Monoterpene Dimers from *Lisianthius seemannii*

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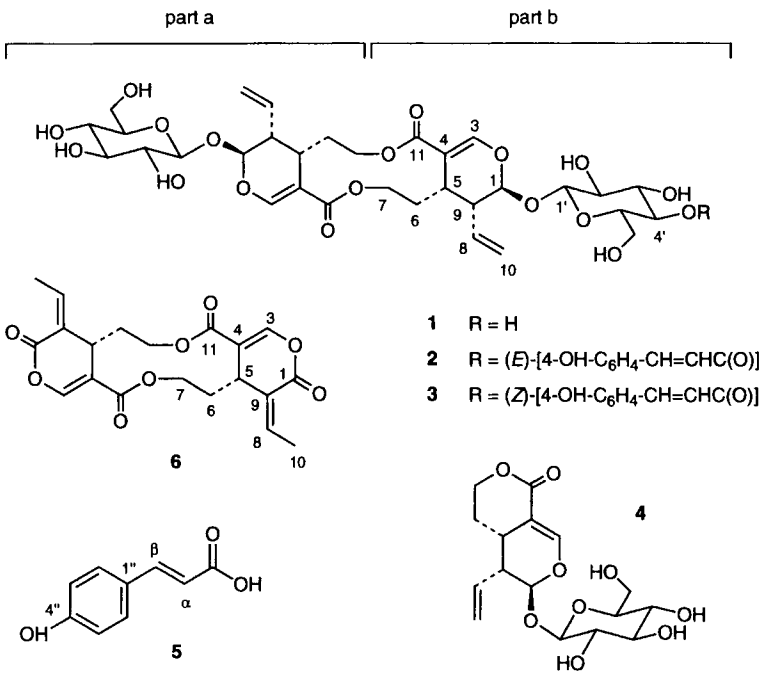
A new acylated dimeric secoiridoid glycoside, seemannoside A (**2**), has been isolated from the aerial parts of *Lisianthius seemannii* (GRISEB) O. KUNTZE (Gentianaceae). The structure was established by spectroscopic analysis (UV, MS, ^{1}H- and ^{13}C-NMR, and 2D-NMR experiments) and chemical reactions as (*E*)-4′-*O*-(*p*-coumaroyl)lisianthioside. The structure of the (*Z*)-isomer (seemannoside B, **3**), also present in the plant, was confirmed by LC/UV/^{1}H-NMR analysis. The active principle, **6**, responsible for the antifungal activity of the apolar extract against *Cladosporium cucumerinum*, has been isolated. Its structure has been established by NMR spectroscopy and X-ray crystallographic analysis as a rare type of aglycone monoterpene dimer.

Introduction. – The flora of Panama, which is amongst the richest in the world, has barely been investigated from a phytochemical viewpoint [1]. In the course of our ongoing investigations of the Gentianaceae, a Panamanian *Lisianthius* species was submitted to both biological and chemical screenings. *Lisianthius* is a small genus of the family, distributed in Central America, in the West Indies, and in the northern part of South America [2]. Less than 30 species have been identified to date, and four species can be found in Panama, namely *L. jefensis* A. ROBYNS et ELIAS, *L. peduncularis* L.O. WMS., *L. seemannii* (GRISEB) O. KUNTZE, and *L. skinneri* (HEMSL.) O. KUNTZE, none of which is used, to our knowledge, in traditional medicine [3]. However, another species, *L. chelonoides* L.f. is used in Peru to treat 'athlete's foot' (also called Hong-Kong disease), while, in Trinidad, the leaves of this species are used against worm-infested wounds of livestock. In South America, other *Lisianthius* species are used to prepare bitter tonics claimed to be febrifuges and anthelmintics [4][5]. Little is known on the chemical constituents of the genus. The only phytochemical investigation in the literature described the presence, in *L. jefensis*, of a new type of dimeric secoiridoid glucoside called lisianthioside (**1**)[1]) [6]. Antimicrobial activity of this latter species against *Staphylococcus aureus* and *Bacillus subtilis* was also reported [7], together with a strong toxicity of *L. jefensis* and *L. skinneri* in a brine shrimp assay [8][1].

In this study, both methanolic and CH_2Cl_2 extracts of *L. seemannii* (GRISEB) O. KUNTZE were investigated by liquid chromatography with photodiode array detection

[1]) For convenience, the biogenetic numbering generally used for secoiridoids is employed throughout the text and in *Tables*. The systematic names are given in the *Exper. Part*.



(LC/DAD-UV) and thermospray-mass-spectrometric detection (LC/TSP-MS). The presence, in the methanolic extract, of compounds presenting relatively high mass ions in the LC/TSP mass spectra was observed. A preliminary screening for different kinds of biological activities has also been performed revealing an antifungal activity against the phytopathogenic fungus *Cladosporium cucumerinum* of the CH_2Cl_2 extract, while this latter was inactive against *Candida albicans*. The targeted isolation of the active principle **6** was then undertaken, together with the isolation of the new acylated monoterpene dimers **2** and **3**. We report here their structure elucidation by the use of chemical reactions, 1D- and 2D-NMR analysis, including COSY, HSQC, and HMBC experiments, X-ray crystallography, and liquid chromatography coupled with NMR (LC/NMR).

