Inocalophyllins A, B and Their Methyl Esters from the Seeds of *Calophyllum inophyllum*

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Fractionation of the ethanolic extract of the seeds of *Calophyllum inophyllum* L. has resulted in the isolation of four novel pyranocoumarin derivatives, designated as inocalophyllins A (1), B (2) and their methyl esters (3, 4) in addition to the known calophyllolide. The structures of these compounds have been determined on the basis of spectroscopic analysis including MS, heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC) and two dimensional incredible natural abundance double quantum transfer experiment (2D-INADEQUATE). Two new methylated products, 5 and 6 were also prepared by methylation of compounds 1 and 2, respectively.

Key words *Calophyllum inophyllum*; Hypericaceae; pyranocoumarin; inocalophyllin A; inocalophyllin B; inocalophyllin methyl ester

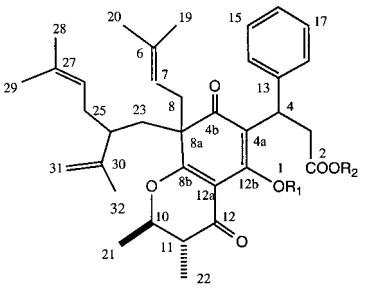
Natural source-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infections have recently been reviewed.[1—3] Coumarins such as calanolides and inophyllums, isolated from plants of the genus *Calophyllum* are novel non-nucleoside inhibitors of HIV type 1 reverse transcriptase. Calanolides A and B were reported to be the most active in the cell-based anti-HIV-1 assay. (+) Calanolide A fully protected human T-lymphoblastic cells from the cytopathogenic effects of HIV-1 and had no cytotoxic effects. The calanolides isomers were also found to be effective against a wide spectrum of drug-resistant HIV strains isolated from patients' T-cells. Due to the novelty of bioactivity, calanolide A was chosen as a candidate for multicenter Phase II clinical trials on patients with HIV infection in the United States and in Malaysia. It was suggested that calanolides may be promising candidates for combination therapy with either nucleoside AZT and/or protease inhibitors against HIV in patients.

Calophyllum inophyllum L. (Hypericaceae) is a tropical evergreen shrub growing along the southern coast of Taiwan. This species was cultivated for ornamental and medicinal purposes. It has been used in Chinese traditional medicine for the treatment of rheumatism, skin infections, wounds, leprous nephritis, pain, eye diseases and inflammations.[4] Extensive chemical investigation of this species has resulted in the isolation of a wide variety of natural products: xanthones, benzodipyranones, coumarins and other interesting bioactive compounds.[5—8] Previous study on leaves and stems of *C. inophyllum* discovered new pyranocoumarins such as inophyllums.[9,10] Very few studies, however, have been carried out on the constituents of its seeds. In our search for a renewable and practical source of calanolides from the seeds of *C. inophyllum* and study of the structures and activity relationship of pyranocoumarins,[11] we herein report the isolation and structural elucidation of four novel pyranocoumarins, designated inocalophyllins A (**1**), B (**2**), inocalophyllin A, B methyl esters (**3**, **4**), from the seeds of this species.

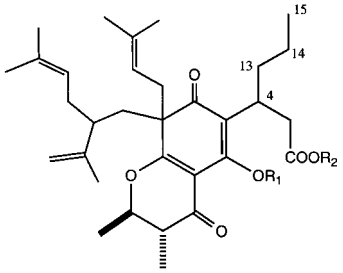
Results and Discussion

The ethanolic extract of *C. inophyllum*, after solvent partition, silica gel and Sephadex LH-20 column chromatography, and preparative TLC purification furnished the known calophyllolide and four new compounds, inocalophyllins A (**1**), B (**2**), inocalophyllin A, B methyl esters (**3**, **4**), which represent a new class of pyranocoumarin derivatives.

