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THE CONSTITUTION OF VERTINOLIDE, A NEW DERIVATIVE OF TETRONIC ACID, PRODUCED BY *VERTICILLIUM INTERTEXTUM*[1]

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Abstract—From the culture of *Verticillium intertextrum* a new tetronic acid derivative, vertinolide, has been isolated as the main constituent of the chloroform extract. Structure **1a** was established for vertinolide by X-ray diffraction analysis. Vertinolide (**1a**) was also transformed to an O-methyl-(**1b**), an O-acetyl- (**1c**), a tetrahydro- (**1d**) and a tetrahydro-O-methyl-derivative (**4**). The spectroscopic properties of **1a**, of its derivatives **1b**, **1c**, **1d** and **4** as well as of three model compounds are compared and discussed.

Verticillium intertextum, when grown in light, produces a yellow material which is released into the nutrient broth. This material might be identical with the substance mentioned earlier[2] as being responsible for the colour of light-grown hyphae of this fungus. Chloroform extraction of the spent culture medium, followed by chromatography, yielded not only a number of yellow compounds,[1] but also a colourless substance that represents a major component of the extract. The present paper deals with the structure of this compound and with some of its derivatives.

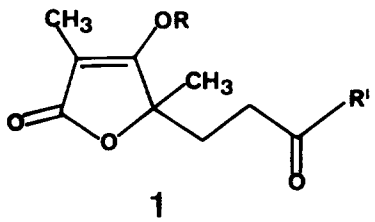
Isolation of vertinolide (**1a**)

The culture filtrate of *V. intertextum* was acidified to pH 3.0 and then extracted several times with chloroform. Chromatography of the extract on Sephadex LH-20 gave a light yellow main fraction between two strongly yellow coloured ones. This intermediate fraction contained 36% of the extract. The residue therefrom, after repeated crystallisation, afforded colourless crystals (5% of the extract), m.p. 149.2–152.3° (dec), $[\alpha]_D^{20} - 25.0°$. We name this compound *vertinolide* (**1a**), corresponding to its origin and its lactone functionality. The systematic name of **1a** according to the IUPAC rules of nomenclature of organic chemistry[3] is (−) - 3 - hydroxy - 2,4 - dimethyl - 4 - (3 - oxo - (E,E) - 4,6 - octadienyl) - 2 - buten - 4 - olide; according to *Chem. Abstr.* (9th and 10th period) it is named (−) - 4 - hydroxy - 3,5 - dimethyl - 5 - (3 - oxo - (E,E) - 4,6 - octadienyl) - 2(5*H*) - furanone. Its structure (without absolute configuration) was determined by X-ray analysis.

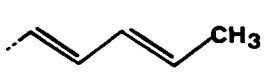
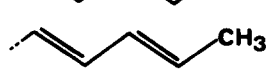
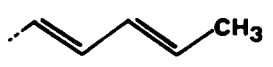
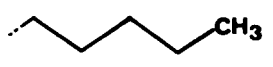
Vertinolide (**1a**) is a metabolic product of the fungus, and not a component of the nutrient broth. As a derivative of tetronic acid (**2c**)[4,5] it belongs to a class of fungal metabolites known as mycotoxins.[6]

X-ray analysis of vertinolide (**1a**) *at ca.* −140°

Vertinolide (**1a**), $C_{14}H_{18}O_4$, M = 250.30, crystallises from methanol as colourless monoclinic crystals, space group $P2_1$, a = 7.288(1), b = 6.509(2), c = 13.838(3) Å, β = 102.57(2)° (from 25 automatically centered reflections of an asymmetric unit), Z = 2. MoK_α-Radiation was used to



1

	R	R'
a	−H	
b	$-CH_3$	
c	$-COCH_3$	
d	−H	

measure the intensities of the 1534 symmetry independent reflections within 54° (2θ) on a Nicolet - R3 - four - circle - autodiffractometer in the ω-scan mode. The intensities were conventionally corrected without absorption correction. The phases were determined by direct methods. The structure was solved by application of the program SHELXTL[7] on an Eclipse S/250 computer.

The atomic parameters of the C- and O-atoms were anisotropically refined. Those of the H-atoms were varied isotropically in the last cycles of the least-squares-refinement after localisation by a difference-Fourier calculation. The 1148 structure factors with $F \geq 4\sigma(F)$, weighted by $1/\sigma^2(F)$, were used to refine the 234 parameters in a blocked matrix (*ca.* 100 parameters/block) to a conventional R-value of 0.048 ($R_w = 0.033$). The ratio of shift to standard deviation averaged finally to 0.001, at maximum 0.005.

A list of atomic coordinates with LS-computed standard deviations is given in Table 1; the absolute configuration was arbitrarily chosen. A complete list of structure factors and atomic parameters can be obtained from one of the authors (J.H.B.). Figure 1 shows a molecular drawing of **1a** with the numbering of the C- and O-atoms as used throughout this paper. There are no unusual bond lengths or angles in the molecule. The lactone ring (C(1)–C(4) and O(4)) and its direct neighbours (O(1), O(3) and C(13)) are coplanar within 0.04 Å. The zig-zag chain with the atoms C(14) and C(4)–C(12) together with O(7) also lie in a plane within 0.05 Å. These two planes are orthogonal to each other. Figure 2 shows the packing diagram in the crystal. The molecules are connected along the b-axis by an intermolecular H-bridge from O(3) to O(1). The less polar side chains form intermediate columns by anti-parallel stacking.

