Journal of Chemistry, Vol. 42 (1), P. 125 - 128, 2004

FLAVONOID GLUCOSIDES FROM THE LEAVES OF *CROTON TONKINENSIS* GAGNEP., EUPHORBIACEAE

Received 14-7-2003

PHAN MINH GIANG[1], JUNG JOON LEE[2], PHAN TONG SON[1]

[1]*Faculty of Chemistry, College of Natural Science, Vietnam National University*
[2]*Anticancer Agent Research Laboratory, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea*

SUMMARY

*Two flavone C-glucosides vitexin (**1**) and isovitexin (**2**) were isolated as the major constituents with the total content of 59.5% of the methanol extract of the Croton tonkinensis leaves along with an minor acylated flavonol O-glucoside kaempferol 3-O-β-D-(6''-O-coumaroyl)glucopyranoside (tiliroside, **3**) (0.9%). The structures of **1**, **2** and **3** were determined on the basis of ESIMS, 1D and 2D NMR spectroscopic data. Although the flavonoid glucosides found exclusively in the ethyl acetate and n-butanol soluble fractions are reported for antioxidative and antiinflammatory activities their contribution to the medicinal properties of C. tonkinensis is demonstrated to be less than the lipid soluble ent-kaurene diterpenoid constituents of this plant.*

In our past studies on the medicinal plant *Croton tonkinensis* Gagnep. (Euphorbiaceae) [1], the phytosterols, the long chain alkyl alcohols and the *ent*-kaurene-type diterpenoids [2] were isolated from the non-polar parts (n-hexane and CH_2Cl_2 soluble fractions) of the methanol extract of the dried leaves of *C. tonkinensis* [3]. The investigation of the methanol extract by reserved-phase high performance liquid chromatography (RP HPLC) coupled with a photodiode array (PDA) detector [4] revealed the presence of two major classes of components: *ent*-kaurene diterpenoids and flavonoid gluco-sides. Of the flavonoids two were quantified as the major (total 59.5%) and one as the minor constituents (0.9%) of the methanol extract which were found to be localized in the polar ethyl acetate and n-butanol soluble fractions. When the ethyl acetate soluble fraction was dissolved in a minimum amount of cool methanol a yellow solid was precipitated. ^{1}H NMR examination of this solid in DMSO-d_6 revealed the presence of a mixture of two major compounds **1** and **2** (2/1 in ratio). A small amount (15 mg) of mixture was subjected to preparative RP HPLC [4] (mobile phase MeOH-H_2O 1 : 1) to afford pure **1** and **2** which were identified as the flavone C-glucosides vitexin (**1**) [5, 6] (R_t 10.2 min) and isovitexin (**2**) [7, 8] (R_t 10.8 min) on the basis of the comparison of their spectroscopic data with the reported values.

Silica gel column chromatographic fractionation of the ethyl acetate soluble fraction eluting with gradient: 100% $CHCl_3 \rightarrow CHCl_3$-MeOH 2 : 1 $\rightarrow$ 100% MeOH, followed by purification by preparative RP HPLC [4] (mobile phase MeOH-H_2O 3 : 2) afforded **3** as a yellow amorphous powder, mp 250 - 252°C. The compound eluted at R_t 15.4 [5] and displayed on-line UV maxima at 199.2, 218 (shoulder), 260, 310.7 nm indicative

