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# BISVERTINOLS: A NEW GROUP OF DIMERIC VERTINOIDS FROM *VERTICILLIUM INTERTEXTUM*†

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Abstract - From the culture medium of *Verticillium intertextum* four new metabolites were isolated, namely bisvertinol (5), dihydrobisvertinol (6), isodihydrobisvertinol (7) and bisvertinolone (8), all derivatives of the ring system D, namely 1,4,4a,6,8-pentahydroxy-4,5a,7,9b-tetramethyl-3,4,4a,5a,9a,9b-hexahydrodibenzofuran. The differences between 5, 6 and 7 are solely due to the side chains: 5 bears two sorbyl groups (CO-A), one at C(9) and the other at C(2), 6 has a sorbyl group (CO-A) at C(9) and a 2,3-dihydrosorbyl group (CO-B) at C(2), while 7 carries CO-A at C(2) and CO-B at C(9). Hydrogenations of 5, 6 and 7 gave the same octahydrobisvertinol (9), which consists of the ring system D carrying two tetrahydrosorbyl groups (CO-C) at C(2) and C(9). Methylation of 5, 6 and 7 with diazomethane afforded the monomethyl ethers 10, 11 and 12, respectively. The ring system of 8 differs from that of 5-7 in that the C(3)-methylene group is replaced by a carbonyl group. Like 5, 8 bears two sorbyl groups at C(2) and C(9). Compounds of the type 5-8 are called bisvertinols.

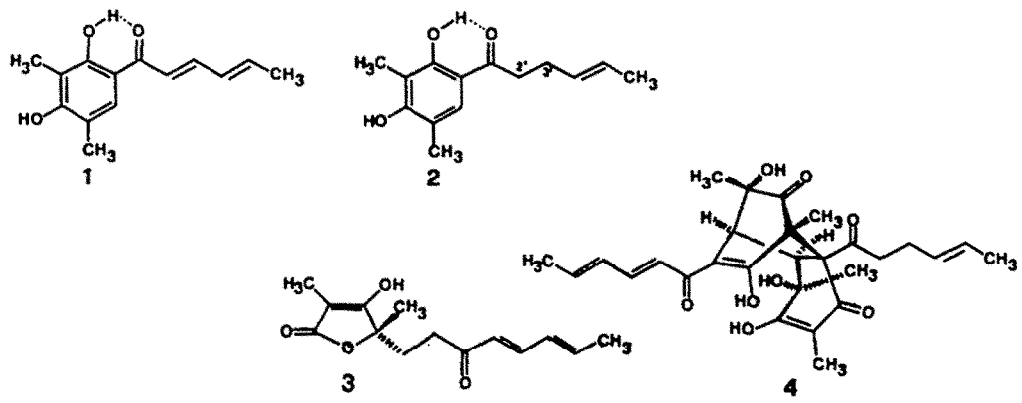
The constitutions of the bisvertinols 5-7 follow from their spectroscopic properties ($^1$H, $^{13}$C NMR, UV, and mass spectra) and from the hydrogenation results. The spectral interpretation led to possible partial structures and their combination was assisted by the computer program CONGEN. The constitution of 8 was derived by comparison of its spectral properties with those of 5. As a model for a part of the structure of 8, 2-[(*E*,*E*)-hexadienoyl]-3-hydroxy-5,5-dimethyl-2-cyclohexen-1-one (17) was prepared.

The bisvertinols 5-8 are closely related to four metabolites, namely sorbicillin (1), 2',3'-dihydrosorbicillin (2), vertinolide (3) and bisvertinoquinol (4), previously isolated from the same organism. The entire group (1-8) from *V. intertextum* is referred to as the vertinoids, which are understood to be hexaketide-derived metabolites with two additional methyl groups, one at C(2) and the other at C(4) (from the carboxylic end) of the $C_{12}$-chain; 1-3 are monomeric ($C_{14}$) and 4-8 are dimeric ($C_{28}$) vertinoids. A scheme is proposed interrelating 1-8 biosynthetically and assigning configurations at several of the chiral centres in 4-8 on the basis of the known (*S*)-configuration of 3.

## INTRODUCTION

In previous publications,[3-5] we have described the isolation and structural assignment of four metabolites from the culture medium of *Verticillium intertextum*, namely the yellow sorbicillin (1), the colourless 2',3'-dihydrosorbicillin (2), the colourless vertinolide (3), and the yellow bisvertinoquinol (4). All four have been proposed to be hexaketides with two additional

† Dedicated to Professor Ralph Raphael, for his sixty-fifth birthday.




methyl groups and thus to be related to each other biosynthetically.[3] According to this view, the first three are monomeric ($C_{14}$)[3-5] and the last dimeric ($C_{28}$).[3,5]

In addition to 1-4, we had noticed other substances in the chloroform extract of the culture medium of V. intertextum.[3] We now describe the isolation of four of them, all yellow-coloured, and present arguments for their constitutions and partially for their configurations. The four new compounds are structurally related to 1-4, so that we refer to the entire group of metabolites as vertinoids. Vertinoids can be defined as hexaketide-derived metabolites where C(2) and C(4) (from the carboxylic end) of the $C_{12}$-chain each bears an additional methyl group. The C(1)-C(6) part of vertinoids with the two extra methyl groups is called the head portion and the C(7)-C(12) is the tail portion (see Fig. 1).

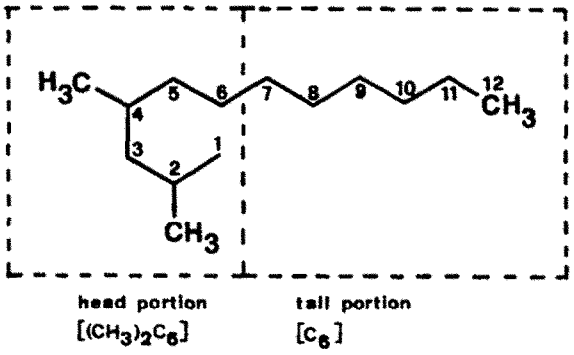


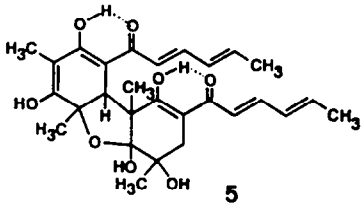

Fig. 1 Generalised 2,4-dimethyl-hexaketide structure of vertinoids

In the vertinoids isolated so far, at least some atoms of the head portion make up a ring, either carbocyclic (as in 1, 2 and 4) or heterocylic (as in 3). In these examples, three C-atoms, namely C(3), C(5) [or C(1)] and C(7) still bear the acetate derived O-atoms, whereas the other three C-atoms, namely C(1) [or C(5)], C(9) and C(11), do not. Furthermore, the tail portion contains a carbonyl group at C(7) and at least one double bond, namely at C(10), C(11), i.e. the tail portion is either a sorbyl or a 2,3-dihydrosorbyl group. Vertinoids may belong to the monomeric ($C_{14}$) or to the dimeric ($C_{28}$) type.

The four new vertinoids to be described here belong to the dimeric group. They are even more closely related to each other (see below) than they are to the other vertinoids 1-4, so we name them bisvertinols [the prefix 'bis' implies the dimeric nature, as was the case with bisvertinoquinol (4)].

## ISOLATION OF FOUR BISVERTINOLS

Three of the bisvertinols were isolated from the previously described[5] fraction c of the Sephadex LH-20 chromatography, performed with the crude chloroform extract of the acidified V. intertextum culture medium. Further fractional chromatography on Sephadex LH-20 and final purification by preparative tlc yielded bisvertinol (5), dihydrobisvertinol (6) and isodihydrobisvertinol (7). The fourth member of the bisvertinols, bisvertinolone (8), was isolated by Sephadex LH-20 chromatography with chloroform of the crude chloroform extract,[4,5] followed by crystallisation from diisopropyl ether. Scheme 1 summarises the isolation procedures for all the vertinoids obtained so far and the diagrams 5-8 give the constitutional formulae of the bisvertinols as proposed in the present paper.



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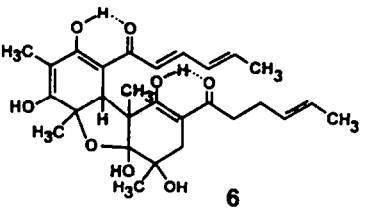

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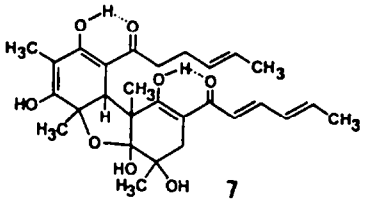

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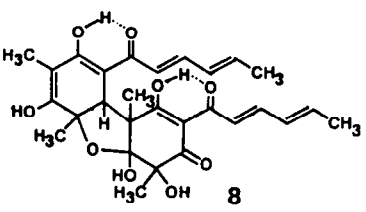

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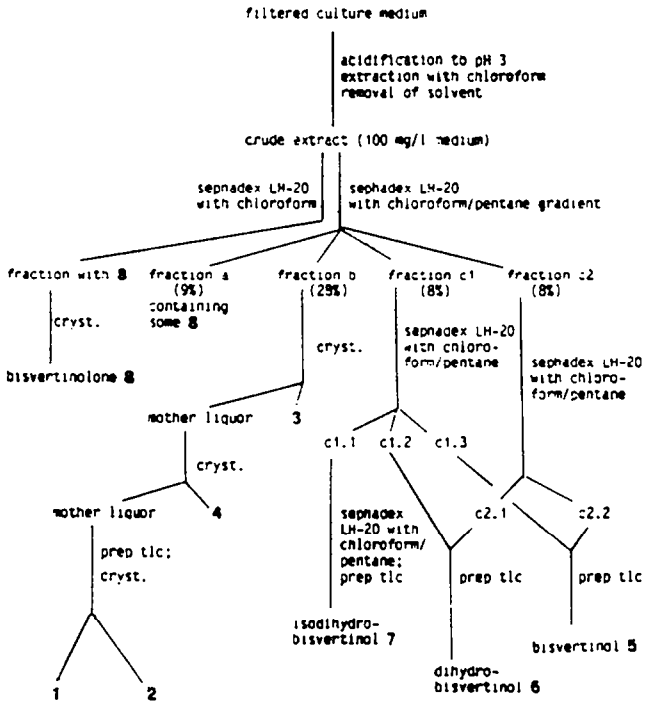



Scheme 1. Isolation of the vertinoids, including the bisvertinols 5-8 from the culture medium of V. intertextum

Bisvertinolone 8 appeared as rosettes with ca. 0.6 moles of the solvent ($^1$H NMR). The other three bisvertinols 5-7 were obtained as amorphous solids resisting crystallisation; analytical tlc and self-consistent NMR spectra showed all of them to be essentially pure. Table 1 summarises the yields and some characteristic properties of the bisvertinols 5-8. For their spectra see Tables 2-7 and the Experimental section.

Table 1 Names, yields and properties of the bisvertinoids 5-8

| Name | Yield[a] % | Physical state | M.p. | $R_f$ value[b] | $[\alpha]_D^{20}$ |
|---|---|---|---|---|---|
| Bisvertinol (5) | 0.23 | yellow-orange amorphous | 139-141° (dec) | 0.31-0.36 | -1467° |
| Dihydro-bisvertinol (6) | 0.13 | yellow amorphous | 104-106.5° (dec) | 0.33-0.37 | -652° |
| Isodihydro-bisvertinol (7) | 0.17 | yellow amorphous | 106-109° (dec) | 0.35-0.40 | -543° |
| Bisvertinolone (8) | 0.05[c] | yellow rosettes | 156-158.3° (dec) | 0.26-0.31 | -1046° |

[a]In % of the original chloroform extract; [b]analytical tlc on silica gel with chloroform/ethanol 94:6; [c]yield after crystallisation; yield of chromatographically pure 8 was 0.20%; [d]containing 0.6 moles of diisopropyl ether.

