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RORIDIN L-2, A NEW TRICHOTHECENE

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Abstract: The structure (2) of roridin L-2 is shown to be a C_{29}-trichothecene that lacks the macrocyclic lactone system usually present in the roridins.

The antitumor activity of certain trichothecenes gives added importance to the search for new members of this interesting group of fungal metabolites. We recently found that several macrocyclic trichothecenes such as roridin E (1) and roridin H[1] were produced during the fermentation of a microorganism identified as a variant of Myrothecium roridum. A major component isolated from the same fermentation beer proved to be a novel C-12' oxygenated C_{29}-trichothecene that lacks the characteristic macrocyclic system spanning C-4 and C-15 of most C_{27} and C_{29}-trichothecenes.[2] Evidence is presented herein to show that this compound, named roridin L-2, has the structure 2.[3]

Roridin L-2 was isolated as a white, homogeneous solid (mp 93-97°; λmax 259 nm (MeOH), ε = 24650; $[\alpha]_D$ + 83.6° (c 1.0, $CHCl_3$); IR(CCl_4) 3600, 3500, 1785, 1750, 1710, 1640, 1600 cm^{-1}) from a concentrate of an ethyl acetate extract of fermentation beer by silica gel chromatography (Waters Prep LC/system-500; elution solvent, 50:50 CH_2Cl_2:EtOAc) followed by reverse phase chromatography on C_{18}-silica gel (elution solvent, 50:50 MeOH:H_2O). Elemental analysis and mass spectral data (M^+ 530) agree with $C_{29}H_{38}O_9$. The isolation of verrucarol (3), identified by mp, IR, and ^{1}H-NMR, from the alkaline hydrolysis (K_2CO_3 in aqueous MeOH, 18 hours at 25°)[4] of roridin L-2 establishes the presence of a verrucarol moiety. The remaining structural features were determined mainly from the NMR data shown in Table 1.

Many of the signals that appear in the ^{1}H and ^{13}C-NMR spectra of roridin E - the diene lactone (C-7' ⟶ C-11'), the 1-hydroxyethyl (C-13' + C-14'), and all of the verrucarol signals - are also present in the spectra of roridin L-2. Notable differences in roridin L-2 spectra include: (1) the absence of a ^{1}H-NMR signal for a C-12' methyl near 2.2 ppm and the presence of a distinctive AB pattern at 4.81 ppm, and (2) the 9-ppm downfield shift of C-3' in the ^{13}C-NMR spectrum. The latter signal, which appears at 167.8 ppm, is indicative of a β-carbon atom of an α,β-unsaturated-γ-lactone,[5] the presence of which in roridin L-2 is corroborated by the two absorptions at 1785 and 1750 cm^{-1} (characteristic of such functions that have an α-H).[6] ^{1}H-NMR and ultraviolet spectral data rule out the involvement of the C-11' carboxyl in such a γ-lactone function, which therefore must be composed of carbons 1' ⟶ 3' plus a 12'-hydroxymethyl group. The isolated 12'-hydrogens thus account for the AB pattern at 4.81 ppm. Because spectral evidence demands that the hydroxyl group at C-4 of verrucarol is esterified (H-4 appears at 6.10 ppm), an ester link between C-4 and C-11' is inferred. The above data reveal that in roridin L-2 the entire assemblage of 14 carbon atoms present in roridin-like trichothecenes emanates from C-4 of verrucarol and terminates as an α,β-unsaturated-γ-lactone at C-1', leaving a free hydroxymethyl group at C-6.

The signals corresponding to the protons on C-15 and C-13' appear with five other proton signals (H-2, 11, 5', and 6') in the region between 3.6 and 3.9 ppm and are difficult to delineate with certainty. Roridin L-2 forms a diacetate whose ^{1}H-NMR spectrum exhibits a 13'-H signal (quintet) at 4.84 ppm and a well-defined AB pattern centered at 4.1 ppm due to the C-15 protons. The Z configuration of the 2'-3' double bond in roridin L-2 corresponds to the same relative configuration of the 2'-3' double bond in roridin E and most related trichothecenes.[7]

Roridin L-2 is the only reported example of a trichothecene in which C-12' has been oxygenated and remains cis to the C-1' carboxyl group, and further, to have a macrocyclic ring "broken" between C-15 and C-1'. The possibility that roridin L-2 is an artifact produced by rearrangement of the unknown 12'-hydroxyroridin E during prolonged isolation procedures was ruled out by the TLC detection of roridin L-2 in a fresh EtOAc extract of a new sample of fermentation broth.