for a flavonol. The molecular formula $C_{30}H_{26}O_{13}$ was deduced from the quasimolecular ions 593 $[M-H]^+$ (ESIMS negative-ion mode) and 617 $[M+Na]^+$ (ESIMS positive-ion mode) showing 18 degrees of unsaturation of **3**. The proton signals at δ_H 6.29 and 6.08 (both 1H, d, J = 1.5 Hz), 7.9 and 6.82 (both 2H, d, J = 8.7 Hz) supported by the correlated carbon signals derived from the HSQC spectrum and the ^{13}C singlet signal at 177 in the ^{13}C decoupled spectrum are indicative of the presence of a 5,7,4'-threesubstituted flavonol nucleus. The anomeric proton signal at δ_H 5.4 with β configuration (1H, d, J = 7.5 Hz), δ_C 106.7 (d) and two proton signals at 4.05 (1H, dd, J = 11.7, 8.3 Hz) and 4.3 (1H, d br, J = 11.7 Hz) (2H-6") are attributable to a glucose moiety in the structure of **3**, the proton and carbon signals in the sugar sequence were assigned on the basis of COSY and HSQC spectra. Consistent with the degree of unsaturation and the ^1H and ^{13}C NMR spectra, the last structural fragment of **3** was ascribed to a *trans-p*-coumaroyl moiety: δ_C 166.2 (s, C=O), δ_H 7.34 (1H), 6.12 (1H) (both d, J = 16 Hz, *trans*-disubstituted double bond), 7.38 and 6.74 (both 2H, d, J = 8.7 Hz, *p*-disubstituted aromatic ring). This was confirmed by the cross peaks observed in HMBC spectrum between H-2''' (δ_H 6.12) and H-3''' (δ_H 7.34) and the carbonyl carbon C-1''' (δ_C 166.2), between H-3''' and C-5''' and C-9''' (δ_C 130.2). Further, the HMBC correlation between the anomeric proton at δ_H 5.4 and C-3 at δ_C 133 placed the sugar moiety at 3-O position, and the connection of the coumaroyl moiety to C-6" of glucose was judged from the HMBC correlation from the two proton signals at δ_H 4.05 (strong) and 4.3 (weak) to the carbonyl carbon signal at δ_C 166.2. Thus, the spectroscopic data were conclusive for the structure of **3** as kaempferol 3-O-β-D-(6''-O-coumaroyl)glucopyranoside (tiliroside) [9]. For the unambiguous assignments of all ^1H and ^{13}C signals the correlations in COSY, HMQC and HMBC spectra were employed.

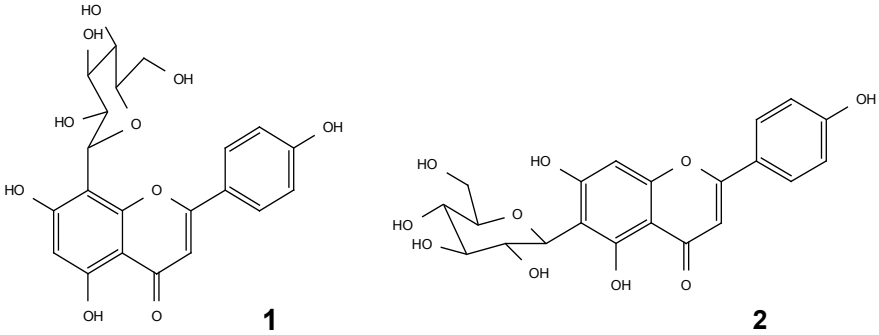

Figure 1: Chemical structures of vitexin (**1**), isovitexin (**2**)

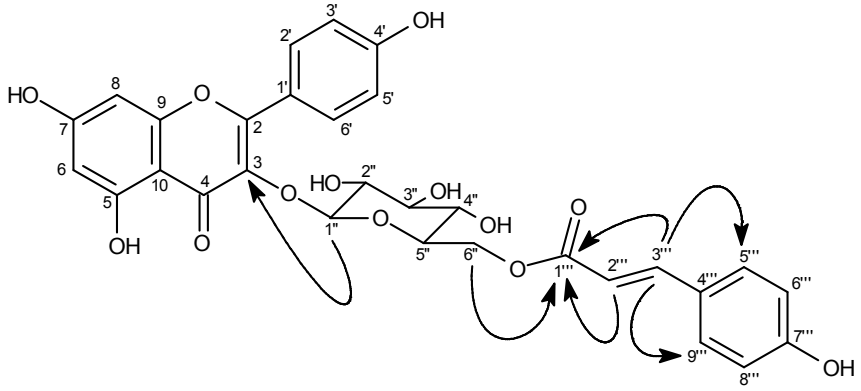

Figure 2: Chemical structure and important H → C correlations in HMBC spectrum of tiliroside (**3**)

