)' | 1.454 |
| C(9)' | C(10)' | 1.355 |
| C(10)' | C(11)' | 1.458 |
| C(11)' | O(5) | 1.367 |
| C(11)' | O(9) | 1.210 |
| C(13)' | C(14)' | 1.493 |
| C(13)' | O(8) | 1.433 |

| Atom | Atom | Atom | Angle (Degrees) |
|------|------|------|-----------------|
| O(1) | C(2) | C(3) | 113.4 |
| O(1) | C(2) | C(12) | 107.6 |
| C(3) | C(2) | C(12) | 101.8 |
| C(2) | C(3) | C(4) | 105.2 |
| C(3) | C(4) | C(5) | 106.0 |
| C(3) | C(4) | O(5) | 106.7 |
| C(5) | C(4) | O(5) | 111.8 |
| | | | |
| C(4) | C(5) | C(6) | 108.5 |
| C(4) | C(5) | C(14) | 113.6 |
| C(4) | C(5) | C(12) | 99.9 |
| C(6) | C(5) | C(12) | 106.6 |
| C(6) | C(5) | C(14) | 114.6 |
| C(12) | C(5) | C(14) | 112.5 |
| C(5) | C(6) | C(7) | 111.5 |
| C(5) | C(6) | C(11) | 110.6 |
| C(5) | C(6) | C(15) | 113.5 |
| C(7) | C(6) | C(11) | 108.2 |
| C(7) | C(6) | C(15) | 109.4 |
| C(11) | C(6) | C(15) | 103.3 |
| C(6) | C(7) | C(8) | 112.3 |
| C(7) | C(8) | C(9) | 112.8 |
| C(8) | C(9) | C(10) | 122.3 |
| C(8) | C(9) | C(16) | 115.6 |
| C(10) | C(9) | C(16) | 112.0 |
| C(9) | C(10) | C(11) | 123.9 |
| O(1) | C(11) | C(10) | 104.7 |
| O(1) | C(11) | C(6) | 113.3 |
| C(6) | C(11) | C(10) | 114.8 |
| O(2) | C(12) | C(2) | 115.2 |
| O(2) | C(12) | C(5) | 117.1 |
| O(2) | C(12) | C(13) | 59.5 |
| C(2) | C(12) | C(5) | 105.0 |
| C(2) | C(12) | C(13) | 125.6 |
| C(5) | C(12) | C(13) | 126.0 |
| O(2) | C(13) | C(12) | 59.7 |
| C(6) | C(15) | O(3) | 109.0 |
| O(3) | C(1)' | O(6) | 125.6 |
| O(3) | C(1)' | C(2)' | 109.8 |
| C(2)' | C(1)' | O(6) | 124.6 |
| C(1)' | C(2)' | C(3)' | 111.5 |
| C(1)' | C(2)' | O(7) | 111.5 |
| C(3)' | C(2)' | O(7) | 110.3 |
| C(2)' | C(3)' | C(4)' | 108.9 |
| C(2)' | C(3)' | C(12)' | 111.0 |
| C(4)' | C(3)' | C(12)' | 113.3 |
| C(3)' | C(4)' | C(5)' | 116.2 |
| C(4)' | C(5)' | O(4) | 108.9 |
| O(4) | C(6)' | C(7)' | 112.5 |
| O(4) | C(6)' | C(13)' | 104.4 |
| C(7)' | C(6)' | C(13)' | 112.4 |
| C(6)' | C(7)' | C(8)' | 124.6 |
| C(7)' | C(8)' | C(9)' | 122.2 |
| C(8)' | C(9)' | C(10)' | 126.3 |
| C(9)' | C(10)' | C(11)' | 121.7 |
| C(10)' | C(11)' | O(5) | 110.3 |
| C(10)' | C(11)' | O(9) | 127.5 |
| O(5) | C(11)' | O(9) | 122.2 |
| C(6)' | C(13)' | C(14)' | 114.6 |
| C(6)' | C(13)' | O(8) | 105.1 |
| O(8) | C(13)' | C(14)' | 111.9 |
| C(2) | O(1) | C(11) | 114.7 |
| C(12) | O(2) | C(13) | 60.8 |
| C(1)' | O(3) | C(15) | 117.2 |
| C(5)' | O(4) | C(6)' | 112.9 |
| C(4) | O(5) | C(11)' | 116.0 |

[a]Based solely on least-squares results. Standard deviations are on the order of 0.006Å for bond lengths and 0.4° for bond angles.

