THE STRUCTURE AND ABSOLUTE CONFIGURATION OF VERTICILLOL, A MACROCYCLIC DITERPENE ALCOHOL FROM THE WOOD OF *SCIADOPITYS VERTICILLATA* SIEB. ET ZUCC. (TAXODIACEAE)

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Abstract—The structure of verticillol diepoxide 4 has been established by direct single crystal X-ray analysis. The structure of verticillol 5 follows from the chemical correlation to the diepoxide 4 as well as from NMR–LIS studies on verticillol which also provide evidence for the conformation of this alcohol. The absolute configuration of verticillol 5 has been assigned on the basis of CD data for the verticillol norketodiepoxide 6a.

1   2   3   4   5   6a X = O   b X = CH₂
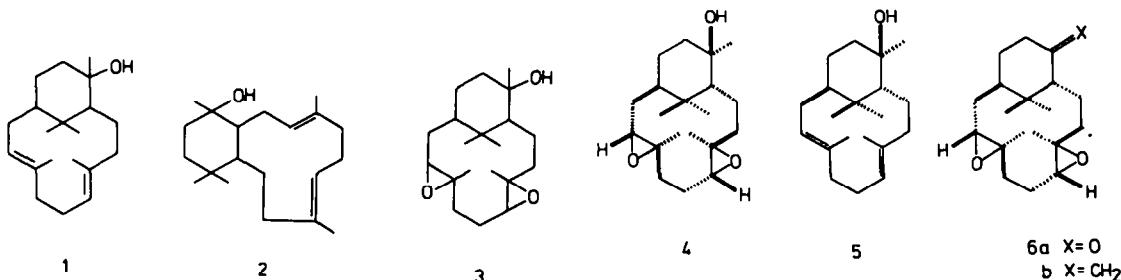
Verticillol is a crystalline diterpene alcohol from the wood of *Sciadopitys verticillata* Sieb. et Zucc. (Taxodiaceae).[1] On the bases of chemical degradation and spectroscopic data a tentative structure 1 has been proposed for this compound[1,2] although an alternative structure 2 has been discussed.[2]

Since verticillol crystallizes with four molecules (space group $P2_1$) and verticillol diepoxide 3 with one molecule (space group $P4_2$) in the asymmetric unit, the structure of this new type of diterpene has been settled by an X-ray structure determination on the diepoxide 3 ($C_{20}H_{34}O_3$, m.p. 159–160°) previously described.[1,2] The cell dimensions of the diepoxide 3 are $a = b = 14.141$ (2) and $c = 9.740(3)$ Å. X-ray intensity data were recorded on a Philips PW 1100 computer-controlled diffractometer. 1453 reflexions were measured out to $\theta = 60°$. 1277 of these were considered to be significant $[\sigma(I)/I \leqslant 0.25]$ and were used in the subsequent refinements. The structure has been solved using the multisolution programme MULTAN of Germain, Main and Woolfson.[3] The structure was refined by a least-squares method with the weighting scheme of Hughes.[4] All hydrogen atoms except H(O20) were included in the

refinement with fixed temperature factor parameters. The atomic scattering factors listed in the *International Tables for X-ray Crystallography*[5] were used for non-hydrogen atoms and those of Stewart, Davidson and Simpson[6] were used for the hydrogen atoms. The final R-value is 0.042.

Table 1 lists the final coordinates and temperature factors of the carbon and oxygen atoms. Positions of the hydrogen atoms are given in Table 2. Figure 1 is a stereoscopic view of the crystal structure. Figure 2 shows the bond lengths and angles and the numbering of the non-hydrogen atoms. Standard deviations are of the order of 0.006 Å for the bonds and 0.4° for the angles. Conformation angles within the rings are given in Table 3. The molecules in the crystal lattice are joined together by hydrogen bonds formed between the water oxygen $O_w(24)$ and the hydroxy-oxygen O(18) and the epoxy O(20) and O(22) oxygens. Figure 3 is a stereoscopic view of the molecular arrangements showing the three independent hydrogen bonds $O_w(24)–O(20)$, 2.85 Å $O_w(24)–O(22^i)$, 2.82 Å, and $O_w(24)–O(18^{ii})$, 2.88 Å (i = $x, y, 1 + z$; ii = $-y, x, \frac{1}{2} + z$).