Roridin L-2 is much less toxic than roridin E or its congeners when administered intraperitoneally to mice and is only moderately active against P388 lymphatic leukemia. (T/C = 100-125 at 8-32 mg/kg.) These findings are in accord with the similar properties of trichoverrin A and B.[2]

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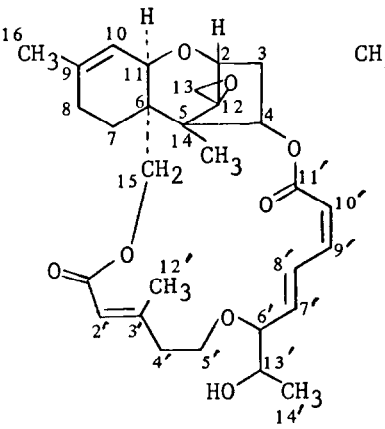
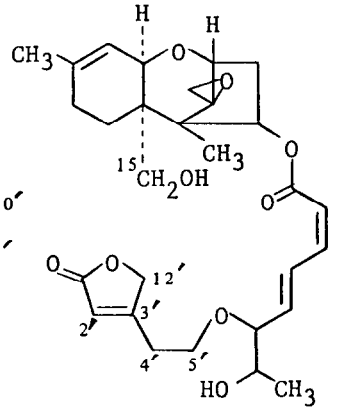
Table 1. NMR Data for Roridin E and Roridin L-2[a]

Position	Roridin E (1)[b]			Roridin L-2 (2)[c]		
	^1H	(J)	^{13}C	^1H	(J)	^{13}C
2	3.82 d		79.3	3.7 m		79.0 d
3A	2.04 ddd		35.8	2.0 m		36.3 t
3B	2.53 dd			2.50 dd	(8,15.5)	
4	6.20 dd	(4,8)	74.2	6.10 dd	(3.5,8)	75.5 d
5			48.4			48.9
6			42.8			44.3
7	2.0 m		21.6	2.0 m		21.2 t
8	2.0 m		27.7	2.1 m		28.1 t[d]
9			140.0			140.6
10	5.5 d	(5)	117.8	5.48 d	(5)	118.9 d[e]
11	3.7 m		67.2	3.7 m		66.9 d
12			65.6			65.7
13A	2.81 d	(4)	48.1	2.81 d	(4)	48.1 t
13B	3.12 d	(4)		3.13 d	(4)	
14	0.82 s		6.7	0.84 s		6.7 q
15A	3.93 d	(12)	63.7	3.65 d	(12)	62.7 t
15B	4.32 d	(12)		3.86 d	(12)	
16	1.71 bs		23.2	1.72 s		23.3 q
1'			165.8[d]			174.2
2'	5.95 q		119.0	5.92 br s	(1.3)	116.7 d
3'			159.0			167.8
4'	2.4-2.7 m		41.3	2.7 m		29.3 t[d]
5'	3.5-4.0 m		69.8	3.7 m		66.4 t
6'	3.7 m		83.8	3.7 m		85.4 d
7'	5.89 dd	(2,15)	138.1	5.86 dd	(7,15.5)	139.1 d
8'	7.51 dd	(11,15)	126.6	7.61 dd	(11.5,15.5)	130.6 d
9'	6.58 t	(11)	143.7	6.61 t	(11.5)	143.5 d
10'	5.73 d	(11)	117.2	5.78 d	(11.5)	118.8 d[e]
11'			166.4[d]			166.5
12'	2.25 d	(1.5)	20.2	A,4.78 dd	(1.5,17.5)	73.6 t
				B,4.84 dd	(1.5,17.5)	
13'	3.7 m		70.5	3.7 m		69.8 d
14'	1.22 d	(6)	18.3	1.14 d	(6)	18.5 q
$(OH)_2$				2.7 m		

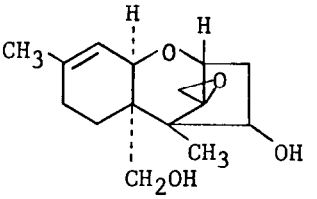
[a] Solvent = $CDCl_3$; chemical shifts are in ppm downfield from Me_4Si.
[b] The ^{13}C and most of the proton chemical shifts, recorded at 20 MHz and 100 MHz, respectively, are taken from reference 8. J(H-H) values and some proton shifts are taken from reference 9.
[c] Proton chemical shifts were recorded at 360 MHz and the ^{13}C-FT-NMR spectrum was taken at 22.63 MHz.
[d,e] Assignments may be interchanged.



1, roridin E



2, roridin L-2



3, verrucarol

References and Notes

1. W. Breitenstein and Ch. Tamm, Helv. Chim. Acta 58, 1172 (1975).
2. B. B. Jarvis, G. Pavanasasivam, C. E. Holmlund, T. DeSilva, G. P. Stahly, and E. P. Mazzola, J. Am. Chem. Soc. 103, 472 (1981) have recently reported trichoverrins A and B which are C_{29}-trichothecenes that lack a bond between C-5' and C-6'.
3. Roridin K is 8α-hydroxyroridin E; B. B. Jarvis et al, J. Org. Chem. 47, 1117 (1982).
4. J. Gutzwiller and Ch. Tamm, Helv. Chim. Acta 48, 177 (1965).
5. H. Shimomura, Y. Sashida, K. Ogawa, and Y. Iitaka, Tetrahedron Letters 22, 1367 (1981).
6. K. Nakanishi, Infrared Absorption Spectroscopy, Holden-Day, Inc. San Francisco, 1962, p. 45.
7. An exception is roridin J where allylic hydroxylation occurs at C-4'; B. B. Jarvis, G. P. Stahly, G. Pavanasasivam, and E. P. Mazzola, J. Antibiotics 33, 256 (1980).
8. M. Matsumoto, H. Minato, K. Tori, and M. Ueyama, Tetrahedron Letters, 4093 (1977).
9. E. Fetz, B. Böhner, and Ch. Tamm, Helv. Chim. Acta 48, 1669 (1965).

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