Inocalophyllin A (**1**), $[\alpha]_D$ −169° (CH_2Cl_2), was obtained as an amorphous solid. The high resolution FAB-MS provided a quasi-molecular ion ($[M+H]^+$) at *m/z* 561.3209, consistent with a molecular formula of $C_{35}H_{45}O_6$. The ^{1}H-NMR spectrum (Table 1) displayed signals for a phenolic proton (δ 12.3, s), a benzyl group (δ 7.38, d; δ 7.17, t; δ 7.07, t), four olefinic protons (δ 4.36, s; δ 4.46, s; δ 4.52, m; δ 4.84, m), a pair of methylene double doublets (δ 3.33, 3.24, H-3), five olefinic methyl singlets (δ 1.63, 1.49×2, 1.42; δ 1.38) and two methyl doublets at δ 1.11 (J=6.8 Hz, Me-22) and at δ



1 $R_1 = R_2 = H$
3 $R_1 = H$, $R_2 = Me$
5 $R_1 = R_2 = Me$



2 $R_1 = R_2 = H$
4 $R_1 = H$, $R_2 = Me$
6 $R_1 = R_2 = Me$

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Table 1. ^{1}H-NMR ($CDCl_3$, 300 MHz)[a] Spectral Data of Compounds **1**—**4**

H	1	2	3	4
3	3.24 (dd, 16.2, 7.2)	2.71 (dd, 16.0, 7.0)	3.20 (dd, 15.8, 9.2)	2.67 (dd, 16.0, 6.6)
	3.33 (dd, 16.3, 8.6)	2.78 (dd, 16.0, 7.5)	3.40 (dd, 15.8, 9.2)	2.85 (dd, 16.0, 8.0)
4	4.82 m	3.44 m	4.88 (t, 7.1)	3.49 m
7	4.84 m	4.87 m	4.88 m	4.90 m
8	1.95 m, 2.10 m			
10	4.03 m	4.07 m	4.05 m	4.12 m
11	2.42 m	2.45 m	2.42 m	2.45 m
13		1.21 m		1.23 m
14	7.38 (d, 7.3)	1.21 m	7.39 (d, 7.4)	1.23 m
15	7.17 (t, 7.3)	0.83 (t, 7.3)	7.21 (t, 7.4)	0.84 (t, 7.2)
16	7.07 (t, 7.3)		7.11 (t, 7.2)	
17	7.17 (t, 7.3)		7.21 (t, 7.4)	
18	7.38 (d, 7.3)		7.39 (d, 7.4)	
19	1.63 s		1.66 s	1.54 s
20	1.49 s	1.62 s	1.53 s	1.62 s
21	1.51 (d, overlap)	1.53 (d, overlap)	1.53 (d, overlap)	1.58 (d, 4.6)
22	1.11 (d, 6.8)	1.15 (d, 6.8)	1.13 (d, 6.8)	1.14 (d, 6.9)
23	1.84 m			
24	1.86 m			
25	2.40 m, 2.46 m		2.42 m, 2.48 m	
26	4.52 m	4.69 t (7.0)	4.56 m	4.72 m
28	1.38 s			
29	1.42 s		1.40 s	
31	4.36 s, 4.46 s	4.55 br s	4.42 s, 4.49 s	4.55 br s
32	1.49 s		1.45 s	1.54 s
12[b]-OH	12.3 s	12.20 s	12.30 s	12.20 s
OMe			3.60 s	3.58 s

a) δ in ppm (J in Hz); TMS as internal standard. *b*) Assignment made by COSY.

