# STRUCTURE OF RORIDIN J, A NEW MACROCYCLIC TRICHOTHECENE FROM *MYROTHECIUM VERRUCARIA*

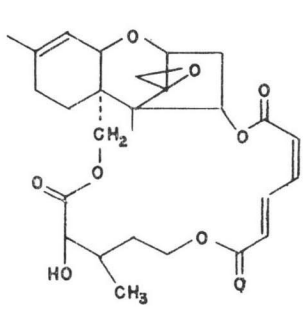
Sir:

During a continuing program to isolate and chemically modify mycotoxins[1], we have isolated and identified a new macrocyclic trichothecene from the fermentation of *Myrothecium verrucaria* (ATCC #24571).* This new mycotoxin was isolated by a combination of column chromatographies (adsorption and partition) and recrystallizations from an ethyl acetate extract of the fermentation brew. The compound has an Rf value (silica gel) just below verrucarin A (**1**), a principal trichothecene produced by this fermentation, but can be separated from **1** by careful chromatography on alumina (hexane-methylene chloride eluent). This new compound which we call roridin J (**2a**) is closely related to roridin H (**3**) but differs in that **2a** possesses an allylic hydroxyl group, and the 2′,3′ double bond in **2a** has the *Z* configuration rather than *E*. Roridin J is the first case established where the configuration of the 2′,3′ double bond in a macrocyclic trichothecene is *Z*.**

The structure assignment for **2** is based on the following data for **2a** and its acetate, **2b**: roridin J (**2a**): $C_{29}H_{36}O_9$; mp 281~285° (from dichloromethane-hexane); M$^+$ 528; $[\alpha]_D^{28}$ +21.8° ($CHCl_3$); $\gamma_{max}^{KBr}$ 3535 (OH), 1715 (C=O), 1645 and 1595 (diene) cm$^{-1}$; $\lambda_{max}^{EtOH}$ 261 nm (log $\epsilon$ 4.28); roridin J acetate (**2b**): $C_{31}H_{38}O_{10}$; mp 230~235°C (from dichloromethane-ether); $[\alpha]_D^{28}$ −40.6° ($CHCl_3$); $\gamma_{max}^{KBr}$ 1745 and 1715 (C=O's), 1655, 1605 (diene) cm$^{-1}$.

Inspection of the proton and carbon-13 NMR spectra of roridin J (**2a**) and roridin J acetate (**2b**) (see Table 1) reveal them to be structurally quite similar to roridin H (**3**) yet different from **3** in one fundamental respect. In terms of similarity, **2a** contains one more oxygen atom than **3**. Formation of a monoacetate derivative **2b** mandates that this extra oxygen atom be present in the form of a hydroxyl group. Its location at C-4′ is demonstrated by the appearance of H-5′ as a 7-Hz doublet in the proton NMR spectrum. In **3** this acetal proton forms the X part of an ABX system.

Roridin J differs significantly from roridin H in that the stereochemistry at C-3′ is reversed. Evidence for a *Z* configuration of the 2′,3′-double bond in **2** derives from observation of a nuclear OVERHAUSER effect (NOE) for H-2′ upon irradiation of the 12′-methyl protons.*** No such NOE was observed for H-2′ in **3**, leading to the conclusion that H-2′ and the 12′-methyl group are situated trans to each other in

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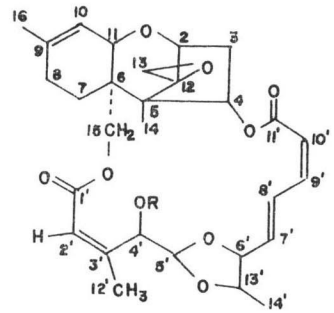
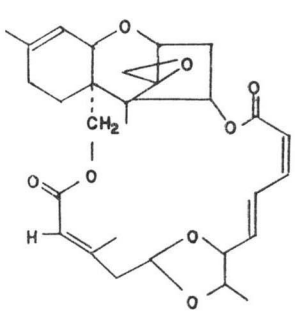



2a, R = H
2b R = Ac

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* This fermentation (200 gal) was carried out under the direction of Mr. RICHARD GEOGHEGAN, Frederick Cancer Research Center, Frederick, Maryland. The details of this procedure will be published in a full paper.

