Pergamon

Tetrahedron, Vol. 53, No. 17, pp. 6195-6208, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00276-7

Eremophilane Derivatives with a Novel Carbon Skeleton from *Ligularia veitchiana*

Yu Zhao[1], Simon Parsons[2], Bruce A. Smart[2], Renxiang Tan[3], Zhongjian Jia[4], Handong Sun[5] and David W. H. Rankin*[2]

[1]Department of Pharmaceutical Biology, Institute of Pharmacy, Johannes Gutenberg-Universität Mainz, D-55099 Mainz, Germany. [2]Department of Chemistry, The University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, U.K. e-mail: d.w.h.rankin@ed.ac.uk. [3]Department of Biology, Nanjing University, Nanjing 210008, P.R. China. [4]Institute of Organic Chemistry, Lanzhou University, Lanzhou 730000, P.R. China. [5]Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P.R. China.

Abstract: Ligulaverin A **1**, an eremophilane derivative with a novel carbon skeleton, was isolated from the medicinal plant *Ligularia veitchiana*, along with four analogues (ligulaverin B **2**, ligulaverin C **3**, ligulaverin D **4** and ligulaverin E **5**). Their structures were elucidated by NMR techniques and X-ray diffraction. The biosynthetic route to this nineteen-carbon skeleton is discussed. © 1997 Elsevier Science Ltd.

INTRODUCTION

More than 20 *Ligularia* species have been used for folk medicines in China.[1,2] Their roots, stems, leaves and flowers are effective anti-inflammation agents, reduce phlegm and relieve coughs, help blood circulation and relieve pain. Chinese pharmacopoeia have recorded that from ancient times they have been used to cure pulmonary tuberculosis, haemoptysis, urinary tract blockages, rheumatism, common cold, pharyngitis and laryngitis, hepatitis, bronchitis and asthma. Among them, *Ligularia veitchiana* (Hemsl.) Greenm. is commonly used for the treatment of influenza, cough, ulcer and tuberculosis,[2] and has therefore been investigated by our group. Several eremophilane derivatives, most of which have an olide ring,[3-7] have been isolated from different parts of the plants; however, we have also isolated a series of components with an unusual structure from the polar part of the EtOAc extract of the whole title plant. NMR spectra showed that although these compounds may be regarded as eremophilane derivatives, they exhibited an apparently novel skeleton. The NMR experiments, including 2D experiments (^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and ^{1}H-^{13}C COLOC), did not provide sufficient information to elucidate their structures and stereochemistries unambiguously. However, we have recently employed single crystal X-ray diffraction to resolve this problem. The results have shown that these are a series of compounds, **1-5**, with a novel 19 skeletal carbons. This skeleton was probably formed by a C_4 unit combining with a common eremophilane skeleton (Fig. 1) at the C-8 and C-12 positions, thereby forming a new 6,6,6-tricyclosystem. The structural elucidation and the possible biopath to this novel skeleton are discussed below. A preliminary account of part of this work has been published.[8]

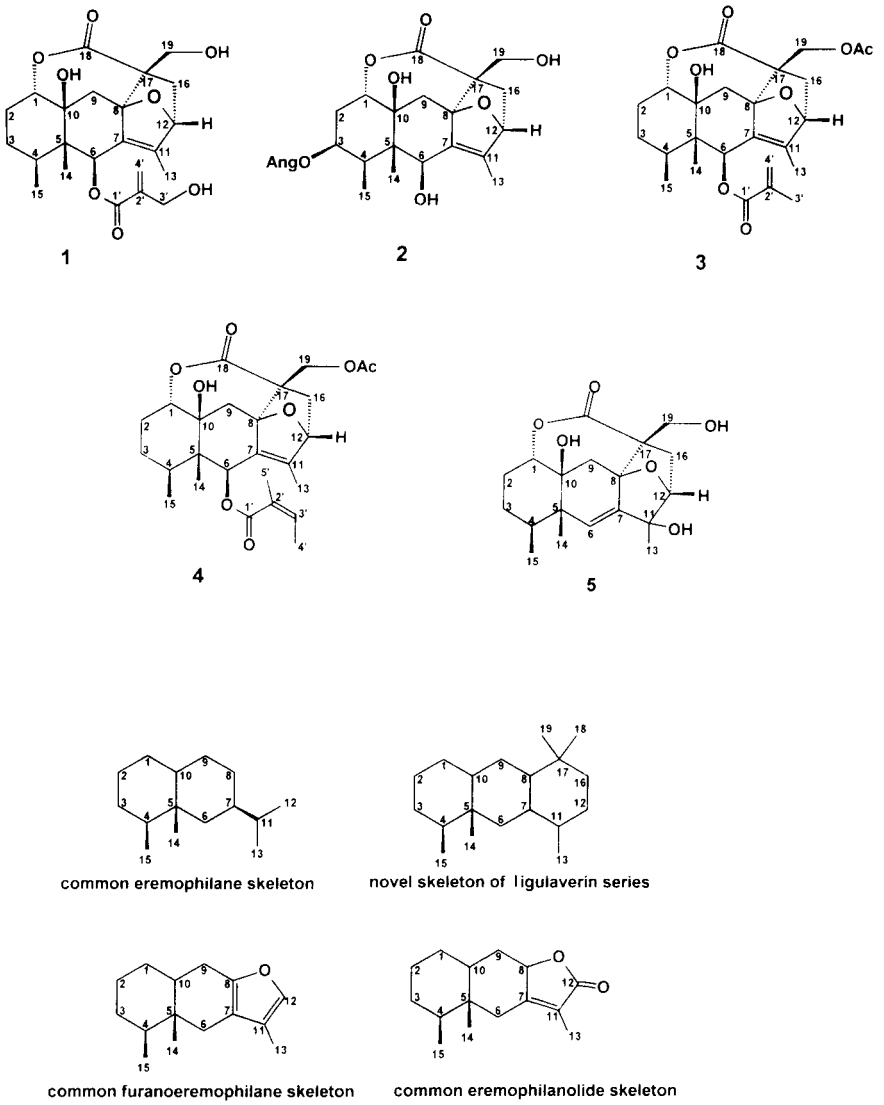


Fig. 1. Comparison of skeletons of eremophilanes and related compounds.

