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VERTISPORIN, A NEW ANTIBIOTIC FROM VERTICIMONOSPORIUM DIFFRACTUM

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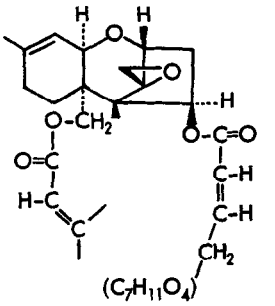
In a continuing search for fungal metabolites having cytotoxic activity, we found that the fungus Verticimonosporium diffractum,[1] strains TM-2098 and TM-2492, produces a new cytotoxic antibiotic, Vertisporin[2](1a). This antibiotic showed limited antifungal activity and inhibited only the growth of Trichophyton asteroides at a concentration of 10 mcg/cm^3. The cytotoxicity effect (ED_{50}) against Hela cells was 0.001 mcg/cm^3.

Vertisporin (1a), a colourless amorphous powder [$C_{29}H_{36}O_{10}$: M^+ 544, m.p. 176-183°: $[\alpha]_D^{26}$ +62.5 (±1.5°), λ_{max}^{EtOH} 216 nm (ε 19,500), $\nu_{max}^{CHCl_3}$ 1723 and 1717 cm^{-1}], has two αβ-unsaturated carboxyl groupings, because a dicarboxylic acid (2a), m.p. 219-232°, was obtained by hydrolysis with an alkali. On acetylation with Ac_2O in pyridine, 1a gave a diacetate (1b), m.p. 145-155°. From these results, we assumed that the remaining four oxygen atoms in 1a are present in ether-linkages. Moreover, ^{1}H-noise-decoupled natural-abundance ^{13}C FT NMR spectra of 1a and 1b in $CDCl_3$ showed twenty-nine and thirty-three ^{13}C signals, respectively; these facts agree with the elemental analysis data.

In the 220-MHz ^{1}H NMR spectrum in $CDCl_3$, 1a exhibited vinyl proton signals at δ 5.76 (1H, d, J = 12.0 Hz) and 6.43 (1H, d-t, J = 12.0 and 8.0 Hz), which were coupled with each other, and at δ 5.72 (1H, br-s). Thus, the presence of groupings A and B was revealed.

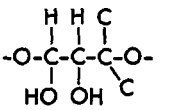
On hydrolysis with $KHCO_3$ in MeOH, 1a gave a diol (3a), m.p. 159-161.5°, together with 2a. Diol 3a and its diacetate (3b), m.p. 84-86.5°, proved to be identical with verrucarol[3] and its acetate, respectively, upon comparison of their IR and ^{1}H NMR spectra. Therefore, vertisporin was classified as a new

cytotoxic compound belonging to the roridin group,[4] and assumed to be represented by formula C.



C

Examination of the ^{1}H-noise-decoupled and single-frequency off-resonance decoupled ^{13}C NMR spectra of 1b and 3b in C_6D_6 leads to a conclusion that seven carbon atoms of the unknown portion in 1a, $-(C_7H_{11}O_4)-$, consist of $-O-\underset{|}{C}H-O-$, 2 x $-\underset{|}{C}H-O-$, $-CH_2-O-$, $\geqslant\underset{|}{C}-O-$, and 2 x $-\underset{|}{C}H_2-$; the ^{13}C signals for 3b were assigned by comparison of the spectrum with those of trichothecanes.[5] Further, in the 100-MHz ^{1}H NMR spectra of 1b in $CDCl_3$, signals due to OAc-bearing carbon atoms appear as two sharp doublets mutually coupled at δ 5.10 and 6.61 (J = 4.0 Hz). These results indicate that 1a has a partial structure D.

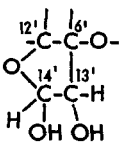


D

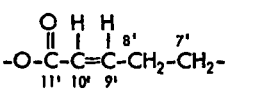
On the other hand, 2a showed absorption bands at 3470 and 1712 cm^{-1} in the IR and an absorption maximum at 222 nm (ε 10,780) in the UV spectra; the low intensity of the UV maximum suggests the disappearance of one αβ-unsaturated carboxyl system in 1a. When 2a was treated with diazomethane, a dimethyl ester (2b), m.p. 129-132.5°, was obtained. The ester has one hydroxy group and one vinyl proton (δ 5.90 in $CDCl_3$) according to IR and ^{1}H NMR spectra. Thus, we assumed that 2a is an addition product of one hydroxy group to the cis αβ-unsaturated carboxyl system.