Results. – Dried powdered aerial parts of *L. seemannii* were extracted at room temperature with solvents of increasing polarity (CH_2Cl_2 and MeOH), and both extracts were analyzed by LC/DAD-UV and LC/TSP-MS. In the methanolic extract, compound **1** was by far the most important constituent (*Fig. 1, c*). This compound could be easily identified as lisianthioside, a dimeric secoiridoid constituted of two sweroside (**4**) moieties, by comparison with an authentic sample. Compounds **2** and **3**, present in smaller amounts, exhibited interesting spectroscopic features. Indeed, in their LC/TSP-MS, similar ions to those of lisianthioside could be observed (*m/z* 734, 572, 393, 359, 197), together with three supplementary ions. The ion at *m/z* 880 corresponded to the molecular ammonium adduct $[M + NH_4]^+$, suggesting a molecular weight of 862 amu, while ions at *m/z* 309 and 505 corresponded to the addition of a coumaroyl part to a glucosyl

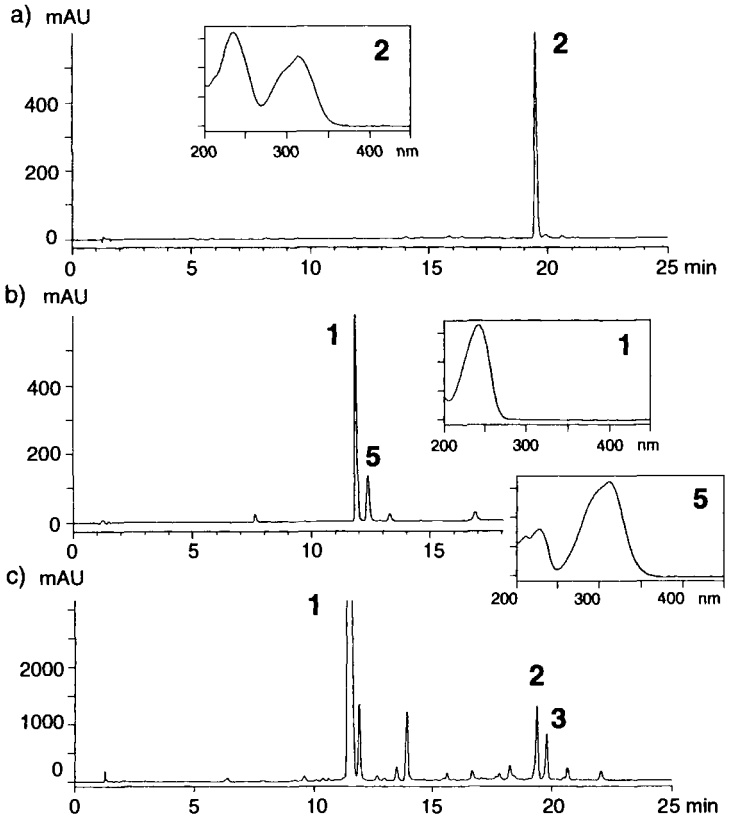


Fig. 1. *LC/DAD-UV Analysis of seemannoside A* (**2**) a) *before and* b) *after alkaline hydrolysis, indicating the presence of lisianthioside* (see **1**) *and a coumaroyl moiety* (see **5**) *in* **2**. c) *LC/DAD-UV Chromatogram of the crude MeOH extract of* L. seemannii *aerial parts*

unit and to a sweroside moiety, respectively. Moreover, their UV spectra differed from that of **1** by presenting a second absorption band (max. at 315 and 310 nm for **2** and **3**, resp.), indicating the presence of an additional aromatic group [9]. No references were found in the literature for compounds corresponding to this molecular weight. Thus, the targeted isolation of **2** and **3** was undertaken by a combination of gel filtration on *Sephadex LH-20* (MeOH), medium-pressure liquid chromatography (MPLC) on *RP-18* ($MeOH/H_2O$), and semi-preparative high performance liquid chromatography on *RP-18* ($MeCN/H_2O$). Pure **2** could be obtained, while **3** rapidly isomerized into **2**, to give a mixture of the two isomers.

Acidic (2N HCl) and enzymatic (*β*-glucosidase) hydrolyses of **2** afforded glucose, while the aglycone apparently decomposed. However, the presence of coumaric acid (**5**) in the organic phase (see *Exper. Part*) was revealed by LC/UV analysis and comparison with standards. An alkaline hydrolysis was then performed and followed by LC/UV. Two hours after the addition of NaOH (see *Exper. Part*) **2** was fully degraded, and the formation of compounds **1** and **5** was observed (*Fig. 1, a* and *b*). Thus, the alkaline hydrolysis indicated that compound **2** consisted of a lisianthioside part combined to a

Table 1. *^{1}H-NMR Data of Compounds* **1**, **2** *and* **6**. δ in ppm relative to TMS, J in Hz.

