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Astragenol-3-*O*-β-D-xylopyranoside, an unusual triterpene type from *Ouratea turnarea* (Ochnaceae)

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ABSTRACT

Some phytochemical investigations were carried on the roots of *Ouratea turnarea*. This work led to the isolation of menisdaurin, epicatechin, β-sitosterol-3-*O*-β-D-glucopyranoside and astragenol-3-*O*-β-D-xylopyranoside, the last compound is reported for the first time from the Ochnaceae family. Their structures were established by direct interpretation of their spectral data, mainly HRESI-MS, 1D and 2D NMR, and by comparison with literature.

INTRODUCTION

Ouratea turnarea (Hook.) Hutch & Dalz is a shrub with a red twig, reaching up to 06 m height, which grows in the woody savannah forests. The ethnomedicinal use of the plant in folk medicine has not yet been reported but other species of the same genus are used to treat rheumatic, whitlow, gonorrhea, gastric pains and are also used against chest pains (Bouquet, 1969; Felicio *et al.*, 2001). Pharmacological studies of some *Ouratea* species have shown that they have good antimicrobial activities (Gangoué *et al.*, 2006). The previous phytochemical studies on this species led to the isolation of one indole alkaloid named serotobenine and many biflavonoids (Abouem *et al.*, 2008).

In this paper, we report the isolation of four known compounds from *Ouratea turnarea* identified as: a triterpene glycoside, astragenol-3-*O*-β-D-xylopyranoside (**1**); a cyanoglycoside, menisdaurin (**2**); a flavonoid, epicatechin (**3**) and a steroid, β-sitosterol-3-*O*-β-D-glucopyranoside (**4**). Meanwhile, further to a work done by Kitagawa et al (1983), the structure elucidation of compound **1** is once more reported. The roots of *O. turnarea* (Hook.) Hutch & Dalz were collected, at Ntui (April 2006) in Centre-Cameroon. This plant material was identified by Mr. Nana Victor (botanist). The voucher sample (No. 10134/SRF/CAM), was deposited at the National Herbarium in Yaoundé, Cameroon.

Previous work

Preliminary studies on this plant revealed the presence of biflavonoids, especially chalcone derivatives and an indole alkaloid only (Abouem *et al.*, 2008); moreover, the genus is characterized by the occurrence of several classes of compounds such as: biflavonoids, flavonoids, nitrile glycosides, simple steroids, terpenoids, lignans, indole alkaloid derivatives and ellegatic acids (Abouem *et al.*, 2008; Bayiha Ba Njock *et al.*, 2011; Felicio *et al.*, 2001; Ngono *et al.*, 2009; Ngono *et al.*, 2011; Pegnyemb *et al.*, 2005; Velandia *et al.*, 2002).

METHODOLOGY

Dried and powdered roots (921g) were extracted with MeOH at room temperature. The extract was concentrated under

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vacuum to yield the residues (brown mass) (258 g). A part of the residues (80 g) was merged in H_2O/MeOH 9:1 and partitioned in hexane, CH_2Cl_2 and AcOEt to afford the hexane-soluble (12 g), CH_2Cl_2-soluble (18 g), AcOEt-soluble (14g) and H_2O-soluble (36g) fractions. The AcOEt-soluble (14g) was submitted to flash chromatography using CH_2Cl_2/MeOH at increasing polarity (from pure CH_2Cl_2 to 100% MeOH) as eluents to provide six main fractions (T1: 2.2 g, T2: 2.1 g ; T3 : 3.6 g ; T4 : 0.2g ; T5 : 5.6 g and T6 : 0.3 g).

Fraction T3 was submitted to a silica gel CC and four sub-fractions (T3a: 0.6 g, T3b: 0.82 g, T3c: 1.3 g, and T3d: 0.82 g) were obtained. T3b was chromatographed over Sephadex LH-20 (MeOH) to afford epicatechin (**3**, 7 mg) (Daniel *et al.*, 2005) when T3d was filtered on silica gel column using CH_2Cl_2/MeOH as mobile phase gradient to afford menisdaurin (**2**, 11 mg) (Ngono *et al.*, 2011)

The analysis of fraction T5, proceeding as above, enabled us to get five sub-fractions (T5a: 0.89 g, T5b: 0.92 g, T5c: 0.8 g, T5d: 1.07 g and T5e: 1.32 g). Sub-fraction T5a was at the beginning subjected to a silica gel column with mixture (CH_2Cl_2/MeOH: 30/1-8/1) and at the end filtered on Sephadex LH-20, using MeOH as mobile phase, to yield sitosterol-3-*O*-β-D-glucopyranoside (**4,** 16 mg) (Ndongo *et al.*, 2010) The last sub-fraction T5e was repeatedly chromatographed over Sephadex LH-20 and preparative TLC (CH_2Cl_2/MeOH: 6/1), producing astragenol-3-*O*-β-D-xylopyranoside (**1**, 6 mg) (kitagawa *et al.*, 1983).

