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New Triterpenoid Isolated from the Roots of *Gouania longipetala* Hemsl

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Abstract

Gouania longipetala Hemsl. (Rhamnaceae) is a medicinal plant from Côte d'Ivoire used to treat many diseases like malaria, gastrointestinal infections and gout. Scientific research of aerial part revealed the presence of triterpenoid compounds, saponins, phenolic compounds and flavonoids and also mentioned antibacterial, antioxydant and anti-inflammatory activities. Despite the various therapeutic uses of the plant, no scientific research mentions the chemical content of the root. So, this phytochemical investigation is made to identify secondary metabolites present in the hydromethanolic extract of its roots. And the research led to the isolation and characterization of three lupane-type triterpenoid compounds: a novel compound derived from gouanic acid, lupeol (1) and betulin (2), which had been previously reported in the literature. The newly discovered lupane-triterpenoid was identified as 1α-hydroxy-lup-20(29)-en-3-oxo-27,28-dioic acid (3). The structures of these compounds were determined based on analyses of spectroscopic data, including 1D-NMR, 2D-NMR and HR-ESI-MS techniques.

Keywords

Gouania longipetala, Rhamnaceae, Lupane-Type Triterpenoids

1. Introduction

Rhamnaceae is a cosmopolitan family of trees, shrubs, climbers, and one herb

comprising approximately 50 genera and 900 species [1]. The genus *Gouania* includes 70 tropical and subtropical species [2]. Previous chemical studies on different species of the genus *Gouania* have led to the isolation and characterization of triterpenoids, alkaloids, flavonoids, saponins and phenolic compounds [2]-[6].

Gouania longipetala Hemsl. is a species that grows in dry, dense forests, forest edges and regrowth areas. This plant is traditionally used to treat many ailments like wounds, gonorrhoea, abdominal pain, lumbago, ophthalmia, conjunctivitis, rickets and malaria, veneral disease, hydrops, swelling, oedema and gout [2] [7] [8]. Scientific investigations reported the antibacterial, antidiabetic [9], antilipidemic, antioxydant and anti-inflammatory effects [10]. Due to the ethnomedicinal importance of *Gouania longipetala*, this study aimed to explore the chemical composition of the hydromethanolic extract of the roots. Thus, three compounds (1 - 3) were isolated, including one new structure (3).

2. Material and Method

2.1. Plant Material

The roots of *Gouania longipetala* were collected in the BESO classified forest at AKOUPE (Latitude: 6, 3546˚ or 6˚21'17" NORTH; Longitude: −3, 6921˚ or 3˚41'32" WEST) in the East region of Côte d'Ivoire, in February 2021. The plant was identified at the floristic center of University Felix Houphouët Boigny (Abidjan, Côte d'Ivoire) under the herbarium number UCJ 014570 in comparison with the herbarium collected by AKE ASSI on 06/04/1966.

2.2. Sample Preparation

The harvested roots were air-dried for 3 weeks at ambient laboratory temperature and protected from sunlight. Then samples were ground using an electric grinder and weighed (4.5 kg).

2.3. Sample Extraction Procedure

One kg of powdered roots was soaked in a mixture of methanol and distilled water (80:20) for 48 hours. Afterward, the mixture was filtered with Whatman No. 1 filter paper. The hydromethanolic extract was then evaporated using a Heidolph rotary evaporator (4000 series), which was set to 40˚C under reduced pressure to obtain the crude sample. The resulting crude extract was oven-dried at 40˚C and weighed (146.1 g). Subsequently, 76 g of the crude extract was dissolved in 500 mL of distilled water and underwent a series of liquid-liquid extractions using solvents with increasing polarity: dichloromethane (3 × 500 mL), ethyl acetate (3 × 500 mL). The remaining residue was then dried. Following the various extractions, we obtained 1.15 g of dichloromethane extract, 5 g of ethyl acetate extract and 62 g of aqueous extract.

2.4. General Experimental Procedures

Fractions were successively purified using open-colon chromatography. Analytical

TLC was performed on precoated silica-gel 60 F254 Merck and spots were observed under UV light at 254 and 365 nm or visualized by spraying the dried plates with sulfuric vanilin, followed by heating. Silica gel 60 (63 - 200 mesh, Merck) was used for column chromatography. NMR experiments were carried out in DMSO-d6 on Bruker Advance DRX III 500 instruments. HR-ESI-MS experiments were performed using a Micromass Q-TOF micro instrument.