H-Atom[a])	**1**[b])	**2**[b])		**6**[c])
	Parts *a* and *b*	Part *a*	Part *b*	Parts *a* and *b*
Monoterpene moieties				
H–C(1)	5.60 (*d*, J=9.3, 2 H)	5.61[d]) (*d*, J=9.3, 1 H)	5.63[d]) (*d*, J=9.3, 1 H)	
H–C(3)	7.43 (*s*, 2 H)	7.43[e]) (*s*, 1 H)	7.44[e]) (*s*, 1 H)	7.41 (*s*, 2 H)
H–C(5)	3.08 (*ddd*, J=11.8, 6.0, 5.1, 2 H)	3.10 (*ddd*, J=11.5, 5.8, 4.5, 1 H)	3.10 (*ddd*, J=11.5, 5.8, 4.5, 1 H)	3.74 (*dd*, J=9.0, 5.8, 2 H)
H_a–C(6)	1.68 (*m*, 2 H)	1.68 (*m*, 1 H)	1.68 (*m*, 1 H)	1.77 (*m*, 2 H)
H_b–C(6)	2.09 (*m*, 2 H)	2.09 (*m*, 1 H)	2.09 (*m*, 1 H)	1.94 (*m*, 2 H)
H_a–C(7)	4.06 (*m*, 2 H)	4.07 (*m*, 1 H)	4.07 (*m*, 1 H)	3.96 (*ddd*, J=12.0, 6.1, 3.8, 2 H)
H_b–C(7)	4.06 (*m*, 2 H)	4.07 (*m*, 1 H)	4.07 (*m*, 1 H)	4.26 (*ddd*, J=12.0, 9.5, 3.0, 2 H)
H–C(8)	5.88 (*ddd*, J=17.3, 10.2, 8.8, 2 H)	5.88 (*ddd*, J=14.5, 8.5, 6.0, 1 H)	5.88 (*ddd*, J=14.5, 8.5, 6.0, 1 H)	6.53 (*q*, J=7.3, 2 H)
H–C(9)	2.64 (*ddd*, J=19.3, 8.8, 6.0, 2 H)	2.66 (*ddd*, J=9.3, 8.5, 5.8, 1 H)	2.66 (*ddd*, J=9.3, 8.5, 5.8, 1 H)	
H_a–C(10)	5.23 (*dd*, J=10.2, 1.8, 2 H)	5.27 (*m*, 1 H)	5.27 (*m*, 1 H)	2.10 (*d*, J=7.3, 6 H)
H_b–C(10)	5.39 (*dd*, J=17.3, 1.8, 2 H)	5.27 (*m*, 1 H)	5.27 (*m*, 1 H)	
Glucose moieties				
H–C(1′)	4.75 (*d*, J=7.5, 2 H)	4.74 (*d*, J=7.5, 1 H)	4.82 (*m*, 1 H)	
H–C(2′)	3.18 (*m*, 2 H)	3.19 (*m*, 1 H)	3.32 (*m*, 1 H)	
H–C(3′)	3.39 (*m*, 2 H)	3.38 (*m*, 1 H)	3.67 (*m*, 1 H)	
H–C(4′)	3.24 (*m*, 2 H)	3.24 (*m*, 1 H)	4.83 (*m*, 1 H)	
H–C(5′)	3.33 (*m*, 2 H)	3.32 (*m*, 1 H)	3.58 (*m*, 1 H)	
H_a–C(6′)	3.92 (*dd*, J=11.7, 2.0, 2 H)	3.92 (*dd*, J=11.7, 2.0, 1 H)	3.56 (*m*, 1 H)	
H_b–C(6′)	3.66 (*dd*, J=11.7, 6.5, 2 H)	3.66 (*m*, 1 H)	3.64 (*m*, 1 H)	
Acyl part				
H–C(α)			6.37 (*d*, J=15.8, 1 H)	
H–C(β)			7.67 (*d*, J=15.8, 1 H)	
H–C(2‴,6‴)			7.47 (*dd*, J=8.5, 2.0, 1 H)	
H–C(3‴,5‴)			6.81 (*dd*, J=8.5, 2.0, 1 H)	

[a]) Biogenetic numbering. [b]) In CD_3OD. [c]) In $CD_3OD/CDCl_3$ 1:1. [d]) [e]) Assignments interchangeable.

Table 2. *^{13}C-NMR Data of Compounds* **1**, **2**, **3**, *and* **6**. δ in ppm relative to TMS.

C-Atom[a])	**1**[b])	**2**[b])		**3**[b])[c])		**6**[d])
	Parts *a* and *b*	Part *a*	Part *b*	Part *a*	Part *b*	Parts *a* and *b*
Monoterpene moieties						
C(1)	97.1	97.2[e])	97.3[e])	97.2[k])	97.3[k])	160.4
C(3)	153.4	153.3[f])	153.4[f])	153.3[l])	153.4[l])	148.5
C(4)	111.6	111.8[g])	111.9[g])	111.8	111.8	114.8
C(5)	33.8	33.9	33.9	33.8	33.8	36.8
C(6)	32.2	32.2	32.2	32.2	32.2	34.8
C(7)	64.2	64.2	64.2	64.2[m])	64.4[m])	61.6
C(8)	135.9	136.0[h])	136.1[h])	136.0[n])	136.1[n])	145.3
C(9)	45.9	46.0	46.0	46.0	46.0	125.7
C(10)	119.1	119.1[i])	119.2[i])	119.1	119.1	15.7
C(11)	169.1	169.2	169.2	169.1	169.1	164.7
Glucose moieties						
C(1′)	100.3	100.4	100.4	100.4	100.4	
C(2′)	74.6	74.8[j])	74.7[j])	74.7[o])	74.8[o])	
C(3′)	77.6	78.0	75.7	78.0	78.0	
C(4′)	71.6	71.7	72.5	71.7	72.4	
C(5′)	78.4	78.5	76.7	78.5	76.7	
C(6′)	62.8	62.9	62.6	62.9	62.6	
Acyl parts						
C=O			168.5		167.3	
C(α)			114.7		116.0	
C(β)			147.3		146.2	
C(1″)			127.1		127.5	
C(2″)			131.3		134.0	
C(3″)			116.9		115.8	
C(4″)			161.5		160.3	