RESULTS AND DISCUSSION

Ligulaverin A 1. The ^{13}C NMR of **1** showed 23 ^{13}C resonances. DEPT experiments differentiate these signals as $3\times CH_3$, $7\times CH_2$, $4\times CH$, $9\times C$ (Table 1). EIMS gave a molecular ion peak at *m/z* 434; combined with the results of elemental analysis, this enabled the molecular formula of **1** to be deduced to be $C_{23}H_{30}O_8$. The

presence of a hydroxymethyl-acryloyl moiety could be distinguished by the typical set of signals at δ_H 6.29 (1H, m), 5.96 (1H, dt, *J*=1.4, 1.6 Hz) and δ_H 4.28 (2H, t, *J*=1.5 Hz) in its ^{1}H NMR spectrum. The corresponding ^{13}C resonances appeared at δ_C 167.07 (C), 134.89 (C), 67.18 (CH_2) and 125.71 (CH_2) (Tables 1 and 2). Thus the remaining 19 carbons formed the skeleton.

As we had previously isolated several eremophilane sesquiterpenes from *Ligularia veitchiana*,[3-6] it was possible to identify methyl groups corresponding to Me-13, Me-14 and Me-15 of an eremophilane skeleton, observed at δ_H 1.60 (3H, d, *J*=2.3 Hz), δ_H 1.14 (3H, s) and δ_H 1.17 (3H, d, *J*=7.4 Hz), respectively, in the ^{1}H NMR spectrum of **1**, which was therefore identified as an eremophilane derivative. Comparing the high frequency chemical shifts of the oxygen-bearing carbons of **1** with those of the structurally similar eremophilane derivatives[3-5] demonstrated that C-10, C-6 and C-1 were oxygenated. These conclusions were supported by the observations that H-9 appeared as a pair of doublets, δ_H 2.42 and 2.38, 1H each, *J*=12.0 Hz (Table 2), and that H-6 also had a high-frequency shift at δ_H 6.29, and exhibited homoallylic coupling with Me-13, *J*=2.3 Hz.[3] Parameters for C-2, C-3, C-4 were similar to those of eremophilane derivatives previously reported.[3-6] However, the commonly appearing olide ring C in the previous eremophilane components was clearly not present in the case of **1**, nor was the C ring a common furan ring (Fig. 1). Instead, it was indicated to be a dihydrofuran by the ^{13}C and ^{1}H NMR data (Tables 1 and 2). Furthermore, C-8 of **1** must be wholly substituted to account for a pair of doublets for H-9 (δ_H 2.42, 1H, d, *J*=12.0 Hz; δ_H 2.38, 1H, d, *J*=12.0 Hz), while the other oxygen-bearing carbon atom of the dihydrofuran ring (C-12) exhibited a trisubstituted methine (δ_H 4.49, 1H, d, *J*=4.6 Hz; δ_C 83.38, CH) (Tables 1 and 2). All these suggest that the environments of C-8 and C-12 have been considerably modified, and are apparently now mono-oxygenated carbons. The ^{1}H-^{13}C COLOC experiment also showed that C-8 was correlated with a pair of high frequency doublets associated with C-19 at δ_H 3.95 (1H, d, *J*=11.0 Hz) and 3.81 (1H, d, *J*=11.0 Hz) (Table 2), while C-12 was correlated with a pair of protons attributable to C-16 at δ_H 2.06 (1H, d, *J*=12.2 Hz) and δ_H 1.84 (1H, dd, *J*=12.2, 4.7 Hz), thus demonstrating that the additional carbon chain did connect to C-8 and C-12. Furthermore, the ^{1}H-^{13}C COLOC spectrum also exhibited several significant correlations between H-16 and C-11/C-8; H-1 and C-9/C-18, etc. There is thus clear evidence for a four carbon chain, which was joined in three places (C-8 with C-17, C-12 with C-16 by C-C bonds, and C-1 with C-18 by means of an ester bridge) with the common fifteen-carbon eremophilane skeleton (Fig. 1 and 2).

Stereochemically, Me-14 and Me-15 are biogenetically β, and H-6 must have the α orientation to allow for the homoallylic coupling with Me-13 (δ_H 1.60, 3H, d, *J*=2.3 Hz). H-1 was deduced to be in the β-orientation by its coupling pattern (1H, dd, *J*=3.3, 2.6 Hz). The α-orientated ester moiety (C-18, C-17 and C-16) could only be connected to C-8 and C-12 also with α-configurations; the alternative of β C-17/β C-16 would involve an impossibly large torsion in formation of the skeleton (Fig. 2). This assignment was in full agreement with the results of the X-ray diffraction study. The asymmetric unit of crystals of **1** contains four molecules, which have essentially identical structures, but differ in the conformations of the substituent hydroxy, hydroxymethyl and hydroxymethylacryloyl groups (Fig. 3).

***Ligulaverin B* 2.** The ^{1}H and ^{13}C NMR spectra of ligulaverin B closely resemble those of ligulaverin A (**1**) (Tables 1 and 2). However, two fine distinctions were visible in its ^{1}H NMR spectrum: a broad quartet due to H-6 appeared at δ_H 4.79 (1H, *J*=2.1 Hz), shifted to low frequency relative to the corresponding resonances of **1** (Table 2), thus suggesting that the 6-hydroxyacryloyl group in **1** had been replaced by a 6-hydroxy group in **2**. Furthermore, another high frequency multiplet was visible at δ_H 5.63 (1H, ddd, *J*=12.5, 5.5, 2.4 Hz),

Table 1. ^{13}C NMR Spectral Data of Compounds **1-5** (100.6 MHz)

