PERGAMON

Phytochemistry 60 (2002) 747–754

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Apotirucallane triterpenoids from *Luvunga sarmentosa* (Rutaceae)

Trinh Phuong Lien[a], Christine Kamperdick[a], Juergen Schmidt[b], Guenter Adam[b], Tran Van Sung[a,*]

[a]*Institute of Chemistry, National Centre for Natural Science and Technology of Vietnam, Hoang Quoc Viet Road, Cau Giay, Hanoi, Viet Nam*
[b]*Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle/Saale, Germany*

Received 28 November 2001; received in revised form 12 April 2002

Abstract

The leaves of *Luvunga sarmentosa* (Bl.) Kurz. yielded eight apotirucallane triterpenoids named luvungins A–G, and 1α-acetoxyluvungin A. Characteristic of the structure are the seven-membered lactone-ring A, the α-hydroxyl or α-acetoxyl group at C-7 and an oxygen bridge in the side chain giving five-, six- or seven-membered rings, respectively. Because of a hemiacetal function at C-21, luvungin C occurred as a mixture of 21-epimers. The structures have been elucidated on the basis of MS and NMR spectral data. In addition, two known coumarins ostruthin (6-geranyl-7-hydroxycoumarin) and 8-geranyl-7-hydroxycoumarin as well as five known triterpenes friedelin, flindissone, melianone, niloticin and limonin were isolated.

Keywords: *Luvunga sarmentosa*; Rutaceae; Apotirucallane triterpenoids; Luvungins A-G; 1α-Acetoxyluvungin A

1. Introduction

Luvunga sarmentosa (Bl.) Kurz. (*Triphasia sarmentosa* Bl., *L. eleutherandra* Dalz., *L. augustifolia* (Oliv.) Tan.) is a shrub growing in the rain forest of Vietnam (Ho, 1992). In Indonesia the sap flowing out of a heated stem of this plant may be rubbed on the gums to treat toothache, whereas the bark and leaves are used to care for pain in the limbs and rheumatism (Perry, 1980). From the stem of *L. sarmentosa*, lupeol, stigmasterol, the coumarins ostruthin, suberosin, and the acridone alkaloids 5-methoxyarborinine, 5-hydroxyarborinine have been isolated (Wijeratne et al., 1992). The leaves have not yet been investigated. In continuation of our search for new biologically active compounds from Vietnamese medicinal plants we now report the isolation and structural elucidation of the constituents of the leaves from this plant.

2. Results and discussion

The leaves of *L. sarmentosa* were extracted with *n*-hexane, ethyl acetate and *n*-butanol, successively. The *n*-hexane and ethyl acetate extracts were repeatedly subjected to column chromatography on silica gel or reversed phase RP-8.

The two isolated coumarins were established as ostruthin (6-geranyl-7-hydroxycoumarin) and 8-geranyl-7-hydroxycoumarin by comparison with reference data (Rashid et al., 1992). The five known triterpenes were shown to be friedelin (Klass et al., 1992), flindissone (Guang-Yi et al., 1988), melianone, nilocitin (Itokawa et al., 1992) and limonin (Sugimoto et al., 1988), by comparison of spectral data. Melianone and niloticin were reported to have moderate cytotoxic activity against V-79, P 388 and KB cells (Itokawa et al., 1992).

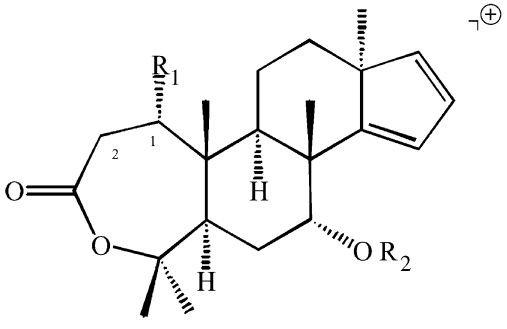
The molecular weight of luvungin A (**1**) of 544 was indicated by the prominent $[M+Na]^+$-peak in the ESI MS at *m*/*z* 567. The elemental composition $C_{32}H_{48}O_7$ was deduced from the $[M-HOAc]^+$-ion at *m*/*z* 484.3163 in the EIMS which gave no molecular ion. The base peak at *m*/*z* 344.2398, $C_{22}H_{32}O_3$ (Fig. 1, **1a**) suggested an easy cleavage of the side chain. The remaining 22 carbons corresponded very well to the above mentioned tetracyclic skeletons of the tirucallane triterpenes flindissone, melianone, niloticin and the apotirucallane triterpene limonin which were also present in this extract. This skeleton was confirmed by analysis of the 2D NMR spectra (HSQC, HMBC, HH-COSY).

* Corresponding author. Tel.: +84-4-756-4794; fax: +84-4-836-1283.
E-mail address: tvs@ich.ncst.ac.vn (T. Van Sung).

