*Phytochemistry*, Vol. 26, No. 9, pp. 2557–2558, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00


# A CHEMOTAXONOMIC STUDY OF FLAVONOIDS IN THE LEAVES OF SIX *TRICHOSANTHES* SPECIES

MASAO YOSHIZAKI, HIROHARU FUJINO, MIHOKO MASUYAMA,* MUNEHISA ARISAWA* and NAOKATA MORITA*

Department of Herbal Garden and *Department of Medicinal Resources, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

(*Revised received* 16 *February* 1987)

**Key Word Index**—*Trichosanthes* spp.; Cucurbitaceae; leaves; flavonoids; chemotaxonomy.

**Abstract**—The 7-, 3'- and 4'-glucosides of luteolin, the 7-glucoside and 6,8-di-*C*-glucoside of apigenin were isolated from *Trichosanthes kirilowii* var. *japonica*. Kaempferol 3,7-di-rhamnoside and 3-glucoside-7-rhamnoside were identified from *T. cucumeroides*, kaempferol 3-galactoside and 3-sophoroside were also identified from *T. anguina*. Quercetin-3-rutinoside was detected from *T. multiloba* and *T. rostrata*. *T. bracteata* afforded luteolin 3'-glucoside and kaempferol 3-rutinoside, and *T. kirilowii* afforded luteolin 7-, 3'- and 4'-glucosides and apigenin 7-glucoside.

## INTRODUCTION

The genus *Trichosanthes* consists of about 60 species, five of which, *T. bracteata* Voiqt, *T. multiloba* Miquel, *T. kirilowii* Maxim. var. *japonica* Kitam., *T. cucumeroides* Maxim. and *T. rostrata* Kitam., are distributed in Japan. Recently, we reported the identification of three phenolic compounds, 11-methoxynoryangonin, vanillic acid and tricin, and steryl-β-D-glucopyranoside from seed of *T. uniflora* Hao [1]. Previously, Nakaoki and Morita have reported the isolation of luteolin 7-*O*-glucoside (**1**) from *T. kirilowii* Maxim. var. *Japonica* Kitam. and kaempferol 3,7-di-*O*-rhamnoside (**6**) from *T. cucumeroides* Maxim. [2]. In this work we have studied the flavonoids of seven *Trichosanthes*, species, the five above plus *T. anguina* L. and *T. kirilowii* Maxim., in order to establish a chemotaxonomic approach to the genus [3].

## RESULTS AND DISCUSSION

Eleven flavonoids (**1**–**11**) were isolated and identified from the leaves of the genus *Trichosanthes T. kirilowii* afforded luteolin 7-*O*-β-D-glucopyranoside (**1**), 4'-*O*-β-D-glucopyranoside (**2**) and 3'-*O*-β-D-glucopyranoside (**4**) and apigenin 7-*O*-β-D-glucopyranoside (**3**). Its variety *japonica* Kitam. afforded **1**–**4** and apigenin 6,8-di-*C*-β-glucopyranoside (**5**). *T. cucumeroides* afforded two kaempferol glucosides, 3,7-di-*O*-α-L-rhamnopyranoside (**6**) and 3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (**7**). *T. anguina* also afforded another two kaempferol glycosides, the 3-*O*-β-galactopyranoside (**10**) and 3-*O*-β-sophoroside (**11**). *T. rostrata* and *T. multiloba* afforded quercetin 3-*O*-β-rutinoside (**8**). From *T. bracteata* (**4**) and kaempferol 3-*O*-β-rutinoside (**9**) were isolated.

These flavonoids were identified as described in the Experimental. Although only a few species have been studied, it seems that *Trichosanthes* can be divided into four groups on the basis of their aglycones (Table 1): one with flavones alone, two with kaempferol alone, three with quercetin alone and four with flavones plus flavonols.

## EXPERIMENTAL

*General experimental procedures.* All mps are uncorr. The $^1$H- and $^{13}$C-NMR spectra were measured at 200 MHz and 50.3 MHz, respectively, and chemical shifts are given in δ (ppm) with TMS as an int. standard.