The structure determination of the diepoxide 4 reveals

Table 1. Positional and anisotropic thermal parameters of the non-hydrogen atoms.

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	4128(3)	2368(3)	655	37(2)	54(2)	93(5)	-4(3)	18(5)	-8(5)
C(2)	3587(3)	2271(3)	-735(6)	45(2)	57(2)	73(5)	-10(4)	27(5)	-3(5)
C(3)	3029(3)	3158(3)	-1081(6)	71(3)	52(3)	79(4)	-22(4)	0(6)	27(5)
C(4)	2361(3)	3459(3)	48(6)	56(3)	45(2)	98(5)	16(4)	-15(6)	22(5)
C(5)	2832(3)	3584(3)	1444(6)	56(3)	42(2)	85(5)	6(4)	15(5)	-2(5)
C(6)	3413(3)	2688(3)	1799(5)	38(2)	39(2)	71(4)	-11(3)	11(5)	2(5)
C(7)	3877(3)	2751(3)	3241(6)	40(2)	48(2)	89(5)	-10(3)	-15(5)	4(5)
C(8)	3278(3)	2385(3)	4428(6)	52(2)	47(2)	76(4)	-17(4)	-18(5)	-8(5)
C(9)	3076(3)	1327(3)	4417(6)	44(2)	51(2)	76(4)	1(3)	1(5)	24(5)
C(10)	2150(3)	1001(3)	3887(6)	44(2)	46(2)	62(4)	0(3)	9(5)	22(5)
C(11)	1897(3)	24(3)	3403(6)	57(3)	51(3)	104(5)	-29(4)	7(6)	24(6)
C(12)	1496(3)	-4(3)	1939(6)	48(2)	47(2)	99(5)	-26(4)	1(5)	-7(6)
C(13)	2192(3)	117(3)	769(5)	45(2)	44(2)	87(5)	0(3)	-11(5)	-15(5)
C(14)	2239(3)	1034(3)	13(6)	43(2)	44(2)	79(5)	-2(4)	-8(5)	-18(5)
C(15)	3010(3)	1336(3)	-953(6)	55(3)	53(2)	76(5)	0(4)	8(5)	-22(5)
C(16)	4547(3)	1408(4)	1032(6)	48(2)	72(3)	131(6)	21(4)	-4(6)	-38(7)
C(17)	4961(3)	3057(4)	457(7)	48(3)	90(4)	134(7)	-39(5)	52(7)	-44(8)
O(18)	2105(2)	3706(2)	2468(5)	59(2)	69(2)	99(4)	33(3)	19(4)	-22(4)
C(19)	3369(4)	4525(3)	1485(7)	86(3)	43(2)	123(6)	-22(4)	-12(7)	2(6)
O(20)	2302(2)	1093(2)	5361(5)	57(2)	55(2)	72(3)	-10(3)	7(4)	20(4)
C(21)	3912(3)	671(4)	4562(7)	47(2)	69(3)	155(7)	23(4)	-6(7)	68(8)
O(22)	1694(2)	228(2)	-533(5)	49(2)	52(2)	93(3)	-11(3)	-26(4)	-24(4)
C(23)	2981(3)	-595(3)	728(6)	62(3)	46(2)	133(6)	19(4)	-3(7)	-14(6)
O_w(24)	567(2)	2411(3)	2018(5)	46(2)	96(3)	90(3)	6(3)	9(4)	30(5)

The β-values refer to the temperature factor expression $\exp\,[-(h^2\beta_{11} + k^2\beta_{22} + l^2\beta_{33} + hk\beta_{12} + hl\beta_{13} + kl\beta_{23})]$. Estimated standard deviations are given in parentheses. Values are $\times 10^4$.

Table 2. Positional parameters of the hydrogen atoms. Values are $\times 10^3$.

