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Ingacamerounol, A New Flavonol and Other Chemical Constituents from Leaves and Stem Bark of *Inga edulis* Mart.

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The genus *Inga* (Fabaceae-Mimosoideae), native in tropical and subtropical America (Mexico to Argentina) comprises about 300 species. Many of them were used by local healers to treat illnesses such as malaria, diarrhoea, ulcers, mouth inflammations and leishmaniasis.[1,2] *Inga edulis* Mart. is native or naturalized throughout South and Central America and is commonly used as a shade tree in coffee and cocoa plantations. It improves degraded soil by increasing soil nitrogen[3] and is used in Cameroon to increase soil fertility. It produced a very high amount of biomass, 61 tons ha^{-1} within 20 months,[4,5] and is known by the population for its sweet fruits and as supplier of fire wood. The leaves of *I. edulis* are used in the folk medicine as anti-inflammatory and anti-diarrheic drugs.[6] In addition, several pharmacological studies reported *in vitro* anti-tumor activities against human tumor cell lines and antioxidant activities of the extracts from leaves and stems of *I. edulis.*[7,8] Previous phytochemical investigations of a methanol-water extract of the leaves revealed the presence of polyphenolic compounds such as gallic acid, catechin, epicatechin, myricetin-3-rhamnopyranoside (**2**), quercetin-3-glucopyranoside, and quercetin-3-rhamnopyranoside (**3**).[8] In a continuing search for bioactive or new secondary metabolites from traditional Cameroonian medicinal plants, we investigated dichloromethane/methanol extracts of the leaves and stem bark of *I. edulis*. We report here on the isolation and structure elucidation of a new flavonol, named ingacamerounol (**1**). The structure of compound **1** was elucidated on the basis of detailed spectroscopic analyses, and by comparison with reported data, and confirmed by X-ray diffraction data. In addition, the known compounds, myricetin-3-*O*-α-L-rhamnopyranoside (**2**), quercetin-3-*O*-α-L-rhamnopyranoside (**3**), hexacosanyl caffeate (**4**), kojic acid (**5**), 1-tetracosanoyl glycerol (**6**), l-(24-hydroxytetracosanoyl) glycerol (**7**), stigmasterol (**8**), and stigmasterol 3-*O*-β-D-glucopyranoside (**9**) were isolated.

The air-dried and powdered leaves and stem bark of *I. edulis* were extracted separately with dichloromethane-methanol (1:1). On successive column chromatography over silica gel, the crude extract from leaves delivered six compounds, namely ingacamerounol (**1**) (Fig. 1), myricetin-3-*O*-

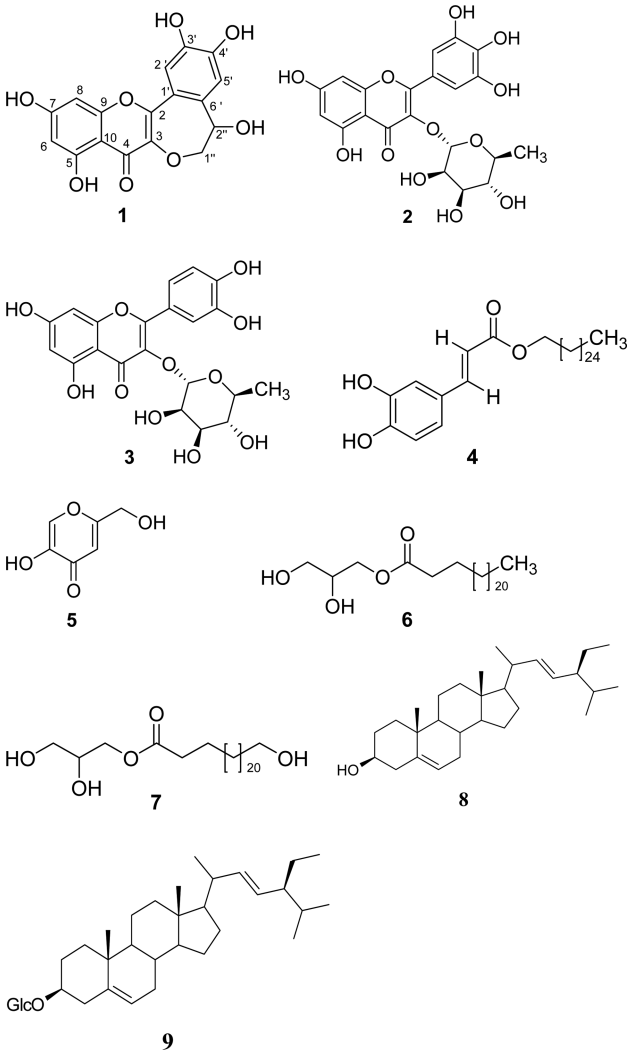

Figure 1. Structures of isolated compounds.

