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CHEMOTAXONOMIC STUDIES OF RUBIACEOUS PLANTS CONTAINING IRIDOID GLYCOSIDES*†

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Key Word Index—Chemotaxonomy; iridoid glycosides; secoiridoid glycosides; Rubiaceae; GC; GC-MS.

Abstract—Thirty-five species of rubiaceous plants have been examined for their iridoids by gas chromatography and/or gas chromatography-mass spectrometry and in some cases by isolation of the glycosides. The results, combined with data from other studies, suggest that these plants can be classified into three groups: (i) subfamily Ixoroideae, members of which contain gardenoside, geniposide and ixoroside; (ii) subfamily Rubioideae, all of which contain asperuloside and/or deacetylasperulosidic acid; (iii) subfamilies Cinchonoideae and Antirheoideae which contain loganin, secoiridoids, and/or indole alkaloids biosynthesized via the latter two glucosides. From this chemotaxonomic point of view, some doubt is thrown on the taxonomic position of *Wendlandia formosana* and plants of *Mussaenda*, two taxa currently placed in the Cinchonoideae but chemically allied to Ixoroideae.

INTRODUCTION

Although studies of iridoid glycosides of the Rubiaceae have a long history, their distribution in this family was not studied before Briggs and Nicholls in 1960 [1] and there was no systematic work until the late 1960's. Only in 1969, Kooiman described the distribution of 'asperulosidic glycosides' in this family [2]. From data mainly obtained by himself and Briggs and Nicholls [1], he found that (i) asperuloside (**1**) and '*Galium* glycosides' occur in almost all the plants of the subfamily Rubioideae, but are not present in other subfamilies; (ii) *Gardenia* glycosides are detectable in some plants of the tribe Gardenieae of the subfamily Ixoroideae, but not in other tribes of the same subfamily and (iii) these three types of glycosides are not found in the other subfamilies such as Cinchonoideae, Urophylloideae (now included in Cinchonoideae), Pomazotoideae (now included in Rubioideae), Guettaroideae (correct name Antirheoideae) and Hillioideae (now included in Cinchonoideae). Based on these findings, he recognized that the distribution of these glucosides corresponds with the classification of Bremekamp [3] with a few exceptions and cited this system as the most reliable. Since then, the classification of Rubiaceae has been improved [4–9]. Furthermore, setting aside the classification Kooiman took, some defects are noticeable in his work from the present point of view. For example, the definition 'asperulosidic glycoside' [asperuloside (**1**), *Galium* glucosides and *Gardenia* glucosides] is obscure. These glucosides can now be more precisely defined, i.e. '*Galium* glucoside' is deacetylasperulosidic acid (**2**) and '*Gardenia* glucoside' is gardenoside (**3**). However, the plants of both genera *Galium* and *Gardenia* are known to contain several other iridoid congeners [10–12]. Kooiman examined plant extracts by paper chromatography using the acidic *p*-anisidine phosphate reagent. In addition, he tested extracts with the Trim and Hill reaction [13], which is similar to hydrochloride reaction used by Briggs and Nicholls [1]. But, it is well known that the former reagent is not specific for iridoid glycosides and the latter reaction is only specific for certain iridoid glycosides such as asperuloside (**1**). Regarding plants belonging to the subfamilies other than Rubioideae and Ixoroideae, Kooiman mentioned the presence of iridoid-related monoterpenoid alkaloids in some plants of the subfamilies Cinchonoideae and Hillioideae (now Cinchonoideae) and also the presence of loganin (**4**) in a few plants. He did not relate these structures to biosynthesis because the biosynthetic pathway of iridoids had not been elucidated at that time. This paper is a chemotaxonomic discussion* on rubiaceous plants based on the results of examination of iridoid

*Dedicated to the Memory of Dr T. Swain.

†Part 61 in the series 'Studies on Monoterpene Glucosides and Related Natural Products'. For Part 60 see Kuwajima, H., Uemura, T., Takaishi, K., Inoue, K. and Inouye, H. (1988) *Phytochemistry* **27** (in press).

