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TRICHOVERRITONE AND 16-HYDROXYRORIDIN L-2,

NEW TRICHOTHECENES FROM MYROTHECIUM RORIDUM

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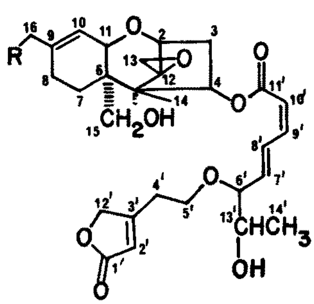
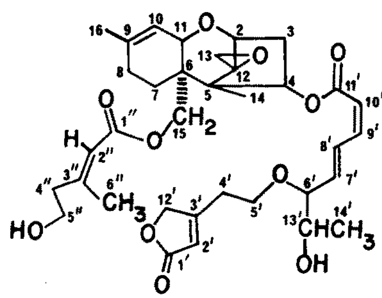
Abstract: Trichoverritone (3), a C-35 trichothecene, and 16-hydroxyroridin L-2 (2) have been isolated from a liquid culture of Myrothecium roridum. The former congener, 3, the largest known trichothecene, also was shown to be produced by a pathogenic strain of M. roridum.

The trichothecene group of secondary fungal metabolites has generated a great deal of interest due to their wide spread occurrence in nature as well as their wide range of bioactivity.[1] With respect to the latter, it is their role as mycotoxins[1d] and as anticancer agents agents[1c] that has generated the most interest in these sesquiterpene antibiotics.

Recently, a novel C-29 trichothecene, roridin L-2 (1), was isolated from a large scale liquid culture of Myrothecium roridum, strain CL-514.[2] Herein, we report the isolation and characterization of 16-hydroxroridin L-2 (2) and the largest heretofore isolated trichothecene, trichoverritone (3), a C-35 trichothecene, from this same culture.[2] Also, we report that M. roridum, strain 81-131, which is a pathogen on Aglaonema sp.,[3] produces trichoverritone (3) and roridin L-2 (1) as well.



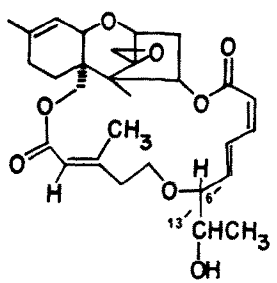
Roridin L-2 (1), R=H
16-Hydroxyroridin L-2 (2), R=OH

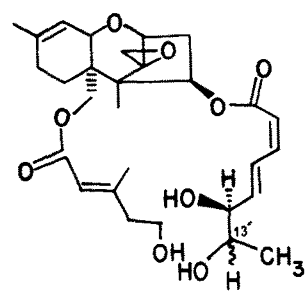


Trichoverritone (3)

Compounds 2 and 3 were isolated from the most polar fraction of a silica gel chromatography of an ethyl acetate extract of the fermentation beer of M. roridum.[2] This crude fraction (17.7 g) in methanol was treated with ferric gel[4] and the resulting organic fraction (5.3 g) was purified further by extensive silica gel chromatography, preparative TLC and reversed phase HPLC. By these methods were isoalted, in order of increasing polarity, trichoverritone (3, 60 mg) and 16-hydroxyroridin L-2 (2, 30 mg).

Trichoverritone was isolated as an oil: λ_{max} 259 nm (MeOH), log ε=4.57; $[\alpha]_D^{25}$ + 45.0 (C=0.2, $CHCl_3$); IR (neat) 3460, 1785, 1750, 1710, 1640, and 1600 cm^{-1}; MS (CI, methane gas reagent), m/e 643.3122 (M^+ + H, calc for $C_{35}H_{46}O_{11}$ + H is 643.3118). Both the mass spectral and ^{13}C NMR data (see Table I) make it evident that trichoverritone (3) is a C-35 compound. Furthermore, in the ^1H NMR spectrum of 3, the two proton doublet at δ 4.82 and the two proton triplet at δ 2.69 are two resonances characteristic of H-12' and H-4', respectively, in roridin L-2 type structures. However, unlike roridin L-2, 3 exhibits a proton resonance at δ 2.20 (3 H, d, J=1.2 Hz) characteristic of a vinyl methyl group in the side chain a found in roridins E (4a) and isoE (4b) and the trichoverrins (5).[5] These data make firm the structure assignment given.





