*Phytochemistry*, Vol. 31, No. 10, pp. 3642–3644, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00
Pergamon Press Ltd

# OLEANANE AND URSANE GLUCOSIDES FROM *RUBUS* SPECIES

XIAO-HONG ZHOU, RYOJI KASAI, KAZUHIRO OHTANI, OSAMU TANAKA*, RUI-LIN NIE,† CHONG-REN YANG,† JUN ZHOU† and KAZUO YAMASAKI‡

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Kasumi, Minami-ku, Hiroshima 734, Japan; *Suzugamine Women's College, Inokuchi, Nishi-ku, Hiroshima 733, Japan; †Kunming Institute of Botany, Academia Sinica, Kunming, Yunnan, China

(*Received in revised form* 24 *February* 1992)

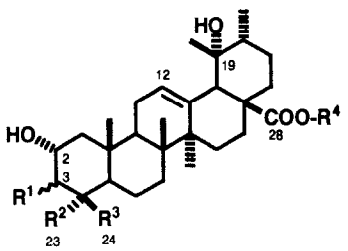
**Key Word Index**—*Rubus accuminatus*; *R. ellipticus*; *R. multibreatus*; Rosaceae; triterpene; oleanane; ursane; glycoside; glucoside.

**Abstract**—A new oleanane-glucoside was isolated from the leaves of three *Rubus* species collected in Yunnan, southern China, along with several known ursane- and oleanane-type triterpene glucosides. The structures of these compounds were established by spectroscopic and chemical means.

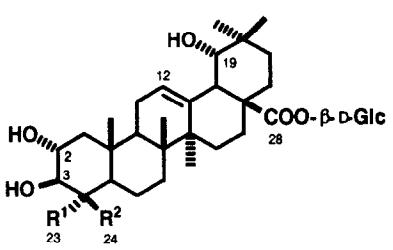
## INTRODUCTION

A sweet kaurene-type diterpene glycoside named rubusoside [1] and a number of related minor glycosides [2, 3] have been isolated from the sweet leaves of *Rubus suavissimus* S. Lee,§ a plant which grows in southern China. From the leaves of *R. chingii* Hu (southern China) [4] and *R. foliolosus* D. Don (south-western China) [5], several labdane-type diterpene glycosides have been isolated. We have studied further the glycosides of a number of other *Rubus* spp. growing in China, Japan and Korea [6, 7]. However, no diterpene glycoside was obtained, but several 19α-hydroxyursane-type triterpene glycosides were isolated. It seems that, based on the higher terpenoid glycoside composition, *Rubus* spp. should be classified into two groups; one containing diterpene glycosides with or without triterpene glycosides, the other containing exclusively triterpene glycosides.

In a continuation of the chemotaxonomical studies of this genus, the present paper deals with the isolation and structure determination of glycosides from leaves of *Rubus accuminatus* Smith in Rees, *R. ellipticus* Smith in Rees and *R. multibreatus* Levl. et Van. collected at Xishuangbanna, Yunnan, southern China.




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| 1 | β-OH | $CH_2OH$ | $CH_3$ | β-D-Glc |
| 2 | β-OH | $CH_3$ | $CH_2OH$ | β-D-Glc |
| 2a | β-OH | $CH_3$ | $CH_2OH$ | H |
| 3 | β-OH | $CH_2OH$ | $CH_2OH$ | H |
| 4 | β-OH | $CH_2OH$ | $CH_2OH$ | β-D-Glc |
| 7 | β-OH | $CH_3$ | $CH_3$ | β-D-Glc |
| 8 | α-OH | $CH_3$ | $CH_3$ | β-D-Glc |




| | $R^1$ | $R^2$ |
|---|---|---|
| 5 | $CH_3$ | $CH_2OH$ |
| 6 | $CH_2OH$ | $CH_3$ |
| 9 | $CH_3$ | $CH_3$ |

‡Author to whom correspondence should be addressed.

§In ref. [1], this sweet plant was tentatively designated as *R. chingii* Hu. However, the subsequent chemotaxonomical study revealed the difference between this and *R. chingii*; the former has been designated as a new species, *R. suavissimus* S. Lee; Lee, S. (1984) *Guihaia* (China) **1**, 17.