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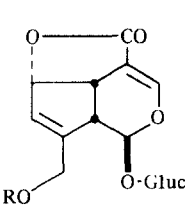
*In this contribution we discuss the taxonomy according to the latest classification system improved by Petit [4], Verdcourt [5] (delimitation of Psychotrieae and Morindeae), Ridsdale [6] (delimitation and subdivision of Naucleeae), Puff [7] (delimitation of Anthospermeae and Paederieae), Robbrecht and Puff [8] (survey of Gardenieae and related tribes) and Robbrecht [9] (new subfamilial classification).



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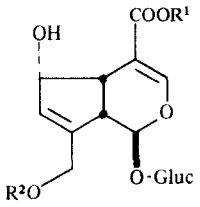

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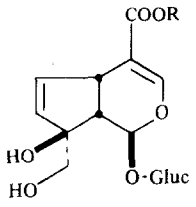
33 R^1 = Me, R^2 = caffeoyl
36 R^1 = H, R^2 = Ac



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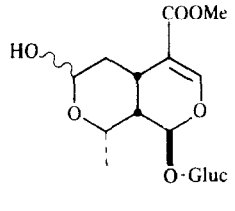

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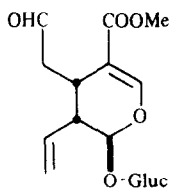

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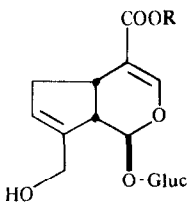

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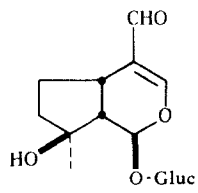

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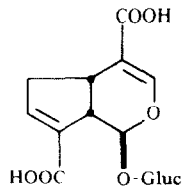

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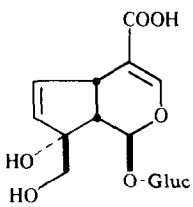

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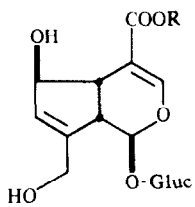

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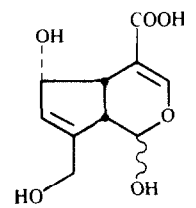

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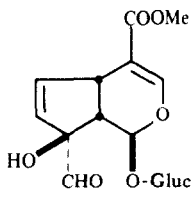

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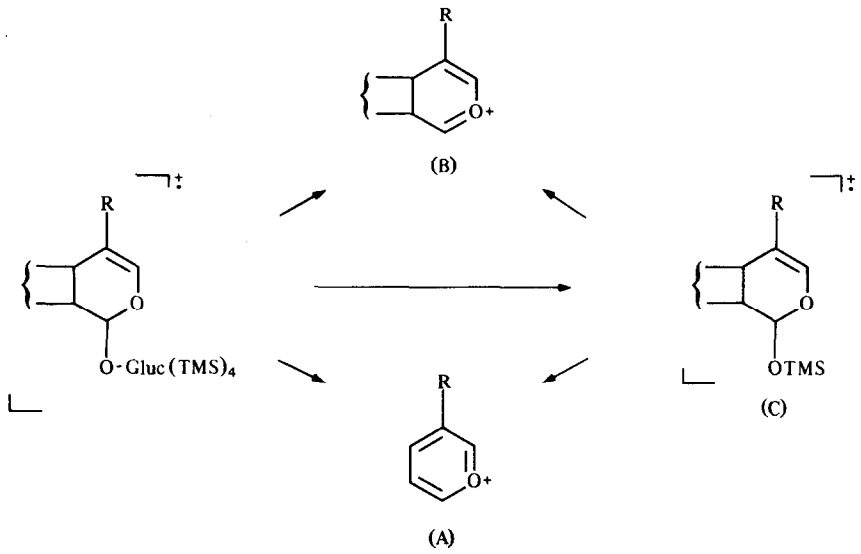
glycosides in many plants of this family by using TLC, GC and GC-MS and in some cases by isolation. The results obtained by us and by other groups in recent years are also considered.

RESULTS AND DISCUSSION

Iridoid series glycosides of 35 plants were examined by means of TLC, GC and GC-MS. Tested plants included three species of the tribe Gardenieae, five of Pavetteae, one of Coffeeae (subfamily Ixoroideae); one of Guettardeae (Antirheoideae); one of Cinchoneae, two of Rondeletieae (Subfamily Cinchonoideae); one of Ophiorrhizeae, two of Hedyotideae, ten of Morindeae, three of Psychotrieae, two of Paederieae and four of Rubieae (subfamily Rubioideae).

