Two New Quinoline and Tris(4-hydroxycinnamoyl)spermine Derivatives from *Microdesmis keayana* Roots

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Purification of a *Microdesmis keayana* hydromethanolic root extract led to the isolation of two new natural compounds, xanthoquininamide (6-hydroxyquinoline-4-carboxamide) and tris(4-hydroxycinnamoyl)spermine (N^5-(*p*-coumaroyl)-N^1,N^{14}-diferuloylspermine) which was named keayanine. Their structures were determined using spectroscopic analyses (ESI-MS, 1D- and 2D-NMR).

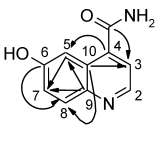
Key words Pandaceae; *Microdesmis keayana*; tris(4-hydroxycinnamoyl)spermine; xanthoquininamide

Microdesmis keayana LÉONARD (Pandaceae) is a tropical shrub, the leaves of which are used in the Ivory Coast against malaria and some other parasites. Its antiplasmodial,[1,2] antitrypanosomal[3] and anthelmintic[4] activities have already been tested. Its roots are also used by traditional healers as a remedy for sexual dysfunction. An aqueous extract of these roots, recently tested, exhibited vasorelaxing properties, explained by an increase in nitric oxide (NO) in the vascular bed, *via* antioxidant properties and stimulation of endothelial nitric oxide synthase (eNOS) mRNA expression.[5] In a previous paper three new tris(4-hydroxycinnamoyl)spermidines have been reported.[6] Continuing investigations on the roots of *M. keayana* have now led to the isolation and structural elucidation of two other new compounds, one quinoline derivative xanthoquininamide **1** and one tris(4-hydroxycinnamoyl)spermine keayanine **2**.

Results and Discussion

Xanthoquininamide (**1**) was obtained as a pale yellow powder soluble in methanol. It showed an $[M+H]^+$ peak at *m*/*z* 189 in the ESI-MS data. The ^{1}H-NMR spectrum in MeOD was very simple and all chemical shifts and couplings which were observed between δ_H 7.3 and δ_H 8.8, including a not-so-common J=4.5 Hz between H-2 and H-3,[7] were consistent for a quinoline derivative which was substituted on C-6 or C-7 (because of the ABX system) and on C-4. Mono- (^{1}H and ^{13}C) and bidimensional (COSY, HMQC, HMBC) NMR experiments, as well as comparison with data of previously described quinine-like alkaloids,[8] made it possible to establish the structure of **1** as 6-hydroxyquinoline-4-carboxamide. ^{1}H- and ^{13}C-NMR chemical shifts are given in Table 1. To our knowledge, this is the first report of both the natural occurrence of **1** and of its complete spectroscopic data, although it has already been synthesised from xanthoquininic acid by amidification.[9]

Compound **2** was characterized both by ESI-MS and NMR data. In the electrospray mass spectrum, a $[M+H]^+$ peak was observed at *m*/*z* 701. Typical fragments were observed on ESI-MS spectrum at *m*/*z* 147 and *m*/*z* 177, corresponding to one coumaroyl and two feruloyl residues respectively, according to the relative heights of both signals. The subtraction of these three elements from the molecular mass of the compound (*m*/*z* 700) gave a mass of 199, compatible with a tri-substituted spermine. The positions of all three acyl moieties were determined by ESI-MS (see Fig. 2)[10]: fragments at *m*/*z* 234 and *m*/*z* 305 were explained by the terminal position of at least one feruloyl moiety (N^1 and/or N^{14}); the second feruloyl residue was thought to be terminal too, as no peak could justify an N^{10} substitution. On the other hand, the absence of fragments at *m*/*z* 204 and *m*/*z* 275 indicates that the coumaroyl moiety was not in terminal position. The observation of a fragment at *m*/*z* 451 corresponds to the fragment bearing both N^1-feruloyl and N^5-coumaroyl after frag-



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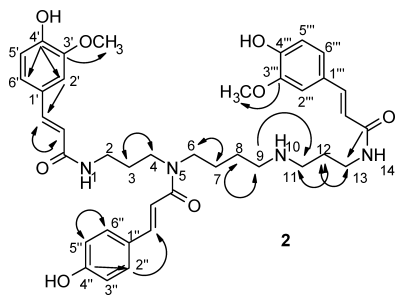

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Fig. 1. Structures of Compounds **1** and **2**

Significant COSY (H↔H) and HMBC (C→H) correlations.