## RESULTS AND DISCUSSION

A methanolic extract of leaves of *R. accuminatus* Smith in Rees collected at Xishuangbanna in Yunnan, China, afforded five compounds, **1**–**5** (in yields of 0.6, 0.026, 0.004, 0.086 and 0.006%, respectively). Compounds **1** and **3**–**5** were identified by means of NMR as known triterpene

glucosides and a free triterpene, i.e. **1**: nigaichigoside F1 isolated from leaves of *R. microphyllus* L.f. and several other *Rubus* spp. [6–8], Loganiaceae [9] and Flacourtiaceae [10], **3**: trachelosperogenin B isolated from *Tracherospermum asiaticum* Nakai (Apocynaceae), **4**: trachelosperoside B-1, β-D-glucosyl ester of **3**, isolated from the same plant [11], **5**: sericoside from *Terminaria sericea* Burch. (Combretaceae) [12].

Another glycoside (**2**), $C_{36}H_{58}O_{11}$ (HR-FABMS), afforded glucose on acid hydrolysis. Hydrolysis of **2** with crude pectinase yielded an aglycone (**2a**) which was identified by inspection of the NMR spectra as hyptatic acid B isolated from *Hyptis capitata* (Labiatae) [13]. The $^{13}C$ signals of **2** in pyridine-$d_5$ [δ177.0 ($CO_2$-βGlc) and 95.8 (anomeric C of ester type βGlc)], indicated the location of a β-glucopyranosyl group of **2** on $CO_2$H-28 (Table 1). The glycoside of the same structure as **2** has been isolated from *Desfontainia spinosa* (Loganiaceae) [9].

Table 1. $^{13}C$ NMR spectral data for compounds **2**, **2a** and **9** and related compounds in pyridine-$d_5$

| C | 2 | 2a | 6* | 7† | 9 |
|---|---|---|---|---|---|
| Aglycone | | | | | |
| 1 | 47.9 | 47.8 | 47.6 | 48.0 | 47.6 |
| 2 | 68.7 | 68.7 | 68.9 | 68.6 | 68.6 |
| 3 | 85.8 | 85.8 | 78.4 | 83.8 | 83.9 |
| 4 | 44.0 | 44.0 | 43.7 | 38.5 | 38.7 |
| 5 | 56.6 | 56.6 | 48.5 | 56.0 | 56.1 |
| 6 | 19.5 | 19.4 | 18.8 | 19.1 | 19.1 |
| 7 | 33.8 | 33.8 | 33.1[a] | 33.5 | 33.2 |
| 8 | 40.6 | 40.4 | 40.4 | 40.6 | 40.3 |
| 9 | 48.0 | 47.9 | 48.2 | 47.9 | 48.5 |
| 10 | 38.3 | 38.3 | 38.6 | 39.9 | 39.9 |
| 11 | 24.2 | 24.4 | 24.3 | 24.2 | 24.3 |
| 12 | 128.3 | 127.9 | 123.5 | 128.2 | 123.5 |
| 13 | 139.3 | 140.0 | 144.4 | 139.5 | 144.0 |
| 14 | 42.1 | 42.1 | 42.2 | 42.3 | 42.2 |
| 15 | 29.2 | 29.3 | 29.1[b] | 29.3 | 29.0 |
| 16 | 26.1 | 26.9 | 28.0 | 26.8 | 28.0 |
| 17 | 48.6 | 48.3 | 46.5 | 48.6 | 46.5 |
| 18 | 54.4 | 54.6 | 44.6 | 54.4 | 44.6 |
| 19 | 72.7 | 72.7 | 81.1 | 72.6 | 81.1 |
| 20 | 42.1 | 42.4 | 35.5 | 42.2 | 35.6 |
| 21 | 26.7 | 26.4 | 29.0[b] | 26.1 | 28.8 |
| 22 | 37.7 | 38.5 | 32.9[a] | 37.8 | 33.1 |
| 23 | 24.4 | 24.2 | 66.7 | 29.5 | 29.5 |
| 24 | 65.7 | 65.7 | 14.2 | 16.8 | 16.9 |
| 25 | 17.4[a] | 17.3[a] | 17.8[c] | 17.1 | 17.7[a] |
| 26 | 17.3[a] | 17.1[a] | 17.4[c] | 17.8 | 17.6[a] |
| 27 | 24.5 | 24.6 | 24.9[d] | 24.6 | 24.9 |
| 28 | 177.0 | 180.7 | 177.3 | 176.9 | 177.3 |
| 29 | 27.0 | 27.1 | 28.7 | 27.0 | 28.8 |
| 30 | 16.7 | 16.8 | 24.7[d] | 17.5 | 24.7 |
| Glucosyl moiety | | | | | |
| 1′ | 95.9 | | 95.9 | 95.8 | 95.9 |
| 2′ | 74.1 | | 74.2 | 74.0 | 74.2 |
| 3′ | 79.0 | | 79.0 | 80.0 | 79.0 |
| 4′ | 71.3 | | 71.2 | 71.1 | 71.1 |
| 5′ | 79.3 | | 79.3 | 79.3 | 79.3 |
| 6′ | 62.4 | | 62.3 | 62.3 | 62.2 |

[a–d]May be interchanged in each column.
*Cited from ref. [15].
†Cited from ref. [10].

