SHORT REPORT

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Alchornealaxine, an Unusual Prenylguanidinyl-epicatechin Derivative from *Alchornea laxiflora* (Benth) Pax and Hoffman

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Abstract : A new epicatechin derivative, alchornealaxine (**1**), was isolated along with nine known compounds, hyperoside, reynoutrin, guaijaverin, taxifolin-3-*O*-β-D-galactopyranoside, taxifolin-3-*O*-β-D-xylopyranoside, byzantionoside B, leeaoside, syringaresinol-β-D-glucoside and β-sitosterol-β-D-glucoside from the leaves of *Alchornea laxiflora*. The structures of these compounds were determined on the basis of spectroscopic methods as well as HR-ESI-TOF-MS analysis.

Keywords: *Alchornea laxiflora*; Euphorbiaceae; Epicatechin derivative; Alchornealaxine.

1. Plant Source

Alchornea laxiflora (Benth) Pax and Hoffman is a shrub belonging to the Euphorbiaceae family which is spread throughout tropical Africa. As its leaves are the commonly used parts in folk medicine both in Nigeria [1,2] and in Cameroon, the present study was undertaken in order to characterize the chemical constituents of the leaves of this plant growing in the western highland of Cameroon. The present repport deals with the isolation and structural determination of ten compounds including an unusual prenylguanidinyl-epicatechine derivative (**1**) (Figure 1), as well as five known flavonoid glycosides (**2**-**6**), two megastigmane glycosides (**7** and **8**), one lignan glucoside (**9**) and *β*-sitosterol-*β*-D-glucoside (**10**).

The leaves of *A. laxiflora* were collected in Foto village located in the Menoua Division of the western region of Cameroon in November 2010. They were identified by the botanists of the Department of Forestry of the University of Dschang, Cameroon, and later authenticated by Mr. Nana of the National Herbarium of Cameroon, Yaoundé, where a voucher specimen (N° 45363/HNC) has been deposited.

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2. Previous Studies

Previous phytochemical and biological studies of the leaves of this plant growing in Nigeria led to the isolation and characterization of flavonoids, mainly quercetin derivatives, as the components responsible both for their antimicrobial [3] and antioxidant [4] activities. Recently, numerous secondary metabolites including fatty acid esters, ceramides, terpenoids and ellagic acid derivatives have been reported from the stem bark of this plant growing in the central region of Cameroon [5].

3. Present Study

Air dried powdered leaves of *A. laxiflora* (2.5 kg) were extracted three times (each for 24 h) with 95% MeOH at room temperature. The filtrate obtained was concentrated under reduced pressure to yield a greenish residue (296 g). Part of this extract (280 g) was suspended in water (1 L) and its successive liquid-liquid partitioning with EtOAc and *n*-BuOH yielded 95 g and 120 g of extracts after evaporation to dryness, respectively. Part of the *n*-BuOH extract (110 g) was subjected to Sephadex LH-20 column chromatography using MeOH as eluent to remove the chlorophyll and oily fraction. The residual fraction (42 g) was further subjected to silica gel 60 (70-230 μm) column chromatography eluting with the mixture EtOAc/MeOH (100:0→70:30) to afford three main fractions (A-C). Fraction A (8 g) was repeatedly chromatographed using silica gel 60 (40-60 μm) column chromatography with increasing amounts of MeOH in EtOAc as eluent to afford compounds **10** (*β*-sitosterol-*β*-D-glucopyranoside), **7** (byzantionoside B) and **8** (leeaoside). Purification of fraction B (18.5 g) using silica gel 60 (40-60 μm) column chromatography with increasing amounts of MeOH in EtOAc as eluent followed by repeated Sephadex LH-20 column chromatography using MeOH as eluent led to the isolation of compounds **2** (hyperoside), **5** (taxifolin-3-*O*-*β*-D-galactopyranoside), **4** (guaijaverin), **6** (taxifolin-3-*O*-*β*-D-xylopyranoside) and **3** (reynoutrin). Fraction C (5.2g) was purified by repeated CC (SiO_2, 40-60 μm) eluting with the mixture EtOAc-MeOH-H_2O (85:15:5) to yield compounds **9** (syringaresinol-*β*-D-glucoside) and **1** (400mg).