Table 1. Flavonoids in the leaves of the *Trichosanthes*

| Flavonoids | Flavones | | | | | Flavonols | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ap. glyc. | | Lu. glyc. | | | Km. glyc. | | | | | Qu. glyc. |
| Plants | 3 | 5 | 1 | 2 | 4 | 6 | 7 | 9 | 10 | 11 | 8 |
| *T. kirilowii* Maxim. | + | | + | + | + | | | | | | |
| var. *japonica* Kitam. | + | + | + | + | + | | | | | | |
| *T. cucumeroides* Maxim. | | | | | | + | + | | | | |
| *T. anguina* Linne | | | | | | | | | + | + | |
| *T. multiloba* Miquel | | | | | | | | | | | + |
| *T. rostrata* Kitam. | | | | | | | | | | | + |
| *T. bracteata* Voiqt | | + | | | | | | + | | | |

Ap., Apigenin; Lu., Luteolin; Km., Kaempferol; Qu., Quercetin.

*Plant materials.* Six *Trichosanthes*, 4 wild species, *T. bracteata* Voiqt, *T. cucumeroides* Maxim., *T. kirilowii* Maxim. var. *japonica* Kitam. and *T. multiloba* Miquel, plus *T. anguina* L. and *T. kirilowii* Maxim. have been grown in our herbal garden since 1980. Each wild species was collected from three habitats in Japan and transplanted, and the latter two species were from garden seed. *T. anguina* L. was introduced from Tsukuba Expermental Station of Medicinal Plants (National Institute of Hygienic Sciences, 1 Hachimandai, Yatabe-cho, Tsukuba 300–21, Japan), *T. kirilowii* Maxim. was derived from Kyoto Herbal Garden. (Pharmacognosy Laboratories, Central Research Division, Takeda Chemical Industries Ltd., Ichijoji, Sakyoku, Kyoto 606, Japan). These plants were collected in August, 1983, plants of *T. rostrata* Kitam. were collected in Kume island, Okinawa, Japan, during the same month. Voucher specimens have been deposited in the herbarium of our university.

*Extraction and isolation of flavonoids.* The fresh leaves of *Trichosanthes* were individually extracted with MeOH. The individual MeOH extract were extracted successively with $Et_2O$, EtOAc and *n*-BuOH. The EtOAc extract of *T. anguina* L. afforded **10** and the *n*-BuOH extract afforded **11**. The EtOAc extract of *T. bracteata* was chromatographed over Sephadex LH-20 to afforded **9** and the *n*-BuOH extract afforded **4**. The EtOAc and *n*-BuOH extracts of *T. cucumeroides* afforded **6** and **7**, respectively. A Sephadex LH-20 CC of the EtOAc extract of *T. kirilowii* afforded three flavonoids, **1**, **2** and **3**, and the column of the *n*-BuOH extract afforded **1** and **4**. Four flavonoids, **1**–**4**, were isolated from the EtOAc extract of *T. kirilowii* var. *japonica* by using Sephadex LH-20 CC, and *C*-glucosylflavone, **5**, was isolated from the *n*-BuOH extract. The EtOAc and *n*-BuOH both extracts of *T. mutiloba* afforded **8**. The flavonol, **8** was also isolated from the EtOAc and *n*-BuOH extracts of *T. rostrata*.

*Luteolin 7-O-β-D-glucopyranoside* (**1**). Yellow micro-needles, mp 240–245°. Identification was established by direct comparison (mmp, UV, IR and $^{13}$C-NMR) with an authentic sample isolated from *Salix gilgiana* Seemen (Salicaceae) [4].