** Previous workers have shown that roridins E, iso-E and H[2] and verrucarin J[3] have the *E* configuration at the 2′, 3′ double bond. However, although satratoxin H[4] also has the *E* configuration at the 2′, 3′ double bond, it nevertheless has the same relative configuration at 2′, 3′ as does roridin J.

*** The NOE experiment was conducted on a Varian EM390 instrument with one minute intervals between sweeps and at a radiating field strength of 0.01 mG. On resonance frequency was set at 2.3 ppm and, as a check, an off frequency resonance experiment at 9.3 ppm also was conducted; the latter experiment showed no change in the integration for H2′, whereas the former gave rise to a 12% enhancement in the integration for H2′.

Table 1. $^{13}C$ and $^{1}H$ NMR data for roridin H (**3**), roridin J (**2a**) and roridin J acetate (**2b**)[a].

| Position | Roridin H (**3**)[g,h] | Roridin J (**2a**) | Roridin J acetate (**2b**) |
|---|---|---|---|
| 2 | 79.0d (3.8d) | 79.2d (3.85d) [5] | 79.2d (3.84d) [5] |
| 3 | 34.8t (b) | 34.7t (2.1m) (2.48dd)[8, 15] | 34.7t (2.1m) (2.47dd) [8, 15] |
| 4 | 74.0d (*ca.* 5.9) | 73.9d (6.0dd) [4,8] | 73.9d (5.96dd) [4,8] |
| 5 | 48.9 | 49.2 | 49.3 |
| 6 | 43.2 | 43.3 | 43.3 |
| 7 | 20.5t (b) | 20.4t (2m) | 21.0t (1.9m) |
| 8 | 27.6t (b) | 27.6t (2m) | 27.6t (1.9m) |
| 9 | 139.9 | 140.4 | 140.4 |
| 10 | 118.6d[e] (5.42d) [4] | 118.6d (5.44d) [5] | 118.6d (5.43d) [5] |
| 11 | 67.6d (3.64) | 67.9d (3.63d) [5] | 67.9d (3.63d) [5] |
| 12 | 65.3 | 65.6 | 65.5 |
| 13 | 47.3t (2.96AB) [4] | 47.9t (2.97AB) [4] | 47.9t (2.97AB) [4] |
| 14 | 7.0q (0.85) | 7.4q (0.87) | 7.4q (0.87) |
| 15 | 63.0t (4.15AB) [12] | 63.4t (4.21AB) [12] | 63.6t (4.21AB) [12 |
| 16 | 22.9q (1.69) | 23.3q (1.74) | 23.3q (1.72) |
| 1′ | 166.0 | 165.9 | 165.8 |
| 2′ | 119.0d[e] (5.67) | 119.8d (5.84d) [1.2] | 122.0d (5.91d) [1.2] |
| 3′ | 154.4 | 155.4 | 151.3 |
| 4′ | 47.7t (2.64m) | 79.8d (3.85d) [7] | 79.7d (4.97d) [7] |
| 5′ | 100.8d (5.58) [3.5,8] | 103.4d (5.24d) [7] | 101.4d (5.42d) [7] |
| 6′ | 81.9d[d] (4.03) | 82.3d (3.87) | 82.3d (4.02) |
| 7′ | 134.6d (5.9m) | 134.5d (5.8d) [15.5] | 134.5d (5.81d) [15.5] |
| 8′ | 126.2d (7.68dd) [11,15.5] | 126.1d (7.70dd) [11.5,15.5] | 126.0d (7.70dd) [11.5,15.5] |
| 9′ | 142.5d (6.55t) [11] | 143.1d (6.54t) [11.5] | 143.3d (6.56t) [11.5] |
| 10′ | 118.9d[e] (5.79d) [11] | 118.9d (5.9d) [11.5] | 118.9d (5.88d) [11.5] |
| 11′ | 166.0 | 166.2[f] | 166.3[f] |
| 12′ | 18.2q[e] (2.27d) [1.5] | 13.1q (2.28d) [1.2] | 13.3q (2.28d) [1.2] |
| 13′ | 76.8d[d] (3.65m) | 76.5d (3.70q) [6] | 76.7d (3.71q) [6] |
| 14′ | 16.3q[e] (1.32d) [6] | 16.0q (1.36d) [6] | 15.9q (1.36d) [6] |
| $CH_3CO$ | | | 20.3q (2.1) |
| $CH_3\underline{C}O$ | | | 169.8 |