Alchornealaxine (**1**): amorphous yellow powder. $(\alpha)_D^{20}$: - 36.7 (c = 0.15, MeOH);
^{1}H NMR (700 MHz, MeOH-d_4): δ (ppm) = 1.12 (3H, d, *J* = 7.0Hz, H-4”), 1.21 (3H, d, *J* = 7.0Hz, H-5”), 1.75 (3H, br *s*, H-4’’’), 1.78 (3H, br *s*, H-5’’’), 2.83 (1H, dd, *J* = 4.0, 16.5Hz, H-4b), 2.84 (1H, m, H-3”), 2.91 (1H, dd, *J* = 4.5, 16.5 Hz, H-4a), 3.90 (1H, m, H-1’’’), 4.27 (1H, m, H-3), 4.84 (1H, s, H-2), 5.35 (1H, m, H-2’’’), 6.13(1H, s, H-6), 6.73 (1H, dd, *J* = 1.8, 8.1Hz, H-6’), 6.75 (1H, d, *J* = 8.1Hz, H-5’), 6.88 (1H, d, *J* = 1.8Hz, H-2’); ^{1}H NMR (700 MHz, DMSO-d_6): δ (ppm) = 1.08 (3H, d, *J* = 7.5Hz, H-4”), 1.10 (3H, d, *J* = 7.5Hz, H-5”), 1.66 (3H, br *s*, H-4’’’), 1.70 (3H, br *s*, H-5’’’), 2.51 (1H, dd, *J* = 3.5, 16.2Hz, H-4*β*), 2.67 (1H, m, H-3”), 2.72 (1H, dd, *J* = 4.0, 16.2 Hz, H-4α), 3.82 (1H, m, H-1’’’), 4.03 (1H, m, H-3), 4.69 (1H, d, *J* = 4.5Hz, OH-3), 4.78 (1H, s, H-2), 5.24 (1H, m, H-2’’’), 6.14(1H, s, H-6), 6.59 (1H, dd, *J* = 1.6, 8.0Hz, H-6’), 6.62 (1H, d, *J* = 8.0Hz, H-5’), 6.79 (1H, d, *J* = 1.6Hz, H-2’), 7.42 (1H, t, *J* = 5.1Hz, NH-1’’’), 8.70 (1H, s, OH-4’), 8.84 (1H, s, OH-3’), 9.35 (1H, s, NH-1’’), 9.56 (1H, s, OH-5), 11.55 (1H, s, =NH); ^{13}C NMR (175 MHz, MeOH-d_4): δ (ppm) = 16.7 (C-3’’’), 19.9 (C-5’’), 20.3 (C-4’’), 24.4 (C-5’’’), 24.9 (C-3’’), 28.2 (C-4), 40.6 (C-1’’’), 65.5 (C-3), 78.7 (C-2), 94.7 (C-6), 98.9 (C-4a), 98.9 (C-8), 113.8 (C-2’), 113.9 (C-1’’), 114.5 (C-5’), 117.7 (C-6’), 118.8 (C-2’’’), 129.7 (C-2”), 130.6 (C-1’), 137.1 (C-3’’’), 144.4 (C-3’), 144.7 (C-4’), 146.1 (C=NH), 154.6 (C-8a), 155.3 (C-7), 157.9 (C-5); HRESI-MS (positive mode): *m/z* 482.2438 $(M+H)^+$, HRESI-MS (negative mode): *m/z* 480.2195 $(M-H)^-$ corresponding to $C_{26}H_{31}O_6N_3$

