Aliphatic Alcohol and Iridoid Glycosides from *Asystasia intrusa*

Tripetch KANCHANAPOOM,*[,a] Somsak RUCHIRAWAT,[b] Ryoji KASAI,[c] and Hideaki OTSUKA[c]

[a]*Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University; Khon Kaen 40002, Thailand:* [b]*Chulabhorn Research Institute, Vipavadee Rangsit Highway; Bangkok 10210, Thailand: and* [c]*Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University; Hiroshima 734-8551, Japan.* Received February 2, 2004; accepted April 1, 2004

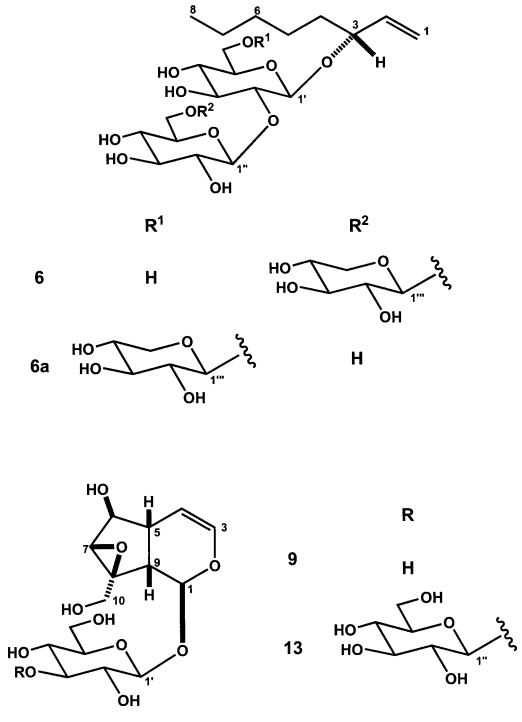
An aliphatic alcohol glycoside (asystoside) and an iridoid diglucoside (3′-*O*-*β*-D-glucopyranosyl-catalpol) were isolated from the aerial part of *Asystasia intrusa* along with benzyl *β*-D-glucopyranoside, zizybeoside I, (6*S*,9*R*)-roseoside, verbascoside, ehrenoside, 6*β*-hydroxyantirrhide, angeloside, catalpol, ajugol, 6-deoxycatalpol, and scutellarioside II. The structural elucidations were based on analyses of physical and spectroscopic data.

Key words *Asystasia intrusa*; Acanthaceae; aliphatic alcohol; iridoid diglucoside; asystoside

As part of our ongoing studies of Acanthaceous plants,[1—4] we investigated the constituents of *Asystasia intrusa* BLUME (Thai name: *Ya-Yaa*), collected from the Botanical Gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. The aerial part of this plant has been used as forage, although there is no mention of its medicinal uses in Thai traditional medicine. Phytochemical investigation has not been carried out in this species. Previous studies on plants in this genus reported iridoid glycosides from *Asystasia bella*.[5,6] The present study deals with the isolation and structural determinations of a new aliphatic glycoside (**6**) and a new iridoid diglucoside (**13**), together with 11 known compounds (**1—5**, **7—12**) from the aerial part of this plant.

Results and Discussion

The methanolic extract was suspended in H_2O and defatted with Et_2O. The aqueous layer was subjected to a Diaion HP-20 column, using H_2O, MeOH and Me_2CO successively. The portion eluted with MeOH was repeatedly chromatographed on columns of silica gel, RP-18, and preparative HPLC-ODS to afford 13 compounds. Eleven were identified as the known compounds benzyl *β*-D-glucopyranoside (**1**),[7] zizybeoside I (**2**),[8] (6*S*,9*R*)-roseoside (**3**),[9] verbascoside (**4**),[10] ehrenoside (**5**),[11] 6*β*-hydroxyantirrhide (**7**),[12] angeloside (**8**),[13] catalpol (**9**),[14] ajugol (**10**),[15] 6-deoxycatalpol (**11**),[16] and scutellarioside II (**12**)[17] by comparison of physical data with values reported in the literature and from spectroscopic evidence.