Plant extracts were purified through carbon column chromatography and subjected to TLC, GC and GC-MS. GC and GC-MS were performed according to our previous method [14] after trimethylsilylation of the glycosides. Almost all iridoid and secoiridoid glycosides show the characteristic mass fragmentation pattern shown in Fig. 1 [14]. In this contribution we especially used the characteristic ion peak A as the index. Asperuloside (**1**) was identified only through TLC and GC, because it is unstable and decomposes during the GC-MS procedure. The results from the above experiments are shown in Table 1.

For seven species which were available in a relatively large quantity, isolation of iridoids was further attempted. The results obtained in our laboratories and other studies are summarized in Table 2. Comparison of the data shown in Tables 1 and 2 clearly indicates the sensitivity and reliability of the above methods. For example, while only one and two iridoids were isolated from *Ophiorrhiza japonica* and *Damnacanthus major*, two and three iridoids respectively were detected in them by means of GC-MS. Furthermore, while no glycoside could be isolated from *Diplospora dubia* (syn. *Tricalysia dubia*), the presence of one iridoid was shown by GC-MS. Iridoid and secoiridoid glycosides have been detected in all plants so far examined with few exceptions. Only in the case of *Coffea arabica* did we fail completely to find iridoids, in spite of several efforts. *Rondeletia odorata* was also nega-



(A1) R = COOMe *m/z* 139
(A2) R = H *m/z* 81
(A3) R = COOTMS *m/z* 197

Fig. 1. Fragmentation pattern of iridoid glucosides

tive, but we need to check this further. Since Jensen and Nielsen found that morroniside (**5**) is detectable only in *Sambucus nigra* young shoots [15], there is always this possibility in other cases, e.g. in *Coffea arabica*. Using only the so-called 'asperulosidic glycosides' as characters and partly depending on the data obtained by other groups, Kooiman designated many species including those of the subfamily Rubioideae as 'asperulosidic glycosides'-negative and he ascribed some of the negative results to the poor quality of the voucher specimens. However, on the basis of the results shown in Tables 1 and 2, it seems that iridoids are present in almost all species and subfamilies of Rubiaceae.

Recently, we found that tarennoside (**6**) and gardenoside (**3**) of cell suspension cultures of *Gardenia jasminoides* are biosynthesized via epiiridodial (**7**) and boschnaloside (**8**) [16, 17], whereas secologanin (**9**) of *Lonicera tatarica* (Caprifoliaceae), several indole alkaloids of *Catharanthus roseus* and cell cultures of *Rauwolfia serpentina* (Apocynaceae) [18] as well as asperuloside (**1**) and some other glucosides of *Galium mollugo* and *G. spurium* are formed via iridodial (**10**) and deoxyloganic acid (**11**), respectively [19]. Jensen *et al.* also verified that asperuloside (**1**) of *Theligonum cynocrambe* (Theligonaceae, syn. Cynocrambaceae; best placed in Rubiaceae subfamily Rubioideae tribe Theligoneae [7, 20], but sometimes considered as a monotypic family near Rubiaceae) is biosynthesized via deoxyloganic acid (**11**) [21]. In addition, it is known that loganic acid (**12**) or loganin (**4**) is at the branch point in the biosynthetic pathway to secologanin (**9**) and indole alkaloids on the one hand and asperuloside (**1**) on the other [22–25]. From these facts, it can be assumed that the iridoids of the Rubiaceae are formed via the main biosynthetic pathway depicted in Fig. 2.

Using this pathway, we can then examine the types of iridoid produced by each subfamily. As regards the subfamily Ixoroideae, which is thought to be the most primitive [2, 7, 26], Kooiman described that some plants of the tribe Gardenieae contained 'asperulosidic glycosides', while some species of this tribe and all species of the tribes Ixoreae and Vanguerieae (now Antirheoideae) lacked them. However, the results presented in Tables 1 and 2 show that all the species of Ixoroideae so far examined (except *Coffea arabica*) contain iridoids. Most of the negative results mentioned by Kooiman can be ascribed to the occurrence of glycosides such as geniposide (**13**), ixoroside (**14**) and ixoside (**15**) which are not detectable by hydrochloride or by the Trim and Hill reagent [13]. Although the types of glycoside [27–36] occurring in this subfamily are more diverse than those of glycosides in the subfamily Rubioideae, their structural features are generally in accord with the biosynthetic pathway via epiiridodial (**7**) [16, 17].