From a methanol extract of leaves of a related plant, *R. multibreatus* Levl. et Van. of Xishuangbanna origin, nigaichigoside F1 (**1**) (see above) and **6** were isolated in yields of 0.25 and 0.013%, respectively.

Compound **6** was identified by means of $^1H$ and $^{13}C$ NMR as arjunglucoside I (4-*epi*-sericoside), a compound previously isolated from *Terminalia arjuna* (Combretaceae) [14] and *Tracherospermum asiaticum* (Apocynaceae) [15].

From a methanol extract of leaves of another plant, *R. ellipticus* Smith in Rees of Xishuangbanna origin, nigaichigoside F1 (**1**), sericoside (**5**) and compounds **7**–**9** were isolated in yields of 0.22, 0.01, 0.026, 0.012 and 0.06%, respectively. Compounds **7** and **8** were identified as known triterpene glucosides, i.e. **7**: glucosyl tormentate isolated from leaves of *Aphloia theiformis* (Vahl.) Benn. (Flacourtiaceae) [10], **8**: kaji-ichigoside F1 isolated from leaves of *Rubus trifidus* Thunb. [6]

The molecular formula of the new glycoside (**9**) was determined as $C_{36}H_{58}O_{10}$ by HR-FABMS. Acid hydrolysis of **9** yielded D-glucose. The $^{13}C$ NMR data established that this glucose was esterified (δG1 95.9), and that the carbon signals for the aglycone moiety were almost superimposable on arjunglucoside I (**6**) except for the A-ring carbons. These on going from **9** to **6** are shifted by +37.2 (C-23), +5.0 (C-4), −5.5 (C-3), −7.6 (C-5) and −2.7 (C-24), respectively. This trend can only be explained by the presence of a hydroxyl on C-23 of **9**. In addition, the carbon signals of the A-ring of **9** were essentially the same as those of the corresponding signals of **7**. It follows that **9** can be formulated as 24-deoxysericoside.

No diterpene glycoside has been isolated from these plants as yet.

## EXPERIMENTAL

General. Mps: uncorr; IR: KBr; $^1H$ (400 MHz) and $^{13}C$ NMR (100 MHz): 40° in pyridine-$d_5$ or $CDCl_3$ using TMS as an int. standard; NOEDS: known methods; EIMS: 70 eV.

*Plant material.* Three species of plant, *Rubus accuminatus* Smith in Rees, *R. ellipticus* Smith in Rees and *R. multibreatus* Levl. et Van were collected at Xishuangbanna in Yunnan, China in December 1989 and the specimens deposited in the Herbarium of Kunming Institute of Botany, Academia Sinica, Kunming, Yunnan, China. The plants were identified by Dr Guoda Tao (Xishuangbanna Tropical Botanic Garden, Academia Sinica, Yunnan, China).

*Extraction and purification.* Powdered, dried leaves (50 g) of *R. accuminatus* were extracted with MeOH. The extract (8.6 g) was suspended in $H_2O$ and defatted with $Et_2O$. The $H_2O$ layer was extracted with 1-BuOH, and the BuOH extract (3.2 g) chromatographed on silica gel (EtOAc–EtOH–$H_2O$, 16:2:1) to give 4 frs, I–IV in order of elution. Fr. II (446 mg) was sepd by CC on RP-8 and then, HPLC (MeOH–$H_2O$, 3:2) to give **5** (3 mg), **2** (13 mg), **3** (2 mg) and **1** (200 mg). Fr.III (371 mg) was applied to a column of LiChroprep RP-8 (55% MeOH) and further sepd by HPLC (TSK gel ODS-120T, 50% MeOH) to give more of **1** (100 mg), and **4** (43 mg).

