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Guaiane dimers from *Xylopia vielana*

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Abstract

From the leaves of *Xylopia vielana* (Annonaceae) two dimeric guaianes named vielanins D and E were isolated and structurally elucidated by mass and NMR spectroscopy. Vielanin D and E consist of bridged ring systems formally representing the Diels–Alder products from the hypothetical guaiane-type monomers. Due to a hemiketal function at C-8′ both compounds occurred as epimeric mixtures.

Keywords: *Xylopia vielana*; Annonaceae; Vielanins A–E; Sesquiterpenoids; Guaiane dimers

1. Introduction

Xylopia vielana belongs to the family Annonaceae and is used in traditional medicine in Vietnam as an emmenagogue and to treat rheumatism, pain and malaria (Do, 2001). Some *Xylopia* species contain dimeric guaianes (Martins et al., 1998) or dimeric diterpenes (Vilegas et al., 1991). Dimeric guaianes which are not derived from guaianolides are rare compounds. In a previous paper we described three such dimers from *X. vielana*, vielanins A–C, which are formally constructed from two hypothetical guaiane monomers by Diels–Alder reaction (Kamperdick et al., 2001). Vielanin A contains a bridged ring system, whereas vielanins B and C can be considered as symmetric dimers with a central cyclobutane ring. In continuation of this work we now describe the isolation and structural elucidation of two guaiane dimers named vielanins D and E (**1**–**2**) from the same plant.

2. Results and discussion

Vielanins D and E (**1**–**2**) were isolated from the EtOAc extract by normal phase and reversed phase chromatography. The similarity of the NMR and mass spectra with those of the previously isolated vielanins A–C (Kamperdick et al., 2001) suggested that they were also dimeric guaianes. The NMR spectra of both compounds showed pairs of close signals due to an isomeric mixture. For vielanin D (**1**) the isomeric ratio was determined as about 87:13 by the ^{1}H integrals. The molecular formula of $C_{32}H_{42}O_7$ and the molecular weight of 538 were obtained by high resolution of the $[M+Na]^+$ peak at *m*/*z* 561.28135 in the ESI MS. The EIMS showed only a weak molecular ion at *m*/*z* 538 (0.3%) and a stronger $[M - CH_3COOH]^+$ peak (13%) at *m*/*z* 478. The most prominent peaks were found at *m*/*z* 234 and 216, which also appeared in the EIMS of vielanin A (Kamperdick et al., 2001) where they represented the [monomer **b** - ketene]$^+$ and [monomer **b** - CH_3COOH]$^+$ fragments. On the basis of these data the second monomer (**a**) was expected to have the molecular weight of 262, which was found as weak fragment peak (6%) and supported by the peaks at *m*/*z* 244 [monomer **a** - H_2O]$^+$ (28%) and 229 [monomer **a** - H_2O - CH_3]$^+$ (39%). These are the same fragments as found for vielanin C. Based on the other vielanins, the carbonyl group in the five-membered ring was expected to be at C-2′ or C-3′. In the HMBC experiment of vielanin D (**1**) no correlations from the carbon signal of the conjugated carbonyl group (δ_C 205.7) with methyl protons at H_3-15′ were found, thus locating the carbonyl group at C-2′. Based on these data the depicted structures of hypothetical monomers **a** and **b** were proposed and

