167. A Novel Type of Dimeric Secoiridoid Glycoside from *Lisianthius jefensis* ROBYNS *et* ELIAS

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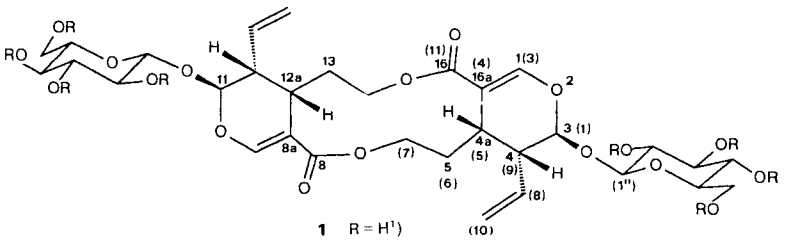
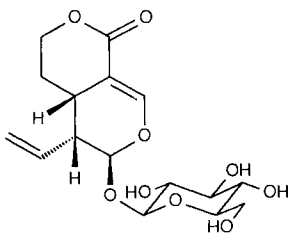
A dimeric secoiridoid glucoside named lisianthioside (**1**) has been isolated from the roots of *Lisianthius jefensis* ROBYNS *et* ELIAS (Gentianaceae). The structure was established by spectroscopic analysis (UV, IR, ^{1}H- and ^{13}C-NMR, DCI-MS) of glycoside **1**, peracetate **1a**, aglycone **3** and the dimethoxy derivatives **4a–c**. Lisianthioside (**1**) is the first representative of a novel type of dimeric secoiridoids. Biogenetically derived from two sweroside (**2**) moieties, the tricyclic aglycone features a symmetrical 12-membered bislactone ring. In contrast to the moderately bitter sweroside (**2**), lisianthioside (**1**) is tasteless.

Introduction. – The flora of the tropical forests in Central America has barely been investigated from a phytochemical standpoint. As a part of our ongoing investigations of medicinally useful plants from Panama [1], we have studied *Lisianthius jefensis* ROBYNS *et* ELIAS (Gentianaceae). The genus *Lisianthius* is geographically restricted to tropical America, and *L. jefensis* ist an endemic species of Panama, found so far only in the hills of Cierro Jefe, Province of Panama [2]. It is a herb or shrub up to 2.5 m tall, growing at the edge of the tropical forest. Some species of the genus, such as *L. chelonoides*, are used in the traditional medicine of Central and South America for the treatment of athlete's foot and worm-infested wounds of livestock [3] [4]. However, no phytochemical investigations of the genus *Lisianthius* has been reported so far. In a study aimed to detect antimicrobial activity in Panamanian plants, ethanolic extracts from the stems and roots of *L. jefensis* displayed antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* when tested at 50 mg/ml [5]. The same extracts were also moderately active in the brine shrimp assay [6], with a LC_{50} of 300 ppm. We wish to report here on the isolation and structure determination of lisianthioside (**1**), a novel type of dimeric secoiridoid glycosides.

Results. – The roots were successively extracted with $CHCl_3$ and MeOH. TLC analysis of the MeOH extract suggested the presence of secoiridoid glycosides. The major compound, named lisianthioside (**1**), was subsequently isolated by liquid-solid chromatography on *Sephadex LH 20* and silica gel. Acid hydrolysis afforded glucose, while the aglycone apparently decomposed. The ^{13}C- and ^{1}H-NMR spectra of **1** (*Tables 1* and *2*) confirmed the presence of a β-D-glucosyl moiety and were indicative of a secoiridoid, the ^{13}C-multiplicities (from DEPT spectra) suggesting a close relationship to sweroside [7] (**2**), a well-known secoiridoid of the Gentianaceae. However, noticeable differences in ^{1}H- and



1 R = H[1])
1a R = Ac



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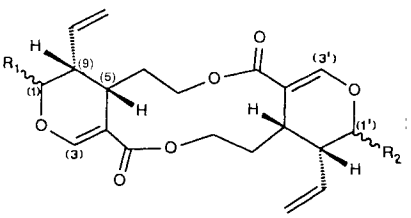

3 $R^1 = R^2 = OH$[1])
4a $R^1 = R^2 = \beta$-MeO
b $R^1 = R^2 = \alpha$-MeO
c $R^1 = \beta$-MeO, $R^2 = \alpha$-MeO

Table 1. *^{13}C-NMR Data of Compounds* **1**, **1a**, *and* **4a**–**c**. δ in ppm.