1.51 (Me-21). The ^{13}C-NMR spectrum of **1** (Table 2) exhibited signals for two conjugated ketone carbonyls (δ 194.6, 196.6), an acid carbonyl (δ 178.6), a benzyl group (δ 143.3, s; 127.6, d; 127.7, d; 125.6, d), seven methyl groups (δ 25.6, $\times$2, 19.1, 18.7, 17.7, 17.5, 9.2) and an additional ten olefinic carbons, of which two are oxygenated singlets (δ 185.6, 166.8) and one is a methylene triplet (δ 112.0). The structure of **1** was deduced using correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond connectivity (HMBC) experiments. The COSY spectrum of **1** established the connectivities of H-3/H-4 (δ 4.82, m), Me-21/H-10 (δ 4.03, m)/H-11 (δ 2.42, m)/Me-22, H-24/H-25 (δ 4.52, m) and H-23/H-29. Because of serious overlapping signals in ^{1}H-NMR spectrum, the structure of **1** was difficult to elucidate until high resolution NMR measurement and detailed analysis of its HMBC spectra. The structure could be divided into three parts, a, b and c (Fig. 1). For part a, the phenolic hydroxyl proton (δ 12.3) was correlated to carbon resonances at δ 105.3 (C-12a, s) and at δ 113.4 (C-4-a, s); the latter was correlated with the methine proton at δ 4.82 (H-4). The H-4 was, in turn, correlated with additional carbons at δ 166.8 (C-12b), 194.6 (C-4b) and δ 178.6 (C-2), together with the methylene carbon at δ 36.1 (C-3). The corresponding methylene protons (H-3) were found to correlate with the acid carbonyl carbon (δ 178.6) and the benzyl carbon at δ 143.3 (C-13, s), which was also correlated with H-4 and H-17. For part b, the proton at δ 4.03 was placed at C-10, based on its correlations with C-21 (δ 19.1, q), C-22 (δ 9.2, q) and C-12 (δ 196.6, s). In the meanwhile, the methyl doublet at δ 1.12 was correlated to those carbons at δ 81.1 (C-10) and δ 43.6 (C-11), whose proton (δ 2.42) was also correlated to the conjugated carbonyl singlet at δ 196.6. The remaining correlations provided the gross structure of part c, which contains three isoprene units. Correlations from the methyl proton signals at δ 1.42 (Me-28) and δ 1.38 (Me-27), and the methylene protons at δ 2.40 and 2.46 (H-24) to the quaternary sp^2 carbon at δ 134.6 and to a tertiary sp^2 carbon at δ 117.2 allowed determination of the side chain C-24/C-25/C-26/C-27/C-28. In a similar manner, the second isoprene unit was deduced from correlations of Me-31 (δ 1.49) and Me-32 (δ 1.63) with the methine carbon at δ 121.9 (C-29) and the quaternary carbon at δ 132.1 (C-30). The third isoprene unit consists of a terminal double bond, whose carbons (δ 112, t, C-19; 146.9, s, C-6) were correlated with a methyl singlet at δ 1.49. The methylene protons at δ 2.10 (H-8) were found to correlate with C-6, C-8a (δ 57.0, s) and C-8b (δ 185.6, s).

Upon methylation with diazomethane compound **1** afforded a dimethylate (**5**), which showed one methyl ester singlet at δ 3.58 and one methyl ether singlet at δ 3.68. The HMBC data of **5** also agreed with the proposed structure of **1**. The correlations of H-8/C-4b, C-8a and H-24/C-8a together with the above mentioned correlation of H-4/C-4b allowed the connection of part a and part c. Although the HMBC spectra of **1** and **5** did not show any correlation between H-10 (δ 4.03) and C-8b due to the low sensitivity, the appearance of a pyranone ring in **1** was reasonable. Apart from assembling the structure **1** by HMBC analysis, the 2D-INADEQUATE (Incredible Natural Abundance Double Quantum Transfer Experiment)[12] of **2** also confirmed the carbon skeleton of **1** and **2**, which showed connectivities between the following salient points: C-8/C-8a, C-8a/C-4b, C-8a/C-8b, C-8a/C-23, C-8/C-7, C-23/C-24, C-24/C-30 (Fig. 2). The stereochemistry at C-10 and C-11 in **1** was deter-

Table 2. ^{13}C-NMR Spectral Data[a] (75 MHz, $CDCl_3$) for Compounds **1**—**4**