2.5. Isolation Procedure of Compounds 1, 2 and 3

The ethyl acetate fraction (5 g) was fractionated on a silica gel system with the hexane/dichloromethane/ethyl acetate/methanol mixture as eluent. This fractionation led to 5 fractions presented in Table 1.

Subsequently, fraction 1 was purified on column chromatography on silica using mobile phase hexane/dichloromethane (80/20) gradient to afford Compound 1 (4.7 mg). Fraction 3 was also purified on column chromatography on silica gel using mobile phase dichloromethane/ethyl acetate (90/10) to afford Compound 2 (3.5 mg) and Compound 3 (5.6 mg).

Table 1. Fractionation of ethyl acetate fraction of *G. longipetala* roots.

Solvents (hexane/dichloromethane/ethyl acetate/methanol)	Fractions	Masse (mg)
50/50/0/0	1	261.7
0/90/10/0	2	559
0/50/50/0	3	546
0/0/99/1	4	632.7
0/0/90/10	5	957.7

3. Results and Discussion

The ethyl acetate fraction of the hydromethanolic extract of the roots of *Gouania longipetala* Hemsl. (Rhamnaceae) was fractionated and purified by successive open-column chromatography, to obtain three lupane-type triterpenoids (1 - 3) (Figure 1). The structural elucidation of the compounds was established through a combined analysis of 1H, 13C, HSQC, COSY, and HMBC NMR spectra. The 1H and 13C NMR spectra provided insights into chemical shifts and coupling constants. The HSQC spectrum allowed us to identify methyl (CH_3), methylene (CH_2), and methyne (CH) groups due to ^{1}H-^{13}C coupling. The COSY spectrum, which involves ^{3}J (^{1}H-^{1}H) coupling, helped us determine the sequences and form the various rings within the structures. Finally, the HMBC spectra, involving ^{3}J, ^{4}J and ^{5}J coupling, enabled us to establish connections between the rings and to define the positions of the angular methyl groups. The ESI-MS mass spectrum is a soft ionization technique and provides only peaks for the molecular ions in negative mode.

3.1. Characterization of Compounds 1 and 2

The 13C-NMR spectrum of Compound 1 revealed 30 carbon signals, including seven

methyl, eleven methylene, six methine, and six quaternary carbons, corresponding to the terpenoid with a lupane skeleton. Notably, a carbon atom attached to the OH group at the C-3 position was observed at 76.2 ppm. The ^{1}H NMR spectrum of Compound 1 shows a doublet at 4.69 ppm (J = 2.6 Hz) integrating a proton attributable to proton H-29b, triplet at 4.55 ppm (J = 2.1 Hz) integrating a proton attributable to proton H-29a, a triplet doublet at 2.97 ppm (J = 10.4 and 5.4 Hz) integrating a proton attributable to proton H-3 and six singletons corresponding to angular methyls: C-23 (0.87 ppm), C-24 (0.65 ppm), C-25 (0.77 ppm), C-26 (0.98 ppm), C-27 (0.91 ppm) and C-28 (0.76 ppm). The total assignment of proton and carbon was confirmed by COSY and HMBC correlations.

Compound 1 was identified as lupeol [11] [12]. Compound 2 structure was deduced from Compound 1 and was identified as betulin [13]-[15]. The 1H-NMR spectrum of Compound 3 shows the same characteristics as that of lupeol. The difference is the presence of two multiplets at 3.08 ppm and 3.51 ppm, each integrating a proton attributable to the hydroxylated carbon C-28 (57.4 ppm).

Their structural elucidations were carried out by HR-ESI-MS, 1D and 2D-NMR analysis and their spectroscopic data corroborated with those in the literature. The structure of Compound 3 is deduced from Compounds 1 and 2.