Roridin E (4a) [6'(R), 13'(R)]
Isororidin E (4b) [6'(S), 13'(S)]

Trichoverrin A (5a) [13'(S)]
Trichoverrin B (5b) [13'(R)]

16-Hydroxyroridin L-2 was isolated as an oil: λ_{max} 261 nm, MeOH, log ε 3.90; $[\alpha]_D^{25}$ + 58.4 (C= 0.19, $CHCl_3$); IR (neat) 3440, 1785, 1750, 1710, 1640, 1600 cm^{-1}; MS (CI, methane gas reagent), m/e 547.2532 (M^+ + H, calc for $C_{29}H_{38}O_{10}$ + H is 547.2543). In addition to the twenty-nine carbon atoms evident in the ^{13}C spectrum (see Table 1), the ^1H NMR spectrum exhibits the two proton AB resonance (dd, J_{AB}=16 Hz and $J_{2',12'}$=1.8 Hz) centered at δ 4.83 (H-12') and a triplet (2H) at δ 2.73 (H-4') characteristic of the lactone side chain in roridin L-2. However, unlike roridin L-2, 2 forms a triacetate. Furthermore, H-16 normally found at ca. δ 1.7 in trichothecenes is a two proton singlet at δ 4.05 in 2 (Table I) and a two proton singlet at δ 4.50 in the triacetate. Collectively, these data make secure the structure assignment given for 2.

Trichoverritone forms a diacetate which exhibits a five line resonance at δ 4.98: H-13', dq, $J_{6',13'}=J_{13',14'}$=6.5 Hz. Also, 16-hydroxyroridin L-2 forms a triacetate which exhibits a five line resonance at δ 5.00: H-13', dq, $J_{6',13'}=H_{13',14'}$=6.4 Hz. Based on similar ^1H NMR data for other trichoverroids,[5] both 2 and 3 have the same configurations at C-6' and C-13', i.e. the asymmetric centers at C-6' and C-13' are either both (S) or both

Table 1. ^{13}C and ^{1}H NMR Data for 16-Hydroxyroridin L-2 (2) and Trichoverritone (3) [a]

Position	2[b]	3[c]
2	79.1	79.1
3α	36.1 (2.53 dd) [8, 15.4]	36.9 (2.59 dd) [7.8, 15]
4	75.4 (6.08 dd) [3.5, 8]	75.4 (5.97dd) [3.4]
5	49.1	48.9
6	44.8	43.3
7	20.9 (2.1 m)	21.5 (~2)
8	23.6 (2.1 m)	28.0 (~2)
9	143.3	140.6
10	119.0 (5.75 d) [5.1]	118.5 (5.47 d) [4.9]
11	66.5	66.9
12	65.5	65.4
13	47.9 (2.98 AB) [4.0]	47.9 (2.98 AB) [4.0]
14	6.5 (0.86 s)	6.8 (0.82 s)
15	62.4	63.0 (4.14 AB) [12.5]
16	66.1 (4.05 s)	23.0 (1.72 s)
1'	174.0	174.1
2'	116.8 (5.90 m)	116.7
3'	167.4	167.2
4'	29.3 (2.73 t) [5.8]	29.3 (2.71 t) [6.1
5'	66.4	66.2
6'	85.4	85.6
7'	139.0 (5.80 dd) [8.3, 15.6]	138.6 (5.80 dd) [8.3, 15.6]
8'	130.5 (7.61 dd) [11.4, 15.6]	131.3 (7.60 dd) [11.6, 15.6]
9'	143.4 (6.62 dd) [11.4, 11.4]	142.8 (6.58 dd) [11.6, 11.6]
10'	118.8 (5.79) [11.4]	117.1 (5.76 d) [11.6]
11'	166.4	165.8
12'	73.6 (4.78 dd) [1.8, 16]; (4.88 dd) [1.8, 16]	73.3 (4.79 d) [1.8]
13'	69.8	69.5
14'	18.5 (1.18 d) [6.4]	18.5 (1.14 d) [5.8]
1"		165.6
2"		119.3 (5.80 m)
3"		157.0
4"		43.7 (2.41 t) [6.1]
5"		59.9
6"		18.9 (2.20 d) [1.2]

[a]Spectra recorded in $CDCl_3$ on an IBM WP-200SY spectrometer. The proton chemical shifts are in parentheses and the $J_{H,H}$ in brackets. ^{13}C Chemical shift assignments were done by comparing proton decoupled spectra with spectra obtained in an INEPT experiment [8] and by comparison with the literature values for roridin L-2 [2] and the trichoverrins. [5] [b]The region of δ 3.5-4.0 in the proton spectrum is a complex pattern of overlapping signals of H's 2,11,15,5',6', and 13'. [c]The region of δ 3.4-3.9 in the proton spectrum is a complex pattern of overlapping signals of H's 2,11,5',6',13', and 5".

(R). It would be of interest to determine these stereochemistries in order to establish the relationship between 2 and 3 and trichoverrin A (5a) [which is C-6' (S), C-13' (S)] [5] and roridins E (4a) [which is C-6' (R), C-13' (R)],[6] and isoE (4b) [which is C-6' (S), C-13' (S)]. [7]

A liquid culture of M. roridum (strain 81-131) [3] was prepared by suspending spores of the fungus in 2 L of aqueous medium containing 40 g of sucrose and various inorganic and organic salts. After ten days, the culture was extracted (EtOAc) to give ca. 200 mg of resin which after extensive chromatography gave 90 mg of trichodermadienediols A and B, [5] 15 mg of trichoverritone (3), and 5 mg of roridin L-2 (1). Production of these metabolites is very sensitive to the degree of aeration.

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