

Flavonols from *Scurrula ferruginea* Danser (*Loranthaceae*)

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Z. Naturforsch. **57c**, 1092–1095 (2002);
received August 6/September 2, 2002

Flavonols, Cytotoxic, *Scurrula ferruginea*

Three natural flavonols compounds have been isolated from the ethyl acetate fraction of *Scurrula ferruginea* Danser (*Loranthaceae*). Besides quercetin and quercitrin, an unusual flavonol glycoside 4'-O-acetylquercitrin was isolated. Structures were determined using spectroscopic methods including UV, NMR and HRMS-EI. The incidence of 4'-O-acetylquercitrin, not previously reported in the *Loranthaceae*, is discussed. Cytotoxic evaluation on four human cancer cell lines showed quercetin to be the most active with IC₅₀ of 35 µM on U251 (human glioblastoma cells).

Introduction

The members of the *Loranthaceae* family (about 74 genera), generally known as mistletoes and mostly distributed in tropics, are semi-parasitic shrubs attached to the hosts by modified roots (Barlow, 1991). Preparations from leaves and stems of several plants belonging to this family have been widely used in traditional medicine as therapeutic herbs (Zee-Cheng, 1997). In Indonesia, *Scurrula* sp. (syn. *Loranthus* sp.) are one of the five components of Benalu Teh[®] used as an infusion in fatigue and in cancer pathologies. Previous studies on *S. ferruginea* Danser exhibited attractive cytotoxic activity (Lohézic-Le-Dévéhat *et al.*, 2002) on U251 glioblastoma cancer cell line. Few phytochemical studies have been conducted on *Scurrula* species. Perseitol (Ishizu *et al.*, 2001), quercitrin, 3-O-xylopyranoside-quercetin (Shibuya *et al.*, 1999) were isolated from *S. fusca*, dihydromyricetin and tannin from *S. liquidambaricolus*

(Shen *et al.*, 1993) but no report was found on *S. ferruginea*.

We report here, from this species, the isolation, the structure elucidation of three flavonols and their cytotoxic activity on human cancer cell lines (U251, K562, DU145, MCF-7).

Material and Methods

General

UV spectra were obtained on the Uvikon 922 (Kontron Instruments) spectrophotometer and FT-IR on a Perkin Elmer 16PC spectrometer. ¹H NMR and ¹³C NMR spectra were recorded in DMSO-*d*₆ using TMS as internal standard on a Bruker DMX 500 WB spectrometer at 500 MHz and 125 MHz, respectively. HRMS-EI spectra were obtained on a Varian Mat 311 mass spectrometer at 70 eV. Silica gel 60 mesh (Merck) was used for column chromatography; TLC was performed using silica gel 60 G with n-hexane:ethyl acetate:methanol:acetic acid (30:80:7:3 v/v). The spots were visualized using UV light (254 and 365 nm) and Neu reagent. Sephadex LH20 was used for Chromatography Column. Reversed phase Semi-preparative HPLC (Hypersil 5 µ, 250 × 10 mm, 28105–120) was used at 2 ml/min flow rate, using a pump constametric (4100 TSP), with 1 ml injection loop and UV spectrophotometer (430A, Kontron Instruments) as detector. Conditions were described concerning isolation.

Extraction and isolation

Dried powdered aerial parts of *S. ferruginea* (1.5 kg) were extracted successively with petroleum ether (3 × 5 l) and ethyl acetate (EA) (12 × 5 l). 60 g out of 87 g EA extract were dissolved in a mixture of acetone/water (3/2) and stored at 4 °C to precipitate liposoluble pigments as a green flocculate (17 g). A portion of the non-precipitated EA residue (25 g) was chromatographed on silica gel column using dichloromethane, ethyl acetate and methanol mixtures of increasing polarity. All collected fractions were analyzed by TLC and gave 8 fractions (A → H).