C#	**1** (CD_3OD)	**2** (Me_2CO-d_6)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)
1	71.67	69.48	70.00	69.17	62.80
2	24.46	29.77	23.55	23.55	25.75
3	24.06	69.33	22.57	22.57	24.20
4	34.38	36.73	32.69	32.69	38.33
5	46.05	48.03	45.24	45.04	38.20
6	84.89	82.29	83.05	83.05	129.16
7	146.75	146.53	146.45	146.13	138.95
8	87.19	86.08	85.35	83.35	85.86
9	41.73	41.76	40.41	40.41	41.86
10	74.85	74.42	74.65	74.65	85.67
11	141.60	138.19	133.20	133.33	54.16
12	83.38	84.33	82.79	82.79	86.50
13	10.38	10.75	10.37	10.30	18.53
14	16.80	16.20	16.78	16.83	15.87
15	17.39	15.79	16.48	15.88	15.53
16	35.46	35.14	35.77	35.77	36.26
17	61.42	60.97	57.37	57.37	61.46
18	176.93	174.27	171.41	171.41	177.92
19	61.56	67.11	66.36	66.36	62.28
OAc	-	-	170.51	170.51	-
	-	-	20.66	20.91	-
1`	167.07	167.49	166.67	166.96	-
2`	134.89	129.05	135.86	126.36	-
3`	67.18	137.83	10.37	140.13	-
4`	125.71	20.74	126.36	18.35	-
5`	-	11.02	-	16.48	-

Chemical shifts δ in ppm.

indicating the presence of an oxygen-bearing methine. The ^1H-^1H COSY experiment showed that this multiplet has a correlation with H-2β (δ_H 1.84, ddd, J=12.6, 2.4, 2.5 Hz), H-2α (δ_H 2.61 br ddd, J=12.4, 12.4, 2.5 Hz), and H-4 (δ_H 2.51, 1H, dq, J=7.4, 5.6 Hz). Moreover, the ^{13}C NMR spectrum of **2** showed that C-3 of **1** (δ_C 24.06, CH_2) had disappeared and been replaced by a high frequency signal at δ_C 69.33 (CH), while C-2 and C-4 of **2** were shifted to high frequency relative to those of **1** (Table 1). All these observations suggested the presence of an oxygen-bearing group at C-3. Meanwhile, a group of signals, which were typically attributable to an angeloyl moiety, were visible at δ_H 6.12 (1H, qq, J=7.2, 1.4 Hz), δ_H 1.98 (3H, dq, J=7.2, 1.5 Hz), and δ_H

Table 2. ^{1}H NMR Spectral Data of Compounds **1-5** (400 MHz)

H#	**1** (CD_3OD)	**2** (Me_2CO-d_6)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)
1ß	5.07 dd (3.3, 2.6)	5.26 dd (3.2, 2.6)	4.80 dd (3.4, 2.9)	4.80 dd (3.4, 2.9)	3.06 br s
2α	2.43 m	2.61 br ddd (12.4, 12.4, 2.5)	2.43 ddt (12.8, 12.8, 2.3)	2.43 ddt (12.8, 12.8, 2.3)	2.08 m
2ß	1.25 m	1.84 ddd (12.6, 2.4, 2.5)	1.34 m	1.34 m	1.72 m
3α	2.45 m	5.63 ddd (12.5, 5.5, 2.4)	2.49 dddd(12.8, 12.8, 3.4, 2.3)	2.49 dddd (12.8, 12.8, 3.4, 2.3)	1.68 m
3ß	1.58 m	-	1.63 m	1.63 m	1.14 m
4	1.50 m	2.51 dq (7.4, 5.6)	1.78 m	1.80 m	1.15 m
6	6.29 m	4.79 br q (2.2)	6.25 q (2.2)	6.29 q (2.3)	5.85 s
9	2.42 d (12.0)	2.50 d (12.6)	2.23 d (12.8)	2.23 d (12.8)	2.67 d (12.6)
9'	2.38 d (12.0)	2.42 d (12.6)	2.57 d (12.9)	2.57 d (12.9)	1.44 d (12.6)
12	4.49 d (4.6)	4.42 d (4.5)	4.58 d (4.5)	4.58 d (4.5)	4.80 s
13	1.60 d (2.3)	1.92 d (2.3)	1.63 d (2.3)	1.64 d (2.4)	1.65 s
14	1.14 s	1.15 s	1.15 s	1.15 s	1.01 s
15	1.17 d (7.4)	1.18 d (7.3)	1.15 d (6.9)	1.15 d (6.9)	0.88 d (7.8)
16	2.06 d (12.2)	2.06 d (12.2)	2.22 d (12.5)	2.22 d (12.5)	2.07 d (12.4)
16'	1.84 dd (12.2, 4.7)	1.78 dd (12.3, 4.5)	2.12 dd (12.5, 4.6)	2.12 dd (12.5, 4.6)	1.63 d (12.4)
19	3.95 d (11.0)	3.94 d(10.8)	4.78 d (11.2)	4.77 d (11.2)	3.90 d (11.6)
19'	3.81 d (11.0)	3.84 d (10.8)	4.08 d (11.2)	4.09 d (11.2)	3.63 d (11.6)
$3''_1$	6.29 m	6.12 qq (7.2, 1.4)	6.17 t (1.6)	6.18 qq (7.3, 1.4)	-
$3''_2$	5.96 dt (1.4, 1.6)	-	5.63 t (1.6)	-	-
4''	4.28 t (1.5)	1.98 dq (7.2, 1.5)	1.97 brs	2.03 dq (7.2, 1.2)	-
5''	-	1.89 dq (1.5, 1.0)	-	1.90 dq (1.2, 1.0)	-
OAc	-	-	2.10 s	2.10 s	-

Chemical shifts δ are in ppm; coupling constants *J* in Hz are in parentheses.

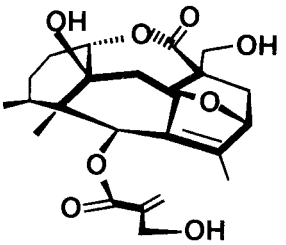


Fig. 2. Cage structure of ligulaverin A **1**

1.89 (3H, dd, *J*=1.5, 1.5 Hz) in the ^{1}H NMR spectrum of **2**, thus indicating that an OAng group was present in the molecule.[3,4] Since the pair of H-9 resonances appeared at δ_H 2.50 (1H, d, *J*=12.6) and δ_H 2.42 (1H, d, *J*=12.6 Hz), retaining similar chemical shifts to those of **1** (Table 2), the possibility of a 10-OAng moiety could be eliminated. Comparisons of the ^{1}H NMR data of the H-19 pair of **2** with those of **1** also ruled out the presence of a 19-OAng group. Thus the angeloyl group could only possibly be connected to C-3, which was consistent with the fact that H-3 exhibited a relatively high frequency signal. Stereochemically, the coupling pattern of H-3 (one large coupling and two small ones) (Table 2) disclosed, with help from a model of the molecule, that H-3 was in an axially α-orientation, while the large substituent (OAng) adopted an equatorial β orientation. Together with a significant fragment due to loss of an angelic acid at *m/z* 348, the EIMS of **2** showed a weak molecular ion peak at *m/z* 448, corresponding to the elemental analysis. The molecular formula could be therefore elucidated as $C_{24}H_{32}O_8$, and the compound was named ligulaverin B.

