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Canarene: A Triterpenoid with a Unique Carbon Skeleton from *Canarium schweinfurthii*

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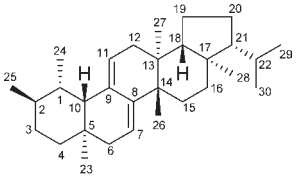
ABSTRACT



Canarene (1)

Canarene (1), a triterpene with an unprecedented carbon backbone, was isolated from *Canarium schweinfurthii*. It is the first member of a new class of triterpenoids, for which the name "canarane" is proposed. 1 showed weak α-glucosidase inhibitory activity, and its structure was unambiguously deduced by single-crystal X-ray diffraction.

The genus *Canarium* (Burseraceae), native to tropical Africa and southern Asia, comprises 75 species. *Canarium schweinfurthii* Engl. is a long (~50 m) tree with a cylindrical bole.[1] It has been used in folk medicine by local people as a stimulant, emollient, and treatment for fever, postpartum pain, and rheumatism.[2] Various biological activities such as analgesic, anti-inflammatory, antimicrobial, and antioxidant have been associated with *C. schweinfurthii*.[3,4] Only lipids from the fruits of *C. schweinfurthii* have been reported to date.[5,6] In a continuation of our search for pharmacologically active natural products, we report here the isolation and structure determination of canarene (**1**),[7] a triterpenoid with an unusual carbon skeleton, from the resin of *C. schweinfurthii*. The possible biogenetic basis of the unique structural features of canarene and its single-crystal X-ray diffraction analysis and α-glucosidase enzyme inhibitory activity are also described.

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(7) Canarene. White crystals from acetone, mp 289 °C, $[\alpha]_D^{28}$ −90.3 (*c* 0.025, MeOH). UV (MeOH) λ_{max}: 248 nm. IR (KBr) ν_{max}: 2976 (CH), 1647 (C=C) cm^{-1}. ^1H and ^{13}C NMR: see Table 1. HRTOF-ESI-MS (positive ion mode): $[M + H]^+$ *m*/*z* 409.3834 (calcd for $[C_{30}H_{48} + H]^+$, 409.3837).

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The resin of *C. schweinfurthii* Engl. was collected from Yaounde, Cameroon, in May 2010 and identified by Prof. Noumi, a botanist at the Department of Biology, University of Yaounde I. A voucher specimen (HNC 25918) was deposited at the Cameroon National Herbarium.

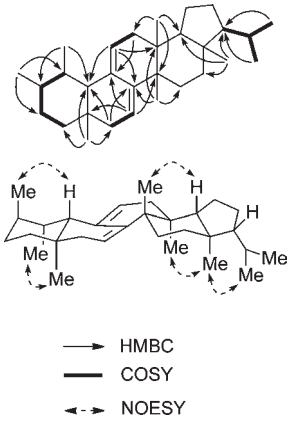


Figure 1. Key COSY, HMBC, and NOESY correlations for canarene (**1**).

Air-dried plant material (100 g) was extracted with dichloromethane at room temperature. The extract (70 g) was subjected to column chromatography (CC) over silica gel (300 g, 60 × 5 cm) and eluted with hexanes followed by hexanes/EtOAc mixtures with increasing proportions of EtOAc to obtain seven fractions (A–G). **1** (75 mg) was obtained from fraction A (200 mg, eluted with 4:1 hexanes/EtOAc) by CC over silica gel (70 g, 60 × 3 cm, eluted with hexanes/acetone gradients). Recrystallization from acetone yielded white crystals.

The molecular formula of **1** was determined to be $C_{30}H_{48}$ from the broadband-decoupled ^{13}C NMR spectrum and HRTOF-ESI-MS spectrum, which exhibited an $[M + H]^+$ peak at m/z 409.3834 (calcd for $[C_{30}H_{48} + H]^+$, 409.3837). The DEPT spectrum was used to identify the ^{13}C NMR resonances as eight methyl, eight methylene, eight methine, and six quaternary carbons. The ^1H and ^{13}C NMR spectra (Table 1) showed characteristic resonances for four secondary methyls [δ_H/δ_C 0.79 (overlapped)/17.5 (CH_3-24), 0.82 (d, $J_{29,22}$ = 6.5 Hz)/21.3 (CH_3-29), 0.87 (d, $J_{25,2}$ = 6.0 Hz)/20.9 (CH_3-25), and 0.90 (d, $J_{30,22}$ = 6.5 Hz)/23.8 (CH_3-30)], four tertiary methyls [δ_H/δ_C 0.76 (s)/30.1 (CH_3-23), 0.95 (s)/28.0 (CH_3-27), 1.00 (s)/23.0 (CH_3-26), and 1.08 (s)/25.9 (CH_3-28)], and four double bonds [δ_H/δ_C 5.18 (t, J = 5.0 Hz)/122.4 (CH-11), 5.19 (t, J = 4.5 Hz)/115.4 (CH-7); δ_C 134.9 (C-9) and 142.4 (C-8)]. The ^1H and ^{13}C NMR chemical shifts (Table 1) were assigned using $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra (Figure 1). The following key unambiguous HMBC correlations were observed: H-7 with C-5, C-9, and C-14; H-11 with C-8, C-10, and C-13; H_3-23 with C-4, C-6, and C-10; H_3-24 with C-2 and C-10; H_3-25 with C-1 and C-3; H_3-26 with C-8, C-13, and C-15; H_3-27 with C-12, C-14, and C-18; H_3-28 with C-16, C-18, and C-21; H_3-29 and H_3-30 with C-21.

