Phytochemistry 69 (2008) 2162–2166

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Phytochemistry and molecular systematics of *Triaenophora rupestris* and *Oreosolen wattii* (Scrophulariaceae)

Søren Rosendal Jensen [a,*], Hong-Qing Li [b], Dirk C. Albach [c], Charlotte H. Gotfredsen [a]

[a] Department of Chemistry, The Technical University of Denmark, Kemitorvet, Build. 201, DK-2800 Lyngby, Denmark
[b] School of Life Science, East China Normal University, Shanghai, China
[c] Institut für Spezielle Botanik, Johannes Gutenberg-Universität Mainz, D-55099 Mainz, Germany

ARTICLE INFO

Article history:
Received 28 March 2008
Received in revised form 15 May 2008
Available online 1 July 2008

Keywords:
Triaenophora
Oreosolen
Scrophulariaceae
Chemotaxonomy
Iridoid glucosides
Scorodioside
Oreosolenoside
Phylogeny

ABSTRACT

The relationships between the genera *Triaenophora*, *Oreosolen* and *Rehmannia* were investigated. All three genera were previously included in tribe Veroniceae which was part of Scrophulariaceae but which is now included in Plantaginaceae. With regard to the content of iridoid glucosides, *Triaenophora rupestris* and the much-investigated *Rehmannia* were almost identical in containing catalpol, ajugol and 6-feruloylajugol. *Oreosolen wattii* was rather different in having compounds typical for the tribe Scrophularieae (Scrophulariaceae), namely aucubin, harpagide, harpagoside as well as two diesters of rhamnopyranosylcatalpol, one of which, here named oreosolenoside, had not previously been described in the literature. These results are consistent with recent analyses based on DNA sequencing and a phylogenetic tree illustrating the taxonomic relationships is presented.

1. Introduction

Rehmannia Libosch. ex Fisch. et Mey. is a small genus of six species (Hong et al., 1998). Five of the six species are endemic to China with *R. glutinosa* (Gaert.) Libosch. ex Fisch. et Mey. extending further to Korea and Japan. Since the latter is important in traditional Chinese medicine, much chemical work has been performed on this species, and we have recently (Albach et al., 2007) extended this with an investigation of the remaining five species. Two other Chinese genera, namely *Triaenophora* and *Oreosolen* have traditionally been regarded as more or less related to *Rehmannia* and placed in Digitalideae (Bentham, 1846; von Wettstein, 1891; Solereder, 1909; Li, 1948; Hong et al., 1998). Together with *Titanotrichum*, *Triaenophora* was segregated from within *Rehmannia* by Solereder (1909), but while the latter two were retained in the Digitalideae, the former was transferred to Gesneriaceae, a relationship later supported by DNA-sequence based analyses (Smith et al., 1997; Albach et al., 2001) Also the chemistry of *Titanotrichum* is consistent with this since *T. oldhamii* (Hemsl.) Soler. is devoid of iridoid glucosides (Jensen, 1996), a characteristic of Gesneriaceae which distinguishes it from most other Lamiales. *Oreosolen*, which was considered to be related to *Ourisia* and *Lafuentea* (vonWettstein 1891) or to genera of Veroniceae (Hallier 1903), was recently moved to a much-reduced Scrophulariaceae by Oxelman et al. (2005). Using DNA sequencing, a sister-group relationship between *Scrophularia* and *Oreosolen* was discovered, and these genera also share a similar floral morphology and leaf architecture. Furthermore, the two genera are sisters to *Verbascum*, together comprising a clade that is predominantly north temperate in distribution, unlike most other lineages in Scrophulariaceae.

Triaenophora and *Oreosolen* are two small genera with only ca. 2 and 1 species, respectively (Hong et al., 1998). *Triaenophora rupestris* (Hemsl.) Soler. is on the "red list" of endangered species in China, and is occasionally found on cliff faces in Hubei and Sichuan, China. *Oreosolen wattii* Hook.f. which is distributed in the Himalayas and Qinghai-Xizang Plateau, is used as a traditional Tibetan medicine for the treatment of fractures, open wounds and sprains. Chemical work on these two genera is very limited. Apparently, *Triaenophora* has not been investigated previously, and only a single work on *O. wattii* has been published (Yu et al., 1996) reporting the presence of verbascoside (**7**) together with four triterpenoid

* Corresponding author. Tel.: +454 525 2103; fax: +454 593 3968.
E-mail address: srj@kemi.dtu.dk (S.R. Jensen).

