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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

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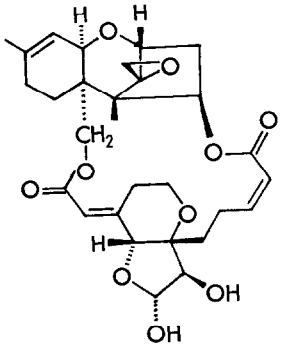
Recently, the structures of vertisporin[1] (1) and baccharin[2] (2), trichothecene epoxides having significantly interesting biological activities, have been determined on the basis of the chemical and spectroscopic evidence and by a direct single-crystal X-ray analysis, respectively. The structure elucidation of these antibiotics has shed a light on the stereochemistry of the side-chain of the roridin group,[3] since an X-ray crystal analysis of p-iodobenzenesulphonate of verrucarin A (3) had only been known so far.[4] We obtained roridin H[5] (4) and six new antibiotics from a species of Cylindrocarpon (strain PF-60),[6] and wish to report here the structural elucidation of four 5-8 of these new metabolites together with a revision of the C-2':C-3' double-bond configurations of roridin E[7] (9) and H (4) mainly by NMR spectroscopy.

Compound 5, named isororidin E (5),[6] $C_{29}H_{38}O_8$, mp 200-202° (EtOAc), was distinguished in its $[\alpha]_D$ value (-65.1°)[6] from that of roridin E (9) (-16°), since its IR, UV, and ^{13}C and ^1H NMR (see the TABLE) were found to be similar to those of 9. Thus, it was assumed that 5 is a stereoisomer or a geometrical isomer of 9 in its side-chain.

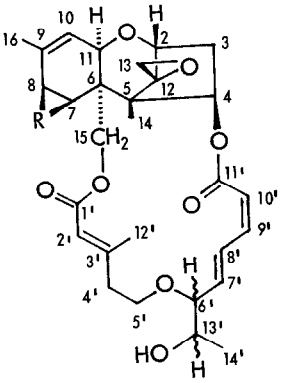
We firstly carried out NOE experiments for the 100-MHz ^1H NMR spectra of 5 and 9, the latter of which was reported to have H-2' and Me-3' in cis-relationship.[3] However, we could not observe any NOE between H-2' and Me-3', surprisingly, whereas we obtained ca. 15% NOE enhancement of the H-10 signal on saturation of the Me-9 signal for both 5 and 9. Therefore, 5 and 9 were found to have the same trans configuration at C-2':C-3', and the configuration of the latter 9 should be revised.

Thus, 5 was hydrolized with 2% K_2CO_3 at room temperature to give verrucarol[8] (10) and carboxylic acids. The acid fraction was esterified with CH_2N_2 and separated by preparative TLC on silica gel into methyl esters 11 and 11', and unidentified two esters. Ester 11 was found to resemble closely the corresponding ester[9] 12 from 9 in UV, IR, and ^1H NMR spectra, but its $[\alpha]_D$ value (-62.3°) was undoubtedly different from that of 12 (+43°). Therefore, 5 was determined to be a stereoisomer of 9 at C-6' or C-13', as the ^1H NMR spectra of 11 and 12 did not coincide. Another methyl ester 11' was assumed to be a C-3':C-4' double-bond isomer of 11 from its spectral data.

Compound 6,[6] $C_{29}H_{36}O_9$, mp 216-219°, resembles 5 in its ^{13}C spectrum except that it showed two O-CH signals at δ_C 50.8 and 56.1 instead of the CH_2 signals at C-7 (δ_C 22.7) and C-8 (27.7) of 5 (see the TABLE). Thus, 6 was assumed to be 7,8-epoxyisororidin E. The magnitudes

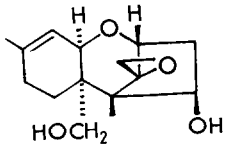


1: Vertisporin[1]



