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Megastigmane and iridoid glucosides from *Clerodendrum inerme*

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Dedicated to Professor Vichiara Jirawongse on the occasion of his 83rd birthday

Abstract

From the aerial parts of *Clerodendrum inerme*, two megastigmane glucosides (sammangaosides A and B) and a iridoid glucoside (sammangaoside C) were isolated together with 15 known compounds. The structural elucidations were based on analyses of physical and spectroscopic data. © 2001 Published by Elsevier Science Ltd.

Keywords: *Clerodendrum inerme*; Verbenaceae; Megastigmane glucoside; Iridoid glucoside; Sammangaosides A–C

1. Introduction

As part of our ongoing study on Thai medicinal plants, we investigated the constituents of *Clerodendrum inerme* Gaertn. (Verbenaceae, Thai name: Sam-ma-nga) collected in the Botanical gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. *C. inerme* is a shrub distributed in South and South-east Asia, Australia and Pacific islands. In Thai traditional medicine, the fresh leaves are externally used for treating skin diseases. In preliminary studies of this plant, flavones (Vendantham et al., 1977) a clerodane diterpene (Achari et al., 1990), a neolignan (Spencer and Flippen-Anderson, 1981), a phenylpropanoid (Fauvel et al., 1989) and iridoid glycosides (Calis et al., 1994a,b) have been isolated. The present study deals with the isolation and structural elucidations of two new megastigmane glucosides (**6**, **7**) and one new iridoid glucoside (**10**), together with 15 known compounds; phenylpropanoid glycosides (**1–3**), phenylethanoid glycosides (**4**, **5**, **15**), iridoid glucosides (**8**, **9**), neolignan glucosides (**11**, **12**), benzyl alcohol glycosides (**13**, **14**), aliphatic glucoside (**16**) and hydroquinone glycosides (**17**, **18**).

2. Results and discussion

The methanolic extract of the aerial part of *C. inerme* was suspended in H_2O and defatted with Et_2O. The aqueous layer was subjected to a column of highly porous copolymer of styrene and divinylbenzene, and eluted with H_2O, MeOH and Me_2O, successively. The fraction eluted with MeOH was repeatedly chromatrographed on columns of silica gel and RP-18, then by HPLC-ODS to afford 18 compounds (**1–18**). Fifteen were identified as known compounds; verbascoside (**1**), isoverbascoside (**2**), leucosceptoside A (**3**) (Miyase et al., 1982), decaffeoylverbascoside (**4**), darendoside B (**5**) (Calis et al., 1993), monomelittoside (**8**) (Chaudhuri and Sticher, 1980), melittoside (**9**) (Swiatek et al., 1981), (7*S*, 8*R*)-dehydrodiconiferyl alcohol 9-*O*-β-glucopyranoside (**11**) (Binns et al., 1987), (7*S*, 8*R*)-dehydrodiconiferyl alcohol 4-*O*-β-glucopyranoside (**12**) (Yoshizawa et al., 1990), benzyl alcohol β-glucopyranoside (**13**) (Miyase et al., 1987), benzyl alcohol β-(2′-*O*-β-xylopyranosyl) glucopyranoside (**14**) (Sudo et al., 2000), salidroside (**15**) (Nonaka et al., 1982), (*Z*)-3-hexenyl-β-glucopyranoside (**16**) (Mizutani et al., 1988) and 2,6-dimethoxy-*p*-hydroquinone 1-*O*-β-glucopyranoside (**17**) (Otsuka et al., 1989), seguinoside K (**18**) (Zhong et al., 1999) by physical data and spectroscopic evidences.

The molecular formula of compound **6** was determined as $C_{19}H_{32}O_8$ by HR–FAB mass spectrometry.