Table 1. ^{1}H (300 MHz) and ^{13}C (125 MHz) NMR and HMBC data for compound **1** in DMSO-d_6

C	δ_C, type	δ_H (*m*, *J* in Hz)	HMBC
2	154.1, C	-	
3	136.4, C	-	
4	176.4, C	-	
5	161.3, C	-	
6	98.4, CH	6.20 (d, 1.8)	C-5; C-7; C-8; C-10
7	164.0, C	-	
8	93.5, CH	6.40 (d, 1.8)	C-6; C-7; C-9; C-10
9	156.4, C	-	
10	104.0, C	-	
1'	118.2, C	-	
2'	114.0, CH	7.30 (s)	C-2; C-1'; C-3'; C-4'; C-6'
3'	148.0, C	-	
4'	144.5, C	-	
5'	114.4, CH	7.09 (1H, s)	C-2";C-1'; C-3'; C-4'; C-6'
6'	135.2, C	-	
1"	80.2, CH_2	4.10 (dd, 7.5, 10.5)	
		4.45 (dd, 4.8, 10.5)	C-2"; C-3; C-6'
2"	68.7, CH	4.70 (dd, 4.8, 7.5)	C-1"; C-1'; C-5'; C-6'

α-L-rhamnopyranoside (**2**),[8] quercetin-3-*O*-α-L-rhamnopyranoside (**3**),[8] kojic acid (**5**),[9] stigmasterol (**8**),[10] and stigmasterol 3-*O*-β-D-glucopyranoside (**9**).[11]

On repeated column chromatography over silica gel, the crude extract obtained from the stem bark afforded five known compounds: hexacosanyl caffeate (**4**),[12,13] 1-tetracosanoyl glycerol (**6**),[14] l-(24-hydroxytetracosanoyl) glycerol (**7**),[15] stigmasterol (**8**), and stigmasterol 3-*O*-β-D-glucopyranoside (**9**).

Compound **1** was obtained as a yellow powder with no optical activity, $[\alpha]_D^{20}$ 0° (*c* 0.25, MeOH) and a yellow fluorescence at λ 365 nm. It reacted positively in the Shinoda test, characteristic of flavonoids. The UV spectrum in MeOH displayed absorption bands at λ_{max} 218, 261 and 363 nm, suggesting a flavonoid skeleton.[16,17] The IR spectrum showed absorption bands of hydroxy (3339 cm^{-1}) and conjugated carbonyl (1648 cm^{-1}) functionalities, and of an aromatic system (1612 and 1557 cm^{-1}). The molecular formula, $C_{17}H_{12}O_8$, implying twelve double bond equivalents, was deduced from NMR data and by HR-ESIMS (see exp. part). The broad-band decoupled ^{13}C NMR spectrum (Table 1) of **1** displayed the expected 17 carbon resonances: By APT and HSQC spectra, 15 carbon signals were accounted for the flavonoid skeleton, among which 11 were due to aromatic quaternary carbons [including one carbonyl function at δ_C 176.4 (C-4) and six oxygenated carbon atoms between δ_C 164.0-148.0]; four aromatic methines were found at δ_C 114.4, 114.0, 98.4 and 93.5. The two remaining signals in the ^{13}C NMR spectrum were an oxymethine (δ_C 68.7) and an oxymethylene (δ_C 80.2). The ^{1}H NMR spectrum exhibited a D_2O-exchangeable 1H singlet at δ_H 12.80 (1H, s), indicating a hydrogen-bound hydroxy group at C-5; four further D_2O exchangeable protons gave signals at δ_H 9.70 (3H, br s) and 5.60 (1H, s). The ^{1}H NMR