Ligulaverin C 3 and D 4. Compounds **3** and **4** were obtained as a mixture (ratio 1:2, by NMR spectra after acetylation). The precursor mixture before acetylation not only failed to separate by column chromatography in several solvent systems but was also difficult to separate on RP-18 plates. The mixture was therefore acetylated (see Experimental Section), but the products (**3** and **4**) remained inseparable. The ^{1}H and ^{13}C NMR spectra were extremely similar to those of **1**. After acetylation, it was observed that the H-19 pair were remarkably shifted to high frequency, to δ_H 4.78 (1H, d, *J*=11.2 Hz) and δ_H 4.08 (1H, d, *J*= 11.2 Hz), while the H-9 pair remained unchanged (Table 2), diagnostically indicating that the acetylation occurred on 19-OH. A model showed that this acetoxy group was spacially near the H-16 pair and thus deshielding H-16 to higher frequency (δ_H 2.22, 1H, d, *J*=12.5 Hz, δ_H 2.12, 1H, dd, *J*=12.5 Hz, 4.6 Hz) relative to those of **1** and **2** (Table 2). The other ^{1}H and ^{13}C chemical shifts were comparable to those of **1**. By the^1 H and^{13} C NMR spectra, the difference between **3** and **4** was clearly demonstrated to be at the C-6 side chains. Since the ratio of the amounts of these two compounds present was 1:2, it was easy to distinguish the different signals of H-6, H-13 and H-4 (Table 2), which were influenced by the C-6 side chain. The minor constituent **3** showed its C-6 side chain to be an acryloyl group [δ_H 6.17 (1H, t, *J*=1.6), δ_H 5.63 (1H, t, *J*=1.6 Hz), δ_H 1.97 (3H, br s)], while **4** (the main constituent) exhibited its C-6 side chain to be an angeloyl group [δ_H 6.18 (1H, qq, *J*=7.3, 1.4 Hz), δ_H 2.03 (3H, dq, *J*=7.2, 1.2 Hz), δ_H 1.90 (3H, dq, *J*=1.2, 1.0 Hz)]. Based on the ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectra, the chemical shifts of these two compounds could be assigned unambiguously (Tables 1 and 2). EIMS spectrometry gave two separate spectra corresponding to different GC peaks (see Experimental Section). The molecular ion peak at *m/z* 460, as well as the significant fragments due to successive losses of water (*m/z* 442)

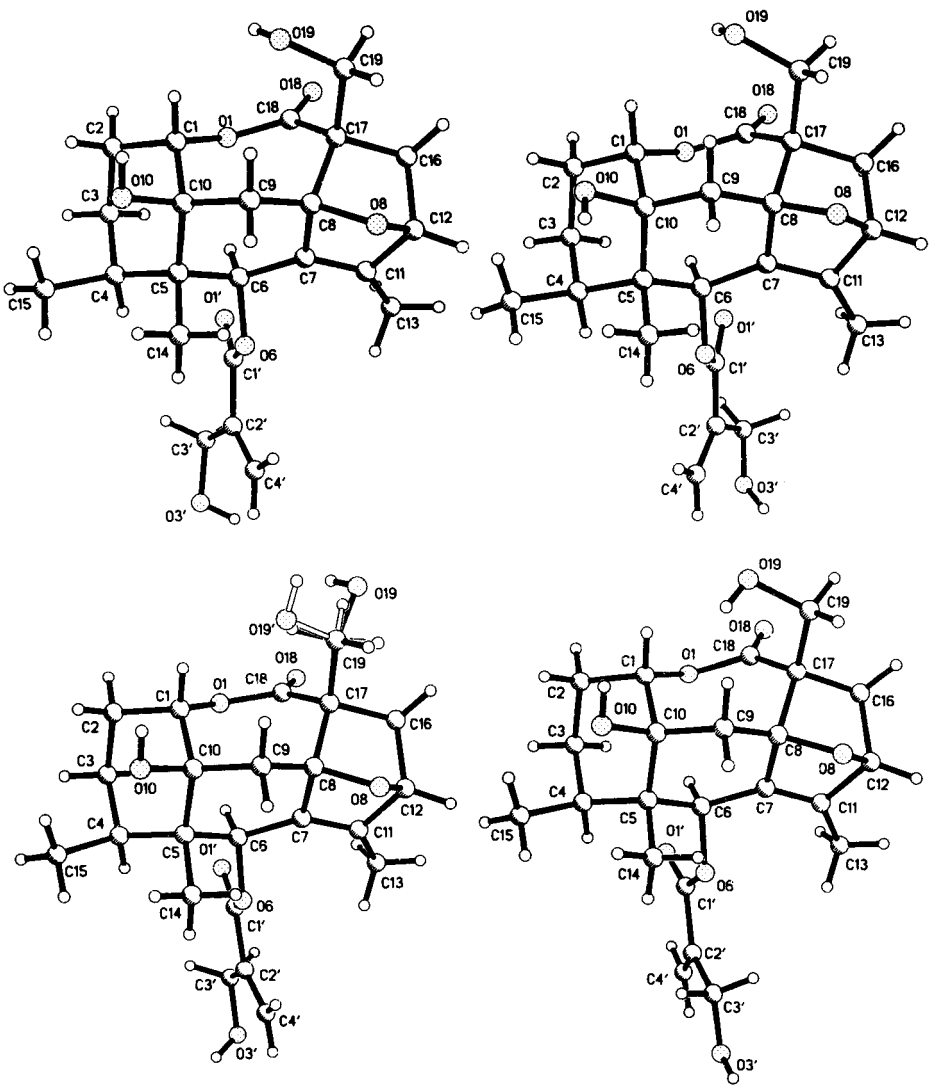


Fig. 3. Perspective views of the four different molecules in the unit cell of ligulaverin A **1**, showing the different conformations adopted.