[a] All spectra were taken in deuteriochloroform solvent. The proton chemical shifts are in parentheses and $^{J}H$, H in brackets. $^{13}C$ NMR spectra were determined on a Varian CFT-20 or FT-80A spectrometer operating at 20 MHz using TMS as an internal reference (0.0 ppm).
The $^{13}C$ NMR signals were assigned using $^{1}H$ single-frequency off-resonance decoupling techniques,[i] chemical shift relations,[i] by comparison with literature data,[h] and by comparison of compounds. $^{1}H$ NMR spectra were determined on a Varian EM-390 spectrometer operating at 90 MHz in the TMS-locked mode.

[b] Not determined.

[c,d,e] These assignments may be interchanged in each column, though by analogy with **2a** and **2b**, the assignments appear correct.

[f] Observed as a narrow doublet in the SFORD spectrum due to coupling to H-9′.

[g] P. TRAXLER and CH. TAMM: Helv. Chim. Acta 53: 1846 (1970).

[h] W. BREITENSTEIN and CH. TAMM: Helv. Chim. Acta 58: 1172 (1975).

[i] F. W. WEHRLI and T. WIRTHLIN, "Interpretation of Carbon-13 NMR Spectra," Heyden & Son, Ltd., Philadelphia, Pennsylvania, 1978.

this compound[2].

Roridin J (**2a**) exhibits substantial *in vivo* activity against P388 mouse leukemia (PS)[5]. In fact, this is the highest reported PS activity observed in a macrocyclic trichothecene lacking oxygen functionality (*e.g.*, $\beta$-9,10-epoxide or $\beta$-8-hydroxyl) in the A-ring[6]. Epoxidation of the 9,10-bond in **2** should lead to a highly PS active

compound in analogy with what has been observed with verrucarin A[1] and roridin A[7].

Acknowledgment

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## References

1) JARVIS, B. B.; G. P. STAHLY & C. R. CURTIS: Antitumor activity of fungal metabolites: Verrucarin $\beta$-9,10-epoxides. Cancer Treat. Rep. 62: 1585~1586, 1978
2) (a) MATSUMOTO, M.; H. MINATO, N. UOTANI, K. MATSUMOTO & E. KONDO: New antibiotics from *Cylindrocarpon* sp. J. Antibiotics 30: 681~682, 1977
(b) MATSUMOTO, M.; H. MINATO, K. TORI & M. UEYAMA: Structures of isororidin E, epoxyisororidin E and epoxy- and diepoxyroridin H, new metabolites isolated from *Cylindrocarpon* species determined by carbon-13 and hydrogen-1 NMR spectroscopy. Revision of C-2′: C-3′ double bond configuration of the roridin group. Tetrahedron Lett. 1977: 4093~4096, 1977
3) TAMM, CH.: private communication.
4) EPPLEY, R. M.; E. P. MAZZOLA, R. J. HIGHET & W. J. BAILEY: Structure of satratoxin H, a metabolic of *Stachybotrys atra*. Application of proton and carbon-13 nuclear magnetic resonance. J. Org. Chem. 42: 240~243, 1977
5) Compound **2a** was tested under the auspices of the U.S. National Cancer Institute: see GERAN, R. I.; N. H. GREENBERG, M. M. MACDONALD, A. M. SCHUMACHER & B. J. ABBOTT: Protocols for Screening Chemicals Agents and Natural Products Against Animal Tumors and Other Biological Systems (Third Edition). Cancer Chemother. Rep., Part 3, 3: 1~103, 1972 Roridin J (**2a**) was toxic at 10 mg/kg and exhibited the following T/C activities (dose level) in PS: 158 (5 mg/kg), 149 (2.5 mg/kg), 140 (1.25 mg/kg), and 125 (0.62 mg/kg).
6) Data made available by J. DOUROS, Head of Natural Products Division of the National Cancer Institute.
7) JARVIS, B. B. & G. P. STAHLY: unpublished results.