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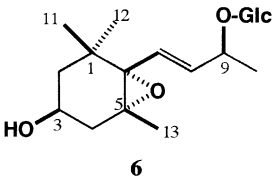
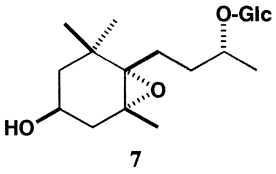
Inspection of the ^{13}C NMR spectrum indicated the presence of one β-glucopyranosyl unit together with 13 carbon signals for the aglycone moiety which suggested it to possess the megastigmane skeleton. Compound **6** showed the same planar structure as 3-hydroxy-5,6-epoxy-β-ionyl-9-*O*-β-glucopyranoside, previously isolated from flue-cured tobacco (Kodama et al., 1981) and the absolute configuration at C-9 was confirmed to be *R* (Sudo et al., 2000). Comparison of the ^{1}H and ^{13}C NMR spectral data of **6** with those of 3-hydroxy-5,6-epoxy-β-ionyl-9-*O*-β-glucopyranoside (Sudo et al., 2000) revealed the same relative arrangement of the ring carbons and protons. The difference of the chemical shifts at C-9 (δ 74.7) and 10 (δ 22.4,) with C-9 (δ 76.9) and C-10 (δ 21.0) of the reported data from the ^{13}C NMR spectrum led to conclude the absolute configuration at C-9 to be *S* (Pabst et al., 1992; Takeda et al., 1997). Therefore, the structure of **6** was assigned as (3*S*, 5*R*, 6*S*, 7*E*, 9*S*)-3-hydroxy-5,6-epoxy-β-ionyl-9-*O*-β-glucopyranoside, named sammangaoside A.



6



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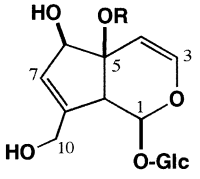


	R
8	**H**
9	**Glc**
10	**Glc (3'–1")-Glc**

The molecular formula of compound **7** was determined as $C_{19}H_{34}O_8$ by HR–FAB mass spectrometry. The ^{1}H and ^{13}C NMR spectral data indicated it to be a megastigmane glucoside. Inspection of the FAB mass spectrometry revealed the two mass units larger than **6** and the signals for the double bond were not observed in the ^{1}H and ^{13}C NMR spectra, indicating the presence of the saturated side chain. The chemical shifts for the signals attributed to the ring were coincident with those of **6**, deduced from the ^{1}H and ^{13}C NMR spectral data. The absolute configuration at C-9 was assigned to be *R* from the chemical shifts at C-9 and 10 (δ 77.8 and 21.8, respectively) (Pabst et al., 1992; Takeda et al., 1997). Consequently, (3*S*, 5*R*, 6*S*, 9*R*)-3-hydroxy-5,6-epoxy-β-dihydroionyl-9-*O*-β-glucopyranoside, was proposed for sammangaoside B.

The molecular formula of compound **10** was determined as $C_{27}H_{42}O_{20}$ by HR–FAB mass spectrometry. The ^{13}C NMR spectrum was very similar to that of **9** (Table 2) except that the ^{13}C NMR signals for one more glucopyranosyl unit were observed. The additional unit was assigned to be attached at C-3″ of the second glucopyranose on C-5 by HMQC and HMBC experiments, in which long-range correlations were observed between (i) H-1‴ (δ 4.54) and C-3″ (δ 88.3), and (ii) H-1″ (δ 4.74) and C-3″ (δ 88.3), C-5 (δ 79.8) as shown in Fig. 1. Moreover, the chemical shifts of C-3″, C-2″ and C-4″ were changed by +10.0,− 0.7 and−1.5 ppm, respectively. Therefore, the structure of compound **10** was elucidated as melittoside 3″-*O*-β-glucopyranoside, named sammangaoside C.

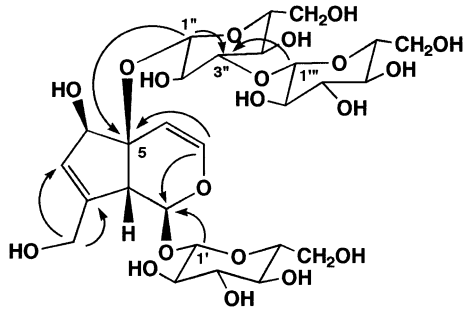
3. Experimental

3.1. General

NMR spectra were recorded in CD_3OD using a JEOL JNM A-400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) and JNM-ECP 500 (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) with tetramethylsilane (TMS) as internal standard. MS were recorded on a JEOL JMS-SX 102 spectrometer. Preparative HPLC was carried out on columns of ODS (150 ×20 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector. The flow rate was 6 ml/min. For CC, silica gel G 60 (Merck), RP-18 (50 μm, YMC) and highly porous copolymer of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co. Ltd) were used. The solvent systems were: (I) EtOAc–MeOH–H_2O (4:1:0.1), (II) EtOAc–MeOH–H_2O (7:3:0.3), (III) EtOAc–MeOH–H_2O (6:4:1), (IV) 15–70% MeOH, (V) 10–50% MeOH, (VI) 10% MeCN, (VII) 20% MeCN, and (VIII) 6% MeCN. The spray reagent used was 10% H_2SO_4 in ethanol.