0031-9422/02/$ - see front matter
PII: S0031-9422(02)00156-5

Characteristic for the skeleton of **1** was the additional oxygen atom in ring A resulting in a seven-membered lactone ring. This was revealed by the carbon shifts at C-4 (δ 85.8) and C-3 (δ 174.8) and by the C-H long-range correlations from H_2-1 and H_2-2 to C-3, and from H_3-28 and H_3-29 to C-4. A hydroxyl group was located at C-7, based on the correlations from H-7 (δ 3.92) to C-5, C-9 and C-30. The double bond between C-14 (δ 161.2) and C-15 (δ_C 119.7, δ_H 5.50) was established from long-range correlations H-15/C-13, H_2-16/C-14, H_3-18/C-14, H_3-30/C-14 and H_2-16/C-15. The remaining signals for the hemiacetal group (δ_C 96.6, δ_H 6.25) and an epoxide moiety (δ_C 66.7/δ_H 2.67 and δ_C 57.2) belonged to the side chain. By comparison of the NMR spectral data of **1** (Tables 1 and 2) with bruceajavanin A (Kitagawa et al., 1994) their structures appeared to have an identical side chain consisting of a five-membered acetal ring connected with an 1,1-dimethyl-ethylenoxide moiety. This was confirmed by long-range correlations from H-21 to C-20, C-22, C-23 and the acetoxycarbon (δ 170.0), from H-23 to C-24 and C-21 and from H_3-26/H_3-27 to C-25 as well as the HH correlations of the spin system H-21/H-20/H_2-22/H-23/H-24. The connection between the side chain and the tetracyclic system between positions 20 and 17 was confirmed from the HMBC correlations H-17/C-20, H-17/C-22 and H-20/C-17. Furthermore, the HH coupling constants of H-7 (3.92, *t*, $J=3.5$ Hz) indicated the equatorial orientation of this proton and the α-configuration of the hydroxyl group. Analysis of NOESY-experiment led to the relative configuration as depicted in Fig. 2. The absence of a NOE between H-20 and H-17 suggested a nearly antiperiplanar arrangement of these two protons. This relative configuration was in correspondence to the stereochemistry of the parent compound tiracallol, which was determined by X-ray analysis (Nes et al., 1984). As in bruceajavanin A, no NOE interactions between the left part (C-20/C-21) and the right part (C-22/C-23) of the tetrahydropyran ring were observed. Because of the very similar chemical shifts of the side chains from **1** and bruceajavanin A, both compounds were assumed to have the same relative configuration.

The EIMS of luvungin B (**2**) showed a weak molecular ion peak at *m/z* 558. Its elemental composition was deduced from the $[M-HOAc]^+$ peak (498.3415, $C_{31}H_{46}O_5$) as $C_{33}H_{50}O_7$. The ^1H and ^{13}C NMR spectra of **2** (Tables 1 and 2) suggested the same skeleton like that of **1** based on the very similar olefinic signals of the Δ^{14} double bond (δ_C 161.6 and 119.7, δ_H 5.48), the lactone function in ring A (δ_C 170.5 and 86.0) and the hydroxyl function at C-7 (δ_C 71.13, δ_H 3.89). This skeleton was confirmed by the HH-correlations and the CH long-range correlations of the methyl groups. An additional acetyl group (δ_C 170.3 and 21.0, δ_H 2.10) was found at C-1 (δ_C 71.07) based on the CH long-range correlations of C-1 to H_3-19 and the HH-correlations between H-1 (δ 4.78) and H_2-2 (δ 3.14). These protons also showed correlations with C-3. The equatorial position of H-1 and thus the α-configuration of the acetoxyl group was deduced from the coupling constant of H-1 wih H_2-3 (*t*, $J=4.1$ Hz). Accordingly, the typical MS fragment of the tetracyclic moiety (Fig. 1, **2a**) appeared at *m/z* 402 with one acetyl group more than that of luvungin A (**1a**). The side chain had a double bond instead of the epoxy function. The two olefinic carbons C-24 and C-25 resonated at δ 124.4 and 137.4 and gave long-range correlations to the two methyl groups H_3-26 (δ 1.74) and H_3-27 (δ 1.72). The five-membered acetal ring connected to C-17 was suggested by the chemical shifts at C-21 (δ_C 108.8, δ_H 4.74) and C-23 (δ_C 73.8, δ_H 4.68) and confirmed by the HH correlations H-21/H20/H-$22^{A/B}$/H-23/H-24 in the HH COSY and by the CH long-range correlations C-23/H-21 and C-17/H-21. Additionally, H-21 gave a correlation peak to the methoxyl carbon at δ 55.5, which was thus located at C-21. The α-configuration of this methoxyl group was deduced from the NOESY experiment by the NOE between H-21 (δ 4.74) and H-17 (δ 1.71). The NOE between H-20 and H_3-18 and between H-21 and H-17 indicated for C-20 and C-17 the same relative configuration as in **1**. The relative configuration of C-23 could not be deduced from the NOESY experiment, but was assumed to be also identical to **1** because of their close structural relationship. The other NOE effects observed



MS fragment	R_1	R_2	*m/z*
1a, 4a	H	H	344
2a, 3a	OAc	H	402
5a, 8a	OAc	Ac	444
6a, 7a	$\Delta^{1(2)}$	Ac	384

Fig. 1. Characteristic MS fragments (**1a**–**8a**) generated by cleavage of the side chain of the luvungins **1–8**.

for **2** resulted in the same configuration of the tetracyclic moiety like **1**.

The molecular mass 602 of **3** was obtained from the $[M+Na]^+$-peak at *m/z* 625 in the ESI MS whereas the molecular formula of $C_{34}H_{50}O_9$ followed from the HR of the $[M-HOAc]^+$-peak in the EIMS showing also the fragment ion **3a** for the tetracyclic moiety at *m/z* 402. This moiety was identical with that of **2**, because of the nearly identical carbon shifts except for C-17. The remaining signals of the side chain were nearly identical to those of **1** resulting in the structure of 1α-acetoxyluvungin A for **3** (Tables 1 and 2).