[a]) Biogenetic numbering. [b]) In CD_3OD. [c]) Measured in a mixture of **2** and **3**. [d]) In $CD_3OD/CDCl_3$ 1:1. [e])–[o]) Assignments interchangeable.

coumaroyl moiety by an ester linkage. The molecular weight of 862 amu was confirmed by the presence of a strong deprotonated molecular ion $[M - H]^-$ at *m/z* 861 in the FAB mass spectrum (negative-ion mode) and by an ammonium adduct ion $[M + NH_4]^+$ at *m/z* 880 in the DCI mass spectrum (positive-ion mode). These MS data, together with the ^{1}H- and ^{13}C-NMR data measured for **2** (*Tables 1* and *2*) corroborated the presence of a coumaroyl moiety and a lisianthioside unit in **2**.

The ^{1}H-NMR spectrum of **2** displayed all the resonances attributable to a bis-monoterpenic skeleton, as found for lisianthioside, but exhibited some differences for the signals of the glycosidic part of the molecule (*Table 1*). Moreover, additional signals, recorded in the region of aromatic protons, confirmed the presence of a cinnamic-acid derivative. In the ^{1}H-NMR spectrum, the four aromatic protons at 6.81 (*dd*, $J = 8.5, 2.0$, 2 H) and 7.47 ppm (*dd*, $J = 8.5, 2.0$, 2 H), and the two (*E*)-olefinic protons at 6.37 (*d*, $J = 15.8$, 1 H) and 7.67 ppm (*d*, $J = 15.8$, 1 H) of a *p*-coumaroyl unit were clearly

discernible. In the sugar region, two distinct anomeric protons could be observed at 4.74 (*d*, $J = 7.5$, 1 H) and 4.82 ppm (*m*, 1 H), while the other chemical shifts did not correspond to two identical glucosyl moieties, suggesting an esterification of the acyl group on one of the glycosidic moieties. This was confirmed by the broad-band decoupled ^{13}C-NMR spectrum and DEPT spectra (*Table 2*), which showed six signals attributable to a non-substituted β-D-glucopyranosyl unit and six resonances corresponding to a β-D-glucopyranosyl moiety esterified at C(4′). For this latter indeed, a deshielding of C(4′) by 0.8 ppm was observed when compared with a non-substituted glucose, while signals corresponding to C(3′) and C(5′) were shifted upfield by 2.3 and 1.8 ppm, respectively. These data, in agreement with those reported in [10], demonstrated, therefore, that C(4′) of one glucosyl was esterified by the *p*-coumaric acid. This esterification was also particularly evident on the HSQC spectrum, where a cross-peak revealed the deshielding of H–C(4′) at 4.83 ppm (hindered in the 1D ^{1}H-NMR spectrum by the solvent signal).

Different chemical shifts were observed in the monoterpenic parts *a* and *b* of the molecule for H–C(1) and H–C(3) in the ^{1}H-NMR spectrum, as well as for C(1), C(3), C(4), C(8), and C(10) in the ^{13}C-NMR spectrum, indicating that the bis-lactone ring of **2** was not symmetrical, contrary to lisianthioside [6]. Nevertheless, both compounds **1** and **2** presented a characteristic *trans*-diaxial relationship of α-configured H–C(1) and β-configured H–C(9) ($J(1,9) = 9.3$ Hz), as reported for lisianthioside. Moreover, as similar couplings were measured for H–C(5) and H–C(9) in **1** and **2**, a β-configuration could be assumed for H–C(9). The relative configuration of the monoterpenic part is then in accord with those described for all secoiridoids isolated to date. Consequently, compound **2** was identified as (*E*)-4′-*O*-(*p*-coumaroyl)lisianthioside, called seemannoside A.

As mentioned above, compound **3** could not be obtained pure due to its rapid conversion to **2**. To obtain the ^{1}H-NMR data of pure **3**, an LC/UV/^{1}H-NMR analysis of a fraction containing both isomers was performed. Indeed, this hyphenated technique is a method of choice for obtaining on-line 1D ^{1}H-NMR spectra of compounds present in complex mixtures [11]. For this analysis, the same LC conditions as for LC/UV/MS were used, except that H_2O was replaced by D_2O. The WET solvent suppression sequence, consisting of a combination of laminar-shifted shaped selective pulses associated with dephasing gradients and selective ^{13}C decoupling [12], was run before each acquisition, in order to remove the residual HOD signal and the resonances of MeCN and its two ^{13}C satellites. For both **2** and **3**, stop-flow LC/^{1}H-NMR spectra were recorded. To obtain a signal-to-noise ratio around 10 for the aromatic protons, 1312 scans were needed for **2** and 1660 scans for **3** (*Fig. 2*).