Since 5-8 could not be brought into a form suitable for X-ray analysis, we had to deduce their structures from spectral data and biosynthetic considerations. In this endeavour, we derived considerable help from our previous work,[3-5] especially from the good spectra of the other four vertinoids, 1-4, the full structures of which (excepting the absolute configuration of 4) were known by synthesis or X-ray analysis.

## THE CONSTITUTION OF THE BISVERTINOLS 5-8

The constitutions 5-8, proposed in the present work for the bisvertinols, are drawn in Scheme 2, in such a way as to show that the differences between them are due to the side chain CO-X at C(2) and C(9) (a sorbyl CO-A or a 2,3-dihydrosorbyl CO-B group) and due to the atom(s) Y at C(3) (Y = $H_2$ or O). Scheme 2 also represents some derivatives of 5-7 obtained in this work.

The major structural evidence stems from the $^1$H and $^{13}$C NMR spectra, which are represented for all four bisvertinols 5-8 in Tables 2-5 together with signal assignments to atoms. The atoms of the eventually derived constitutions are labelled by the numbering shown in Scheme 3. Where individual assignments were not possible, groups of signals were assigned to groups of atoms. Whenever immediately evident, groups of atoms were assembled into partial structures. Thus Tables 2 and 3 collect the signals of the side chain CO-X (X = A or B) and in Tables 4 and 5 certain (smaller) partial structures of the rest of the molecules are given letter names, namely E-M. The partial structures E-M were subsequently assembled to larger partial structures and all partial structures were finally combined to the constitutions of the whole molecules.

Our arguments for the constitution of the bisvertinols 5-8 are now presented stepwise, each step being indicated by a subtitle. Judicious use is made of the information, at any step, that certain data have already been interpreted in a preceding step. We begin with the bisvertinols 5-7, since their closer structural relation permits simpler conclusions; the slightly different bisvertinolone (8) will be treated later.

Table 2. $^1$H NMR signals[a] of the side chains at C(2) and C(9) (sorbyl = CO-A or 2,3-dihydrosorbyl = CO-B) of the bisvertinols 5-8

| Bisvertinol (5)[b] | | Dihydrobisvertinol (6)[c] | | Isodihydrobisvertinol (7)[d] | | Bisvertinolone (8)[e] | |
|---|---|---|---|---|---|---|---|
| Signals of A-CO-C(2) | Atom assignment | Signals of B-CO-C(2) | Atom assignment | Signals of A-CO-C(2) | Atom assignment | Signals of A-CO-C(2) | Atom assignment |
| 6.48/d (15)[f] | H-C(11) | 2.6-2.4/m[g] | $H_2$-C(11) | 6.4-6.1/m | H-C(11) | 7.41/d (15) | H-C(11) |
| 7.27/dxd (15, 11)[h] | H-C(12) | 2.4-2.2/m | $H_2$-C(12) | 7.4-7.2/m | H-C(12) | 7.58/dxd (15, 10)[h] | H-C(12) |
| 6.6-6.2/m | H-C(13) | 5.6-5.4/m | H-C(13) | 6.4-6.1/m | H-C(13) | 6.4-6.25/m | H-C(13) |
| | H-C(14) | | H-C(14) | | H-C(14) | | H-C(14) |
| 1.9-1.8/m | $CH_3$-C(14) | 1.64/d (4.7) | $CH_3$-C(14) | 1.9-1.8/m | $CH_3$-C(14) | 1.92/d (5) | $CH_3$-C(14) |
| Signals of A-CO-C(9) | | Signals of A-CO-C(9) | | Signals of B-CO-C(9) | | Signals of A-CO-C(9) | |
| 6.30/d (15)[f] | H-C(20) | 6.42/d (15) | H-C(20) | 2.9-2.2/m | $H_2$-C(20) | 6.40/d (15) | H-C(20) |
| 7.23/dxd (15, 11)[h] | H-C(21) | 7.29/dxd (15, 11) | H-C(21) | | $H_2$-C(21) | 7.33/dxd (15, 11)[h] | H-C(21) |
| 6.6-6.2/m | H-C(22) | 6.31/dxd (15, 11) | H-C(22) | 5.6-5.3/m | H-C(22) | 6.4-6.25/m | H-C(22) |
| | H-C(23) | 6.10/dxq (15, 6) | H-C(23) | | H-C(23) | 6.13/dxq (15, 7) | H-C(23) |
| 1.9-1.8/m | $CH_3$-C(23) | 1.88/d (6) | $CH_3$-C(23) | 1.62/d (4.4) | $CH_3$-C(23) | 1.88/dxd (7, 1) | $CH_3$-C(23) |

[a]Chemical shifts as δ in ppm, the integration of every signal corresponds to the number of protons indicated in the assignment; some signal assignments were supported by decoupling (see in the Experimental); for atom numbering see Scheme 3; [b]360 MHz, $CD_3OD$, r.t.; [c]200 MHz, $CDCl_3$, r.t.; [d]200 MHz, $CDCl_3$, -15°; [e]400 MHz, $CDCl_3$, r.t.; [f, h]assignment of signals bearing the same superscript within one column might be exchanged; [g]signal lies under the three proton m, which includes one of the signal of $H_2$-C(3) in the ring system (see Table 4).

Table 3. $^{13}$C NMR signals[a] of the side chains at C(2) and C(9) (sorbyl = CO-A or 2,3-dihydrosorbyl = CO-B) of the bisvertinols 5-8

| Bisvertinol (5)[b] | | Dihydrobisvertinol (6)[c] | | Isodihydrobisvertinol (7)[c] | | Bisvertinolone (8)[c] | |
|---|---|---|---|---|---|---|---|
| Signals of A-CO-C(2) | Atom assignment | Signals of B-CO-C(2) | Atom assignment | Signals of A-CO-C(2) | Atom assignment | Signals of A-CO-C(2) | Atom assignment |
| 194.4/s[d] | C(10) | 199.3/s[d] | C(10) | 192.5/s[d] | C(10) | 196.2/s[x] | C(10) |
| 121.4/d | C(11) | 36.9/t[x] | C(11) | 120.2/d | C(11) | 121.8/d[e] | C(11) |
| 143.0/d[f] | C(12) | 27.4/t[x] | C(12) | 142.7/d | C(12) | 148.1/d[f] | C(12) |
| 132.2/d[g] | C(13) | 126.0/d[g] | C(13) | 130.8/d[g] | C(13) | 131.3/d[g] | C(13) |
| 139.1/d[h] | C(14) | 130.8/d[g] | C(14) | 140.2/d[g] | C(14) | 139.4/d[h] | C(14) |
| 19.1/q[y] | C(15) | 18.5/q[y] | C(15) | 19.0/q[y] | C(15) | 18.5/q[y] | C(15) |
| Signals of A-CO-C(9) | | Signals of A-CO-C(9) | | Signals of B-CO-C(9) | | Signals of A-CO-C(9) | |
| 193.0/s[d] | C(19) | 191.8/s[d] | C(19) | 191.5/s[d] | C(19) | 191.0/s[x] | C(19) |
| 121.4/d | C(20) | 120.0/d | C(20) | 32.7/t[x] | C(20) | 119.9/d[e] | C(20) |
| 140.4/d[f] | C(21) | 138.7/d | C(21) | 29.9/t[x] | C(21) | 143.8/d[f] | C(21) |
| 132.0/d[g] | C(22) | 129.0/d[g] | C(22) | 126.2/d[g] | C(22) | 130.8/d[g] | C(22) |
| 137.2/d[h] | C(23) | 137.2/d[g] | C(23) | 129.3/d[g] | C(23) | 137.3/d[h] | C(23) |
| 18.9/q[y] | C(24) | 18.0/q[y] | C(24) | 18.1/q[y] | C(24) | 18.7/q[y] | C(24) |

[a]Chemical shifts (δ in ppm) from proton noise decoupled spectra, multiplicity from off resonance spectra; for atom numbering see Scheme 3; [b]25.2 MHz, $CD_3OD$, r.t.; [c]25.2 MHz, $CDCl_3$, r.t.; [d-h]assignment of signals bearing the same superscript within one column may be exchanged; [x,y]assignment of signals bearing the same superscript may be exchanged within one column and with identically labelled signals of the same compound in Table 5.

Table 4. $^1$H NMR signals[a] of the ring system **D** of the bisvertinols **5-8** and assignments to partial structures

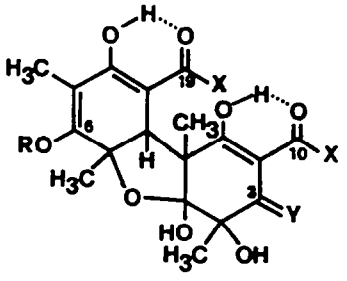
| Bisvertinol (5)[b] | Dihydrobis-vertinol (6)[c] | Isodihydrobis-vertinol (7)[d] | Bisvertinolone (8)[e] | Atom assignment | Partial structure |
|---|---|---|---|---|---|
| -[f] | 16.27/s[g,h] | 16.83/s[g,h] | 17.71/s[g,h] | HO-C(8) | E |
| -[f] | 7.50/s,br.[g] | 8.46/s,br.[g] | 6.19/s,br.[g] | HO-C(6) | E |
| 1.44/s[i] | 1.56/s[i] | 1.52/s[i] | 1.49/s[i] | $CH_3$-C(7) | E |
| -[f] | 16.05/s[g,h] | 16.28/s[g,h] | 16.35/s[g,h] | HO-C(1) | F |
| -[f] | 4.18/s,br.[g,j] | 4.50/s,br.[g,j] | 4.51/s,br.[g,j] | HO-C(4) | G |
| -[f] | 3.20/s,br.[g,j] | 3.84/s,br.[g,j] | 4.12/s,br.[g,j] | HO-C(4a) | H |
| 3.63/s | 3.67/s | 3.58/s | 3.75/s | H-C(9a) | I |
| 1.41/s[i] | 1.49/s[i] | 1.48/s[i] | 1.47/s[i] | $CH_3$-C(4) | L |
| 1.27/s[i] | 1.35/s[i] | 1.37/s[i] | 1.45/s[i] | $CH_3$-C(5a) | L |
| 1.20/s[i] | 1.29/s[i] | 1.32/s[i] | 1.38/s[i] | $CH_3$-C(9b) | L |
| 2.72/d (14.5) | 2.71/d (14.5) | 2.83/d (14) | - | $H_2$-C(3) | M |
| 2.43/d (14.5) | 2.6-2.4/m[k] | 2.62/d (14) | - | | M |

[a-e]See Table 2; [f]signal not visible in $CD_3OD$; [g]removed by exchange with $D_2O$; [h-j]assignment of signals bearing the same superscript within one column may be exchanged; [k]signal lies under the three-proton m, which includes the signal of $H_2$-C(11) in the side-chain (see Table 2).