The molecular formula of luvungin C (**4**) was deduced from the $[M]^+$-peak at *m/z* 486.3365 in the EIMS to be $C_{30}H_{46}O_5$. The NMR spectra (Tables 1 and 2) revealed that the sample was an isomeric mixture exhibiting many close pairs of signals. From the integrals of the ^1H spectrum the content of the major compound was determined as 55–60%. Despite this, the spectra were similar to those of **1** showing for the tetracyclic moiety the lactone group of ring A (δ_C 85.97/86.02 and 174.96/174.93), the $\Delta^{14(15)}$ double bond (δ_C 161.50/161.22 and 119.52/119.98), as well as the hydroxyl group at C-7 (δ_C 71.61, δ_H 3.91). Also the hemiacetal function of the side chain was indicated (δ_C 101.96/97.04 and δ_H 5.29/5.32), but no acetyl or methoxyl group, leaving the hemiacetal hydroxyl group unsubstituted and prone to epimerization at C-21, which explains the existence of two isomers. A double bond (δ_C 124.47/127.57 and 137.24/135.99) was found instead of the epoxide function in the side chain. Analysis of the 2D NMR spectra and comparison with **1** led to confirmation of the structure with the tetracyclic moiety identical with that of **1** and assignment of the two epimers. The configuration of C-20 was established by analysis of NOE effects (from the NOESY experiment) between H_3-18 (δ 1.09/1.03) and H-20 (δ 2.37/2.20). The major isomer of the epimeric mixture was the 21α-hydroxyl compound, revealed by the NOE effects between H-21 (δ 5.29) and H-17 (δ 1.74) and the absence of NOE effects between H-21 and H_3-18 (δ 1.09) and H-20 (δ 2.37). On the other hand, the 21β-hydroxyfunction of the minor isomer was deduced from NOE interactions of H-21 (δ 5.32) with H_3-18 (δ 1.03) and H-20 (δ 2.20) and missing effects of H-17 (δ 2.01). Other effects were not detectable because of heavy overlapping of the signals. The very good correspondence of the ^1H shifts of **4** with those of flindissone and flindissol (Guang-Yi et al., 1988) supported identical configuration of the side chain.

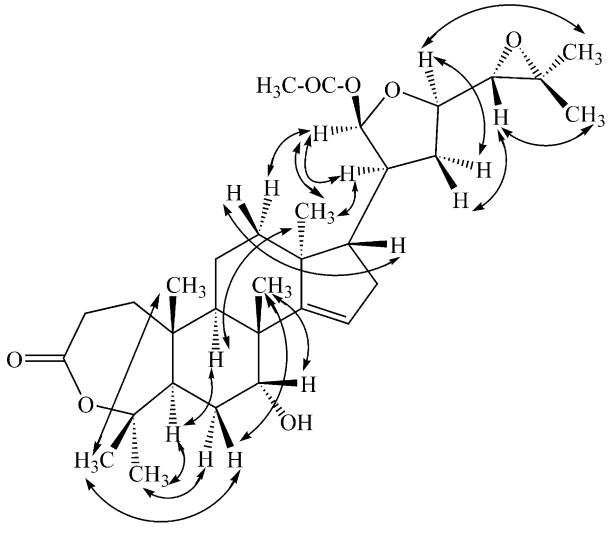


Fig. 2. Significant NOE effects observed for luvungin A (**1**).

The elemental compositions of luvungin D (**5**) and luvungin E (**6**) were established as $C_{34}H_{52}O_9$ (M = 604) and $C_{32}H_{48}O_7$ (M = 544), respectively, by HRMS of their $[M-H_2O]^+$-peaks. The NMR spectra of both compounds (Tables 3 and 4) also exhibited the characteristic signals of the luvungin skeleton with a lactone group in ring A and a $\Delta^{14(15)}$ double bond. Compound **5** additionally showed NMR signals of two acetoxyl groups, which were both located at the tetracyclic moiety because its fragment ion **5a** appeared in the EIMS at *m/z* 444. Interpretation of the 2D-NMR spectra of **5** (HH-COSY, HSQC, HMBC), which showed only a few C–H long-range correlations, together with comparison of the carbon shifts with **2** located the two acetoxyl groups at C-1 and C-7. This was revealed by the very similar shifts of **5** and **2** for ring A. In ring B, the additional acetoxyl group caused acetylation shifts of +3.4 ppm (C-7), −0.6 ppm (C-6) and −2.2 ppm (C-8). The remaining signals for the side chain were in good agreement with those of 3-episapelin A (Itokawa et al., 1992) with a tetrahydropyran ring. This structure was further confirmed by the C–H long range correlations from H_3-26 and H_3-27 to C-24, from H-24 to C-25 and C-23. The NOE effects (obtained from the NOESY experiment) between H-1 (δ 4.85) and H_3-19 (δ 1.14) and between H-7 (δ 5.14) and H_3-30 (δ 1.18) proved the α-configuration of both acetoxyl groups. Further NOE effects between H-24 (δ 2.88) and H_3-26 (δ 1.30)/Hα-22 (δ 1.52)/Hα-21 (δ 3.42) and between H-20 (δ 1.88) and Hα-21 (δ 3.42)/H_3-18 (δ 0.92), on the one hand, and between H-23 (δ 3.85) and H_3-27 (δ 1.25)/Hβ-22 (δ 2.02), on the other hand, resulted in the depicted relative configuration, which is identical to that of 3-episapelin A. The small coupling constants (J = 2.4 and < 1 Hz) of H-20 (δ 1.86) with Hα-22 (δ 3.42, *dd*, J = 11.6 and 2.4 Hz) and Hβ-22 (δ 3.92, *dd*, J = 12.1 and < 1 Hz) revealed that the six-membered ring did not assume the usual chair conformation suggested by the presence of two bulky 1,4-*cis* substitutents.

The ^1H NMR spectrum of luvungin E (**6**) was very similar to that of **5**, especially the close correspondence of the proton and carbon shifts of the side chain which established their identities in this part of the molecule.