The molecular formula of compound **6** was determined to be $C_{25}H_{44}O_{15}$ by negative high-resolution (HR)-FAB mass spectrometry. The ^{1}H- and ^{13}C-NMR spectra revealed the presence of three sugar units from the anomeric proton signals at δ 4.32 (d, J=7.6 Hz), 4.43 (d, J=7.6 Hz), and 4.62 (d, J=7.8 Hz), and from the carbon signals at δ 101.8, 104.9, and 105.3. Acid hydrolysis gave D-xylose and D-glucose, identified by TLC and comparison of the optical rotation with that of authentic samples. The negative FAB-MS exhibited the characteristic fragment ions of a linear sugar chain at m/z 451 [M−pentose]$^-$ and 289 [M−pentose−hexose]$^-$, indicating that xylose is a terminal sugar connected to an inner glucose. The distortionless enhancement by polarization transfer (DEPT) experiments indicated the presence of one methyl (δ 14.5), five methylenes (δ 23.7, 25.6, 33.1, 35.8, 116.9), as well as two methines (δ 83.7, 140.6) of the aglycone moiety, which could be assigned to (3*R*)-1-octen-3-ol (matsutake alcohol) by comparing the spectral data with those in the literature.[2,18] The chemical shifts of compound **6** were almost the same as those of ebracteatoside B (**6a**), previously isolated from *Acanthus ebracteatus*,[2] except for the difference in the chemical shifts of the sugar chain (Table 1). The sugar moiety was identified as a *β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl unit by heteronuclear multiple-bond connectivity (HMBC), in which long-range correlations were found between i) H-1‴ (δ 4.32, d, J=7.6 Hz) and C-6″ (δ 69.5), and C-5‴ (δ 66.8); ii) H-1″ (δ 4.62, d, J=7.8 Hz) and C-2′ (δ 82.4); and iii) H-1′ (δ 4.43, d, J=7.6 Hz) and C-3 (δ 83.7) and C-2′ (δ 82.4), as shown in Fig. 1. Consequently, the structure of compound **6** was concluded to be (3*R*)-1-octen-3-ol-3-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside, namely asystoside.

The aliphatic alcohol glycosides, which have the aglycone (3*R*)-1-octen-3-ol or its derivatives, are rarely found from plant sources. Only a few studies identified (3*R*)-1-octen-3-

* To whom correspondence should be addressed. e-mail: trikan@kku.ac.th

Table 1. ^{13}C-NMR Spectral Data of **6a** and **6** (100 MHz, CD_3OD)

No.	**6a**	**6**
Aglycone		
1	116.9	116.9
2	140.5	140.6
3	83.8	83.7
4	35.7	35.8
5	25.5	25.6
6	32.9	33.1
7	23.6	23.7
8	14.5	14.5
Glc-1′	101.7	101.8
2′	82.3	82.4
3′	77.5[a)]	77.6[a)]
4′	71.0	71.1[b)]
5′	77.5[a)]	78.2[a)]
6′	69.3	62.9
Glc-1″	104.8	104.9
2″	75.9	76.1
3″	77.8[a)]	78.0[a)]
4″	71.4	71.1[b)]
5″	78.0[a)]	76.8
6″	62.7	69.5
Xyl-1‴	105.1	105.3
2‴	74.7	74.8
3‴	77.5[a)]	77.7[a)]
4‴	71.4	71.6[b)]
5‴	66.7	66.8

a, b) Assignments may be interchanged in each column.

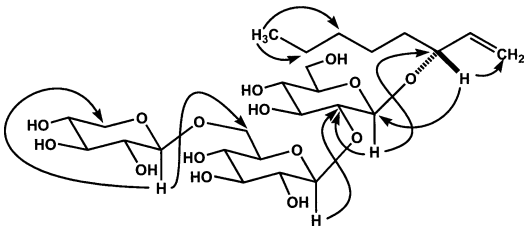


Fig. 1. Significant HMBC Correlations of Compound **6**

ol-3-*O*-β-D-glucopyranoside from *Mentha spicata*,[18] (3*R*)-1-octen-3-ol-3-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside from *M. spicata*,[18] *Barleria lupulina*,[1] and *Barleria strigosa*,[4] ebracteatosides B—D from *Acanthus ebracteatus*,[2] and ilicifolioside B from *Acanthus ilicifolius*.[19]