C-Atom[a])	**1**[b])	**1a**[c])	**4a**[c])	**4b**[c])	**4c**[c])	
C(1)	95.5	96.9[d])	100.9	101.6	100.9[e])	101.8[f])
C(3)	161.7	151.3	152.4	150.6	152.1	150.9
C(4)	110.1	111.3	110.2	111.5	110.6	111.4
C(5)	32.2	31.2	32.2	29.9	32.3	29.9
C(6)	30.8	31.1	31.8	29.0	32.0	29.1
C(7)	62.5	62.9	62.9	64.1	63.0	64.0
C(8)	135.0	133.0	133.8	134.6	133.8	134.6
C(9)	43.8	44.2	45.1	43.9	45.2	44.0
C(10)	118.5	119.8	119.3	118.9	119.3	119.1
C(11)	166.5	166.9	167.2	167.6	167.3	167.6
C(1″)	99.1	96.1[d])				
C(2″)	73.0	70.7				
C(3″)	76.7	72.0				
C(4″)	70.1	68.2				
C(5″)	77.4	72.6				
C(6″)	61.3	61.6				
CH_3COO		170.5, 170.2, 169.4, 169.1				
CH_3COO		20.7, 20.6 (3 ×)				
CH_3O			56.8	56.7	56.9	56.7

[a]) 'Biogenetic' numbering[1]).
[b]) In (D_6)DMSO.
[c]) In $CDCl_3$.
[d]) Assignments interchangeable.
[e]) C(1) to C(11).
[f]) C(1′) to C(11′).

[1]) The 'biogenetic' numbering (indicated in parentheses in the structural formula) generally used for secoiridoids has been employed throughout the text and *Tables* for reasons of convenience.

Table 2. *^{1}H-NMR Data of Compounds* **1** *and* **1a**. δ in ppm rel. to TMS (= 0 ppm), J in Hz.

H-Atom[a])	**1**[b])	**1a**[c])
H–C(1)	5.52 (*d*, J = 9.3)	5.25 (unres.)
H–C(3)	7.39 (*s*)	7.39 (*s*)
H–C(5)	3.00 (*m*)	3.04 (*ddd*, J = 6.0, 5.5, 4.5)
2 H–C(6)	1.60 (*m*), 1.97 (*m*)	1.68 (*m*), 1.80 (*m*)
H_a–C(7)	3.97 (*m*)	4.16 (unres.)
H_b–C(7)		4.03 (br. *dd*, J = 9.5, 9.5)
H–C(8)	5.81 (*ddd*, J = 18.0, 10.3, 8.0)	5.69 (*ddd*, J = 18.0, 10.2, 8.2)
H–C(9)	2.58 (*ddd*, J = 9.3, 8.0, 5.0)	2.59 (*ddd*, J = 8.5, 8.5, 6.0)
H_a–C(10)	5.21 (*dd*, J = 10.3, 1.9)	5.30 (unres.)
H_b–C(10)	5.30 (*dd*, J = 18, 19)	
H–C(1″)	4.59 (*d*, J = 7.7)	4.95 (*d*, J = 7.7)
H–C(2″)	2.97 (*dd*, J = 8.6, 7.7)	5.02 (*dd*, J = 8.0, 8.0)
H–C(3″)	3.20 (*dd*, J = 8.6, 8.6)	5.24 (*dd*, J = 9.5, 8.0)
H–C(4″)	3.04 (*dd*, J = 9.4, 8.6)	5.12 (*dd*, J = 9.5, 9.5)
H–C(5″)	3.19 (*ddd*, J = 9.4, 6.2, 1.8)	3.73 (*dt*, J = 9.5, 3.0)
H_a–C(6″)	3.71 (*dd*, J = 11.7, 1.8)	4.25 (unres.)
H_b–C(6″)	3.38 (*dd*, J = 11.7, 6.2)	
CH_3COO		2.01, 2.03, 2.09 (2 ×)

[a]) 'Biogenetic' numbering[1]).
[b]) In (D_6)DMSO.
[c]) In $CDCl_3$.