Table 1. Fractional atomic coordinates of vertinolide (**1a**) with e.s.d.s in units of the last significant digit

Atom	X	Y	Z
C(1)	.1010(4)	.6445(6)	.8975(2)
O(1)	.1765(3)	.8000	.9376(2)
C(2)	.1470(4)	.4305(6)	.9183(2)
C(3)	.0183(4)	.3216(5)	.8551(2)
O(3)	-.0117(3)	.1199(4)	.8409(2)
H-O(3)	.069 (5)	.037 (6)	.882 (3)
C(4)	-.1200(4)	.4574(6)	.7880(2)
O(4)	-.0514(3)	.6626(4)	.8205(2)
C(5)	-.1151(4)	.4351(6)	.6788(2)
H_α-C(5)	-.201 (4)	.535 (4)	.643 (2)
H_β-C(5)	-.160 (4)	.305 (5)	.659 (2)
C(6)	.0779(5)	.4577(7)	.6557(2)
H_α-C(6)	.166 (4)	.360 (5)	.690 (2)
H_β-C(6)	.131 (5)	.592 (7)	.672 (3)
C(7)	.0790(4)	.4357(6)	.5465(2)
O(7)	-.0673(3)	.4182(5)	.4844(1)
C(8)	.2646(4)	.4393(7)	.5207(2)
H-C(8)	.373 (5)	.436 (8)	.570 (2)
C(9)	.2823(4)	.4296(6)	.4270(2)
H-C(9)	.170 (3)	.421 (6)	.377 (2)
C(10)	.4573(4)	.4395(6)	.3936(2)
H-C(10)	.573 (4)	.432 (6)	.438 (2)
C(11)	.4586(4)	.4372(6)	.2974(2)
H-C(11)	.340 (4)	.432 (6)	.255 (2)
C(12)	.6312(5)	.4476(8)	.2546(3)
H_α-C(12)	.749 (4)	.461 (6)	.306 (2)
H_β-C(12)	.636 (5)	.322 (6)	.209 (3)
H_γ-C(12)	.622 (6)	.566 (7)	.213 (3)
C(13)	.3075(5)	.3615(6)	.9996(2)
H_α-C(13)	.415 (4)	.385 (6)	.986 (2)
H_β-C(13)	.293 (5)	.386 (7)	1.060 (2)
H_γ-C(13)	.328 (6)	.214 (8)	.995 (3)
C(14)	-.3178(4)	.4325(6)	.8048(2)
H_α-C(14)	-.390 (4)	.536 (5)	.773 (2)
H_β-C(14)	-.316 (3)	.452 (6)	.874 (2)
H_γ-C(14)	-.366 (5)	.292 (6)	.778 (3)

Physical properties of vertinolide (**1a**)

The spectroscopic properties of vertinolide (**1a**) and of some of its transformation products **1b–d** and **4** (for the transformations see next section) are collected in Tables 2 (UV and IR), 3 (^{1}H NMR) and 4 (^{13}C NMR). These properties reflect the presence in **1a** of the 4 - hydroxy - 3,5 - dimethyl - 2(5*H*) - furanon - 5 - yl substructure (**2a** = substructure **A**) and the (E,E) - 2,4 - hexadienon - 1 - yl substructure (**3a** = substructure **B**), the two of them linked together by a dimethylene bridge. This is evident by a comparison of these properties with those of two model compounds, namely α-methyltetronic acid (**2b**)[8]

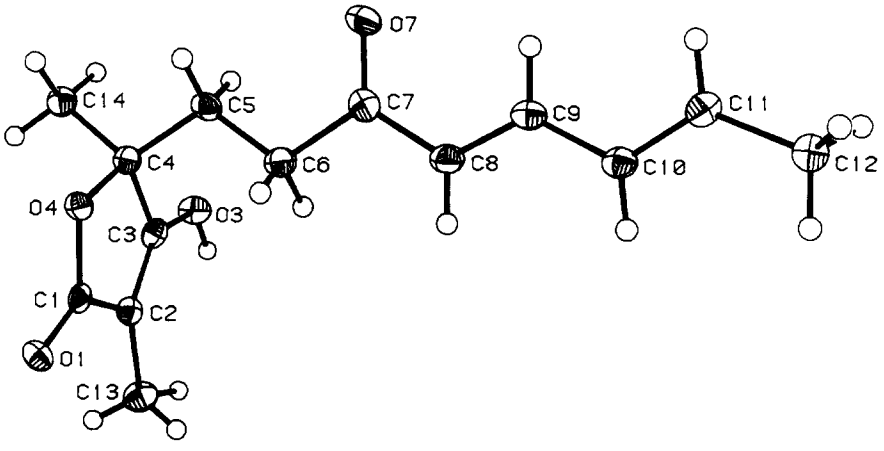


Fig. 1. Molecular drawing of vertinolide (**1a**, absolute configuration chosen arbitrarily) with the numbering of the atoms as used throughout this paper. The H-atoms are drawn with an arbitrary radius, whereas the other atoms are represented by their 50% probability ellipsoids of vibration.