3.2. Plant material

The aerial part of *Clerodendrum inerme* was collected in August, 1999 from the Botanical gardens, Faculty of



10

Fig. 1. The HMBC correlations of compound **10**.

Pharmaceutical Sciences, Khon Kaen University, Thailand. The identification of the plant was confirmed by Professor Vichiara Jirawongse, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher sample is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

3.3. Extraction and isolation

The dried aerial part (880 g) of *C. inerme* was extracted with hot MeOH. After removal of the solvent by evaporation, the residue (90.0 g) was defatted with Et_2O. The aqueous layer was subjected to column chromotography, using highly porous copolymer of styrene and divinylbenzene, eluted with H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH (17.5 g) was subjected to silica gel column chromotography (systems I, II and III, respectively) affording seven fractions. Fraction 2 (3.12 g) was applied to a RP-18 column using system IV, then followed by prep. HPLC–ODS (system VI and VII) to provide compounds **1** (456 mg), **2** (183 mg), **3** (10 mg), **4** (9 mg), **5** (5 mg), **6** (11 mg), **7** (6 mg), **11** (5 mg), **13** (13 mg), **14** (5 mg), **15** (8 mg), **16** (24 mg), **17** (13 mg) and **18** (4 mg). Fraction 4 (1.74 g) was further separated on a RP-18 column (system IV) and purified by prep. HPLC–ODS (system VII) to give compound **12** (7 mg). Fraction 5 (2.73 g) was similarly subjected to RP-18 column (system IV), then followed by prep. HPLC–ODS (system VII) to afford compounds **8** (50 mg). Fraction 6 (4.8 g) was purified by using columns of RP-18 (system V) and prep. HPLC–ODS (system VIII) to give compounds **9** (1.0 g) and **10** (10 mg).

3.4. Sammangaoside A (6)

Amorphous, $[\alpha]_D^{20}$ −99.1° (MeOH, *c* 0.8); ^{1}H NMR (CD_3OD): δ 6.04 (1H, *d*, *J* = 15.6 Hz, H-7), δ 5.56 (1H, *dd*, *J* = 15.6, 7.6 Hz, H-8), δ 4.52 (1H, *m*, H-9), δ 4.27 (1H, *d*, *J* = 7.5 Hz, H-1′ Glc), δ 3.73 (1H, *m*, H-3), δ 2.28 (1H, *dd*, *J* = 14.2, 1.7 Hz, H-4e), δ 1.61 (1H, *dd*, *J* = 14.2, 9.3 Hz, H-4a), δ 1.56 (1H, *bd*, *J* = 10.7 Hz, H-2e), δ 1.27 (3H, *d*, *J* = 6.3 Hz, H-10), δ 1.24 (3H, *s*, H-13), δ 1.20 (1H, *bt*, *J* = 11.0 Hz, H-2a), δ 1.08 (3H, *s*, H-11), δ 0.93 (3H, *s*, H-12); ^{13}C NMR (CD_3OD): Table 1; Negative HR–FAB–MS, *m*/*z*: 387.2039 $[M–H]^-$ ($C_{19}H_{31}O_8$ requires 387.2019)

3.5. Sammangaoside B (7)

Amorphous, $[\alpha]_D^{20}$ −35.0° (MeOH, *c* 0.4); ^{1}H NMR (CD_3OD): δ 4.30 (1H, *d*, *J* = 7.8 Hz, H-1′ Glc), δ 3.76 (1H, *m*, H-9), δ 3.67 (1H, *m*, H-3), δ 2.21 (1H, *dd*, *J* = 14.0, 1.8 Hz, H-4e), δ 1.98 (1H, *m*, H-7), δ 1.77 (1H, *m*, H-8), δ 1.63 (1H, *m*, H-8), δ 1.59 (1H, *dd*, *J* = 14.0, 9.2 Hz, H-4a), δ 1.57 (1H, *m*, H-7), δ 1.47 (1H, *bd*, *J* = 12.6 Hz, H-2e), δ 1.35 (3H, *s*, H-13), δ 1.23 (3H, *d*, *J* = 6.2 Hz, H-10), δ 1.18 (3H, *s*, H-11), δ 1.16 (1H, *dd*, *J* = 12.6, 11.0 Hz, H-2a), δ 1.03 (3H, *s*, H-12); ^{13}C NMR (CD_3OD): Table 1; Negative HR–FAB–MS, *m*/*z*: 389.2142 $[M–H]^-$ ($C_{19}H_{33}O_8$ requires 389.2175).