However, in the luvungin skeleton of **6** one acetoxyl group was missing, which was also revealed by the mass of the fragment **6a** at *m*/*z* 384. Instead, the NMR spectra showed additional signals for a disubstituted double bond (δ_C 120.1/δ_H 5.89 *d*, J = 12.4 Hz and δ_C 156.3/δ_H 6.51 *d*, J = 12.4 Hz). The high deshielding of one of the carbons suggested its conjugation to the lactone group. This was confirmed by comparison of the carbon shifts of ring A to those of proceranone (Sondengam et al., 1981) resulting in the depicted structure of luvungin E (**6**).

Luvungin F (**7**) displayed only a weak molecular ion at *m*/*z* 544 in its EIMS. The elemental composition $C_{32}H_{48}O_7$ was established by high resolution of the $[M-H_2O]^+$-peak at *m*/*z* 526.3273. Luvungin F (**7**) (Tables 3 and 4) had the same tetracyclic moiety as luvungin E (**6**), which was proposed from the nearly identical carbon shifts except for C-17 and confirmed by the CH long-range correlations. For the side chain the HH correlations H-23 (δ 3.80)/H-24 (δ 3.42)/H_2-22 (δ 1.97 and 1.60) and H_2-21 (δ 3.60, 3.49)/H-20 (δ 1.89), and the CH long-range correlations from H-24 (δ 3.42) to C-25 (δ 76.2), C-23 (δ 67.8) and C-22 (δ 37.9) and from H_3-26 (δ 1.31) and H_3-27 (δ 1.15) to C-25 and C-24 (δ 80.6) were observed. All these correlations corresponded very well to the six-membered ring in the side chain of **6**. But the carbon shifts were different. These findings could only be explained by a different location of the ether bridge, which in **6** connected C-21 and C-24. One already known compound with a similar side chain containing an ether bridge between C-21 and C-25 was hispidone (Itokawa et al., 1992). Its ^{13}C shifts of the side chain were in very close correspondence to those of **7**, thus proving **7** to also have this seven-membered ring. The connection between the tetracyclic moiety and the side chain at C-17 and C-20 could not be confirmed by CH long-range correlations due to the overlapping of two proton signals of H-20 and H-17 (δ 1.89 and 1.90), but was gained from NOE's between H-20/H_3-18, Hα-21/H_3-18 and Hα-21/H-20. Further NOE's between H_3-27/Hβ-21, H_3-27/H-24 and H-24/Hβ-22, on the one hand, and between H_3-26/H-23, H-23/Hα-22 and H-23/H-20, on the other hand, together with the coupling constant of 9.1 Hz between H-23 and H-24 indicated that these two carbinol protons were nearly axial, thus the two hydroxyl groups were nearly equatorial. More NOE effects of this compound and the relative configuration were depicted in Fig. 3.

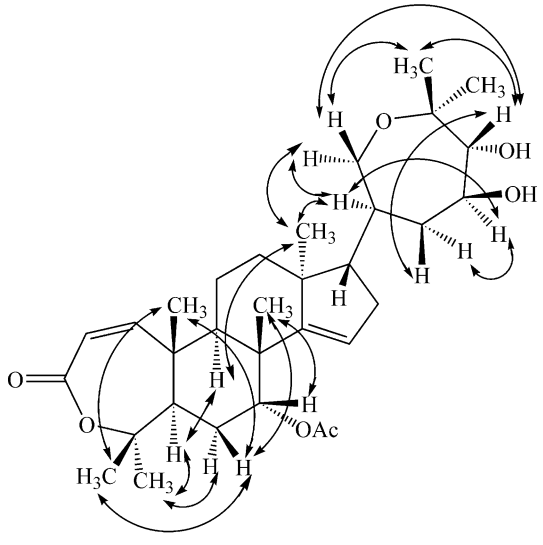


Fig. 3. Significant NOE effects observed for luvungin F (**7**).

The molecular mass of 604 and the elemental composition $C_{34}H_{52}O_9$ of luvungin G (**8**) were obtained from the $[M+Na]^+$-ion in the ESI–MS and the HR of the $[M-H_2O]^+$-peak in the EIMS. The fragment ion **8a** was found at *m*/*z* 444 suggesting two *O*-acetyl groups at the tetracyclic moiety. The carbon shifts in this part were nearly identical with those of **5** except for C-17 ($\Delta\delta$ 1.9)

Table 1
^{13}C NMR spectroscopic data of compounds **1–4** in $CDCl_3$ (for **4** shifts of the minor component in parentheses)

	1[a]	**2**[b]	**3**[a]	**4**[a]
1	37.5	71.07	71.0[c]	37.63 (37.52)
2	31.88	34.9	34.8[d]	31.94
3	174.8	170.5[c]	170.5	174.96 (174.93)
4	85.8	86.0	85.9	85.97 (86.02)
5	46.1	42.7	42.7	45.99[c] (46.22)
6	27.9	26.9	26.9	27.85
7	71.6	71.13	71.1[c]	71.61
8	43.8	44.0	43.9	43.84
9	41.1	34.0	33.8	41.36 (41.30)
10	40.2	44.4	44.3	40.09 (40.15)
11	16.5	16.2	16.1	16.54 (16.61)
12	32.4	32.4	32.1	32.72 (33.09)
13	46.3	46.8	46.4	46.65 (46.30)
14	161.2	161.6	161.4	161.50 (161.22)
15	119.7	119.7	119.9	119.52 (119.98)
16	35.1	34.7	35.0[d]	34.71 (35.10)
17	52.6	57.8	52.4	57.87 (52.77)
18	19.7	18.7	18.9	19.53 (19.97)
19	16.3	15.0	14.9	16.39 (16.29)
20	44.2	47.1	44.0	46.04[c] (48.23)
21	96.6	108.8	96.5	101.96 (97.04)
22	31.3	38.7	31.3	39.10 (35.25)
23	79.7	73.8	79.7	74.20 (75.70)
24	66.7	124.4	66.6	124.47 (127.57)
25	57.2	137.4	57.2	137.24 (135.99)
26	19.3	25.9	19.3	25.76
27	24.9	18.4	24.9	18.29 (17.92)
28	31.91	34.4	33.4	31.84
29	26.0	23.7	23.6	26.05 (25.95)
30	26.9	27.7	27.8	26.72 (26.79)
$OCOCH_3$	170.0	170.3[c]	169.9	
$OCOCH_3$			169.8	
$OCOCH_3$	21.5	21.0	21.4	
$OCOCH_3$			20.8	
OCH_3		55.5		