Atom	x	y	z	Atom	x	y	z
H(C2)	411	218	-152	H1(C16)	409	104	130
H1(C3)	342	368	-121	H2(C16)	501	152	176
H2(C3)	269	313	-192	H3(C16)	482	114	24
H1(C4)	184	302	22	H1(C17)	470	372	3
H2(C4)	200	400	-11	H2(C17)	539	269	-1
H(C6)	294	213	188	H3(C17)	527	321	125
H1(C7)	407	340	348	H1(C19)	371	463	73
H2(C7)	448	241	320	H2(C19)	373	456	220
H1(C8)	261	276	447	H3(C19)	289	505	141
H2(C8)	368	249	531	H1(C21)	368	0	475
H(C10)	174	152	358	H2(C21)	429	85	557
H1(C11)	135	-12	393	H3(C21)	428	75	378
H2(C11)	250	-33	347	H1(C23)	277	-115	80
H1(C12)	103	50	180	H2(C23)	346	-46	151
H2(C12)	118	-69	178	H3(C23)	328	-57	-9
H(C14)	185	152	34	H1(O_w24)	28	222	265
H1(C15)	276	141	-170	H2(O_w24)	12	241	141
H2(C15)	347	87	-100				
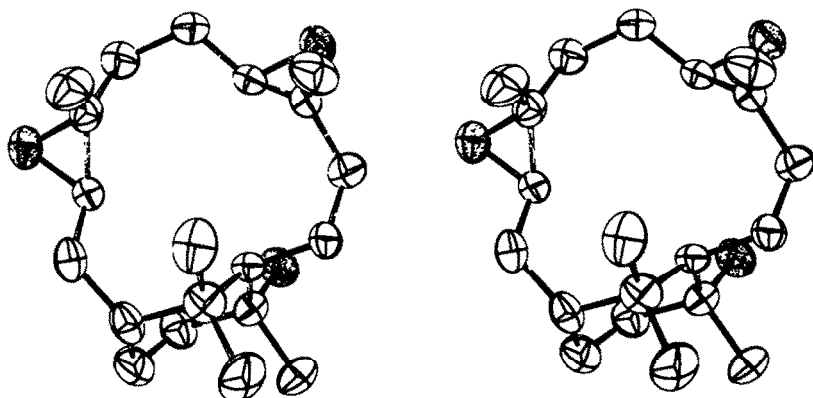
Fig. 1. A stereoscopic view of verticillol diepoxide 4. Oxygen atoms are shaded.

(a).

(b).

C(2)-C(1)-C(16) 108.7° C(8)-C(9)-C(10) 118.3°
C(6)-C(1')-C(17) 113.5° O(20)-C(9)-C(21) 112.8°
C'(4)-C(5)-C(19) 109.8° C(12)-C(13)-C(14) 119.8°
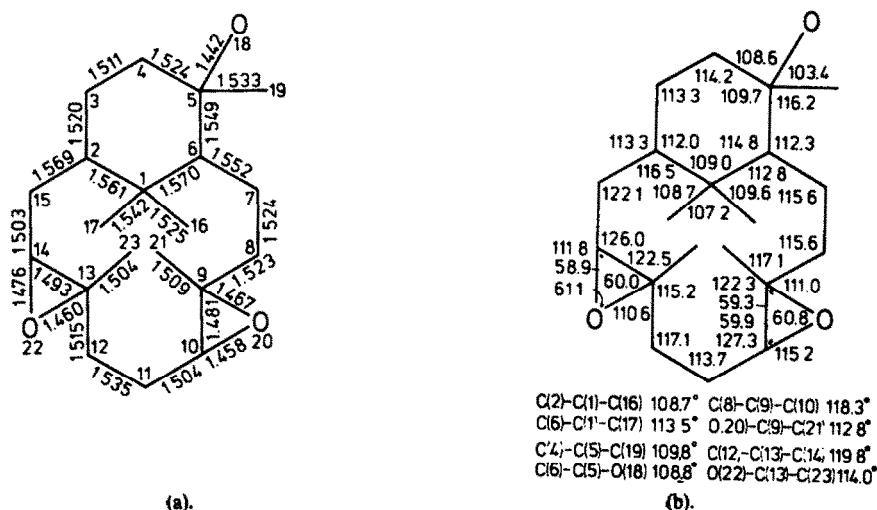C(6)-C(5)-O(18) 108.8° O(22)-C(13)-C(23)114.0°