Table 1
^{13}C NMR spectral data of compounds **6** and **7** (CD_3OD, 100 MHz)

C	6	7
1	35.9	36.6
2	47.9	47.9
3	64.5	64.4
4	41.6	42.6
5	68.1	67.4
6	71.1	70.8
7	129.6	27.9
8	136	34.4
9	74.7	77.8
10	22.4	21.8
11	30.1	29.5
12	25	25.9
13	20.7	21.3
1′	101.3	103.8
2′	75	75.3
3′	78.3	78.2
4′	71.8	71.7
5′	78.1	77.8
6′	62.8	62.8

Table 2
^{13}C NMR spectral data of compounds **9** and **10** (CD_3OD, 100 MHz)

C	9	10[a]
1	94.2	93.8
3	143.4	143.4
4	105.2	105
5	80	79.8
6	79.9	79.6
7	128.1	128
8	147.3	147.6
9	51.5	51.4
10	60.9	60.9
1''	98.1	98
2''	74.8	74.9
3''	78.4	78.2a
4′	71.6	71.6b
5′	77.1	77.2
6′	32.7	62.8c
1″	99.6	99.3
2″	75.1	74.4 (−0.7)
3″	78.2	88.3 (+10.0)
4″	70.8	69.3 (−1.5)
5″	78.1	77.8
6″	62.1	62
1‴		105.2
2‴		75.5
3‴		78.5a
4‴		71.7b
5‴		77.8
6‴		62.6c

[a] a–c, assignment may be interchangeable.

3.6. Sammangaoside C (**10**)

Amorphous, $[\alpha]_D^{20}$ −36.1° (MeOH, *c* 0.7); ^{1}H NMR (CD_3OD): δ 6.65 (1H, *d*, *J* = 6.7 Hz, H-3), δ 5.79 (1H, *bs*, H-7), δ 5.63 (1H, *d*, *J* = 3.7 Hz, H-1), δ 5.15 (1H, *dd*, *J* = 6.4, 1.1 Hz, H-4), δ 4.74 (1H, *d*, *J* = 7.8 Hz, H-1″ Glc), δ 4.59 (1H, *d*, *J* = 7.8 Hz, H-1′ Glc), δ 4.54 (1H, *d*, *J* = 7.8 Hz, H-1‴ Glc); ^{13}C NMR (CD_3OD): Table 2; Negative HR–FAB–MS, *m*/*z*: 685.2204 $[M–H]^-$ ($C_{27}H_{41}O_{20}$ requires 685.2191).

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References

Achari, B., Chaudhuri, C., Saha, C.R., Dutta, P.K., Pakrashi, S.C., 1990. A clerodane diterpene and other constituents of *Clerodendron inerme*. Phytochemistry 29, 3671–3673.

Binns, A.N., Chen, R.H., Wood, H.N., Lynn, D.G., 1987. Cell division promoting activity of naturally occurring dehydrodiconiferyl glucosides: do cell wall components control cell division? Proceedings of the National Academy Sciences of the United States of America 84, 980–984.

Calis, I., Hosny, M., Yuruker, A., 1994. Inerminosides A1, C and D, three iridoid glycosides from *Clerodendrum inerme*. Phytochemistry 37, 1083–1085.

Calis, I., Hosny, M., Yuruker, A., Wright, A.D., Sticher, O., 1994. Inerminosides A and B, two novel complex iridoid glycosides from *Clerodendrum inerme*. Journal of Natural Products 57, 494–500.

Calis, I., Saracoglu, I., Basaran, A.A., Sticher, O., 1993. Two phenethyl alcohol glycosides from *Scutellaria orientalis* subsp. *pinnatifida*. Phytochemistry 32, 1621–1623.