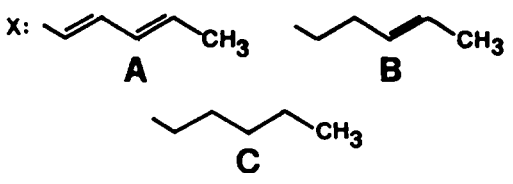
Table 5. $^{13}$C NMR signals[a] of the ring system **D** of the bisvertinols **5-8** and assignments to partial structures

| Bisvertinol (5)[b] | Dihydrobis-vertinol (6)[c] | Isodihydrobis-vertinol (7)[c] | Bisvertinolone (8)[c] | Atom assignment | Partial structure |
|---|---|---|---|---|---|
| 178.6/s[d] | 181.0/s[d] | 178.9/s[d] | 185.5/s[d] | C(6) | E |
| 110.2/s[e] | 108.8/s[e] | 109.2/s[e] | 110.7/s[e] | C(7) | E |
| 168.9/s[d] | 167.8/s[d] | 177.6/s[d] | 169.9/s[d] | C(8) | E |
| 107.0/s[e] | 105.5/s[e] | 105.9/s[e] | 107.1/s[e] | C(9) | E |
| 7.2/q | 7.0/q | 6.9/q | 7.0/q | C(18) | E |
| 168.0/s[d] | 166.8/s[d] | 166.2/s[d] | 163.8/s[d] | C(1) | F |
| 105.8/s[e] | 103.5/s[e] | 104.1/s[e] | 103.9/s[e] | C(2) | F |
| 102.3/s[e] | 100.8/s[e] | 99.9/s[e] | 99.8/s[e] | C(4a) | G |
| 80.2/s[f] | 79.2/s[f] | 79.7/s[f] | 79.8/s[f] | C(4) | G |
| 74.0/s[f] | 73.8/s[f] | 73.8/s[f] | 79.2/s[f] | C(5a) | H |
| 54.6/d | 52.8/d | 54.2/d | 54.4/d | C(9a) | I |
| 60.2/s | 56.3/s | 58.3/s | 59.8/s | C(9b) | K |
| 25.8/q[y] | 25.5/q[y] | 25.3/q[y] | 25.7/q[y] | C(16) | L |
| 22.7/q[y] | 22.1/q[y] | 22.4/q[y] | 22.9/q[y] | C(17) | L |
| 20.0/q[y] | 18.9/q[y] | 19.3/q[y] | 19.1/q[y] | C(25) | L |
| 36.2/t | 35.7/t[x] | 35.5/t[x] | 199.6/s[x] | C(3) | M[g] |

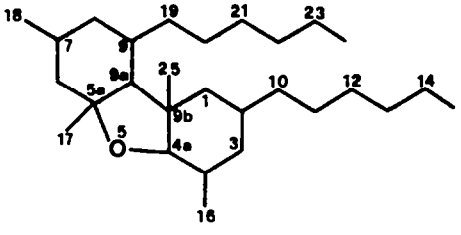
[a-c]See Table 3; [d-f]assignment of signals bearing the same superscript within one column may be exchanged; [g]the partial structure **M** is the $CH_2$ group in **5-7**, but the CO group in **8**; [x,y]assignment of signals bearing the same superscript may be exchanged within one column and with identically labelled signals of the same compound in Table 3.



| | RO–C(6) | X–C(10) | X–C(19) | Y=C(3) |
|---|---|---|---|---|
| 5 | H | A | A | $H_2$ |
| 6 | H | B | A | $H_2$ |
| 7 | H | A | B | $H_2$ |
| 8 | H | A | A | O |
| 9 | H | C | C | $H_2$ |
| 10 | $CH_3$ | A | A | $H_2$ |
| 11 | $CH_3$ | B | A | $H_2$ |
| 12 | $CH_3$ | A | B | $H_2$ |




Scheme 2. Constitutional formulae of the bisvertinols 5-8, of octahydrobisvertinol (9) and of the bisvertinol monomethyl ethers 10-12



Scheme 3. Numbering of the molecular skeleton of the bisvertinols 5-8 and of their derivatives 9-12 (cf. Scheme 2)

Composition of the bisvertinols 5-7:

The compositions $C_{28}H_{34}O_8$ for 5 and $C_{28}H_{36}O_8$ for 6 and 7 follow from $^{13}C$ NMR and mass spectra. The $^{13}C$ NMR spectra (see Tables 3 and 5) show 28 separate signals in the case of 6 and 7; in the case of 5, 27 signals are seen, with one signal more intensive (121.4 ppm, presumably coincidence of the C(11) and C(20) resonances) than the others. The mass spectra (see Experimental) of 6 and 7 exhibit (relatively weak) $M^+$ peaks while that of 5 contains only fragment peaks; the monomethyl ethers 10, 11 and 12, however, derived from 5, 6 and 7, respectively, with diazomethane, show strong $M^+$ peaks corresponding to $C_{29}H_{36}O_8$ for 10 and to $C_{29}H_{38}O_8$ for 11 and 12.

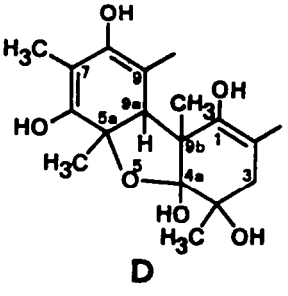
Side chains of 5–7: The bisvertinols 5-7 possess side chains which have so far been found typical for vertinoids: 5 has two non-equivalent sorbyl groups CO-A and 6 and 7 each contain one sorbyl CO-A and one 2,3-dihydrosorbyl CO-B group (A - 1,3-pentadienyl, B - 3-pentenyl, see Scheme 2). These side chains are immediately recognised in the $^1H$ and $^{13}C$ NMR spectra of 5-7 (see Tables 2 and 3)

by comparison with the spectra of the previously known vertinoids 1-4 (cf. Table 1 in Ref. 5). The presence of the side chains CO-A and CO-B is independently indicated by relatively intensive MS peaks at m/z 95 ($COC_5H_7^+$) for CO-A with 5-7 and at m/z 97 ($COC_5H_9^+$) for CO-B with 6 and 7 (see Experimental; a weak peak at m/z 97 with 5 might be due to a minor impurity).

The common ring system D in 5-7: The existence of a central molecular portion, in common to three of the bisvertinols, namely 5, 6 and 7, is suggested when the elements of the side chains known to be present are subtracted from each of the three molecular formulae. This leads to the same partial composition $C_{16}H_{20}O_6$ of the central portions in all three bisvertinols 5-7. (Later it will be shown that the central portion of bisvertinolone (8) differs from that of 5-7 only in one functionality.)

That these central portions of 5-7 are identical not only in composition, but also in constitution and configuration, follows from catalytic hydrogenations of 5-7, which yielded the same octahydrobisvertinol 9 (see Scheme 2) as evidenced by analytical tlc, UV and MS (see Experimental). The hydrogenations had converted all side chains into tetrahydrosorbyl groups (CO-C = hexanoyl) as shown by the mass spectrum of 9 with its intensive fragment peak at m/z 99 ($COC_5H_{11}^+$) and none at m/z 95 or 97. While no $M^+$ peak was observed for 9, several fragment peaks between m/z 253 and m/z 165 support the contention that the hydrogenations of 5-7 had not touched their central molecular portions; they are the ones at m/z 181 and 165, which also appear in 5-7 (see Experimental), and the ones at m/z 252 and 236, which have their analogues in 5 (248 and 232), in 6 (248 and 234) and 7 (250 and 232), see Table 6.

We shall now argue that the central molecular portion of 5-7 and 9 is 1,4,4a,6,8-pentahydroxy-4,5a,7,9b-tetramethyl-3,4,4a,5a,9a,9b-hexahydro--dibenzofuran (partial formula D, $C_{16}H_{20}O_6$), substituted at C(2) and C(9). In deriving its constitution from the partial structures E-M we shall refer to it as the "ring system D" of the bisvertinols.



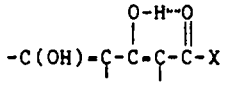

D

Partial structures E-M of 5-7: The existence of the partial structures E-M in the ring system D is derived from NMR observations (see Tables 4 and 5). In doing this, we exclude all but one of the NMR signals assigned to each of the side chains CO-A and CO-B (see Tables 2 and 3) from consideration. The non-excluded signals belong to the side chain carbonyl groups; they are needed here to show that these carbonyl groups are part of 1,3-dicarbonyl functions, of which the other parts belong to D.

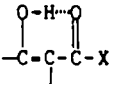
We first draw some conclusions from the immediate environment of the six O-atoms of D in 5-7. Five of these O-atoms belong to hydroxyl groups ($^1H$ NMR exchange experiments with $D_2O$) leaving one in an ether type function. Three of the (five) hydroxyls are part of 1,3-dicarbonyl enols, two chelated (two $^1H$ NMR singlets at a very low field) and the third non-chelated ($^1H$ NMR singlet between 8.5 and 7.5 ppm). The two chelated enols give rise to a positive $FeCl_3$ test (see Experimental) and the non-chelated enol can be methylated selectively with

diazomethane to yield the monomethyl ethers 10-12 (see Scheme 2). The two chelated enolised 1,3-dicarbonyl functions of 5-7 have to involve the carbonyl groups of the side chains (no other carbonyl groups are present according to $^{13}C$ NMR). At least one of them involves the sorbyl group CO-A with its conjugating unsaturation, which makes it responsible for the yellow colour of 5-7 (cf. the yellow colour of 1 and 4). The non-chelated enolised 1,3-dicarbonyl function in 5-7 must be in conjugation with one of the two chelated enol functions (no NMR evidence for any other function as driving force for the enolisation). Since our spectral information from solutions is not able to decide the direction of enolisation of 1,3-dicarbonyl functions we have arbitrarily chosen to represent the O-atoms in the side chains as belonging to the carbonyl groups and the others to the enols (cf. the corresponding uncertainty with bisvertinoquinol 4 [5]).

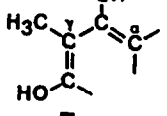
Thus two partial structures are present in 5-7, one a doubly enolised 1,3,5-tricarbonyl function



(X = A or B, see Scheme 2) and one a singly enolised 1,3-dicarbonyl function



The C-atoms of these two functions give rise to the expected $^{13}C$ NMR signals (see Table 5), namely to three enol type singlets between 181 and 166 ppm and to three vinyl type singlets between 110 and 104 ppm (for the signals of CO-X, see Table 3). In the 1,3,5-tricarbonyl function, a methyl group must also be present, which is located between two O-atoms in a W-type arrangement (for similar methyl signals in 1-4 and in a model compound, see Ref. 4 and 5). Since such an arrangement precludes chelation the methyl group must be located at C(γ) of the 1,3,5-tricarbonyl function with a S-cis conformation of the 1,3-butadienyl system. Thus, we formulate two trivalent partial structures, the double enol E and the single enol F within the ring system D. In each case the free valence at C(α) cis to the OH carries one of the side chain CO-X (X = A or B); the two other free valences do not carry an H-atom.



E

F

The remaining two of the (five) hydroxyls belong to aliphatic carbinol functions ($^1H$ NMR singlets between 4.5 and 3.2 ppm). The sixth O-atom is linked as ether function to two quadriligant C-atoms (no further carbonyl or enol signal in the $^{13}C$ NMR and no indication of a peroxide functionality). The C-atom neighbours of this ether O-atom and of the two carbinol O-atoms give rise to only three $^{13}C$ NMR signals in a reasonable chemical shift range, all of them singlets, one around 100 ppm, (doubly oxygenated aliphatic C-atom) and the other two between 80 and 74 ppm (singly oxygenated aliphatic C-atoms). Thus, the partial structures G, a hemiacetal, and H, a tertiary carbinol, can be formulated with none of their free valences carrying an H-atom. The partial structures E-H together with the side chains CO-X (X = A or B, see Scheme 2) attached to E and F account for the environment of all eight O-atoms in the bisvertinols 5-7.