TABLE 2.    Atomic Coordinates[a] for Roridin A.

| Atom | x | y | z | $B_{eq}(A^2)$[b] |
|------|-----|-----|-----|-----|
| O(1)............... | 0.5196(3) | 0.1810(4) | 0.7967(3) | 3.2(1) |
| O(2)............... | 0.3902(3) | 0.1450(4) | 0.4352(4) | 4.3(1) |
| O(3)............... | 0.7776(3) | 0.4098(4) | 0.5433(3) | 3.8(1) |
| O(4)............... | 1.0831(3) | 0.3809(4) | 0.0684(3) | 3.9(1) |
| O(5)............... | 0.6980(3) | 0.1083(4) | 0.4000(3) | 3.5(1) |
| O(6)............... | 0.9240(4) | 0.5031(5) | 0.6654(4) | 5.7(2) |
| O(7)............... | 0.9634(4) | 0.6048(4) | 0.4308(4) | 4.7(1) |
| O(8)............... | 1.2525(4) | 0.2296(5) | −.1555(4) | 6.3(2) |
| O(9)............... | 0.8715(4) | 0.2030(5) | 0.3673(4) | 4.1(1) |
| C(2)............... | 0.4990(5) | 0.1238(5) | 0.6737(5) | 3.4(1) |
| C(3)............... | 0.6254(5) | 0.0774(5) | 0.6296(5) | 3.6(1) |
| C(4)............... | 0.6882(5) | 0.1525(5) | 0.5359(5) | 3.1(1) |
| C(5)............... | 0.5921(5) | 0.2389(5) | 0.5269(5) | 3.3(1) |
| C(6)............... | 0.6277(4) | 0.3056(4) | 0.6567(5) | 3.1(1) |
| C(7)............... | 0.5282(5) | 0.3879(5) | 0.6629(5) | 3.7(1) |
| C(8)............... | 0.5606(5) | 0.4532(5) | 0.7891(5) | 4.3(1) |
| C(9)............... | 0.5881(5) | 0.3999(5) | 0.9213(5) | 3.9(1) |
| C(10).............. | 0.6185(5) | 0.3078(5) | 0.9238(5) | 3.7(1) |
| C(11).............. | 0.6282(4) | 0.2484(5) | 0.7951(5) | 3.2(1) |
| C(12).............. | 0.4638(4) | 0.1886(5) | 0.5542(5) | 3.3(1) |
| C(13).............. | 0.3306(5) | 0.2215(5) | 0.5088(6) | 4.4(1) |
| C(14).............. | 0.5843(5) | 0.2894(5) | 0.3858(5) | 3.7(1) |
| C(15).............. | 0.7682(5) | 0.3464(5) | 0.6613(5) | 3.5(1) |
| C(16).............. | 0.5817(6) | 0.4573(6) | 1.0539(6) | 5.6(2) |
| C(1')............. | 0.8650(5) | 0.4797(5) | 0.5581(6) | 3.8(1) |
| C(2')............. | 0.8838(5) | 0.5232(5) | 0.4178(5) | 3.4(1) |
| C(3')............. | 0.9411(5) | 0.4516(5) | 0.3193(5) | 3.5(1) |
| C(4')............. | 0.9625(5) | 0.5013(5) | 0.1808(5) | 3.8(1) |
| C(5')............. | 0.9654(5) | 0.4379(5) | 0.0544(5) | 4.2(1) |
| C(6')............. | 1.0925(5) | 0.3167(5) | −.0445(5) | 3.9(1) |
| C(7')............. | 1.0019(5) | 0.2329(5) | −.0369(5) | 3.7(1) |
| C(8')............. | 0.9422(5) | 0.2078(5) | 0.0767(5) | 3.8(1) |
| C(9')............. | 0.8540(5) | 0.1267(5) | 0.0774(5) | 3.8(1) |
| C(10')............. | 0.7868(5) | 0.0976(5) | 0.1858(5) | 4.1(1) |
| C(11')............. | 0.7941(5) | 0.1429(5) | 0.3227(5) | 3.6(1) |
| C(12')............. | 1.0671(5) | 0.4051(6) | 0.3387(6) | 4.8(1) |
| C(13')............. | 1.2387(5) | 0.2874(6) | −.0348(6) | 5.0(2) |
| C(14')............. | 1.2852(6) | 0.2373(7) | 0.0971(6) | 7.4(2) |