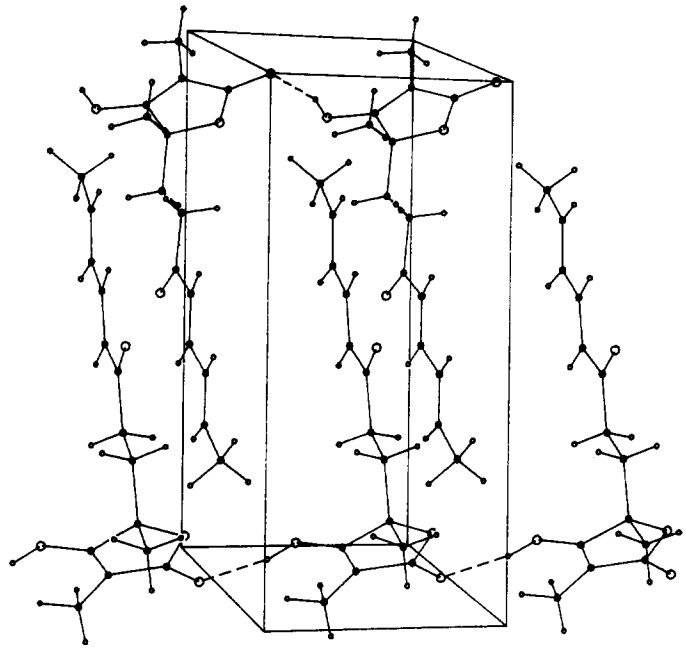
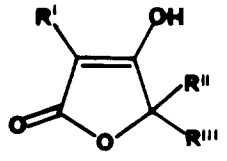


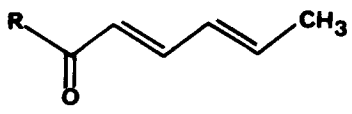
Fig. 2. Packing diagram of the molecules of vertinolide (**1a**) in the crystal.

(short: the lactone **2b**) and of (E,E) - sorbyl - methyl - ketone (**3b**) (short: the dienone **3b**). Since these properties are of considerable diagnostic value for structures of type **1a** they are discussed in some detail as follows:



2

	R^I	R^{II}	R^{III}
a	$-CH_3$	$-CH_3$	—
b	$-CH_3$	—H	—H
c	—H	—H	—H



3

	R
a	—
b	$-CH_3$

(1) The pK_a value of **1a** (5.4) and of its tetrahydro derivative **1d** (5.6) is due to the enolized 3-oxo-butan-4-olide functionality of substructure **A**; it falls in the same range as that of the lactone **2b** and of other derivatives of tetronic acid (pK_a 1.8–6.7).[5,9]

(2) The UV spectrum of **1a** (see Table 2) shows two absorptions, a shoulder at 230 nm due to substructure **A** and a maximum at 270 nm due to substructure **B**. The additional shoulder at about 256 nm expected for **A** (see Ref. 10 for this absorption in the lactone **2b**) is hidden under the maximum of **B**, but it becomes visible after hydrogenation of the double bonds in the side chain (see UV spectrum of **1d**). The UV spectrum of **1a** is pH dependent: In basic solution only one, rather intensive maximum at 261 nm is present; this is due to superimposition of the unaffected maximum of **B** (the UV spectrum of the dienone **3b**[11] is not pH dependent) and the base-shifted maximum of **A** around 255 nm (as is also found with **1d** and the lactone **2b**). Acidification regenerates the original spectrum of **1a**.

(3) In the IR spectrum of **1a** (see Table 2), substructure **A** is responsible for a band at 1740 (lactone carbonyl), a shoulder at 1690 and a strong band at 1671 cm^{-1} (enol), similar bands being found with the lactone **2b**.[12] Substructure **B** contributes to the band at 1671 cm^{-1} by a relatively weak absorption and exhibits two further double bond bands at 1638 and 1594 cm^{-1}, all three being similar to the ones of the dienone **3b**.[11]

(4) In the ^1H NMR spectrum of **1a** (see Table 3), the substructure **B** is easily recognised by the similarity of some of its signals (for four vinyl protons and for the secondary olefinic methyl group) with those of the dienone **3b**[13] and by the disappearance of these signals in the product **1d** of hydrogenation of **1a**. Three singlets in the ^1H NMR spectrum of **1a** have to be assigned to substructure **A**: a low field, D_2O-exchangeable broad one due to the enol proton, which is replaced by a methyl

Table 2. IR and UV absorptions of vertinolide (**1a**), of its derivatives **1b–d**, of **4** and of the model compounds **2b** and **3b**

Compound	IR ($CHCl_3$)[a] in cm^{-1}						Ref. (IR)	UV (CH_3OH) : max in nm (ε) neutral	with OH^-	with H_3O^+	Ref. (UV)
1a	1740m		1690sh	1671s	1638m	1594m	b	230 (9900)[c] 270 (28460)	261 (36850)	229 (14350) 272 (25300)	b
1b	1743m		1690sh	1670s	1640m	1598m	b	230 (13760) 274 (23920)	unchanged	unchanged	b
1c	1788s 1756s		1690m	1660w	1640m	1596m	b	213 (13100) 273 (26300)	260 (37570)	229 (14230) 274 (25460)	b
1d	1740m	1715-1685m, br.		1670s			b	229 (10000) 256 (4670)[c]	257 (18910)	228 (12060)	b
2b	1751m		1690sh	1676s			12	229 (11010)[d] 256 (4670)[c]	256 (19400)	228 (12940)	b
3b				1670	1645	1595	11	271 (22500)[e]	unchanged	unchanged	11
4		1710-1685m, br.				1593s	b	264 (15700)	unchanged	unchanged	b

[a] All peaks from 1800 to 1500 cm^{-1} are reported; [b] this work; [c] shoulder; [d] in [10], only a maximum at 228 nm (ε = 12023) was reported for **2b**; [e] in C_2H_5OH.

