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Note

Structures of new secofriedelane and friedelane acids from *Calophyllum inophyllum* of French Polynesia

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Three new friedelane-type triterpenoids, 3,4-secofriedelan-3,28-dioic acid (1), 27-hydroxyacetate canophyllic acid (2) and 3-oxo-27-hydroxyacetate friedelan-28-oic acid (3), were isolated from the leaves of *Calophyllum inophyllum* (Clusiaceae) grown in French Polynesia. Their structures were established by the concerted application of 2D NMR techniques including gs-COSY, gs-HMQC and gs-HMBC. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; COSY; HMQC; HMBC; *Calophyllum inophyllum*; Clusiaceae; friedelane; 3,4-secofriedelane; triterpene

INTRODUCTION

The genus *Calophyllum*, belonging to the Clusiaceae family, contains many species of evergreen trees widespread in the tropical regions of Asia, America and Africa.[1] *Calophyllum inophyllum*, one of the most abundant of this genus, locally called 'Tamanu' in French Polynesia, having a sacred symbol for the Polynesian culture, was used as a common ingredient in traditional folk medicine.[2] Moreover, plants from the genus *Calophyllum* are known as a source of friedelane triterpenes.[3] As a part of phytochemical investigation of *C. inophyllum* collected in French Polynesia, we report the occurrence of three new friedelane-type triterpenoids isolated from the ethyl acetate extract of the leaves of *C. inophyllum*: 3,4-secofriedelan-3,28-dioic acid (**1**), 27-hydroxyacetate canophyllic acid (**2**) and 3-oxo-27-hydroxyacetate friedelan-28-oic acid (**3**) (Scheme 1). Their structures were established by the concerted application of 2D NMR experiments (gs-COSY, gs-HMQC and gs-HMBC).

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EXPERIMENTAL

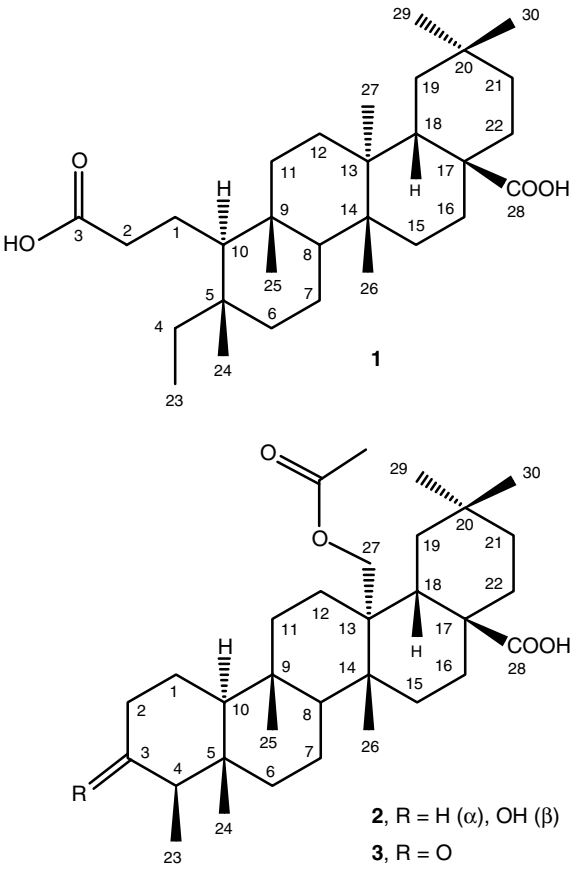
General procedures

Melting-points (uncorrected) were obtained on a Electrothermal IA 9100 system. IR spectra were obtained with KBr discs using a Jasco FT/IR-460 Plus spectrometer. HPLC was performed using an HP 1100 pump with a Varian Dynamax Si column (250 × 21.4 mm i.d., film thickness 5 μm), a refractometric detector and a solvent flow-rate of 10 ml min^{-1} (isocratic conditions). Mass spectra were measured on a Sciex (Thornill, ON, Canada) API III Plus triple-quadrupole mass spectrometer equipped with an atmospheric pressure ionization (API) source, via an ionspray interface.

NMR spectra

NMR spectra were recorded in CD_3OD solutions at 300 K using a Bruker Avance DRX 500 spectrometer equipped with a Bruker CryoPlatform and a 5mm cryo TXI probe. The temperature of the probe and preamplifier was 30 K. Chemical shifts were referenced to CD_3OD: $\delta_H = 3.31$ ppm, $\delta_C = 49.0$ ppm.[4] Resonance multiplicities for ^{13}C signals were established via the acquisition of DEPT spectra. For two-dimensional experiments, Bruker microprograms using gradient selection (gs) were applied.

gs-COSY spectra[5] were obtained with an F_2 spectral width of 10 ppm and 2 K data points and an F_1 spectral width of 256 t_1 increments with sine-bell windows in both dimensions. The gs-HMQC spectra[6] resulted from



Scheme 1. Structures of triterpenes **1**–**3**.

