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Phytochemical Constituents of the Stem Barks of *Uvaria tortilis*, an Endemic Annonaceae from Côte d'Ivoire

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Abstract

Uvaria tortilis is a lianescent annonaceous species endemic to the Côte d'Ivoire where it is used to treat amenorrhea and as uterotonic. Thus, the MeOH crude extract of its stem barks was investigated in order to determine the chemical composition. A new C-benzyl hydroxlated, uvriatortilisin (**1**), together with six known compounds, dichamanetin (**2**), β-sitosterol (**3**), stigmasterol (**4**), chamanetin (**5**), uvaretin (**6**) and (-)-epicatechin (**7**), were isolated. Structure of these compounds was elucidated using spectroscopic techniques, including IR, UV, MS and NMR (1D and 2D). Certain compounds could be considered to have chemotaxonomic value.

Keywords

Uvaria tortilis, Annonaceae, C-Benzyl, Uvriatortilisin

1. Introduction

The use of natural products as therapeutic agents has become increasingly popular. The World Health Organization estimates that 80% of the world's developing countries used locally the medicinal plants in primary healthcare, because they are easily accessible and less expensive [1]. The genus Uvaria, family Annonaceae, comprises over 100 species distributed in the tropical regions of Asia, New Guinea, New Caledonia, Oceania, Africa and Madagascar. It is mainly composed of shrubs, lianas or sarmentose bushes, most often with unduly stellate hairs [2]. Several species have been reputed to possess medicinal properties and are reported to be used in the treatment of urinary disorders, fevers, jaundice,

rheumatic affections, skin diseases, malaria, stomach disorders, dysentery, infections, especially those related to sexually transmitted diseases, tumours and mental illness [3] [4]. From a chemical viewpoint, studies carried out on this genus mainly reported the presence of alkaloids, flavonoids, chalcones, polyoxygenated cyclohexenes, xanthenes and terpenoids [3] [4] [5]. This chemodiversity is endowed with varied and significant pharmacological traits, including antimalarial, cytotoxic, antifungal, α-glucosidase inhibitory, antitubercular, antitrypanosomal and antileishmanial compounds [3] [4]. In our ingoing to search new bioactive metabolites from Ivorian medicinal plants [6] [7] [8], we investigated *Uvaria tortilis*, a lianescent species with alternate simple leaves endemic to the Côte d'Ivoire. This species grows only in the undergrowth of the tropical forests and savannahs where it is used to treat amenorrhea and as uterotonic [9] [10]. Previous phytochemical studies realized on the essential oils of its stem barks revealed the presence of γ-terpinene, β-caryophyllene and germacrene D as major components [2]. The present study reports the results of a phytochemical investigation of the stem barks of *U. tortilis* which resulted in the isolation and identification of one new C-benzyl (**1**) together with six (**2 - 7**) known compounds.

2. Experimental

2.1. General Methods

Chromatography columns were carried out on silica gel (Merck, 40 - 230 mesh) or Sephadex® LH-20. Thin layer chromatography (TLC) was carried out on aluminum plates coated with silica gel $60F_{254}$ (Merck) and revealed under UV light (254 and 366 nm) and/or with vanillin-H_2SO_4, Lieberman (Acetic anhydride-H_2SO_4) and Fast Blue B reagents. The 1D NMR spectra (^1H, ^{13}C) and 2D NMR spectra (COSY, HSQC, HMBC and NOESY), were recorded in the CD_3OD or $CDCl_3$ on a Bruker AC-400 spectrometer operating at 400 MHz for ^1H spectra and 100 MHz for ^{13}C. Low resolution mass spectra, APCIMS and ESIMS, were acquired using a Bruker Esquire-LC_00040 spectrometer. HRESIMS spectra were recorded with a Bruker Esquire LC_00040 spectrometer.

2.2. Plant Material

The stem barks of *U. tortilis* were collected in March 2019 at Toumodi (6˚33'28"N, 5˚01'03"W). The species was later authenticated by the botanist at the Centre National de Floristique (CNF), Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire). A voucher specimen (OAT-Ut-19) has been also deposited in the Herbarium. The collected plant material was washed, cut into small pieces and dried for two weeks.