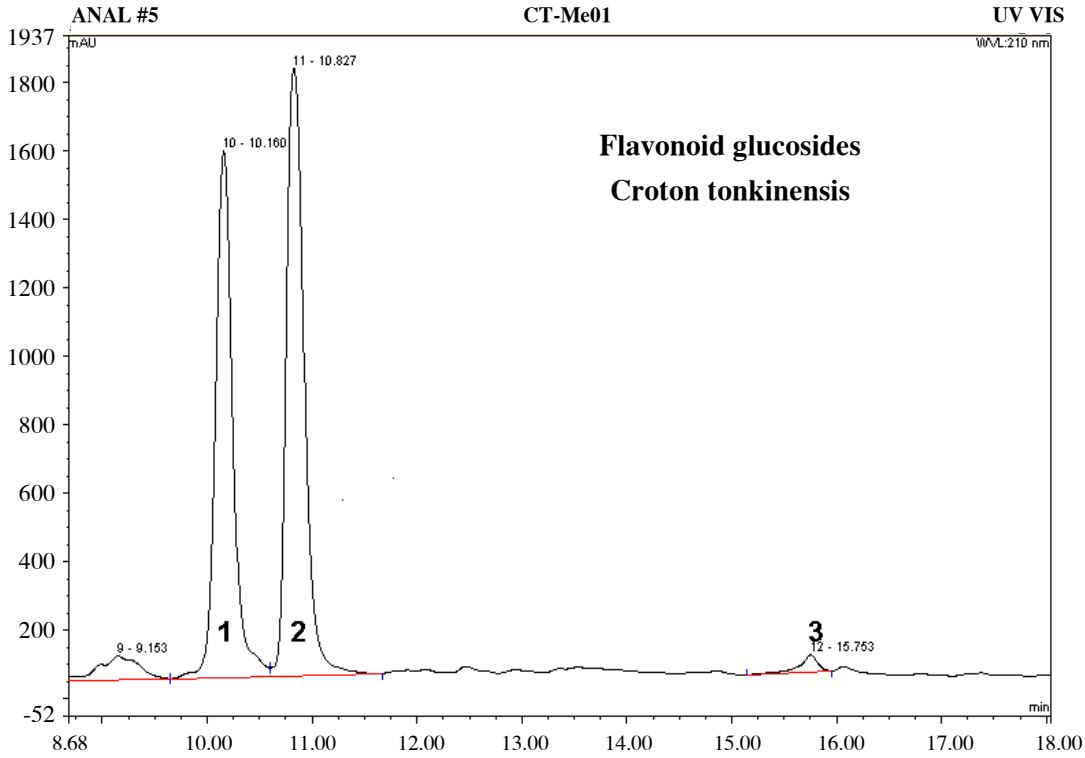

Figure 3: HPLC chromatogram (flavonoid part) of the methanol extract of *C. tonkinensis*

Tiliroside (kaempferol 3-O-β-D-(6''-O-coumaroyl)glucopyranoside) (**3**). Yellow amorphous powder, mp 250 - 252°C. UV max: 199.2, 218 (sh), 260, 310.7 nm; ESIMS 593 $[M-H]^+$, 617 $[M+Na]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.5 (1H, s, 5-OH), 7.9 (1H, d, J = 8.7 Hz, H-2', H-6'), 7.38 (1H, d, J = 8.7 Hz, H-5''', H-9'''), 7.34 (1H, d, J = 16 Hz, H-3'''), 6.82 (1H, d, J = 8.7 Hz, H-3', H-5'), 6.74 (1H, d, J = 8.7 Hz, H-6''', H-8'''), 6.29 (1H, d, J = 1.5 Hz, H-8), 6.12 (1H, d, J = 16 Hz, H-2'''), 6.08 (1H, d, J = 1.5 Hz, H-6), 5.4 (1H, d, J = 7.5 Hz), 4.3 (1H, d br, J = 11.7 Hz), 4.05 (1H, dd, J = 11.7 Hz, 8.3 Hz), 3-3.8 (4H, m); ^{13}C-NMR (300 MHz, DMSO-d_6): δ 177 (s, C-4), 166.2 (s, C-1'''), 164.7 (s, C-7), 164.3 (s, C-10), 161 (s, C-5), 159.9 (s, C-7'''), 159.9 (s, C-4'), 156.3 (s, C-9), 156.2 (s, C-2), 144.7 (d, C-3'''), 133 (s, C-3), 130.8 (d, C-2', C-6'), 130.2 (d, C-5''', C-9'''), 124.9 (s, C-4'''), 120.7 (s, C-1'), 115.8 (d, C-6''', C-8'''), 115.1 (d, C-3', C-5'), 113.6 (d, C-2'''), 106.7 (d, C-1''), 99 (d, C-6), 94 (d, C-8), 76 (d, C-3''), 74 (2d, C-2'', C-5''), 69.9 (d, C-4''), 63 (t, C-6'').

Taking into account the high contents of the polar vitexin (27.2%), isovitexin (32.2%) and tiliroside (0.9%) in the whole leave methanol extract (Figure 3) the correlation between the biological activites of the flavonoid glucosides and medicinal properties of *C. tonkinensis* [1] should be put under discussion. While isovitexin and vitexin displayed moderate antioxidative activity [10, 11] tiliroside was reported to possess a potent anticomplementary activity [12] and to inhibit induced histamine released in rat mast cells which could be considered as evidence for the antiinflammatory effect of tiliroside [13]. In consideration of the association of tumor promotion with oxidative and inflammatory tissue damage [14], it would be worthwhile to determine the possible chemopreventive effects on carcinogenesis. However, it is noticeable that the glycosilation of flavonoids often results in

the reduction of biological activities regardless of the types of aglycones and types of linkage (C- or O-glycosides) possibly due to their hydrophilicity and consequent diminished ability to penetrate cell membrane and steric hindrance caused by their bulky glycosyl residue [15] as demonstrated with vitexin [16] and isovitexin [10]. Finally, it is important to underline that in our bioassays involving the inhibition of the transcription factor NF-κB and iNOS-dependent NO production the flavonoid glucosides were non-active. Consistently, in our antiplasmodial tests against the *Plasmodium falciparum* strains the lipid soluble components showed much more pronounced inhibitory activity than the water soluble components of *C. tonkinensis* [17].