0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.05.010

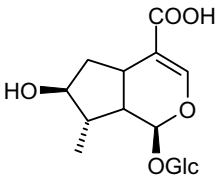
saponins, namely mimengosides A and B and buddlejasaponins I and Ia.

2. Results and discussion

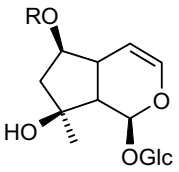
Dry material of *T. rupestris* from two different localities was investigated, and these were more or less identical in chemical content. Thus, the sugar fraction consisted mainly by sucrose and glucose with a little mannitol present, while the main iridoid constituents were catalpol (**5**), and ajugol (**2**) accompanied by verbascoside (**7**). Minor constituents were epiloganic acid (**1**) and two iridoid esters 6-feruloyl ajugol and 6-(*p*-coumaroyl) ajugol (**2a** and **2b**). Such a profile of compounds is almost the same as that found in *Rehmannia* species (Albach et al., 2007) and the two ajugol esters **2a** and **2b** have so far only been reported from *Rehmannia glutinosa* (Nishimura et al., 1989). Thus, in chemical content, *Triaenophora* cannot be distinguished from *Rehmannia*, which agrees with the close relationship based on DNA sequence data (Fig. 1).

The result from *O. wattii* was quite different. The sugar fraction from this plant contained apparently only sucrose and glucose, while the main iridoids were aucubin (**4**), harpagide (**3**) as well as the 6-*O*-rhamnopyranosylcatalpol esters scorodioside (**6a**) and oreosolenoside (**6b**), of which the latter was new. Minor constituents were catalpol (**5**), methylcatalpol (**5a**) and harpagoside (**3a**).

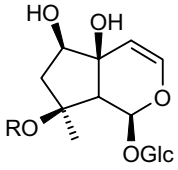
Oreosolenoside (**6b**) was obtained as a mixture of *E/Z* isomers and consequently, the specific rotation was not measured. The elemental formula was established to be $C_{32}H_{40}O_{17}$ by HR-ESIMS. The NMR spectra (Table 1) were assigned using 1D and 2D techniques. The preparation was clearly a mixture of *E/Z* isomers, as seen by the two sets of ^{1}H resonances at δ 6.42 (*d*, 16.0) and δ 5.88 (*d*, 12.8). The ^{13}C NMR spectrum showed that all the signals arising from the aromatic part were doubled, while the remaining signals were not. This part of the spectrum was very similar to that of scorodioside (**6a**) from *Scrophularia scorodonia* L. (Fernandez et al., 1995), which is a 3″-acetyl-2″-cinnamoyl diester of 6-α-*O*-rhamnopyranosylcatalpol (see Table 1), except for the signals arising from the aromatic part. From the spectrum, however, it was obvious that this part of **6b** was a *p*-coumaroyl moiety, and therefore the compound was an isomer of the two known diesters: scrophuloside A_2 (a 2″-acetyl-4″-*p*-coumaroyl diester) and scrophuloside A_3 (a 3″-acetyl-4″-*p*-coumaroyl diester) found in *Scrophularia nodosa* L. (Miyase and Mimatsu, 1999). The ^{1}H NMR spectrum was consistent with the presence of a 2″,3″-diester of 6-α-*O*-rhamnopyranosylcatalpol, since the 2″- and the 3″-protons were observed at δ 5.4 and δ 5.2, respectively, which is 1.2 and 0.8 ppm, downfield from that of the unesterified iridoid (Taskova et al., 2006). Besides these signals, all the aromatic, the 6″-Me and the 6- and 7-proton were doubled. The positions of the acetyl groups of the *E/Z*-forms were determined by the HMBC spectrum. In this, correlations between the 2″-protons at δ 5.39 and δ 5.35 and the aromatic carbonyl signals at δ 168.1 and δ 167.0 from the *E*- and the *Z*-forms, respectively, could be seen. Likewise, we found correlations between the 3″-protons at δ 5.16 and the aliphatic carbonyl signal at δ 172.3. This established the structure of the isolate to be 6-*O*-(3″-acetyl-2″-*p*-(*E/Z*)-coumaroyl-rhamnopyranosyl)-catalpol, and we have named the compound oreosolenoside. Regarding the presence of *E*- and *Z*-mixtures of *p*-coumaroyl esters in our isolates, we are uncertain whether one or the other exists in a pure form in the plants. We know however, that these forms easily isomerise either by gentle heating or by the influence of daylight since in one case we obtained the pure ester **2b** from *T. rupestris*, and this had isomerised completely to the 1:1 mixture by standing for a month on the laboratory bench.



