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FLAVONOIDS FROM *FISSISTIGMA ACUMINATISSIMA*

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SUMMARY

From the leaves of Fissistigma acuminatissima growing in Vietnam, two flavonoids, catechin and isorhamnetin-3-o-rutinoside were isolated. Their structures were characterized on the basis of MS and NMR spectra data and comparison with reported data.

Keywords: *Annonaeae; Fissistigma acuminatissima; catechin; isorhamnetin-3-o-rutinoside.*

I - INTRODUCTION

The genus *Fissistigma* is a large tribe with ca.70 species in the Annonaceae family [1]. The decoctions of some *Fissistigma* species have been used in Southeast Asia as traditional medicines for treatment of infections and enhancement of blood circulation [2]. *Fissistigma acuminatissima* (Vietnamese name Cách thư nhọn or Lãnh công lá nhọn) is climbing shrub growing in the north Vietnam [1]. In continuation of our investigation on the constituents of *F. acuminatissima* we now report the isolation and structural elucidation of two flavonoids, catechin (**1**) and isorhamnetin-3-o-rutinoside (isorhamnetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosid, **2**). The structures of these compounds were determined by MS, NMR techniques and comparison of those spectral data with reported data.

II - EXPERIMENTAL

1. General

FT-IR: Nicolet IMPACT 410. EIMS (70 eV): MS5989B. NMR: Bruker Avance 500, 499.84 MHz (^{1}H-) and 125 MHz (^{13}C-, ^{13}C-DEPT). TMS (δ = 0.0, ^{1}H) and CD_3OD (δ = 49.0, ^{13}C) were references. CC: Silica gel 60, 0.06 – 0.2 mm (Merck) for the first column; Sephadex LH-20 and silicagel 60, 40 – 63 μm (Merck) for the following columns. TLC: silicagel 60 F_{254} (Merck).

2. Plant material

Leaves of *F. acuminatissima* were collected in Nghe An province, Vietnam in November 2004. The species was identified by Dr. Ngo Van Trai, Institute of Materia Medica, Hanoi. A voucher specimen is deposited in the Herbarium at the same Institute.

3. Extraction and isolation

The dried and powdered leaves of *F. acuminatissima* (1.4 kg) were extracted with 95% aqueous MeOH at room temperature. MeOH was evaporated *in vacuo*, and the aq. solution was partitioned with *n*-hexane followed by EtOAc and *n*-BuOH. The organic solutions were evaporated *in vacuo* to afford 50; 10 and 15 g extracts, respectively. The *n*-hexane extract was chromatographed over silica gel with gradient $CHCl_3$/MeOH (95:5→30:70) to give 40 fractions (Fr-1→Fr-40).

*a) Catechin (**1**)*

The crude compound **1** (15 mg) was isolated

from fraction 2 (Fr-2) by CC (sephadex LH-20, MeOH) and further purified by column chromatography on silicagel ($CHCl_3$-MeOH, 70:30); FT-IR (KBr) ν_{max}, cm^{-1}: 3405 (OH), 2924, 1612, 1518, 1463, 1287, 1141; EI-MS 70 eV, *m/z* (rel. int.): 290 $[M]^+$ (42), 271 (4), 152 (40), 139 (100), 123 (69), 110 (39), 97 (32), 55 (76); ^{1}H- and ^{13}C-NMR data, see table 1.

Table 1: ^{1}H- and ^{13}C-NMR data of compounds **1** and **2** (125/500 MHz in CD_3OD)

Position	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
2	82.85	4.59, d (7.5)	158.91	
3	68.81	3.99, m	135.46	
4	28.50	*ax* 2.53, dd (16.1, 8.1) *eq* 2.87, dd (16.1, 5.4)	179.36	
5	157.56		163.02	
6	96.33	5.95, d (2.3)	100.01	6.24, d (2.1)
7	157.82		166.14	
8	95.54	5.88, d (2.3)	94.94	6.44, d (2.1)
9	156.91		158.54	
10	100.86		105.70	
1'	132.23		123.04	
2'	115.28	6.86, dd (1.9)	116.13	7.96, d (2.1)
3'	146.24		150.86	
4'	146.22		148.36	
5'	116.11	6.78, d (8.1)	114.60	6.94, d (8.5)
6'	120.05	6.74, dd (8.1, 1.9)	124.02	7.65, dd (8.5, 2.1)
Gluc 1"			102.52	5.25, d (7.4)
2"			77.39	3.4 - 3.5, m
3"			78.19	3.4 - 3.5, m
4"			69.79	3.2 - 3.3, m
5"			75.91	3.2 - 3.3, m
6"			68.54	3.4 - 3.5, m 3.84 dd (1.5, 11.5)
Rham 1'"			104.39	4.56, br s
2'"			72.09	3.62, dd (1.6, 4.3)
3'"			72.30	3.4 - 3.5, m
4'"			73.85	3.4 - 3.5, m
5'"			72.09	3.4 - 3.5, m
6'"			17.87	1.12, d (6.2)
OMe			56.81	3.97 s
OH		4.56 br s		

NMR: Bruker Avance 500, 499.84 MHz (^{1}H-) and 125 MHz (^{13}C-, ^{13}C-DEPT). TMS (δ = 0.0, ^{1}H) and CD_3OD (δ = 49.0, ^{13}C) were references. CC: Silica gel 60, 0.06 - 0.2 mm (Merck) for the first column; Sephadex LH-20 and silica gel 60, 40 - 63 µm (Merck) for the following columns. TLC: silica gel 60 F_{254} (Merck).