Acknowledgements: *This work was partly supported by International Foundation for Science (Stockholm, Sweden, Grant No. F/2841-2 to Phan Minh Giang). The working facilities provided by Anticancer Agent Research Laboratory, Korea Research Institute of Bioscience and Biotechnology, Korea, to Phan Minh Giang are gratefully acknowledged.*

REFERENCES

1. Vo Van Chi. Dictionary of Vietnamese Medicinal Plants. Publishing House Medi-cine (Ho Chi Minh City) (1997).
2. (a) Phan Tong Son, Van Ngoc Huong, Phan Minh Giang, C. Taylor Walter. Vietnam J. Chem., Vol. 37, No. 1-2 (1999). (b) Phan Tong Son, Phan Minh Giang, C. Taylor Walter. Austr. J. Chem., Vol. 53, P. 1003 - 1005 (2000). (c) Phan Minh Giang, Jung Joon Lee, Phan Tong Son. Vietnam J. Chem., Vol. 41, No. 1 (2003).
3. The air-dried leaves were collected in the suburbs of Hanoi, and identified by a botanist Prof. Vu Van Chuyen (Hanoi College of Pharmacy, Hanoi in 2002.
4. RP HPLC: Dionex HPLC system with a P580 pump, an ASI-100 automated sample injector and a PDA-100 photodiode array detector. Analytical condition: YMC ODS-H80 column (150 × 4.6 mm I.D., S-4 μm), sample injection size 10 μl, mobile phase gradient 20 - 100% MeOH in HPLC grade H_2O, run time 25 min, flow rate 1 ml/min. Preparative condition: YMC ODS-H80 column (150 mm × 20 mm I.D., S-4 μm), flow rate 6 ml/min).
5. G. F. Pauli and P. Junior. Phytochemistry, Vol. 38, P. 1245 - 1250 (1995).
6. P. -C. Zhang and S. -X. Xu. J. Asian Nat. Prod. Research, Vol. 5, P. 131 - 136 (2003).
7. M. Haribal and J. A. A. Renwick. Phytochemistry, Vol. 47, P. 1237 - 1240 (1998).
8. G. T. Maatooq, S. H. El-Sharkawy, M. S. Afifi, and J. P. N. Rosazza. Phytochemistry, Vol. 44, P. 187 - 190 (1997).
9. N. Backhouse, C. Delporte, R. Negrete, S. A. San Feliciano, and J. L. Lopez-Perez. Phytother. Res., Vol. 16, 562 - 566 (2002).
10. C. -M. Lin, C. -T. Chen, H. -H. Lee, and J. -K. Lin. Planta Med., Vol. 68, P. 365 - 367 (2002).
11. R. Aquino, S. Morelli, M. R. Lauro, S. Abdo, A. Saija, and A. Tomaino. J. Nat. Prod., Vol. 64, P. 1019 - 1023 (2001).
12. K. Y. Jung, S. R. Oh, S. H. Park, I. S. Lee, K. S. Ahn, J. J. Lee, and H. K. Lee. Biol. Pharm. Bull., Vol. 21, P. 1077 - 1078 (1998).
13. T. Tsuruga, Y. Ebizuka, J. Nakajima et al.. Chem. Pharm. Bull., Vol. 39, P. 3265 - 3271 (1991).
14. Y. -J. Surh. Food and Chemical Toxicology, Vol. 40, P. 1091 - 1097 (2002).
15. H. K. Kim, B. S. Cheon, Y. H. Kim, S. Y. Kim, and H. P. Kim. Biochem. Pharmacol, Vol. 58, P. 759 - 765 (1999).
16. A. Basile, S. Giordano, J. A. Lopez-Saez, and R. C. Cobianchi. Phytochemistry, Vol. 52, P. 1479 - 1482 (1999).
17. Phan Tong Son, Le Huyen Tram, Phan Minh Giang. Vietnam J. Chem., Vol. 40, P. 53 - 57 (2003).