Fig. 2. (a) Bond lengths (Å) in verticillol diepoxide 4. (b) Bond angles in verticillol diepoxide 4.
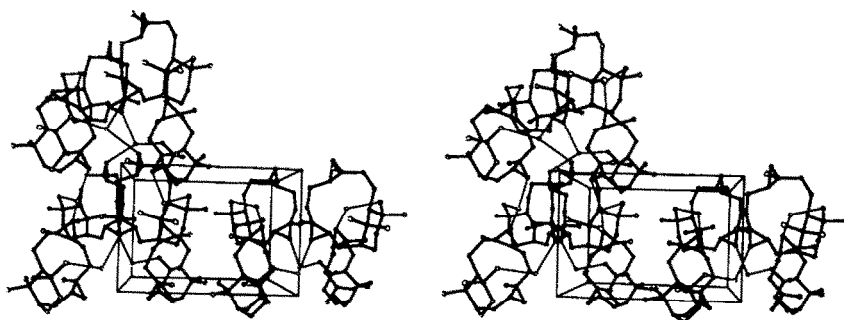
Fig. 3. Packing diagrams for verticillol diepoxide 4 with a horizontal, c vertical and b downwards perpendicular to the plane of the paper.

the prescence of water in the crystals. This was also confirmed by an elemental analysis of the diepoxide monohydrate sample ($C_{20}H_{34}O_3 \cdot H_2O$).

Considering that the formation of the diepoxide 4 proceeds in a normal way, the parent diene-alcohol, verticillol 1, will have an E,E arrangement of the double bonds. Verticillol will therefore be represented by structure 5.

The NMR shifts of some protons in verticillol were easily and firmly assigned and the paramagnetic induced shifts (ΔEu) were computed[7,8] assuming a similar con-

formation of verticillol to that of verticillol diepoxide 4 in its crystalline state. There is a good agreement (correlation coefficient 0.99) between observed and calculated ΔEu values (Table 4) which not only confirms structure 5 but also establishes the conformation (Fig. 4) of verticillol.

The coupling constants of the olefinic protons of verticillol and of the oxirane protons of the diepoxide (Experimental) give further support for the assignment of the conformations of verticillol 5 and its diepoxide 4.

The absolute configuration of verticillol 5 was deter-

Table 3. Intra-ring torsion angles (°).

C(6) - C(1) - C(2) - C(3)	52.3	C(6) - C(7) - C(8) - C(9)	66.1
C(1) - C(2) - C(3) - C(4)	-54.2	C(7) - C(8) - C(9) - C(10)	-99.2
C(2) - C(3) - C(4) - C(5)	54.1	C(8) - C(9) - C(10)- C(11)	161.1
C(3) - C(4) - C(5) - C(6)	-51.2	C(9) - C(10)- C(11)- C(12)	-124.7
C(4) - C(5) - C(6) - C(1)	52.0	C(10)- C(11)- C(12)- C(13)	75.3
C(5) - C(6) - C(1) - C(2)	-52.9	C(11)- C(12)- C(13)- C(14)	-104.5
C(15)- C(2) - C(1) - C(6)	-80.4	C(12)- C(13)- C(14)- C(15)	166.4
C(2) - C(1) - C(6) - C(7)	176.9	C(13)- C(14)- C(15)- C(2)	-123.3
C(1) - C(6) - C(7) - C(8)	-139.3	C(14)- C(15)- C(2) - C(1)	58.1

Table 4. Observed and calculated Eu(fod)₃-induced proton chemical shifts for verticillol 5.

Proton No.	ΔEu values*	
	Observed	Calculated
CH₃(19)	17.85	14.23
CH₃(17)	5.49	5.05
CH₃(16)	4.28	4.17
C(10)-H	6.34	4.93
C(14)-H	3.64	4.64
CH₃(21)	1.78	2.40
CH₃(23)	1.56	1.41

*ΔEu values are given in ppm. Calculated values are computed using the equation of McConnell and Robertson[7] (see also Ref[8]).
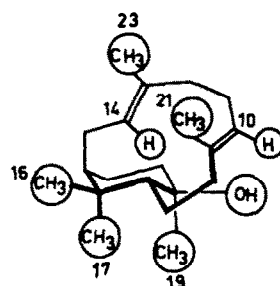

Fig. 4. Stereostructure of verticillol 5 in its most probable conformation. Encircled groups or atoms refer to those observed in the NMR–LIS analysis (see Table 4).