Their structures were established by direct interpretation of their spectral data, mainly HRESIMS, 1D NMR (^{1}H, ^{13}C, DEPT) and 2D NMR (COSY, NOESY, HSQC and HMBC), and by direct comparison with the literature data.

EXPERIMENTAL

Optical rotations were measured on a Perkin–Elmer 341 polarimeter. NMR spectra were run on a Brüker instrument equipped with a 5 mm ^{1}H and ^{13}C probe operating at 400 and 100 MHz, respectively, with TMS as internal standard. ^{1}H assignments were made using 2D-COSY and NOESY (mixing time 500 ms) while ^{13}C assignments were made using 2D-HSQC and HMBC experiments.

For this latter, the delay was 70 ms. Melting points were measured on a Büchi apparatus and are uncorrected. IR data were measured on a JASCO FTIR-300E spectrometer with KBr pellets. Silica gel 70–230 mesh (Merck) and Sephadex LH-20 were used for column chromatography while precoated aluminium sheets silica gel 60 F254 were used for TLC.

The HR-ESI mass spectra were run on an Applied Biosystems API Q-STAR PULSAR. The EIMS was recorded on a JEOL JMSD-300 instrument. The solvent systems were: CH_2Cl_2/MeOH at increasing polarity (from pure CH_2Cl_2 to 100% MeOH).

RESULTS AND DISCUSSION

The structure of compound **1** was established as astragenol-3-*O*-β-D-xylopyranoside by ^{1}H and ^{13}C NMR and by the HRESI-MS analysis that presented the quasi molecular peak at *m/z* 621.4102 ([M-H]$^-$), calc. 621.4081, consistent with the molecular formula $C_{35}H_{58}O_9$ (Kitagawa *et al.*, 1983) according to other analyses, compound **1** was identified as an isomer of cycloartan type triterpene glycoside; this hypothesis was confirmed by direct comparison of its NMR data (Table 1) with those of known cycloartan derivatives (Kitagawa *et al.*, 1983). Extensive analysis of 1D and 2D NMR spectra indicated the presence of eight tertiary methyl groups instead of the seven ones usually observed in a cycloartan unit; their chemical shifts appeared at δ_H : 1.04 ; 1.23 ; 1.35 ; 1.38 ; 1.40 ; 1.42 ; 1.43 ; 1.70 ppm. An olefin broad triplet proton was remarkable at δ_H 5,99 ppm, coupling with a carbon at δ_C 120.3 (C-11) as an indication of the absence of the cyclopropane ring (Fig. 1).

A quaternary carbon was observed at δ_C 139.1 ppm (C-9) such as two oxymethine protons at δ_H 3.39 (H-3) and 3.96 (H-24) ppm, with the last one coupling in HMBC with carbons at δ_C 86.2 (C-20), 37.3 (C-22), 27.1 (C-23), hence displaying a tetrahydrofuran ring in compound **1**; these chemical shifts are typical signals of astragenol skeleton **(**Kitagawa *et al.*, 1983) The ^{1}H NMR spectrum showed also an anomeric signal at δ_H 4,83 ppm (d, J = 7.4 Hz) which correlates with the carbon atom at δ_C 107.8 ppm; this anomeric proton was determined to have β-orientation based on his relatively large $^3J_{H\text{-}1,\ H\text{-}2}$ value of 7.4 Hz.

The sugar moiety was identified as xylose by analysis of its EIMS after acid hydrolysis of compound **1**, showing characteristic fragment peaks of this sugar at *m/z*= 132 et 148 (Debella *et al.*, 2000). Based on the above evidence, this compound which was previously isolated from the roots of *Astragalus membranaceus* has been identified as astragenol-3-*O*-β-D-xylopyranoside.

Ouratea species have been described as reservoirs of terpenoids, isoflavonoids, flavonoid glycosides and more frequently biflavonoids which are considered as chemical markers of this genus (Carvalho *et al.*, 2008; Felício *et al.*, 2004; Moreira *et al.*, 1999; Suzart *et al.*, 2007). Previous phytochemical studies on this species led to the isolation of many biflavonoids and an indole alkaloid (Abouem *et al.*, 2008) in accordance with the chemical profile of this genus. The isolation of an unusual triterpenoid, reported in this work for the first time from the Ochnaceae family, suggests in the first hand the occurrence of interspecific variety within some species of the *Ouratea* genus and in the other hand, the taxonomic replacement of several species which are rich in nitrogenous and other compounds (Abouem *et al.*, 2008; Njock et al., 2011). The frequent occurrence of triterpenoids suggests that they may be important metabolites of some Ochnaceae species (Sani *et al.*, 2011).