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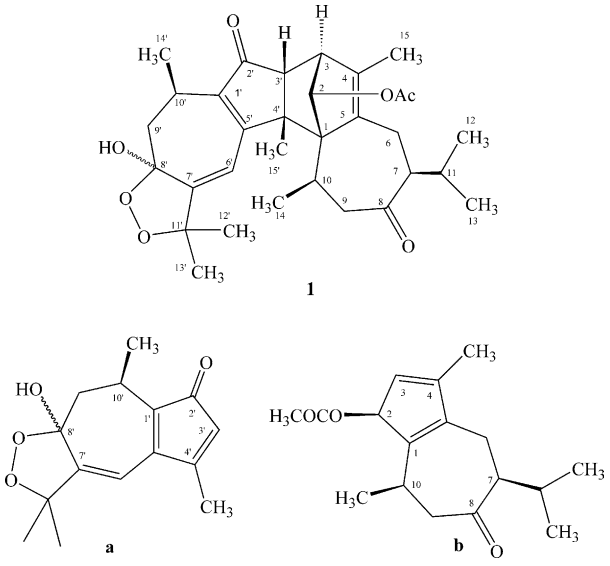
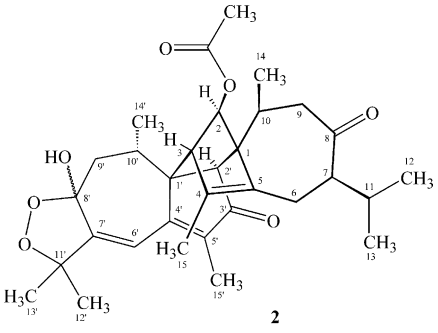
supported by the similarity of the chemical shifts with the corresponding moieties of vielanin A and C. These monomeric units then may form the Diels–Alder-like product to give the 1-3′/3-4′ or the 3-3′/1-4′ connected dimer. The strong CH long-range correlation in the HMBC experiment between C-1 (δ 65.5) and the methyl group at C-4′ (H_3-15′, δ 1.55) proposed the 3-3′/1-4′ connected dimer. The resulting constitution for vielanin D (**1**) was confirmed by full analysis of the CH long-range correlations (Table 1). Comparison of the carbon shifts of both isomers showed the largest differences at C-8′ ($\Delta\delta$ -1.8) and C-9′ ($\Delta\delta$ +6.5) which revealed the hemiacetal C-8′ as the epimerisation center.

The relative configuration of **1** was deduced by analysis of the NOE difference spectra. According to the only possible *cis*-connection between the central norbornene system and the *fused* cyclopentenone (C-1′–C-5′), strong NOE effects between H-3′ (δ 2.51) and H_3-15′ (δ 1.55) were observed. Both groups additionally gave interactions to H-2 (δ 5.01) resulting in the depicted relative configuration for the central norbornene system (Fig. 1). In this figure the central norbornene moiety lies in the background and the tips of both seven-membered rings are located at the front. Furthermore, H-10 (δ 2.31) showed NOE effects on H_3-15′ (δ 1.55) and on H-6′ (δ 5.84), whereas for the methyl group connected to C-10 (H_3-14, δ 1.22) an interaction with H-2 (δ 5.01) was observed. These facts revealed that the methyl group at C-4 is as depicted in Fig. 1 at the back (dashed bonds) and H-10 at the front side (bold bonds) of the seven-membered ring. The high $^3J_{HH}$ coupling of about 11 Hz between H-10 (δ 2.31) and H-9^B (δ 3.08) proposed the nearly *trans*-diaxial relationship of these two protons. In accordance with such a structure an NOE effect between the *cis* protons H-10 and H-9^A (δ 1.93) was found. Because the observed interaction between H-10 and H-6^A (δ 1.72) across the seven-membered ring is only possible if both protons are axial on the same side of the ring H-6^A (δ 1.72) must have axial conformation at the front of the seven-membered ring. The multiplicity of H-6^A (*br d*, J = 16.8 Hz) proposed the absence of a *trans* diaxial coupling partner and that the isopropyl group instead of H-7 was *trans* to H-6^A. This was further supported by the NOE effects from the isopropyl group to other protons located at the back, such as the effects from H_3-13 (δ 0.95) on H-6^B (δ 2.52) and H_3-15 (δ 1.60) and from H_3-12 (δ 0.80) on H-9^B (δ 3.08). These data indicated a *cis* configuration of the methyl and isopropyl substituents at C-10 and C-7. In the second seven-membered ring (C-5′–C-10′) the high coupling constants of H-9′^{A} (δ 1.67, *t*, J = 13.4 Hz) revealed the *trans*-diaxial relationship to H-10′ (δ 2.86) and the equatorial conformation of the methyl group H_3-14′ (δ 1.22). Furthermore, the α-configuration of H-10′ was proposed based on its NOE interaction with H_3-15 (δ 1.60), which had only weak intensity because of the larger distance.