Table 1. ^{1}H- and ^{13}C-NMR Data of Compound **1** in CD_3OD

Position	δ_C	δ_H (mult; *J*, Hz)	HMBC[a)]
2	146.0 d	8.69, d (4.5)	3
3	118.9 d	7.53, d (4.5)	2
4	140.8 s	—	5
5	106.0 d	7.54, d (2.8)	7
6	156.8 s	—	8
7	122.6 d	7.40 dd, (9.1, 2.8)	5
8	129.7 d	7.95, d (9.1)	—
9	143.2 s	—	5, 7
10	126.0 s	—	3, 8
$CONH_2$	171.1 s	—	3

a) HMBC correlations are from proton(s) stated to the indicated carbon.

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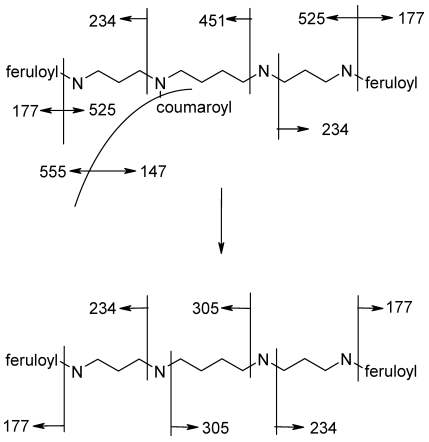

Fig. 2. ESI-MS Fragmentation Scheme for Compound **2**

mentation between C-9 and N^{10}.

With ^{1}H- and ^{13}C-NMR spectra the structure suggested by ESI-MS spectra was confirmed. In the ^{1}H-NMR spectrum, the coupling constant of the doublets around δ_H 6.50 and δ_H 7.45 (J=16, 7 Hz) made it possible to establish the (*E*) configuration of all three hydroxycinnamic moieties.[11] The ^{13}C-NMR spectrum confirmed the presence of two feruloyl and one coumaroyl moieties. NMR attributions of the signals corresponding to the methylenes of spermine were established by bidimensional NMR experiments (COSY, HMQC, HMBC) and by comparing with previously published data.[12] ^{1}H- and ^{13}C-NMR chemical shifts are given in Table 2. Consequently, the structure of compound **2** was unambiguously established as N^1,N^{14}-di-(*E*)-feruloyl-N^5-(*E*)-*p*-coumaroyl-spermine. This is a new compound in the plant kingdom which is named keayanine.

On a biosynthetic point of view it is interesting to note that it is the first report of both quinoline and spermine derivatives in the same plant. Biological activities of the isolated compounds and their possible part in the traditional use of *Microdesmis keayana* will be studied soon.

Experimental

General Experimental Procedures Electrospray-ionspray mass spectra (ESI-MS) were obtained on an API3000 (Perkin-Elmer Sciex) in the Laboratoire d'Application de Spectrométrie de Masse, at the University of Lille 2. Nuclear magnetic resonance (NMR) spectra were recorded in MeOD on a Bruker Avance 300 spectrometer operating at 300 MHz for ^{1}H- and 75 MHz for ^{13}C-NMR and analysed with the software Topspin 1.3, in the Laboratoire d'Application de RMN (LARMN), at the same university.

UV spectra were recorded on a Philips PU8720 UV–visible spectrometer. IR spectra were recorded on a Vector 22 Bruker spectrometer. High performance liquid chromatography (HPLC) was performed on a Shimadzu apparatus, chromatograms were recorded and analysed with the software LC solution.

Plant Material *M. keayana* was collected in the rain forest around Yopougon, a suburb of Abidjan in the Ivory Coast. Plant identification was confirmed by Pr. Laurent Aké Assi at the National Floristic Centre of the University of Abidjan. The roots were dried for several weeks at 37 °C. A voucher specimen (Mk5901) has been deposited in the herbarium of the Department of Pharmacognosy and Botany (Herbarium code: LIP), University of Lille 2, France.