3.2. Characterization of Compound 3

Compound 3 appears as white amorphous crystals. Its structure is deduced from that of Compounds 1 and 2. Indeed, the analysis of the ^{1}H, ^{13}C (**Table 2**) and HSQC NMR spectra shows similarities between the two compounds and suggests that Compound 3 is also a lupane-type triterpene. In the ^{1}H NMR spectrum, we observe a broad singlet at 4.58 ppm and a doublet at 4.69 ppm (J = 1.45 Hz) corresponding to the exocyclic ethylenic protons H-29a and H-29b, respectively. There is also a triplet of doublets at 3.01 ppm (J = 10.5, 2.73 Hz) integrating for one proton H-19, a multiplet at 2.29 ppm integrating for one proton H-13, and five singlets corresponding to the five angular methyl groups. Three of these signals integrate for 3 protons each at 0.99 ppm (H-23), 0.89 ppm (H-2), and 1.64 ppm (H-30), and one signals at 0.95 ppm integrate for six protons corresponding to methyl groups 25 and 26.

Furthermore, the HMBC spectrum shows correlation peaks with the carbons C-30 (18.43 ppm) and H-19 (3.00 ppm).

The ^{13}C NMR spectrum of Compound 3 shows signals at 204.4 (C-3), 176.3 (C-27), and 177.1 (C-28) ppm (**Table 2**). These high chemical shift values suggest that these carbons bear a ketone and an acid functional groups, respectively. Indeed, the HMBC spectrum reveals correlations between the signal at 204.4 ppm and the protons H-2a (1.66 ppm), H-2b (1.79 ppm), H-1 (2.62 ppm) and H-5 (0.96 ppm). These different correlations allow for the assignment of carbon C-3. Carbon C-28 (177.1 ppm) is attributed through the HMBC spectrum, by its correlations with protons H-16a (1.25 ppm), H-16b (2.22 ppm), H-18 (1.51 ppm), and H-22b (1.82 ppm). Carbon C-27 (176.3 ppm) is also attributed to its HMBC correlations with

proton H-13 (2.29 m), H-15a (1.24 m) and H-15b (1.89 m). The important COSY and HMBC correlations are reported in Figure 2. Compound 3 was assigned to the molecular formula $C_{30}H_{43}O_6$ from its negative HR-ESI-MS, which exhibited a molecular ion peak $[M-H]^-$ at m/z 499.3062 and was identified as 1*α*-hydroxy-lup-20(29)-en-3-oxo-27, 28-dioic acid.

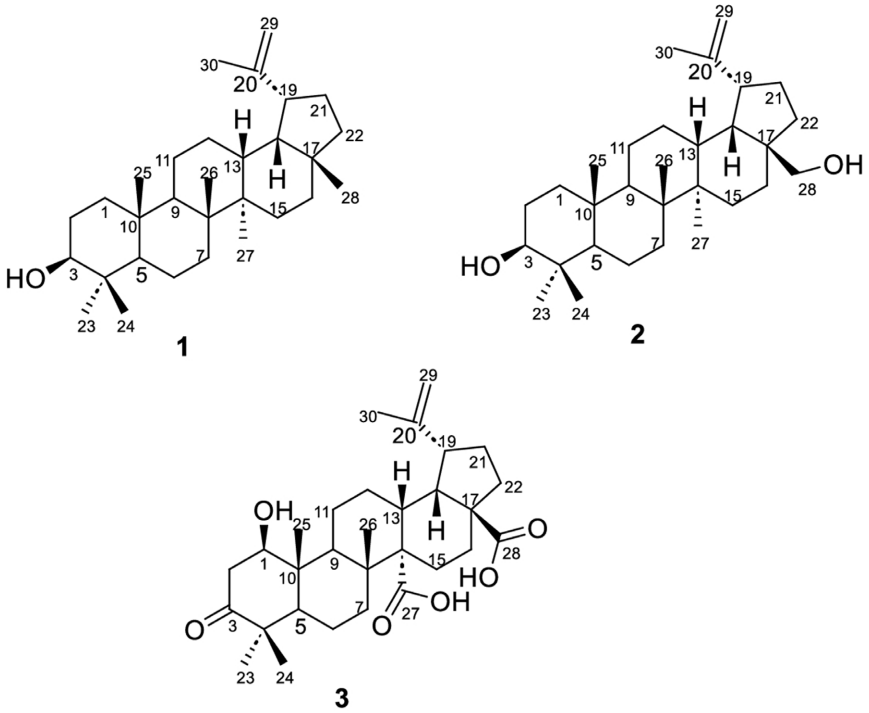

Figure 1. Isolated compounds from *Gouania longipetala* roots.