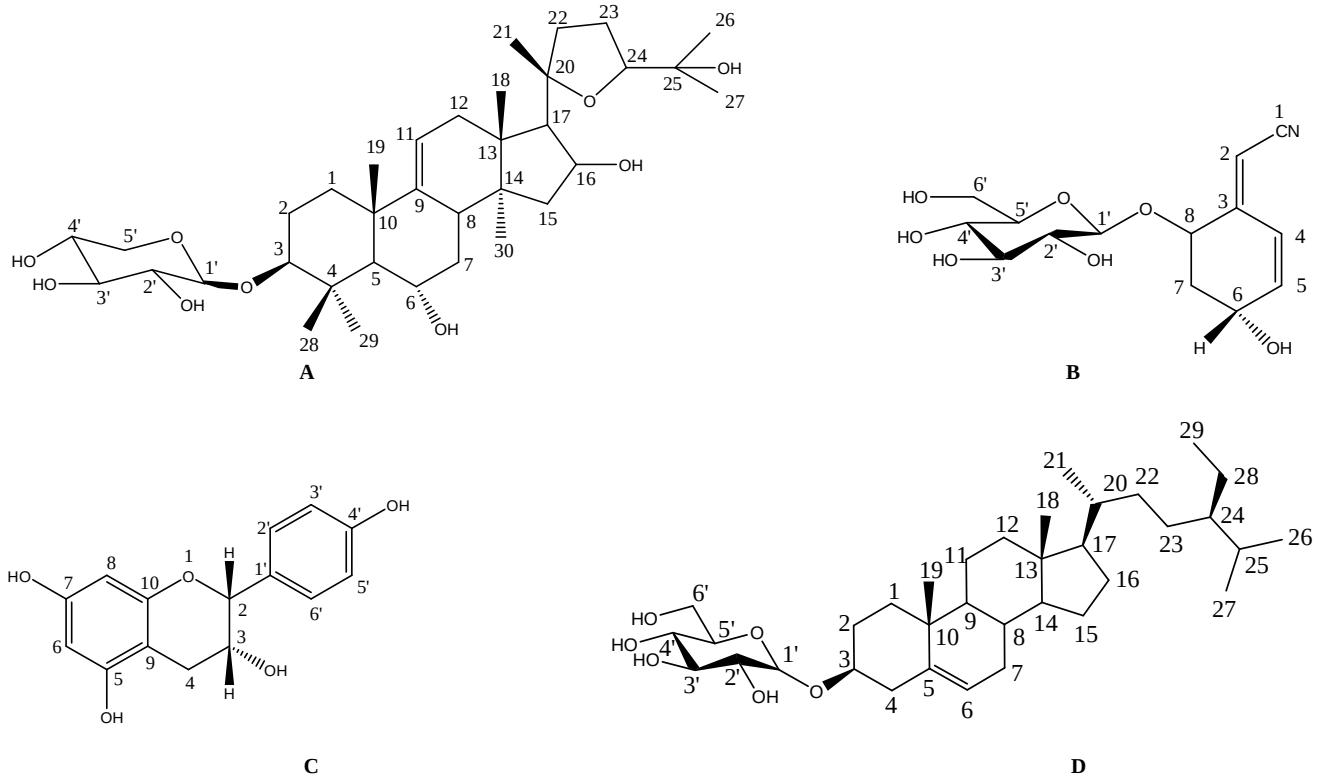

Fig. 1: Compounds isolated from the roots of *O. turnarea*.

Table 1 : ^{1}H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) data of compound **1** (δ in ppm, J in Hz)[a].

Position	δ_C	*m*	δ_H	*m*	*J*
1	32.3	*t*	-		-
2	30.2	*t*	-		
3	89.6	*d*	3.39	*dd*	4.5; 11.1
4	42.7	*s*	-		-
5	52.6	*d*	-		
6	79.1	*d*	3.94	*m*	
7	34.0	*t*	-		
8	45.4	*t*	-		
9	139.1	*s*	-		-
10	29.1	*s*	-		-
11	120.3	*d*	5.34		-
12	33.3	*t*	-		-
13	46.8	*s*	-		-
14	47.1	*s*	-		-
15	45.7	*t*	-		-
16	76.6	*d*	5.66	*ddd*	5.2; 7.8 ; 7.9
17	58.0	*d*	2.62	*d*	8.6
18	20.8	*q*	1.42	*s*	
19	27.3	*q*	1.38	*s*	-
20	86,2	*s*	-		-
21	27.2	*q*	1.40	*s*	-
22	37.3	*t*	-		-
23	27.1	*t*	-		-
24	83.3	*d*	3.96		-
25	71,3	*s*	-		-
26	28.5	*q*	1.43	*s*	-
27	27.2	*q*	1.35	*s*	-
28	28.5	*q*	1.70	*s*	-
29	16.9	*q*	1.23	*s*	-
30	20.3	*q*	1.04	*s*	
1’	107.8	*d*	4.83	*d*	7.4
2’	75.8	*d*	4.05	*d*	
3’	78.7	*d*	4.20	*d*	
4’	71.4	*d*	4.17	*d*	
5’	67.2	*t*	4.35	*t*	
			3.64		

[a] Assignments were based on 1D and 2D NMR experiments

CONCLUSION

Species of the *Ouratea* genus are known for their content of biflavonoids, cyanoglycosides, steroids and terpenoids as major secondary metabolites and our phytochemical study on the roots of *O. turnarea* corroborates this assertion. Our study also showed that some chemical differentiations can appear in the bosom of the genus because of the wide variety of the species.

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