^{13}C-chemical shifts of the atoms involved in the lactone ring and significantly different coupling constants for the protons attributable to the monoterpene moiety (see *Tables 1* and *2* and [8]) were readily apparent. Furthermore, the molecular weight (716 daltons from DCI-MS) was inconsistent with a simple secoiridoid glycoside. Further MS data suggested **1** to be a symmetrical secoiridoid dimer with the molecular formula $C_{32}H_{44}O_{18}$. Thus, the most reasonable structure hypothesis was that of a dimer derived from sweroside, with the two monomers forming a twelve-membered symmetrical bislactone ring, a structure confirmed by spectral analysis of some derivatives (see below).

The broad-band decoupled ^{13}C-NMR spectrum and DEPT spectra of **1** (*Table 1*) show signals of 1 ester carbonyl (166.5 ppm), 2 quaternary olefinic C-atoms at 161.7 and 110.1 ppm, 1 acetal C-atom (99.5 ppm), a vinylic side chain (135.0 and 118.5 ppm), 1 O-bearing CH_2 group (62.5 ppm), 1 additional sp^3 CH_2 group (30.8 ppm), and 2 sp^3 CH groups (32.2 and 43.8 ppm), together with signals attributable to a hexosyl moiety. In the DCI-MS, quasimolecular ions at m/z 734 ($[M + NH_4]^+$) and 717 ($[M + H]^+$) appear. Fragment ions at m/z 572 ($[M + NH_4 - 162]^+$), 555 ($[M + H - 162]^+$), 410 ($[M + NH_4 - 324]^+$), and 393 ($[M + H - 324]^+$) are indicative of a successive elimination of two hexosyl units. The peaks at m/z 376 ($[M + NH_4 - 358]^+$) and 359 ($[M + H - 358]^+$) result from a symmetrical cleavage of the molecule, and a successive elimination of a hexosyl moiety leads to the intense fragment ion at m/z 197.

Enzymatic hydrolysis of **1** with β-glucosidase readily yielded aglycone **3** as amorphous powder. The presence of two cyclic hemiacetal groups in the molecule, susceptible to isomerisation in solution, rendered the compound not amenable to NMR-spectral analysis[2]). The DCI-MS of **3**, however, was in accordance with the proposed structure (strong pseudomolecular ions at m/z 410 ($[M + NH_4]^+$) and 393 ($[M + H]^+$), bislactone-

[2]) Mutarotation was observed: an $[\alpha]_D^{25} = -100$ was measured for a freshly prepared methanolic solution of **3**, which decreased towards 0 within a few h.

cleavage fragments at *m/z* 214 and 196). Upon acetylation of **1**, the octaacetate **1a** was obtained. Unambiguous confirmation of the structure of lisianthioside (**1**) was established by an X-ray crystallographic analysis of **1a** the results of which will be published elsewhere. Methanolysis of lisianthioside (**1**) with 1% methanolic H_2SO_4 yielded a mixture of three compounds **4a–c** which were separated by semi-prep. HPLC on silica gel. The structures of these methoxy derivatives were established by MS and NMR data.

The EI-MS of **4a** exhibits M^+ at *m/z* 420 ($C_{22}H_{28}O_8$). A *s* at 3.57 ppm in the ^{1}H-NMR spectrum (*Table 3*) confirms the presence of a MeO group. A $J(1,9)$[1]) of 9.1 Hz is indicative of α-configuation for both H–C(1) and H–C(1′) as in the parent compound **1**. The β-configuration of H–C(1) and H–C(1′) in dimethoxy compound **4b** is deduced from a $J(1,9)$ of 2.6 Hz, indicative of a equatorial-axial relationship of the two vicinal protons. Compound **4c** shows two distinct signals for H–C(1) and H–C(1′), with $J(1,9)$ of 8.9 and 2.8 Hz, respectively. Thus, **4c** is the 1α,1′β(or 1β,1′α) isomer.