Table 6. Major peaks above 230 m/z in the mass spectra of the bisvertinoids **5–8** and of octahydrobisvertinol (**9**), together with interpretation

| Vertinoid and derivative (molecular weight) | Major peaks above 230[a] (m/z /%) | Elemental composition of primary and secondary fragments[b] | Interpretation of primary and secondary fragments: Partitioning of the primary and secondary fragment composition into composition of side chains and half ring systems | Description of major fragments in terms of participating structure portions |
|---|---|---|---|---|
| **5** (498) | 250/14 | $C_{14}H_{18}O_4$[c] | $(COC_5H_7)$-$(C_8H_{11}O_3)$ | (CO-**A**)-(**O**) |
| | 248/7 | $C_{14}H_{16}O_4$[c] | $(COC_5H_7)$-$(C_8H_9O_3)$ | (CO-**A**)-(**N**) |
| | 232/36 | $C_{14}H_{16}O_3$[d] | $(COC_5H_7)$-$(C_8H_{11}O_3 - H_2O)$ | (CO-**A**)-(**O**- $H_2O$) |
| **6** (500) | 252/4 | $C_{14}H_{20}O_4$[c] | $(COC_5H_9)$-$(C_8H_{11}O_3)$ | (CO-**B**)-(**O**) |
| | 248/12 | $C_{14}H_{16}O_4$[c] | $(COC_5H_7)$-$(C_8H_9O_3)$ | (CO-**A**)-(**N**) |
| | 234/15 | $C_{14}H_{18}O_3$[d] | $(COC_5H_9)$-$(C_8H_{11}O_3 - H_2O)$ | (CO-**B**)-(**O**- $H_2O$) |
| **7** (500) | (251/10 | $C_{14}H_{19}O_4$[e] | same as 250 + H) | |
| | | $C_{14}H_{18}O_4$[c] | $(COC_5H_7)$-$(C_8H_{11}O_3)$ | (CO-**A**)-(**O**) |
| | | | $(COC_5H_9)$-$(C_8H_9O_3)$ | (CO-**B**)-(**N**) |
| | 232/61 | $C_{14}H_{16}O_3$[d] | $(COC_5H_7)$-$(C_8H_{11}O_3 - H_2O)$ | (CO-**A**)-(**O**- $H_2O$) |
| **8** (512) | 264/30 | $C_{14}H_{16}O_5$[c] | $(COC_5H_7)$-$(C_8H_9O_4)$ | -[f] |
| | 248/10 | $C_{14}H_{16}O_4$[c] | $(COC_5H_7)$-$(C_8H_9O_3)$ | (CO-**A**)-(**N**) |
| | 246/7 | $C_{14}H_{14}O_4$[d] | $(COC_5H_7)$-$(C_8H_9O_4 - H_2O)$ | -[f] |
| **9** (506) | (253/7 | $C_{14}H_{21}O_4$[e] | same as 252 + H) | |
| | 252/42 | $C_{14}H_{20}O_4$[c] | $(COC_5H_{11})$-$(C_8H_9O_3)$ | (CO-**C**)-(**N**) |
| | 236/16 | $C_{14}H_{20}O_3$[d] | $(COC_5H_{11})$-$(C_8H_{11}O_3 - H_2O)$ | (CO-**C**)-(**O**- $H_2O$) |

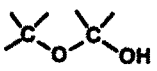
[a] for the entire list of MS peaks, see Experimental; [b] composition corresponds to the m/z values on the same line; [c] primary MS fragment; [d] secondary MS fragment; [e] primary MS fragment plus one H-atom; [f] analogous to (CO-**A**)-(**O**) and (CO-**A**)-(**O**- $H_2O$), respectively, in which a $CH_2$ group in **O** has been replaced by a C=O group.

Table 7. UV spectra of the bisvertinols **5-8** and of octahydrobisvertinol (**9**)

| Compound | $\lambda_{max}$ ($\varepsilon$) in $CH_3OH$ | in $CH_3OH$ basic[a] | in $CH_3OH$ acidic[b] | chromophores |
|---|---|---|---|---|
| **5** | 400 (26500) | 418 (21400) | 400 (31150) | chr. 1: (CO-**A**)-(**E**) |
| | 316sh (16000) | 357 (22000) | | chr. 2: (CO-**A**)-(**F**) |
| | 301 (16550) | 301sh (15000) | 301 (17300) | |
| | 273 (17700) | 273 (18400) | 273 (17400) | |
| | 233 (12250) | 234 (15050) | 224 (10750) | |
| **6** | 387 (20050) | 406 (19400) | 379 (21150) | chr. 1: (CO-**A**)-(**E**) |
| | 304 (11600) | 305 (12850) | 305 (11400) | chr. 4: (CO-**B**)-(**F**) |
| | 270 (15550) | 274 (15900) | 270 (16000) | |
| | 228 (9100) | 233 (11450) | 224 (8700) | |
| **7** | 354 (18400) | 342 (19600) | 370 (18800) | chr. 3: (CO-**B**)-(**E**) |
| | 312 (15850) | 312sh (17300) | 312 (13700) | chr. 2: (CO-**A**)-(**F**) |
| | 300 (15850) | 300 (17500) | 300 (13550) | |
| | 250sh (8400) | 240sh (7500) | 252sh (9800) | |
| **8** | 362 (31400) | 394 (16800) | not determined | |
| | 305sh (18300) | | | |
| | 271 (20000) | 275 (30900) | | |
| | 231 (18800) | 240sh (18700) | | |
| **9** | 334sh (10500) | 340sh (9700) | not determined | chr. 3: (CO-**C**)-(**E**) |
| | 305 (12300) | 299 (13400) | | chr. 4: (CO-**C**)-(**F**) |
| | 276sh (8800) | | | |
| | 234 (5300) | 236 (4900) | | |

[a] Addition of one drop 0.1 N NaOH to the $CH_3OH$ solution in the cuvette;

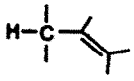
[b] addition of two drops 0.1N HCl to the basic solution (see [a]) in the cuvette.



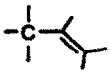
G

H

The other partial structures identifiable from the NMR spectra are an allylic methine group I ($^1$H NMR singlet ~3.7 ppm and a $^{13}$C NMR doublet ~54 ppm), a quaternary allylic C-atom K ($^{13}$C NMR singlet ~58 ppm), three teriary methyl groups L (three $^1$H NMR 3-proton singlets between 1.5 and 1.2 ppm, the fourth already assigned to E, and three $^{13}$C NMR quartets between 26 and 19 ppm). Finally, a methylene group linked to two C-atoms with no H-atom attached to each of them ($^1$H NMR AB-system at 2.8-2.4 ppm and a $^{13}$C NMR triplet ~36 ppm) makes up partial structure M. None of the free valences indicated in I-M carries an H-atom (no corresponding multiplicities nor vinyl H-signals).



I



K

L(3x)

M

The eight partial structures E-M, together with the side chains CO-X (X = A or B), account for the entire set of $^1$H and $^{13}$C NMR signals of the bisvertinols 5-7. The atoms explicitly drawn as letters in E-M correspond to $C_{16}H_{20}O_6$, which has been shown above to be the molecular formula of the ring system D.

The half ring systems N and O: In order to construct the constitution of the ring system D by combining the partial structures E-M and in order to locate each of the side chains CO-A or CO-B properly at the two enol systems E and F we now take into consideration three further sets of data of the bisvertinols 5-7 and (partially) of their octahydro derivative 9: they are their mass spectra (see Table 6 and Experimental), their UV spectra (see Table 7) and a consideration of their origin (co-metabolites of the vertinoids 1-4).

First we show that the ring system D consists of two similar halves. This possibility is suggested by the mass spectra of the bisvertinols 5-7 and of octahydrobisvertinol (9): in all these cases, peaks of high intensity (major peaks) appear only in m/z regions of approximately half the molecular weights and below; the molecular peaks are weak or even absent, see above. Of special interest for our argument are the major peaks above 230 m/z (see Table 6). Among them there are two peaks for fragments near, or at, half of the molecular weight (primary peaks, due to the primary fragments) and one peak situated lower by about m/z = 18 (secondary peak, due to the secondary fragment). The m/z-values of the two primary peaks (250 and 248 with 5, 252 and 248 with 6, 254 (not visible, but see below) and 252 with 9) or of twice the single primary peak (250 with 7, disregarding the odd-number peak at 251 assumed to be due to H-uptake) always sum up to the corresponding molecular weight (498 for 5, 500 for 6 and for 7, 506 for 9). The secondary peaks (232 with 5 and 7, 234 with 6, 236 with 9) always correspond to fragments after a splitting off of $H_2O$ from the heavier of the primary fragments.

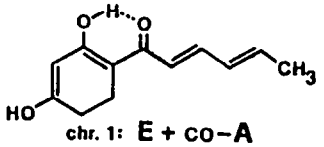
Each of the primary fragments has to contain one of the side chains and approximately half of the ring system D (see interpretation of major fragments in Table 6). These approximate halves will be called the "half ring systems", one

of them N and the other O. In 5 (with two sorbyl side chains), N and O must carry a sorbyl group CO-A each, so that the observed difference of 2 m/z between the two primary peaks has to be due to a weight difference of 2 amu between N and O. We shall call the lighter one N. Subtraction of the side chain atoms from the primary fragments gives us the compositions of the two half ring systems, namely $C_8H_9O_3$ for N and $C_8H_{11}O_3$ for O. In the bisvertinols 6 and 7, both types of side chains, CO-A and CO-B, are present (see Scheme 2 and Tables 2 and 3). The difference of 4 m/z between the two primary peaks of 6 (see Table 6) indicates that here the sorbyl group CO-A is attached to N and the 2,3-dihydrosorbyl group CO-B to O (CO-B and O are each 2 amu heavier than CO-A and N). As expected from this interpretation, only one primary peak is observed in the MS of 7 (CO-A + O has the same weight as CO-B + N). We further note that, in all three bisvertinols 5-7, it is always only the O-containing primary MS fragment which loses $H_2O$, so that $H_2O$ must be split off from the O-portion and not from the N-portion nor from either of the side chains. The above reported MS observations with 9 are also consistent with a loss of $H_2O$ from the O-portion, if it is assumed that the (CO-C + O)-fragment (m/z 254, not observed) rapidly loses $H_2O$ to give the (CO-C + O - $H_2O$)-fragment (m/z 236, observed). From all this information regarding the half ring systems N and O, together with the information derived earlier that the common ring system D is the same for 5-7 and 9, we conclude that the structures of both N and O are the same in 5-7 and 9 and that, consequently, the MS fragmentation of D is independent of the side chains attached to it.

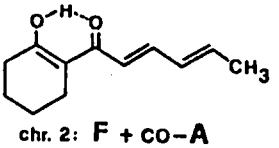
Each half ring system, N ($C_8H_9O_3$) and O ($C_8H_{11}O_3$), has to contain one of the enols, E or F, and two of the four methyls belonging to the ring system D, one methyl groups present in E and three as L, since otherwise the number of O-atoms and the C to H ratio in N and O would not agree with their composition.

We now resort to the UV spectra of 5-7 in order to deduce which of the half ring systems, N or O, contains which of the enols, E or F, and also where the side chains are located with respect to these enols. First we note (see Table 7) that the wavelength of the highest UV maximum decreases in going from bisvertinol (5, 400 nm) over dihydrobisvertinol (6, 387 nm) and isodihydrobisvertinol (7, 354 nm) to octahydrobisvertinol (9, 334 nm). We conclude that the lengths of the UV-chromophores responsible for the highest maximum decrease in going from 5 via 6 and 7 to 9.

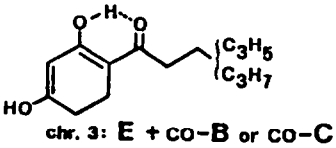
Partial structures containing chromophores of different lengths can indeed be constructed on the assumption that the side chains CO-X 5-9 are attached at C(α) of the double enol E and at C(α) of the single enol F. The combinations of interest are shown here as chr. 1, 2, 3 and 4. The lengths of these chromophores decrease in the order 1 > 2 > 3 > 4.