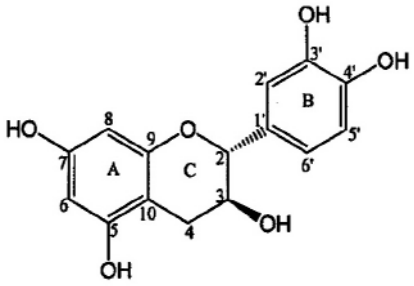
b) Isorhamnetin-3-o-rutinoside (*narcissoside*, **2**)

Compound **2** (18 mg) was isolated as brown powder from Fr26-28 by CC (sephadex LH-20, MeOH) and further purified by column chroma-

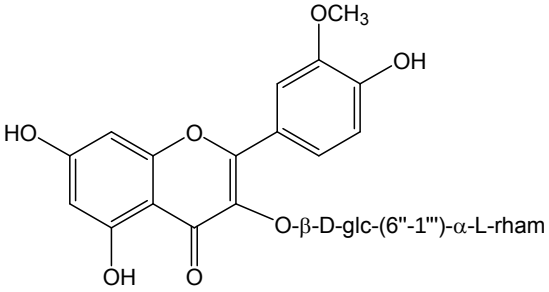
tography on silica gel ($CHCl_3$-MeOH, 80:20); FT-IR (KBr) ν_{max}: 3388, 2925, 1652, 1600, 1506, 1451, 1358, 1061 cm^{-1}; EI-MS 70 eV, *m/z* (rel. int.): 316 $[M\text{-gly}]^+$ (100), 301 $[M\text{-Me}]^+$ (15), 286 $[M\text{-}30]^+$ (11), 153 (8), 128 (14), 85 (19), 60 (26); ^1H- and ^{13}C-NMR data, see table 1.

III - RESULTS AND DISCUSSION

The flavonoids were obtained from the MeOH extract of the leaves *via* partition between *n*-hexane followed by EtOAc and *n*-BuOH. The *n*-hexane extract was chromatographed over silica gel with gradient $CHCl_3$-MeOH and further purified by column chromatography on silica gel to afford **1** and **2**.



1: Catechin



2: Isorhamnetin-3-o-rutinoside

The molecular formula of compound **1** ($C_{15}H_{14}O_6$) was deduced from combined analysis of EI-MS at *m/z* 290 $[M]^+$, ^1H- and ^{13}C-DEPT NMR spectra (*table 1*). The EI-MS of **1** shows the base peak of the A-ring and the B-ring fragments (*m/z* 139 and 152) due to a *Retro-Diels-Alder* cleavage, indicating the presence of a flavan-3-ol with two hydroxy groups in each A- and B-ring [3]. The ^1H-NMR spectrum exhibited a doublet at δ 4.59 (H-2), two doublets of doublet multiplet at δ 3.99 (H-3), 2.53 (H-4*ax*), 2.87 (H-4*eq*), as well as five aromatic protons. The ^1H- and ^{13}C-NMR spectrum data of **1** are identical with those in literature [4, 5]. Catechin and its analogs showed antitumor and antioxidant activities [3, 4].

The EI-MS of compound **2** gave a peak at *m/z* 316 $[M\text{-glycone}]^+$, combination with ^{13}C-NMR and DEPT spectra leading to the formula $C_{16}H_{12}O_7$ for the aglycone. The aglycone moiety was identified as flavone-3-ol from the characteristic of molecular formula and aromatic signals in the ^1H- and ^{13}C-NMR spectra. The ^1H-NMR spectrum showed one methoxy group (δ_H3.97, δ_C56.81) and five aromatic protons. The signals from δ_H 3.3 - 5.3 are assigned of two sugar protons. The β-D-glucopyranose was identified by anomeric signals at δ_H5.25 (d, J = 7.4 Hz) δ_C104.39 and the rhamnopyranose was identified by methyl signal at δ_H 1.12 (d, J = 6.2 Hz) δ_C17.87 (table 1). The ^1H- and ^{13}C-NMR spectra of sugar moiety were identical with those of quercetin-3-o-rutinoside [6] and the aglycon moiety were identical with those of isorhamnetin [7], therefore the structure of **2** was determined as 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranyloxy-4',5,7-trihydroxy-3'-methoxyflavone (isorhamnetin-3-o-rutinoside, narcissoside). Isorhamnetin-3-o-rutinoside was isolated for the first time from the flowers of *Narcissus tazetta* and then was found in *Lilium aurantum*, *Herniaria glabra* [6 - 8].

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