The molecular formula of compound **13** was determined to be $C_{21}H_{32}O_{15}$ by negative HR-FAB mass spectrometry. Analysis of the ^{13}C-NMR spectral data revealed the presence of two β-glucopyranosyl units in addition to nine carbon signals in the aglycone moiety. Acid hydrolysis provided D-glucose, identified by TLC and comparison of the optical rotation with that of an authentic sample. DEPT experiments indicated that compound **13** contains one methylene (δ 61.4), seven methines (δ 39.0, 43.5, 62.5, 79.5, 95.3, 104.0, 141.7), and one quaternary carbon (δ 66.2) in the aglycone part, corresponding to an iridoid. The chemical shift at δ 95.3 was characteristic of an acetal group at C-1. The methine signals at δ 141.7 and 104.0 were assigned to a disubstituted olefin group at C-3 and C-4. The chemical shifts at δ 62.5 and 66.2 belonged to an epoxyl group on C-7 and C-8 of the cyclopentanopyran ring. The ^{13}C-NMR spectral data were very similar to those of catalpol (**9**) except that the signals of one more β-D-glucopyranosyl unit were observed (Table 2). This additional unit was located at C-3′ since the chemical shifts of C-3′, C-2′, and C-4′ were changed by +8.4, −0.7, and −1.7, respectively. The chemical shifts of the sugar moiety were also in agreement with the reported data for the β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl unit.[3] Therefore the structure of compound **13** was elucidated to be 3′-*O*-β-D-glucopyranosyl-catalpol.

Table 2. ^{13}C-NMR Spectral Data of **9** and **13** (100 MHz, CD_3OD)

No.	**9**	**13**
Aglycone		
1	95.3	95.3
3	141.8	141.7
4	104.0	104.0
5	39.1	39.0
6	79.6	79.5
7	62.6	62.5
8	66.2	66.2
9	43.6	43.5
10	61.7	61.4
Glc-1′	99.7	99.4
2′	74.9	74.2
3′	78.7	87.1
4′	71.8	70.1
5′	77.7	77.7[a)]
6′	63.0	62.7[b)]
Glc-1″		105.1
2″		75.5
3″		78.0[a)]
4″		71.5
5″		78.1[a)]
6″		62.6[b)]

a, b) Assignments may be interchanged in each column.

The biological activities of the isolated compounds have not been investigated. However, the biological and pharmacologiced activities of phenylethanoids and naturally occurring iridoids have been reviewed.[20,21] Further investigations of the isolated compounds are in progress.

Experimental

General Procedures NMR spectra were recorded in CD_3OD using a JEOL JNM α-400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR). MS values were obtained on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a union PM-1 digital polarimeter. For column chromatography, silica gel 60 (70—230 mesh, GE0049, Scharlau Chemie S. A.), RP-18 (50 μm, YMC), and Diaion HP-20 (Mitsubishi Chemical Industries Co. Ltd.) were used. Preparative HPLC was carried out on an ODS column (150×20 mm i.d., YMC) with a Shimadzu RID-6A refractive index detector. The flow rate was 6 ml/min. The solvent systems were: I) EtOAc–MeOH (9 : 1); II) EtOAc–MeOH–H_2O (40 : 10 : 1); III) EtOAc–MeOH–H_2O (70 : 30 : 3); IV) 10—50% aqueous MeOH; V) 2.5% aqueous MeCN; VI) 4% aqueous MeCN; VII) 5% aqueous MeCN; VIII) 10% aqueous MeCN; IX) 15% aqueous MeCN; and X) 20% aqueous MeCN. The spraying reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

Plant Material *A. intrusa* Blume was cultivated and collected in August 2003 from the Botanical Garden, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand. The plant was identified by Mr. Bamrung Thavinchiua, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher sample (KKU 0046) is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and Isolation Dried whole *A. intrusa* (1.3 kg) was extracted three times with hot MeOH (5 l for each extraction, under reflux). The solvent was concentrated *in vacuo* to give a greenish powder (143.3 g). This residue was suspended in H_2O and defatted with Et_2O three times (1 l each). The aqueous layer was applied to a column of Diaion HP-20 and eluted with