Chaudhuri, R.K., Sticher, O., 1980. Globularifolin, a new acyl iridoid glucoside from *Globularia corifolia*. Helvetica Chemica Acta 63, 117–120.

Fauvel, M.T., Gleye, J., Andary, C., 1989. Verbascoside: a constituent of *Clerodendrum inerme*. Planta Medica 55.

Kodama, H., Fujimori, T., Kato, K., 1981. Isolation of a new terpene glucoside, 3-hydroxy-5,6-epoxy-β-ionyl-β-D-glucopyranoside from flue-cured tobacco. Agricultural and Biological Chemistry 45, 941–944.

Miyase, T., Koizumi, A., Ueno, A., Noro, T., Kuroyanagi, M., Fukushima, S., Akiyama, Y., Takemoto, T., 1982. Studies on the acyl glycosides from *Leucoseptrum japonicum* (Miq.) Kitamura et Murata. Chemical and Pharmaceutical Bulletin 30, 2732–2737.

Miyase, T., Ueno, A., Takizawa, N., Kobayashi, H., Karasawa, H., 1987. Studies on the glycosides of *Epimedium graniflorum* Morr. var. *thunbergianum* (Miq.) Nakai. Chemical and Pharmaceutical Bulletin 35, 1109–1117.

Mizutani, K., Yuda, M., Tanaka, O., Saruwatari, Y.-I., Fuwa, T., Jia, M-R, Ling, Y.-K., Pu, X.-F., 1988. Chemical studies on Chinese traditional medicine dangshen. I. Isolation of (*Z*)-3-(*E*)-2-hexenyl β-D-glucosides. Chemical and Pharmaceutical Bulletin 36, 2689–2690.

Nonaka, G.-I., Nishimura, H., Nishioka, I., 1982. Tannins and related compounds IV. Seven new phenol glucoside gallates from *Quercus stenophylla* Makino. Chemical and Pharmaceutical Bulletin 30, 2061–2067.

Otsuka, H., Takeuchi, M., Inoshiri, S., Sato, T., Yamasaki, K., 1989. Phenolic compounds from *Coix lachryma-jobi*. var. *ma-yuen*. Phytochemistry 30, 883–886.

Pabst, A., Barron, D., Semon, E., Schreier, P., 1992. Two diastereomeric 3- oxo-α-ionol β-D-glucosides from raspberry fruit. Phytochemistry 31, 1649–1652.

Spencer, G.F., Flippen-Anderson, J.L., 1981. Isolation and X-ray structure determination of a neolignan from *Clerodendron inerme* seeds. Phytochemistry 20, 2757–2759.

Sudo, H., Ide, T., Otsuka, H., Hirata, E., Takushi, A., Shinzato, T., Takeda, Y., 2000. Megastigmane, benzyl and phenethyl alcohol glycosides, and 4,4′-dimethoxy-β-truxinic acid catalpol diester from the leaves of *Premna subscandens* Merr. Chemical and Pharmaceutical Bulletin 48, 542–546.

Swiatek, L., Lehmann, D., Chudhuri, R.K., Sticher, O., 1981. Occurrence of milittoside in the seeds of *Plantago media*. Phytochemistry 20, 2023–2324.

Takeda, Y., Zhang, H., Matsumoto, T., Otsuka, H., 1997. Oosio, Y, Honda, G Tabata, M., Fujita, T, Sun, H., Sezik, E, Yesilada, E., Megastigmane glycosides from *Salvia nemorosa*. Phytochemistry 44, 117–120.

Vendantham, T.N.C., Subramanian, S.S., Harborne, J.B., 1977. 4′-Methylscutellarein and pectolinarigenin from *Clerodendron inerme*. Phytochemistry 16, 294.

Yoshizawa, F., Deyama, T., Takizawa, N., Usmanghani, K., Ahmad, M., 1990. The constitutents of *Cistanche tubulosa* (Schrenk) Hook.f. II. Isolation and structures of a new phenylethanoid glycoside and a new neolignan glycoside. Chemical and Pharmaceutical Bulletin 38, 1927–1930.

Zhong, X.-N., Otsuka, H., Ide, T., Hirata, E., Takeda, Y., 1999. Hydroquinone diglycosides acyl esters from the leaves of *Myrsine seguinii*. Phytochemistry 52, 923–927.