Extraction and Isolation Root powder (400 g) was defatted with 2×500 ml of light petroleum and extracted by a MeOH–H_2O (1 : 1) mixture (2×500 ml, 48 h), affording a 10.5 g extract, which was then divided between equal volumes of water and ethyl acetate (2×50 ml). The aqueous fraction was extracted by *n*-butanol, to obtain a 2.6 g residue. This residue was chromatographed on silica gel with CH_2Cl_2–MeOH, yielding fractions A—I, the most important being B (148 mg) and F (234 mg), eluted with CH_2Cl_2–MeOH (8 : 2) and (6 : 4) respectively.

Table 2. ^{1}H- and ^{13}C-NMR Data of Compound **2** in CD_3OD

Position	δ_C mult.	δ_H (J in Hz)	HMBC[a]
2	45.7 t	3.52, m	—
3	28.9 t	1.85, m	—
4	47.5 t	3.46, m	—
6	49.2 t	3.54, m	—
7	30.7 t	1.89, m	—
8	27.3 t	1.58, m	9
9	50.0 t	2.68, m	11
11	47.6 t	2.68, m	12, 13
12	30.0 t	1.76, m	11, 13
13	38.1 t	3.33, m	11, 12
1′	127.8 s	—	5′, α′
2′	111.5 d	7.09, m	6′, β′
3′	149.5 s	—	5′, OCH_3
4′	150.6 s	—	2′, 6′
5′	116.7 d	6.76, d (8.1)	6′
6′	123.4 d	7.01, m	2′, β′
α′	118.3 d	6.43, d (15.7)	β′
β′	142.4 d	7.45, d (15.7)	2′, 6′
CONH	169.5 s	—	α′, β′
OCH_3	56.4 q	3.86, s	—
1″	127.3 s	—	3″–5″, α″
2″–6″	131.0 d	7.43, d (8.9)	β″
3″–5″	117.0 d	6.73, d (8.9)	—
4″	161.4 s	—	2″–6″
α″	118.3 d	6.43, d (15.7)	β″
β″	144.6 d	7.50, d (15.7)	2″–6″
CONH	169.2 s	—	α′, β′
1‴	127.8 s	—	5‴, α‴
2‴	111.5 d	7.09, m	6‴, β‴
3‴	149.5 s	—	5‴, OCH_3
4‴	150.6 s	—	2‴, 6‴
5‴	116.7 d	6.76, d (8.1)	6‴
6‴	123.4 d	7.01, m	2‴, β‴
α‴	118.3 d	6.43, d (15.7)	β‴
β‴	142.4 d	7.45, d (15.7)	2‴, 6‴
CONH	169.5 s	—	α‴, β‴, 13 (CON^{14}H)
OCH_3	56.4 q	3.86, s	—

a) HMBC correlations are from proton(s) stated to the indicated carbon.

Fraction B was purified successively on silica gel and by gel filtration on Sephadex LH-20 by elution with MeOH–H_2O (6 : 4). This purification process yielded compound **1** (58 mg).

Compound **2** was first purified, from fraction F, by two successive steps on Sephadex LH-20: MeOH–H_2O (8 : 2) then MeOH–H_2O (4 : 6). It was isolated as a pure compound (95 mg) by HPLC on a Nucleosil 5 μm C18 column (15 cm×4.6 mm), following a gradient of H_2O (acidified to pH 2.9 with H_3PO_4) and MeOH (7 : 3 to 3 : 7 in 30 min) with detection at 310 nm.

Xanthoquininamide (**1**): Pale yellow powder; UV λ_{max} nm (MeOH) (log ε): 204.9 (4.17), 232.2 (3.94), 270.1 (3.91), 308.1 (3.23); IR (KBr) cm^{-1}: 3430, 3361, 3161, 2934, 2868, 1667, 1608, 1478, 1423, 805, 785; for ^{1}H- and ^{13}C-NMR spectroscopic data, see Table 1; ESI-MS m/z=377 $[2M+H]^+$, 189 $[M+H]^+$, 172, 161, 146, 144, 118.

Keayanine (**2**): Yellow powder; UV λ_{max} nm (MeOH) (log ε): 218 (4.78), 232 (4.72), 293 (4.64), 315 (4.68); IR (KBr) cm^{-1}: 3385, 2926, 1655, 1584, 1514, 1425, 1254, 1162, 1124, 1029, 973, 812; for ^{1}H- and ^{13}C-NMR spectroscopic data, see Table 2; ESI-MS m/z=701 $[M+H]^+$, for more details, see Fig. 2.

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