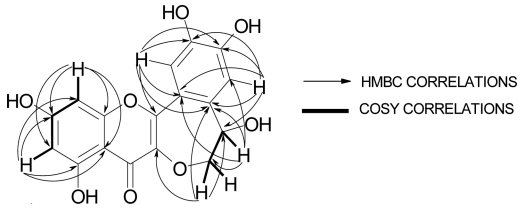

Figure 2. HMBC and COSY correlations for compound **1**.

spectrum also showed signals for two *meta*-coupled aromatic protons at δ_H 6.20 (1H, d, J = 1.8 Hz) and 6.40 (1H, d, J = 1.8 Hz), suggesting that the ring A had two oxygenated positions, consistent with a 5,7-dihydroxy substitution, and two uncoupled protons at δ_H 7.09 and 7.30 in accordance with a 1',3',4',6'-tetrasubstituted B-ring.[18] In addition, there were resonances of an ABX-type spin system, integrating for one proton each at δ_H 4.10 (dd, J = 7.5, 10.5 Hz), 4.45 (dd, J = 4.8, 10.5 Hz) and 4.70 (dd, J = 4.8, 7.5 Hz), assigned to two diastereotopic oxymethylene protons and one oxymethine, respectively. The ^{1}H-^{1}H COSY spectrum (Fig. 2) confirmed the ABX system and allowed the identification of the sequence O-CH_2-CH-O, which connected the C-3 and C-6' positions of the flavonoid unit, according to HMBC correlations. In fact, cross peaks were observed between the two diastereotopic oxymethylene protons (δ_H 4.10 and 4.45) and C-3 (δ_C 136.4) and C-6' (δ_C 135.2), and between the oxymethine proton (δ_H 4.70) and C-1' (δ_C 118.2), C-5' (δ_C 114.4) and C-6' (δ_C 135.2). The position and direction of the sequence O-CH_2-CH-O were confirmed by the X-ray crystallographic structure determination (Fig. 3).

The missing CD effect (Fig. 4) and the missing optical rotation suggested that **1** is racemic at the single chiral center C-2". Compound **1** is a new quercetin-derived flavonol, which we named ingacamerounol (Fig. 1).

The crude extract and isolated compounds were tested *in vitro* for their cytotoxicity against brine shrimps (*Artemia salina*) but no significant activity was observed.[19] They were tested also against the bacteria *Staphylococcus aureus* and *Escherichia coli*, the yeast *Candida albicans* and the plant-

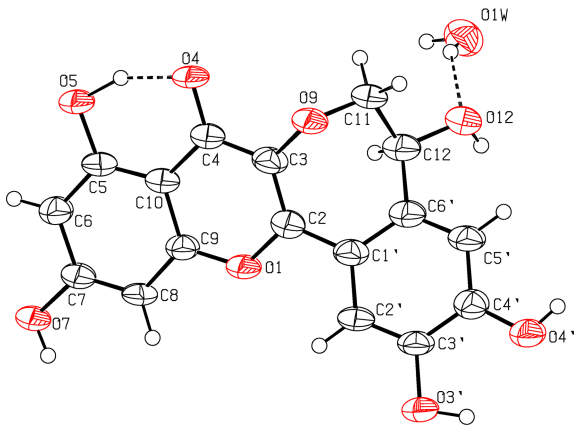

Figure 3. Thermal ellipsoid plot from the single-crystal structure determination with ellipsoids at 50% probability, illustrating the connectivity of **1**.

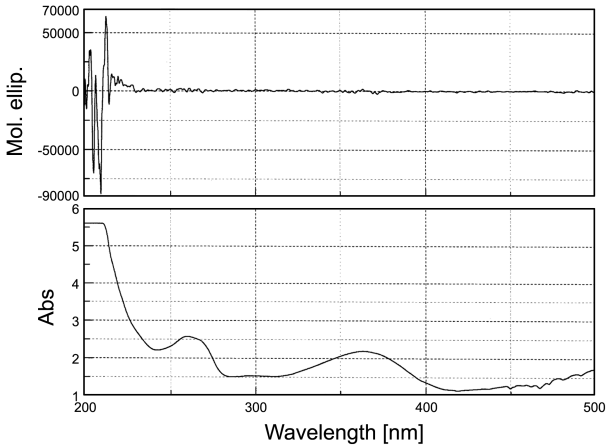

Figure 4. CD spectrum of **1** measured in MeOH.