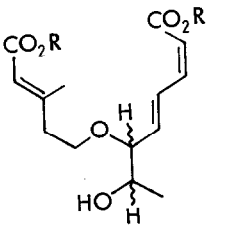
5: R = H_2: Isororidin E
6: R = O
9: R = H_2: Roridin E[7] (Stereoisomer)

5(9) ⟶

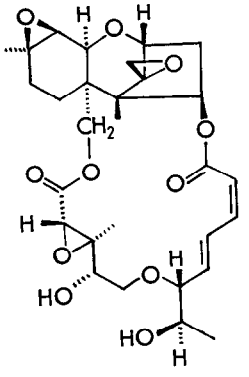


10: Verrucarol

+

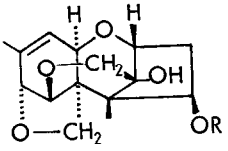


R = H
11 (12): R = Me

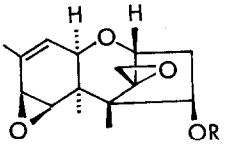


2: Baccharin[2]

6 ⟶

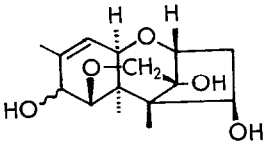


13: R = H
13a: R = Ac



14:[9] R = CO-CH=CH-CH_3
15: R = H

⟶



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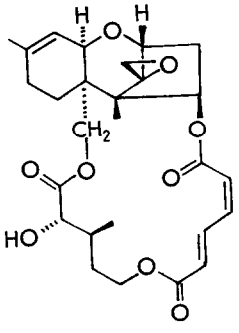
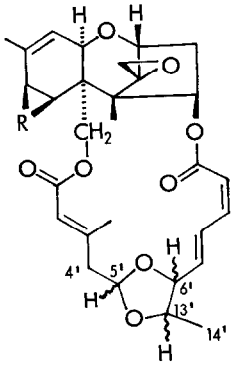


3: Verrucarin A[3,4]



4: R = H_2: Roridin H[5]
7: R = O

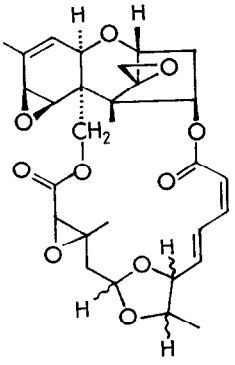


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TABLE. ^{13}C and ^{1}H NMR Spectral Data in $CDCl_3$,[a] δ_C (±0.1), δ_H (±0.02, in parentheses), and $J_{H,H}$ (±0.2, in square bracket)[b]