The methanolic extract of the leaves of *A. laxiflora* was suspended in H_2O and partitioned with EtOAc and n-BuOH, respectively. The n-BuOH soluble fraction was separated by a combination of chromatographic procedures to provide a new epicatechin derivative (**1**) (Figure 1) and nine known compounds, hyperoside (**2**) [6], reynoutrin (**3**) [7,8], guaijaverin (**4**) [9], taxifolin-3-*O*-*β*-D-galactopyranoside (**5**) [10], taxifolin-3-*O*-*β*-D-xylopyranoside (**6**) [11], byzantionoside B (**7**) [12],

leeaoside (**8**) [13], syringaresinol-*β*-D-glucoside (**9**) [14], and *β*-sitosterol-*β*-D-glucoside (**10**) [15] which were identified by comparison of their spectroscopic data with literature values.

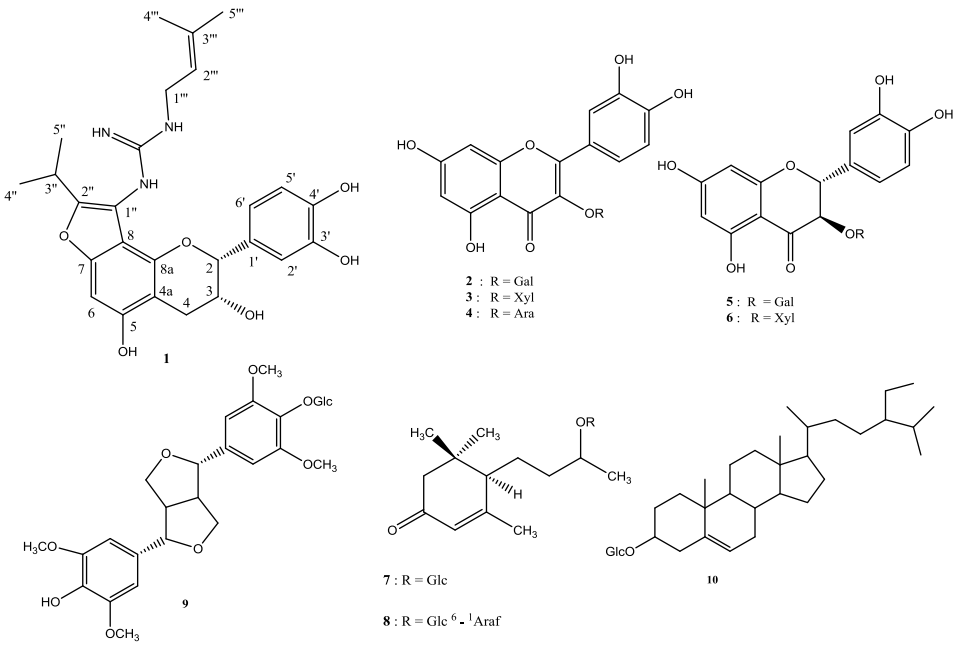

Figure 1. Chemical structures of compounds isolated from *A. laxiflora* (Glc = *β*-D-glucopyranosyl, Gal = *β*-D-galactopyranosyl, Xyl = *β*-D-Xylopyranosyl, Ara = α-L-arabinopyranosyl, Araf = α-L-arabinofuranosyl)