The LC/^{1}H-NMR spectrum of **2** was in total accord with the one obtained by the conventional method, although slight differences could be noticed for the chemical-shift values due to the solvent (MeCN/D_2O for on-line measurements). The LC/^{1}H-NMR spectrum recorded for **3** was very similar to that measured for **2**, as far as the monoterpenic and glycosidic parts of the molecule are concerned. However, the resonances corresponding to the acyl moieties (5–8 ppm) reflected the structural differences of the two isomers **2** and **3**, as shown in *Fig. 2*. Indeed, olefinic protons at 6.47 (*d*, $J = 16.1$, H–C(α)) and 7.78 ppm (*d*, $J = 16.1$, H–C(β)) in **2** were shifted upfield to 5.95 (*d*, $J = 12.2$, H–C(α)) and 7.11 ppm (*d*, $J = 12.2$, H–C(β)), respectively, in **3**. These

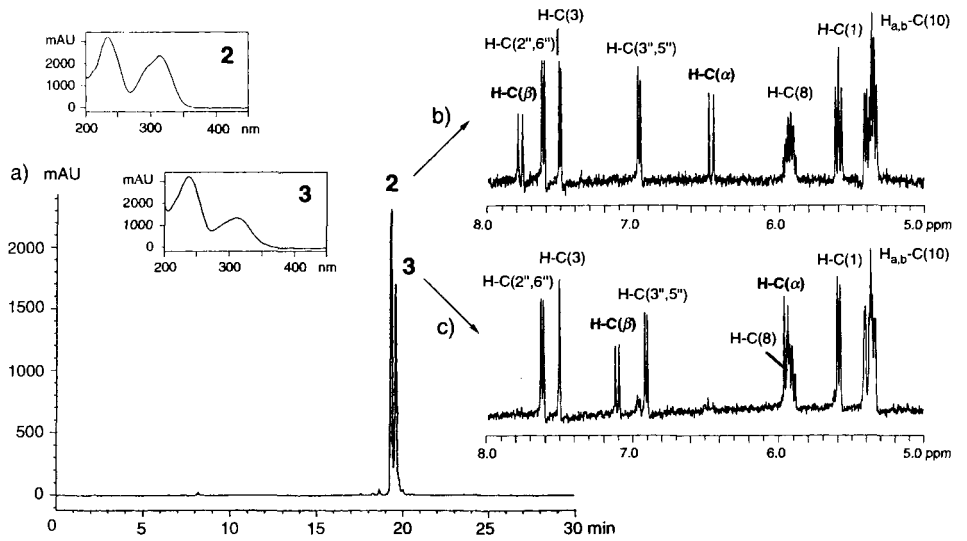


Fig. 2. a) *LC/DAD-UV Chromatogram of a mixture of both compounds* **2** *and* **3** *and stop-flow LC/^{1}H-NMR spectra* (region of aromatic and olefinic protons) *obtained on-line for the* b) (E)- *and* c) (Z)-isomers

latter chemical-shift values, together with the values of the coupling constants were characteristic of (*Z*)-olefinic protons. For both compounds **2** and **3**, four aromatic protons, forming an *AA'XX'* system, were clearly discernible and corresponded to the phenyl part of a *p*-coumaroyl unit [13]. The presence of a (*Z*)-*p*-coumaroyl moiety in **3** was thus established, and **3** was finally identified as (*Z*)-4'-*O*-(*p*-coumaroyl)-lisianthioside, called seemannoside B.

The antifungal activity of the CH_2Cl_2 extract of the aerial parts of *L. seemannii*, revcaled by bioautographic TLC assay with *C. cucumerinum* [14], was linked to a strong UV active spot (R_f 0.52 (SiO_2; petroleum ether/AcOEt 1:1)). On consideration of the LC/UV chromatogram (recorded at 254 nm), compound **6** appeared to be the major constituent of this latter extract and was consequently assumed to be the active principle. The LC/TSP mass spectra of **6** exhibited an intense ion at *m/z* 406 $[M + NH_4]^+$, suggesting a molecular weight of 388 amu. This result, together with the UV spectral data (maxima at 220 and 252 (sh) nm) did not correspond to any compound reported to date in the family. The desired isolation of **6** was thus undertaken and was performed by a combination of open-column chromatography on silica gel (step gradient elution, petroleum ether/AcOEt) and gel filtration on *Sephadex LH-20* ($CHCl_3$/MeOH 1:1).

The broad-band decoupled ^{13}C-NMR spectrum and DEPT spectra of **6** showed only ten distinct resonances (*Table 2*): two ester C=O groups (160.4 and 164.7 ppm), two ternary olefinic C-atoms (148.5 and 145.3 ppm), two quaternary olefinic C-atoms (114.8 and 125.7 ppm), one CH_2O group (61.6 ppm), one sp^3-CH group (36.8 ppm), one sp^3-CH_2 group (34.8 ppm), and a Me group (15.7 ppm). Further MS data (EI and DCI) suggested **6** to be a symmetrical dimer with the molecular formula $C_{20}H_{20}O_8$. This hypothesis was confirmed by the ^{1}H-NMR spectrum, which exhibited signals corresponding to ten distinct H-atoms (for each monomer), amongst which resonances at 2.10

(*d*, $J = 7.3$, 6 H) and 6.53 ppm (*q*, $J = 7.3$, 2 H), showed clearly the presence of an ethylidene side chain (*Table 1*). The structure of **6**, together with the full assignment of the C-atoms, was then established by further 2D-NMR experiments (HSQC, HMBC).