Table 3. ^1H NMR signals of vertinolide (**1a**), of its derivatives **1b–d**, of **4** and of the model compounds **2b** and **3b** [δ in ppm (J in Hz)]

Compound	Solvent	RO-C(3)	CH_3-C(2)	CH_3-C(4)	$H_2C(5)$	$H_2C(6)$	H-C(8)	H-C(9)	H-C(10)	H-C(11)	CH_3-C(11)	Ref.
1a	$CDCl_3$	9.80/br. s[a]	1.68/s	1.48/s	2.4-2.1/m	2.8-2.4/m	6.06/d (15.4)	7.19/dxd (15.4 & 9.6)	6.4-6.1/m		1.89/d (5.4)	b
1b	$CDCl_3$	4.12/s[c]	1.99/s	1.43/s	2.2-2.0/m	2.7-2.3/m	6.07/d (15.5)	7.15/dxd (15.5 & 10)	6.3-6.2/m		1.88/d (5.0)	b
1c	$CDCl_3$	2.32/s[d]	1.74/s	1.48/s	2.3-2.0/m	2.7-2.45/m	6.05/d (15.5)	7.14/dxd (15.5 & 9.6)	6.3-6.2/m		1.89/d (5.4)	b
1d	CD_3OD	-[e]	1.66/s	1.42/s	2.1-1.95/m	2.5-2.25/m	2.42/t[f] (7.2)	1.6-1.45/m[f]	1.4-1.2/m[g]		0.90/t (6.0)	b
2b[h]	DMSO-d_6	11.70/br. s[a]	1.60/t (0.5)	4.59/q[f] (0.5)								10
3b[h]	CCl_4						5.90/d (16)	7.0/m	6.05/m		1.80/d (6.0)	13
4	$CDCl_3$	4.01/s[i]	1.58/s	1.42/s	2.2-2.0/m	2.5-2.3/m	2.5-2.3/m[f]	1.5-1.4/m[f]	1.4-1.2/m[g]		0.89/t (6.0)	b

[a] R = H; [b] this work (200 MHz); [c] R = CH_3; [d] R = $COCH_3$; [e] R = D; [f] CH_2-signal; [g] 2 x CH_2-signal; [h] the signals of **2b** and **3b** are placed in the columns of corresponding signals of **1a**; [i] CH_3O-C(1).

singlet in **1b** and by an acetyl singlet in **1c**, as well as two high field ones due to the methyl groups at C(2) and C(4). These methyl assignments are derived from chemical shift considerations and from the shift-displacements due to enol methylation and acetylation as well as from selective decouplings in the ^{13}C NMR spectrum of **1a** (see below). Finally, two complicated multiplets partially simplifiable by mutual decoupling are assigned to the two diastereotopic proton-pairs at C(5) and C(6) in the dimethylene group of **1a**.

(5) The proton noise decoupled ^{13}C NMR spectrum of **1a** shows separate signals for each of the fourteen carbon atoms (see Table 4). The six signals due to substructure **B** are assigned by comparison with the corresponding data of the dienone **3b**.[14] The signals of the dimethylene group are recognised by their chemical shifts and their triplet multiplicity in the proton off resonance spectrum. Of the remaining six signals, five are at chemical shifts comparable to those found with the lactone **2b** and the last one, a quartet in the off resonance spectrum, must be assigned to the methyl group at C(4). Of particularly diagnostic value among the ^{13}C-signals of the lactone **2b** and of **1a** is one at extremely high field; it must be assigned to the carbon atom of a methyl group which lies in the α-position of an enolised cyclic β-dicarbonyl system (see Ref. 15 for a similar case). Of the two signals in a middle field region, plausible for C(2) and C(4), the one at lower field must be due to C(2) because it is a singlet in both vertinolide (**1a**) and the lactone **2b**, whereas the other one, due to C(4), is a singlet in **1a**, but a triplet in **2b**. Of the two signals for both **1a** and **2b** in the low field region characteristic for carboxyl- and

Table 4. ^{13}C NMR signals at 25.14 MHz of vertinolide (**1a**), of its derivatives **1b–d**, of **4** and of the model compounds **2b** and **3b** [δ in ppm from proton noise decoupled spectra; multiplicity from off resonance spectra]

Compound	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)	C(8)	C(9)	C(10)	C(11)	C(12)	C(13)	C(14)	RO-C(3)	Ref.
1a[a]	173.9 s	97.2 s	176.6 s	82.6 s	31.7 t	34.8 t	199.1 s	128.6 d	143.5 d	131.4 d	140.7 d	18.7 q	6.2 q	23.6 q	-	b
1b[a]	173.7 s	96.8 s	176.1 s	82.3 s	31.8 t	34.6 t	199.0 s	128.5 d	143.4 d	131.3 d	140.9 d	18.8 q	8.5 q	23.8 q	59.7 q	b
1c[a]	171.5 s	113.9 s	166.3[c] s	83.9 s	31.2 t	34.5 t	198.7 s	128.6 d	143.5 d	131.3 d	140.8 d	18.7 q	8.7 q	23.2 q	168.3[c] s & 20.5 q	b
1d[a]	174.1 s	96.8 s	176.8 s	82.5 s	32.0 t	36.8 t	209.4 s	42.0 t	24.0 t	31.1 t	23.1 t	14.2 q	6.2 q	23.6 q	-	b
2b[d,e]	173.1 s	94.8 s	175.6 s	66.7 t									6.0 q			b
3b[e,f]							197.7 s	128.3 d	143.3 d	130.0 d	139.7 d	18.3 q				14
4[a]	180.0 s	92.1 s	200.0 s	87.0 s	32.1 t	36.7 t	209.3 s	42.9 t	24.1 t	30.9 t	23.1 t	14.2 q	4.1 q	22.1 q	56.5[g] q	b

[a] In CD_3COCD_3; [b] this work; [c] assignment may be interchanged; [d] in DMSO-d_6; [e] the signals of **2b** and **3b** are placed in the columns of corresponding signals of **1a**; [f] in $CDCl_3$; [g] CH_3O-C(1).