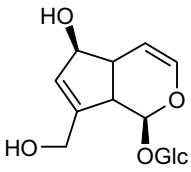
1; Epiloganic acid



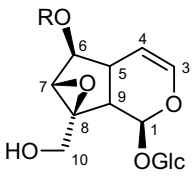
2; R=H; Ajugol
2a; R=Feruloyl
2b; R=*p*-Coumaroyl



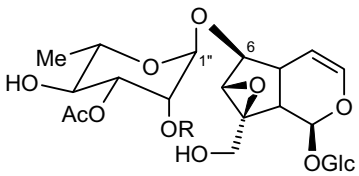
3; R=H; Harpagide
3a; R=Bz; Harpagoside



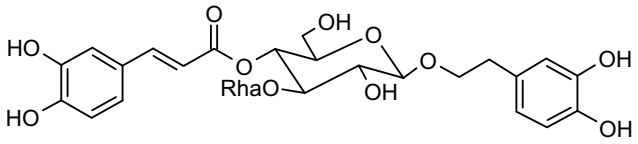
4; Aucubin



5; R=H; Catalpol
5a; R=Me; Methylcatalpol



6a; R=*E*-Cinnamoyl
6b; R=*E/Z*-Coumaroyl



7; Verbascoside

Together with the four triterpenoid saponins previously reported from *O. wattii* (Yu et al., 1996), the iridoid composition found in the present work is consistent with a taxonomic position in Scrophulariaceae close to *Scrophularia* and *Verbascum*. Thus, the triterpenes found have also been reported from several species of both these genera as well as from some species of *Buddleja* (Tschesche et al., 1980; Klimek et al., 1992; Calis et al., 1993; Yamamoto et al., 1991, 1993; Hartleb and Seifert, 1995; Emam et al., 1996, 1997; Giner et al., 1998, 2000; Tatli et al., 2004; Arrif et al., 2006). With regard to the iridoids, most of those found in *O. wattii*, namely harpagide (**3**), harpagoside (**3a**), aucubin (**4**), catalpol (**5**), methylcatalpol (**5a**) and scorodioside (**6a**) have also been found in *S. scorodonia* and several other species (De Santos Galindez et al., 2001). With little variation, almost the same iridoid profile has been reported from *V. thapsus* L. (Warashina et al., 1991) and other species of that genus. Thus, *Oreosolen* is chemically very similar to *Scrophularia* and *Verbascum* as it is also indicated by a phylogenetic analysis based on combined DNA sequence data from

