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

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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 IC_{50} 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. ^{1}H NMR and ^{13}C 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 floculate (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-

nent **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-H_2O (4:6), during 141 min and methanol-H_2O (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. ^{1}H NMR (500.14 MHz, in DMSO-d_6): δ (ppm) 0.7 (3H, d, $J^3_{H\text{-}5''}$ = 6 Hz, CH_3-6″), 2.0 (3H, s, C$\underline{H}_3$-CO), 3.39 (1H, dq, $J^3_{H\text{-}4''}$ = 9 Hz, $J^3_{H\text{-}6''}$ = 6 Hz, H-5″), 3.74 (1H, dd, $J^3_{H\text{-}4''}$ = 9.7 Hz, $J^3_{H\text{-}2''}$ = 3 Hz, H-3″), 4.03 (1H, sl, H-2″), 4.71 (1H, t, $J^3_{H\text{-}3''}$ = 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, $J^3_{H\text{-}6'}$ = 8.3 Hz, H-5′), 7.24 (1H, dd, $J^3_{H\text{-}5'}$ = 8.3 Hz, $J^4_{H\text{-}2'}$ = 2 Hz, H-6′), 7.30 (1H, sd, $J^3_{H\text{-}6'}$ = 1.9 Hz, H-2′), 12.61 (1H, s, OH-5). ^{13}C 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, C$\underline{H}_3$-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, $J^3_{H\text{-}6}$ = 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, $J^3_{H\text{-}6'}$ = 3 Hz, C-2′), 120.4 (sd, $J^3_{H\text{-}5'}$ = 9.7 Hz, C-1′), 120.9 (dd, J^1 = 163 Hz, $J^3_{H\text{-}2'}$ = 7.3 Hz, C-6′), 133.9 (sd, $J^4_{H\text{-}6'=H\text{-}2'}$ = 2.4 Hz, C-3), 145.2 (sm, C-3′), 148.4 (sm, C-4′), 156.4 (sd, $J^2_{H\text{-}8}$ = 3.6 Hz, C-8a), 157.4 (sm, C-2), 161.2 (sl, C-5), 164.3 (sl, C-7), 169.8 (sm, CH_3-C$\underline{O}$O), 177.5 (s, C-4). HRMS-EI ($C_{23}H_{22}O_{12}$) *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 IC_{50} (μM). 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 $C_{15}H_{10}O_7$. ^{1}H NMR and ^{13}C NMR spectra confirmed the well-known structure of quercetin (Markham, 1989).

The molecular formula of compound **2** was established as $C_{21}H_{20}O_{11}$ based on high resolution measurements (HRMS-EI). The structure of compound **2** was deduced from spectroscopic data of **1** and the ^{1}H NMR data of the aglycone of **2** were consistent with a quercetin derivative. The ^{1}H 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 ($C_{23}H_{22}O_{12}$) 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 carbonyle band to the high frequenties (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 ^{1}H NMR and ^{13}C 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 (C$\underline{H}_3$) and to the two signals at 20.8 (CO-C$\underline{H}_3$) and 169.8 ppm (C$\underline{O}$). It was confirmed by the upfield shift of C$\underline{H}_3$-6″ and downfield shift of H-4″, H-5″ and H-3″. The position of the acetylation was

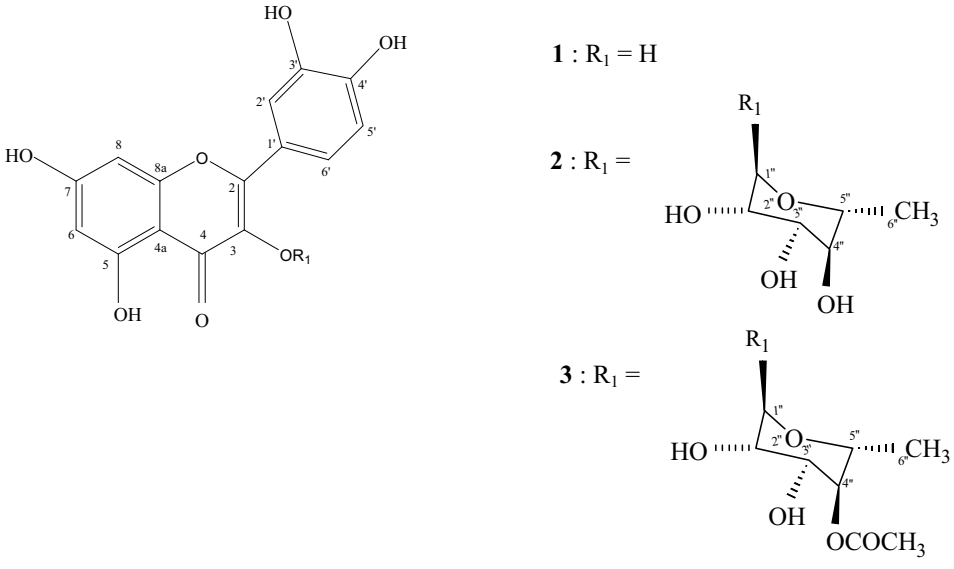

Fig. 1. Structure of quercetin (**1**), quercitrin (**2**) and 4″-O-acetylquercitrin (**3**).

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 phyllogenetically linked genera of *Scurrula*, *Taxillus*, *Dendrophtoe* 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 μM) while quercitrin and its acetylated derivative remained inactive (IC_{50} > 200 μM). Many studies have been conducted on quercetin which has been found to be one of the most active flavonol as anticancer agent with gen-

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

Isolated compound	Human Cancer Cell line (IC_{50}/μM)			
	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

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 preclinical 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.