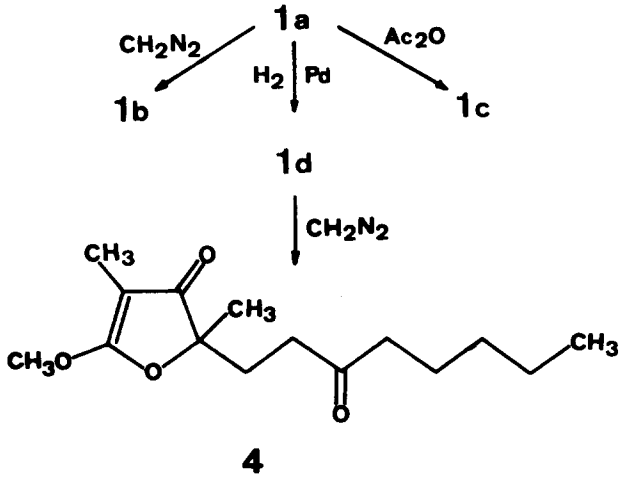
enol-carbon atoms, the one at slightly higher field is attributed to C(1) because it is a quartet (J = 4.5 Hz) in the fully coupled spectrum of **1a** due to vicinal coupling with only the three protons of the C(2)-methyl group, whereas the lower field signal of C(3) is a complex multiplet due to vicinal coupling with eight protons. Selective decoupling of the ^{13}C NMR spectrum of **1a** by irradiation at the frequency of the CH_3-C(4) proton resonance (1.48 ppm), which generates a singlet at 23.0 ppm (CH_3-C(4)) and diminishes the coupling constants of 24.4 and 32.2 Hz in the quartets at 6.2 (CH_3-C(2)) and 18.5 ppm (CH_3-C(11)), respectively, permits the assignment of the ^1H and ^{13}C NMR signals to the three methyl groups of **1a** as given above.

Transformations of Vertinolide (**1a**)

In order to evaluate the chemical properties of vertinolide (**1a**) a few reactions were performed, as shown in the scheme. Diazomethane and acetic anhydride–pyridine reacted with the enol function of **1a** to give the monomethyl ether **1b** and the monoacetate **1c** (MS evidence), respectively. The constitution of these derivatives follows from their spectral properties in comparison with those of vertinolide (**1a**): The UV spectrum of the ether **1b** is practically identical with that of **1a**, but pH independent, that of the acetate **1c** shows a typical hypsochromic shift of the lower wave length maximum assigned to substructure A (see Ref. 16). In basic solution the acetate **1c** is hydrolysed, so that the UV absorption behaviour of **1a** is observed.

In the IR spectra of the ether **1b** and of the acetate **1c**, the enol OH band is missing; in that of the acetate **1c**, the lactone carbonyl band is shifted to higher frequency (see Ref. 17). The ^1H and the ^{13}C NMR spectra of the ether **1b** and of the acetate **1c** show the expected new signals of the O-methyl group and the O-acetyl group. Of interest is the upfield shift of the signals of C(1) and C3) and the downfield shift of C(2) in the ^{13}C NMR spectrum of the acetate **1c** compared with that of vertinolide (**1a**) (see also Ref. 15).

Catalytic hydrogenation of vertinolide (**1a**) caused two equivalents of hydrogen to be consumed to give the



Scheme 1.

tetrahydro derivative **1d** (MS evidence), in which substructure **B** is saturated. This follows from the corresponding changes in all the spectral properties assigned to substructure **B** in **1a** (see Tables 2–4).

Methylation of the tetrahydro derivative **1d** with diazomethane afforded two products (tlc) of which only the major one, **4**, was isolated. Its constitution as a furanone-derivative, i.e. as the product of monomethylation (MS evidence) at the lactone oxygen atom, was deduced from its spectral properties in comparison to those of its precursor **1d**: The properties associated with the saturated side chain (tetrahydro substructure **B** and dimethylene group) are practically identical with those of **1d**. In contrast, with respect to the spectral properties of substructure **A**, **4** differs considerably from both **1d** and **1b**: The UV and the IR spectra are comparable to those of other tetronic acid derivatives methylated at the lactone oxygen atom.[9,15] The difference in the position of the ring C, C double bond in **1d** and **4** expresses itself clearly in the ^{13}C NMR chemical shifts of C(1) and C(3) (see Table 4).

EXPERIMENTAL

Identity and culture of fungus

The organism producing vertinolide (**1a**) was originally isolated as a laboratory contaminant; its identity with *Verticillium intertextum*[1] was established by W. Gams, Centraalbureau voor Schimmelcultures, Baarn, Holland. The fungus was grown in static liquid culture on 2.4% potato-dextrose Difco for 21 d in a constant temperature room (27°) under fluorescent lamps. For details of inoculum preparation see Ref. 18. The yield (per l) was *ca.* 5 mg of vertinolide.