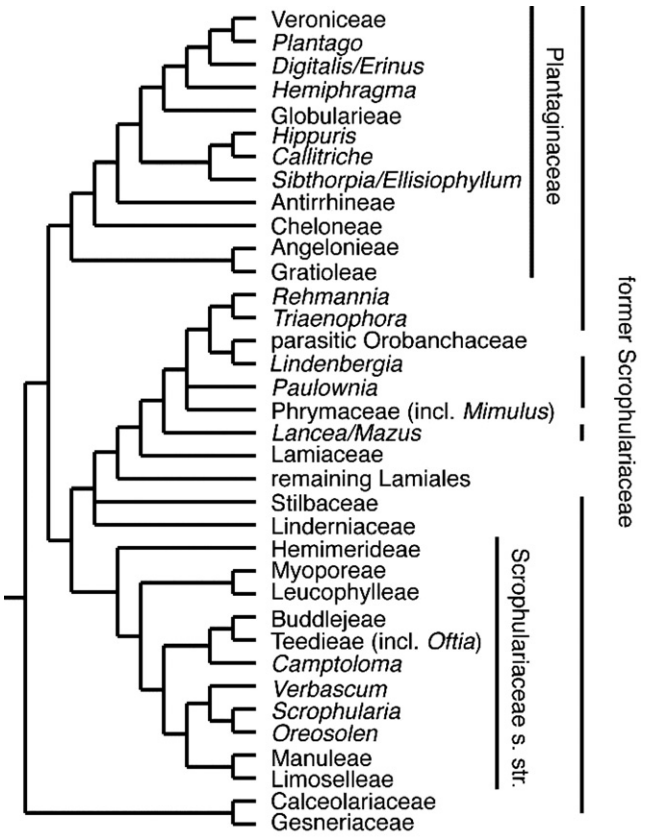


Fig. 1. Phylogenetic relationships among former Scrophulariaceae and Lamiales based on DNA sequences from the ITS region, the *trnL-trnL-trnF*-regions and the *rps16* intron. Data from Albach et al. (2005), Oxelman et al. (2005) and unpublished results.

Albach et al. (2005), Oxelman et al. (2005) and Albach et al. (in preparation) (see Fig. 1). In conclusion (1) *Triaenophora* and *Oreosolen* are not related to Veroniceae and (2) the chemical results for both species investigated in this work are remarkably congruent with the phylogenetic results.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova-500 MHz in CD_3OD using the solvent peak as the internal standard. Dry plant material was extracted by blending in EtOH. The mixture was brought to the boiling point and then left to stand at room temperature for some days. After filtering, the extract was taken to dryness and partitioned in H_2O–Et_2O (25 ml each); the aqueous layer was concentrated to give the crude extract, which was chromatographed using a Merck Lobar RP-18 column size B. The initial eluent was H_2O followed by H_2O:MeOH mixtures (15:1 to 4:5) and finally by MeOH. The content of sugars in the polar fraction was estimated by the intensity of the signals in ^{13}C NMR spectrum. LC-HR ESIMS was performed on an Agilent HP 1100 Liquid Chromatograph equipped with a BDS-C18 reversed phase column running a water–acetonitrile (50 ppm TFA in water) gradient. The LC was coupled to a LCT of a TOF MS (Micromass, Manchester, UK) operated in the positive electrospray ion mode using 5-leucineenkephalin as lock mass. The isolated compounds were identified by their ^{1}H and ^{13}C NMR spectra by comparison with spectra of known standards: **1**, **2**, **4**, **5**, **5a** and **7** (Taskova et al., 2006); ajugol esters **2a** and **2b** (Nishimura et al., 1989); **3** and **3a** (Chaudhuri et al., 1980); scorodioside (**6a**) (Fernandez et al., 1995).

3.2. Plant material

Material of *T. rupestris* was collected by HL in Jianshi, Hubei, China in July 2006 (voucher: Hongqing LI 2006998) and in Xingshan, Hubei, China in September 2007 (voucher: Hongqing LI 2007901). *O. wattii* was collected by Yu-Hu Wu in Zaduo County, Qinghai, China in Aug. 2005 (voucher: Wuyuhu-001). Vouchers are deposited in Herbarium of East China Normal University (HSNU).

3.2.1. Triaenophora rupestris

Dry leaf material from Jianshi (55 g) in EtOH (250 ml) for 7 days gave the crude extract (4.73 g) which upon chromatography gave the sugar fraction (1.2 g; mainly sucrose and glucose with ca. 20% of mannitol), followed by catalpol (**3**; 1.43 g), ajugol (**2**; 260 mg), a fraction with mainly descaffeoylverbascoside (30 mg), epiloganic acid (**1**; 45 mg), verbascoside (**7**; 550 mg), isoacteoside (130 mg) and a fraction with mainly 6-feruloyl ajugol (**2a**; 80 mg). Rechromatography of the latter gave pure **2a**.

3.2.2. Triaenophora rupestris

Dry leaves and stem material from Xingshan (26 g) in EtOH (150 ml) for 10 days gave the crude extract (0.6 g) which upon chromatography gave the sugar fraction (190 mg), followed by catalpol (**3**; 200 mg), ajugol (**2**; 50 mg), verbascoside (**7**; 40 mg), and a fraction with a mixture of 6-feruloyl ajugol and 6-(p-coumaroyl) ajugol (**2a** and **2b**; 15 mg). Rechromatography of the latter gave pure **2b**.