The high resolution of the $[M+Na]^+$ peak of the second compound vielanin E (**2**) at m/z 561.28149 in the ESI-TOF-MS gave the same molecular formula $C_{32}H_{42}O_7$ as found for vielanin D (**1**). The ^{1}H NMR spectra also showed an epimeric mixture with the ratio of 67–68% for the major epimer determined from the ^{1}H integrals. Also the EI MS gave the same fragments with similar intensities as found for vielanin D (**1**) (see Experimental). However, the carbon shifts of vielanin E (**2**) were different from those of vielanin D (**1**), and especially one methyl group resonated only at δ 8.5, which was characteristic for C-15′ of vielanin A (Kamperdick et al., 2000). Strong CH Long-range correlations in the HMBC of **2** from the conjugated carbonyl group in the five-membered ring (δ_C 206.1) to H_3-15′ (δ_H 1.66) located this carbonyl group at C-3′ like in vielanin C (Kamperdick et al., 2000) instead of C-2′ like in vielanin D (**1**). These data suggested the same skeleton for vielanin E (**2**) and vielanin C (**3**) except for the peroxide ring and the location of the double bond at C-6′ instead



Scheme 1.



Scheme 2.

Table 1
NMR spectroscopic data of vielanin D (**1**) in $CDCl_3$ at 125/500 MHz (shifts of the minor compound in brackets, if detected)[be]