Chemical methods

Column chromatography was performed on Sephadex LH-20. Preparative thin layer chromatography (prep tlc) was carried out using Merck silica gel 60 PF_{254} on hand coated plates 20 × 20 cm (10 g per plate) and analytical thin layer chromatography (anal tlc) using Merck DC Fertigplatten Kieselgel 60 F_{254} (10 × 20 cm, layer 0.25 mm). The spots were detected under UV light of 254 nm. UV spectra were determined on a UVIKON 810 spectrophotometer. Maxima and shoulders are reported in nm (ϵ) in Table 2. IR spectra were obtained on a Perkin–Elmer 257 grating infrared instrument; the frequencies of all well resolved peaks between 1800 and 1500 cm^{-1} are listed in Table 2. The ^1H NMR spectra were measured at 200 MHz on a Varian XL 200 FT. or at 360 MHz on a Bruker HXS-360 FT. spectrometer and the ^{13}C NMR spectra at 25.14 MHz on a Varian XL 100 spectrometer. Internal TMS served as reference ($\delta = 0$ ppm). The data of the ^1H NMR spectra (see also Table 3) are reported as chemical shifts/multiplicity, coupling constants J in Hz, number of protons H (interpretation). For double resonance experiments only those signals are reported which were changed upon irradiation. For the ^{13}C NMR spectra (see also Table 4) the chemical shifts are taken from proton noise decoupled spectra with multiplicities from off resonance spectra.

Mass spectra were obtained on a Varian MAT 711 apparatus. All peaks above *m/z* 150 with an intensity >1% relative to the base peak and those between *m/z* 150 and 40 with an intensity >10% are reported as *m/z* values/intensity (interpretation and, in cases of high resolution measurement, calculated *m/z* value). The melting points (m.p.) were determined on a Mettler FP5 + FP52 apparatus, the $[\alpha]_D$ values on a Perkin–Elmer 241 polarimeter and the pK_a values by ultramicro potentiometric titration of 7×10^{-5} M solutions in CH_3OH with 0.1 N NaOH.

Isolation of vertinolide (**1a**)

The spent culture medium (pH 5.5–6.5) was separated from the mycelium by filtration through glass fibre paper, acidified to pH 3.0 with 5N HCl and then extracted several times with chloroform (one-third of the aqueous volume) until no further colouring of the organic layer was observed. Evaporation of the chloroform solution at 20°/15 Torr and drying of the residue at 20°/0.1 Torr left a dark brown oil (100 mg per liter broth). 4.46 g of this crude residue was chromatographed on 300 g Sephadex LH-20 in a 4.5 × 60 cm column with $CHCl_3$/pentane 2:1 at 4° in the dark (flow rate 1.3 ml/min). A polarity gradient was used changing the solvent ratio to 3:1 after 2000 ml eluate was collected. The fractions were controlled by anal tlc ($CHCl_3/C_2H_5OH$ 94:6). The dark red residue (1.60 g), obtained after evaporation and drying at 20°/15 Torr of those fractions (1900–2850 ml) which contained vertinolide (**1a**), was crystallised from $CH_2Cl_2/(C_2H_5)_2O$ at −20° to give 370 mg pale yellow crystals, m.p. 138–146° (dec). Repeated recrystallisations from $CH_2Cl_2/(C_2H_5)_2O$, acetone/$(C_2H_5)_2O$ and finally acetone yielded 221 mg of pure vertinolide (**1a**) (5% of the crude residue) as colourless prisms, m.p. 149.2–152.3° (dec); $[\alpha]_D^{20} = -25.0°$ (c = 0.05, $CHCl_3$; $pK_a = 5.43$; anal tlc ($CHCl_3/C_2H_5OH$ 94:6) R_f 0.25 UV, IR, ^1H and ^{13}C NMR see tables 2–4. ^1H NMR (360 MHz, $CDCl_3$) double resonance: Irradiation at 7.19 (H-C(9)) gave: 6.35/simplified m (H-C(10), H-C(11)) and 6.06/d, J = 2.0 (H-C(8)). Irradiation at 6.35 (H-C(10), H-C(11)) gave: 7.19/d, J = 15.4 (H-C(9)) and 1.89/s (CH_3-C(11)). Irradiation at 6.06 (H-C(8)) gave: 7.19/dxm, J = 9.6 (H-C(9)). Irradiation at 2.70 (H_α-C6)) gave: 2.6–2.4/simplified m (H_β-C(6)) and 2.4–2.2/simplified m (H_2-C(5)). Irradiation at 2.50 (H_β-C(6)) gave: 2.8–2.6/simplified m (H_α-C(6)) and 2.4–2.2/simplified m (H_2-C(5)). Irradiation at 2.30 (H_2-C(5)) gave: 2.70/d, J = 14 (H_α-C(6)) and 2.50/d, J = 14 (H_β-C(6)). Irradiation at 1.89 (CH_3-C(11)) gave: 6.35/d, J = 15.4 (H-C(11)) and 6.25/dxd, J = 15:4 & 9.6 (H-C(10)). ^{13}C NMR (DMSO-d_6, coupled): 198.4/m (C(7)); 176.4/m (C(3)); 173.2/q, J = 4.5 (C(1)); 142.6/dxm, J = 152.6 (C(9)); 140.4/dxm, J = 155.1 (C(11)); 130.3/dxm, J = 155.0 (C(10)); 127.5/dxm, J = 156.6 (C(8)); 94.7/q, J = 6.6 (C(2)); 81.2/m (C(4)); 33.7/txm, J = 127.2 (C(6)); 30.5/txm, J = 129.9 (C(5)); 23.0/q, J = 128.9 C(14)); 18.5/qxd, J = 126.9 & 5.0 (C(12)); 6.2/q, J = 128.4 (C(13)). ^{13}C NMR (DMSO-d_6) heteronuclear double resonance: Irradiation at 1.48 (CH_3–C(4)) gave: 23.0/s (C(14)), 18.5/q, J_{red} = 32.2 (C(12)) and 6.2/q, J_{red} = 24.4 C(13)). MS (70 eV): 250/1 (M^+); 235/3 ($M^+ - CH_3$); 151/3; 108.0575/29 (C_7H_8O, calc. 108.0574); 99/13; 95.0495/100 (C_6H_7O, calc. 95.0496); 67/34; 41/20. (Found: C, 67.37; H, 7.19. Calc. for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25%).