3.2.3. Oreosolen wattii

Dry leaves and stems (36 g) in EtOH (250 ml) for 11 days gave the crude extract (2.0 g) which was chromatographed to give the sugar fraction (410 mg; mainly sucrose and glucose), followed by catalpol (**5**; 80 mg), a 3:2:1 mixture of 3,4-dihydroaucubin, aucubin and 6-hydroxyantirrhinoside (40 mg), pure aucubin (**4**; 320 mg), harpagide (**3**; 180 mg), methylcatalpol (**5a**; 10 mg), a fraction with mainly verbascoside (**7**; 10 mg), a fraction (80 mg) with acylated rhamnopyranosylcatalpol mixture (including some **6b**) which was not further separated and finally harpagoside (**3a**, 20 mg).

3.2.4. Oreosolen wattii

Dry fruits (18 g) in EtOH (100 ml) for 10 days gave the crude extract (1.8 g) which was chromatographed to give the sugar fraction (370 mg; mainly sucrose and glucose), followed by catalpol (**5**; 10 mg), 3,4-dihydroaucubin (8 mg), aucubin (**4**; 110 mg), harpagide (**3**; 30 mg), methylcatalpol (**5a**; 70 mg), a fraction A (150 mg) and scorodioside (**6a**, 6-*O*-(3-acetyl-2-cinnamoyl-rhamnopyranosyl)-catalpol; 50 mg).

A part (90 mg) of fraction A was rechromatographed to give oreosolenoside (6-*O*-(3″-acetyl-2″-*p*-(E/Z)-coumaroyl-rhamnopyranosyl)-catalpol; **6b**; 50 mg).

3.3. Oreosolenoside (*6b*)

Amorphous solid: LC-HR ESIMS *m/z*: 714.2636 $[M+NH_4]^+$; ($C_{32}H_{44}NO_{17}$ requires 714.2609); ^{1}H and ^{13}C NMR data in Table 1.

Table 1
NMR data (CD_3OD) for oreosolenoside (**6b**) and the model scorodioside **6a**

Atom	Oreosolenoside (**6b**)				Scorodioside (**6a**)	
	^1H		^{13}C		^1H	^{13}C
Isomer	*E*	*Z*	*E*	*Z*	*E*	*E*
Agluc						
1	5.12 (obsc.)		95.1		5.10 (*d*, 9.5)	95.1
3	6.41 (obsc.)		142.3		6.39 (*dd*, 6.0; 1.6)	142.4
4	5.10 (obsc.)		103.3		5.11 (*dd*, 6; 4)	103.3
5	2.51 (*m*)		37.1		2.48 (*m*)	37.2
6	4.09 (*br. d*, 8.7)	4.07 (*br. d*, 8.9)	84.3		4.05 (*br. d*, 8)	84.3
7	3.70 (*br. s*)	3.69 (*br. s*)	59.4		3.67 (*br. s*)	59.3
8			66.5			66.5
9	2.61 (*t*-like, 8.5)		43.2		2.58 (*dd*, 9.5; 7.8)	43.2
10	4.18 (*d*, 13.1)		61.4		4.15 (*d*, 13.2)	61.4
	3.84 (*d*, 13.1)				3.81 (*d*, 13.2)	
Glc						
1′	4.81 (*d*, 7.9)		99.6		4.78 (*d*, 7.9)	99.7
2′	3.27 (obsc.)		74.7		3.26 (obsc.)	74.8
3′	3.44 (*t*, 9.0)		77.6		3.40 (*t*, 8.9)	77.6
4′	3.30 (obsc.)		71.6		3.26 (obsc.)	71.8
5′	3.30 (obsc.)		78.5		3.27 (obsc.)	78.6
6′	3.95 (br. *d*, 11.7)		62.8		3.91 (*dd*, 12.3; 1.6)	62.9
	3.65 (obsc.)					
Rha						
1″	5.08 (br. *s*)	5.04 (br. *s*)	97.7		5.07 (br. *s*)	97.7
2″	5.39 (br. *s*)	5.35 (br. *s*)	71.2		5.37 (*dd*, 3; 1.5)	71.1
3″	5.15 (obsc.)		73.1		5.15 (*dd*,10.1; 3)	73.2
4″	3.65 (obsc.)		71.6		3.63 (obsc.)	71.3
5″	3.89 (obsc.)		70.2		3.87 (obsc.)	70.2
6”	1.38 (*d*, 6.1)	1.32 (*d*, 6.2)	18.0		1.35 (*d*, 6.2)	17.9
Aroyl						
1‴			127.0	127.5		135.5
2‴/6‴	7.71 (2H; *d*, 8.5)	7.52 (2H; *d*, 8.5)	131.4	133.8	7.64 (*m*, 2H)	129.4
3‴/5‴	6.86 (2H; *d*, 8.5)	6.80 (2H; *d*, 8.5)	116.9	115.9	7.43 (*m*, 3H)	130.1
4‴			161.4	161.1	7.43 (*m*, H)	131.8
β	7.70 (*d*, 16.0)	6.98 (*d*, 12.8)	147.6	147.3	7.75 (*d*, 16.0)	147.3
α	6.42 (*d*, 16.0)	5.88 (*d*, 12.8)	114.3	115.7	6.60 (*d*, 16.0)	118.1
CO			168.1	167.0		167.5
Acetyl						
CO			172.3			172.2
Me	2.05	2.03	20.9		2.02	20.9