256 × 1024 data matrix size with 2–16 scans per t_1 depending on the sample concentration, an inter-pulse delay of 3.2 ms and a 5 : 3 : 4 gradient combination. gs-HMBC spectra[7] were measured using a pulse sequence optimized for 10 Hz (inter-pulse delay for the evolution of long-range couplings 50 ms) and the same gradient ratios.

Plant material

Leaves of *C. inophyllum* were collected on Tahiti and Moorea islands, French Polynesia, in November 2001. A voucher specimen (collection FL 11/2001) was deposited at the herbarium of the University of French Polynesia.

Extraction and isolation

The air-dried leaves of *C. inophyllum* (2 kg) were successively extracted with hexane, AcOEt and MeOH in a Soxhlet apparatus. The soluble part of the AcOEt extract in CH_2Cl_2 gave a dark-green wax (104 g). This fraction was submitted to flash chromatography on silica gel (240–300 mesh) with hexane, AcOEt and MeOH as solvents. Early fractions eluted with hexane yielded four known friedelanes: friedelin (**4**),[8,9] canophyllol (**5**),[8] canophyllol acetate (**6**)[10] and canophyllic acid (**7**).[11,12]

The fraction eluted with AcOEt (28 g) was submitted to medium-pressure liquid chromatography on silica gel using a stepwise gradient from a mixture of hexane–AcOEt (85 : 15) to AcOEt, yielding 150 fractions. Fractions having similar R_f values on silica gel TLC [hexane–acetone (60 : 40)] were combined and gave 11 fractions. Fractions 1 and 2 were further chromatographed by HPLC using a Varian Dynamax Si column (250 × 21.4 mm i.d., film thickness 5 μm) with isooctane–AcOEt (80 : 20) as eluent under isocratic conditions. Three fractions (A, B and C) were obtained from this chromatographic technique. Each fraction was further purified by preparative silica gel TLC [hexane–dioxane (60 : 40)] to yield **1** (3 mg), **2** (5 mg) and **3** (2 mg).

3,4-Secofriedelan-3,28-dioic acid (**1**): $C_{30}H_{50}O_4$, amorphous powder, m.p. 292–294 °C, $[\alpha]_D^{25}$ +13 (*c* 0.65, MeOH); ESI (positive ion) MS, m/z $[M + H]^+$ (475), $[M + NH_4]^+$ (492), $[M + Na]^+$ (497), $[M + K]^+$ (513); IR, ν_{max} (cm^{-1}) (KBr) 3448, 2938, 1699, 1456, 1224.

27-Hydroxyacetate canophyllic acid (**2**): $C_{32}H_{52}O_5$, amorphous powder, m.p. 238–241 °C, $[\alpha]_D^{22}$ +18 (*c* 0.54, MeOH); ESI (positive ion) MS, m/z $[M + H]^+$ (517), $[M + NH_4]^+$ (534), $[M + Na]^+$ (539), $[M + K]^+$ (555); IR, ν_{max} (cm^{-1}) (KBr) 3444, 2946, 1691, 1242.

3-Oxo-27-hydroxyacetate friedelan-28-oic acid (**3**): $C_{32}H_{50}O_5$, amorphous powder, m.p. 233–236 °C, $[\alpha]_D^{22}$ +1.5 (*c* 0.26, MeOH); ESI (positive ion) MS, m/z $[M + H]^+$ (515), $[M + NH_4]^+$ (532), $[M + Na]^+$ (537), $[M + K]^+$ (553); IR, ν_{max} (cm^{-1}) (KBr) 3449, 2945, 1712, 1241.

RESULTS AND DISCUSSION

Extraction of the leaves of *C. inophyllum* with AcOEt by silica gel colum chromatography, followed by preparative HPLC and TLC, yielded compounds **1**–**3**. Structural determinations are based on the NMR spectral assignments, which were confirmed by 2D experiments (gs-COSY, gs-HMQC and gs-HMBC). The ^1H and ^{13}C chemical shifts are given in Table 1.

Compound **1** was obtained as a white amorphous solid. The molecular formula $C_{30}H_{50}O_4$ was assigned from the molecular ion at m/z $[M + H]^+$ 475 in ESIMS, in combination with its ^{13}C NMR data. The ^{13}C NMR spectrum gave a total of 30 separated resonances and the DEPT spectrum showed the presence of seven methyls, 12 methylenes, three methines and eight quaternary carbons including two acid carbonyls. Acid functions were also indicated by absorption bands at 1699 and 3448 cm^{-1} in the IR spectrum.