Extraction and chromatography of 2.4% potato-dextrose Difco in the same manner as described here did not afford any **1a**.

Derivatives of vertinolide (**1a**)

(−) - 3 - *Methoxy* - 2,4 - *dimethyl* - 4 - (3 - *oxo* - (*E,E*) - 4,6 - *octadienyl*) - 2 - *buten* - 4 - *olide* (**1b**). A suspension of 78.2 mg (0.31 mmol) vertinolide (**1a**) in 5 ml $(C_2H_5)_2O$ was treated with 18 ml 1.4% solution of CH_2N_2 in $(C_2H_5)_2O$ (7.0 mmol) at −10° for 30 min. After evaporation of the solvent the residue was worked up by preparative tlc (4 plates with $CHCl_3/C_2H_5OH$ 99:1). The product from a band at R_f 0.6 was recrystallised from $(C_2H_5)_2O$/pentane at −20° to give 38.9 mg (48%) **1b** as colourless needles, m.p. 59.0–61.5°; $[\alpha]_D^{20} = -13.2°$ (c = 0.1, $CHCl_3$); anal tlc ($CHCl_3/C_2H_5OH$ 94:6) R_f 0.8. UV, IR, ^1H and ^{13}C NMR see Tables 2–4. MS (70 eV): 266/5 ($M^+ + 2H$); 264/5 (M^+); 249/2; 223/1; 221/2; 220/1; 198/2; 197/12; 191/1; 181/3; 179/3; 170/1; 169/1; 165/2; 159/1; 157/1; 155/4; 153/3; 152/2; 151/2; 142/20; 141/17; 109/10; 108/77; 95/100; 71/13; 69/15; 67/34; 65/10; 59/17; 57/22; 55/23; 45/16; 43/45; 41/52. (Found: C, 67.99; H, 7.72. Calc. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63%)

(−) - 3 - *Acetoxy* - 2,4 - *dimethyl* - 4 - (3 - *oxo* - (*E,E*) - 4,6 - *octadienyl*) - 2 - *buten* - 4 - *olide* (**1c**). A solution of 32.0 mg (0.128 mmol) vertinolide (**1a**) in 3 ml pyridine–acetic anhydride 1:1 was kept at r.t. in the dark for 74 h. After evaporation and drying at 20°/0.02 Torr for 3 h 37.0 mg (100%) **1c** remained as colourless crystals, m.p. 83.2–85.5. $[\alpha]_D^{20} = -42.5°$ (c = 0.04, $CHCl_3$); anal tlc ($CHCl_3/C_2H_5OH$ 94:6) R_f 0.8. UV, IR, ^1H and ^{13}C NMR see tables 2–4. MS (70 eV): 292/2 (M^+); 251/2; 250/10 ($M^+ - CH_2CO$); 249/1; 235/1; 183/2; 177/1; 167/5; 151/1; 150/2; 123/17; 108/65; 99/25; 95/82; 83/13; 67/32; 43/100; 41/31. An analytical sample, recrystallised from $(C_2H_5)_2O$/pentane, had m.p. 82.0–85.1°. (Found: C, 65.42; H, 6.72. Calc. for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90%)

(+) - 3 - *Hydroxy* - 2,4 - *dimethyl* - 4 - (3 - *oxo* - *octyl*) - 2 - *buten* - 4 - *olide* (**1d**). To a suspension of 18 mg 10% Pd/C in 15 ml CH_3OH, which had been stirred under a H_2 atmosphere at r.t. for 1 h, 37.5 mg (0.15 mmol) vertinolide (**1a**) was added. The H_2 consumption stopped after 7.95 ml (2.2 molar equivalents) were absorbed within 15 min. The catalyst was filtered, washed with CH_3OH and the filtrate evaporated to dryness. The oily residue was worked up by prep tlc (3 plates with $CHCl_3/C_2H_5OH$ 9:1) to yield 38.0 mg of crude product. Crystallisation from $(C_2H_5)_2O$/pentane at −20° gave 34.4 mg (90%) **1d** as colourless crystals, m.p. 61.7–63.9°; $[\alpha]_D^{20} = +19.2°$ (c = 0.05, $CHCl_3$); pK_a = 5.60; anal tlc ($CHCl_3/C_2H_5OH$ 94:6) R_f 0.25. UV, IR, ^1H (CD_3OD) and ^{13}C NMR see Tables 2–4. MS (70 eV): 254/1 (M^+); 230/1; 225/1; 211/1; 199/3; 198/2; 197/1; 183/2; 181/3; 171/14; 155/2; 153/6; 128/18; 99/49; 97/23; 71/31; 56/10; 55/16; 43/100; 41/20. (Found: C, 66.42; H, 8.91. Calc. for $C_{14}H_{22}O_4$: C, 66.12; H, 8.72%).