Oxidation of 2b with dipyridine chromium (VI) oxide complex gave a five-membered ring lactone (4), $\nu_{max}^{CHCl_3}$ 1800 cm^{-1}. From this result, the partial structure D can reasonably be extended to E.

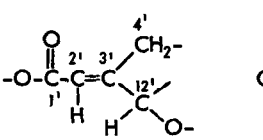
Further detailed double- and triple-resonance experiments for the 100-MHz ^{1}H NMR spectra of 1b both in $CDCl_3$ and C_6D_6 provided the following information. The presence of a $-CH_2-CH_2-$ or a $-CH_2-CH-CH-$ fragment and a $-CH=C\begin{matrix}CH_2-\\CH-O-\end{matrix}$ fragment can be expected by examinations of signals due to the groupings A and B, respectively, by the decoupling and INDOR techniques. Thus, partial structures A and B in formula C can be extended to F and G, respectively. In addition, the fact that the 15% enhancement in signal intensities due to the nuclear Overhauser effects was observed between the H-2' signal (δ 5.81 in



E



F



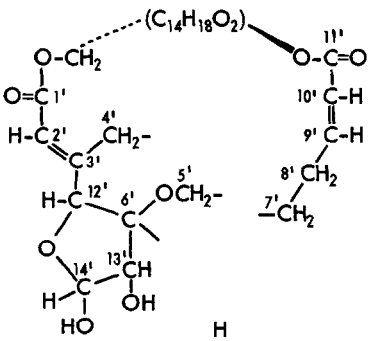
G

Table. ^{13}C and ^{1}H NMR Spectral Data on Vertisporin Diacetate (1b) and Verrucarol Diacetate (3b) in $CDCl_3$ and C_6D_6 (in parentheses)[a]

Carbon No.	δ(C)[b] 1b	δ(C)[b] 3b	δ(H) 1b	δ(H) 3b
2	(79.0d)[c]	(79.0d)[c]	3.82d(3.66t)	3.80d(3.67d)
3	(35.0t)	(36.7t)	f	f
4	(74.0d)	(75.4d)	~5.8br(5.71t)	3.24dd(5.81dd)
5	(49.7s)	(48.9s)	-	-
6	(43.2s)	(43.4s)	-	-
7	(20.7t)	(21.4t)	f	f
8	(27.7t)	(28.1t)	f	f
9	(138.4s)	(138.7s)	-	-
10	(119.9d)	(119.7d)	5.40d(5.32d)	5.39dd(5.36d)
11	(67.6d)[c]	(66.8d)[c]	3.57d(3.21d)	3.75d(3.50d)
12	(65.3s)	(65.2s)	-	-
13	(47.2t)	(47.3t)	2.77d(2.42d) 3.09d(2.69d)	2.79d(2.43d) 3.09d(2.69d)
14	(8.0q)	(6.8q)	0.80s(0.87s)	0.79s(0.81s)
15	(64.7t)	(63.7t)	3.97d(4.02d) 4.24d(4.27d)	4.05d(4.10d) 4.15d(4.18d)
16	(23.0q)	(23.0q)	1.70s(1.45s)	1.70s(1.49s)
1'	(165.7s)[d]		-	
2'	(118.5d)		5.81s(5.80s)	
3'	(152.6s)		-	
4'	(22.8t)[e]		f	
5'	(64.9t)		f	
6'	(86.7s)		-	
7'	(23.6t)[e]		f	
8'	(26.4t)[e]		f	
9'	(149.4d)		6.41dt(5.99dt)	
10'	(121.1d)		5.82dd(5.68dd)	
11'	(166.0s)[d]		-	
12'	(86.0d)		4.19s(4.26s)	
13'	(75.7d)		5.10d(5.33d)	
14'	(97.5d)		6.61d(6.92d)	

[a] All ^{13}C FT NMR spectra were measured with a Varian NV-14 FT NMR spectrometer at 15.1 MHz [δ(C), ±0.1 ppm]; 220-MHz and 100-MHz ^{1}H NMR spectra were taken with a Varian HR-220, courtesy of Dept. of Hydrocarbon Chem., Kyoto Univ., and a Varian HA-100 spectrometer, respectively [δ(H), ±0.02 ppm; J, ±0.5 Hz]. [b] Multiplicities were obtained by single-frequency off-resonance decoupling (SFORD) experiments. The δ(C) values in $CDCl_3$ are almost the same as those in C_6D_6; however, SFORD experiments were not done in $CDCl_3$. Data on OAc are not shown. [c] Detailed SFORD experiments in C_6D_6 revealed that the assignments of the C-2 and C-11 signals in trichothecanes reported by Hanson, et al.[5] were reversed. [d,e] These assignments are interconvertible. [f] Not exactly determinable.