In fraction H (21 g), a main component **2** was present. In fraction E (2 g), another main compo-

ment **1** was detected. In the fractions F (250 mg) and G (550 mg), **1** and **3** were present. A portion of the fraction H (188 mg) and the fraction E (200 mg) were deposited on Sephadex LH-20 (20 g) and eluted with methanol to afford **2** (144 mg) and **1** (168 mg) respectively. Fraction F (260 mg) was chromatographed on Sephadex LH-20, eluted with methanol to give F1 and F2 which were purified by semi-preparative RP18 using methanol-H₂O (4:6), during 141 min and methanol-H₂O (6:4) during 37 min then methanol 100%, with a 2 ml/min flow rate. Two compounds were isolated **1** (20 mg) and **3** (70 mg). The structure of isolated constituents were elucidated based on chemical evidences and spectral analyses.

4''-O-acetylquercitrin (3) = (3-[(4-O-acetyl-6-deoxy- α -L-mannopyranosyl)oxy]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one). UV λ_{\max} MeOH nm: 259, 353. IR (cm⁻¹) ν 800–815, 1390, 1503, 1602, 1731, intense band about 3194. ¹H NMR (500.14 MHz, in DMSO-*d*₆): δ (ppm) 0.7 (3H, d, $J^3_{\text{H-5''}} = 6$ Hz, CH₃-6''), 2.0 (3H, s, CH₃-CO), 3.39 (1H, dq, $J^3_{\text{H-4''}} = 9$ Hz, $J^3_{\text{H-6''}} = 6$ Hz, H-5''), 3.74 (1H, dd, $J^3_{\text{H-4''}} = 9.7$ Hz, $J^3_{\text{H-2''}} = 3$ Hz, H-3''), 4.03 (1H, sl, H-2''), 4.71 (1H, t, $J^3_{\text{H-3''}} = 10$ Hz, H-4''), 5.23 (1H, sl, H-1''), 6.22 (1H, sl, H-6), 6.40 (1H, sl, H-8), 6.90 (1H, d, $J^3_{\text{H-6'}} = 8.3$ Hz, H-5'), 7.24 (1H, dd, $J^3_{\text{H-5'}} = 8.3$ Hz, $J^4_{\text{H-2'}} = 2$ Hz, H-6'), 7.30 (1H, sd, $J^3_{\text{H-6'}} = 1.9$ Hz, H-2'), 12.61 (1H, s, OH-5). ¹³C NMR (125.76 MHz, in DMSO-*d*₆): δ 17.0 (q, $J^1 = 128.5$ Hz, C-6''), 20.8 (q, $J^1 = 128.5$ Hz, CH₃-COO), 67.8 (dm, $J^1 = 143$ Hz, C-5''), C-3''), 69.8 (d, $J^1 = 128$ Hz, C-2''), 73.04 (dm, $J^1 = 128$ Hz, C-4''), 93.6 (dd, $J^1 = 165$ Hz, $J^3_{\text{H-6}} = 3.6$ Hz, C-8), 98.7 (dl, $J^1 = 163.5$ Hz, C-6), 101.4 (d, $J^1 = 176$ Hz, C-1''), 103.9 (s, C-4a), 115.4 (d, $J^1 = 159$ Hz, C-5'), 115.6 (dd, $J^1 = 159$ Hz, $J^3_{\text{H-6'}} = 3$ Hz, C-2'), 120.4 (sd, $J^3_{\text{H-5'}} = 9.7$ Hz, C-1'), 120.9 (dd, $J^1 = 163$ Hz, $J^3_{\text{H-2'}} = 7.3$ Hz, C-6'), 133.9 (sd, $J^4_{\text{H-6'}} = 2.4$ Hz, C-3), 145.2 (sm, C-3'), 148.4 (sm, C-4'), 156.4 (sd, $J^2_{\text{H-8}} = 3.6$ Hz, C-8a), 157.4 (sm, C-2), 161.2 (sl, C-5), 164.3 (sl, C-7), 169.8 (sm, CH₃-COO), 177.5 (s, C-4). HRMS-EI (C₂₃H₂₂O₁₂) *m/z* = 489.103.