It was clearly visible that the structure of **6** derived from lisianthioside, containing a similar twelve-membered bis-lactone ring. Nevertheless, the structure of the monomer differed from sweroside by the presence of an ethylidene side chain at C(9) (instead of a vinyl side chain) and by the oxidation of the acetal function at C(1) into a lactone. Crystals obtained from $CHCl_3$/MeOH were subjected to X-ray analysis (*Fig. 3*). The structure of **6** was thus confirmed, and the relative configuration at C(5) was determined. This compound has already been reported in [15] under the name *longi*. Nevertheless, the latter, which was not described as a natural product, had not been fully characterized, but the crystallographic data reported (unit cell parameters, space group) are in accord with those obtained for **6**. In the TLC bioautographic assay, the minimal amount inhibiting growth of *C. cucumerinum* was 0.75 μm (propiconazole: 0.1 μg). The antifungal activity of **6** was also determined by dilution assays using solid media [16]. A minimum inhibition concentration (MIC) value of 100 μg ml^{-1} was measured (propiconazole: 1 μg ml^{-1}).

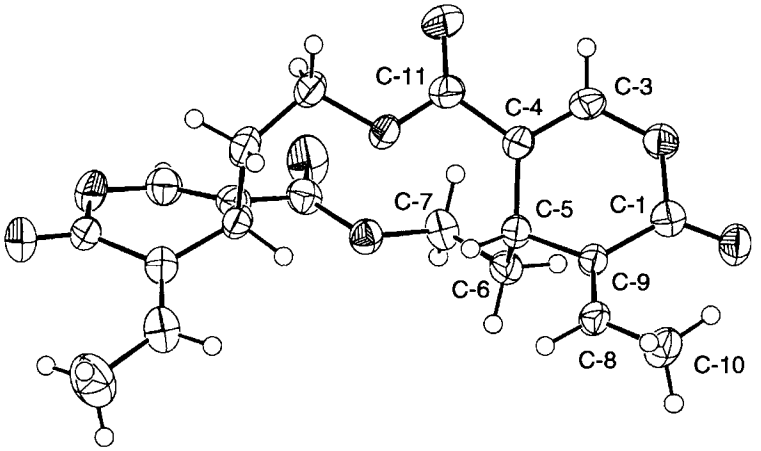


Fig. 3. *A perspective view of molecule* **6** (thermal ellipsoids at 50% probability level). Biogenetic numbering.

Discussion. – Chemical screening by LC/UV/MS has shown its efficacy for the detection and isolation of new natural products. The technique also led to the detection of a hitherto unknown, acylated bis-secoiridoid dimer. LC/NMR is still a recent hyphenated technique in the phytochemical field, but appears to be a tool of choice for obtaining 1D ^{1}H-NMR spectra of compounds which cannot be isolated as pure substances. In the case of *L. seemannii*, the presence of both (*E*)- and (*Z*)-isomers was demonstrated from on-line data generated by LC/NMR analysis of a fraction enriched in compounds of interest. These metabolites encountered in the genus *Lisianthius* constitute a class of substances which has no equivalent in any other family. Indeed, true bis-secoiridoids are extremely rare, and the only similar compound is centauroside, isolated from *Erythrea centaurium* (Gentianaceae) [17]. Other dimeric, trimeric, and even branched

tetrameric iridoid glycosides have been reported [18–20], but none of them possesses a similar twelve-membered bis-lactone ring. Although lisianthioside, and seemannosides A and B did not show any activity against our biological targets, these structures may have activities in other bioassays. Indeed, macrocyclic lactones are the skeletons of numerous pharmacologically useful substances. It is also interesting to mention that all of the three dimers were tasteless, while the crude MeOH extract of *L. seemannii* was relatively bitter. Other constituents must be responsible for this property.

The antifungal activity of **6** was particularly interesting for two reasons, although the MIC value against *C. cucumerinum* was much higher than the one exhibited by propiconazole. On one hand, the activity of **6** was selective, as no effect has been observed against the human pathogenic fungus *Candida albicans*. On the other hand, a parallel can be drawn between the antifungal activity of apolar extracts and the use of some *Lisianthius* species in traditional medicine to treat athlete's foot, an infection due to dermatophytes of the genus *Tricophyton*. The next step will be to verify the presence of antifungal compounds in *L. chelonoides* and investigate whether **6** is active against *Tricophyton*.

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Experimental Part

General. M.p.: *Mettler-PF-80/82* hot-stage apparatus; uncorrected. α_D: *Perkin-Elmer-241* polarimeter; $[\alpha]_D^{20}$ (*c* in g sample in 100 ml solvent, solvent used). TLC: Silica gel *60* F_{254} sheets (*Merck*); detection at 254 and 366 nm and with *Godin* reagent [21]. Column chromatography (CC): silica gel *60* (63–200 μm, 600 × 35 mm i.d., *Merck*) and *Sephadex LH-20* (*Pharmacia*, 650 × 50 mm i.d.). Medium-pressure liquid chromatography (MPLC): home-packed *LiChroprep RP-18* column (15–25 μm, 460 × 36 mm i.d., *Merck*). Semi-prep. HPLC: *SymmetryPrep RP-18* column (7 μm, 150 × 19 i.d., *Waters*). ^{1}H- and ^{13}C-NMR spectra: *Varian UNITY INOVA 500* at 499.87 and 125.70 MHz, resp.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz; complete attribution by 2D experiments (COSY, HSQC, HMBC). MS: *Finnigan MAT TSQ 700* triple quadrupole instrument; *m/z* (rel. intensity in %); IE, ionization energy 70 eV; DCI (NH_3), positive-ion mode; FAB with *70-BioProbe* accessory (*Finnigan MAT*), source temp. r.t., probe tip 50°, FAB gun 4 kV and 1.2 mA, Xe gas used for bombardment, negative-ion mode.