The spectral features indicated that **1** was a secotriterpene, with the A ring opened, and with two acid carboxylic functions at C-3 and C-28.[13,14] With the observations of the C,H correlations in the HMBC and HMQC experiments it was possible to identify **1** as 3,4-secofriedelan-3,28-dioic acid (Table 1). The starting point of the assignment of the ^1H and ^{13}C of **1** was the position of the methylene group [CH_2-2 (δ_H 2.29 t, J = 7.0 Hz; δ_C 38.93)], detected through HMBC, by the correlation between H-2 and the carboxylic acid carbon C-3 (δ_C 177.88), the methylene carbon C-1 (δ_C 22.55) and the CH-10 (δ_C 60.83), indicating that **1** has the unit $CHCH_2CH_2COOH$. Furthermore, the methyl protons at δ_H 0. 81 (t, J = 7.3 Hz) attached to C-23 showed correlations with CH_2-4 (δ_C 37.17) and C-5 (δ_C 38.84), confirming that the A ring is opened. Correlations of other methyl protons furnished the main connectivities of the four rings B–E of the triterpene core: CH_3-24 (δ_H 0.81) with C-4, C-5, C-6 and C-10; CH_3-25 (δ_H 0.89) with C-8, C-9, C-10 and C-11; CH_3-26 (δ_H 0.82) with C-8, C-13, C-14 and C-15; CH_3-27 (δ_H 1.04) with

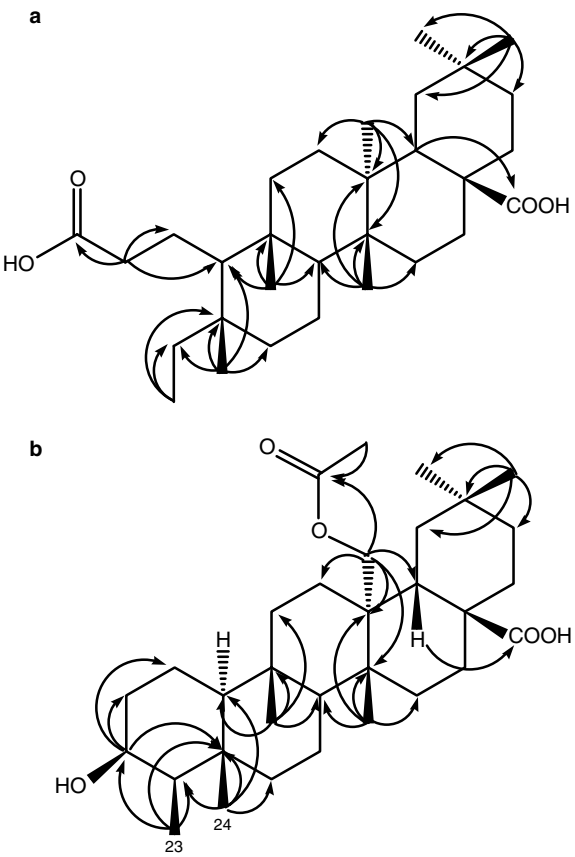
Table 1. ^{1}H and ^{13}C NMR chemical shifts of friedelanes **1**–**3** (CD_3OD)

	1		**2**		**3**	
Position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	1.56, m; 1.49, m	22.55	1.58, m; 1.35, m	17.11	1.95, m; 1.66, m	23.42
2	2.29, t	38.93	1.85,dm; 1.49, m	36.20	2.38, td; 2.27,dd	42.20
3	—	177.88	3.67, q	73.10	—	215.94
4	1.37, m; 1.13, m	37.17	1.20, m	50.77	2.37, q	58.99
5	—	38.84	—	39.03	—	43.18
6	1.51, m; 1.20, m	39.96	1.74, m	42.87	1.65, m; 1.32, m	42.00
7	1.40, m; 1.35, m	19.06	1.41, m; 1.33, m	18.77	1.45, m	19.37
8	1.34, m	54.32	1.33, m	54.73	1.47, m	54.39
9	—	40.38	—	38.48	—	38.74
10	0.87, t	60.83	0.89, t	62.71	1.62, m	60.13
11	1.41; 1.26	36.21	1.50, m	37.53	1.50, m	37.46
12	1.45; 1.38	32.01	1.98, dm; 1.35, m	26.18	2.02, m; 1.41, m	26.04
13	—	38.84	—	39.36	—	39.26
14	—	39.96	—	43.83	—	43.83
15	1.43, m; 1.19, m	33.83	1.50, m; 1.15, m	32.71	1.55 m; 1.18, m	32.76
16	2.34, dd; 1.69	30.82	1.45, m	37.13	1.46, m	37.18
17	—	45.84	—	45.85	—	45.95
18	2.44, dd	39.09	2.49, dd	39.52	2.54, dd	39.47
19	1.41, m; 1.18, m	35.97	1.60, m; 1.17, m	36.79	1.50, m; 1.18, m	36.83
20	—	29.40	—	29.25	—	29.27
21	1.42, m; 1.27, m	33.66	1.20, m	33.59	1.32, m; 1.20, m	33.60
22	1.49, m; 1.38, m	37.10	2.47, dd; 1.60, m	30.73	2.50, dd; 1.61, m	30.78
23	0.81, t	7.94	0.91, d	12.15	0.83, d	7.13
24	0.81, s	19.89	0.96, s	16.92	0.72, s	15.01
25	0.89, s	18.11	0.92, s	18.49	0.94, s	18.07
26	0.82, s	21.27	0.88, s	21.98	0.91, s	22.15
27	1.04, s	19.14	4.48, d	66.33	4.53, d	66.25
			4.43, d		4.48, d	
28	—	182.69	—	182.18	—	183.81
29	1.03, s	30.31	1.03, s	29.94	1.03, s	29.99
30	0.93, s	35.03	0.90, s	35.00	0.90, s	35.03
CO	—	—	—	173.08	—	173.08
CH_3	—	—	2.04, s	21.22	2.05, s	21.51