In the plants of Rubioideae, almost uniform distribution of asperuloside (**1**) was observed and, even if this glucoside was not detectable, deacetylasperulosidic acid (**2**) was. These glucosides would be formed via iridodial [19]. In many cases, **1** was accompanied by other glycosides, as indicated by fragment ion A in GC-MS experiments. Thus, along with **1**, monotropein (**16**) was isolated from *Damnacanthus major* as well as *Morinda umbellata* and paederoside (**17**), and scandoside (**18**) were found in *Paederia scandens* [37–39].

Co-occurrence of glucosides of tarennoside (**6**)-genipo-

Table 1. Plants examined by GC and GC-MS for asperuloside (1) and iridoid glycosides

Subfamily	Tribe	Species	Asperuloside (1) TLC	Asperuloside (1) GC*†	Iridoid glycosides GC-MS‡§
Ixoroideae	Gardenieae	*Gardenia globosa* Hochst.	–	–	3
		Randia sinensis (Lour.) Schult.	–	–	2
		Diplospora dubia (Lour.) Masamune (syn. *Tricalysia dubia* (Lindl.) Ohwi	–	–	1
	Pavetteae	*Ixora casei* Hance	–	–	1
		I. japonica DC.	–	–	1
		I. odorata Hook.	–	–	2
		I. macrothyrsa Teism.	–	–	1
		Tarenna kotoensis Lam. Kan. et Sas. var. *gyokushinka* (Ohwi) Masamune	–	–	2
	Coffeeae	*Coffea arabica* L.	–	–	–
Antirheoideae	Guettardeae	*Guettarda speciosa* L.	–	–	2
Cinchonoideae	Cinchoneae	*Cinchona succirubra* Pavon ex Klotzsch	–	–	2
	Rondeletieae	*Rondeletia odorata* Jacq.	–	–	–
	Condamineae	*Bikkia* sp.	–	–	2
Rubioideae	Ophiorrhizeae	*Ophiorrhiza japonica* Blume	–	–	3
	Hedyotideae	*Hedyotis biflora* (Lin.) Lam. var. *parviflora* Hooker et Arnot	–	Δ	2
		Pentas lanceolota K. Schum.	+	+	–
	Morindeae	*Lasianthus plagiophyllus* Hance	+	+	–
		L. obliquinervis Merr.	+	+	–
		L. fordii Hance	+	+	–
		L. cyanocarpus Jack	+	+	–
		L. curtisii King et Gamble	–	Δ	–
		Damnacanthus major Sieb. et Zucc.	+	+	3
		D. minutispinus Koidz.	+	+	–
		D. macrophyllus Siebold	+	+	3
		Morinda citrifolia L.	+	+	3
		M. umbellata L.	+	+	–
	Psychotrieae	*Psychotria serpens* L.	+	+	1
		P. rubra (Lour) Poir.	+	+	–
		P. manillensis Bartl. ex DC.	+	+	1
	Paederieae	*Serrisa japonica* Thunb.	+	+	3
		Mitchella undulata Sieb. et Zucc.	+	+	2
	Rubieae	*Galium verum* L. var. *asiaticum* Nakai	+	+	4
		G. kikumugura Ohwi	–	–	3
		Rubia cordifolia L. var. *munjista* Miq.	–	Δ	1
		R. tinctorum L.	–	Δ	1

*Same results were obtained on 1.5% OV-1 and 3% OV-17.

†Δ shows the presence of peaks corresponding to deacetylasperulosidic acid (**2**) (on 2m column).

‡The number of substances (=number of peaks on GC) which showed the fragment ion A.