[a]Standard deviations, given in parentheses, are based solely on least-squares parameters.
[b]The $B_{eq}$ values are calculated according to the formula $B_{eq}=4/3 \sum_i \sum_j \beta_{ij} \, a_i \cdot a_j$ where the $\beta$'s are the anisotropic thermal factors.

The fact that the baccharinoids and roridins A and iso-A all possess the $(R)$ configuration at C6'[2] suggests that the $(R)$ stereochemistry at C6' is going to

[z]Another baccharinoid for which the structure has been determined by an X-ray diffraction study is *i* (28). Allylic oxidation (SeO₂) of roridin A gives 8β-hydroxyroridin A which is epimeric with *i* at C2' (29). Compound *i* was isolated from *B. megapotamica* along with a diastereomer which is epimeric with *i* at C13'. See footnote (10) in reference (12).



*i*
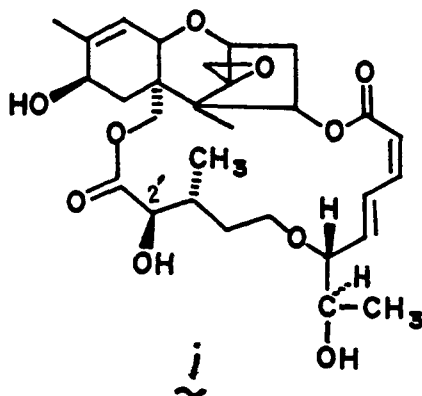
prove common to all of the roridins and related compounds. The biosynthetic route from the trichoverrins (7) to the roridins appears to involve ring-closure with inversion of configuration at C6'. It is tempting to propose that trichoverrins A and B first form roridins E and iso-E with inversion of configuration at C6', and these isomers are subsequently elaborated further to the various diastereomeric pairs of roridins and baccharinoids. However, this suggestion must be abandoned, at least in part, because a recently completed single-crystal X-ray analysis of isororidin E has shown this compound to be C6' (S), C13'(S) (30). We currently are examining more closely the biosynthetic pathways leading from the trichoverrins to the macrocyclic trichothecenes.

## EXPERIMENTAL

GENERAL.—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 Perkin-Elmer 552 spectrophotometer. Optical rotations were recorded on a Perkin-Elmer 241 automatic polarimeter. 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 internal standard. The $^{13}$C nmr signals were assigned using $^1$H single-frequency off-resonance decoupling techniques, 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. 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 (31) was carried out on silica gel 60, 230–400 mesh (E. Merck or Whatman LPS–2). Medium pressure liquid chromatography (mplc) (32) was carried out on either Licroprep 60 (E. Merck) or Whatman LPS–1 silica gel. High performance liquid chromatography (hplc) was performed with an Altex model 332 Gradient Liquid Chromatograph. Preparative separations were carried out on either a Whatman Magnum 9 (10/15) semipreparative Partisil column, or a Whatman Magnum 20 preparative Partisil column.

ISORORIDIN A (4a).—Partition chromatography of a 48 g portion of the mycelium extract of a large scale (760 liters) fermentation of *M. verrucaria* (16) gave a fraction (10 g) rich in roridin A (4). This fraction was subjected to flash chromatography on 300 g of silica gel with 3% methanol in methylene chloride to give three principal fractions: A, B, and C, in the order of elution. Fraction A (600 mg) was composed of roridin D and roridin K acetate (16). Fraction C (1.0 g) was composed mostly of trichothecenes of lower R$_f$ than that of roridin A. Fraction B (4.1 g) was crystallized from methylene chloride-hexane to give 2.2 g of roridin A. The mother liquor was subjected to preparative hplc (2% MeOH/CH$_2$Cl$_2$) to give 15 mg of verrucarin L (16), 700 mg of roridin A (4), and 30 mg of isororidin A (4a) in their order of elution.