$CDCl_3$ and 5.80 in C_6D_6) and the H-12' singlet (δ 4.19 in $CDCl_3$ and 4.26 in C_6D_6) in 1b confirmed the stereochemical relationship shown as partial structure G. Since the ^{13}C NMR spectra, as mentioned above, indicated the presence of one more $-CH_2-O-$ group in the unknown portion of 1a, $-(C_7H_{11}O_4)-$, formula C should be as represented by formula H. It is

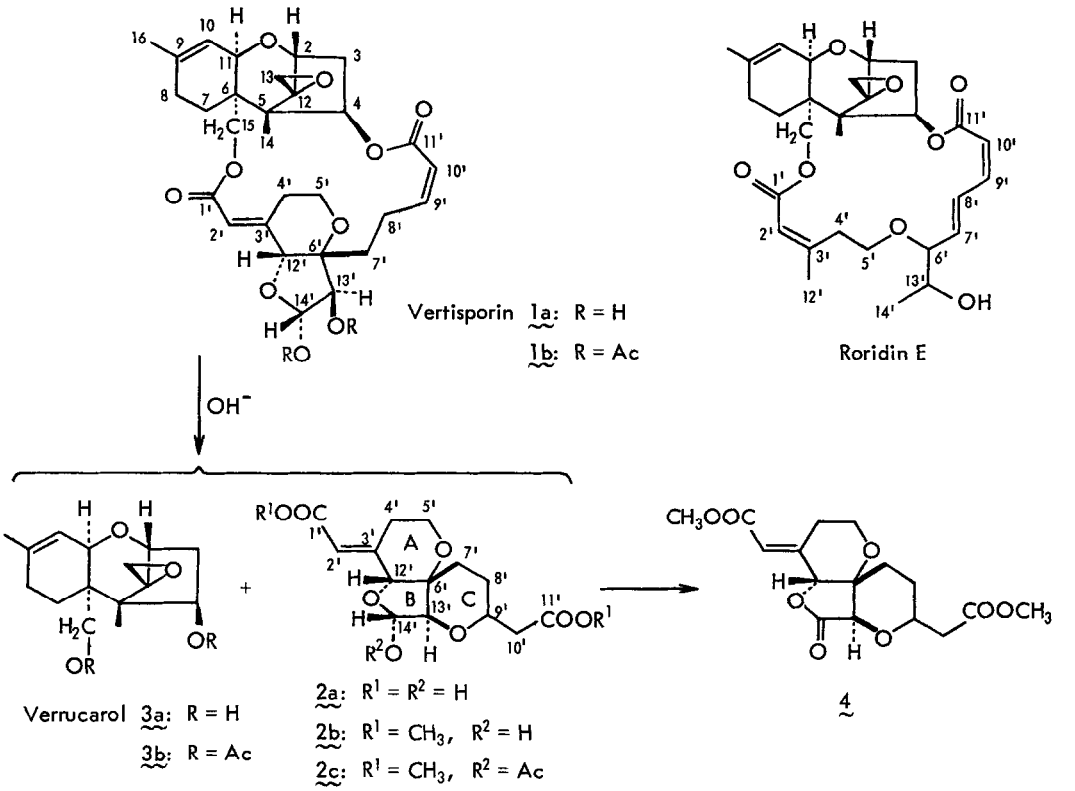


H

problematical whether C-4' and C-6' bind with C-5' and C-7', respectively, or C-4' and C-5' bind with C-6' and C-7', respectively. However, in the latter case a four-membered ring must be formed; this cannot be in harmony with the present results.

On the bases of the above results and the consideration of a biosynthetic route similar to that of roridins, formula 1a is derived for the plane structure of vertisporin.

Since the CD spectrum of 2b showed a positive $\pi-\pi^*$ Cotton effect, $[\theta]_{227}^{MeOH}$ +42,000, due to the



αβ-unsaturated ester chromophore, the configuration of H-12' is β.[6] Furthermore, the 100-MHz ^{1}H NMR spectrum of an acetate 2c in $CDCl_3$ showed four singlet signals at δ 5.93 (H-2'), 4.30 (H-12'), 3.88 (H-13'), and 6.04 (H-14'), indicating that the dihedral angles between H-2' and H-12' and between H-13' and H-14' are about 0° and 90°, respectively. Examination of molecular models shows that the only stereostructure having cis-A/B and cis-B/C ring junctures, and an α-OAc at C-14' satisfies the above results. Therefore, the absolute configuration of vertisporin is elucidated to be structure 1a.

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