[a] 75 MHz.
[b] 125 MHz.
[c,d] Signals are interchangeable in each column.

and C-12 ($\Delta\delta$ 0.8), whereas the shifts of the side chain were identical with **7** (Tables 3 and 4). The relative configuration of the two hydroxyl groups was confirmed by the fact that **8** was able to form a methylboronate, which was analysed by GC–MS ($[M]^+$, m/z 568).

3. Experimental

3.1. General experimental procedures

Mps. uncorr.; $[\alpha]_D^{20}$: JASCO DIP 1000 polarimeter; IR: Bruker IFS 28; UV: Kontron Uvikon 940; EIMS (AMD 402, AMD Intectra GmbH): 70 eV (DIS), HR-EIMS (resolution ca. 5000); ESI-MS (TSQ 7000, Finnigan, positive ion mode, electrospray voltage 4.5 kV, syringe pump); ^{1}H and 2D spectra were recorded on a Varian UNITY 500 spectrometer at 499.83 MHz. ^{13}C $\{^1$H$\}$ and APT spectra were recorded on a Varian Gemini 300 spectrometer at 75.5 MHz. Chemical shifts were referenced to internal TMS ($\delta = 0$, ^{1}H) and $CHCl_3$ ($\delta = 77.0$, ^{13}C), respectively.

3.2. Plant material

Leaves and branches of *L. sarmentosa* (Blume) Kurz. were collected in Vinh Phuc province, Vietnam, on August 1997. The species was identified by Mr. Ngo Van Trai, Institute of Materia Medica, Hanoi. A voucher specimen (Nr. 2–27/8/97) was deposited in the herbarium of this institute.

3.3. Extraction and isolation

The plant material (820 g) was dried at room temp., ground and extracted 3× for 12 h with 95% MeOH at room temp. MeOH was evapd. in vacuo, and the aq. solution extracted with *n*-hexane, followed by EtOAc and *n*-BuOH (each 3×). The solvents were evapd. in vacuo. The *n*-hexane extract (8 g) was fractionated on silica gel with *n*-hexane–EtOAc (2:8) increasing the amount of EtOAc to 100% (19 frs). Frs 2, 3, 15–16 were crystallised from *n*-hexane/acetone yielding flindissone (40 mg), friedelin (11 mg) and melianone (100 mg), respectively. Frs 13–14 were subjected to silica gel chromatography using *n*-hexane–acetone (8:2) to afford ostruthin (50 mg) and nilocitin (200 mg). The EtOAc extract (13 g) was applied to a silica gel column, eluted with *n*-hexane–acetone (7:3), increasing the ratio of acetone to 100% (11 frs). Frs 3, 4, 5 and 6 were purified by column chromatography on silica gel and then reversed-phase RP-8 giving 8-geranyl-7-hydroxycoumarin (10 mg), luvungin B (**2**, 5 mg), and luvungin C (**4**, 10 mg), respectively. Frs 7–8 were separated on

Table 2
^{1}H NMR spectroscopic data of compounds **1–4** in $CDCl_3$ (for **4** shifts of the minor component in parentheses)

	1[a]	**2**[b]	**3**[b]	**4**[b]
1	1.76; 1.53	4.78 *t* (4.1)	4.74 *dd* (5.1/2.9)	1.78; 1.56
2	2.66; 2.61	3.14 *d* (4.1)	3.13 *m*	2.68; 2.62
5	2.36	2.71 *d* (10.5)	2.71 *dd* (11.0/<1.5)	2.38
6	α: 1.82; β: 1.89	α: 2.02; β: 1.82		1.89; 1.82
7	3.92 *t* (3.5)	3.89 *br s*	3.90 *m*	3.91 *br s*
9	2.05	2.53 *m*	2.51 *dd* (11.4/7.9)	2.08
11	1.74; 1.56	1.50; 1.47		1.74; 1.59
12	α: 1.61; β: 1.31	α: 1.80; β: 1.46		2.00; 1.50 (1.83; 1.58)
15	5.50 *dd* (3.0/1.7)	5.48 *br s*	5.52 *br s*	5.47 *m* (5.49 *m*)
16	2.26–2.18	2.16; 2.11		2.17; 2.12
17	1.94	1.71		1.74 (2.01)
18	1.02 *s*	0.99 *s*	0.98 *s*	1.09 *s* (1.03 *s*)
19	1.101[c] *s*	1.14 *s*	1.13[c] *s*	1.10 *s*
20	2.36	2.35 *m*		2.37 (2.20)
21	6.25 *d* (4.1)	4.74 *d* (3.3)	6.23 *d* (4.1)	5.29 *d* 3.3 (5.32 *d* 3.6)
22	α: 2.08; β: 1.70	2.05; 1.24		2.09; 1.30 (2.00; 1.60)
23	3.93 *ddd* (10.0/7.4/6.8)	4.68 *ddd* (10.4/8.4/4.8)	3.92 *m*	4.84 *ddd* (4.74 *td*)
24	2.67 *d* (7.6)	5.14 *d* (8.5)	2.67 *d* (7.4)	5.13 *d* (8.5) (5.22 *d* (9.1))
26	1.29 *s*	1.74 *s*	1.29 *s*	1.73 *s*
27	1.33 *s*	1.72 *s*	1.33 *s*	1.72 *s* (1.70 *s*)
28	1.49 *s*	1.50 *s*	1.50 *s*	1.49 *s*
29	1.43 *s*	1.50 *s*	1.50 *s*	1.44 *s*
30	1.103[c] *s*	1.11 *s*	1.12[c] *s*	1.10 s
$COCH_3$	2.06 *s*	2.10 *s*	2.08 *s*; 2.05 *s*	
OCH_3		3.38 *s*		