	δ_C	δ_H (*J* in Hz)	HMBC[a]	NOE[i]
1	65.52 [66.20]	–	H-3, H-6^B, H-9^A, H-9^B, H-10, H_3-14, H-3′, H_3-15′	
2	86.26 [85.92]	5.01 *s* [4.99 *s*]	H-3, H-10 (w), C$\underline{H}_3$-CO-O (w)	H-3, H_3-14, H-3′, H_3-15′
3	51.19 [51.52]	3.22 *d* (3.7) [3.22]	H_3-15, H-3′	H-2, H_3-15, H-3′
4	136.84 [136.84]	–	H-2, H-6^B, H_3-15, H-3′	
5	132.76 [ca 132.4]	–	H-2, H-3, H-6^B, H_3-15	
6	25.56 [25.18]	A 1.72 br *d* (16.8) [1.53]	–	
		B 2.52 *dd* [b] (ca 15; ca 4) [2.52]		[c] H-6^A, H-7, H_3-13, H_3-15
7	60.96 [60.96]	1.99 *m*	H-6^B, H-9^A/H-11, H_3-12, H_3-13	
8	215.58 [215.82]	–	H-6^B, H-7 (w), H-9^A/H-11, H-9^B	
9	47.86 [47.74]	A 1.93 *m*[d] [1.98]	H-10 (w), H_3-14	
		B 3.08 *t* (11.4) [3.08]		H-2 (w) H-9^A, H_3-12 (w), H_3-14 (w), C$\underline{H}_3$-CO-O (w)
10	31.98 [32.39]	2.31 *dq* (10.8; 6.6) [2.43]	H-9^A/H-11, H-9^B, H_3-14	H-2 (w), H-6^A (w), H-9^A [e], H_3-14, H-6′, H_3-15′ (w)
11	27.37 [27.59[f]]	1.93 *m* [ca.1.98]	H-7 (w), H_3-12, H_3-13	
12	21.06 [21.06]	0.80 *d* (6.4) [0.82 *d* (6.7)]	H_3-13	H-7, H-9^B, H-11, H-13, H_3-12′/13′ (w), C$\underline{H}_3$-CO-O (w)
13	20.73 [20.61]	0.95 *d* (6.4) [0.96 *d*]	H-7 (w), H_3-12	H-6^B, H-7, H-11, H-12, H_3-15, H_3-12′/13′ (w)
14	21.23 [21.23]	1.22[g] *d* (6.7) [1.22]	–	
15	14.80 [14.63]	1.60 *s* [1.59]	–	H-3, H-6^B, H_3-13, H-10′ (w)
1′	146.68 [146.72]	–	H-6′, H-9′^{B}, H-10′, H_3-14′	
2′	205.70 [205.70]	–	H-3′	
3′	55.55 [54.99]	2.51 *d* (4.3) [2.53 *d*]	H_3-15′	[c] H-2, H-3, H_3-15′
4′	53.29 [52.82]	–	H-3, H-6′, H_3-15′	
5′	162.08	–	H-3′, H-10′, H_3-15′	
6′	113.38 [112.82]	5.84 *s* [5.80 *s*]	–	H-6^A (w), H-10, H_3-12′/13′, H_3-15′
7′	162.55 [161.05[h]]	–	H-6′, H-9′^{A} (w), H-9′^{B}, H_3-12′/H_3-13′	
8′	102.94 [104.71]	–	H-6′, H-9′^{A}, H-9′^{B}, H_3-14′ (w)	
9′	39.32 [32.78]	A 1.67 *t* (13.4) [1.65]	H-10′, H_3-14′	
		B 2.13 *dd* (14.0; 6.7) [2.19]		
10′	27.88 [ca 27.4]	2.86 *dqui* (13.2; 6.6) [2.89]	H-9′^{A}, H-9′^{B}, H_3-14′	H_3-15 (w), H-9′^{B}, H_3-14′
11′	85.99 [85.61]	–	H-6′, H_3-12′/H_3-13′	
12′	27.14 [27.59[f]]	1.47 *s* [1.43]	H_3-13′	
13′	23.87 [24.71]	1.47 *s* [1.55]	H_3-12′	
14′	18.51 [18.78]	1.22[g] *d* (6.7) [1.29 *d* (7.3)]	H-9′^{A}, H-10′	
15′	23.97 [22.51]	1.55 *s* [1.51]	H-3′	H-2, H-10, H_3-14, H-3′, H-6′
CH_3-$\underline{C}$O-O	170.33 [170.33]	–	H-2 (w), C$\underline{H}_3$-CO-O	
C$\underline{H}_3$-CO-O	21.23 [21.23]	2.04 *s* [2.05]	–	

(w) = weak

[a] The HMBC correlations to H-6^B (δ 2.52) were overlapping with those to H-3′ (δ 2.51) and its isomer (δ 2.53).

[b] The multiplicity of the signal at δ 2.52 (H-6^B of the major epimer) could not be determined from the ^{1}H NMR spectrum due to overlapping with H-3′ (δ 2.51 for the major and 2.53 for the minor component). It was obtained from the response signal in the NOE difference spectrum with irradiation at H_3-15 (δ 1.60).

[c] In the NOE difference spectra, the signals at δ 2.51 (H-3′) and at δ 2.52 (H-6^B) were too close together. This, upon irradiation of H-3′ the responses of both protons were observed together and assigned according to the known constitution.

[d] The multiplicity of this proton H-9^B (δ 1.93) which overlapped with H-11 could be gained from the NOE difference spectrum with irradiation at H-10 (δ 2.31), where it appeared as broad doublet (*J* ca 12 Hz).

[e] The assignement of this NOE interaction to H-9^A and not to H-11, which both resonated at δ 1.93 was deduced from the doublet splitting of the response signal.

[f] Assignement not confirmed.

[g] The methyl groups H_3-14 and H_3-14′ appeared as two doublets at δ 1.224 and 1.226.

[h] The isomer signal at δ 161.05 may also belong to C-5′.