*Luteolin 4′-O-β-D-glucopyranoside* (**2**). Yellow needles, mp 190–195°. $^1$H-NMR (DMSO-$d_6$): δ4.89 (1H, *d*, *J* = 6.9 Hz, anomeric H), 6.20 (1H, *d*, *J* = 2.0 Hz, 6-H), 6.50 (1H, *d*, *J* = 2.0 Hz, 8-H), 6.83 (1H, *s*, 3-H), 7.24 (1H, *d*, *J* = 8.8 Hz, 5′-H), 7.49 (1H, *d*, *J* = 1.9 Hz, 2′-H), 7.53 (1H, *dd*, *J* = 1.9 and 8.8 Hz, 6′-H). $^{13}$C-NMR (DMSO-$d_6$): δ60.79 (*t*, 6″-C), 69.87 (*d*, 4′-C), 73.29 (*d*, 2″-C), 75.93 (*d*, 3′-C), 94.09 (*d*, 8-C), 99.02 (*d*, 6-C), 101.37 (*d*, 1″-C), 103.91 (*s*, 10-C), 104.10 (*d*, 3-C), 116.21 (*d*, 2′-C), 118.60 (*d*, 5′-C), 124.90 (*s*, 1′-C and *d*, 6′-C), 147.12 (*s*, 3′-C), 148.68 (*s*, 4′-C), 157.52 (*s*, 9-C), 161.62 (*s*, 5-C), 163.37 (*s*, 7-C), 164.45 (*s*, 2-C), 181.93 (*s*, 4-C). Identification was established by UV and $^1$H and $^{13}$C-NMR standard procedures [5, 6].

*Apigenin 7-O-β-D-glucopyranoside* (**3**). Yellow needles, mp 238–240°. Identification was established by direct comparison (mmp, UV, IR and $^{13}$C-NMR) with an authentic sample isolated form *Vicia unijuga* Al. Br. (Leguminosae) [7].

*Luteolin 3′-O-β-D-glucopyranoside* (**4**). Yellow needles, mp 221–224°. $^1$H-NMR (DMSO-$d_6$): δ4.91 (1H, *d*, *J* = 6.8 Hz, anomeric H), 6.20 (1H, *d*, *J* = 2.0 Hz, 6-H), 6.53 (1H, *d*, *J* = 2.0 Hz, 8-H), 6.82 (1H, *s*, 3-H), 6.98 (1H, *d*, *J* = 8.3 Hz, 5′-H), 7.67 (1H, *dd*, *J* = 1.5 and 8.3 Hz, 6′-H), 7.80 (1H, *d*, *J* = 1.5 Hz, 2′-H). Identification was established by UV and $^1$H- and $^{13}$C-NMR standard procedures [5, 6, 8].

*Apigenin 6,8-di-C-β-glucopyranoside* (**5**). Yellow needles, mp 258–260°. $^1$H-NMR (DMSO-$d_6$): δ4.77 (1H, *d*, *J* = 9.8 Hz, anomeric H), 4.82 (1H, *d*, *J* = 9.8 Hz, anomeric H), 6.81 (1H, *s*, 3-H), 6.93 (2H, *d*, *J* = 8.3 Hz, 3′- and 5′-H), 8.03 (2H, *d*, *J* = 8.3 Hz, 2′- and 6′-H), 9.36 (1H, *br s*, OH), 10.37 (1H, *br s*, OH), 13.71 (1H, *br s*, 5-OH). $^{13}$C-NMR (DMSO-$d_6$): 59.91 (*t*, 6″-C), 61.28 (*t*, 6‴-C), 69.09 (*d*, 4″-C), 70.65 (*d*, 4‴-C), 71.00 (*d*, 2‴-C), 71.97 (*d*, 2″-C), 73.29 (*d*, 1‴-C), 74.13 (*d*, 1″-C), 77.88 (*d*, 3″-C), 78.91 (*d*, 3‴-C), 80.91 (*d*, 5‴-C), 81.93 (*d*, 5″-C), 102.68 (*d*, 3-C), 103.95 (*s*, 10-C), 105.37 (*s*, 8-C), 107.57 (*s*, 6-C), 115.92 (*d*, 3′ and 5′-C), 121.58 (*s*, 1′-C), 129.10 (*d*, 2′ and 6′-C), 155.17 (*s*, 9-C), 158.69 (*s*, 5-C), 160.94 (*s*, 4′-C), 161.33 (*s*, 7-C), 164.26 (*s*, 2-C), 182.47 (*s*, 4-C). Identification was established by UV and $^1$H and $^{13}$C-NMR standard procedure [5, 6].