[a] 500 MHz.
[b] 300 MHz.
[c] Assignment exchangeable.

silica gel with solvents of increasing polarity (5–20% acetone in $CHCl_3$) to give the crude luvungin G (**8**), luvungin D (**5**) and luvungin A (**1**), which were then purified by reversed-phase RP-8 chromatography with MeOH–H_2O (7:3) yielding 40, 10 and 70 mg of these compounds, respectively. Frs 9–11 were subjected to silica gel chromatography, eluted with a gradient of $CHCl_3$-acetone (from 9:1 to 8:2) to give fractions containing limonin, 1α-acetoxyluvungin A (**3**), luvungin E (**6**) and luvungin F (**7**) (in order of increasing polarity). The fraction containing luvungin E (**6**) gave, after crystallisation from acetone, 100 mg pure compound, whereas the other fractions were separated with MeOH–H_2O (6:4) on RP-8 to afford 30 mg of limonin, 12 mg of 1α-acetoxyluvungin A (**3**) and 12 mg of luvungin F (**7**).

3.3.1. Luvungin A (*1*)

Needles: mp 187–189°C (*n*-hexane–acetone). R_f 0.26 ($CHCl_3$–acetone 9:1). $[\alpha]_D^{27.3}$ −1.20° (MeOH; *c* 0.33). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3551 (OH), 2932, 2872, 1737 (ester), 1714 (lactone), 1458, 1392, 1374, 1235, 1114, 1005, 953, 886. ESI–MS: *m/z* 567 $[M+Na]^+$. EI–MS (70 eV) *m/z* (rel. int.): 526 $[M-H_2O]^+$ (6), 484.3163 $[M-60]^+$ (calc. 484.3189, $C_{30}H_{44}O_5$) (8), 413 (12), 395 (9), 384 (11), 344.2398 **1a** (calc. 344.2351, $C_{22}H_{32}O_3$, (100), 268 (16), 159 (21), 145 (19), 99 (28), 91 (22), 81 (24), 69 (26), 55 (43).

Table 3
^{13}C NMR spectroscopic data of compounds **5–8** in $CDCl_3$ (75 MHz)

	5	6	7	8
1	70.9	156.3	156.1	70.9
2	34.9	120.1[a]	119.7	34.9
3	170.4	167.8	167.8	170.4
4	85.6	84.9	85.0	85.6
5	44.0	49.2	49.2	44.07
6	26.3	27.5	27.4	26.3
7	74.5	74.7	74.5	74.4
8	41.8	42.1	42.1	41.8
9	35.8	41.1	40.9	35.7
10	44.1	43.9	44.0	44.14
11	16.5	18.6	18.4	16.5
12	34.7	35.4	34.4	33.9
13	46.1	46.2	46.1	46.1
14	158.9	158.5	158.7	159.1
15	119.5	120.0[a]	119.5	119.1
16	34.8	34.9	35.0	34.9
17	52.0	52.4	54.1	53.9
18	19.2	20.8	20.3	18.9
19	15.2	15.9	15.9	15.2
20	35.7	35.8	36.3	36.4
21	69.9	70.0	64.1	64.2
22	36.1	36.2	37.9	37.8
23	64.3	64.3	67.8	68.0
24	86.4	86.6	80.6	80.6
25	74.0	74.2	76.2	76.2
26	28.4	28.6	22.4	22.3
27	23.8	24.0	26.2	26.2
28	34.3	32.0	31.9	34.3
29	23.5	26.2	26.2	23.5
30	27.1	26.9	26.9	27.2
$O\underline{C}OCH_3$	170.0	170.2	170.2	170.1
$O\underline{C}OCH_3$	169.8			169.8
$OCO\underline{C}H_3$	20.8	21.1	21.2	21.1
$OCO\underline{C}H_3$	21.1			20.8

[a] Signals are interchangeable.

3.3.2. Luvungin B (*2*)

Amorphous: R_f 0.55 ($CHCl_3$–acetone 9:1). $[\alpha]_D^{28.1}$ −40.1° (MeOH, *c* 0.326). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3546 (*br*, OH), 2932, 2873, 1735 (ester), 1715 (lactone), 1623 (C=C), 1456, 1393, 1376, 1235, 1118, 1025. EI–MS (70 eV) *m/z* (rel. int.): 558 $[M]^+$ (2), 540 $[M-H_2O]^+$ (2), 526 $[M-MeOH]^+$ (21), 498.3415 (calc. 498.3346, $C_{31}H_{46}O_5$) $[M-HOAc]^+$ (100), 403 $(\mathbf{2a}+H)^+$ (24), 402 **2a** (14), 362 (13), 342 (11), 328 (26), 159 (36), 145 (50), 133 (58), 119 (51), 107 (60), 95 (72), 81 (54), 69 (71).

3.3.3. 1α-Acetoxyluvungin A (*3*)

Oil: R_f 0.25 ($CHCl_3$–acetone 9:1). $[\alpha]_D^{29.2}$ −18.0° (MeOH; *c* 1.00). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3546 (OH), 2988, 2935, 2873, 1733 (ester), 1715 (lactone), 1393, 1375, 1234, 1191, 1118, 1025, 925, 889. ESI–MS: *m/z* 625 $[M+Na]^+$. EI–MS (70 eV) *m/z* (rel. int.): 542.3269 (calc. 542.3243, $C_{32}H_{46}O_7$, $[M-HOAc]^+$ (100), 527 $[M-HOAc-CH_3]^+$ (23), 524 $[M-HOAc-H_2O]^+$ (27), 509 (7), 483 (20), 469 (19), 429 (31), 411 (41), 402 **3a** (42), 393 (91), 375 (36), 369 (42), 351 (33), 342 [**3a**-HOAc]$^+$ (35).