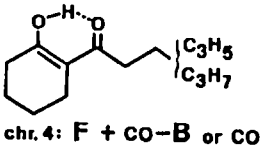
chr. 1: E + CO-A



chr. 2: F + CO-A

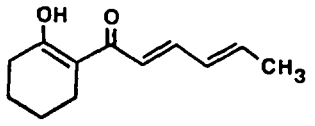


chr. 3: E + CO-B or CO-C



chr. 4: F + CO-B or CO-C

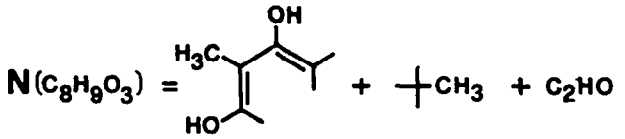
A consistent picture is obtained as follows: Compound 5 with two sorbyl groups must have chr. 1 and 2 and thus absorbs at the highest wavelength (400 nm), 6 with one sorbyl and one dihydrosorbyl group must have chr. 1 and 4 since it absorbs at the next lower wavelength (387 nm), 7 with the sorbyl and the dihydrosorbyl groups exchanged must have chr. 2 and 3 since it absorbs at still lower wavelength (354 nm), and 9 with two tetrahydrosorbyl groups must have chr. 3 and 4 and thus absorbs at the lowest wavelength (334 nm). In agreement with the hypothesis underlying these conclusions, we find that a model substance containing just chr. 2, namely 2-[(E,E)-2,4-hexadienoyl]cyclohexanone (13) absorbs at 354 nm, ε = 20,200 (cf. ref. 5), comparing well with the value for 7,

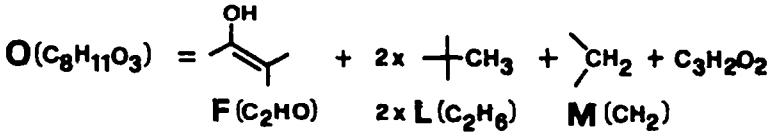



13

also containing chr. 2 (together with chr. 3). The assignment of these chromophores to 5-7 and 9 are shown in the last column of Table 7. A further agreement supporting this assignment can be derived from the shift of the highest UV-maximum in 5-7 and 9 on addition of base, which are Δλ = +18 nm for 5, +19 nm for 6, -12 nm for 7, and +6 nm for 9, if it is assumed that a proton of a non-chelated enol is abstracted in preference to one of a chelated enol: In that case the deprotonation affects the highest UV maximum in 5, 6 (containing chr. 1) and 9 (containing chr. 3) causing a bathochromic shift; in 7, however, the deprotonation affects the shorter chromophore (chr. 3), thus giving the impression of a hypsochromic shift (see Table 7). The UV evidence just submitted shows that the 2,3-dihydrosorbyl group (CO-B) is associated with E in 7 and with F in 6 (Table 7). The previously mentioned MS evidence showed that CO-B is associated with the half ring system N in 7 and with O in 6 (Table 6). This proves that the double enol E is part of N and the single enol F part of O. This is in accord with the corresponding analysis of the partial structures containing the sorbyl group (CO-A) in 5, 6 and 7 and the tetrahydrosorbyl group (CO-C) in 9.

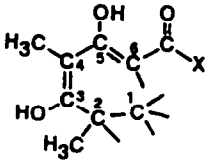
In addition since one methyl group is part of E, the half ring system N contains only one of the tertiary methyl groups L, whereas O has two of them. The methylene group M also must be part of the half ring system O, since only its two H-atoms can be the reason for the 2 amu greater weight of the primary MS fragment containing O than the one of the fragment containing N.



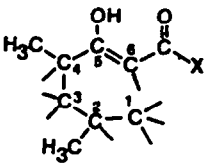



In order to generate structural proposals for the half ring system N and O, we now combine the partial structures derived so far with structural features derivable from the information that all previously isolated vertinoids (including the dimeric ones) contain unrearranged 2,4-dimethylated hexaketide carbon skeletons (see Fig 1). We assume that two such C-skeletons are also present in the bisvertinols. For our explanation we use the numbering shown in Fig. 1: in N, C(3) to C(6) with the methyl group at C(4) must be the double enol partial structure E since it is substituted at C(6) with the side chain CO-X. In that case the other methyl group L has to be linked to C(2). This leads to a preliminary structural proposal P, which is part of the half ring system N together with its side chain CO-X (X = A in 5 and 6, X = B in 7). Only one H-atom and one O-atom have not been located, but it is known that neither C(2) nor C(6) do carry an H-atom.

In the same way, a preliminary structural proposal Q can be formulated, which is part of the half ring system O together with its side chain CO-X (X = A in 5 and 7, X = B in 6). The single enol F becomes C(5) and C(6) since it carries the side chain, one of the two tertiary methyl groups L linked to C(2) and the other to C(4). Only four H-atoms and two O-atoms have not been located.



P



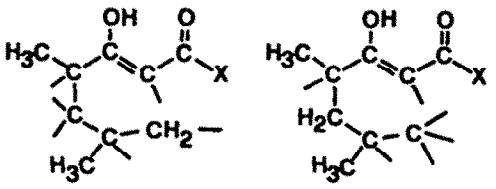
Q

Having P and Q we obtain the collapsed molecular formula $H_5PQO_3$ of the bisvertinols 5-7. We subsequently used the CONGEN computer program[6] to generate all possible combinations of P (= $C_8H_8O_2(COX)$), Q (= $C_8H_7O(COX)$), the 5 H-atoms and the 3 O-atoms.

Generalisation of a proposal for the constitution of the bisvertinols 5-7: The molecular formula of the bisvertinols 5-7 was defined as $C_{16}H_{20}O_6(COX)_2$ with X (the $C_5$-side chain portion A and B) taken as a monovalent atom type. Taking P and Q as superatoms, we obtained for the collapsed formula $H_5PQO_3$ 235 intermediate structures. This number was reduced to 101 with the constraint that P is linked (by any bond order) to at least one O-atom and Q to at least two O-atoms, thus specifying the molecular formula as $H_5(PO)(QO_2)$. Inspection showed a number of these structures to be peroxides, which can be excluded because the bisvertinols do not exhibit any of the correspondingly expected behaviour. This left 68 intermediate structures.

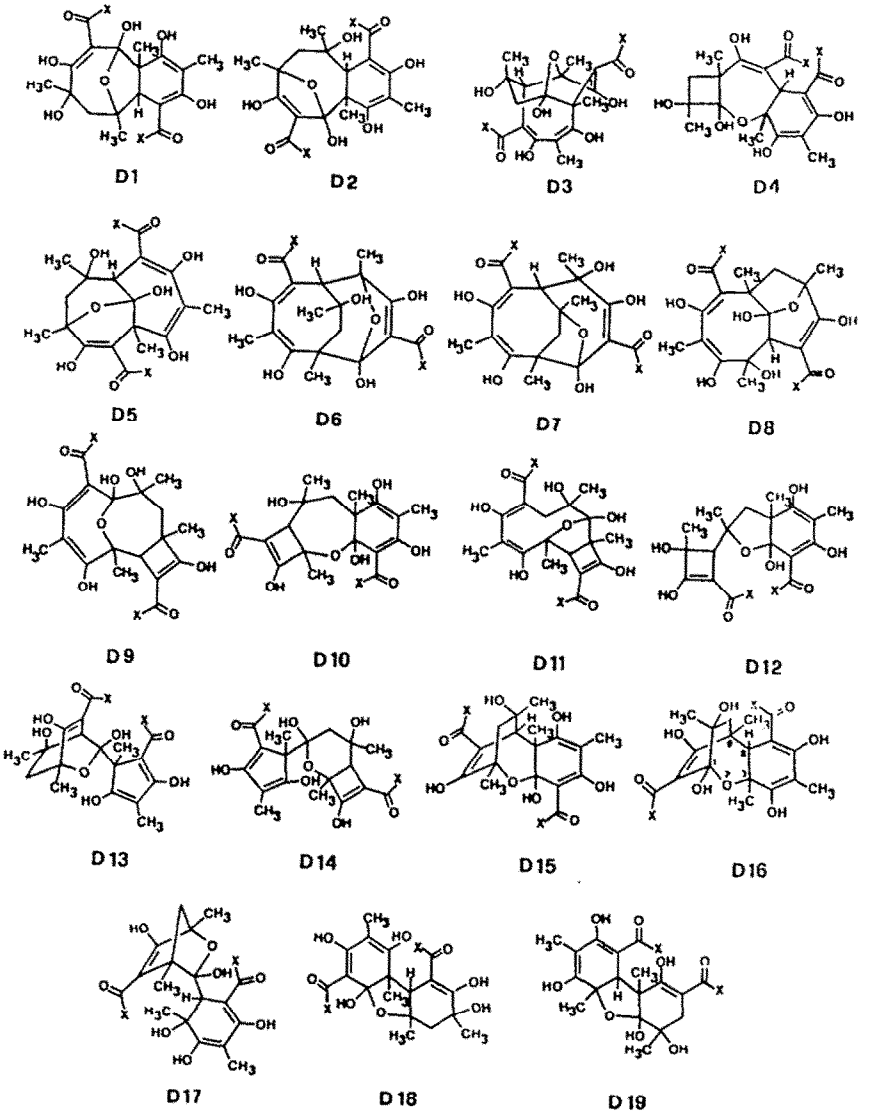
The superatoms P and Q were now imbedded with the constraints that the resulting structures must contain the partial structures G (a hemiacetal), H (a teriary carbinol), I (an allylic C-H group) and K (an allylic tertiary atom). This led to 26 chemical constitutions of the composition $C_{16}H_{20}O_6(COX)_2$.

At this point of the analysis, the occurrence of the methylene group (partial structure M) as C(1) or C(3) of O (and thus in Q) was taken into consideration, leading to two redefined preliminary structures for Q, namely Q1 and Q2.



Q1 Q2

By selecting (in the SURVEY subcommand mode of CONGEN) structures with the requirement that they must contain the partial structures built up from P plus Q1 or from P plus Q2*, the number of candidate constitutions for the bisvertinols 5-7 was reduced to 19 (see Scheme 4).



D1 D2 D3 D4

D5 D6 D7 D8

D9 D10 D11 D12

D13 D14 D15 D16

D17 D18 D19

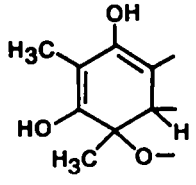
Scheme 4. CONGEN[6] generated proposals for the constitution of the common ring system D with attached side chains CO-X of the bisvertinols 5-7

* In order to prevent the survey procedure to identify atoms of Q1 or Q2 with atoms of P in the structures to be surveyed, the partial structure P had to be included in the two definitions of the partial structures that were used in the logical OR-combination of the SELECT command of SURVEY.

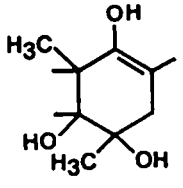
The 19 structures D1-D19 were inspected with respect to their likelihood to cleave easily into two almost equal primary MS fragments (see Table 6), both of which must be relatively stable species as ions and as neutral losses (both intensive peaks with 5-7). Easy cleavage might be expected for the hemiacetal C, OR-bond (in partial structure G) and for a doubly allylic C,C-bond. Only 5 structures are candidates for this type of fragmentation, namely D15-D19. Of these, D17 can be eliminated because its fragmentation (without any H-transfer) would not produce primary fragments of the composition $C_8H_9O_3$(COX) and $C_8H_{11}O_3$(COX) (cf. Table 6).

The molecular models of the remaining candidate structures D15, D16, D18 and D19 in form of all stereoisomers were inspected with respect to the proximity of their $CH_2$ group to the H(α) of one of their sorbyl side chains (CO-X = CO-A), since the observed $^1$H NMR deshielding (~1 ppm) of H-C(11) in 8 must be due to the fact (see below in the section on bisvertinolone 8) that the C=O group in 8 has replaced the $CH_2$ group in 5, 6 and 7. The criterion of this $CH_2$ to H-C(11) proximity is met with for all stereoisomers of D19, but not for those of the other candidates, except that it can not be quite excluded for the (1R*, 3R*, 8S*, 9R*)-stereoisomer of D16. We exclude this last mentioned structure since its $CH_2$ group is not allylic. The allylic position of $CH_2$, as found in D19, fits better with the observed $^1$H NMR chemical shift of this group in 5-7 (around 2.6 ppm, see Table 4). Thus D19 remains as the general constitution of the bisvertinols 5-7 with CO-X = CO-A or CO-B (cf. Scheme 2).