Position	Roridine E (9)	Isororidin E (5)	7β,8β-Epoxyisororidin E (6)	Roridin H (4)[5,10]	7β,8β-Epoxyroridin H (7)	7β,8β,2',3'-diepoxyroridin H (8)
2	79.3 (3.82d)	79.2(3.84d)	79.2 (3.90d)	79.0 (3.8d)	79.3 (3.91d)	79.2(3.90d)
3	35.8 (2.04ddd) (2.53dd)	36.6(2.03ddd) (2.58dd)	36.6 (2.06ddd) (2.55dd)	34.8 (c)	35.0(2.15ddd) (2.40dd)	34.8(2.26ddd) (2.45dd)
4	74.2 (6.20dd)	75.3(6.35dd)	74.6 (6.30dd)	74.0(∿5.9)	73.6 (5.87dd)	73.9(5.89dd)
5	48.4	48.5	47.0	48.9	48.2	48.5
6	42.8	42.6	44.0	43.2	44.2	44.7
7	21.6 (c)	22.7(c)	50.8 (3.34dd) [4.0,3.2[f]]	20.5 (c)	50.9 (3.75dd) [4.0,3.0[f]]	50.9(3.62dd) [3.9,3.0[f]]
8	27.7 (c)	27.7(c)	56.1 (3.17dd) [4.0,2.1[g]]	27.6 (c)	57.2 (3.17dd) [4.0,2.1[g]]	56.3(3.18dd) [3.9,1.9[g]]
9	140.0	140.1	138.2	139.9[d]	138	138.6
10	117.8 (5.47dm)	118.9(5.50dm)	123.0 (5.75dq)	118.6[d](5.42d)	123.0 (5.69d)	122.4(5.67dm)
11	67.2 (3.89dm)	66.7(4.09d)	66.7 (4.21dd)	67.6 (3.64)	67.9 (4.08m)	67.3(3.75dd)
12	65.6	65.7	65.7	65.3	65.5	65.3
13	48.1 (2.81d) (3.12d)	47.7(2.83d) (3.15d)	48.3 (2.99d) (3.21d)	47.3 (2.96dd)	47.9[e](2.98d) (3.18d)	48.0(2.97d) (3.18d)
14	6.7 (0.79s)	6.4(0.80s)	7.2 (1.02s)	7.0 (0.85s)	8.2 (1.12s)	8.5(1.12s)
15	63.7 (3.93d) (4.32d)	64.5(4.06d) (4.16d)	62.9 (3.79d) (4.07d)	63.0 (4.15dd)	65.8 (3.41d) (4.43d)	65.6(4.44s)
16	23.2[d](1.71bs)	23.2(1.71bs)	21.9[d](2.00d)	22.9 (1.69s)	22.0 (1.98d)	22.0(2.01d)
1'	165.8[d]	166.3	165.9[d]	166.0[d]	165.9[d]	167.8
2'	119.0 (5.95q)	119.5(5.83q)	119.1 (5.80q)	119.0[d](5.67s)	118.7[d](5.69q)	59.2(3.29s)
3'	159.0	158.0	159.0	154.4	155.6	60.8
4'	41.3 (c)	40.0(c)	40.1 (c)	47.7 (2.64d)	47.7[e](2.32m) (2.41m)	44.0(1.57dd) (2.32dd)
5'	69.8 (c)	67.0(c)	67.0 (c)	100.8 (5.58dd)	101.0 (5.54dd)	101.1(5.37dd)
6'	83.8 (3.70m)	83.2(3.74m)	83.3 (3.75m)	81.9 (4.03) [6]	82.0 (4.06ddd) [8.0,2.3,[h]2.0[i]]	82.7(4.17ddd) [8.0,2.2,[h]1.8[i]]
7'	138.1 (5.89dd) [16.0,2.0[h]]	135.2(5.71dd) [16.0,6.0[h]]	135.5 (5.77dd) [15.9,∿5[f]]	134.6 (5.9m)	135.4 (5.95dd) [15.4,2.3[h]]	134.6(5.98ddd) [15.5,3.0,[j]2.2[h]]
8'	126.6 (7.51dd) [16.0,11.2]	131.0(7.55dd) [16.0,11.0]	130.5 (7.54dd) [15.9,11.0]	126.2 (7.68dd) [15.5,11]	126.2 (7.76ddd) [15.4,11.4,2.0[i]]	126.8(7.60ddd) [15.5,11.3,1.8[i]]
9'	143.7 (6.56dd)	142.0(6.60dd)	142.4 (6.62dd)	142.5[d](6.55t)	143.4[d](6.58dd)	142.7(6.62dd)
10'	117.2[d](5.73d)	117.1(5.82d)	116.7[d](5.83d)	118.9[d](5.79d)	118.4[d](5.79d)	119.0(5.88d)
11'	166.4[d]	166.3	166.2[d]	166.0	165.9	166.9
12'	20.2 (2.25d)	19.8(2.22d)	20.0 (2.24d)	18.2 (2.27s)	18.4 (2.28d)	17.1(1.60s)
13'	70.5 (3.7m)	69.6(3.7m)	69.7 (3.7m)	76.8 (3.65m)	77.1 (c)	76.3(3.7m)
14'	18.3 (1.19d)	18.5(1.17d)	18.5 (1.16d)	16.3 (1.32d)	16.5 (1.34d)	15.8(1.33d)