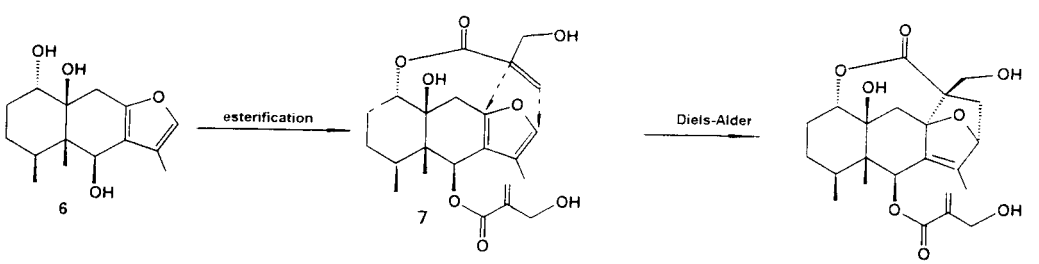
and acryloyl (*m/z* 356), apparently arose from **3**, which was named ligulaverin C. The molecular ion peak at *m/z* 474, along with the diagnostic fragments at *m/z* 456 $[M\text{-}H_2O]^+$ and *m/z* 374 $[M\text{-}HOAng]^+$ were in agreement with the proposed structure of **4**, which has the trivial name ligulaverin D.

Ligulaverin E 5. The EIMS spectrum showed the molecular ion peak of **5** at *m/z* 350. Using this information,

combined with the DEPT spectrum and the results of elemental analysis, its molecular formula was deduced to be $C_{19}H_{26}O_6$. The ^{13}C NMR spectrum of **5** exhibited only 19 carbon signals (Table 1), which clearly indicated the lack of side chains. Furthermore, a tri-substituted double bond was visible in the ^{13}C NMR spectrum of **5** (δ_C 129.16 and 138.95). In addition, the characteristic doublet of Me-13 in compounds **1-4** was not present in the ^1H NMR spectrum; instead, a high-frequency singlet at δ_H 1.65 (3H) was observed (Table 2). This indicated that the 7,11-double bond in **1-4** no longer existed in **5**. Scrutiny of the ^{13}C and ^1H NMR spectra allowed the assignment of a 6,7-double bond (Tables 1 and 2). An OH group (12-OH) was therefore required since the Me-13 resonance described above appeared as a high-frequency singlet. Combination of the information given by ^1H-^1H COSY and ^1H-^{13}C COSY spectra led to the proposed structure, whose ^1H NMR spectrum nevertheless showed many slight differences to those of **1-4** (Table 1). The low-frequency shifted ^1H signals of H-2α, H-3α, H-3ß, H-4, as well as those of H-9' and Me-15 (Table 2), could be attributed to the existence of the 6,7-double bond, while the changed coupling constants of the H-19 pair (δ_H 2.07, 1H, d, J=12.4 Hz; δ_H 11.63, 1H, d, J=12.4 Hz) and H-12 (a singlet instead of a doublet), probably can be attributed to the fact that the 7,11-double bond in ring C (compounds **1-4**) would produce different skeletal torsions to those arising from the 6,7-double bond in ring B in the case of **5**. The ^{13}C NMR spectrum also showed slight differences, mainly at C-4, C-5, C-10, C-11, and C-13 (Table 1), which were also in agreement with the proposed change.

Biosynthesis of ligulaverins. The ring structure of these new compounds is unprecedented, although they are clearly related to the well-known eremophilanes shown in Fig. 1, and particularly to the furanoeremophilane. The most reasonable route to ligulaverin A is *via* euryopsol **6**, the 1,6,10-trihydroxy derivative of furanoeremophilane, which has previously been isoltaed from *Euryops* spp. (Compositae).[9] Formation of the 1,6-bis(hydroxy-methacrylate) ester, **7**, followed by a Diels-Alder reaction, would then yield ligulaverin A (Scheme 1). Of course, whether esterification at the 6 position takes place before or after the Diels-Alder reaction is merely a matter of speculation, as are questions concerning the relationships between modes of formation of the five different ligulaverins.

The proposed Diels-Alder reaction is of interest, because pericyclic reactions are rare, although not unknown, in biological systems. The contention that enzyme-catalysed Diels-Alder reaction do occur naturally has been the subject of considerable controversy,[10] and tentative evidence for a true enzyme-catalysed Diels-Alder reaction *in vivo* has only recently emerged, from cell-free studies of the biosynthesis of the solanapyrones.[11] In this case, the possibility that compound 1 is an artifact produced during the extraction and



Scheme 1. Probable biosynthetic route to ligulaverin A 1.

isolation procedures can be ruled out by the fact that the isolation conditions did not involve the use of elevated temperatures. A non-enzymatic Diels-Alder reaction, with **1** being produced by the effect of solar heating of **7** in the plant tissue, must also be considered, but this is inconsistent with the observation that these compounds are concentrated in the roots. We are therefore left with the conclusion that at least one of the family of ligulaverin metabolites is the product of a true enzymatic Diels-Alder'ase process. This important matter will be studied further.

To investigate further whether this process can be regarded as enzymatic, calculations at the AM1 level[12] were performed using the Gaussian 94 program.[13] Geometry optimisations were performed on both ligulaverin A **1** and its postulated precursor **7**, to give an estimate of the energy of reaction. The product was calculated to be 15.3 kJ mol^{-1} *less* stable than the precursor, which would indicate that this is not a reaction which could occur spontaneously. Although the energy difference given by the AM1 calculations should not be regarded as being absolutely accurate, the calculations do support the suggestion that the process is enzymatic; there must, of course, be at least one other concurrent reaction, to provide the energy to drive the process. Attempts to model the transition state were unfortunately not successful, but it is clear that this must be substantially higher in energy than reagent and product, so a catalyst must be involved for the reaction to proceed at a reasonable rate at ambient temperature.