Acknowledgements

We thank Yu-Hu Wu (Institute of Tibetan Plateau Research, Chinese Acadmy of Sciences) for providing samples, and Kristian Fog Nielsen (BioCentrum, The Technical University of Denmark) for recording the mass spectrum. The study was supported by National Science Foundation of China (Project No. 30770136 to Hong-Qing Li).

References

Albach, D.C., Soltis, P.S., Soltis, D.E., Olmstead, R.G., 2001. Phylogenetic analysis of asterids based on sequences of four genes. Ann. Missouri Bot. Gard. 88, 163–210.

Albach, D.C., Meudt, H.M., Oxelman, B., 2005. Piecing together the “new” Plantaginaceae. Am. J. Bot. 92, 297–315.

Albach, D.C., Li, H.Q., Zhao, N., Jensen, S.R., 2007. Molecular systematics and phytochemistry of *Rehmannia* (Scrophulariaceae). Biochem. Syst. Ecol. 35, 293–300.

Arrif, S., Benkhaled, M., Long, C., Lavaud, C., David, B., 2006. Glycosylated iridoids and a triterpene saponin from *Verbascum ballii* (Batt.). M. Qaiser. Biochem. Syst. Ecol. 34, 259–262.

Bentham, G., 1846. Scrophulariaceae. In: de Candolle, A.P. (Ed.), Prodromus Systematis Naturalis Regni Vegetabilis, vol. 10. V. Masson, Paris, pp. 180–586.

Calis, I., Zor, M., Basaran, A.A., Wright, A.D., Sticher, O., 1993. Ilwensisaponins A, B, C, and D: triterpene saponins from *Scrophularia ilwensis*. Helv. Chim. Acta 76, 1352–1360.

Chaudhuri, R.K., Afifi-Yazar, F.U., Sticher, O., Winkler, T., 1980. Carbon-13 NMR spectroscopy of naturally occurring iridoid glucosides and their acylated derivatives. Tetrahedron 36, 2317–2326.

De Santos Galindez, J., Fernandez Matellano, L., Diaz Lanza, A.M., 2001. Iridoids from *Scrophularia* genus. Z. Naturforsch. 56c, 513–520.

Emam, A.M., Moussa, A.M., Faure, R., Favel, A., Delmas, F., Elias, R., Balansard, G., 1996. Isolation and biological study of a triterpenoid saponin, mimengoside A, from the leaves of *Buddleja madagascariensis*. Planta Med. 62, 92–93.

Emam, A.M., Diaz-Lanza, A.M., Matellano-Fernandez, L., Faure, R., Moussa, A.M., Balansard, G., 1997. Biological activities of buddlejasaponin isolated from *Buddleja madagascariensis* and *Scrophularia scorodonia*. Pharmazie 52, 76–77.

Fernandez, L., Diaz, A.M., Ollivier, E., Faure, R., Balansard, G., 1995. An iridoid diglycoside isolated from *Scrophularia scorodonia*. Phytochemistry 40, 1569–1571.

Giner, R.M., Villalba, M.L., Del Carmen Recio, M., Mainez, S., Gray, A.I., Rios, J.L., 1998. A new iridoid from *Scrophularia auriculata* ssp. *pseudoauriculata*. J. Nat. Prod. 61, 1162–1163.