LC/UV Analysis. Reversed-phase HPLC of the crude extracts: *Waters 600-MS* solvent-delivery system and on-line UV *Hewlett-Packard-1050* series photodiode array detector; *NovaPak RP-18* column (4 μm, 150 × 3.9 mm i.d., *Waters*), MeCN/H_2O gradient 5:95 → 65:35 containing 0.05% CF_3COOH in 50 min, flow rate 1 ml/min, detection at 254 nm, recording of UV spectra between 190 and 600 nm.

LC/MS Analysis. For LC/TSP-MS analyses, and aq. buffer of 0.5M of NH_4OAc was added post-column (0.2 ml/min) to induce ionizaton (*Waters 590-MS pump*). A *Thermospray 2* (*Finnigan MAT*) interface was used with the following conditions: source temp. 280°, vaporizer 100°, aerosol 280–360°, electron multiplier voltage 1800 V, dynode 15 kV, filament off, positive-ion mode. MS detection with a *Finnigan MAT TSQ 700* triple quadrupole instrument, spectra (150–900 amu) recording every 2 s.

LC/NMR Analysis. A *Varian UNITY INOVA 500* instrument equipped with a $^1H\{^{13}C\}$ pulse field gradient indirect detection microflow LC/NMR probe (60 μl, 3 mm i.d.) was used. Reversed-phase HPLC of the fraction containing compounds **2** and **3** was carried out on a *Varian* modular HPLC system, comprising a *Varian 9012* pump, a *Valco* injection valve, and a *Varian 9050* UV detector.

Plant Material. Whole plants of *Lisianthius seemannii* (GRISEB) O. KUNTZE were collected in 1994 in the vicinity of the Panama Channel and were authenticated by Prof. *Mireya D. Correa* (Smithsonian Tropical Research Institute, Panama). A voucher specimen has been deposited at the Herbarium of the University of Panama.

Extraction and Isolation. 205 g of dry powdered aerial parts of *L. seemannii* were extracted successively at r.t. with CH_2Cl_2 (3 × 1000 ml) and MeOH (3 × 1000 ml), and afforded 4.2 g (3.5%) and 36.5 g (30.4%) of extracts, resp. A portion (15 g) of the MeOH extract was fractionated by gel filtration on *Sephadex LH-20* with MeOH. Ten fractions were collected (1–10). *Fr. 4* afforded 1.5 g of **1** after purification on *Sephadex LH-20* with MeOH. A portion of *Fr. 6* (500 mg) was subjected to MPLC (*RP-18*; MeOH/H_2O 45:55, flow rate 3 ml/min) and yielded

26 mg of **2**/**3**. Purification was then achieved by semi-prep. HPLC (*RP-18*; MeCN/H_2O 27:73, flow rate 6 ml/min) affording 18 mg of **2**, while **3** could not be isolated in pure form. A portion (2 g) of the CH_2Cl_2 extract was subjected to CC (silica gel, step gradient elution, petroleum ether/AcOEt 3:1 → 1:1). The total elution volume was 3.5 l at a flow rate of 4.5 ml/min. Fractions showing antifungal activity were collected together. After purification on *Sephadex LH-20* (MeOH/$CHCl_3$ 1:1), 60 mg of **6** were obtainced.

Acid Hydrolysis of **2**. The sample (2 mg) was refluxed in 1N HCl (10 ml) for 4 h. The mixture was extracted with AcOEt, the org. layer analyzed by TLC (SiO_2; $CHCl_3$/MeOH 7:3) and HPLC (*RP-18*; MeCN/H_2O 5:95 → 65:35 in 50 min) and the aq. phase adjusted to pH 7 with $NaHCO_3$. After freeze drying, the residue was extracted with pyridine and analyzed for sugar by TLC (SiO_2; AcOEt/H_2O/MeOH/AcOH 13:3:3:4, detection with *p*-anisidine phthalate) [22].

Alkaline Hydrolysis of **2**. The sample (1 mg) was dissolved in 0.5 ml of MeOH with a drop of 1N NaOH and stirred at 40° for 2 h. HPLC (same system as above, injection without purification) of the mixture afforded **1** and **5**.

(3R,4S*,4aR*,11R*,12S*,12aR*)-4,12-Diethenyl-3,11-bis[(β-D-glucopyranosyl)oxy]-4,4a,5,6,12,12a,13,14-octahydro-3H,8H,11H,16H-dipyrano[3,4-c:3',4'-i][1,7]dioxacyclododecin-8,16-dione 3''-[(E)-3-(4-Hydroxyphenyl)prop-2-enoate]* (= *(E)-4''-O-(p-Coumaroyl)lisianthioside* [1]) = *Seemannoside A*; **2**). White powder. M.p. 134–136°. $[\alpha]_D^{20} = -192$ ($c = 0.2$, MeOH). TLC (SiO_2, $CHCl_3$/MeOH/H_2O 65:35:5): R_f 0.70, *Godin* reagent: purple coloration. HPLC (*Novapak RP-18*; MeCN/H_2O 5:95 → 65:35): t_R 19.4. UV (MeOH): 236, 290 (sh), 315. ^{1}H- and ^{13}C-NMR (CD_3OD): *Tables 1* and *2*, resp. FAB-MS: 861 ($[M - H]^-$). DCI-MS: 880 ($[M + NH_4]^+$), 863 ($[M + H]^+$), 734 ($[M + NH_4 - 146]^+$), 717 ($[M + H - 146]^+$), 572, 505 ($[M + H - 196 - 162]^+$), 410, 359, 309 ($[M + H - 196 - 196 - 162]^+$), 197.