Powdered, dried leaves (50 g) of *R. multibreatus* were extracted with MeOH. The extract (9.0 g) was suspended in $H_2O$ and defatted with $Et_2O$. The $H_2O$ layer was extracted with 1-BuOH, and the BuOH extract (3.1 g) chromatographed on silica gel

(EtOAc–EtOH–$H_2O$, 16:2:1–14:2:1–12:2:1) to give 4 frs I–IV in order of elution. Fr. II (174 mg) was separated by chromatography on a column of LiChroprep RP-8 (50% MeOH), and then by HPLC (TSKgel ODS-120T, 30 cm × 21.5 mm; MeOH–$H_2O$, 11:9) to give fr. IIa (42 mg), which was acetylated with $Ac_2O$–pyridine (1:1) and then sepd by HPLC (TSKgel ODS-120T, 80% MeOH) to give **6Ac** and **1Ac**, both of which were refluxed with 0.4% $NaHCO_3$ for 40 min to afford **6** (6 mg) and **1** (122 mg), respectively.

Powdered, dried leaves (50 g) of *R. ellipticus* were extracted with MeOH. The extract (8.8 g) was suspended in $H_2O$ and defatted with $Et_2O$. The $H_2O$ layer was extracted with 1-BuOH, and the BuOH extract (2.2 g) chromatographed on silica gel (EtOAc–EtOH–$H_2O$, 16:2:1–12:2:1–5:2:1) to give 2 frs. Fr. I (211 mg) was sepd on a column of RP-8 (60% MeOH) to give **1** (82 mg) and a mixture, which was subjected to HPLC (TSKgel ODS-120T, MeOH–$H_2O$, 2:1) to give fr. Ia (26 mg) and **7** (13 mg). Fr. Ia was sepd by Lichrosorb Si 60 ($CHCl_3$–MeOH–$H_2O$, 30:10:1) to give **8** (6 mg) and **9** (3 mg). From fr. II of the first silica gel CC, **1** (28 mg) and **5** (5 mg) were obtained.

*Identification of known compounds.* Compounds **1** and **3–8** were identified by comparison of their $^1H$ and $^{13}C$ NMR data and/or optical rotation and/or mp with those of the following reference compounds: niga-ichigoside F1 (**1**): needles (from MeOH–$H_2O$), mp 230–231°, $[\alpha]_D^{25}$ +6° (MeOH; *c* 1.0). [6]; trachelosperogenin B (**3**) [11]; trachelosperoside B-1 (**4**) [11]; sericoside (**5**): $[\alpha]_D^{25}$ +19° (MeOH; *c* 0.37) [12]; arjunglucoside I (**6**): $[\alpha]_D^{25}$ +13° (MeOH; *c* 0.46) [12, 13]; glucosyl tormentate (**7**): $[\alpha]_D^{25}$ +6° (MeOH; *c* 0.84) [13], and kaji-ichigoside F1 (**8**): $[\alpha]_D^{25}$ +23° (MeOH; *c* 1.02) [6].

4-epi-*Niga-ichigoside F1* (**2**). Powder, $[\alpha]_D^{25}$ −10° (MeOH; *c* 0.80). FABMS (neg.) *m/z*: 665 $[M-H]^-$, 563 $[M-H-Glc]^-$; HR-FABMS (neg.) *m/z*: 665.3886 ($C_{36}H_{58}O_{11}-H$ requires *m/z* 665.3901); $^1H$ NMR (pyridine-$d_5$): δ3.73 (1H, *ddd*, *J* = 4.4, 9.5, 11.2 Hz, H-2), 3.54 (1H, *d*, *J* = 9.5 Hz, H-3), 5.45 (1H, *t*, *J* = 3.5 Hz, H-12), 2.92 (1H, *s*, H-18), 1.07 (3H, *d*, *J* = 6.6 Hz, $H_3$-30), 1.04, 1.15, 1.38, 1.57, 1.64 (3H × 5, each *s*, *tert*-Me × 5), 6.28 (1H, *d*, *J* = 8.1 Hz, H-1 of Glc); $^{13}C$ NMR: Table 1.

*Enzymic hydrolysis of compound* **2**. Crude pectinase (Tanabe 10 mg) was added to a soln of **2** (11 mg) in 50 mM acetate buffer (pH 5.6, 2 ml) and the mixt. incubated for 4 days at 37°. The reaction mixt. was extracted with 1-BuOH (2 ml). D-Glc in the aq. layer was detected by GLC [16]. The 1-BuOH extract was deionized with Dowex 50W X8 ($H^+$ form) and chromatographed on silica gel ($CHCl_3$–MeOH, 5:1) to give **2a** (4 mg).