Compound **1** was isolated as an amorphous yellow powder and showed a molecular ion peak of 482.2438 in the ESI-TOF-MS (positive mode) corresponding to a molecular formula of $C_{26}H_{31}O_6N_3$. The NMR spectrum displayed characteristic signals for an epicatechin moiety [16]. Nevertheless, besides the tri-substituted aromatic ring system, only one aromatic singlet proton at *δ* 6.13 could be observed, suggesting a substitution of the epicatechin moiety in position C-6 or C-8. The substituent was clearly characterized by one prenyl residue comprising of two olefinic methyl groups at *δ* 1.78 (3H, s, H-5’’’) and *δ* 1.75 (3H, s, H-4’’’), as well as one olefinic proton at *δ* 5.35 (m, H-2’’’) coupling with a methylene group at *δ* 3.90 (2H, m, H-1’’’). This prenyl moiety had to be nitrogen bound due to the chemical shift of the methylene carbon at *δ* 40.6 (C-1’’’). Furthermore, two methyl doublets at *δ* 1.12 (3H, d, *J* = 7.0 Hz, H-4’’) and *δ* 1.21 (3H, d, *J* = 7.0 Hz, H-5’’) were observed, that coupled to a methine proton at *δ* 2.84 (m, H-3’’), which is typical for an isopropyl residue. In the HMBC spectrum, this methine proton showed long-range correlations to two quaternary olefinic carbons at *δ* 129.7 (C-2’’) and 113.9 (C-1’’), respectively. Taking into account the molecular formula, the substituent had to contain one further carbon atom (*δ* 146.1, C=NH), as well as three nitrogens. Therefore, it had to be a guanidine derivative substituted with two prenyl residues, one of which was bound to the epicatechin moiety as demonstrated in Figure 1. As H-6 of the epicatechin moiety showed HMBC correlations (Figure 2) to C-4a, C-5 and C-7, whereas H-2 showed HMBC correlations to C-8a, the linkage of the substituent was determined to be in position C-8.

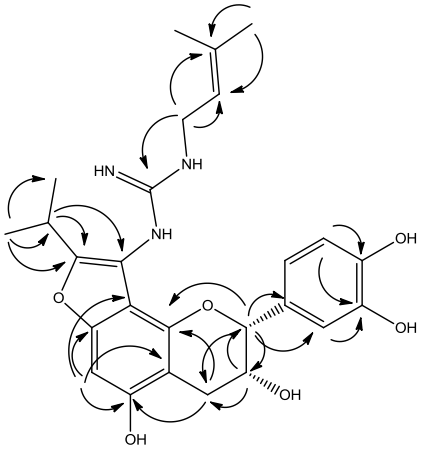

Figure 2. Selected HMBC correlations of alchornealaxine (**1**).

Guanidine alkaloids like *N*,*N*,*N*-triisopentenylguanidine have been reported in various *Alchornea* species such as *A. glandulosa* [17], *A. cordifolia* [18], and *A. javanensis* [19] and seem to be a chemotaxonomic marker of these species. Although this alkaloid has not been isolated during the present research work, compound **1** seems to derive biogenetically from the condensation of epicatechin (**2**) and *N*,*N*,*N*-triisopentenylguanidine (**3**) followed by a subsequent loss of one isopentenyl moiety as proposed on scheme 1. Catechin derivatives on the other hand have been reported only once from *Alchornea* species up to now [20]. Furthermore it could be deduced from this study that the bioactivity of this plant as used in Cameroonian folk medicine against microbial diseases might be attributed to its flavonoid components as has been shown for that growing in Nigeria.

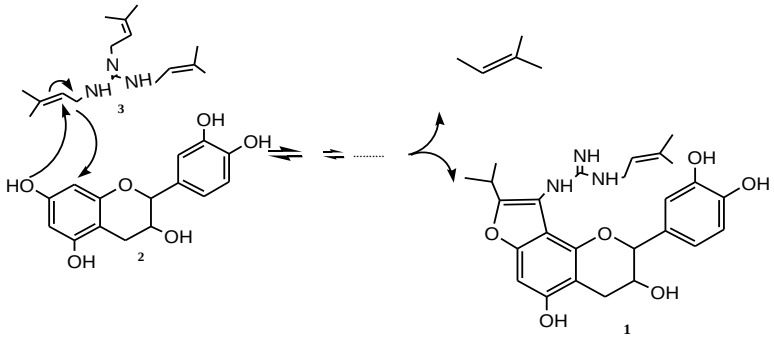

Figure 3. Proposed biogenetically formation of compound 1 from epicatechin (**2**) and *N*,*N*,*N*-triisopentenylguanidine (**3**).

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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