§—Not examined.

side (**13**)-gardenoside type (**3**) and deacetylasperulosidic acid (**2**)-asperuloside (**1**) type are sometimes observed both in Ixoroideae and Rubioideae. Thus geniposide (**13**) and the methyl ester of deacetylasperulosidic acid (**2**) exist in *Gardenia jasminoides* [11], derivatives (**19** and **20**) of **2** and **3** occur in *Randia canthioides* and *R. formosa* [27, 28] and asperuloside (**1**) and gardenosidic acid (**21**) in *Galium mollugo* [10]. In these cases, gardenoside (**3**) type glycosides would be formed via iridodial (**10**) and asperuloside (**1**) type glycosides via epiiridodial (**7**). Gardenosidic acid (**21**) of *G. mollugo* [10] would be formed via iridodial (**10**), deoxyloganic acid (**11**) and geniposidic acid (**22**), since 10-hydroxyloganin (**23**) and secogalioside (**24**) are also produced in this plant. Further, deacetylasperulosidic acid (**2**) of *Gardenia jasminoides* would be formed through epiiridodial (**7**), since this has already demonstrated in cell cultures of this plant [16, 17]. The question whether the main pathway via iridodial (**10**) or epiiridodial (**7**) is characteristic of some tribe or subfamily or both pathways exist together in the same tribe or subfamily remains to be proved.

With regard to subfamilies other than Ixoroideae and Rubioideae, it is well known that plants of Cinchonoideae contain several indole- and *Cinchona* alkaloids, all of which prove to be biosynthesized via secologanin (**9**) [40]. In addition, loganin (**4**), sweroside (**25**) and morroniside (**5**), the biogenetic congeners of **9**, have been isolated from two *Adina* species (tribe Naucleeae) [14]. Furthermore,

Table 2. Iridoids and secoiridoids isolated from rubiaceous plants

Ixoroideae		
Gardenieae		
(Gardeniinae)	*Gardenia jasminoides* Ellis f. *grandiflora Makino* [11, 12]	geniposide (**13**), gardenoside (**3**), shanzhiside (**30**), gardoside (**31**), scandoside methyl ester (**32**), deacetylasperulosidic acid methyl ester, etc.
	Randia canthioides Champ. ex Benth. [27]	10-dehydrogardenosid (**20**), deacetylasperulosidic acid aglucone (**19**)
	R. formosa Schum. [28]	10-caffeoyldeacetyldaphylloside (**33**)
	Genipa americana L. [29, 30]	genipin (**34**), geniposidic acid (**22**), etc.
(Diplosporinae)	*Diplospora dubia* (Lour.) Masamune (syn. *Tricalysia dubia* (Lindl.) Ohwi)*	—
Pavetteae	*Ixora chinensis* Lam. [31]	ixoroside (**14**), ixoside (**15**), geniposidic acid (**22**)
	Tarenna kotoensis Lam. Kan. et Sas. var. *gyokushinka* (Ohwi) Masamune [32]	tarennoside (**6**)
	T. graveolens (S. Moore) Bremek. [33]	ixoside (**15**), ixoside 11-methyl ester, 6-feruoylshanzhiside
Coffeeae	*Coffea arabica* L.*	—
Hypobathreae	*Feretia apodanthera* Del. [34]	gardenoside (**3**), scandoside methyl ester (= feretoside) (**32**)
Antirheoideae		
Vanguerieae	*Canthium subcordatum* DC. [35, 36]	shanzhiside methyl ester (**29**)
Guettardeae	*Guettarda speciosa* L.*	loganic acid (**12**), secologanin (**9**)
Cinchonoideae		
Naucleeae§		
(Naucleinae)		
	Nauclea diderrichii (De Wild.) Merr. [41, 42]	naucledal (**26**), diderroside (**27**)
(Adininae)	*Adina racemosa* (Sieb. et Zucc.) Miq. [14]	loganin (**4**), secologanin (**9**), sweroside (**25**)
	A. pilulifera (Lam.) Franch. ex Drake [14]	morroniside (**5**)
Rondeletieae	*Wendlandia formosana* Cowan [43]	tarennoside (**6**), gardenoside (**3**) geniposidic acid (**22**)
Isertieae	*Mussaenda parviflora* Miq. [44]	mussaenoside (**28**), shanzhiside methyl ester (**29**)
	M. shikokiana Makino [44]	mussaenoside (**28**), shanzhiside methyl ester (**29**)
	M. arcuata Poir. [33]	shanzhiside methyl ester (**29**), 8-acetylshanzhiside methyl ester shanzhiside methyl ester gentiobioside
Rubioideae		
Ophiorrhizeae§	*Ophiorrhiza japonica* Blume*	sweroside (**25**)
Hedyotideae	*Hedyotis diffusa* Willd. [45]	asperuloside (**1**), 6-*O*-*p*-coumaroylscandoside methyl ester
Morindeae	*Damnacanthus major* Sieb. et Zucc.*	asperuloside (**1**), monotropein (**16**)
	Morinda umbellata L.*	asperuloside (**1**), monotropein (**16**)
	M. citrifolia L.*	asperuloside (**1**), deacetylasperulosidic acid (**2**)
	M. lucida Benth. [46]‡	oruwacin (**35**)
Rubieae	*Galium mollugo* L. [10]	asperuloside (**1**), gardenosidic acid (**21**), monotropein (**16**), secogalioside (**24**), etc.
	G. verum L. var. *asiaticum* Nakai [47, 48]	v_1, v_2, v_3 iridoids (asperuloside (**1**) derivatives)
	Rubia tinctorum L. [49]	asperulosidic acid (**36**), deacetylasperulosidic acid (**2**)
	R. peregrina L. [49]	asperulosidic acid (**36**), deacetylasperulosidic acid (**2**)
Paederieae	*Paederia scandens* (Lour.) Merr.[37, 38]	asperuloside (**1**), paederoside (**17**), scandoside (**18**)
Anthospermeae	*Coprosma* spp. [1]	asperuloside (**1**)
Theligoneae	*Theligonum cynocrambe* L. [21]	asperuloside (**1**)