H_2O, MeOH, and Me_2CO successively. The fraction eluted with MeOH (20.2 g) was concentrated to dryness and subjected to a silica gel column using solvent systems I, II, and III. Six fractions were collected. Fraction 2 (2.3 g) was applied to a column of RP-18 using solvent system IV to give 10 fractions. Fractions 2-3 and 2-4 were combined and purified by preparative HPLC-ODS with solvent system VIII to afford compounds **1** (34 mg) and **3** (97 mg). Fraction 2-5 was subjected to preparative HPLC-ODS with solvent system IX to give compound **12** (41 mg). Fraction 2-6 was purified by HPLC-ODS with solvent system X to provide compound **4** (73 mg). Fraction 3 (4.2 g) was subjected to a column of RP-18 using solvent system IV, affording nine fractions. Fraction 3-1 was purified by preparative HPLC-ODS with solvent system V to give compounds **7** (32 mg), **8** (49 mg), and **9** (124 mg). Fraction 3-2 was further purified by preparative HPLC-ODS with solvent system VI to provide compound **10** (124 mg). Fraction 3-3 was purified by preparative HPLC-ODS with solvent system VII to obtain compound **11** (23 mg). Fraction 3-4 was subjected to preparative HPLC-ODS with solvent system VIII to afford compound **2** (52 mg), and fraction 3-7 was purified by preparative HPLC-ODS with solvent system IX to provide compound **5** (108 mg). Similarly, fraction 4 (3.9 g) was separated on a column of RP-18 using solvent system IV to give 12 fractions. Fraction 4-1 was purified by preparative HPLC-ODS with solvent system V to obtain compound **13** (55 mg). Finally, fraction 4-8 was purified by preparative HPLC-ODS using solvent system X to provide compound **6** (38 mg).

Compound **6:** Amorphous powder, $[\alpha]_D^{27}$ +7.7° (c=1.56, MeOH); ^{1}H-NMR (CD_3OD): δ:5.86 (1H, ddd, J=17.1, 10.2, 7.3 Hz, H-2), 5.22 (1H, br d, J=17.1 Hz, H-1), 5.13 (1H, br d, J=10.2 Hz, H-1), 4.62 (1H, d, J=7.8 Hz, H-1″ Glc), 4.43 (1H, d, J=7.6 Hz, H-1′ Glc), 4.32 (1H, d, J=7.6 Hz, H-1‴ Xyl), 4.12 (1H, dd, J=13.4, 6.8 Hz, H-3), 1.66 (1H, *m*, H-4), 1.49 (1H, *m*, H-4), 1.22—1.39 (6H, *m*, H-5, 6, 7), 0.90 (3H, t, J=6.8 Hz, H-8); ^{13}C-NMR (CD_3OD): Table 1. Negative FAB-MS *m*/*z* 583 $[M-H]^-$, 451 $[M-Xyl]^-$, 289 $[M-Xyl-Glc]^-$. Negative HR-FAB-MS, *m*/*z*: 583.2589 ($C_{25}H_{43}O_{15}$ required 583.2601).

Compound **13**: Amorphous powder, $[\alpha]_D^{27}$ −74.9° (c=3.41, MeOH); ^{1}H-NMR (CD_3OD): δ: 6.34 (1H, d, J=5.4Hz, H-3), 5.07 (1H, dd, J=5.4, 4.6 Hz, H-4), 5.02 (1H, d, J=9.8 Hz, H-1), 4.77 (1H, d, J=7.8 Hz, H-1′ Glc), 4.59 (d, J=7.8 Hz, H-1″ Glc), 4.11 (1H, d, J=13.1 Hz, H-10a), 3.92 (1H, br d, J=8.0 Hz, H-6), 3.80 (1H, d, J=13.1 Hz, H-10b), 3.45 (1H, br s, H-7), 2.53 (1H, dd, J=9.8, 8.3 Hz, H-9), 2.27 (1H, m, H-5); ^{13}C-NMR (CD_3OD): Table 2. Negative HR-FAB-MS, *m*/*z*: 523.1677 ($C_{21}H_{31}O_{15}$ required 523.1663).