mined by a CD study of the verticillol norketodiepoxide 6a. This ketone was obtained by dehydration of the diepoxide 4 using phosphoryl chloride in pyridine to yield the crystalline anhydroverticillol diepoxide 6b, which was degraded to the norketone 6a by a low temperature ozonolysis in methylene chloride. The ozonides were decomposed by steam distillation. The norketone 6a exhibits a strong positive Cotton effect,

CD, $(\theta)_{292}$ + 5240. As shown by the octant diagram (Fig. 5), a positive $n \rightarrow \pi^*$ CD maximum is expected when the norketone possesses the absolute configuration depicted in 6a and is assumed to have a conformation similar to that observed for the diepoxide 4 and verticillol 5. The main contribution to the Cotton effect derives from the 12-membered ring with its substituents situated in the rear positive octant. The gem-dimethyl group will have a weak designate behaviour[9] and will make no significant contribution to the observed CD.
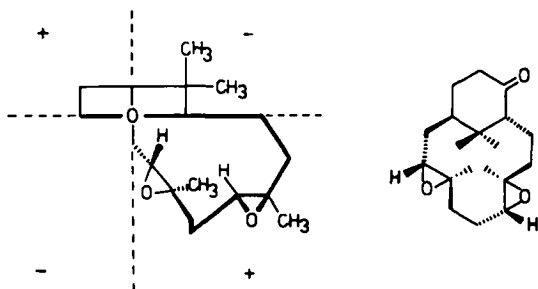
It is interesting to note that the proposed absolute

(-)-Cembrene A

(+)-Cembrene (Thunbergene)

Cembranes

(+)-Verticillol
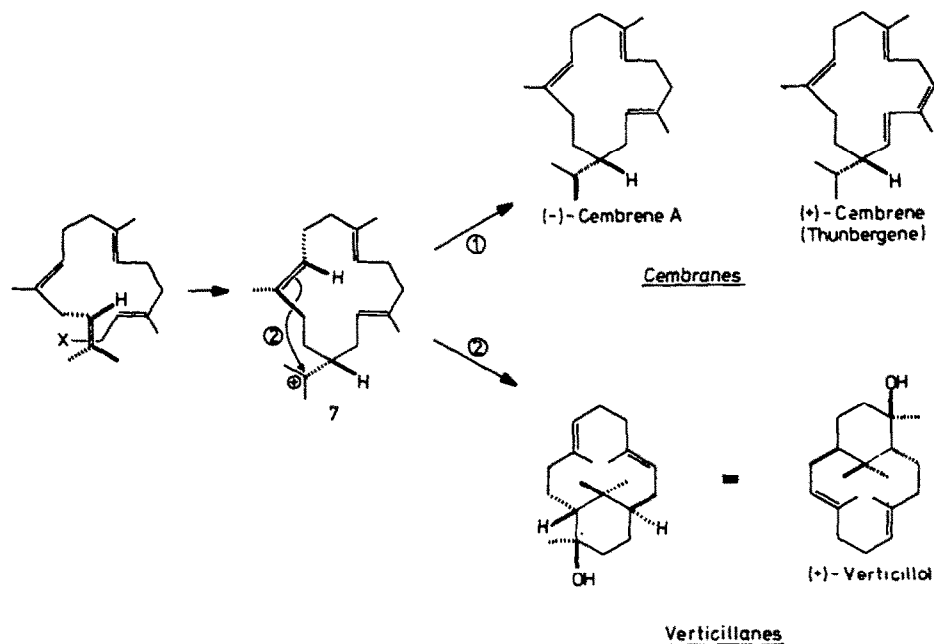
Verticillanes

Fig. 5. Octant diagram of verticillol norketodiepoxide 6a.

configuration of verticillol 5 correlates biogenetically with that of the cembrane type of diterpenes,[10-15] which are also conifer wood constituents. This correlation is shown in the formulae scheme. The two types of diterpenes may be derived from a common precursor 7.

EXPERIMENTAL

M.ps are taken on a Kofler micro-hot stage and are not corrected. Light petroleum refers to a fraction with b.p. 40–60°. NMR spectra (ca. 5% CCl$_4$ solutions) are recorded on a Varian A-60A instrument (60 MHz). Chemical shifts are given in δ-units, ppm relative to TMS (internal standard). The following abbreviations are used: q, quartet; tr, triplet; d, doublet; s, singlet; b, broad; u, the peak position is uncertain. Mass spectra (MS) are recorded on an LKB 9000 instrument (direct inlet). .