C#.	1	2	3	4
2	178.6 s	179.2 s	173.5 s	174.0 s
3	36.1 t	37.7 t	36.1 t	37.7 t
4	34.9 t	30.1 d	35.3 d	30.6 d
4a	113.4 s	112.5 s	113.7 s	112.6 s
4b	194.6 s	195.0 s	194.6 s	194.9 s
6	134.6 s	134.6 s	134.7 s	134.6 s
7	117.2 d	117.5 d	117.4 d	117.7 d
8	41.6 t	41.5 t	41.6 t	41.4 t
8a	57.0 s	57.0 s	57.1 s	57.0 s
8b	185.6 s	185.5 s	185.7 s	185.4 s
10	81.1 d	81.1 d	81.2 d	81.1 d
11	43.6 d	43.7 d	43.8 d	43.8 d
12	196.6 s	196.6 s	196.8 s	196.7 s
12a	105.3 s	105.4 s	105.6 s	105.7 s
12b	166.8 s	167.3 s	166.9 s	167.2 s
13	143.3 s	34.6 t	143.7 s	34.8 t
14	127.6 d	20.9 t	127.8 d	21.0 t
15	127.7 d	14.1 q	127.8 d	14.2 q
16	125.6 d		125.7 d	
17	127.7 d		127.8 d	
18	127.6 d		127.8 d	
19	17.5 q	17.7 q	17.6 q	17.8 q
20	25.6 q	25.7 q	25.8 q	25.7 q
21	19.1 q	19.1 q	19.2 q	19.2 q
22	9.2 q	9.3 q	9.4 q	9.3 q
23	41.5 t	41.3 t	41.6 t	41.4 t
24	44.2 d	44.3 d	44.4 d	44.4 d
25	33.9 t	34.0 t	34.0 t	34.0 t
26	121.9 d	121.9 d	122.1 d	122.0 d
27	132.1 s	132.3 s	132.3 s	132.3 s
28	25.6 q	25.6 q	25.7 q	25.7 q
29	17.7 q	17.7 q	17.9 q	17.8 q
30	146.9 s	147.1 s	147.0 s	147.2 s
31	112.0 t	112.0 t	112.4 t	112.2 t
32	18.7 q	18.8 q	18.7 q	18.7 q
OMe			51.4 q	51.2 q

a) Mutiplicities were obtained from HSQC.

mined by comparison of chemical shifts and coupling constants of **1** with the previous reported pyranocoumarins such as calanolide A, inophyllum C and inophynones, suggesting that they had the same relative stereochemistry at C-10 and C-11.[9,10]

Inocalophyllin B (**2**) was obtained as an amorphous powder, $[\alpha]_D$ −95° (CH_2Cl_2). A molecular formula of $C_{32}H_{46}O_6$ was established for a molecular ion ($[M]^+$) at *m/z* 526 in its electron impact (EI)-MS and *quasi*-molecular ions at *m/z* 549 ($[M+Na]^+$) and *m/z* 527 ($[M+H]^+$) in its FAB-MS. The ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) of **2** resembled those of **1** except that the signals for the benzyl group in **1** were missing in **2**. Instead, signals for a *n*-propyl group (δ 0.83, t, 14.1, q, CH_3; 1.21, m, 20.9, t; 34.6, t, CH_2CH_2) were observed at C-4 position in **2**. Detailed analysis of the HMBC data of **2** revealed that correlation spots of **1** and **2** were similar. Notable differences appeared only in the side chain at C-4 position. The EI-MS fragmentation of compounds **1** and **2** also displayed similar fragment ions, including the base peak at *m/z* 69 ($C_5H_9^+$). Other important fragments appeared at *m/z* 55 ($C_4H_7^+$), *m/z* 135 ($C_{10}H_{15}^+$), *m/z* 335, 390 and *m/z* 457 (Fig. 3). Methylation of **2** yielded a product (**6**), which showed one carbomethyl ester at δ 3.59 (s) and one aromatic methyl ether at δ 3.98 (s) in the ^{1}H-NMR spectrum. All the spectral data of **2** agreed with the

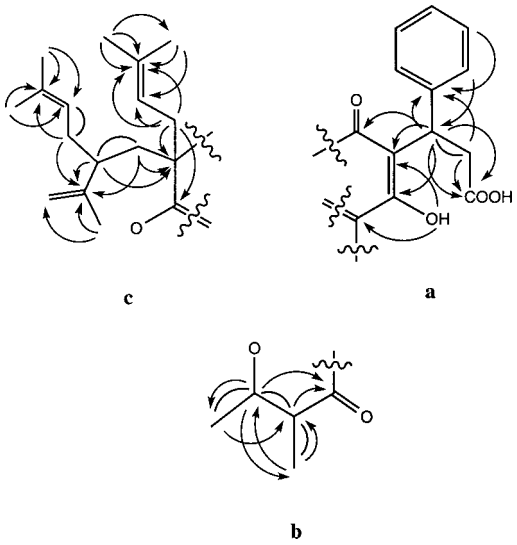

Fig. 1. Partial Structures of Inocalophyllin A (**1**), HMBC (Arrow) and COSY (Curve)