Isororidin A (4a) was recrystallized from methylene chloride-hexane to give clear spherical crystals: mp 183–185°; $[\alpha]^{25}$D +6.7° (c, 3.3, CHCl$_3$); ms (chemical ionization, methane gas reagent), $m/e$ 533.2743 (M$^+$+H, calcd. 533.2754); $^1$H nmr (CDCl$_3$) δ 0.83 (3H, s, 14–H), 1.09 (3H, d, $J$=7 Hz, 12'–H), 1.16 (3H, d, $J$=7 Hz, 14'–H), 1.75 (3H, s, 16–H), 2.48 (1H, dd, $J$=8 and 15 Hz, 3α–H), 2.96 (2H, AB, $J$=4 Hz, 13–H), 3.60 (1H, d, $J$=5 Hz, 11–H), 3.86 (1H, d, $J$=5 Hz, 2–H), 4.11 (1H, dd, $J$=3 and 6 Hz, 2'–H), 4.44 (2H, s(br), 15–H), 5.44 (1H, d, $J$=5 Hz, 10–H), 5.80 (1H, m, 4–H), 5.80 (1H, d, $J$=11 Hz, 10'–H), 6.00 (1H, dd, $J$=2 and 15.5 Hz, 7'–H), 6.66 (1H, dd, $J$'s=11 Hz, 9'–H), 7.64 (1H, dd, $J$=11 and 15.5 Hz, 8'–H); $^{13}$C nmr (CDCl$_3$) δ 7.5 q (C14), 14.4 q (C12'), 17.9 q (C14'), 20.3 t (C7), 23.3 q (C16), 27.7 t (C8), 33.3 t (C4'), 34.9 t (C3), 37.0 d (C3'), 43.8 s (C6), 47.8 t (C13), 49.4 s (C5), 64.4 t (C15), 65.3 s (C12), 67.2 d (C11), 69.4 t (C5'), 70.0 d (C13'), 74.4 d (C4), 75.5 d (C2'), 79.1 d (C2), 82.6 d (C6'), 117.3 d (C10'), 118.3 d (C10), 126.4 d (C8'), 139.0 d (C7'), 140.9 s (C9), 143.9 d (C9'), 166.6 s (C11'), 174.9 s (C1').

HYDROGENATION OF RORIDINS A and ISO-A.—Roridin A (4) (30 mg) was dissolved in 10 ml of ethanol and allowed to take up 6.5 ml of hydrogen gas at atmospheric pressure (ambient temperature) while in contact with 9 mg of 10% palladium on carbon. The mixture was filtered and solvent removed to give a glassy solid which was crystallized from ether-hexane to give 20 mg of 8: mp 181–181.5° (lit. (17) 181–182°); $[\alpha]^{25}$D +26 ±3° (c, 5.0, CHCl$_3$) (lit. (17)$[\alpha]^{25}$D +23 ±1° (c, 1.488, CHCl$_3$).

In a similar fashion, 15 mg of isororidin A (4a) gave 11 mg of 7',8',9',10'-tetrahydroisororidin A (8a): mp 170–171°; $[\alpha]^{25}$D +9.8 ±0.5° (c, 7.33, CHCl$_3$); ms (chemical ionization, methane gas reagent), $m/e$ 537.3025 (M$^+$+H, calcd. 537.3052); $^1$H nmr (CDCl$_3$) δ 0.73 (3H, s, 14–H), 0.96 (3H, d, $J$=8 Hz, 12'–H), 1.12 (3H, d, $J$=7 Hz, 14'–H), 1.72 (3H, s, 16–H), 3.01 (2H, AB, $J$=4 Hz, 13–H), 3.62 (1H, d, $J$=5 Hz, 11–H), 3.83 (1H, d, $J$=5 Hz, 2–H), 4.11 (1H, dd, $J$=3 and 6 Hz, 2'–H); 4.30 (2H, AB, $J$=12 Hz, 15–H), 5.42 (1H, d, $J$=5 Hz, 10–H) and 5.80 (1H, dd, $J$=5 and 8 Hz, 4–H).