[i] Obtained from the NOE difference spectra.

of C-7′. This structure was supported by a good correspondence of the proton and carbon shifts of **2** with the equivalent moieties of vielanins A and C (Kamperdick et al., 2000). Finally it was confirmed by full analysis of the CH long-range correlations (Table 2). The similarity of the carbon and proton shifts of the identical moieties of vielanin E (**2**) and C (Kamperdick et al., 2000) also suggested their identical relative configuration. The cross peaks in the NOESY experiment H-2/H-2′, H-2/H-3, H-2/H-10′, H-6′/H_3-15 confirmed this proposed

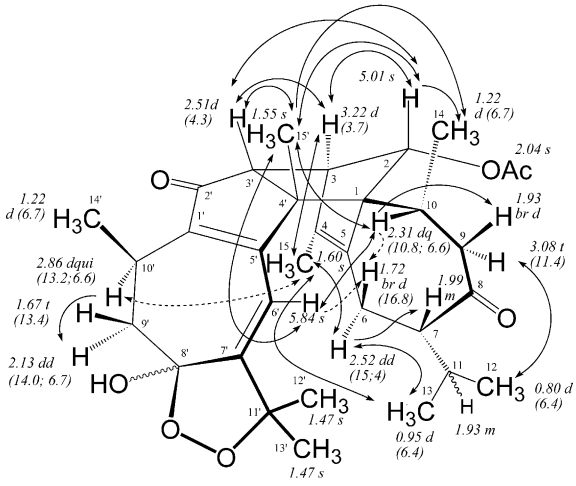


Fig. 1. Significant NOE interactions observed for the major epimer of vielanin D (**1**). Weak effects are drawn as dashed lines.

configuration for the central norbornene moiety. Furthermore, H-2 showed NOE effects on H_3-14 and no effects on H-10, which meant that the methyl group H_3-14 is β. The observed NOE interaction across the seven-membered ring between H-10 and H-7 is only possible, if both protons are axial, so that the isopropyl group must be β. This configuration was confirmed by NOE effects from the acetyl group on the methyl groups H_3-12 and H_3-13. In contrast to vielanin C, where the β-configuration of the H_3-14′ methyl group was based on the NOE effect between H-2 and H_3-14′ and the absence of an effect between H-2 and H-10′ (Kamperdick et al., 2000), in vielanin E (**2**) H-2 and H-3 showed NOE effects on H-10′ instead of effects on H_3-14′ indicating the α-configuration of this methyl group. The cross peak H-3/H-9′^{B} identified H-9′^{B} as the 9′β-proton. Accordingly H-9′α showed NOE effects to the α-methyl group H_3-14′. In the ^{1}H spectra of vielanin E (**2**) the large difference in the chemical shifts of H-3 for the two epimers, which resonated at δ 3.05 for the major and δ 3.97 for the minor epimer, was striking. The higher chemical shift of H-3 in the minor epimer is due to the deshielding effect of the hydroxyl group that is located close to H-3 and in the same plane in the case of the β-configuration of the hydroxyl group. Thus the major epimer was identified as the 8′α-hydroxy compound.

Comparison of the deduced relative configurations of vielanin D (**1**: rel. 2S, 7S, 10R, 10′R) and E (**2**: rel. 2R, 7R, 10S, 10′S) gave the identical relative configuration for both compounds, which supported the correctness of the results.

The hypothetical guaiane-type monomers, which formally function as educts of a Diels–Alder-reaction to give the dimers, are unstable, but synthons of them may be the biosynthethic precursors of the vielanins. However, no guaiane monomers were found in *n*-hexane, EtOAc or *n*-butanol extracts. This supported the view that the vielanins were real biosynthetic metabolites and not artefacts formed from monomers during extraction procedures.

Additionally, from the *n*-hexane extract, β-sitostenone (24*R*-stigmast-4-en-3-one) was isolated and identified by comparison of the ^{13}C NMR shifts with reference data from the known compound isolated from mature wheat straw (*Triticum aestivum*) (Gaspar et al., 1993).