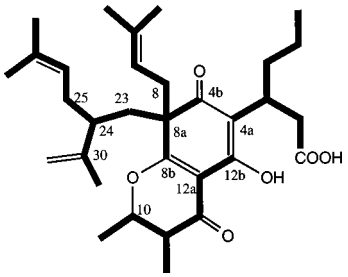

Fig. 2. Carbon–Carbon Correlations (2D-INADEQUATE) of Compound **2**

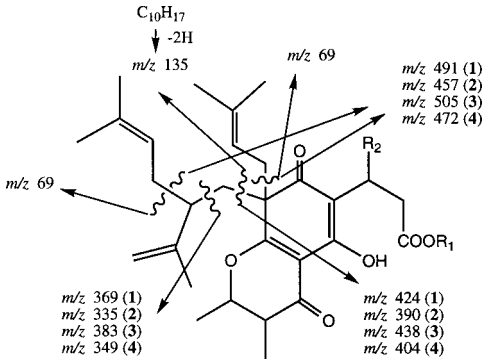

Fig. 3. EI-MS Fragmentation of Compounds **1**—**4**

structural assignment of **2**.

Inocalophyllin A methyl ester (**3**), $[\alpha]_D$ −122° (CH_2Cl_2), was obtained as an amorphous solid. The molecular formula of $C_{36}H_{46}O_6$ was derived from a *quasi*-molecular ion ($[M-H]^-$) at *m/z* 573.3242 in the negative high resolution (HR)-FAB-MS of **3**, clearly indicating that the molecule was 14 mass units larger than **1**. The ^{1}H- and ^{13}C-NMR data of **3** were superimposable with those of **1** except that **3** contained an extra methyl ester at δ 3.60 and δ 51.4, suggesting that it was a close analogue of **1**. This finding was supported by the HMBC spectrum of **3**, in which the methoxyl proton was correlated with the carbonyl C-2 at δ 173.5. The EI-MS fragmentation pattern also was consistent with the proposed

structure of **3** (Fig. 3). The similar chemical shifts, coupling constants and specific rotation of **3** and **1** were suggestive of their identical stereochemistry.

Inocalophyllin B methyl ester (**4**), $[\alpha]_D$ −138° (CH_2Cl_2) had the molecular formula $C_{33}H_{48}O_6$ as deduced from a combination of EI- and FAB-MS, as well as HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra (Tables 1, 2) displayed signals similar to those of **2** and **3**, suggesting that it was a close analogue of **2** and **3**. However, in its EI-MS spectrum, compound **4** was 14 mass units greater than that of **2**, indicating the presence of a methyl ester in **4** instead of an acidic group in **2**. Indeed, the ^{1}H- and ^{13}C-NMR data of **4** exhibited a methyl ester group at δ 3.58, 174.0 and 51.2, further supporting the structure assigned for this compound. The fragment ions at *m/z* 55, *m/z* 69, *m/z* 349 and *m/z* 472 in **4** also confirmed its structure (Fig. 3). On the basis of all spectral evidence, the structure was established as **4**.

The structures established for **1**—**6** represent a new class of pyranocoumarin derivatives. They contain an isoprene unit and a monoterpene group at C-8a position of the unique pyranocoumarin ring system. The appearance of these novel compounds and calophyllolide in the seeds of *Calophyllum inophyllum* is quite unusual. It is of significance that inocalophyllins A—D may play a role in protecting the seeds of *Calophyllum inophyllum* from feeding birds and animals. On the other hand, the relationship of inocalophyllins A—D and calanolides may be of interest from the biogenetic point of view.