Table 3. *^{1}H-NMR Data of Methoxy Derivatives* **4a–c**[a])

H-Atom[b])	**4a**	**4b**	**4c**	
H–C(1)	4.86 (*d*, *J* = 9.1)	4.89 (*dd*, *J* = 2.6, 0.7)	4.84 (*d*, *J* = 8.9)[c])	4.91 (*dd*, *J* = 2.8, 0.8)[d])
H–C(3)	7.47 (*s*)	7.44 (*d*, *J* = 1)	7.47 (*s*)	7.44 (*d*, *J* = 0.9)
H–C(5)	3.04 (*ddd*, *J* = 6.9, 5.1, 5)	2.86 (*ddddd*, *J* = 7.3, 6.5, 2.3, 1, 0.7)	3.03 (*ddd*, *J* = 6.7, 5.4, 3.4)	2.88 (*ddddd*, *J* = 7, 7, 3, 0.9, 0.8)
H_a–C(6)	1.67 (*dddd*, *J* = 15, 9.5, 5, 3.8)	1,77 (*dddd*, *J* = 15.3, 11.4, 3.8, 3)	1.70–1.95 (unres.)	1.70–1.95 (unres.)
H_b–C(6)	1.91 (*dddd*, *J* = 15, 6.8, 6, 0.7)	2.54 (*dddd*, *J* = 15.3, 7.3, 4, 2)		2.51 (*dddd*, *J* = 15, 7.3, 4.9, 2.3)
H_a–C(7)	4.19 (*ddd*, *J* = 11.3, 6, 3.5)	4.25 (*ddd*, *J* = 11.4, 4, 3.8)	4.24 (unres.)	4.24 (unres.)
H_b–C(7)	4.02 (*ddd*, *J* = 11.3, 9.5, 2.7)	3.86 (*ddd*, *J* = 11.4, 11.4, 2)	3.99 (*ddd*, *J* = 11.2, 10, 2.9)	3.89 (*ddd*, *J* = 11.1, 11.1, 2.1)
H–C(8)	5.75 (*ddd*, *J* = 17.4, 10.1, 8.7)	5.98 (*ddd*, *J* = 17.3, 10.1, 9.1)	5.74 (*ddd*, *J* = 17.4, 10, 8.8)	6.00 (*ddd*, *J* = 17.3, 10.2, 9.2)
H–C(9)	2.59 (*ddd*, *J* = 9.1, 8.7, 5.1)	2.64 (*dddd*, *J* = 9.1, 6.5, 2.6, 0.7)	2.64 (unres.)	2.64 (unres.)
H_a–C(10)	5.27 (*m*)	5.22 (*ddd*, *J* = 10.1, 1.8)	5.21–5.31 (unres.)	5.21–5.31 (unres.)
H_b–C(10)		5.25 (*ddd*, *J* = 17.3, 1.8, 0.7)		
CH_3O	3.57 (*s*)	3.42 (*s*)	3.57 (*s*)	3.44 (*s*)

[a]) In $CDCl_3$; δ in ppm rel. to TMS (= 0 ppm), *J* in Hz.
[b]) 'Biogenetic' numbering[1]).
[c]) H–C(1) to H–C(10).
[d]) H–C(1′) to H–C(10′).

The ^{1}H-NMR data of lisianthioside (**1**) and its derivatives **1a** and **4a** are characteristic for a *trans*-diaxial relationship of α-configurated H–C(1) and β-configurated H–C(9) ($J(1,9)$ = 8.5–9.3 Hz) and indicate a small dihedral angle (*ca.* 30°) between H–C(9) and β-configurated H–C(5) ($J(5,9)$ = 5–6 Hz; see *Tables 2* and *3*). According to NOE difference measurements on **4a** (see *Exper. Part* and below), the dihydropyran ring adopts the same sofa conformation as in lisianthioside octaacetate (**1a**) in the solid state

where C(9) is located above the best plane through the remaining five ring atoms. Interestingly, the dihydropyran ring of monomeric secoiridoids like sweroside (**2**) has a different conformation, with H–C(1) and H–C(9) in equatorial positions ($J(1,9)$ = 1.5–1.8 Hz) [7] [8]. These conformational differences are also reflected in the absence of an allylic coupling constant $J(3,5)$ in the case of **1**, **1a**, and **4a** as compared to sweroside (**2**) and its peracetate ($J(3,5)$ = 2.0–2.5 Hz [7] [8]). They can be explained by the constraints exerted by the six-membered lactone ring of **2**, while the flexible twelve-membered bislactone ring of **1**, **1a**, and **4a** allows the dihydropyran ring to adopt a conformation with the substituents at C(1) and C(9) in the more favorable pseudoequatorial positions.