*Kaempferol 3,7-di-O-α-L-rhamnopyranoside* (**6**). Yellow needles, mp 226–230°. Identification was established by direct comparison (mmp, UV, IR and $^{13}$C-NMR) with an authentic sample [2].

*Kaempferol 3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside* (**7**). Yellow needles, mp 249–252°. Identification was established by direct comparison (mmp, UV, IR and $^{13}$C-NMR) with an authentic sample isolated from *Vicia faba* L. (Leguminosae) [7].

*Quercetin 3-O-β-rutinoside* (**8**). Yellow needles, mp 188–191°. Identification was established by direct comparison (mmp, UV, IR and $^{13}$C-NMR) with an authentic sample [9].

*Kaempferol 3-O-β-rutinoside* (**9**). Yellow needles, mp 191–194°. Identification was established by direct comparison (mmp, UV, IR and $^{13}$C-NMR) with an authentic sample isolated from *Clitoria ternatea* L. (Leguminosae) [10].

*Kaempferol 3-O-β-D-galactopyranoside* (**10**). Yellow micro-needles, mp 240–245°. Identification was established by direct comparison (mmp, UV, IR and $^{13}$C-NMR) with an authentic sample isolated from *Liquidamber formosana* Hance (Hamamelidaceae) [9].

*Kaempferol 3-O-β-sophoroside* (**11**). Yellow needles, mp 194–200°. $^1$H-NMR (DMSO-$d_6$): δ4.30 (1H, *br*, OH), 4.64 (1H, *d*, *J* = 7.5 Hz, anomeric H), 5.08 (1H, *br*, OH), 5.30 (1H, *br*, OH), 5.72 (1H, *d*, *J* = 7.5 Hz, anomeric H), 6.20 (1H, *d*, *J* = 2.5 Hz, 6-H), 6.43 (1H, *d*, *J* = 2.5 Hz, 8-H), 6.92 (2H, *d*, *J* = 9.0 Hz, 3′ and 5′-H), 8.08 (2H, *d*, *J* = 9.0 Hz, 2′ and 6′-H), 10.15 (1H, *br s*, OH), 10.82 (1H, *br s*, OH), 12.62 (1H, *s*, OH). Identification was established by UV and $^1$H and $^{13}$C-NMR standard procedure [5, 6, 8].

*Acknowledgements*—The authors thank Mr S. Sawasaki, Faculty of Medicine, University of Okinawa, for collection of *T. rostrata* and also Mr M. Morikoshi, Analytical Center of our university, for measurement of $^{13}$C-NMR.

## REFERENCES

1. Arisawa, M., Yoshizaki, M. and Morita, N. (1985) *Shoyakugaku Zasshi* **39**, 316.
2. Nakaoki, T. and Morita, N. (1957) *Yakugaku Zasshi* **77**, 108.
3. Yoshizaki, M., Fujino, H., Masuyama, M., Arisawa, M. and Morita, N. (1984) *The Papers of the 31st Annual Meeting of the Japanese Society of Pharmacognosy, Tokyo, Japan*, 87.
4. Morita, N., Shimizu, M., Arisawa, M. and Kitanaka, S. (1974) *Yakugaku Zasshi* **94**, 875.
5. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
6. Markham, K. R., Chari, V. M. and Mabry, T. J. (1982) *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds) p. 19. Chapman & Hall, London.
7. Arisawa, M., Takakuwa, T. and Handa, K. (1971) *Yakugaku Zasshi* **91**, 587.
8. Markham, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.
9. Arisawa, M., Hamabe, M., Sawai, M., Hayashi, T., Kizu, H., Tominori, T., Yoshizaki, M. and Morita, N.′ (1984) *Shoyakugaku Zasshi* **38**, 216.
10. Morita, N., Arisawa, M., Nagase, M., Hsu, H.-Y. and Chen, Y.-P. (1977) *Yakugaku Zasshi* **97**, 649.