Experimental

Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and Hitachi U-3210 spectrophotometers, respectively. EI-MS and FAB-MS were recorded on a VG Quattro 5022 mass spectrometer. FAB-MS spectra were taken on a JEOL JMS-SX 102 mass spectrometer. The ^{1}H-, ^{13}C-NMR, distortionless enhancement by polarization transfer (DEPT), COSY, HSQC, HMBC and nuclear Overhauser effect spectroscopy (NOESY) experiments were recorded on a Bruker FT-300 and a Varian FT-500 spectrometer. The 2D-INADEQUATE spectrum was measured on a Bruker FT-600 NMR instrument.

Plant Material The seeds of *Calophyllum inophyllum* were collected in February 2000, in Kaohsiung, Taiwan. The plant was identified by one of the authors (Y. C. Shen). A voucher specimen has been deposited in the Institute of Marine Resources, National Sun Yat-sen University.

Extraction and Isolation The fresh seeds (210 g) were ground and extracted with EtOH (850 ml×2). The combined EtOH extracts were concentrated to a brown residue (79 g). After diluting with H_2O (500 ml), the resulting suspension was partitioned with an equal volume of EtOAc. The EtOAc-soluble fraction was concentrated under vacuum to give a residue (15 g), which was applied to a silica gel column (400 g) and eluted with a mixture of *n*-hexane/EtOAc of increasing polarity (10 : 1; 7 : 1; 5 : 1; 4 : 1; 3 : 1; 2 : 1) to give nine fractions, I (1.85 g), II (836 mg), III (4.7 g), IV (1 g), V (2.06 g), VI (2.65 g), VII (2.24 g) and VIII (1.41 g). Fraction VII (2.24 g) was rechromatographed on a silica gel (35 g) column and eluted with the solvent mixture *n*-hexane–$CHCl_3$–MeOH (150 : 150 : 1; 50 : 50 : 1; 20 : 20 : 1; 10 : 10 : 1; 5 : 5 : 1) to yield inocalophyllin A (**1**, 357 mg). Fraction V (2.06 g) was chromatographed on a silica gel (60 g) column and eluted with the solvent mixture *n*-hexane–$CHCl_3$–MeOH (100 : 100 : 1; 50 : 50 : 1; 40 : 40 : 1; 15 : 15 : 1) to yield inocalophyllin B (**2**, 570 mg). Fraction VI (2.65 g) was chromatographed on a silica gel (50 g) column and eluted with *n*-hexane–acetone (100 : 1; 50 : 1; 25 : 1; 10 : 1; 7 : 1; 5 : 1) to yield three fractions A (31 mg), B (9 mg) and C (12.4 mg). Fractions A, B and C were applied, respectively, to a silica gel TLC plate using *n*-hexane/EtOAc (65 : 35 or 3 : 1) as solvent to give inocalophyllin A methyl ester (**3**, 8.4 mg), inocalophyllin B methyl ester (**4**, 2.4 mg) and calophyllolide (2.7 mg).[13)]

Inocalophyllin A (**1**): Amorphous solid; $[\alpha]_D^{25}$ −169° (c=0.05, CH_2Cl_2); UV λ_{max} (log ε) (MeOH) nm: 220 (3.92), 314 (3.46); IR (neat) ν_{max} 3745, 2983, 2964, 1714, 1707, 1620, 1556, 1446, 1417, 1377, 1288, 1142 cm^{-1}; ^{1}H- and ^{13}C-NMR: Tables 1 and 2, respectively; FAB-MS *m/z*: 561 $[M+H]^+$, 583 $[M+Na]^+$; EI-MS *m/z* (rel. int.) 561 ($[M+H]^+$, 2) 560 ($[M]^+$, 1.5), 545 ($[M-CH_3]^+$, 1), 491 ($[M-C_5H_9]^+$, 1.5), 457 (5), 437 (1, $[M-C_5H_9-C_4H_7+H]^+$), 424 (4, $[M-C_{10}H_{17}+H]^+$), 390 (12), 369 (51, $[M-C_{10}H_{17}-C_4H_7+H]^+$), 351 (28), 335 (63), 317 (32), 309 (15), 275 (36), 135 (11, $C_{10}H_{15}$), 107 (30), 69 ($[C_5H_9]^+$, 100), 55 ($[C_4H_7^+]$, 38); HR-FAB-MS *m/z* 561.32091 ($[M+H]^+$, Calcd for $C_{35}H_{45}O_6$, 561.32161).