* Results of the present work.

§ Otherwise, several species of Naucleeae and Ophiorrhizeae are known to contain alkaloidal glycosides.

‡ Only the petrol extract of leaves were examined.

— Iridoids were not isolated.

naucledal (**26**), a modified aglucone of sweroside (**25**), and diderroside (**27**) have also been found from *Nauclea diderrichii* (tribe Naucleeae) [41, 42]. On the other hand, we have already isolated some glucosides characteristic of species of Ixoroideae such as gardenoside (**3**), tarennoside (**6**) and geniposidic acid (**22**) from *Wendlandia formosana* (tribe Rondeletieae) [43] which has been placed in the subfamily Cinchonoideae following Bremekamp's proposal [3]. According to the above arguments, this finding, however, seems to be very peculiar and would suggest the necessity of changing the taxonomic position of *Wendlandia*. The genus is unusual in the Rondeletieae in that it is confined to the Old World and centred in southeastern Asia, whereas the major part of the tribe occurs in the

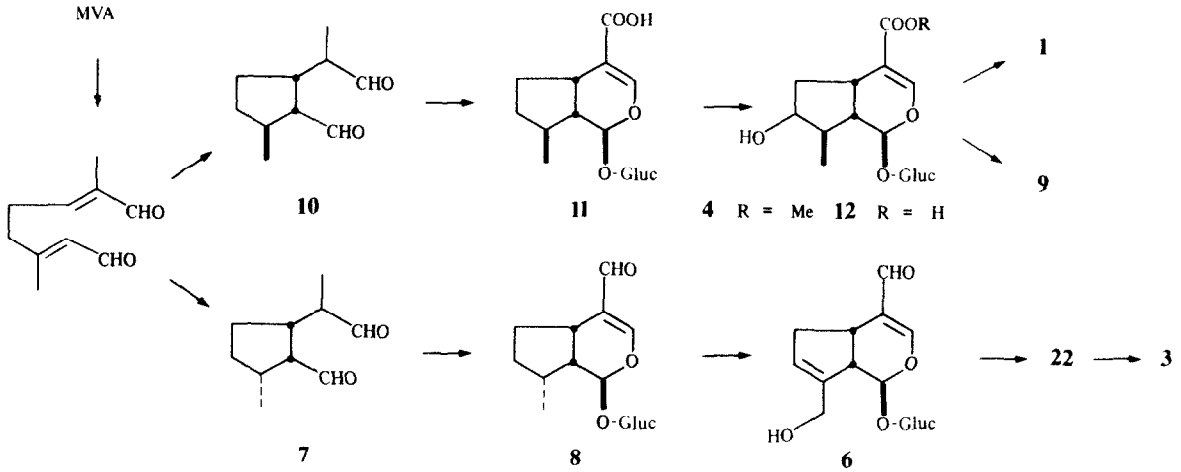
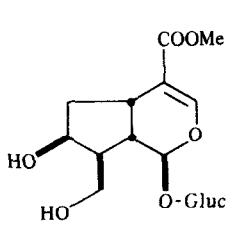