3. Experimental

3.1. General experimental procedures

M.p. are uncorrected. Optical rotation $[\alpha]_D$: JASCO DIP 1000. UV: KONTRON UVIKON 940. FTIR: Bruker IFS 28. EIMS: AMD 402, 70 eV. ESI MS: Finnigan TSQ 700. HR-ESI MS: QStar Pulsar (Applied Biosystems). NMR: Varian Gemini 300, Unity 500, Bruker Avance 500. CC: silica gel 60, 0.06–0.2 mm (Merck) for the first column, silica gel 60, 40–63 μm (Merck) or Lichroprep RP-18, 25–40 μm (Merck) for the following columns. Prep. TLC: precoated plates, silica gel 60, F_{254}, thickness 1 mm (Merck).

3.2. Plant material

Leaves and twigs of *Xylopia vielana* (Lour.) Tan. Pierre ex Fin. & Gagn. were collected in August 1997 in Vinh Phuc, North Vietnam, and identified by Ngo Van Trai, Institute of Materia Medica, Hanoi. A voucher specimen (No. 2240) is deposited at this same Institute.

3.3. Extraction and isolation

The EtOAc extract of the dried leaves (1.54 kg) was prepared and separated to 16 fractions with increasing polarity as described earlier. Fr 6 (1.7 g, eluted with $CHCl_3$–MeOH 8:2) was separated on silica gel (160 g, 63–200 μm) with *n*-hexane–acetone (8:2) giving 149 fractions. Frs 37–40 (145 mg) were fractionated by reversed phase chromatography (RP18) using MeOH–H_2O (7:3) to yield 31 mg of vielanin D (**1**). Frs 50–59 (316 mg) were further separated by silica gel chromatography using *n*-hexane–acetone (7:3) to give 204 mg, which were purified by silica gel column chromatography with *n*-hexane–EtOAc (65:35) and yielded 23 mg vielanin E (**2**).

Dried ground leaves (0.5 kg) and dried ground twigs (1.2 kg) were extracted together in the same manner to give *n*-hexane (9 g), EtOAc (19.5 g) and *n*-butanol (60 g) extracts. The *n*-hexane extract was fractionated on silica gel eluting with a gradient of *n*-hexane and acetone to give 100 frs (100 ml each). Frs 18–20 (212 mg) were repeatedly subjected to CC using silica gel with *n*-hexane–acetone (96:4) and silica gel with *n*-hexane–EtOAc–MeOH

Table 2
NMR data of vielanin E (**2**) in $CDCl_3$ at 125/500 MHz (shifts of the minor compound in brackets, if detected)