3.3.4. Luvungin C (*4*)

Oil: R_f 0.26 ($CHCl_3$–acetone 9:1). $[\alpha]_D^{28.5}$ −20.3° (MeOH; *c* 0.66). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3549 (OH), 2935, 2872, 1711 (lactone), 1622 (C=C), 1453, 1392, 1375, 1295, 1265, 1236, 1114, 985. EI–MS (70 eV) *m/z* (rel. int.): 486.3365 $[M]^+$ ($C_{30}H_{46}O_5$, calc. 486.3345) (16), 468 $[M-H_2O]^+$ (38), 450 $[M-2H_2O]^+$ (10), 440 $[M-H_2O-CO]^+$ (52), 344 **4a** (41), 311 (21), 268 (22), 173 (41), 159 (60), 145 (68), 133 (63), 119 (65), 107 (72), 99 (87), 81 (64), 69 (77), 55 (100).

3.3.5. Luvungin D (*5*)

Needles: mp 181–183°C (acetone). R_f 0.22 ($CHCl_3$/acetone 9:1). $[\alpha]_D^{28.3}$ −42.0° (MeOH, *c* 1.00). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3490 (*br*, OH), 2989, 2934, 2860–2830, 1730 (ester), 1716 (lactone), 1375, 1236, 1121, 1073, 1025. ESI–MS: *m/z* 627 ($[M+Na]^+$). EI–MS (70 eV) *m/z* (rel. int.): 586.3482 (calc. 586.3506, $C_{34}H_{50}O_8$) $[M-H_2O]^+$ (57), 568 $[M-2H_2O]$ (15), 526 $[M-2H_2O-H_2C=C=O]$ (15), 484 $[M-2H_2O-2H_2C=C=O]$ (9), 466 (10), 445 $[\mathbf{5a}+H]^+$ (36), 444 **5a**, (100), 426 (11), 384 [**5a**-HOAc]$^+$ (25), 324 (21), 281 (11), 266 (12), 253 (14) 84 (67), 83 (100).

3.3.6. Luvungin E (*6*)

Amorphous: R_f 0.24 ($CHCl_3$–acetone 9:1). $[\alpha]_D^{28.8}$ 21.06° (MeOH; *c* 0.50). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3490 (*br*,

Table 4
^{1}H NMR spectroscopic data of compounds **5–8** in $CDCl_3$

	5[a]	**6**[b]	**7**[a]	**8**[b]
1	4.85 *dd* (6.7/1.2)	6.51 *d* (12.4)	6.50 *d* (12.5)	4.84 *dd* (5.8/2.2)
2	α: 3.17 *dd* (15.5/1.6) β: 3.12 *dd* (15.5/6.6)	5.89 *d* (12.4)	5.88 *d* (12.5)	3.13–3.14
5	2.51 *dd* (12.9/2.7)	2.47 *dd* (13.2/3.3)	2.47 *dd* (13.1/3.1)	2.50 *m*
6	α: 1.94; β: 1.89		α: 1.82; β: 1.97	
7	5.14 *dd* (3.5/1.2)	5.17 *br s*	5.17 *t* (2.7)	5.14 *br s*
9	2.53 *dd* (11.1/6.3)		2.19 *dd* (11.8/6.0)	
11	ca. 1.50; ca.1.45		1.77	
12	α: 1.83; β: 1.51		1.84; 1.66	
15	5.31 *dd* (4.0/1.4)	5.34 *m*	5.30 *dd* (3.4/1.2)	5.28 *m*
16	2.28 *ddd* (13.5/5.4/3.7); 1.97		2.26 *ddd* (14.6/5.9/3.7); 1.98	
17	1.97		1.90	
18	0.92 *s*	0.96 *s*	0.96 *s*	0.93 *s*
19	1.14 *s*	1.26[c] *s*	1.26 *s*	1.137[c] *s*
20	1.86		1.89	
21	α: 3.42 *dd* (11.6/2.4) β: 3.92 *br d* (12.1)	3.44 *dd* (11.6/2.2) 3.95 *d* (12.1)	α: 3.49 *dd* (12.8/4.0) β: 3.60 *dd* (12.8/2.8)	α: 3.48 *dd* (11.8/2.8) β: 3.59 *d* (11.8)
22	α: 1.52; β: 2.02		α: 1.97; β: 1.60	
23	3.85 *ddd* (11.0/9.2/4.7)	3.87 *m*	3.80 *td* (9.1/3.0)	3.80 *td* (9.2/3.0)
24	2.88 *d* (9.2)		3.42 *d* (9.2)	3.42 *d* (8.8)
26	1.30 *s*	1.32 *s*	1.16 *s*	1.17 *s*
27	1.25 *s*	1.28 *s*	1.31 *s*	1.30 *s*
28	1.40 *s*	1.37 *s*	1.37 *s*	1.39 *s*
29	1.50 *s*	1.45 *s*	1.45 *s*	1.50 *s*
30	1.18 *s*	1.20[c] *s*	1.18 *s*	1.143[c] *s*
$COCH_3$	2.11 *s*; 1.99 *s*	1.98 *s*	1.98 *s*	2.10 *s*; 1.99 *s*

[a] 500 MHz.
[b] 300 MHz.
[c] Assignment exchangeable.