In structure D19, the half ring systems N and O (as shown) are readily recognisable. Thus both molecular halves (CO-X)-(N) and (CO-X)-(O) contain a

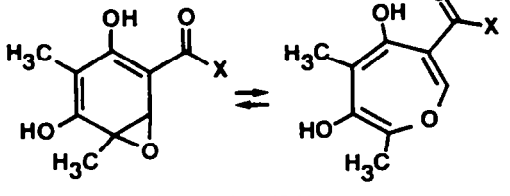



**N**

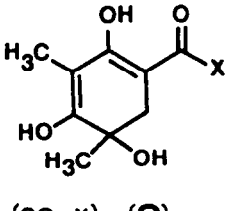



**O**

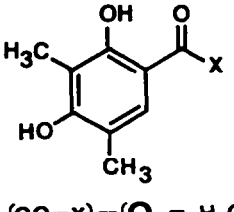
6-membered ring, a structural feature not demanded explicitly by any spectroscopic evidence. This ring size, however, permits a ready interpretation of the high intensities of the primary and secondary MS peaks (see Table 6), as follows: 1) The lighter primary fragment (CO-X)-(N) could be an arene-epoxide ⇌ oxepine (m/z 248 from 5, 248 from 6, 250 from 7 and 252 from 9). 2) The heavier primary fragment (CO-X)-(O) could be a 5-hydroxy-cyclohexa-1,3-diene (m/z 250 from 5, 252 from 6, 250 from 7, not observed from 9). 3) The secondary fragment (CO-X)-(O - $H_2O$) could be sorbicillin (1, m/z 232 from 5 and 7), dihydro-sorbicillin (2, m/z 234 from 6) or tetrahydrosorbicillin (m/z 236 from 9).




(CO–X)–(**N**)




(CO–X)–(**O**)



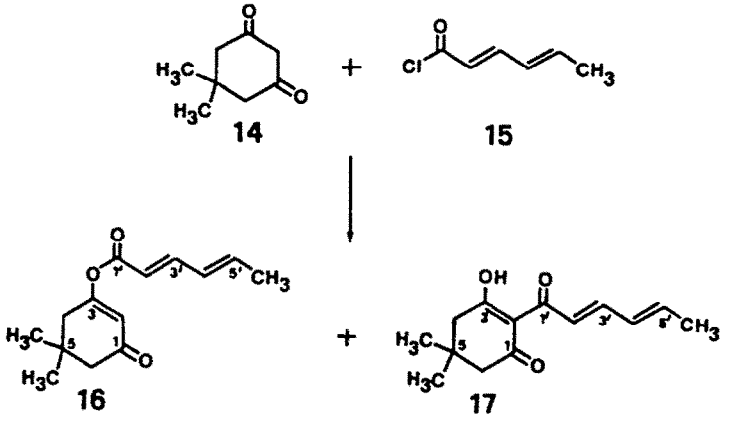

(CO–X)–(**O** – $H_2O$)

The constitution of bisvertinolone (8): Bisvertinolone (8) has the composition $C_{28}H_{32}O_9$ as evidenced by its MS (see Experimental) and NMR spectra (see Tables 2-5). The molecule bears twice the sorbyl side chain CO-A (see Tables 2 and 3). Subtracting the side chain elements (twice $COC_5H_7$) from the molecular formula of 8 leads to $C_{16}H_{18}O_7$, i.e. to a composition of the central molecular portion of 8, which differs from that of the ring system D of the bisvertinols 5-7 ($C_{16}H_{20}O_6$) only in the presence of an additional O-atom instead of 2 H-atoms.

Comparison of the $^1H$ and $^{13}C$ signals assigned to the central molecular portion of bisvertinolone (8) with those assigned to the ring system D of the bisvertinols 5-7 (see Tables 4 and 5) reveals striking similarities: all signals are practically identical, except that the two $^1H$ NMR doublets ($\delta$ = 2.4-2.6 ppm) and the $^{13}C$ NMR triplet ($\delta$ = 35.5-36.2 ppm) of the $CH_2$ group (partial structure M) in 5-7 are missing in 8, and that the (C=O)-$^{13}C$ NMR singlet (one of the three between $\delta$ = 191 and 200 ppm) in 8 is missing in 5-7. We conclude that 5-7 and 8 have the same ring system D, except that a $CH_2$ group 5-7 has been replaced with a C=O group in 8. Thus bisvertinolone (8) is the C(3)-oxo derivative of bisvertinol (5).

In the $^1H$ NMR spectrum of bisvertinolone (8) we note that the signal of H($\alpha$) of one of its sorbyl side chains (CO-A) appears at unusual low field (7.41 ppm instead of 6.5-6.3 ppm as for instance with 5, see Table 2). We assign this signal to H-C(11) (H($\alpha$) of the sorbyl side chain at C(2)) since this H-atom is located in the deshielding cone of the C(3)-carbonyl group of 8 if the C(10)-C(15)-side chain is kept in a zigzag conformation coplanar with C(1), C(2) and C(3) by chelation. This $^1H$ NMR deshielding observation was used above for the selection of D19 as bisvertinol constitution from the CONGEN generated structural proposals D1-D19 (see Scheme 4).

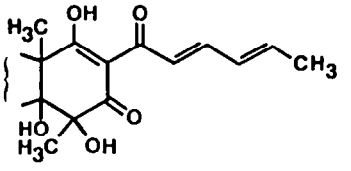
The same effect was observed with the model compound 17, prepared (8%) together with 16 (22%) by sorbylation of dimedone (14) (see Scheme 5). In 17, the H($\alpha$) of the sorbyl side chain absorbs at ~7.5 ppm in the $^1H$ NMR spectrum, i.e. ~1.2 ppm higher than the corresponding H-atom in 2-[(E,E)-hexadienoyl]-cyclohexanone (13), $\delta$ = 6.27 ppm (cf. Ref. 5).




Scheme 5. Synthesis of 2-[(E,E)-2,4-hexadienoyl]-3-hydroxy-5,5-dimethyl-2-cyclohexen-1-one (17) as a model for one of the molecular halves in 8 (cf. Scheme 6)

The above structural arguments for 8 are supported by the mass spectrum (see Table 6) with its two major peaks (m/z = 264 and 248) analogous to the ones observed for 5-7, which correspond to the same type of primary fragments, now differing in weight by 16 amu. This is because the heavier primary fragment (see Scheme 6) contains an O-atom in the case of 8 instead of 2 H-atoms in the case of

5, where the heavier primary fragment (O + CO-A) was 2 amu heavier than the lighter one (N + CO-A). Accordingly, the lighter primary fragment of 8 has the same weight (and thus composition) as the lighter primary fragment of 5.




Scheme 6. Molecular half in bisvertinolone (8) corresponding to the heavier primary fragment

## CONFIGURATIONS OF THE BISVERTINOLS

The CD spectra of all four bisvertinols 5-8 are rather similar; they show a strong negative Cotton effect in the region of 420-380 nm (see Fig. 2). In view

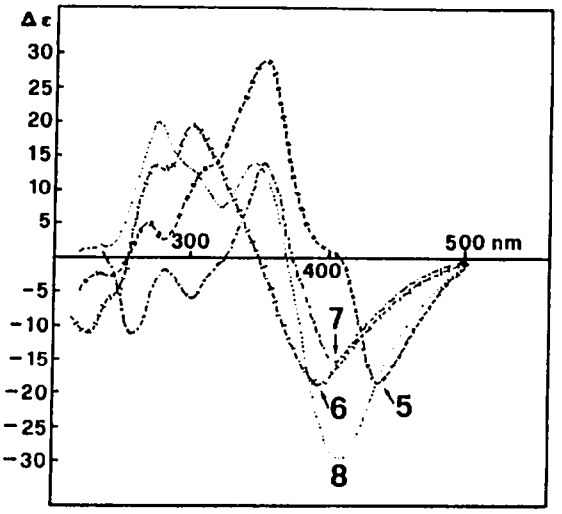


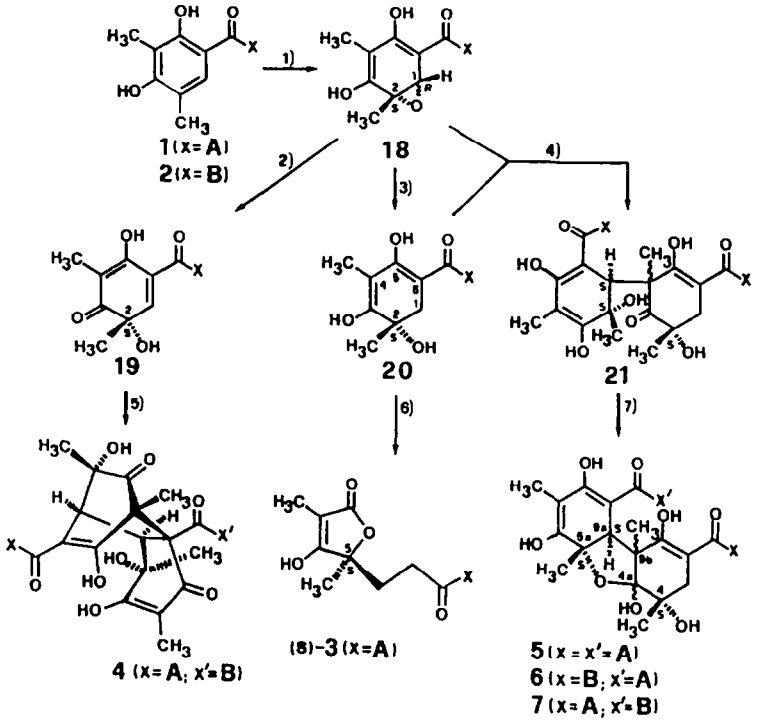

Fig. 2. CD spectra of bisvertinol (5, -"-"-"-"-), of dihydrobisvertinol (6,-'-'-'-'-'-), of isodihydrobisvertinol (7, -.-.-.-.-.), and of bisvertinolone (8, .....) in methanol.

of the constitutional similarity of the ring systems this indicates similar, if not identical, geometrical relations of atoms and groups around the chiral centres in these molecules and thus suggests the same absolute configuration. A proposal for the absolute configuration of the bisvertinols 5-8 is made in the following section on the basis of biosynthetic considerations, which also lead to arguments on some relative configurations.

## BIOSYNTHETIC CONSIDERATIONS

The bisvertinols have been shown above to consist of two halves, namely the half ring systems N and O, each together with its side chain CO-X. These two halves are closely related to the known monomeric vertinoids, i.e. sorbicillin (1) and 2,3-dihydrosorbicillin (2); they have the same C-skeleton but carry an additional O-atom at C(2). A vertinoid structure, also oxygenated at C(2), has been proposed as the monomeric precursor of the dimeric vertinoid bisvertinoquinol (4),[2,3,5] namely the quinol 19 (see Scheme 7). We now consider a common biosynthetic precursor for the monomeric building blocks of all the dimeric vertinoids. Such a common monomeric intermediate, $C_8H_9O_3$(CO-X), becomes particularly attractive if the differences in the side chains (CO-X = sorbyl CO-A or 2,3-dihydrosorbyl CO-B) and in the ring member C-Y (C-Y = $CH_2$ or C=O) of the

vertinoids (see Scheme 2) are assumed to be due to secondary modifications either before or after the dimerisation. In addition, the monomeric vertinoid 3, vertinolide = $C_8H_{11}O_3(CO\text{-}X)$, also contains an additional O-atom and might, therefore, also be derived from the same putative precursor. Such a precursor $C_8H_9O_3(CO\text{-}X)$ could well be the epoxide 18, derivable from sorbicillin (1) or 2'3'-dihydrosorbicillin (2) by an epoxidation (step 1) of the benzene ring (see Scheme 7), a process proposed as a key step in the biosynthesis of penicillic acid from orsellinic acid,[7] and of tyrosine from phenylalanine (NIH shift).[8] H-Migration in 18 with opening of the epoxide ring (step 2) would lead to the quinol 19 and hence to bisvertinoquinol (4) by Diels-Alder dimerisation (step 5). Reductive opening of the epoxide 18 by cleavage of its O,C(1) bond (step 3) would generate 20. Intramolecular attack of the O-atom of the C(2)-OH group onto the carbonyl C-atom (C(5)), followed by cleavage of the C(5), C(6)-bond (step 6), could convert 20 into vertinolide (3). Finally, nucleophilic attack of C(4) in 20 onto C(1) of 18 with epoxide ring opening (step 4) might generate the vertinoid dimer 21, a hemiacetal tautomer (step 7) of the bisvertinols 5-7.