2.3. Extraction and Isolation of Compounds

The stem barks powder (400 g) of *Uvaria tortilis* were extracted in a Soxhlet apparatus with methanol. After solvent evaporation, the crude extract (MeOH, 46.8 g) was suspended in water and then partitioned sequentially using hexane

and ethyl acetate to yield hexane (Hex) and ethyl acetate (Ae) sub-extracts. The hexane sub-extract (Hex, 20 g) was fractionated on a silica gel column using a C_6H_{14}/EtOAc elution (90:10 to 60:40) to yield three fractions (F_1 to F_4). Fraction F_3 (5.0 g) was further chromatographed on a silica gel using C_6H_{14}/EtOAc (90:20) to provide ten sub-fractions (F_{31} to F_{310}). Sub-fraction F_{35} (230 mg), in crystals form, was first washed several times with hexane and then with dichloromethane before being purified on silica gel ($CHCl_3$/CH_2Cl_2/EtOAc, 30:60:10) and Sephadex LH-20 (CH_2Cl_2/MeOH, 2:1) columns, yielding the inseparable compounds **3** and **4** (1:1; 13.3 mg). Sub-fraction F_{39} (570 mg) was successfully purified on Sephadex LH-20 (CH_2Cl_2/MeOH, 2:1) and silica gel (C_6H_{14}/$(CH_3)_2CO$, 80:30) columns to yield compounds **1** (4.1 mg) and **2** (13.2 mg). The ethyl acetate sub-extract (15.3 g) was fractionated on a silica gel column, eluted with C_6H_{14}/AcOEt/MeOH/Formic acid (50:50:0:0 to 0:90:10:2), to give five fractions (F_1 to F_5). Fraction F_1 (1.15 g) was chromatographed over silica gel, eluted with C_6H_{14}/$(CH_3)_2CO$ (70:30) to afford six sub-fractions (F_{11} to F_{16}). Sub-fraction F_{12} (483.2 mg) was submitted to a flash chromatography over silica gel, eluted by C_6H_{14}/CH_2Cl_2/EtOAc (20:75:5), to afford compound **5** (7.5 mg) and two sub-fractions (F_{111} to F_{112}). Sub-fraction F_{112} (222.5 mg) was purified on Sephadex LH-20 column (CH_2Cl_2/MeOH, 2:1) to yield compound **6** (12.3 mg). Fraction F_4 (390.0 mg), purified on Sephadex LH-20 eluted with CH_2Cl_2/MeOH (2:1), yielded compound **7** (52.7 mg).

3. Results and Discussion

The crude MeOH extract of the stem barks of *U. tortilis* was sequentially partitioned using hexane and ethyl acetate to provide the corresponding sub-extracts. These sub-extracts individually fractioned using various chromatographic techniques resulted in the isolation of the new C-benzyl derivative, uvriatortilisin (**1**), together with the six known compounds: dichamanetin (**2**) [11], chamanetin (**5**) [12], uvaretin (**6**) [13], (-)-epicatechin (**7**) [14], β-sitosterol (**3**) and stigmasterol (**4**) [15] (**Figure 1**). The structures of the isolated compounds were elucidated by spectroscopic techniques including UV, IR, NMR and MS analysis. These compounds were isolated four thirst time from *U. tortilis*.

Compound **1** was obtained as an amorphous solid. The HRESIMS exhibited an ion peak at *m/z* 501.1917 $[M + H]^+$ (calcd for $C_{30}H_{29}O_7$, 501.1913), indicating the molecular formula of $C_{30}H_{28}O_7$ that corresponding to seventeen degrees of hydrogen deficient. The UV spectrum showed an absorption maximum at 267 and 262 nm, and the IR spectrum displayed bands for hydroxy and carbonyl functional groups at 3340 and 1721 cm^{-1}, respectively. The ^1H NMR spectrum (**Table 1**) demonstrated resonances for eight aromatic protons of two 1,2-disubstitued benzenes [δ_H 7.61 (1H, dd, J = 7.6 and 1.9 Hz), 7.44 (1H, dd, J = 7.6 and 1.8 Hz), 7.10 (2H, ddd, J = 8.6, 8.0 and 1.8 Hz), 6.90 (2H, m) and 6.84 (2H, dd, J = 8.0 and 1.1 Hz)], five aromatic protons of a mono-substituted benzene [δ_H 7.29 (2H, m), 7.24 (2H, m) and 7.21 (1H, m)], a methoxy group [δ_H 3.74 (3H, s)], two coupled methylene groups [δ_H 3.42 and 3.01 (each, 2H, t, J = 7.8 Hz], and two

isolated methylene groups [δ_H 3.95 and 3.88 (each, 2H, s)], together with signal for a chelated hydroxy group [δ_H 13.57 (1H, s)]. The ^{13}C NMR and Dept 135 spectra (Table 1) showed resonances for 30 carbons including an ester carbonyl

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data in $CDCl_3$ of compound **1**.