The conformation adopted by the bislactone ring of **1** and **1a** could not be studied by ^{1}H-NMR, since the relevant signals are overlapping or unresolved (*Table 2*). A detailed analysis of the chemical shifts and vicinal coupling constants of H–C(5), CH_2(6), and CH_2(7) of dimethoxy derivative **4a**, however, was possible (*Table 3*) and suggested the presence of a preferred solution conformation (see *Fig.*). It corresponds to the solid conformation of the aglycone moiety in peracetate **1a** as established by X-ray diffraction analysis. The preferred solution conformation of **4a** was corroborated by a series of NOE difference measurements. While the symmetry of **4a** and large interatomic distances rendered impossible the measurement of NOE's across the macrocycle, highly informative NOE's were observed between the protons of the dihydropyran ring, the vinylic side chain, and the CH_2 protons involved in the macrocycle.

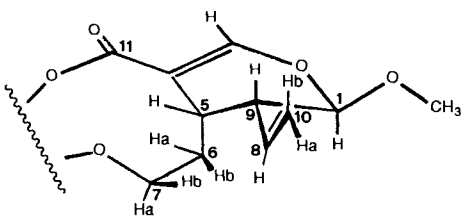

Figure. *Preferred solution conformation of dimethoxy derivative* **4a**, *as deduced from* ^{1}*H-NMR data* (see text). Only one half of the symmetric dimer is shown. 'Biogenetic' numbering.

Relatively large chemical-shift differences are observed in the ^{1}H-NMR spectrum of **4a** for the diastereotopic H-atoms at C(6) and C(7) which show the following relevant vicinal coupling constants: J(6a, 7a) = 3.5, J(6a, 7b) = 9.5, J(6b, 7a) = 6, and J(6b, 7b) = 2.7 Hz. The NOE's observed for the protons of the 'lower' face of the molecule, in particular a strong enhancement at H–C(1) upon irradiation of H_b–C(6), and NOE's at H–C(8) upon irradiation of H_b–C(6) and H_b–C(7), establish the spatial vicinity of these four protons. Irradiation of H–C(9) results in an enhancement at H_b–C(10), confirming the orientation of the vinyl moiety relative to the dihydropyran ring. The enhancement at H–C(5) on irradiation of H_b–C(7) establishes the proximity of these two protons and thus the spatial arrangement within the bislactone ring.

As far as can be estimated from chemical shifts, vicinal coupling constants, and variable-temperature measurements (213–303 K), the solution conformation of the aglycone moiety in peracetate **1a** should be identical with that of dimethoxy derivative **4a**. However, the conformation of both the dihydropyran ring and the macrocycle of the two dimethoxy derivatives **4b** and **4a** differs, as evidenced by the appearance of an allylic coupling constant $J(3,5)$ for **4b** and significant differences in chemical shifts and vicinal coupling constants of the protons at C(5), C(6), C(7), and C(9) (*Table 3*). That the

modification of the configuration at C(1) has a major effect on the conformation of the entire aglycone moiety has to be considered as indicative of the relative flexibility of the macrocycle lacking stabilizing intramolecular H-bonds.

Discussion. – Lisianthioside (**1**) is the first representative of a new type of dimeric secoiridoids in which two monomers are symmetrically linked through ester moieties to form a twelve-membered bislactone ring. It formally contains two molecules of sweroside (**2**), one of the secoiridoid glucosides most frequently encountered in Gentianaceae. While a few dimeric [9–15], trimeric [16], and, more recently, even branched tetrameric iridoid-secoiridoid glycosides [17] have been reported, pure bis-secoiridoids are a extremely rare type of natural products [18]. It appears that centauroside, isolated from *Erythraea centaurium* (Gentianaceae) [19] would be the only other bis-secoiridoid described so far. It is also rare in iridoids that formation of a macrocyclic ring occurs to form a new skeleton. To our knowledge, jasminin [9] and the closely related jasmisnyroside [10], jasminin 10″-*O*-*β*-D-glucoside [15] and jasmoside [16], all of them containing a secoiridoid glucoside moiety and an additional monoterpene unit, are the only other iridoids similar to lisianthioside (**1**) in that respect.