(+)-Verticillol 5. Crude (+)-verticillol was obtained from a neutral fraction of a petroleum ether-soluble extract of the wood of Sciadopitys verticillata Sieb. et Zucc.[1,2,16] Repeated recrystalization from light petroleum gave a pure sample: m.p. 104–105°; $[\alpha]_D + 168°$ (CHCl$_3$, c 1.5); IR (KBr), characteristic absorption bands at 3630 (in CCl$_4$) and 1161 cm^{-1} (tertiary –OH group), 1385 and 1365 cm^{-1} (geminal dimethyl group), 1660 and 830 cm^{-1} (trisubstituted double bond); UV, ϵ 9500 at 208 nm (EtOH); NMR, 5.65 bd (1H, J 12 cps, olefinic proton), 4.89 bd (1H, J 7 cps, olefinic proton), 1.50 bs (3H, olefinic methyl group), 1.48 bs (3H, olefinic methyl group), 1.21 s (3H, methyl group on carbinol carbon), 0.77 s and 0.69 s (each 3H, geminal dimethyl group); MS (70 eV) m/e (rel. intensity), 290 (M$^+$, 2), 257 (100), 121 (91), 133 (75), 93 (75), 119 (69), 81 (63), 134 (56), 161 (53), 91 (51), 105 (50); other characteristic fragments at 272 (34), 229 (23), 216 (7), 201 (22), 189 (37), 175 (23), 147 (36), 69 (36), 55 (42), 41 (68) (Found: C, 82.7; H, 11.6. C$_{20}$H$_{34}$O requires: C, 82.7; H, 11.8%).

Verticillol diepoxide 4. Monoperphthalic acid in ethyl ether (5 ml solution containing 2.1 mmole active oxygen determined by iodometric titration) was added to (+)-verticillol (5, 145 mg, 0.5 mmole) dissolved in a small amount of ethyl ether (ca. 0.5 ml) at 0°. The mixture was kept in a refrigerator (+5°) for 28 h when 0.96 mmole of active oxygen had been consumed. The organic solution was washed with aqueous sodium bicarbonate (10%) and with water, and then dried over sodium sulphate. Evaporation of the solvent under reduced pressure yielded verticillol diepoxide (4, yield 90%) which was recrystallized from methanol: m.p. 159–160°; $[\alpha]_D + 68.7°$ (CHCl$_3$, c 1.8); IR (KBr), characteristic absorption bands at 3420, 1672, 1420, 1393, 1375, 1233, and 1086 cm^{-1}; UV, ϵ 150 at 205 nm (EtOH); NMR, 3.45 tr (1H, J$_1$ = J$_2$ = 6 cps, oxirane proton), 2.93 bd (1H, J 7 cps, oxirane proton), 1.27 s (9H, three methyl groups attached to carbon also carrying oxygen), 0.96 s and 0.85 s (each 3H, geminal dimethyl group) (Found: C, 70.8; H, 10.6; O, 18.6. C$_{20}$H$_{34}$O$_3$·H$_2$O requires: C, 70.5; H, 10.7; O, 18.8%).

Anhydroverticillol diepoxide 6b. Phosphoryl chloride (0.5 ml) was added dropwise to an ice cooled solution of verticillol diepoxide (4, 215 mg) in pyridine (10 ml) and the reaction mixture was kept in a refrigerator (+5°) for 28 h. Light petroleum and ice water were added and the water phase was then acidified with aqueous hydrochloric acid. The organic phase was washed with water until it gave a neutral reaction and then dried (Na$_2$SO$_4$).