	δ_C	δ_H (*J* in Hz)	HMBC[a]	NOE effects[b]
1	64.51 [63.98]	–	H-3, H-6^B, H-9α, H-9β, H_3-14, H-2′	
2	86.73 [85.96]	4.849 *d* (1.4) [4.892 *d* (1.4)]	H-3, H-10 (w)	H-3, H_3-14, H-2′, H-10′
3	55.40 [57.02]	3.048 *d* (1.3) [3.965 *d* (1.4)]	H_3-15, H_3-15′ (w)	H-2, H_3-15, H-9′β, H-10′
4	136.26 [136.89]		H-2, H-3 (w), H-6^A, H-6^B/H-7, H_3-15	
5	132.47 [132.32]		H-2, H-3, H-2′, H-6^A, H-6^B/H-7, H_3-15	
6	26.69 [25.42]	A: 1.83 *dm* (ca. 12) B: 2.313 *dm* (15.2; 7.0) [ca 2.38]	H_3-15	H-6^B
7	57.29 [57.99]	ca 2.39 [ca 2.34]	H-6^A, H-9β, H_3-12, H_3-13	
8	213.98 [214.35]		H-6^A (w), H-6^B/H-7, H-9α, H-9β	
9	48.15 [47.85]	α: 2.157 *dd* (13.7; 2.5) [2.143 *dd* (12.4; 1.5)] β: 2.719 *dd* (13.7; 12.5) [2.772 *t* (ca. 12.8)]	H-10 (w), H_3-14	H-9β, H-10, H_3-14 H-9α, C$\underline{H}_3$-CO-O (w)
10	28.45 [28.68]	2.86 *m*	H-9α, H-9β, H_3-14, H-2′	H-9α, H-7, H_3-14, H-2′
11	27.85 [27.78]	ca 1.96	H-6^A, H-6^B, H_3-12, H_3-13	
12	21.21 [21.28]	0.813 *d* (6.6) [0.813]	H_3-13	H-7, H-9β (w), C$\underline{H}_3$-CO-O
13	20.16 [20.18]	0.920 *d* (6.6) [0.916 *d* (6.4)]	H_3-12	H-6^A (w), H-6^B/H-7, H_3-12, H_3-15, C$\underline{H}_3$-CO-O
14	17.18 [17.33]	1.086 *d* (6.8) [1.095 *d* (6.7)]	H-9α, H-10	H-2, H-10, H-9a, H-2′
15	14.34 [13.96]	1.501 *s* [ca 1.464 *s*]	H-3 (w)	H-3, H-6^B, H-6′
1′	58.39 [57.61]		H-6′, H-2 (w), H-9′α, H_3-14′	
2′	54.32 [55.17]	2.477 *s* [2.472 *s*]	H-3	[e] H-2, H-10, H_3-14, H_3-14′
3′	206.13 [206.35]		H-2′, H_3-15′	
4′	143.01 [142.50]		H-6′, H_3-15′	
5′	161.45 [160.65]		H-2′, H-10′, H_3-15′	
6′	114.60 [115.23]	5.923 *s* [6.116 *s*]	-	H_3-15, H_3-12′, H_3-13′, H_3-15′
7′	159.41 [159.23]		H-6′, H-9′α, H-9′β[c], H_3-12′, H_3-13′,	
8′	104.73 [103.88]		H-9′α, H-9′β, H-6′, H-10′	
9′	34.27 [39.34]	α: 2.217 *dd* (14.3; 4.1) [1.881 *dd* (15.1;5.6)] β: 2.517 *dd* (14.7; 3.4) [2.70 *dd* (15.1; 6.5)]	H-10′ (w), H_3-14′	H_3-14′ (w), H-9′β H-3, H-9′α, H_3-14′
10′	34.76 [34.02]	ca. 2.48 [2.561 *br q* (6.5)]	H-3[d], H-2′, H-9′α[d], H-9′β[c], H_3-14′,	H-2, H-9′β[f], H_3-14′
11′	85.06 [85.49]		H-6′, H_3-12′, H_3-13′	
12′	28.37 [28.22]	1.475 *s* [1.504 *s*]	H_3-13′	H_3-13′
13′	24.80 [25.23]	1.554 *s* [1.522 *s*]	H_3-12′	H_3-12′
14′	17.03 [19.38]	1.155 *d* (7.2) [0.945 *d* (7.2)]	H-9′β, H-10′	H-2′, H-9′α, H-10′
15′	8.45 [8.38]	1.657 *s* [1.695 *s*]		H-6′
CH_3-C$\underline{O}$-O	170 65 [170.70]		H-2, C$\underline{H}_3$-CO-O	
C$\underline{H}_3$-CO-O	21.17 [21.17]	2.001 *s* [2.012 *s*]		H-9β, H_3-12′, H_3-13′

[a] Some correlations of close or overlapping signals were not resolved in the HMBC such as correlations to H-6^B (δ 2.32, 2.38) and H-7 (d 2.39, 2.34), correlations to H-2′ (δ 2.477) and H-10′ (δ 2.48), and correlations from C-14 (δ 17.18, 17.33) and C-14′ (δ 17.03).

[b] Obtained from the NOESY experiment, mixing time 0.45 sec.

[c] This correlation was only observed for the minor epimer.

[d] This correlation was stronger for the minor epimer.