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of 3 in DMSO-d6.

Position	^{1}H (*δ*, ppm) (m; J, Hz)	^{13}C (*δ*, ppm)	Position	^{1}H (*δ*, ppm) (m; J, Hz)	^{13}C (*δ*, ppm)
1	2.63 dd (7.8, 2.19)	60.3	16	2.22 m, 1.25 m	33.7
2	1.79 m, 1.66 m	35.7	17	-	54.8
3	-	204.4	18	1.51 m	50.8
4	-	37.3	19	3.00 td (10.48, 2.73)	46.3
5	0.96 m	57.9	20	-	149.5
6	1.29 m	17.6	21	1.81 m, 1.29 m	29.6
7	1.55 m, 1.31 m	36.1	22	1.82 m, 1.29 m	36.2
8	-	39.8	23	0.99 s	31.3
9	1.86 m	44.5	24	0.89 s	25.7
10	-	51.1	25	0.95 s	19.1
11	1.54 m	22.7	26	0.95 s	16.8
12	2.02 m, 1.54 m	24.7	27	-	176.4
13	2.29 m	38.8	28	-	177.1
14	-	58.3	29	4.69 d (1.45), 4.58 brs	109.7
15	1.89 m, 1.24 m	27.2	30	1.64 s	18.3

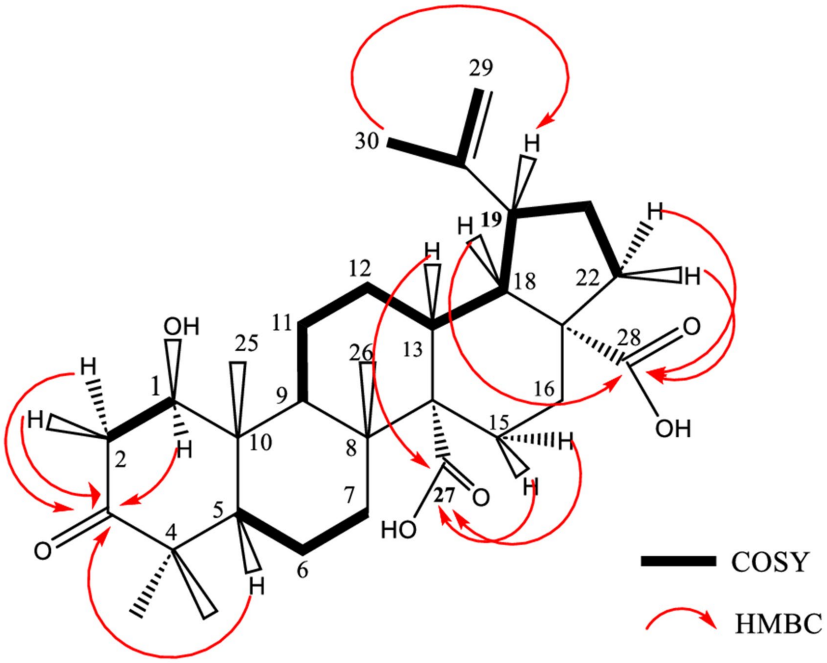

Figure 2. Important correlations observed in the COSY and HMBC spectra of Compound 3.

Lupeol (1) : ^{13}C NMR (125 MHz, DMSO); δ_C (ppm): 37.8 (C-1), 26.6 (C-2), 76.2 (C-3), 38.98 (C-4), 54.4 (C-5), 17.44 (C-6), 33.3 (C-7), 39.8 (C-8), 49.3 (C-9), 36.2 (C-10), 19.9 (C-11), 24.2 (C-12), 37.1 (C-13), 41.9 (C-14), 26.5 (C-15), 34.6 (C-16), 42.0 (C-17), 47.3 (C-18), 46.9 (C-19), 149.9 (C-20), 28.7 (C-21), 38.9 (C-22), 27.6 (C-23), 15.3 (C-24), 15.4 (C-25), 15.2 (C-26), 13.84 (C-27), 17.3 (C-28), 109. (C-29), 18.5 (C-30).