1) epoxidation; 2) H-migration; 3) reduction; 4) $S_N2$ reaction; 5) Diels-Alder reaction; 6) ring cleavage and lactonisation; 7) intramolecular hemiacetal formation.

Scheme 7. Proposed biosynthetic interrelations between the vertinoids 1-7 and suggested configurations of 4-7

The biosynthetic Scheme 7 suggests the absolute and some relative configurations of the chiral vertinoids 4-7: 1) Since (S)-configuration (at C(5)) has been established (by total synthesis) for 3[9] the Scheme suggests that 18 and 20 have the (S)-configuration at C(2). 2) Since this configuration is most likely retained in the rearrangement of 18 to 19 and in the dimerisation to bisvertinoquinol (4), the latter must possess the absolute configuration as drawn in 4 (which is accidentally the same as the one shown in our previous publications[3,5]), the relative configurations being known by X-ray. 3) The reaction between C(4) of 20 with C(1) of 18 may be of $S_N2$-type (inversion) at C(1), so that 5-7 receive the (S)-configuration at C(9a). 4) The configurations

at C(2) of both 18 and 20 are unaffected in their dimerisation via 21 to 5-7, so that the latter have the (S)-configuration also at C(4) and C(5a). 5) The configurations at C(4a) and C(9b) of 5-7 have to remain unpredicted.

Since it has already been shown above that bisvertinolone (8) is the C(3)-oxo derivative of bisvertinol (5), the configurations just deduced for 5-7 also apply to 8.

## EXPERIMENTAL

### General Remarks

For the details of origin and cultivation of V. intertextum (ATCC 46284) and for chemical methods, instruments and abbreviations see Refs. 4 and 5.

### Isolation of bisvertinol (5), dihydrobisvertinol (6) and isodihydrobisvertinol(7)

The spent culture medium of V. intertextum was extracted with $CHCl_3$ and the crude extract (8.44 g) was chromatographed on Sephadex LH-20 as described in Ref. 5, however, partitioning the fraction c, mentioned there, into two subfractions, c1 (5100-6500 ml) and c2 (6500-7500 ml). The residue from the subfraction c1 (0.75 g) was chromatographed on 50 g Sephandex LH-20 at 4° in the dark in a 2.5 x 50 cm column with 1960 ml $CHCl_3$/pentane 1:1 and then 200 ml $CHCl_3$/pentane 33:17 at a flow rate of 0.33 ml/min. Three subfractions were collected after inspection by anal tlc in $CHCl_3/C_2H_5OH$ 47.3: c1.1 (1300-1600 ml), containing 7, c1.2 (1600-1800 ml), containing 6, and c1.3 (1960-2150 ml), containing 5. The intermediate eluate between subfraction c1.2 and c1.3 (1800-1960 ml) contained a small amount of 5 and 6 and was set aside.

Subfraction c2 (0.70 g) was chromatographed under the same conditions as fraction c1, but with $CHCl_3$/pentane 3:2, to give the subfractions c2.1 (1600-1800 ml), containing 6, and c2.2 (1950-2200 ml), containing 5. The intermediate fraction (1800-1950 ml), containing some 5 and 6, was set aside.

The residue of subfraction c1.1 was again chromatographed on 100g Sephadex LH-20 at 4° in the dark with $CHCl_3$/pentane 3:2 in a 1.5 x 125 cm column at a flow rate of 0.4 ml/min and yielded a main fraction with crude 7 (anal tlc in $CHCl_3/C_2H_5OH$ 47:3). The residue therefrom was purified on 2 prep tlc plates with $CHCl_3/C_2H_5OH$ 47:3. The yellow zone at $R_f$ 0.38 was eluted with $CHCl_3/CH_3OH$ 5:1, the solvent removed in vacuo and the residue dissolved in $CHCl_3$. This solution was filtered, evaporated and dried at r.t./0.05 Torr to give 15.0 mg (0.17% of the crude extract) 7 as a yellow amorphous solid, m.p. 106.0-109.0° (dec), which showed a green-brownish colour reaction with $FeCl_3$ in $H_2O/C_2H_5OH$; $[\alpha]_D^{20}$ = -543° (c = 1.55, $CHCl_3$). UV see Table 7. CD ($CH_3OH$): 233 (+1.5), 240 (0), 259 (-11.0), 282 (-1.5), 302 (-5.9), 322 (0), 352 (+14.0), 372 (0), 404 (-14.4). IR ($CHCl_3$): 3550m, 3450-3100m; 2980m; 2930m; 2880w; 2860w; 1713w; 1600m sh; 1640s sh; 1606s; 1570s sh; 1540s sh. $^1H$ and $^{13}C$ NMR see Table 2-5. $^1H$ NMR (200 MHz, -15°, $CDCl_3$) double resonance: Irradiation at 6.3 (H-C(11), H-C(13) and H-C(14)) gave: 1.89/s ($CH_3$-C(14)). MS (70eV): 500/1 ($M^+$); 482/2 ($M^+ - H_2O$); 251/10 ($C_{14}H_{19}O_4^+$); 250/45 ($C_{14}H_{18}O_4^+$); 233/15 ($C_{14}H_{19}O_4^+ - H_2O$); 232/61 ($C_{14}H_{18}O_4^+ - H_2O$); 231/16; 217/57 ($C_{13}H_{13}O_3^+$); 207/14; 191/26 ($C_{11}H_{11}O_2^+$); 189/17; 182/11; 181/91 ($C_9H_9O_4^+$); 179/19; 175/18; 166/11; 165/75 ($C_9H_9O_3^+$); 164/15; 161/13; 154/35; 153/12; 151/13; 149/13; 139/13; 138/17; 137/18; 136/42; 135/28; 123/18; 122/11; 121/21; 109/21; 108/20; 107/24; 97/12 ($COC_5H_9^+$); 95/82 ($COC_5H_7^+$); 91/27; 83/34; 81/15; 79/37; 78/10; 77/35; 69/37; 68/13; 67/66; 66/13; 65/33; 57/22; 55/58; 53/38; 52/12; 52/12; 51/23; 43/100; 41/92.

The subfractions c1.2 and c2.1 were combined and the residue thereform was purified on 4 prep tlc plates with $CHCl_3$/pentane/$CH_3CN$/$CH_3OH$ 23:10:10:7. The yellow zone at $R_f$ 0.60 was treated as described above for the isolation of 7 and gave 11.0 mg (0.13% of the crude extract) 6 as a yellow amorphous solid, m.p. 104.0-106.5° (dec), which showed a green-brownish colour reaction with $FeCl_3$ in $H_2O/C_2H_5OH$; $[\alpha]_D^{20}$ = -652° (c = 1.0, $CHCl_3$). UV see Table 7. CD ($CH_3OH$): 225 (-11.2), 245 (-5.2), 255 (0), 273 (+13.8), 300 (+19.6), 346 (0), 392 (-18.6). IR ($CHCl_3$): 3540 m; 3450-3100m; 2980m; 2920m; 2850m; 1710m; 1670m; 1615s; 1555s br. $^1H$ and $^{13}C$ NMR see Table 2-5. $^1H$ NMR (200 MHz, $CDCl_3$) double resonance: Irradition at 7.29 (H-C(21)) gave: 6.42/s (H-C(20)) and 6.31/d, J = 15 (H-C(22)); irrad. at 6.10 (H-C(23)) gave: 6.2-6.4/simplified m (H-C(20) and H-C(22)); irrad. at 5.5 (H-C(13) and H-C(14)) gave: 2.3/t, J = 6 ($H_2C$(12)) and 1.64/s ($CH_3$-C(14)). MS (70 eV): 500/0.5 ($M^+$); 252/4 ($C_{14}H_{20}O_4^+$); 248/12 ($C_{14}H_{16}O_4^+$); 234/15 ($C_{14}H_{20}O_4^+ - H_2O$); 216/19; 195/12; 194/16; 191/11; 183/12; 181/15 ($C_9H_9O_4^+$); 180/29; 166/12; 165/81 ($C_9H_9O_3^+$); 152/18; 151/14; 140/14; 139/21; 138/34; 137/21; 128/15; 125/10; 123/17; 110/19; 109/18; 101/10; 99/17; 97/26; ($COC_5H_9^+$); 95/40 ($COC_5H_7^+$); 83/19; 81/10; 79/11; 69/74; 68/11; 67/21; 57/26; 55/77; 53/15; 43/100; 41/61.

The subfractions c1.3 and c2.2 were combined and the residue therefrom was purified on 8 prep tlc reversed phase plates with $H_2O/CH_3OH/CH_3CN$ 11:7:3. The red-orange zone at $R_f$ 0.55 was extracted with $CH_3OH$, the solvent was removed in vacuo and the residue was further purified on 4 prep TLC plates with $CHCl_3/C_2H_5OH$

9:1. The orange zone was extracted with $CHCl_3/CH_3OH$ 5:1 and then treated as described above for the isolation of 6 and 7 and gave 19.5 mg (0.23% of the crude extract) 5 as a yellow-orange amorphous solid, m.p. 139.0-141.0 (dec), $[\alpha]_D^{20}$ which showed a greenish-brown colour reaction with $FeCl_3$ in $H_2O/C_2H_5OH$; $[\alpha]_D^{20}$ = 1467° (c = 0.05, $CHCl_3$). UV see Table 7. CD ($CH_3OH$): 245 (-2.9), 255 (0), 269 (+5.2), 310 (+13.2), 352 (+28.6), 410 (0), 436 (-18.2). IR ($CHCl_3$): 3600-3100m; 2980m; 2930m; 2850m; 1720m; 1670s; 1620m br.; 1560m br.; $^1H$ (200 MHz, $CD_3OD$ and $^{13}C$ NMR see Table 2-5. $^1H$ NMR (360 MHz, $CD_3OD$) double resonance: Irradiation at 7.25 (H-C(12) and H-C(21) gave: 6.48/s and 6.30/s (H-C(11) and H-C(20)); irrad. at 6.48 (H-C(11) or H-C(20)) gave: 7.23/d, J = 10 (H-C(12) or H-C(21)); irrad. at 2.72 ($H_\alpha$-C(3)) gave: 2.43/s ($H_\beta$-C(3)); irrad. at 1.88 ($CH_3$-C(14) and $CH_3$-C(23)) gave: 6.18/d, J = 15 and 6.09/d J = 15 (H-C(14) and H-C(23)). MS (70 eV): 250/14 ($C_{14}H_{18}O_4^+$); 248/7 ($C_{14}H_{16}O_4^+$); 232/36 ($C_{14}H_{18}O_4^+ - H_2O$); 231/10; 217/41; 207/11 ($C_{13}H_{13}O_3^+$); 191/19 ($C_{11}H_{11}O_3^+$); 189/15; 181/7 ($C_9H_9O_4^+$); 180/18; 175/10; 165/26 ($C_9H_9O_3^+$); 164/11; 161/13; 152/13; 149/28; 138/10; 137/10; 136/25; 124/10; 123/15; 122/14; 121/12; 109/12; 108/19; 107/19; 97/14 ($COC_5H_9^+$); 96/13; 95/100 ($COC_5H_7^+$); 91/14; 83/18; 79/15; 77/17; 71/11; 69/35; 67/45; 65/15; 57/23; 55/31; 53/10; 43/85; 41/58. Found: C, 66.07; H, 6.59; Calc. for $C_{28}H_{34}O_8$: C, 67.45; H, 6.87%.