Table 1. NMR Data for Canarene (**1**)[a]

		δ_H (J in Hz)[b]	
no.	δ_C, mult. in $CDCl_3$	in $CDCl_3$	in acetone-d_6
1	37.2, CH	0.78	0.81
2	38.2, CH	0.87	0.89
3	30.9, CH_2	1.57, 1.65	1.63, 1.71
4	39.0, CH_2	1.24, 1.36	1.27, 1.39
5	33.5, C	–	–
6	33.0, CH_2	2.40 br. d (18.0), 1.50	2.44 br. d (17.5), 1.53
7	115.4, CH	5.19 t (4.5)	5.19 br. d (4.5)
8	142.4, C	–	–
9	134.9, C	–	–
10	56.7, CH	1.23	1.29
11	122.4, CH	5.18 t (5.0)	5.17 br. d (5.0)
12	36.8, CH_2	2.40 br. d (18.0), 1.63	2.44 br. d (17.5), 1.67
13	37.5, C	–	–
14	39.0, C	–	–
15	27.6, CH_2	1.19, 1.23	1.24, 1.76
16	37.4, CH_2	1.59, 1.63	1.62, 1.69
17	40.5, C	–	–
18	58.3, CH	1.45	1.52
19	28.1, CH_2	1.05, 1.62	1.09, 1.68
20	31.2, CH_2	1.23, 1.44	1.27, 1.45
21	62.0, CH	1.21	1.26
22	28.2, CH	1.69	1.72
23	30.1, CH_3	0.76 s	0.78 s
24	17.5, CH_3	0.79	0.82
25	20.9, CH_3	0.87 d (6.0)	0.89 d (6.0)
26	23.0, CH_3	1.00 s	1.04 s
27	28.0, CH_3	0.95 s	0.95 s
28	25.9, CH_3	1.08 s	1.13 s
29	21.3, CH_3	0.82 d (6.5)	0.85 d (6.5)
30	23.8, CH_3	0.90 d (6.5)	0.91 d (6.5)

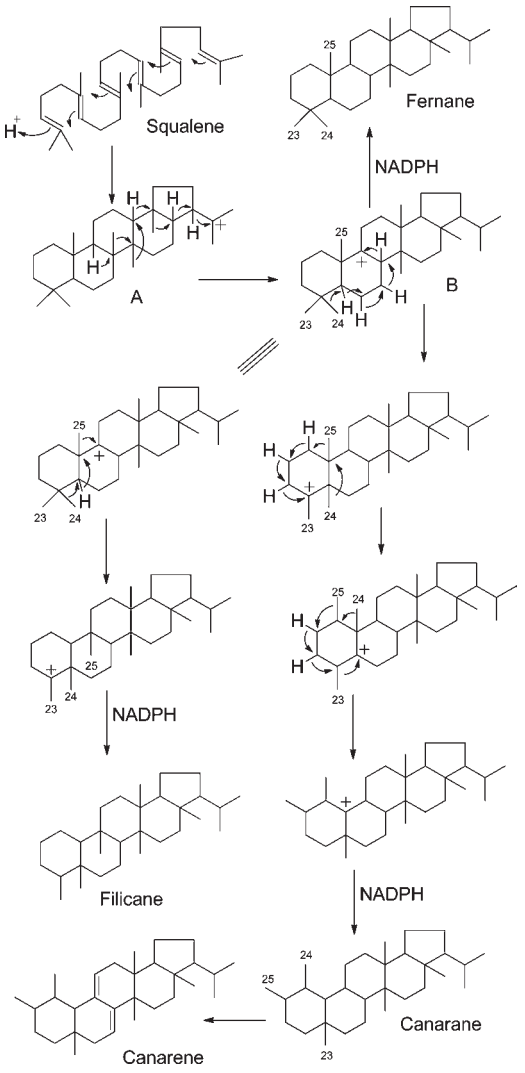
[a] ^1H and ^{13}C NMR data were recorded at 500 and 100 MHz, respectively. Chemical shifts (δ) are in ppm. [b] The multiplicity was not clear for some signals because of overlap.