Cytotoxic activity

Cytotoxicity assays were carried out on four human cancer lines : U251 (NCI strain), K562 (CCL-243, ATCC), DU145 (HTB-81, ATCC),

MCF-7 (HTB-22, ATCC) according to the procedure described previously (Bezivin *et al.*, 2002) and expressed as IC₅₀ (μ M). Each experiment was repeated three times. The positive control (Doxorubicin) for the cytotoxicity was purchased from Dakota Pharm.

Results – Discussion

We carried out an isolation of main constituents of ethyl acetate fraction of *Scurrula ferruginea* Danser by Chromatography Column on silica gel and Sephadex LH-20 for compounds **1–3**. Further purification by preparative HPLC on C18 afforded **3**. Compounds **1–3** (Fig. 1) were obtained as yellow amorphous powders. The UV procedure (Mabry *et al.*, 1970) suggested a 3-OH free flavonol for compound **1** but a 3-OH substituted flavonol for compounds **2** and **3**.

HRMS-EI measurement of **1** gave the molecular formula C₁₅H₁₀O₇. ¹H NMR and ¹³C NMR spectra confirmed the well-known structure of quercetin (Markham, 1989).

The molecular formula of compound **2** was established as C₂₁H₂₀O₁₁ based on high resolution measurements (HRMS-EI). The structure of compound **2** was deduced from spectroscopic data of **1** and the ¹H NMR data of the aglycone of **2** were consistent with a quercetin derivative. The ¹H NMR spectrum also showed the presence of a rhamnosyl unit with an α -linkage characterized with the anomeric proton at 5.26 ppm and the weak magnitude of the coupling constant $J_{1''2''}$ (Agrawal, 1992). The structure of compound **2** was elucidated as quercitrin.

The molecular formula (C₂₃H₂₂O₁₂) of **3** given by HRMS-EI indicated its close structure with **2**. The FT-IR spectrum of **3** was very similar to those of **2** except the shift of the carbonyl band to the high frequencies (1731 cm⁻¹) and the lack of the 1565 cm⁻¹ band but the novel one at 1390 cm⁻¹ suggested an acetylation of the quercitrin sugar moiety (Carpena *et al.*, 1980). The ¹H NMR and ¹³C NMR data of compound **3** confirmed the presence of quercitrin with an acetyl group on rhamnose which can be assigned to a signal at 2 ppm (CH₃) and to the two signals at 20.8 (CO-CH₃) and 169.8 ppm (CO). It was confirmed by the upfield shift of CH₃-6'' and downfield shift of H-4'', H-5'' and H-3''. The position of the acetylation was

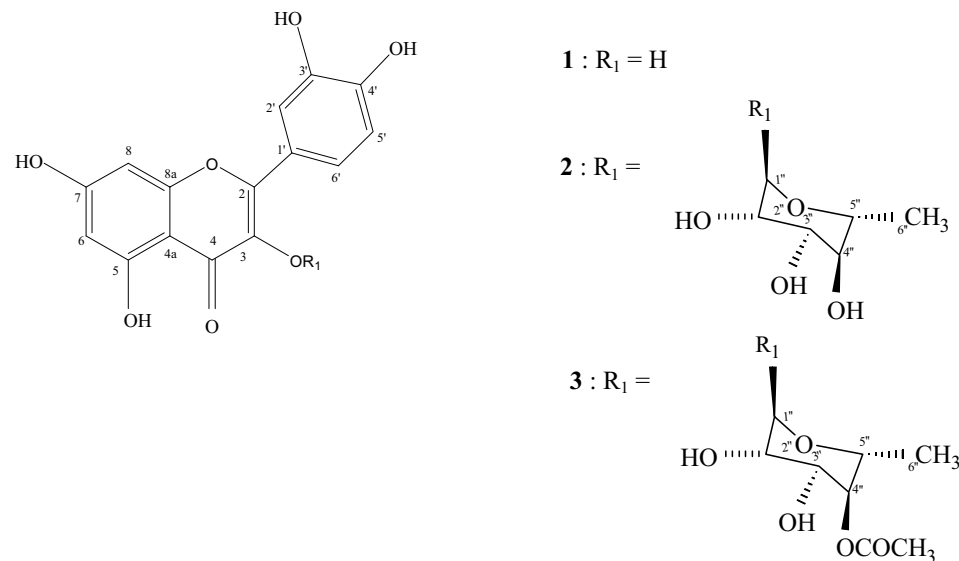


Fig. 1. Structure of quercetin (**1**), quercitrin (**2**) and 4''-O-acetylquercitrin (**3**).