(−) - 5 - *Methoxy* - 2,4 - *dimethyl* - 2 - (3 - *oxo* - *octyl*) - 3(2H) - *furanone* (**4**). A solution of 35 mg (0.14 mmol) **1d** in 10 ml 1.4% solution of CH_2N_2 in $(C_2H_5)_2O$ (3.8 mmol) was kept at 0° for 5 min. After evaporation of the solvent the residue was worked up by prep tlc (1 plate with $CHCl_3/C_2H_5OH$ 9:1) to give two fractions with R_f 0.75 and 0.82. The material from the more polar fraction was crystallised from $(C_2H_5)_2O$/pentane at −20° to yield 26 mg (44%) **4** as fine colourless needles, m.p. 35.2–36.3°; $[\alpha]_D^{20}$ = −35.1° (c = 0.13, $CHCl_3$). UV, IR, ^1H and ^{13}C NMR see Tables 2–4. MS (70 eV): 269/5 ($M^+ + H$); 268/26 (M^+); 250/4; 240/1; 235/1; 226/1; 225/6; 213/1; 212/8; 208/3; 207/2; 197/4; 193/2; 191/2; 185/1; 182/12; 180/2; 179/3; 170/6; 169/3; 166/1; 165/6; 157/1; 156/4; 155/28; 154/2; 153/12; 152/6; 151/1; 142/88; 138/18; 137/19; 127/14; 109/15; 99/44; 97/11; 83/44; 82/11; 71/63; 69/10; 55/28; 43/100; 41/41. (Found: C, 66.89; H, 8.91. Calc. for $C_{15}H_{24}O_4$: C, 67.14; H, 9.01%).

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REFERENCES

[1]Preliminary Communication, L. S. Trifonov, A. S. Dreiding, L. Hoesch and D. M. Rast, *Helv. Chim. Acta* **64**, 1843 (1981).

[2]I. Isaac and R. R. Davies, *Trans. Brit. Mycol. Soc.* **38**, 143 (1955).

[3]IUPAC, *Nomenclature of Organic Chemistry*, 1979 Edn. Pergamon Press, Oxford (1979).

[4]P. M. Boll, *Acta Chem. Scand.* **22**, 3245 (1968); W. B. Turner, *Fungal Metabolites*, pp. 284, 355. Academic Press, London (1971); F. Bohlmann and M. Grenz, *Tetrahedron Letters* 3623 (1971); J. L. Bloomer, F. E. Kappler and G. N. Pandey, *J. Chem. Soc. Chem. Commun.* 243 (1972); K. Kobayashi and T. Ui, *Tetrahedron Letters* 4119 (1975).

[5]L. J. Haynes and J. R. Plimmer, *Q. Rev. Chem. Soc.* **14**, 292 (1960).

[6]A. Ciegler, D. W. Detroy and E. B. Lillehoj, *Patulin, penicillic acid, and other carcinogenic lactones, in Microbial Toxins* (Edited by A. Ciegler, S. Kadis and S. J. Agil), Vol. VI, p. 409–434. Academic Press, New York (1971); D. M. Wilson, *Patulin and Penicillic Acid*, in *Mycotoxins and Other Fungal Related Food Problems*, (Edited by J. V. Rodricks) p. 90–109. *Adv. in Chemistry Series* 149, American Chemical Society, Washington D. C. (1976).

[7]G. M. Sheldrick (1980), SHELXTL, Revision 2.5, *An integrated system for solving, refining and displaying crystal structures from diffraction data*, Göttingen, Germany.

[8]L. Wolff and C. Erbstein, *Justus Liebigs Ann. Chem.* **288**, 16 (1895).

[9]W. Hofheinz and P. Schönholzer, *Helv. Chim. Acta* **60**, 1367 (1977).

[10]A. Svensen and P. M. Boll, *Tetrahedron* **29**, 4251 (1973); in addition to the reported maximum in the UV spectrum of **2b**, we observed with our sample (prepared according to Ref. 8) a shoulder at 256 nm (ϵ = 4670).

[11]J. P. Schirmann and J. Dreux, *C. R. Acad. Sci. Ser. C* **262**, 652 (1966).

[12]N. M. Chopra, W. Cocker, B. E. Cross, J. T. Edward, D. H. Hayes and H. P. Hutchinson, *J. Chem. Soc.* 588 (1955). In addition to the bands reported in the IR spectrum of **2b**, we observed with our sample (prepared according to Ref. 8) a shoulder at 1690 cm^{-1}.

[13]Y. Leraux and E. Vauthier, *C. R. Acad. Sci. Ser. C* **271**, 1333 (1970).

[14]R. Hollenstein and W. von Philipsborn, *Helv. Chim. Acta* **55**, 2030 (1972).

[15]G. Alfano, G. Cimino and S. De Stefano, *Experientia* **35**, 1136 (1979).

[16]A. I. Scott, *Interpretation of Ultraviolet Spectra of Natural Products*, p. 58. Pergamon Press, Oxford (1964).

[17]L. A. Duncanson, *J. Chem. Soc.* 1207 (1953); J. Lehmann and H. Wamhoff, *Justus Liebigs Ann. Chem.* 1287 (1974).

[18]G. E. Pfyffer and D. M. Rast, *Exp. Mycol.* **4**, 160 (1980).