Secoiridoids are responsible for the bitter taste of many Gentianaceae plants, some of them having medicinal use as bitter tonics. Sweroside (**2**) itself is only moderately bitter, but its trihydroxydiphenylcarboxylic ester amaropanin is among the most bitter natural product known [20] [21]. It is, therefore, noteworthy that lisianthioside (**1**) is absolutely tasteless. While the functional groups required for an interaction with the taste receptors are present [21], either the conformational difference of the dihydropyrane ring, compared to the monomeric secoiridoids like sweroside (**2**), or steric hindrance due to the dimeric nature of **1** apparently do not allow for a binding to the receptor site. Chemotaxonomic investigations of other *Lisianthius* species and isolation of biologically active constituents are in progress.

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Experimental Part

General. TLC: silica gel GF_{254} coated Al sheets *(Merck)*; detection by UV_{254} light after staining with *Godin* reagent. Anal. HPLC: *Knauer LiChrosorb* silica column (7 μm, 4.6 × 250 mm i.d.), *Spectra-Physics-8700* pump, *HP-1040A* photodiode array detector with *HP-85B* computer and *HP-7470A* plotter; hexane/i-PrOH 95:5, 2 ml·min^{-1}. Semiprep. HPLC: *Waters-M6000* pump, *Knauer LiChrosorb* silica column (7 μm, 16 × 250 mm i.d.); hexane/i-PrOH 95:5, 10 ml·min^{-1}; detection at 226 nm. M.p.: *Mettler-FP-80/82* hot-stage apparatus. UV spectra: *Varian-DMS-100S* spectrometer. IR spectra: *Philips-PU-9716* spectrometer. Optical rotation: *Perkin-Elmer-241-MC* polarimeter. NMR experiments: *Varian-VXR-200* instrument; TMS (^{1}H-NMR) or solvent signal (^{13}C-NMR) as reference; ^{1}H,^{1}H connectivities through COSY experiments; ^{13}C multiplicities from DEPT experiments; NOE difference measurements: alternating irradiation of the individual lines of the *m* according to [22], total irradiation time 5 s; for each irradiated proton, spectra at several decoupler power levels were recorded; 256 or 512 transients, in alternate blocks of 16 transients preceeded by 4 dummy scans, were aquired for enhanced and control spectra; variable-temperature measurements on peracetate **1a** were performed in CD_2Cl_2.

Plant Material. The plant material was collected at Cierro Jefe, Province of Panama, and authenticated by Prof. *Mireya D. Correa.* A voucher specimen has been deposited at the Herbarium of the University of Panama.

Extraction and Isolation. Powdered root (20 g) of *L. jefensis* was extracted at r.t. successively with CH_2Cl_2 and MeOH. A portion (2.0 g) of the MeOH extract was submitted to gel chromatography on *Sephadex LH 20* with MeOH. Five fractions were collected. *Fraction 2* (740 mg) was further separated on a silica-gel column with $CHCl_3$/MeOH (8:2→7:3) to afford **1** (310 mg).

Acid Hydrolysis of **1**. Glycoside **1** (2 mg) was refluxed in 2N HCl for 2 h, the mixture extracted with AcOEt, the org. layer analyzed by TLC (SiO_2, $CHCl_3$/MeOH 9:1), and the aq. phase adjusted to pH 6 with $NaHCO_3$. After freeze drying, the residue was extracted with pyridine and analyzed for sugars by TLC on silica gel (AcOEt/MeOH/H_2O/AcOH 65:15:15:20, detection with *p*-anisidine phthalate).

*(3*R*,*4*S*,*4a*R*,*11*R*,*12*S*,*12a*R*)*-4,12-Diethenyl-3,11-bis[(β-*D*-glucopyranosyl)oxy]-4,4a,5,6,12,12a,13,14-octahydro-3*H,*8*H,*11*H,*16*H-dipyrano[3,4-*c*:3′,4′-*i*][1,7]dioxacyclododecene-8,16-dione* (= *Lisianthioside*; **1**). White, amorphous powder. M.p. 202–205°. TLC (SiO_2, $CHCl_3$/MeOH 7:3): R_f 0.30. $[\alpha]_D^{25} = -173$ ($c = 0.43$, MeOH). UV (MeOH): 238 (4.36). IR (KBr): 3500–3300, 2900, 1670, 1615, 1380, 1295, 1160, 1070, 985, 920, 760. ^{1}H-NMR: *Table 2.* ^{13}C-NMR: *Table 1.* DCI-MS (NH_3, pos.-ion mode): 734 ($[M + NH_4]^+$), 717 ($[M + H]^+$), 572 ($[M + NH_4 - 162]^+$), 555 ($[M + H - 162]^+$), 410 ($[M + NH_4 - 324]^+$), 393 ($[M + H - 324]^+$), 376 ($[M + NH_4 - 358]^+$), 359 ($[M + H - 358]^+$), 197 ($[M + H - 520]^+$), 180.