OH), 2934, 2864, 1719 (*br*, ester), 1691 (*br*, lactone), 1631 (C=C), 1375, 1255, 1171, 1127, 1110, 1074, 921. EI–MS (70 eV) *m*/*z* (rel. int.): 544 $[M]^+$ (3), 526.3272 (calc. 526.3294, $C_{32}H_{46}O_6$) $[M-H_2O]^+$ (100), 508 $[M-2H_2O]$ (21), 486 (6), 468 (22), 455 (14), 426 (15), 384 **6a** (75), 324 [**6a**-HOAc]$^+$ (48), 157 (28), 59 (23).

3.3.7. *Luvungin F (7)*

Oil: R_f 0.17 ($CHCl_3$–acetone 9:1). $[\alpha]_D^{25.1}$ 18.5° (MeOH; *c* 1.00). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3580–3370 (OH), 2987, 2935, 2875, 2836, 1719 (ester), 1689 (lactone), 1631 (C=C), 1457, 1374, 1256, 1236, 1128, 1078, 1036, 1025, 986, 921. ESI–MS: *m*/*z* 567 $[M+Na]^+$, 545.3 $[M]^+$. EI–MS (70 eV) *m*/*z* (rel. int.): 544 $[M]^+$ (2), 526.3273 (calc. 526.3294, $C_{32}H_{46}O_6$ $[M-H_2O]^+$) (11), 508 [M-2 H_2O] (2), 486 (6), 468 (37), 454 (25), 426 (16), 411 (28), 393 (24), 384 **7a** (56), 325 (44), 324 [**7a**-HOAc]$^+$ (35), 241 (59), 223 (43), 159 (66), 157 (76), 145 (60), 131 (50), 119 (47), 105 (49), 91 (44) 59 (100).

3.3.8. *Luvungin G (8)*

Oil: R_f 0.15 ($CHCl_3$–acetone 9:1). $[\alpha]_D^{26.9}$ −29.9° (MeOH; *c* 1.00). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3550–3400 (OH), 2985, 2935, 2876, 1730–1715 (ester, lactone), 1457, 1375, 1255–1236, 1121, 1075, 1025, 986. ESI–MS: *m*/*z* 627 $[M+Na]^+$. EI–MS (70 eV) *m*/*z* (rel. int.): 586.3424 (calc. 586.3505, $C_{34}H_{50}O_8$) $[M-H_2O]^+$ (18), 571 $[M-H_2O-CH_3]^+$ (18), 528 (7), 514 (24), 453 (12), 444 **8a** (20), 393 (20), 335 (15), 253 (17), 197 (20), 171 (19), 159 (22), 145 (25), 131 (20), 113 (87), 101 (29), 84 (40), 69 (24), 59 (100).

Acknowledgements

Two of us (T.P.L. and C.K.) are indebted to the Deutscher Akademischer Austauschdienst (DAAD), Bonn, Germany, and the Alexander von Humboldt-foundation, Bonn, Germany, respectively, for a grant.

References

Guang-Yi, L., Gray, A.I., Waterman, P.G., 1988. Tirucallane and oleanane triterpenes from the resin of *Avcoumea lelaineana*. Phytochemistry 27, 2283–2286.

Ho, P.H., 1992. Cay co Vietnam (An Illustrated Flora of Vietnam), Vol. 2. Mekong Printing, Santa Ana, Canada.

Itokawa, H., Kishi, E., Morita, H., Takeya, K., 1992. Cytotoxic quassinoids and tirucallane-type triterpens from the woods of *Eurycoma longifolia*. Chemical and Pharmaceutical Bulletin 40, 1053–1055.

Kitagawa, I., Mahmud, T., Simanjuntak, P., Hori, K., Uji, T., Shibuya, H., 1994. Indonesian medicinal plants. VIII. Chemical structures of three new triterpenoids, bruceajavanin A, dihydrobruceajavanin A,

and bruceajavanin A, and a new alkaloidal glycoside, bruceacanthinoside, from the stems of *Brucea javanica* (Simaroubaceae). Chemical and Pharmaceutical Bulletin 42, 1416–1421.

Klass, J., Tinto, W.F., McLean, S., Reynolds, W.F., 1992. Friedelan triterpenoids from *Peritassa compta*: complete ^{1}H and ^{13}C assignment by 2D NMR spectroscopy. Journal of Natural Products 55, 1626–1630.

Nes, W.D., Wong, R.Y., Benson, M., Landrey, J.R., Nes, W.R., 1984. Rotational isomerism about the 17(20)-bond of steroids and euphoids as shown by crystal structures of euphol and tirucallol. Proceedings of the National Academy of Sciences of the United States of America 81, 5896–5900.

Perry, L.M., 1980. Medicinal Plants of East and Southeast Asia. MIT Press, Cambrige.

Rashid, M.A., Armstrong, J.A., Gray, A.I., Waterman, P.G., 1992. Novel *C*-geranyl-7-hydroxycoumarins from the aerial parts of *Eriostemon tomentellus*. Zeitschrift für Naturforschung 47b, 284–287.

Sondengam, B.L., Kamga, C.S., Kimbu, S.F., Connolly, J.D., 1981. Proceranone, a new triterpenoid from *Carapa procera*. Phytochemistry 20, 173–174.

Sugimoto, T., Miyase, T., Kuroyanagi, M., Ueno, A., 1988. Limonoids and quinolone alkaloids from *Evodia rutaecarpa* BENTHAM. Chemical and Pharmaceutical Bulletin 36, 4453–4461.

Wijeratne, E.M.K., Bandara, B.M.R., Gunatilaka, A.A.L., Tezuka, Y., Kikuchu, T., 1992. Chemical constituents of three Rutaceae species from Sri Lanka. Journal of Natural Products 55, 1261.