Crystal structure and hydrogen bonding. Crystal data for ligulaverin A **1** have been given in the preliminary account of this work,[8] and atomic coordinates, thermal parameters, bond lengths and angles are available from the Cambridge Crystallographic Data Centre (CCDC); any request to CCDC for this material should quote the full citation for reference 1 and the reference number 182/249.

A striking and possibly significant feature of the structure of ligulaverin A is the relationship between its cage and that of the anticancer agent, Taxol® [14,15] (Fig. 4). Taxol was the first compound to be discovered which owed its activity to its ability to bind to β-tubulin,[16] although since then two other naturally occurring compounds, epothilone[17] and discodermolide,[18, 19] have been found to have similar activity. There is now the possibility that the ligulaverin framework could be incorporated into a family of derivatives which could be of chemopharmaceutical significance.

There are four molecules in the asymmetric unit, differing mainly in the conformations adopted by the hydroxy, hydroxymethyl and hydroxymethylacryloyl substituents (Fig. 3). The most surprising feature is that, in just one of the four molecules (molecule 4), the C=O bond in the hydroxymethylacryloyl group is *syn* with respect to the C=C bond in the group, whereas in the other three molecules the C=O and C=C bonds are

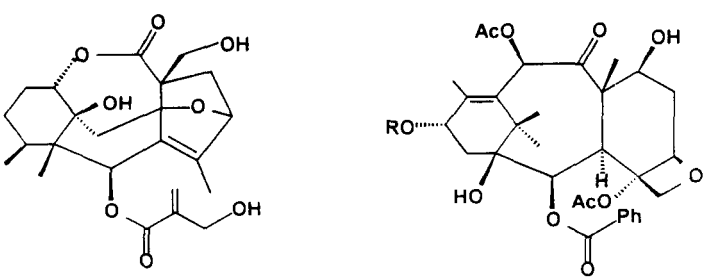


Fig. 4. Structural comparison of ligulaverin A **1** and Taxol

mutually *anti* - the usual arrangement. This has to be attributed to the consequential favourable effect on hydrogen bonding, which is a significant but complex feature of the crystal structure.

The arrangement of the molecules in the crystal is best described as consisting of four chains, each made up of molecules of one of the four types in the asymmetric unit. Alternating chains of molecules 1 and 2 form a strongly hydrogen-bonded layer, as do those of molecules 3 and 4, but there is only a single hydrogen bond per asymmetric unit between layers.

The chains of molecules of type 1 are linked together by hydrogen bonds between the hydrogen atom of the 10-hydroxy group and the hydroxy group oxygen atom in the hydroxy methacrylate ester group, while the links between the type 2 molecules use the hydrogen atom of the latter ester hydroxy group and the oxygen atom of the third hydroxy group, that bound to C(19). In contrast, the chains of molecules of type 4 have no hydrogen-bond links, but those of type 3 are linked through the same hydroxy groups as those of type 1, but in this case there is a double interaction involving all four atoms (Fig. 5).

The type 1 and type 2 chains are held together by three hydrogen bonds per asymmetric unit, as shown in Fig. 6, and there are also up to three hydrogen bonds joining the chains of types 3 and 4 (Fig. 7), although the average number of links is only 2.7, due to disorder of the C(19)H_2OH group in molecule 1. Four of these six inter-chain hydrogen bonds use the lactone carbonyl oxygen atoms in all four molecules, while the other two involve the oxygen atom in the five-membered rings. These oxygen atoms are not utilised in any other hydrogen bonds.

The final hydrogen bond is the only one between the layers, and links the 3'-hydroxy group hydrogen of molecule 1 and the ester carbonyl oxygen of molecule 4. It is the involvement of the 3'-hydroxy group in this interaction which precludes the possibility of repetition in the molecule 1 chains of the four-centre double hydrogen bond found in the molecule 3 chains. Finally, we note that the existence of only weak interactions between layers accounts for the formation of crystals as very thin flakes, crystal growth occurring primarily as extension of existing layers, rather than the formation of new ones.

Overall, therefore, 10.7 of the 12 hydroxy group hydrogen atoms are involved in hydrogen bonding. The molecules are joined into chains entirely by links between two hydroxy groups, the chains are formed into layers by bonds using the lactone carbonyl and furan oxygen atoms, and the inter-layer contact uses the ester carbonyl oxygen. All the hydrogen bond data are listed in Table 3.

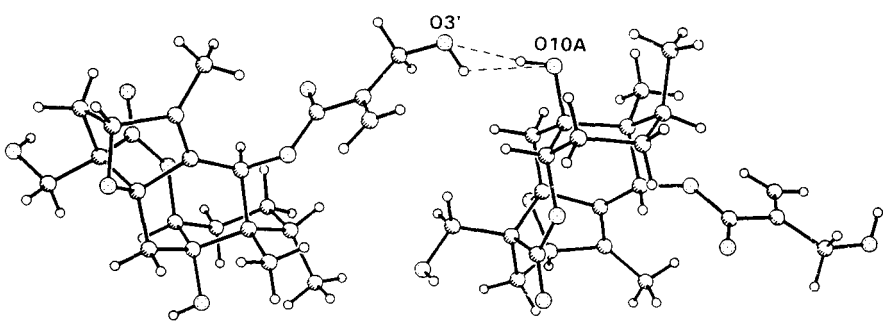


Fig. 5. Double hydrogen bond between molecules of types 3 and 4.