N° Atome	^{13}C (δ, ppm)	^{1}H (δ, ppm) m (J, Hz)	COSY	HMBC
1	141.2	-		
2	128.4	7.24 m		C-2/C-6, C-4
3	128.4	7.29 m		C-1, C-3/C-5
4	126.0	7.22 m		
5	128.4	7.29 m		C-1, C-3/C-5
6	128.4	7.24 m		C-2/C-6, C-4
7	31.1	3.01 t (7.8)	H-8	C-1, C-2/C-6, C-8
8	43.9	3.42 t (7.8)	H-7	C-1, C-7, C-9
9	179.0	-		
1'	109.0	-		
2'	161.3	-		
3'	111.9	-		
4'	158.7	-		
5'	113.6	-		
6'	159.1	-		
7'	23.6	3.88 s		C-4', C-5', C-6', C-1'', C-2'', C-6''
8'	23.0	3.95 s		C-2', C-3', C-4', C-1''', C-2''', C-6'''
1''	126.2	-		
2''	152.6	-		
3''	115.7	6.84 dd (1.1; 8.0)	H-4''	C-1'', C5''
4''	128.0	7.10 ddd (1.6; 8.0; 8.6)	H-3'', H-5''	C2'', C6''
5''	121.1	6.90 m	H-4'', H-6''	C1'', C3''
6''	131.6	7.44 dd (1.6; 7.6)	H-5''	C1'', C2'', C4''
1'''	126.3	-		
2'''	152.6	-		
3'''	115.6	6.84 dd (1.1; 8.0)	H-4'''	C-1''', C5'''
4'''	127.8	7.10 ddd (1.6; 8.0; 8.6)	H-3''', H-5'''	C2''', C6'''
5'''	121.2	6.90 m	H-4''', H-6'''	C1''', C3''
6'''	132.2	7.61 dd (1.9; 7.6)	H-5'''	C1''', C2''', C4'''
6'-OMe	63.7	3.74 s		C-6'
2'-OH	-	13.57 s		C-1', C-2', C-3'

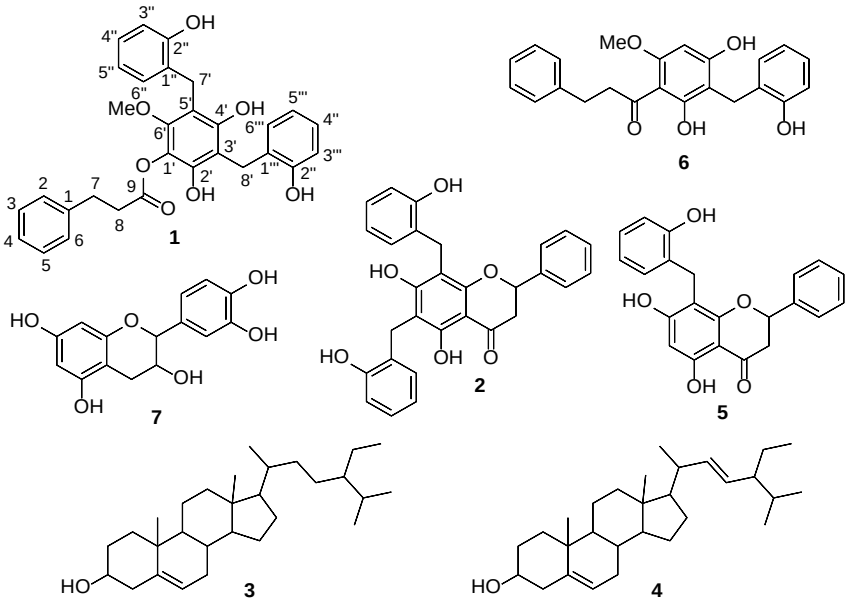

Figure 1. Isolated compounds from stem barks of *Uvaria tortilis*.