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Agrawal P. (1992), NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry **31**, 3307–3310.

Agullo G., Gamet-Payrastre L., Fernandez Y., Anciaux N., Demigne C. and Remesy C. (1996), Comparative effects of flavonoids on the growth, viability and metabolism of a colonic adenocarcinoma cell line (HT29 cells). Cancer Lett. **105**, 61–70.

Asen S. (1977), 2″-O-acetylquercitrin from *Azalea* flowers. Phytochemistry **16**, 147–148.

Barlow B. (1991), Provisional key to the genera of *Loranthaceae* and *Viscaceae* of the flora Malesiana region. Flora Malesiana Bulletin **10**, 335–338.

Belt J., Thomas J., Buchsbaum R. and Racker E. (1979), Inhibition of lactate transport and glycolysis in Ehrlich ascites tumor cells by bioflavonoids. Biochemistry **18**, 3505–3511.

Bezivin C., Lohézic F., Sauleau P. and Boustie J. (2002), Cytotoxic activity of Tricholomatales, determined with murine and human cancer cell lines. Pharmaceutical Biology **40**, 196–199.

Carpena O., Baleriola C. and Mataix J. (1980), Etude spectrophotométrique de flavonoides acétylés. I. Flavonols et glycosides de flavonols. Bulletin de liaison, groupe polyphénols **9**, 340–347.

Cos P., Calomme M., Sindambiwe J.-B., Bruyne T. D., Cimanga K., Pieters L., Vlietinck A. J. and Van Den Berghe D. V. (2001), Cytotoxicity and lipid peroxidation-inhibiting activity of flavonoids. Planta Med. **67**, 515–519.

Danieli B., Luisetti M., Sampognaro G., Carrea G. and Riva S. (1997), Regioselective acylation of polyhydroxylated natural compounds catalyzed by *Candida antarctica* lipase B (Novozym 435) in organic solvents. J. Mol. Catalysis B: Enzymatic **3**, 193–201.

Ferry D., Smith A., Malkhandi J., Fyfe D., Detakats P., Anderson D., Baker J. and Kerr D. (1996), Phase I clinical trial of the flavonoid quercetin: pharmacokinetics and evidence for *in vivo* tyrosine kinase inhibition. Clin. Cancer Res. **2**, 659–668.

Fossen T., Larsen A., Kiremire B. T. and Andersen O. (1999), Flavonoids from blue flowers of *Nymphaea caerulea.* Phytochemistry **51**, 1133–1137.

Fuchino H., Konishi S., Satoh T., Yagi A., Saitsu K., Tatsumi T. and Tanaka N. (1996), Chemical evaluation of *Betula species* in Japan. II. constituents of *Betula platyphylla var.japonica.* Chem. Pharm. Bull. **44**, 1033–1038.

Ishizu T., Tsujino E., Winarno H., Ohashi K. and Shibuya H. (2001), A complex of perseitol and K+ ion from *Scurrula fusca* (Loranthaceae). Tetrahedron Lett. **42**, 6887–6889.

Koganov M., Dueva O. V. and Tsorin B. L. (1999), Activities of plant-derived phenols in a fibroblast cell culture model. J. Nat. Prod. **62**, 481–483.

Li W., Shen F. and Weber G. (1999), Ribavirin and quercetin synergistically downregulate signal transduction and are cytotoxic in human ovarian carcinoma cells. Oncol. Res. **11**, 243–7.

Lohézic-Le-Dévéhat F., Bakhtiar A., Bézivin C., Amoros M. and Boustie J. (2002), Antiviral and cytotoxic activities of some Indonesian plants. Fitoterapia, accepted 16th May.

Mabry T., Markham K. and Thomas M. (1970), The systematic identification of flavonoids. Berlin–Heidelberg–New York, Verlag, Springler Publ. 353.

Markham K. (1989), Flavones, flavonols and their glycosides. Methods in Plant Biochemistry. J. Harborne. 2. ed. London. **1**, 197–232.

Molnar J., Beladi I., Domonkos K., Födeak S., Boda K. and Veckenstedt A. (1981), Antitumor activity of flavonoids on NK/ly ascites tumor cells. Neoplasma **28**, 11–18.

Ossipov V., Nurmi K., Loponen J., Prokopiev N., Haukioja E. and Pihlaja K. (1995), HPLC isolation and identification of flavonoids from white birch *Betula pubescens* leaves. Biochem. Syst. Ecol. **23.**

Shen C., Chang Y. and Ho L. (1993), Nuclear magnetic resonance studies of 5,7-dihydroxyflavonoids. Phytochemistry **34**, 843–845.

Shibuya H., Ohasshi K. and Kitagawa I. (1999), Search for pharmacochemical leads from tropical rainforest plants. Pure Appl. Chem. **71**, 1109–1113.

Tanaka N., Murakami T., Saiki Y., Chen C. and Gomez L. (1978), Chemische Untersuchungen von *Pteris grandifolia* L. Chem. Pharm. Bull. **26**, 3580–3582.

Tilney P. and Lubke R. (1974), A chemotaxonomic study of twelve species of the family *Loranthaceae.* J. Sth African Botany **40**, 315–322.

Zee-Cheng R. (1997), Anticancer research on *Loranthaceae* plants. Drugs Future **22**, 519–530.

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