Isolation of bisvertinolone (8)

The crude $CHCl_3$ extract (1.7 g) of the spent culture medium of V. intertextum was chromatographed on 150 g Sephadex LH-20 in a 4 x 50 cm column with $CHCl_3$/petrol ether (30-60°) 3:1 at r.t. using a flow rate of ca. 5.5 ml/min. The solvent was changed to pure $CHCl_3$ after 2090 ml solvent was collected. From a strong yellow fraction after a faint yellow one 298 mg of a yellow-orange oil were isolated which was again chromatographed on 50 g Sephadex LH-20 in a 2.5 x 40 cm column with $CHCl_3$ at r.t. using a flow rate as above. The first yellow fraction was evaporated and yielded 71 mg (6.5% of the $CHCl_3$ extract) of a yellow oil. This was combined with 200 mg material obtained separately in the same way, again three times chromatographed on 50 g Sephadex LH-20 in 2.5 x 40 cm columns with $CHCl_3$ at r.t. using a flow rate as above and yielded, each time taking the first yellow fraction, 12.5 mg (0.20%) of a yellow amorphous solid, m.p. 127.5-135.5° (dec). Crystallisation from diisopropyl ether yielded after standing for 3 weeks at -20° 3 mg (0.05%) 8 in yellow rosettes, m.p. 156-158.3° (dec); $[\alpha]_D^{20}$ = -1046° (c = 0.91, $CHCl_3$). UV see Table 7. CD ($CH_3OH$): 274 (+20.1), 300 (+12.9), 345 (+14.0), 368 (0), 408 (-29.2). IR ($CHCl_3$): 3430w br.; 3030w; 2930w; 2855w; 1738w; 1670m; 1605s; 1565m; 1515m. $^1H$ and $^{13}C$ NMR see Table 2-5. $^1H$ NMR (400 MHz, $CDCl_3$) double resonance: Irradiation at 7.58 (H-C(12)) gave: 7.41/s (H-C(11)) and 6.4-6.25/simplified m (H-C(13), H-C(22) and H-C(23)); irrad. at 7.33 (H-C(21)) gave: 6.40/s (H-C(20)) and 6.4-6.25/simplified m (H-C(13), H-C(14) and H-C(22)); irrad. at 6.30 (H-C(13), H-C(14), H-C(20), H-C(22) and H-C(23) gave: 7.58/d, J = 15 (H-C(12)); 7.33/d, J = 15 (H-C(21); 1.90/s ($CH_3$-C(14)) and 1.88/d, J = 7 ($CH_3$-C(23)); irrad. at 6.13 (H-C(23)) gave 1.88/s ($CH_3$-C(23)); irrad. at 1.90 ($CH_3$-C(14) and $CH_3$-C(23)) gave: 6.4-6.25/simplified m (H-C(13), H-C(14) and H-C(22) and 6.13/d, br., J = 15 (H-C(23)). MS (70 eV): 512/5 ($M^+$); 264/30 ($C_{14}H_{16}O_5^+ - H_2O$); 248/10 ($C_{14}H_{16}O_4^+$); 246/7 ($C_{14}H_{16}O_5^+ - H_2O$); 231/12; 222/16; 221/65; 206/10; 204/14; 181/13 ($C_9H_9O_4^+$), 154/17; 153/100; 147/10; 137/20; 95/66 ($COC_5H_7^+$); 83/26; 77/20; 69/21; 67/60; 65/23; 57/15; 55/17; 53/15; 43/75; 41/50. HR-MS: 264.0995 (calc. for $C_{14}H_{16}O_5$: 264.0998); 221.0809 (calc. for $C_{12}H_{13}O_4$: 221.0813); 153.0183 (calc. for $C_7H_5O_4$: 153.0188).

Methylation of the bisvertinols 5, 6 and 7

Samples of 2 mg of the bisvertinols 5, 6 and 7 each in 1 ml diethyl ether were treated with 3 ml 1.4% solution of diazomethane at r.t. for 5 min. Evaporation of the solvent in vacuo afforded the respective monomethyl ethers as yellow oils, which showed in each case a green-brownish colour reaction with $FeCl_3$ in $H_2O/C_2H_5OH$ and gave a single spot in the anal tlc with $CHCl_3/C_2H_5OH$ 47:3.

The monomethyl ether 10 from 5: $R_f$ 0.45. MS (70 eV): 514/1 ($M^+$ + 2 H); 513/4 ($M^+$ + H); 512/13 ($M^+$); 497/1 ($M^+ - CH_3$); 494/1 ($M^+ - H_2O$); 263/16; 247/18; 245/22; 232/10; 231/14; 195/15; 95/100 ($COC_5H_7^+$); 67/34; 43/35; 41/28.

The monomethyl ether 11 from 6: $R_f$ 0.47. MS (70 eV): 516/3 ($M^+$ + 2 H); 515/10 ($M^+$ + H); 514/30 ($M^+$); 499/1 ($M^+ - CH_3$); 496/1 ($M^+ - H_2O$); 263/36; 247/24; 246/12; 245/28; 231/21; 205/10; 195/34; 194/12; 179/17; 167/10; 165/22; 151/14; 139/10; 138/11; 137/11; 97/11 ($COC_5H_9^+$); 95/100 ($COC_5H_7^+$); 69/25; 67/26; 55/29; 43/52; 41/31.

The monomethyl ether 12 from 7: $R_f$ 0.50. MS (70 eV): 516/15 ($M^+$ + 2 H); 515/20 ($M^+$ + H); 514/60 ($M^+$); 499/4 ($M^+ - CH_3$); 496/2 ($M^+ - H_2O$); 265/38 ($C_{15}H_{21}O_4^+$); 250/16; 249/10; 247/20; 246/11; 233/14; 232/15; 231/10; 230/14; 221/11; 217/12; 215/11; 205/11; 195/27; 189/13; 181/19; 180/10; 179/63; 168/11; 165/21; 153/14; 151/11; 149/11; 139/10; 137/17; 136/15; 135/11; 125/13; 123/16; 121/14; 109/13; 108/20; 107/19; 99/12; 97/55 ($COC_5H_9^+$); 96/11; 95/100 ($COC_5H_7^+$); 91/25; 83/19; 81/11; 79/19; 77/19; 69/64; 67/58; 65/20; 57/15; 55/52; 53/18; 45/14; 43/98; 41/73.

Hydrogenation of the bisvertinols 5, 6 and 7

Samples of 3 mg of the bisvertinols 5, 6 and 7 each in 1 ml $CH_3OH$ were hydrogenated at r.t. under an atmosphere of $H_2$ in the presence of 2 mg Pd/C for 1 hr. The filtrate after filtration from the catalyst was evaporated to dryness in vacuo and gave a pale yellow oil. This product, octahydrobisvertinol (9), showed in each case a single spot in the anal. tlc with $CHCl_3/C_2H_5OH$ 47:3 at $R_f$ 0.35 and a green-brownish colour reaction with $FeCl_3$ in $H_2O/C_2H_5OH$. The UV spectra and the mass spectra of the three products were identical. UV, see Table 7. MS (70 eV): 252/18 ($C_{14}H_{20}O_4^+$); 236/12 ($C_{14}H_{22}O_4^+ - H_2O$); 193/12; 182/12; 181/100 ($C_9H_9O_4^+$); 180/15; 165/70 ($C_9H_9O_3^+$); 138/10; 110/13; 99/27 ($COC_5H_{11}^+$); 71/23; 57/10; 55/22; 43/44; 41/21.

Sorbylation of 5,5-dimethyl-1,3-cyclohexanedione (14)

To a solution of 1.40 g (10 mmol) 14 in 6 ml dry pyridine 1.43 g (11 mmol) sorbyl chloride (15) was added dropwise at 0° within 10 min. After stirring the reaction mixture at r.t. for 96 hr, ether (20 ml) was added and the precipitate filtered and washed with ether. The combined filtrate was evaporated to dryness in vacuo and the residue dried for 1 hr at r.t/0.01 Torr. The residue was dissolved in 25 ml ether and extracted with 0.1 N NaOH (2 x 25 ml). The organic layer was washed with water, the solvent evaporated to dryness and the residue chromatographed on 4 prep tlc plates with hexane/ether/acetone 6:3:1. Extraction of the UV active zone with $CHCl_3$, evaporation to dryness and bulb-to-bulb distillation at 160°/0.02 Torr of the residue gave 0.52 g (22%) 3-[(E,E)-2,4-hexadienoyloxy]-5,5-dimethyl-2-cyclohexen-1-one (16) as a colourless oil. UV ($CH_3OH$): 272 (32200), 230 sh (10000). IR (film): 3020w; 2950m; 2860m; 1730s; 1670s; 1640s; 1610s. $^1H$ NMR (200 MHz, $CDCl_3$): 7.41-7.27/m, 1 H (H-C(3')); 6.28-6.18/m, 2 H (H-C(4') and H-C(5')); 5.95/t, J = 1.1, 1 H (H-C(2)); 5.81/dxd, J = 15.0 and 0.8, 1 H (H-C(2')); 2.45/d, J = 1.1, 2 H ($H_2C(4)$); 2.27/s, 2 H ($H_2C(6)$); 1.90/d, J = 4.7, 3 H ($CH_3$-C(5')); 1.1/s, 6 H (2 x $CH_3$-C(5)). $^{13}C$ NMR (25.1 MHz, $CDCl_3$): 199.4/s (C(1)); 168.4/s and 163.6/s (C(3) and C(1')); 148.1/d (C(3')); 141.8/d (C(5')); 129.6/d (C(4')); 117.1/d and 116.1/d (C(2) and C(2')); 50.8/t and 42.3/t (C(4) and C(6)); 33.1/s (C(5); 28.2/q (2 x $CH_3$-C(5)); 18.8/q ($CH_3$-C(5')). MS: 206/2 ($M^+ - CO$); 96/11; 95/100 ($COC_5H_7^+$); 67/35; 41/23. Found: C, 71.71; H, 7.72. Calc for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74%.

The alkaline aqueous layer was acidified with 5% HCl and extracted with ether. The residue after removal of the organic solvent in vacuo was chromatographed on 4 prep tlc plates with hexane/ether/acetone 6:3:1. Extraction of the UV active zone with $CHCl_3$, evaporation of the solvent and recrystallisation of the residue from ether/hexane gave 0.18 g (8%) 2-[(E,E)-hexadienoyl]-3-hydroxy-5,5-dimethyl2-cyclohexen-1-one (17) as long lemon-yellow prisms, m.p. 93.5-95.0°. The product showed a brown-orange colour reaction with $FeCl_3$ in $H_2O/C_2H_5OH$. UV ($CH_3OH$): 343 (22600), 316 sh (15800), 249 (10200); UV after addition of one drop of 0.1 NaOH: 330 (8900), 272 (32650); after acidification the original UV spectrum was recovered. IR ($CHCl_3$): 3080w; 2990w; 2950m; 2930m; sh; 2860w; 1655s; 1635s; 1600s. $^1H$ NMR (200 MHz, $CDCl_3$): 18.32/s, 1 H (OH); 7.60-7.52/m, 2 H (H-C(2') and H-C(3')); 6.40-6.20/m, 2 H (H-C(4') and H-C(5')); 2.53/s, 2 H and 2.39/s, 2H ($H_2C(4)$ and $H_2C(6)$); 1.91/d, J = 5.4, 3 H ($CH_3$-C(5')); 1.08/s, 6 H (2 x $CH_3$-C(5)). $^{13}C$ NMR (25.1 MHz, $CDCl_3$): 201.3/s (C(1)); 195.4/s (C(1')); 187.4/s (C(3)); 146.6/d (C(3')); 141.9/d (C(5')); 131.3/d (C(4')); 123.9/d (C(2')); 110.6/s (C(2)); 53.1/t and 48.7/t (C(4) and C(6)); 30.3/s (C(5)); 28.2/q (2 x $CH_3$-C(5)); 18.9/q ($CH_3$-C(5')). MS: 235/9; 234/56 ($M^+$); 233/21; 220/15; 219/100 ($M^+ - CH_3$); 216/16; 193/43; 167/45; 163/35; 135/15; 121/17; 107/12; 95/50 ($COC_5H_7^+$); 83/65; 69/14; 67/31; 65/12; 55/15; 43/20; 41/35. Found: C, 71.84; H, 8.12. Calc for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74%.

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