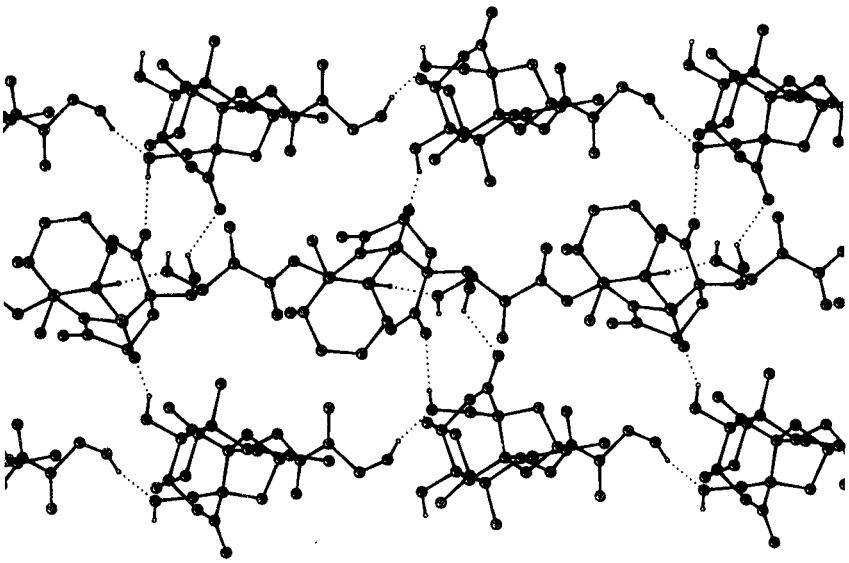


Fig. 6. Layers of molecules of types 1 (centre chain) and 2 (outer chains).[20]

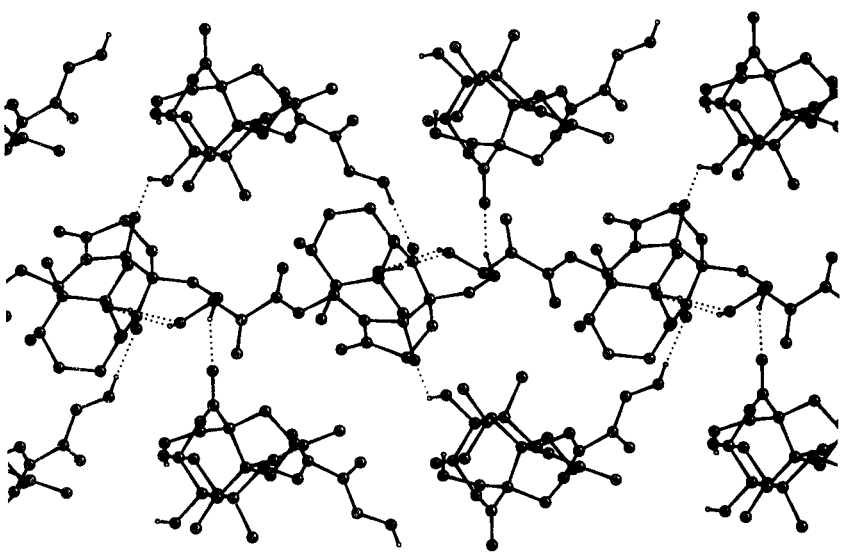


Fig. 7. Layers of molecules of types 3 (centre chain) and 4 (outer chains).[20]

Table 3. Hydrogen bonding in ligulaverin A **1**

Hydrogen	Oxygen	Sym. operation	*r*(O-H)/Å	*r*(H···O)/Å	<OH···O/°	*r*(O···O)/Å
H(10) - 1	O(3') - 1	-x+1, y-½, -z+1	0.840	1.945	173.2	2.781
H(3') - 2	O(19) - 2	-x, y-½, -z+1	0.840	1.962	162.5	2.774
H(3') - 3	O(10) - 3	-x+1, y-½, -z+2	0.840	2.328	116.3	2.803
H(10) - 3	O(3') -3	-x+1, y+½, -z+2	0.840	1.969	172.0	2.803
H(19) - 1	O(18) - 2	-x+1, y-½, -z+1	0.840	2.139	126.2	2.721
H(10) - 2	O(8) - 1	-x, y+½, -z+1	0.840	2.169	133.5	2.814
H(19) - 2	O(18) - 1	-x+1, y+½, -z+1	0.840	2.253	119.1	2.761
H(19) - 3a	O(18) - 4	-x, y+½, -z+2	0.840	2.146	128.7	2.750
H(3') - 4	O(18) - 3	x, y, z	0.840	1.870	164.7	2.689
H(10) - 4	O(8) - 3	-x+1, y-½, -z+2	0.840	2.091	127.4	2.685
H(3') - 1	O(1') - 4	-x, y+½, -z+1	0.840	2.051	145.5	2.785

EXPERIMENTAL

General procedures. Mps: uncorr.; EIMS: direct inlet, 70 eV. ^{1}H (400 MHz) and ^{13}C NMR spectra (100.6 MHz) with TMS as int. standard; IR: KBr.

Plant material. Whole plants of *L. veitchiana* (3.5 kg) were collected in August 1988. They were identified by Prof. R.N. Zhao at the Dept. of Pharmcognosy, Medicinal College of Lanzhou, Lanzhou 730000, P.R. China; the voucher specimen is deposited there.

Extraction and isolation. The extraction and column chromatography of the crude extract has been described previously.[3,6] From the last fraction (3.2 g), *viz.* F_{10} of the EtOAc extract,[6] 420 mg of crude **1** was crystallized, in addition to 1β-hydroxy-6,9-diene-8-oxo-eremophil-(12)-oic acid, which had been isolated previously.[6] Further recrystallization from MeOH gave 280 mg of pure, crystalline **1**. The mother liquor was evaporated (2.5 g), and then fractionated using a Si gel column (300 g, 200-300 mesh), using $CHCl_3$-Me_2CO 10:1-0:1 for gradient elution (250 ml each eluate); eluates 8 and 9 were evaporated to afford 84 mg of **2**. Eluates 10-15 contained mainly **1** and the precursors of **3**/**4**. The dried material from this fraction (840 mg) was separated on a 50 g Si H column, employing C_6H_6-MeOH 6:1-1:1 as elution (50 ml each eluate). Repeated purification by prep. TLC of eluates 7-9 (68 mg) using aluminium-based Si GF_{254} plates (C_6H_6-MeOH 5:1, 3 developments) yielded a mixture (44 mg) of precursors of **3** and **4**. The mixture was dissolved in 2 ml of Ac_2O and 1 ml of pyridine, and then left overnight at room temperature. The solution was then evaporated under vacuum and purified by preparative TLC (C_6H_6-MeOH 8:1, 3 developments). A single band containing 26 mg of **3** and **4** was obtained. Eluates 19-21 were found to contain ligulaverin E (**5**). The combined solution was evaporated and the residue (63 mg) was subjected to three GF_{254} plates ($CHCl_3$-MeOH 10:1, 3 developments); 40 mg of **5** (a gum) was thus purified.