Octaacetate **1a**. Compound **1** (40 mg) was kept in pyridine/Ac_2O 1:1 (4 ml) at r.t. for 16 h. The mixture was poured into ice-H_2O and the precipitate filtered off and recrystallized from MeOH/Et_2O: **1a** (43 mg). M.p. 113–115°. $[\alpha]_D^{25} = -107$ ($c = 0.31$, $CHCl_3$). UV (MeOH): 236. IR (KBr): 2940, 1730, 1690, 1615, 1425, 1360, 1220, 1060, 760. ^{1}H-NMR: *Table 2.* ^{13}C-NMR: *Table 1.* DCI-MS (NH_3, pos.-ion mode): 1070 ($[M + NH_4]^+$).

*4,12-Diethenyl-4,4a,5,6,12,12a,13,14-octahydro-3,11-dihydroxy-3*H,*8*H,*11*H,*16*H-dipyrano[3,4-*c*:3′,4′-*i*][1,7]dioxacyclododecene-8,16-dione* (**3**). Compound **1** (50 mg) was dissolved in acetate buffer pH 4.9 (10 ml) and incubated with emulsin at 35° for 2 d. The precipitate was filtered off and washed with H_2O: **3** (20 mg) as white, amorphous material. M.p. 70–73°. UV (MeOH): 238. IR (KBr): 3400, 2900, 1685, 1675, 1615, 1380, 1295, 1195, 1160, 1070, 985, 920, 760. DCI-MS (NH_3, pos.-ion mode): 410 ($[M + NH_4]^+$), 393 ($[M + H]^+$), 375 ($[M + H - 18]^+$), 214 ($[M + NH_4 - 196]^+$), 197 ($[M + H - 196]^+$), 179, 153.

*4,12-Diethenyl-4,4a,5,6,12,12a,13,14-octahydro-3,11-dimethoxy-3*H,*8*H,*11*H,*16*H-dipyrano[3,4-*c*:3′,4′-*i*][1,7]dioxacyclododecene-8,16-diones* (**4a–c**). Compound **1** (50 mg) was dissolved in abs. MeOH (10 ml) containing 1% H_2SO_4 and refluxed for 1 h. The mixture was poured into ice/H_2O and extracted with $CHCl_3$. The residue of the org. phase (23 mg) consisting of **4a–c** was separated by semiprep. HPLC (see *General; ca.* 5-mg portions) to yield **4a** (4 mg), **4b** (4.5 mg), and **4c** (8 mg) as amorphous waxy materials. Capacity factors k': 0.70 (**4b**), 1.45 (**4c**), and 2.60 (**4a**).

*(3*R*,*4*R*,*4a*S*,*11*R*,*12a*S*)*-Isomer* **4a**. $[\alpha]_D^{25} = -150$ ($c = 0.24$, MeOH). UV (MeOH): 239. IR (KBr): 2940, 2910, 1685, 1620, 1290, 1175, 1100, 915, 760. ^{1}H-NMR: *Table 3.* NOE: H–C(1)/CH_3O–C(1), H_b–C(6), H–C(8); H–C(5)/H_b–C(7), H–C(9); H_b–C(6)/H–C(1), H–C(8); H_b–C(7)/H–C(5), H–C(8); H–C(8)/H–C(1), H_b–C(6), H_b–C(7); H–C(9)/H–C(5), H_b–C(10); H_b–C(10)/H–C(9). ^{13}C-NMR: *Table 1.* EI-MS: 420 (0.1, M^+), 388 (4), 356 (0.5), 211 (19), 179 (7), 127 (8), 109 (5), 84 (100).