*Compound* **2a** (*hyptatic acid B*). Powder, $[\alpha]_D^{25}$ +15° (MeOH; *c* 0.53). $^1H$ NMR (MeOH-$d_4$): δ3.79 (1H, *ddd*, *J* = 4.4, 9.4, 11.3 Hz, H-2), 3.06 (1H, *d*, *J* = 9.4 Hz, H-3), 5.29 (1H, *t*, *J* = 3.5 Hz, H-12), 2.50 (1H, *s*, H-18), 3.40, 4.04 (1H × 2, each *d*, *J* = 11.0 Hz, $H_2$-24), 0.93 (3H, *d*, *J* = 6.6 Hz, $H_3$-30), 0.77, 0.98, 1.19, 1.25, 1.33 (3H × 5, each *s*, *tert*-Me × 5). These values were essentially the same as ref. [13], except H-24$_b$ (δ4.40 in [13] may be misprint of 4.04); $^{13}C$ NMR: Table 1.

*24-Deoxy-sericoside* (**9**). Powder, $[\alpha]_D^{25}$ +15° (MeOH; *c* 0.20). FABMS (negative) *m/z*: 649 $[M-H]^-$, 487 $[M-H-Glc]^-$; HR-FABMS (negative) *m/z*: 649.3970 ($C_{36}H_{58}O_{10}-H$ requires *m/z* 649.3951); $^1H$ NMR (pyridine-$d_5$): δ3.40 (1H, *d*, *J* = 11.0 Hz, H-3), 5.53 (1H, *t*, *J* = 3.4 Hz, H-12), 3.45 (1H, *br s*, H-18), 3.62 (1H, *d*, *J* = 4.2 Hz, H-19), 0.99, 1.05, 1.11, 1.16, 1.19, 1.34, 1.62 (3H × 7, each *s*, *tert*-Me × 7), 6.39 (1H, *d*, *J* = 8.1 Hz, H-1 of Glc); $^{13}C$ NMR: Table 1.

*Acknowledgement*—The authors are grateful to Dr Guoda Tao (Xishuangbanna Tropical Botanic Garden, Academia Sinica, Yunnan, China) for identification of the plant material. Financial support for this work was provided by a grant under the Mombusho International Scientific Research Program (No. 03044103, 1991).

## REFERENCES

1. Tanaka, T., Khoda, H., Tanaka, O., Chen, F.-H., Chou, W.-H. and Leu, J.-L. (1981) *Agric. Biol. Chem.* **45**, 2165.
2. Hirono, S., Chou, W., Kasai, R., Tanaka, O. and Tada, T. (1990) *Chem. Pharm. Bull.* **38**, 1743.
3. Ohtani, K., Aikawa, Y., Kasai, R., Chou, W., Yamasaki, K. and Tanaka, O. (1992) *Phytochemistry* **31** (submitted).
4. Tanaka, T., Kawamura, K., Kitahara, T., Kohda, H. and Tanaka, O. (1984) *Phytochemistry* **23**, 615
5. Ohtani, K., Yang, C.-R., Miyajima, C., Zhou, J. and Tanaka, O. (1991) *Chem. Pharm. Bull.* **39**, 2443.
6. Seto, T., Tanaka, T., Tanaka, O. and Naruhashi, N. (1984) *Phytochemistry* **23**, 2829.
7. Ohtani, K., Miyajima, C., Takahasi, T., Kasai, R., Tanaka, O., Hahn, D.-R. and Naruhashi, N. (1990) *Phytochemistry* **29**, 3275.
8. Shigenaga, S., Kouno, I. and Kawano, N. (1985) *Phytochemistry* **24**, 115.
9. Houghton, P. J. and Lian, L. M. (1986) *Phytochemistry* **25**, 1939.
10. Gopalsamy, N., Vargas, D., Guého, D., Ricaud, C. and Hostettmann, K. (1988) *Phytochemistry* **27**, 3593.
11. Abe, F and Yamauchi, T. (1987) *Chem. Pharm. Bull.* **35**, 1748.
12. Bombardelli, E., Bonati, A., Gabetta, B. and Mustich, G. (1974) *Phytochemistry* **13**, 2559.
13. Yamagishi, T., Zhang, D.-C., Chang, J.-J., McPhail, D. R., McPhail, A. T. and Lee, K.-H. (1988) *Phytochemistry* **27**, 3213.
14. Honda, T., Murae, T., Tsuyuki, T., Takahashi, T. and Sawai, M. (1976) *Bull. Soc. Chem. Jpn* **49**, 3213.
15. Abe, F. and Yamauchi, T. (1987) *Chem. Pharm. Bull.* **35**, 1833.
16. Ohshima, R., Kumanotani, J. and Watanabe, C. (1983) *J. Chromatogr.* **259**, 159.