[e] The signals of H-2′ (δ 4.477) and H-10′ (δ 2.48) were not resolved in the NOESY experiment. The assignement of the correlations to H-2′ or H-10′ was made by comparison with the NOE difference spectra.

[f] The cross peak from H-10′ (δ 2.48) to H-9′^{B} (δ 2.517), which was expected according to the rel. configuration, could not be identified for the major epimer due to overlapping. It was found with weak intensity for the minor epimer (δ 2.56 and 2.70).

(98:2:0.3). Final purification was achieved by preparative TLC using *n*-hexane–EtOAc–MeOH (4.9:0.1:0.1) two times to yield 15 mg β-sitostenone.

3.3.1. Vielanin D (1)

Amorphous, m.p. 213–215 °C (*n*_hexane–EtOAc). $[\alpha]_D^{25}$-45° ($CHCl_3$, c 0.5). UV λ_{max}^{EtOH} nm (log): 294 (3.92), 204 (4.09). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3567 (w, broad), 3360 (w, br), 2963 (m), 2936 (w), 2874 (w), 1735 (m-s), 1689 (s), 1586 (w), 1455 (w), 1375–1365 (m), 1284 (w), 1242 (s), 1045 (m). HR ESI-TOF-MS (*m*/*z*): 561.28135 $[M+Na]^+$ ($C_{32}H_{42}O_7Na$ requires 561.28227), 539.30011 $[M+H]^+$ ($C_{32}H_{43}O_7$ requires 539.30033). EIMS *m*/*z* (rel. int.): 538 $[M]^+$ (0.3), 521 [M - OH]$^+$ (1.3), 496 [M - ketene]$^+$ (0.9), 478 [M - CH_3COOH]$^+$ (13), 462 (21), 461 (15), 445 (22), 444 (18), 420 (16), 419 (10), 276 [monomer **b**]$^+$ (7), 262 [monomer **a**]$^+$ (6), 244 [monomer **a** - H_2O]$^+$ (28), 234 [monomer **b** - ketene]$^+$ (88), 229 [monomer **a** - H_2O - CH_3]$^+$ (39), 216 [monomer **b** - CH_3COOH]$^+$ (100), 174 (17), (173) (17), 159 (19), 135 (39), 108 (20), 91 (16), 69 (25), 55 (20).

3.3.2. Vielanin E (2)

Amorphous, m.p. 220-222 °C (MeOH). $[\alpha]_D^{26}+31°$ ($CHCl_3$, c 0.5). UV λ_{max}^{EtOH} nm (log): 292 (4.21), 280 (4.15), 204 (4.21). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3565 (w), 2965 (m),

2936 (w), 2874 (w), 1723 (m), 1685 (s), 1595 (w), 1457 (w), 1441 (w), 1383 (m), 1365 (m), 1247 (s), 1130 (w), 1036 (w), 958 (w). HR ESI-TOF-MS (*m*/*z*): 561.28149 $[M+Na]^+$ ($C_{32}H_{42}O_7Na$ requires 561.28227). EIMS *m*/*z* (rel. int.): 520 $[M - H_2O]^+$ (4.3), 478 $[M - CH_3COOH]^+$ (4.6), 460 $[M - CH_3COOH - H_2O]^+$ (4.6), 276 [monomer **b**]$^+$ (3.2), 262 [monomer **a**]$^+$ (2.9), 245 (20), 244 [monomer **a** - H_2O]$^+$ (26), 234 [monomer **b** - ketene]$^+$ (82), 235 (16), 233 (16), 232 (14), 231 (15), 216 [monomer **b** - CH_3COOH]$^+$ (100), 215 (23), 174 (16), 159 (9), 135 (18), 69 (12).

3.3.3. β-Sitostenone (24R-stigmast-4-en-3-one)

EIMS $[M]^+$ *m*/*z* 412. The compound was identified by comparison of the carbon shifts measured in $CDCl_3$ with reference data (Gaspar et al., 1993).

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