carbon (δ_C 179.0), eleven quaternary aromatic carbons (δ_C 161.3, 159.1, 158.7, 152.6 (2C), 141.2, 126.3, 126.2, 113.6, 111.9 and 109.0), thirteen aromatic methine carbons (δ_C 132.2, 131.6, 128.4 (4C), 128.0, 127.8, 126.1, 121.2, 121.1, 115.7 and 115.6), four methylene carbons (δ_C 43.9, 31.1, 23.6 and 23.0), and a methoxy carbon (δ_C 63.7). These NMR data were similar with those of diuvaretin [16] except for the replacement of the signal of *α*, *β*-unsatured carbonyl (δ_C near 205) with signal for ester carbon unit (δ_C 179.0). Thus, the aromatic protons of the 1,2-disubstitued benzenes at δ_H 7.61, 7.44, 7.10, 6.90 and 6.84 were assigned to H-6‴, H-6", H-4"/H-4‴, H-5"/H-5‴ and H-3"/H-3‴, respectively, due to their multiplicities and the HMBC correlations of H-6‴ to C-2‴ (δ_C 152.6), C-4‴ (δ_C 127.8), C-8' (δ_C 23.0) and C-1' (δ_C 133.5); of H-6" to C-2" (δ_C 152.6), C-4" (δ_C 128.0) and C-7' (δ_C 23.6); of H-4"/H-4‴ to C-2"/C-2‴ and C-6" (δ_C 132.1)/C-6‴ (δ_C 131.6); of H-5"/H-5‴ to C-1" (δ_C 126.2)/C-1‴ (δ_C 126.3) and C-3" (δ_C 115.7)/C-3‴ (δ_C 115.6); and H-3"/H-3‴ to C-1"/C-1‴ and C-5" (δ_C 121.1)/C-5‴ (δ_C 121.2). The mono-substituted benzene was further identified to be the aromatic ring of a phenyl propanoic ester group that was confirmed by interpretation of the 1D- and 2D-NMR spectroscopic data of **1**, including COSY, HSQC and HMBC spectroscopy experiments (**Figure 2**). The methoxy group was assigned to C-6' (δ_C 159.1) according to the HMBC correlation observed between the signal at δ_H 3.74 and C-6' as well as C-6' with the methylene protons of one of the two C-benzyl groups at δ_H 3.88 (H-7') that also presented HMBC cross peaks with C-5' (δ_C 113.6) and C-4' (δ_C 158.7). The other C-benzyl group was located at C-3' because the signal of its methylene protons at δ_H 3.95 (H-8') showed HMBC correlations with C-2' (δ_C 161.3), C-3' (δ_C 111.9) and C-4' (δ_C 158.7). The HMBC correlations of the signal of proton of the chelated OH (δ_H 13.57) with those of C-1', C-2' (δ_C 161.3) and C-3' (δ_C 111.9) allowed to locate this OH group at C-2'.

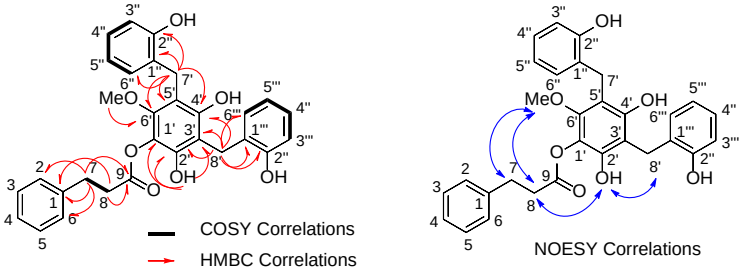

Figure 2. Important 2D correlations of compound **1**.

Further, NOE effects observed between protons of the methylene at δ_H 3.01 (H-7) and 3.94 (H-8) with protons of the methoxy and the chelated OH suggested that the ester group is located at C-1' (**Figure 2**). Compound **1** was thus elucidated as 2,4-dihydroxy-3,5-bis(2-hydroxybenzyl)-6-methoxyphenyl 3-phenylpropanoate that we called uvariatortilisin. Compounds, such as **1**, **2**, **5** and **6**, containing one or two hydroxylated C-benzyl groups, have been mainly isolated from Annonaceae species, precisely in the genera Cleistochlamys [11], Melodorum [17], Sphaerocoryne [18], Uvaria [5] [13] and Xylopia [19]. All these genera belong to Annonoideae sub-family [20]. Thus, the natural occurrence limited of these compounds can be considered as characteristic chemotaxonomical value of Annonoideae sub-family that supporting phylogenetic relationships between its genera.

4. Conclusion

Phytochemical investigation of the MeOH extract of stem barks of *Uvaria tortilis* resulted in the isolation of uvariatortilisin (**1**), a novel phenolic 3-phenylpropanoic acid ester derivative containing two C-benzyl hydroxylated groups, together with dichamanetin (**2**), chamanetin (**5**), uvaretin (**6**), (-)-epicatechin (**7**), *β*-sitosterol (**3**) and stigmasterol (**4**). All these compounds were isolated for the first time from *Uvaria tortilis*. Certain of them, with C-benzyl hydroxylated groups in their structure, could be have a chemotaxonomic importance that would support phylogenetic relationships between genera Cleistochlamys, Melodorum, Sphaerocoryne, Uvaria and Xylopia, all from Annonoideae sub-family.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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