Conclusive evidence for the structure of canarene was obtained by single-crystal X-ray diffraction analysis (Figure 2).[8] The molecule crystallized in the orthorhombic system with space group $P2_12_12_1$. The unit cell parameters were found to be a = 7.2941(2) Å, b = 11.5690(3) Å, and c = 29.1980(8) Å with all three angles equal to 90°. The asymmetric unit contained one independent molecule. The structure is composed of five trans-fused rings that exist in chair, half-chair, half-chair, chair, and envelope conformations, respectively. The final R and R_w factors for the data with $I > 2\sigma$ were measured as 0.0368 and 0.1046,

(8) The crystallographic data (CCDC 821643) can be obtained free of charge from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. Fax: +44-1223-336033. E-mail: deposit@ccdc.cam.ac.uk; Internet: www.ccdc.cam.ac.uk/conts/retrieving.html.

respectively. The bond lengths and angles were in the normal ranges.[9] The figure was plotted using the ORTEPII program.

Scheme 1. Plausible Biogenetic Pathway toward the Canarane Skeleton



Canarene (**1**) belongs to a new class of triterpenoids for which the name "canarane" is proposed. A plausible biogenetic route toward the novel skeleton of canarene is shown in Scheme 1. Like the closely related triterpene classes fernane and filicane (Figure 3), canarane seems to be derived from squalene through carbocation intermediates (A and B) by the usual hydride/methyl shifts,[10] as shown in Scheme 1. When the canarane skeleton is compared to the skeletons (Figure 3), the C-23, C-24, and C-25 methyl groups are found to be migrated to C-5, C-1, and C-2, respectively, in canarane.

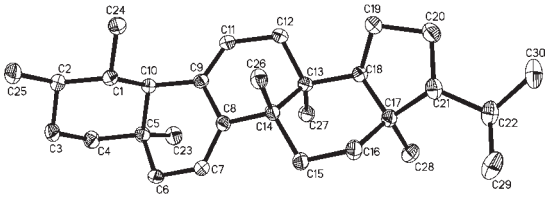


Figure 2. ORTEP drawing of the final X-ray structure of canarene (**1**) with atom labels and 30% probability displacement ellipsoids. H atoms are not shown.

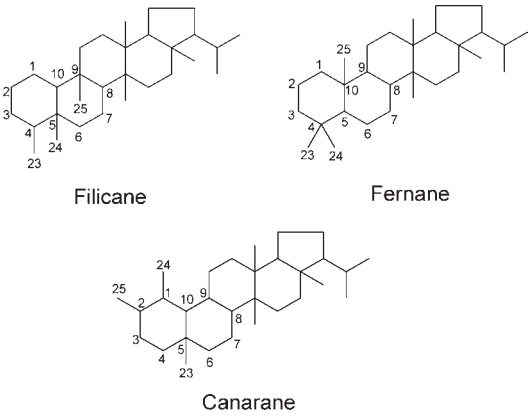


Figure 3. Canarane, fernane, and filicane skeletons.

1 was evaluated for α-glucosidase[11] inhibitory activity in a dose-dependent manner. It showed weak inhibitory activity of the enzyme, with an IC_{50} value of 471.0 ± 5.4 μM. The α-glucosidase inhibition assay was performed following the slightly modified method of Matsui et al.[12] α-Glucosidase, an important membrane-bound enzyme, catalyzes the hydrolysis of disaccharides into monosaccharides, which are absorbed in the gut. Inhibition of α-glucosidase is an important intervention to delay the absorption of glucose and control postprandial hyperglycemia in diabetic patients. The inhibition of the enzyme activity was measured spectrophotometrically at pH 6.9 and 37 °C using 0.7 mM *p*-nitrophenyl-α-D-glucopyranoside (PNP-G) as a substrate and 0.02 U/mL enzyme in 50 mM sodium phosphate buffer containing 100 mM NaCl. 1-Deoxynojirimycin was used as a positive control. The increment in absorption at 400 nm due to the hydrolysis of PNP-G by α-glucosidase was monitored continuously with a spectrophotometer (Spectra Max, Molecular Devices, Sunnyvale, CA).

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Supporting Information Available. ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC, and UV spectra of canarene (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.