*(3*R*,*4*S*,*4a*R*,*11*R*,*12*S*,*12a*R*)*-Isomer* **4b**. $[\alpha]_D^{25} = +42$ ($c = 0.28$, MeOH). UV (MeOH): 236. IR (KBr): 2950, 2920, 1690, 1625, 1435, 1370, 1280, 1265, 1175, 1110, 1075, 1055, 910, 765. ^{1}H-NMR: *Table 3.* ^{13}C-NMR: *Table 1.* EI-MS: 420 (0.5, M^+), 388 (1), 356 (1.5), 211 (24), 179 (13), 127 (9), 109 (8), 84 (100).

*(3*R*,*4*R*,*4a*S*,*11*S*,*12*R*,*12a*S*)*-Isomer* **4c**. $[\alpha]_D^{25} = -46$ ($c = 0.37$, MeOH). UV (MeOH): 237. IR (KBr): 2940, 2920, 1690, 1620, 1280, 1260, 1175, 1110, 1080, 915, 765. ^{1}H-NMR: *Table 3.* ^{13}C-NMR: *Table 1.* EI-MS: 420 (0.3, M^+), 388 (3), 356 (0.6), 211 (22), 179 (11), 127 (9), 109 (5), 84 (100).

REFERENCES

[1] M. Maillard, M. Hamburger, M.P. Gupta, K. Hostettmann, *Planta Med.* **1989,** *55,* 281.
[2] T.S. Elias, A. Robyns, *Ann. Missouri Bot. Garden* **1975,** *62,* 61.
[3] J.F. Morton, 'Atlas of Medicinal Plants of Middle America, Bahamas to Yucatan', Charles C. Thomas, Springfield, Ill., 1981, p. 669.
[4] W. Wong, *Econ. Bot.* **1976,** *30,* 103.
[5] M.P. Gupta, P.N. Solis, C. Miranda, O. Montenegro, R. Martinez, L.A. Varela, M.A. Correa, *Rev. Med. Panama* **1988,** *13,* 70.

[6] B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobson, D. E. Nichols, J. L. McLaughlin, *Planta Med.* **1982,** *45,* 31.
[7] H. Inouye, T. Yoshida, Y. Nakamura, S. Tobita, *Tetrahedron Lett.* **1968,** 4429; H. Inouye, S. Ueda, Y. Nakamura, *Chem. Pharm. Bull.* **1970,** *18,* 1856.
[8] T. A. van Beek, P. P. Lankhorst, R. Verpoorte, A. Baerheim-Svendsen, *Planta Med.* **1982,** *44,* 30.
[9] T. Kamikawa, K. Inoue, T. Kubota, M. C. Woods, *Tetrahedron* **1970,** *26,* 4561.
[10] Z. He, C. Yang, *Yunnan Zhiwa Yanjin* **1989,** *11,* 55 (*CA: 111,* 228945).
[11] T. Sevenet, C. Thal, P. Potier, *Tetrahedron* **1971,** *27,* 663.
[12] S. R. Jensen, S. E. Lyse-Petersen, B. J. Nielsen, *Phytochemistry* **1979,** *18,* 273.
[13] F. Murai, M. Tagawa, S. Matsuda, T. Kikuchi, S. Uesato, H. Inouye, *Phytochemistry* **1985,** *24,* 2329.
[14] B. Podanyi, R. S. Reid, A. Kocsis, L. Szabo, *J. Nat. Prod.* **1989,** *52,* 135.
[15] T. Tanahashi, N. Nagakura, H. Kuwajima, K. Takaishi, K. Inoue, H. Inouye, *Phytochemistry* **1989,** *28,* 1413.
[16] K. Inoue, T. Tanahasi, H. Inouye, *Phytochemistry* **1985,** *24,* 1299.
[17] T. Tanahashi, N. Nagakura, K. Inoue, H. Inouye, *Tetrahedron Lett.* **1988,** *29,* 1793.
[18] P. Junior, *Planta Med.* **1990,** *56,* 1.
[19] S. Tagaki, M. Amaki, E. Yunioka, T. Nishimura, K. Sakina, *Yakugaku Zasshi* **1982,** *102,* 313.
[20] K. Münzing-Vasirian, Dissertation, Munich, 1974.
[21] H. Wagner, 'Pharmazeutische Biologie. Drogen und ihre Inhaltsstoffe', 4th edn., G. Fischer, Stuttgart, 1988, pp. 111–114.
[22] M. Kinns, J. K. M. Sanders, *J. Magn. Reson.* **1984,** *56,* 518.