thought to be on C-4'' as this carbon (δ 73.04 ppm) is shifted downfield (+1.9 ppm) while adjacent carbons C-5'' and C-3'' were shifted upfield (-2.3 and -2.7 ppm respectively) (Markham, 1989; Danieli *et al.*, 1997). Thus, compound **3** was identified as 4''-O-acetylquercitrin for which NMR assignments were ascertained on the basis of fine long-range coupling constant measurements. Data corresponded to those previously described (Fuchino *et al.*, 1996) excepted the ^{13}C NMR assignments of C-8a (δ 156.4 ppm) and C-2 (δ 157.4 ppm) and of C-5' (δ 115.4 ppm) and C-2' (δ 115.6 ppm) which have been inverted.

While quercitrin and quercetin are common flavonols in higher plants, the acetylated derivatives are very unusual. The 2''-O-acetylquercitrin was isolated from azalea flowers (Asen, 1977), the 3''-O-acetylquercitrin from a fern (Tanaka *et al.*, 1978) and aquatic flowers (Fossen *et al.*, 1999). The 4''-O-acetylquercitrin was previously isolated from

Betula species (Ossipov *et al.*, 1995; Fuchino *et al.*, 1996) and from *Pteris grandifolia* (Tanaka *et al.*, 1978). The taxonomic marker of the *Loranthaceae* family was quercitrin (Tilney *et al.*, 1974) already isolated from three phylogenetically linked genera of *Scurrula*, *Taxillus*, *Dendrophloe* and *Helixanthera* but to our knowledge it was the first isolation of the 4''-O-acetylquercitrin in this family. Further studies would be of taxonomic interest to know if the 4''-O-acetylquercitrin could be a specific marker of *Scurrula* genus.

Cytotoxic activities of the three isolated compounds were evaluated on four human cancer cell lines (Table I). Quercetin was the only biological active compound, specially on the U251 glioblastoma cells ($IC_{50} = 35 \mu M$) while quercitrin and its acetylated derivative remained inactive ($IC_{50} > 200 \mu M$). Many studies have been conducted on quercetin which has been found to be one of the most active flavonol as anticancer agent with gen-

Isolated compound	Human Cancer Cell line ($IC_{50}/\mu M$)			
	U251	K562	DU145	MCF-7
Quercetin (1)	35	69	205	> 331
Quercitrin (2)	> 222	> 222	> 222	> 222
4''-O-acetylquercitrin (3)	> 204	> 204	> 204	> 204
Doxorubicin	0.022	8.8	0.13	0.25

Table I. Cytotoxicity of compounds **1**, **2** and **3**.

istein (Agullo *et al.*, 1996). Although quercetin has been tested alone *in vitro* or *in vivo* on many cancer lines (Li *et al.*, 1999) and has reached the pre-clinical stage in association with cisplatin (Ferry *et al.*, 1996), it has never been tested on U251. However, the glycosylation of the OH-3 group seemed to decrease the cytotoxic activity which is in agreement with previous results (Agullo *et al.*, 1996) in the field of antioxidant (Cos *et al.*, 2001), antiproliferative (Koganov *et al.*, 1999) and enzyme inhibition (Belt *et al.*, 1979; Molnar *et al.*, 1981) activities.

Acknowledgments

We are grateful to Dr Amri Baktiar who provided and authenticated the *Loranthaceae Scurrula ferruginea* Danser, to the ARC (Association pour la Recherche sur le Cancer) and the Ligue Régionale de l'Ille et Vilaine de Lutte contre le Cancer for the grants they provided to conduct this study, to Dr P. Guenot for mass measurements and to J.F. Cupiff and M. Le Roch for performing NMR spectra.

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Nachdruck – auch auszugsweise – nur mit schriftlicher Genehmigung des Verlages gestattet
Satz und Druck: AZ Druck und Datentechnik GmbH, Kempten