Giner, R.M., Villalba, M.L., Recio, M.C., Manez, S., Cerda-Nicolas, M., Rios, J., 2000. Anti-inflammatory glycoterpenoids from *Scrophularia auriculata*. Europ. J. Pharm. 389, 243–252.

Hallier, E., 1903. Über Abgrenzung und Verwandtschaft der Sippen der Scrophulariaceen. Bull. Herb. Boiss. 3, 181–207.

Hartleb, I., Seifert, K., 1995. Triterpenoid saponins from *Verbascum songaricum*. Phytochemistry 38, 221–224.

Hong, D.Y., Yang, H., Jin, C.L., Holmgren, N.H., 1998. Scrophulariaceae. In: Wu, Z.Y., Raven, P.H. (Eds.), Flora of China. Science Press, Beijing, pp. 1–212.

Jensen, S.R., 1996. Phenylethanoid glycosides in *Sanango racemosa* and in the family Gesneriaceae. Phytochemistry 43, 777–783.

Klimek, B., Lavaud, C., Massiot, G., 1992. Saponins from *Verbascum nigrum*. Phytochemistry 31, 4368–4370.

Li, H.L., 1948. A revision of the genus *Rehmannia*. Taiwania 1, 71–82. Plates I–V.

Miyase, T., Mimatsu, A., 1999. Acylated iridoid and phenylethanoid glycosides from the aerial parts of *Scrophularia nodosa*. J. Nat. Prod. 62, 1079–1084.

Nishimura, H., Sasaki, H., Morota, T., Chin, M., Mitsuhashi, H., 1989. Chemical and biological studies on Rehmanniae radix. Part 3. Six iridoid glycosides from *Rehmannia glutinosa*. Phytochemistry 28, 2705–2709.

Oxelman, B., Kornhall, P., Olmstead, R.G., Bremer, B., 2005. Further disintegration of Scrophulariaceae. Taxon 54, 411–425.

Smith, J.F., Brown, K.D., Carroll, C.L., Denton, D.S., 1997. Familial Placement of *Cyrtandromoea, Titanotrichum* and *Sanango*, three problematic genera of the Lamiales. Taxon 46, 65–74.

Solereder, H., 1909. Über die Gattung *Rehmannia*. Ber. Deutsch. Bot. Ges. 27, 390–404.

Taskova, R.M., Gotfredsen, C.H., Jensen, S.R., 2006. Chemotaxonomy of Veroniceae and its allies in the Plantaginaceae. Phytochemistry 67, 286–301.

Tatli, I.I., Akdemir, Z.S., Bedir, E., Khan, I.A., 2004. Saponin, iridoid, phenylethanoid and monoterpene glycosides from *Verbascum pterocalycinum* var. *mutense*. Turk. J. Chem. 28, 111–122.

Tschesche, R., Sepulveda, S., Braun, T.M., 1980. Triterpenes. XXXIII. Saponin of *Verbascum phlomoides* blossoms. Chem. Ber. 113, 1754–1760.

von Wettstein, R., 1891. Scrophulariaceae. In: Engler, A., Prantl, K. (Eds.), Die natürlichen Pflanzenfamilien IV, vol. 3b. Engelmann, Leipzig, pp. 39–107.

Warashina, T., Miyase, T., Ueno, A., 1991. Iridoid glycosides from *Verbascum thapsus* L. Chem. Pharm. Bull. 39, 3261–3264.

Yamamoto, A., Miyase, T., Ueno, A., Maeda, T., 1991. Buddlejasaponins I-IV, four new oleanane-triterpene saponins from the aerial parts of *Buddleja japonica* Hemsl. Chem. Pharm. Bull. 39, 2764–2766.

Yamamoto, A., Miyase, T., Ueno, A., Maeda, T., 1993. Scrophulasaponins II-IV, new saikosaponin homologs from *Scrophularia kakudensis* Franch. Chem. Pharm. Bull. 41, 1780–1783.

Yu, G., Li, X., Wang, Y., Liu, Y., Yang, C., 1996. Spermicidal saponins from *Oreosolen wattii*. Yunnan Zhiwu Yanjiu 18, 229–233.