Acid Hydrolysis of Compounds 6 and 13 Compound **6** (25 mg) was dissolved in 5% HCl and heated at 90°C for 2 h. After cooling, the reaction mixture was extracted with Et_2O. The aqueous layer was neutralized with saturated $NaHCO_3$ and concentrated to dryness. The residue was applied to a silica gel column, using solvent system II, to obtain D-xylose (4 mg, $[\alpha]_D^{27}$ +20.0°) and D-glucose (7 mg, $[\alpha]_D^{27}$+49.5°) in comparison with authentic samples. By the same method, compound **13** (22 mg) gave D-glucose (5 mg, $[\alpha]_D^{27}$+51.2°).

Acknowledgments This study was financially supported by a Grant-in-Aid for Scientific Research (No. MRG-4680108) from the Thailand Research Fund and Ministry of University Affairs. We also thank Mr. Bunlert Khamya of the Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University, for help in obtaining the plant material.

References

1) Kanchanapoom T., Kasai R., Yamasaki K., *Phytochemistry*, **58**, 337—341 (2001).
2) Kanchanapoom T., Kasai R., Picheansoonthon C., Yamasaki K., *Phytochemistry*, **58**, 811—817 (2001).
3) Kanchanapoom T., Kasai R., Yamasaki K., *Phytochemistry*, **60**, 769—771 (2002).
4) Kanchanapoom T., Noiarsa P., Ruchirawat S., Kasai R., Otsuka H., *Chem. Pharm. Bull*., **52**, 612—614 (2004).
5) Jensen H. F. W., Jensen S. R., Nielsen B. J., *Phytochemistry*, **27**, 2581—2589 (1988).
6) Demuth H., Jensen S. R., Nielsen B. J., *Phytochemistry*, **28**, 3361—3364 (1989).
7) Miyase T., Ueno A., Takizawa N., Kobayashi H., Karasawa H., *Chem. Pharm. Bull*., **35**, 1109—1117 (1987).
8) Okamura N., Yagi A., Nishioka I., *Chem. Pharm. Bull*., **29**, 3507—3514 (1981).
9) Otsuka H., Yao M., Kamada K., Takeda Y., *Chem. Pharm. Bull*., **43**, 754—759 (1995).
10) Miyase T., Koizumi A., Noro, T., Kuroyanagi M., Fukushima S., Akiyama Y., Takemoto T., *Chem. Pharm. Bull*., **30**, 2732—2737 (1982).
11) Lahloub M. F., Gross G.-A., Sticher O., Winkler T., Schulten H.-R., *Planta Med*., **52**, 352—355 (1986).
12) Otsuka H., *Phytochemistry*, **33**, 617—622 (1993).
13) von Poser G. L., Damtoft S., Schripsema J., Henriques A. T., Jensen S. R., *Phytochemistry*, **46**, 371—373 (1997).
14) Otsuka H., Kubo N., Yamasaki K., Padolina W. G., *Phytochemistry*, **28**, 513—515 (1989).
15) Nishimura H., Sasaki H., Morota T., Chin M., Mitsuhashi H., *Phytochemistry*, **28**, 2705—2709 (1989).
16) Damtoft S., Jensen S. R., Nielsen B. J., *Phytochemistry*, **24**, 2281—2283 (1985).
17) Sudo H., Ide T., Otsuka H., Hirata E., Takushi A., Takeda Y., *Phytochemistry*, **46**, 1231—1236 (1997).
18) Yamamura S., Ozawa K., Ohtani K., Kasai R., Yamasaki K., *Phytochemistry*, **48**, 131—136 (1998).
19) Wu J., Zhang S., Xiao Q., Li Q., Huang J., Long L., Huang L., *Phytochemistry*, **63**, 491—495 (2003).
20) Jimenez C., Riguera R., *Nat. Prod. Res*., **11**, 591—606 (1994).
21) Ghisalberti E. L., *Phytomedicine*, **5**, 147—163 (1998).