Fig. 2. Biosynthetic pathway of rubiaceous iridoid glycosides



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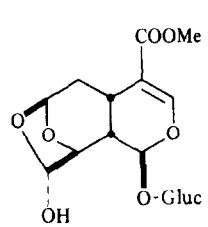
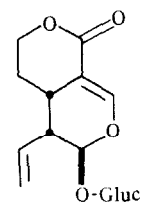


24



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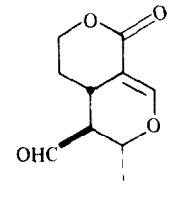
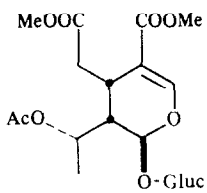


26



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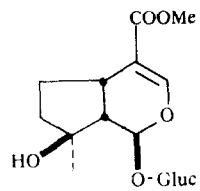

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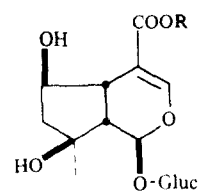
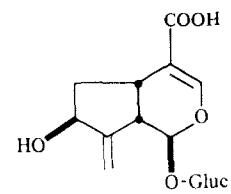


29 R = Me
30 R = H



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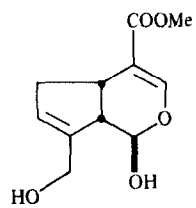
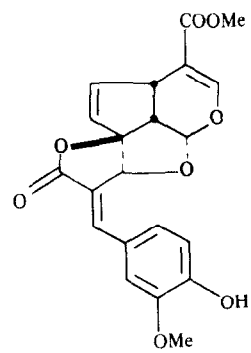


34



35

New World; various morphological features (capsular fruits, etc.), however, make a position in the Ixoroideae seem highly unlikely but do not preclude a very distant relationship. It was also found that mussaenoside (**28**) and the methyl ester (**29**) of shanzhiside (**30**), a glucoside occurring in *Gardenia* plants, occur in plants of the genus *Mussaenda* (tribe Isertieae) [33, 44] which is also referred to the subfamily Cinchonoideae. These findings may either indicate the diversity of the glycosides in taxa of this subfamily or may point to a distant relationship to the Gardenieae s.l. (morphological links between the Isertieae and Gardenieae s.l. have been presumed by various authors; of the two taxa, the Isertieae are considered to be the more derived group [8]). Isolation of sweroside (**25**) from an *Ophiorrhiza* species (tribe Ophiorrhizeae) is also remarkable since its presence seems to point to a close affinity to the subfamily Cinchonoideae. The Ophiorrhizeae are nowadays either associated with or included in the tribe Hedyotideae. Some elements of the Hedyotideae, in turn, are considered to form a link between the subfamilies Rubioideae and Cinchonoideae [9]; our findings are thus not in grave contradiction to the proposed position of the genus/tribe. The occurrence of loganin (**4**) and secologanin (**9**) in *Guettarda speciosa* (tribe Guettardeae, subfamily Antirheoidea) would suggest a position of the subfamily closer to the Cinchonoideae than to the Ixoroideae and Rubioideae; there, however, does not seem to be much evidence from other disciplines to support this.

EXPERIMENTAL

Plant materials. All the plants described below were collected by us and identified by Mr G. Murata (Faculty of Science, Kyoto University): *Lasianthus plagiophyllus, L. obliquinervis, L. fordii, L. cyanocarpus, L. curtisii Damnacanthus minutispinus, Morinda citrifolia* and *M. umbellata*, (collected in Okinawa Island); *Randia sinensis, Diplospora dubia* (syn. *Tricalysia dubia*), *Tarenna kotoensis, Guettarda speciosa, Hedyotis biflora* var. *parvifolia, Morinda citrifolia, Psychotria serpenes, P. rubra* and *P. manillensis* (collected in Ishigaki Island); *Damnacanthus major* (in Fukuoka Pref.); *Ophiorrhiza japonica* (Kagoshima Pref.). Other plants were obtained in the following Botanical Gardens: Kyoto Herbal Garden of Takeda Chemical Industries, Ltd, Kyoto Botanical Garden and Honolulu Botanical Garden.