pathogenic fungi *Rhizoctonia solani*, *Aphanomyces cochlioides* and *Pythium ultimum*, and against the microalgae *Chlorella vulgaris*, *C. sorokiniana*, and *Scenedesmus subspicatus*. Only the crude extract of leaves showed moderate activity against *A. cochlioides*, with an inhibition zone of 14 mm diameter at concentrations of 400 μg/paper disk.

Experimental Section

General. UV/Vis spectra were recorded in neutral, acidic (HCl), and basic (NaOH) MeOH on a Varian CARY (3E) UV/Vis spectrophotometer. The CD spectrum was recorded on a Jasco J-810 spectropolarimeter. The optical rotation was recorded in MeOH on a Jasco P-2000 polarimeter. IR spectra were recorded on a Jasco FT/IR-4100 Fourier-transform infrared spectrometer type A. The ^{1}H NMR spectra were recorded on Varian Mercury-300 (300.141 MHz), Varian VNMRS-300 (300.536 MHz), Varian Inova-500 (499.8 MHz) and Inova-600 (599.744 MHz) spectrometers equipped with 3 mm probes; ^{13}C NMR spectra were measured at 125.707 MHz and 150.821 MHz relatively to TMS as internal standard; shifts are reported as δ values. Electrospray-ionization mass spectrometry (ESIMS) and high-resolution mass spectra (HRESIMS) were recorded on a micrOTOF time-of-flight mass spectrometer (Bruker Daltonics, Bremen/Germany), as well as on an Apex IV7 Tesla Fourier-transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, MA/USA). Flash and column chromatography (CC): silica gels (SiO_2; 230-400 and 70-230 mesh; Merck). Pre-coated silica gel 60 F_{254} aluminum sheets were used for TLC with different mixtures of *n*-hexane, ethyl acetate, dichloromethane and methanol as eluents; spots were detected using UV lamps (254 and 365 nm) or by spraying with 50% H_2SO_4 followed by heating at 50-100 °C.

Plant Material. The stem bark and leaves of *I. edulis* were collected in January 2010 at Mbalmayo in the Centre Region of the Republic of Cameroon. The plant was identified by Mr. V. Nana, botanist at the National Herbarium (Yaounde, Cameroon), where a voucher specimen (no. 65644 HNC) has been deposited.

Ingacamerounol (1): Yellow powder; molecular formula $C_{17}H_{12}O_8$; $[\alpha]_D^{20}$ 0° (*c* 0.25, MeOH); UV/Vis (MeOH): λ_{max} (log ε) 218 (4.34), 261 (4.27), 297 (3.86) and 363 (4.22) nm. - UV/Vis (HCl): λ_{max} (log ε) 216 (4.38), 261 (4.28), 298 (3.88) and 363 (4.22) nm. - UV/Vis (NaOH): λ_{max} (log ε) 221 (4.44), 273 (4.38), 323 (3.92) and 408 (4.36) nm; IR λ_{max} 3339, 3081, 1648, 1612, 1557, 1030 cm^{-1};^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data, see Table 1; MS ((+)-ESI) *m/z*: 367.1 $[M+Na]^+$, 711.1 $[2M+Na]^+$, 1055.2 $[3M+Na]^+$, 1399.3 $[4M+Na]^+$. - MS ((−)-ESI) *m/z*: 343.0 $[M-H]^-$, 687.1 $[2M-H]^-$, 1031.1 $[3M-H]^-$. - HRMS((+)-ESI) *m/z*: 367.0423 (calcd. 367.0424 for $C_{17}H_{12}NaO_8$, $[M+Na]^+$) and HRMS ((−)-ESI) *m/z*:343.0458 (calcd. 343.0459 for $C_{17}H_{11}O_8$, $[M-H]^-$).

Suporting Information. Details on isolation, ^{1}H and ^{13}C NMR spectra, X-ray data of compound **1** together with the biological activities are available as Supporting Information. And the publication cost of this paper was supported by the Korean Chemical Society.

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