C-12, C-13, C-14 and C-18; CH_3-29 (δ_H 1.03) and CH_3-30 (δ_H 0.93) with C-19, C-20 and C-21. Finally, the signal at δ_H 2.44, attributed to the CH-18 group, showed correlation with the second carboxylic group C-28. Scheme 2(a) shows the significant two- and three-bond $^{13}C-^{1}H$ correlations observed in the HMBC spectrum. The above information allowed us to establish the structure of **1** as 3,4-secofriedelan-3,28-dioic acid.

Compound **2** was obtained as a white amorphous solid. The molecular formula was deduced to be $C_{32}H_{52}O_5$ by a combination of ^{13}C NMR, DEPT (8 C, 5 CH, 12 CH_2 and 7 CH_3) and ESIMS data. The IR spectrum displayed absorption bands at 1702 and 3400 cm^{-1} due, to carbonyl and hydroxyl groups, respectively. In the ^{13}C NMR spectrum the low-field resonances at δ_C 182.18 and 173.08 suggested the presence of two carbonyl groups. Two oxygenated carbons were located at δ_C 73.10 (CH) and 66.33 (CH_2). Moreover, ^{1}H and ^{13}C NMR data revealed the presence of an acetate function (δ_C 173.08, 21.22; δ_H 2.04). The tertiary methyl (δ_C 12.15; δ_H 0.91, J = 6.3 Hz) suggested a friedelane skeleton for this compound.[8] Structural and spectral assignments of **2** were then achieved by the concerted application of gs-COSY, gs-HMBC and gs-HMQC experiments. The key observations from the HMBC spectrum were the correlations from the ^{1}H methyl signals: CH_3-23 (δ_H 0.91) with C-3, C-4 and C-5; CH_3-24 (δ_H 0.96) with C-4, C-5, C-6 and C-10; CH_3-25 (δ_H 0.92) with C-8, C-9, C-10 and C-11; CH_3-26 (δ_H 0.88) with C-8, C-13, C-14 and C-15; CH_3-29 (δ_H 1.03) and CH_3-30 (δ_H 0.90) with C-19, C-20 and C-21. Moreover, the diastereotopic methylene protons assigned to C-27 showed ^{2}J and ^{3}J correlation peaks with C-12, C-13, C-14 and C-18. Further analysis of the HMBC spectrum indicated the linkage of an acetate group at C-27. Finally, concerted analysis using COSY, HMQC and HMBC spectra furnished the other ^{1}H and ^{13}C chemical shifts of **2**. The structure of **2** therefore be established as 27-hydroxyacetate canophyllic acid [Table 1 and Scheme 2(b)].

The molecular formula for **3** was found to be $C_{32}H_{50}O_5$ by a combination of ^{13}C NMR (32 carbons), DEPT (9 C, 4



Scheme 2. Partial HMBC correlations (H → C) for (a) 3,4-secofriedelan-3,28-dioic acid (**1**) and (b) 27-Hydroxyacetate canophyllic acid (**2**).

CH, 12 CH_2 and 7 CH_3) and ESIMS data. The IR spectrum of **3** displayed characteristic bands for a hydroxyl group (3420 cm^{-1}) and carbonyl functions (1755 cm^{-1}). The ^1H and ^{13}C NMR data of **3** are very close to those of **2**. However, in contrast to **2**, the ^{13}C NMR spectrum of **3** shows a carbonyl function at δ_C 215.94, which was assigned to C-3 on the basis on long-range correlation peaks with H-23. The complete ^1H and ^{13}C assignment (Table 1) established the structure of **3** as 3-oxo-27-hydroxyacetate friedelan-28-oic acid.

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