Inocalophyllin A Dimethylate (**5**): Methylation of inocalophyllin A (**1**, 50 mg) with excess CH_2N_2 (5 °C) and usual work-up gave inocalophyllin A dimethylate (**5**, 41 mg); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.23 (1H, dd, J=16.3, 8.5 Hz, H-3A), 3.29 (1H, dd, J=16.3, 8.0 Hz, H-3B), 4.82 (1H, m, H-4), 4.87 (1H, m, H-7), 2.04, 2.11 (2H, m H-8), 4.02 (1H, m, H-10), 2.37 (1H, m, H-11), 1.64 (1H, s, H-19), 1.52 (1H, s, H-20), 1.48 (3H, d, J=6.3 Hz, H-21), 1.12 (1H, d, J=6.9 Hz, H-22), 4.35 (1H, t, J=8 Hz, H-26), 1.35 (1H, s, H-28), 1.37 (1H, s, H-29), 4.46 (1H, s, H-31A), 4.48 (1H, s, H-31B), 1.57 (1H, s, H-32), 3.58 (3H, s, COOMe), 3.68 (3H, s, 1-OMe); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 173.1 (s, C-2), 36.8 (t, C-3), 36.4 (s, C-4), 122.8 (s, C-4a), 198.0 (s, C-4b), 134.5 (s, C-6), 117.2 (t, C-7), 42.7 (t, C-8), 57.6 (s, C-8a), 183.0 (s, 8b), 80.8 (d, C-10), 45.4 (d, C-11), 188.8 (s, C-12), 108.4 (s, 12a), 168.2 (s, 12b), 142.8 (s, C-13), 127.8 (d, C-14, 18), 127.9 (d, C-15, 17), 125.8 (d, C-16), 17.4 (q, C-19), 25.7 (q, C-20), 19.0 (q, C-21), 10.5 (q, C-22), 40.7 (t, C-23), 44.8 (d, C-24), 33.3 (t, C-25), 122.0 (d, C-26), 132.1 (s, C-27), 25.4 (q, C-28), 17.8 (q, C-29), 146.4 (s, C-30), 112.6 (t, C-31), 18.3 (q, C-32), 60.5 (q, 1-OMe), 51.3 (q, COO<u>C</u>H$_3$).

Inocalophyllin B (**2**): Amorphous solid; $[\alpha]_D^{25}$ −95° (c=0.05, CH_2Cl_2); IR (neat) ν_{max} 3737, 2989, 2964, 1707, 1660, 1622, 1554, 1437, 1417, 1379, 1290, 1142 cm^{-1}; ^{1}H- and ^{13}C-NMR data: Tables 1 and 2, respectively; FAB-MS *m/z*: 527 $[M+H]^+$, 549 $[M+Na]^+$; EI-MS *m/z* (rel. int.) 527 ($[M+H]^+$, 6), 526 ($[M]^+$, 2), 511 (1, $[M-CH_3]^+$), 457 ($[M-C_5H_9]^+$, 6), 390 ($[M-C_{10}H_{17}+H]^+$, 11), 335 ($[M-C_{10}H_{17}-C_4H_7-H]^+$, 99), 317 ($[M-C_{10}H_{17}-C_4H_7-H_2O-H]^+$, 56), 275 (48), 233 (17), 177 (16), 135 (11, $C_{10}H_{15}$), 69 ($[C_5H_9]^+$, 100), 55 ($[C_4H_7^+]$, 35).