Evaporation of the solvent gave a crude crystalline residue which was dissolved in a small amount of light petroleum and absorbed on to a silica gel column (Merck, Kieselgel 60). Light petroleum containing ethyl ether (15%) eluted a main fraction (176 mg, yield 85%), which on recrystallization from methanol followed by sublimation under reduced pressure (0.01 mm Hg, 120°) gave anhydroverticillol diepoxide 6b: m.p. 160°; $[\alpha]_D + 30.3°$(CHCl$_3$, c 2.0); IR (KBr), characteristic absorption bands at 1643 and 910 cm^{-1} (vinylidene group), 1385 and 1363 cm^{-1} (geminal dimethyl group); UV, ϵ 6300 at 206 nm (EtOH); NMR, 4.90 bs and 4.53 bs (each 1H, binylidene protons), 3.20 q (1H, J$_1$ 7.5 cps, J$_2$ 4.5 cps, oxirane proton), 2.50 u (1H, oxirane proton), 1.26 s (6H, two methyl groups attached to carbon also carrying oxygen), 1.00 s and 0.78 s (each 3H, geminal dimethyl group) (Found: C, 79.3; H, 10.9. C$_{20}$H$_{32}$O$_2$ requires: C, 78.9; H, 10.6%).

Verticillol norketodiepoxide 6a. Anhydroverticillol diepoxide (6b, 273 mg) in methylene chloride (8 ml) was saturated with ozone at −70°. Most of the solvent was evaporated under reduced pressure. The residue was subjected to steam distillation for 15 min in order to decompose the ozonides and evaporate the formaldehyde formed. The residue was then partitioned between ethyl ether and aqueous sodium bicarbonate (5%). The organic phase was washed with water, dried (Na$_2$SO$_4$), concentrated and absorbed on to a silica gel column (Merck, Kieselgel 60). Light petroleum containing ethyl ether (15%) and methanol (1%) eluted first a minor product (6 mg) and then a main product which was characterized as the verticillol norketodiepoxide (6a, 26 mg, yield 10%). The two products were homogeneous according to TLC (SiO$_2$, Merck HF 254) using ethyl ether (20%) and methanol (1%) in light petroleum (minor product, R$_f$ 0.35; verticillol norketodiepoxide 6a, R$_f$ 0.25; anhydroverticillol diepoxide 6b, R$_f$ 0.50). The norketone 6a was further purified by crystallization from ethyl ether/light petroleum followed by sublimation under reduced pressure (0.01 mm Hg, 92°): m.p. 138.5–139.5°; $[\alpha]_D + 58.6°$(CHCl$_3$, c 0.15); CD, $(\theta)_{292} + 5240$ (CH$_3$CN, c' 0.83 · 10^{-3}); IR (CCl$_4$), characteristic absorption bands at 1710, 1390, 1385, 1368 cm^{-1}; NMR, 3.27 q (1H, J$_1$ 8 cps, J$_2$ 3.5 cps, oxirane proton), 2.55 bd (1H, J 7.5 cps, oxirane proton), 1.35 s and 1.27 s (each 3H, two methyl groups attached to carbon also carrying oxygen), 1.07 s and 0.83 s (each 3H, geminal dimethyl group); MS (20 eV) m/e (rel. intensity) 306 (M$^+$, 4), 95 (100), 121 (91), 120 (88), 125 (86), 109 (80), 85 (75), 291 (75), 111 (71), 123 (70), 124 (70); other characteristic fragments at 288 (7), 273 (29), 263 (28), 245 (18), 231 (8), 221 (14), 205 (17), 191 (18), 177 (40), 159 (40), 153 (33), 149 (33), 137 (60), 135 (67) (Found: C, 74.1; H, 9.8. C$_{19}$H$_{30}$O$_3$ requires: C, 74.5; H, 9.9%).

The minor ozonization product (TLC, R$_f$ 0.35), appears to be an epimer of the norketone 6a, since the two compounds exhibit similar spectroscopic properties (IR and NMR). This minor compound was found to be identical with the verticillol norketodiepoxide previously described by us in a preliminary communication[1]: m.p. 151–152.5°, $[\alpha]_D + 170.3°$ (CHCl$_3$, c 2.2). Due to lack of material this compound has not been further investigated.

Oxidation of anhydroverticillol diepoxide 6b with osmium tetroxide followed by cleavage of the intermediate diol by periodate yielded the norketone 6a. A product corresponding to the minor ozonization product (TLC, R$_f$ 0.35) could not be detected in the reaction mixture.

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[16]A full account of our investigation on the neutral wood extractives of *Sciadipitys verticillata* Sieb. et Zucc. is in preparation.