OXIDATION OF 8 AND 8a TO METHYL KETONE 9.—Tetrahydroisororidin A (8a, 11 mg), 4 mg of anhydrous sodium acetate, and 6 mg of pyridinium chlorochromate (PCC, Aldrich) were stirred for 2h in 1 ml of methylene chloride. The mixture was filtered, concentrated and charged onto one 0.25 mm silica gel plate (20 x 20 cm). The plate was developed with 2% methanol in methylene chloride. Extraction of the major band followed by crystallization

from ether gave 5.6 mg of **9**: mp 201–202°; [α]²⁵D –3.5±1.0 (c, 2.88, CHCl₃); ms (chemical ionization, methane gas reagent) *m/e* 535.2887 (M⁺+H, calcd. 535.2903); ¹H nmr (CDCl₃) δ 0.86 (3H, s, 14–H), 0.96 (3H, d, *J*=7 Hz, 12'–H), 1.72 (3H, s, 16–H), 2.18 (3H, s, 14'–H), 3.00 (2H, AB, *J*=4 Hz, 13–H), 3.86 (1H, d, *J*=5 Hz, 2–H), 4.11 (1H, dd, *J*=3 and 6 Hz, 2'–H), 4.30 (2H, AB, *J*=12 Hz, 15–H), 5.44 (1H, d, *J*=5 Hz, 10–H), and 5.82 (1H, dd, *J*=5 and 8 Hz, 4–H).

In a similar fashion, **8** was oxidized with PCC to give **9**, identical to the sample isolated above.

PYRIDINIUM DICHROMATE OXIDATION OF RORIDINS TO VERRUCARINS.—To a solution of 40 mg (0.08 mmol) of roridin A (**4**) in 4 ml of DMF was added 60 mg of pyridinium dichromate (PDC) (**18**). The mixture was stirred for 8 hours and poured into 30 ml of water. The aqueous mixture was extracted with ether which was dried (MgSO₄) and concentrated to give 25.3 mg (70%) of verrucarin A (**1**). In similar reactions, roridins E (**6**) and iso-E (**6a**) gave verrucarin J (**3**), isororidin A gave verrucarin A, roridin D (**5**) gave verrucarin B (**2**), and roridin K acetate (**16**) gave verrucarin L acetate (**16**).

Roridin A (**4**) crystallizes in the monoclinic space group P2₁ with a=10.197(3) Å, b=14.079(4) Å, c=9.606(2) Å, β=94.6(1)° and z=2 (i.e., one molecule per asymmetric unit). 1890 independent reflections were collected on a NICOLET P3F automatic diffractometer using CuKα radiation with a graphite monochromator on the incident beam out to a 2 theta maximum of 112°. The structure was solved by direct methods (33), using the MULTAN78 system of computer programs (34) to obtain a partial structure which was then developed into the full structure by recycling with the tangent formula (35). The structure was refined by full-matrix least-squares methods using program ORI XLS3 on the full set of 1890 reflections (36). The function minimized was $\Sigma w(F_{|0|}-|F_2|)^2$ where the weights, w (derived from estimated standard deviations of observed intensity), were calculated according to Gilardi (37). All data were used in the refinement. Twenty-eight of the 40 hydrogen atoms were located in difference maps. The 12 methyl hydrogens were put in at calculated positions. Hydrogen coordinates with isotropic thermal factors were then included in the final cycles of refinement as constant parameters. The final R-factors (agreement between observed and calculated structure factors) were R=4.87% and Rw=5.17%. The goodness-of-fit parameter (standard deviation of an observation of unit weight) at the conclusion of the refinement was 1.7. Refined coordinates for the non-hydrogen atoms are listed in table 2.³

## ACKNOWLEDGMENT

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³Tables of anisotropic thermal parameters for the nonhydrogen atoms, coordinates for hydrogen atoms and comparisons between observed and calculated structure factors are available upon request made to J. L. Flippen-Anderson.

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