Ligulaverin A [12-oxa-1ß,8,9aß-trimethyl-5ß-hydroxymethyl-4α,5-ethano-7ß,10aß-epoxy-9ß-hydroxylmethylacryloyl-1,2,3,4,4a,5,6,7,9,9a,10,10a-dodecahydro-anthracen-13-on] (1). Colourless plates, mp. 198-199° (MeOH); IR ν_{max}(KBr) cm^{-1}: 3469 (OH), 2998, 2972, 2952, 2930, 1751, 1730, 1695, 1657, 1380, 1278, 1250, 1221, 1167, 1141, 1096, 966, 915, 838, 795. Found: C, 63.49; H, 6.88. $C_{23}H_{30}O_8$ calc.: C, 63.59; H, 6.91%; EIMS *m/z* (rel. int.): 434 (M^+, 3), 416 (M-H_2O^+, 17), 350 (11), 332 (29), 314 (8), 304 (7), 265 (7), 248 (40), 230 (100), 213 (40), 188 (30), 170 (32), 162 (27), 124 (51), 85 (62), 57 (14).

Ligulaverin B [12-oxa-1ß,8,9aß-trimethyl-2ß-angeloyl-5ß-hydroxymethyl-4α,5-ethano-7ß,10aß-epoxy-9ß-hydroxy-1,2,3,4,4a,5,6,7,9,9a,10,10a-dodecahydro-anthracen-13-on] (2). Colourless gum; IR ν_{max} (KBr) cm^{-1}: 3474 (OH), 2995, 2930, 1748, 1727, 1704, 1666, 1456, 1381, 1276, 1220, 1172, 1144, 1089, 980, 932, 844; Found: C, 64.24; H, 7.06. $C_{24}H_{32}O_8$ calc.: C, 64.29; H 7.14%; EIMS *m/z* (rel. int.): 448 (M^+, 4), 430 (M-H_2O^+, 10), 348 (M-HOAng$^+$, 14), 330 (15), 302 (23), 287 (5), 274 (8), 259 (10), 241 (13), 230 (28), 217 (14), 203 (16), 190 (5), 170 (53), 143 (20), 124 (47), 100 (34), 85 (65), 57 (18), 55 (24), 29 (100).

Ligulaverin C [12-oxa-1ß,8,9aß-trimethyl-5ß-acetoxymethyl-4α,5-ethano-7ß,10aß-epoxy-9ß-methylacryloyl-1,2,3,4,4a,5,6,7,9,9a,10,10a-dodecahydro-anthracen-13-on] (3) and ligulaverin D [12-oxa-1ß,8,9aß-trimethyl-5ß-acetoxymethyl-4α,5-ethano-7ß,10aß-epoxy-9ß-angeloyl-1,2,3,4,4a,5,6,7,9,9a,10,10a-dodecahydro-anthracen-13-on] (4). Colourless gum; IR ν_{max} (KBr) cm^{-1}: 3489 (OH), 2994, 2974, 2953, 2932, 2662, 1758, 1717, 1698, 1639, 1452, 1383, 1361, 1219, 1177, 1151, 1038, 989, 846, 509; EIMS *m/z* (rel. int.): [peak A, minor compound **3**]: 460 (M^+, 1), 442 (M-H_2O^+, 6), 374 (M-H_2O-68$^+$, 11), 356 (374- H_2O^+, 2), 331 (374-Ac$^+$, 12), 316 (20), 314 (356-Ac$^+$,6), 296 (13), 268 (8), 230 (12), 213 (11), 173 (4), 162 (6), 127 (28), 95 (4), 91 (7), 83 (100), 69 (38), 55 (44), 43 (90); [peak B, major compound **4**]: 474 (M^+, 0.8), 456 (M-H_2O^+, 9), 374 (M-H_2O-82$^+$, 12), 331 (12), 316 (32), 314 (30), 297 (23), 296 (28), 286 (4), 268 (7), 259 (3), 230 (23), 213 (20), 173 (5), 162 (8), 127 (28), 81 (100), 69 (38), 43 (80).

Ligulaverin E [12-oxa-1ß,8,9aß-trimethyl-8-hydroxy-5ß-hydroxymethyl-4α,5-ethano-7ß,10aß-epoxy-1,2,3,4,4a,5,6,7,8,9a,10,10a-dodecahydro-anthracene-13-on] (5). Colourless gum; IR ν_{max} (KBr) cm^{-1}: 3458 (OH), 2966, 2951, 2875, 1774, 1719, 1668, 1655, 1577, 1455, 1381, 1321, 1185, 1117, 1088, 1061, 1021, 938, 731, 512. Found: C, 65.25; H, 7.37. $C_{19}H_{26}O_6$ calc.: C, 65.14, H, 7.43%; EIMS *m/z* (rel. int.): 350 (M^+, 0.6), 332 (M-H_2O^+, 2), 317 (M-H_2O-Me$^+$, 3), 302 (38), 286 (22), 275 (5), 271 (8), 243 (8), 229 (10), 205 (10), 201 (7), 177 (11), 163 (23), 128 (19), 109 (25), 91 (42), 85 (46), 69 (40), 55 (41), 43 (100).

X-Ray structure analysis of 1: *Crystal data.* $C_{23}H_{30}O_8$, M=434.47, monoclinic, $P2_1$, a=12.179(4), b=19.598(7), c=18.769(5) Å, ß=108.16(2)°. At convergence $R1$=7.09% [based on F and 4792 unique data with $F>4\sigma(F)$]. Full details are given in reference 8.

Acknowledgements: The authors thank The Royal Society of London, The Royal Society of Edinburgh and The National Science Foundation of China for their financial support. One of the authors (Dr. Y. Zhao) also wishes to express his gratitude to the Alexander von Humboldt-Stiftung for affording an opportunity to continue researching in Mainz University. We thank Dr. R.L. Baxter, University of Edinburgh, for helpful discussions and advice and Prof. Dr. J. Stöckigt (Universität Mainz) for his suggestions and encouragement during preparation of the manuscript.

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(*Received in UK* 11 *February* 1997; *revised* 12 *March* 1997; *accepted* 13 *March* 1997)