[a] Natural-abundance ^{1}H-noise-decoupled ^{13}C FT NMR spectra were measured by a Varian NV-14 FT NMR (15.087 MHz) or a CFT-20 (20 MHz) spectrometer in 8-mm tubes using TMS as an internal reference (δ_C 0). The ^{13}C signals were assigned using ^{1}H single-frequency off-resonance decoupling techniques, and known chemical-shift rules, and by comparisons with the literature data,[1,10,13,14] and from compound to compound. ^{1}H NMR spectra were recorded on a Varian HA-100 spectrometer operating at 100 MHz in the TMS-locked mode. [b] Shown only where necessary for the structure elucidation. [c] Not determined. [d,e] These assignments may be interchanged in each vertical column. [f] $J_{7\alpha,11\alpha}$. [g] $J_{8\alpha,10}$. [h] $J_{6',7'}$. [i] $J_{6',8'}$. [j] $J_{7',13'}$.

of long-range spin-couplings between H-7 and H-11 (3.2 Hz) and between H-8 and H-10 (2.1 Hz) determined by double and triple resonance experiments at 100-MHz ^{1}H NMR (see the TABLE) showed that the epoxide ring is attached to the β-position.

When 6 was hydrolized with 2% K_2CO_3 in MeOH for 6 days at room temperature, it gave carboxylic acids and an alcohol 13. The acid fraction was esterified with CH_2N_2 and separated by preparative TLC to give two methyl esters, which were confirmed to be 11 and 11' by comparison with their spectral and $[\alpha]_D$ data. Alcohol 13 was obtained as colorless crystals, $C_{15}H_{20}O_5$, mp >300° (sublim. at 200°), M^+ 280, and gave a monoacetate 13a, which has a free tertiary OH. Crotocol (15) was obtained by hydrolysis of crotocin (14)[9] and converted into a diol 16 through the opening of the 12,13-epoxide ring induced by the cleavage of the 7β,8β-epoxide on heating in 5% NaOH. Alcohol 13 may be obtained by the same double ring-opening reaction as in the case of 16. The ^{1}H NMR spectrum of 13a showed no signals due to the two epoxide rings. Detailed double and triple resonance experiments revealed the structure of 13a as expected. As a result, 6 was determined to be 7β,8β-epoxyisororidin E. In this case also, we could observe no NOE between H-2' and Me-3'.

Compound 7, $C_{29}H_{34}O_9$, amorphous powder,[6] and compound 8, $C_{29}H_{34}O_{10}$, mp 291-293° (decomp.),[6] were assumed to be roridin H derivatives from their ^{13}C and ^{1}H NMR spectral comparisons. Signals due to the central trichothecene moieties of 7 and 8 were found to be identical with that of 6, and those due to the side-chain of 7 are also identical with those of 4 (see the TABLE).[5,10] Therefore, 7 was revealed to be 7β,8β-epoxyroridin H. In this case also, no NOE was observed between H-2' and Me-3' as well as those in 4. The ^{13}C and ^{1}H spectra of 8 resemble those of 7 except for the signals due to C-2', C-3', C-4', C-12', and the corresponding protons. These spectral differences are quite similar to those between 9 and its 2',3'-epoxide, i.e., roridin D.[10,11] Therefore, 8 was assigned to 7β,8β,2',3'-diepoxyroridin H.

As a conclusion, we suggest based on the δ_H- and J-values obtained from the H-6' and H-7' signals (see the TABLE) that 9 has the same absolute configurations at C-6'(R) and C-13'(R) with those of 2 ($J_{6',7'}$ = 2 Hz),[12] and that 5 and 6 have the (6'S) and (13'R)-configurations ($J_{6',7'}$ = 6 Hz). It should be emphasized that the C-2':C-3' double-bond configuration must be revised at least in roridins E and H,[3] whereas it should not in vertisporin[1] and probably satratoxin H.[13]

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(12) For 2, $\delta_{H-7'}$ 5.98, $\delta_{H-8'}$ 7.48, $\delta_{H-9'}$ 6.60, $J_{6',7'}$ 2 Hz.[2]
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