Inocalophyllin B Dimethylate (**6**): Methylation of inocalophyllin B (**2**, 45 mg) with excess CH_2N_2 (5 °C) and usual work-up gave inocalophyllin B dimethylate (**6**, 30 mg); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.85 (1H, m, H-3A), 2.67 (1H, m, H-3B), 3.47 (1H, m, H-4), 4.87 (1H, m, H-7), 2.05, 2.10 (2H, m H-8), 4.05 (1H, m, H-10), 2.40 (1H, m, H-11), 1.47 (1H, s, H-19), 1.62 (1H, s, H-20), 1.53 (3H, d, J=6.3 Hz, H-21), 1.15 (1H, d, J=6.8 Hz, H-22), 4.69 (1H, t, J=7.3 Hz, H-26), 1.51 (1H, s, H-28), 1.54 (1H, s, H-29), 4.55 (1H, br s, H-31), 1.62 (1H, s, H-32), 3.59 (3H, s, COOMe), 3.98 (3H, s, 1-OMe); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 174.1 (s, C-2), 36.8 (t, C-3), 30.1 (s, C-4), 111.1 (s, C-4a), 195.0 (s, C-4b), 134.6 (s, C-6), 117.5 (t, C-7), 41.4 (t, C-8), 57.1 (s, C-8a), 184.6 (s, 8b), 81.0 (d, C-10), 43.7 (d, C-11), 190.1 (s, C-12), 106.6 (s, 12a), 169.0 (s, 12b), 34.6 (t, C-13), 20.9 (t, C-14), 14.1 (q, C-15), 17.6 (q, C-19), 25.1 (q, C-20), 19.1 (q, C-21), 9.3 (q, C-22), 41.3 (t, C-23), 44.3 (d, C-24), 34.0 (t, C-25), 121.9 (d, C-26), 132.3 (s, C-27), 25.7 (q, C-28), 17.7 (q, C-29), 147.1 (s, C-30), 112.0 (t, C-31), 18.8 (q, C-32), 61.0 (q, 1-OMe), 53.2 (q, COO<u>C</u>H$_3$).

Inocalophyllin A Methyl Ester (**3**): Amorphous solid; $[\alpha]_D^{25}$ −122° (c=0.05, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) nm: 222 (3.90), 315 (3.45) ; IR (neat) ν_{max} 3752, 2983, 2927, 2916, 1734, 1716, 1622, 1556, 1541, 1456, 1435, 1417, 1375, 1288, 1165 cm^{-1}; ^{1}H- and ^{13}C-NMR data: Tables 1 and 2, respectively; FAB-MS *m/z*: 575 $[M+H]^+$, 597 $[M+Na]^+$; EI-MS *m/z* (rel. int.) 574 (0.1, $[M]^+$), 559 (0.3, $[M-CH_3]^+$), 505 (3, $[M-C_5H_9]^+$), 451 (1, $[M-C_5H_9-C_4H_7+H]^+$), 438 (5, $[M-C_{10}H_{17}+H]^+$), 383 (43, $[M-C_{10}H_{17}-C_4H_7+H]^+$), 365 (3), 351 (27), 349 (22), 309 (20), 275 (23), 253 (13), 221 (29), 121 (81), 107 (15), 91 (21), 83 (28), 69 (100, $C_5H_9^+$), 55 (39, $C_4H_7^+$); negative HR-FAB-MS *m/z* 573.3242 ($[M-H]^+$, Calcd for $C_{36}H_{45}O_6$, 573.3239).

Inocalophyllin B Methyl Ester (**4**): Amorphous solid; $[\alpha]_D^{25}$ −138° (c=0.05, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) nm: 314 (3.45); IR (neat) ν_{max} 3743, 2981, 2927, 2920, 1736, 1660, 1622, 1556, 1543, 1454, 1435, 1417, 1377, 1288, 1167, 1142 cm^{-1}; ^{1}H- and ^{13}C-NMR data: Tables 1, 2, respectively; FAB *m/z*: 563 $[M+Na]^+$, 541 $[M+H]^+$; EI-MS *m/z*: 541 (2, $[M+H]^+$), 540 (3, $[M]^+$), 525 (1, $[M-CH_3]^+$), 472 (5, $[M-C_5H_9]^+$), 404 (9, $[M+C_{10}H_{17}+H]^+$), 349 (55, $[M-C_{10}H_{17}-C_4H_7-H]^+$), 317 (33, $[M-C_{10}H_{17}-C_4H_7-MeOH]^+$), 275 (24), 233 (12), 217 (10), 177 (13), 135 (4, $C_{10}H_{15}$), 107 (7), 69 (100, $C_5H_9^+$), 55 (15, $C_4H_7^+$); HR-FAB-MS *m/z* 541.3533 ($[M+H]^+$, Calcd for $C_{33}H_{49}O_6$, 541.3532).

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