(3R,4S*,4aR*,11R*,12S*,12aR*)-4,12-Diethenyl-3,11-bis[(β-D-glucopyranosyl)oxy]-4,4a,5,6,12,12a,13,14-octahydro-3H,8H,11H,16H-dipyrano[3,4-c:3',4'-i][1,7]dioxacyclododecin-8,16-dione 3''-[(Z)-3-(4-Hydroxyphenyl)prop-2-enoate]* (= *(Z)-4''-O-(p-Coumaroyl)lisianthioside* [1]) = *Seemannoside B*; **3**). TLC (SiO_2; $CHCl_3$/MeOH/H_2O 65:35:5): R_f 0.70, *Godin* reagent: purple coloration. HPLC (*Novapak RP-18*; MeCN/H_2O 5:95 → 65:35): t_R 19.8. UV (MeOH): 236, 290 (sh), 310. LC/^{1}H-NMR (D_2O/MeCN *ca.* 70:30[2]): 5.81 (*d*, $J = 9.7$, 2 H–C(1)); 7.50 (*s*, 2 H–C(3)); 3.14 (*m*, 2 H–C(5)); 1.73 (*m*, 2 H_a–C(6)); 4.10 (*m*, 4 H–C(7)); 5.92 (*m*, 2 H–C(8)); 2.69 (*m*, 2 H–C(9)); 5.33–5.41 (*m*, 4 H–C(10)); 4.84 (*d*, $J = 8.8$, H–C(1')*; 4.88 (*d*, $J = 8.8$, H–C(1'')*; 3.14–3.91 (*m*, 12 H, glucose moieties); 5.95 (*d*, $J = 12.2$, H–C(α)); 7.11 (*d*, $J = 12.2$, H–C(β)); 7.61 (*d*, $J = 8.3$, H–C(2''',6''')); 6.91 (*d*, $J = 8.3$, H–C(3''',5''')). ^{13}C-NMR (CD_3OD): *Table 2*. TSP-MS: 880 ($[M + NH_4]^+$), 863 ($[M + H]^+$, weak), 734 ($[M + NH_4 - 146]^+$), 572, 505 ($[M + H - 146 - 162]^+$), 393, 359, 309 ($[M + H - 554]^+$), 197.

(4Z,4aR,12Z,12aR*)-4,12-Diethylidene-4,4a,5,6,12,12a,13,14-octahydro-3H,8H,11H,16H-dipyrano[3,4-c:3',4'-i][1,7]dioxacyclododecin-3,8,11,16-tetrone* (**6**). Colorless crystals from $CHCl_3$/MeOH. M.p. 207–208°. $[\alpha]_D^{20} = -157$ ($c = 0.2$, MeOH). TLC (SiO_2; petroleum ether/AcOEt 1:1): R_f 0.52, *Godin* reagent: purple coloration. HPLC (*Novapak RP-18*; MeCN/H_2O 5:95 → 65:35): R_f 36.3 min. UV (MeOH): 220, 252 (sh). ^{1}H- and ^{13}C-NMR (CD_3OD/$CDCl_3$): *Tables 1* and *2*, resp. TSP-MS: 406 ($[M + NH_4]^+$). DCI-MS: 406 ($[M + NH_4]^+$), 298. EI-MS: 388 (44, $M^{+\cdot}$), numerous peaks under *m/z* 200.

Crystallographic Data for **6**. $C_{20}H_{20}O_8$, orthorhombic space group $P2_12_12_1$, $a = 9.877(12)$, $b = 12.393(2)$, $c = 15.239(3)$ Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$. $V = 1865.3(5)$ Å^3, $Z = 4$, 1986 reflections measured, 1986 independent reflections, 1610 observed reflections [$I > 2\sigma(I)$], final $R_1 = 0.0501$, $Rw_2 = 0.0921$ mm^{-1}, goodness of fit 1.210, residual density max./min. 0.239/ – 0.175 eÅ^{-3}. Absorption coefficient $\mu = 0.067$ mm^{-1}; no correction for absorption was applied.

Suitable crystals of **6** were grown from $CHCl_3$/MeOH as colorless blocks. The crystal analyzed had the following dimensions 0.76 × 0.32 × 0.23 mm and ϱ of 1.382 g · cm^{-3}. Intensity data were collected at 223 K on a *Stoe AED2* four-circle diffractometer using MoK_α graphite monochromated radiation ($\lambda = 0.71073$ Å) with $\omega/2\Theta$ scans in the Θ range 2.12–25.49°. The structure was solved by direct methods using the programme SHELXS-86 [23]. The refinement and all further calculations were carried out using SHELXS-93 [24]. All of the H-atoms were included in calculated positions and allowed to ride on the corresponding C-atom. The non-H-atoms were refined anisotropically, using weighted full-matrix least-squared on F^2.

The bond lengths and angles were normal within experimental error. No attempt was made to determine the absolute configuration of the molecule. Full tables of atomic parameters and bond lengths and angles may be obtained from the *Cambridge Crystallographic Data Center*, 1 Union Road, Cambridge CB2 1EZ, UK, on quoting the full journal citation.

[2]) *: Assignments interchangeable.

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