^{1}H NMR (500 MHz, DMSO), δ_H (ppm) **:** 0.82 (m, H-1a), 1.56 (m, H-1b), 1.43 (m, H-2), 2.96 (m, H-3), 0.63 (m, H-5), 1.34 (m, H-6a), 1.46 (m, H-6b), 1.33 (m, H-7), 1.24 (m, H-9),), 1.36 (m, H-11), 1.04 (m, H-12a), 1.63 (m, H-12b), 1.62 (m, H-13), 0.96 (m, H-15a), 1.63 (m, H-15b), 1.35 (m, H-16a), 1.44 (m, H-16b), 1.32 (m, H-18), 2.37 (m, H-19), 1.26 (m, H-21a), 1.86 (m, H-21b), 1.18 (m, H-22a), 1.34 (m, H-22b), 0.87 (s, H-23), 0.65 (s, H-24), 0.77 (s, H-25), 0.98 (s, H-26), 0.91 (s, H-27), 0.76 (s, H-28), 4.54 (brs, H-29a), 4.68 (d, J = 2.49 Hz, H-29b), 1.64 (s, H-30).

Betulin (2): ^{13}C NMR (125 MHz, DMSO), δ_C (ppm) : 37.7 (C-1), 26.7 (C-2), 76.3 (C-3), 38.01 (C-4), 54.3 (C-5), 17.5 (C-6), 33.3 (C-7), 39.9 (C-8), 49.3 (C-9), 36.2 (C-10), 19.8 (C-11), 24.3 (C-12), 36.3 (C-13), 41.7 (C-14), 26.2 (C-15), 28.5 (C-16), 46.9 (C-17), 47.6 (C-18), 46.8 (C-19),149.90 (C-20), 28.8 (C-21), 33.3 (C-22), 27.6 (C-23), 15.3 (C-24), 15.4 (C-25), 15.2 (C-26), 14.1(C-27), 57.4 (C-28), 109.1 (C-29), 18.3 (C-30).

^{1}H NMR (500 MHz, DMSO), δ_H (ppm): 0.83 (m, H-1a), 1.56 (m, H-1b), 1.44 (m, H-2), 2.97 (m, H-3), 0.63 (m, H-5), 1.33 (m, H-6a), 1.45 (m, H-6b), 1.32 (m, H-7), 1.24 (m, H-9), 1.15 (m, H-11a), 1.31 (m, H-11b), 0.97 (m, H-12a), 1.58 (m,

H-12b), 1.61 (m, H-13), 0.91 (m, H-15a), 1.62 (m, H-15b), 1.22 (m, H-16a), 1.87 (m, H-16b), 1.48 (m, H-18), 2.38 (m, H-19), 1.27 (m, H-21a), 1.86 (m, H-21b), 1.86 (m, H-22), 0.87 (s, H-23), 0.65 (s, H-24), 0.76 (s, H-25), 0.96 (s, H-26), 0.91 (s, H-27), 3.08 (m, H-28a), 3.51 (m, H-28b), 4.53 (brs, H-29a), 4.66 (brs, H-29b), 1.63 (s, H-30).

4. Conclusions

This study presents a comprehensive study on the extraction, isolation, and identification of three molecules, including lupeol, betulin and a new Compound 3, for our knowledge, from the roots of *Gouania longipetala.* The successful isolation and characterization of these compounds shed light on the chemical composition of *Gouania longipetala*. Triterpenes are known for their antibacterial, anticancer and antimalarial activities, and the presence of these triterpenoids could explain the traditional use of *Gouania longipetala*. The presence of 1α-hydroxy-lup-20(29)-en-3-oxo-27,28-dioic acid could explain the different biological activities, but also be the source of new biological activities.

These findings contribute to the growing body of knowledge on traditional medicinal plants in Côte d'Ivoire and provide a basis for further research and development of natural remedies derived from *Gouania longipetala* Hemsl.

Authors' Contributions

APY-K directed the project. LV-N, KJ-MK, SCS and ZLEA conducted all experiments and analyzed the RMN data. KVN and ACK collected the plant material and ACK isolated compounds and wrote the article. All authors discussed the results and commented on the manuscript.

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Conflicts of Interest

The authors declare that there is no competing interest related to this manuscript.

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