Examination of iridoids in plant material by TLC, GC and GC-MS. Finely powdered leaves (5–10 g) were extracted with hot H_2O (50–100 ml × 3). The combined extract was subjected directly to a charcoal (5–10 g) column. The column was eluted well with H_2O and then with MeOH (1.1). The methanolic eluate was evapd *in vacuo* to give a powder which was subjected to TLC, GC and GC-MS. TLC was carried out on silica gel G (Merck) using $CHCl_3$–MeOH (7:3) as the eluent. Spots were visualized by exposure to I_2 vapour or by spraying with a mixture of anisaldehyde (0.5 ml), conc. H_2SO_4 (0.5 ml), HOAc (few drops) and EtOH (9 ml) followed by heating. Trimethylsilylation of the above extract was carried out after drying up. A part (*ca* 3 mg) of the dried powder was treated with a mixture (300 μl) consisting of dry pyridine (10 ml), hexamethyldisilazane (2 ml), and trimethylchlorosilane (1 ml) at 80° for 10 min. The reaction mixture was evapd *in vacuo* and the residue was dissolved in $CHCl_3$ (30 μl). An aliquot (2–4 μl) of the solution was injected to GC. GC was run on a Shimadzu Gaschromatograph GC-6AM. Conditions for GC: detector, FID; carrier gas, N_2 at 50 ml/min; column, 2 m × 3.5 mm or 0.5 m × 3.5 mm i.d. packed with 1.5% OV-1 or 3% OV-17 on 80–100 mesh Shimalite WAW/BMCS; column temp., 260° or 240°. For extracts of plants, which were thought to contain asperuloside (**1**) according to the results of TLC, 0.5 m columns were also used. GC-MS was run on a Shimadzu-LKB-9000 GC-MS spectrometer. Conditions for GC-MS; carrier gas, He at 25 ml/min; temperature of ion source, 280°; accelerating voltage, 3.5 kV; ionization voltage, 70 eV; trapping current, 60 μA.

Isolation of loganic acid (**12**) *and secologanin* (**9**) *from* Guettarda speciosa. Dried leaves (400 g) were extracted with hot MeOH (3.6 l × 3). The methanolic extract was treated in the same way as above and the concd aq. extract was chromatographed on charcoal (150 g) with H_2O–EtOH as eluent with increasing EtOH content. The eluate with H_2O–EtOH (7:3) gave on concn *in vacuo* a residue (6.71 g) including loganic acid (**11**). A portion (516 mg) of this residue gave on acetylation [Ac_2O–Py] followed by methylation with ethereal CH_2N_2 and subsequent purification through chromatography on Si gel (20 g) with Et_2O as eluent loganin pentaacetate (83 mg), mp 139–141°, $[\alpha]_D^{25}$ −75° ($CHCl_3$, *c* 1.23) [identified by mmp and ^{1}H NMR]. The eluate with H_2O–EtOH (1:1) gave on concn *in vacuo* a residue (2.55 g). A portion (1.60 g) of this residue was subjected to silica gel (70 g) chromatography with $CHCl_3$–MeOH with increasing MeOH content and the residue of the faster eluate was purified by repeated prep. TLC (silica gel; $CHCl_3$–MeOH (9:1)) to give secologanin dimethyl acetal (33 mg) as an amorphous powder [identified by ^{1}H NMR].

Isolation of sweroside (**25**) *from* Ophiorrhiza japonica. Dried aerial parts of the plant (750 g) were extracted with hot EtOH (10 l × 3), the extract was concd *in vacuo* and the residue was taken in H_2O (total 1.5 l). After washing with AcOEt (0.5 l × 2), the aq. extract was concd *in vacuo* to ca. 0.5 l. and chromatographed on a charcoal (200 g) column with H_2O–EtOH as eluent with increasing EtOH content. The eluate with H_2O–EtOH (3:2) was concd *in vacuo* and the residue (260 mg) was chromatographed on silica gel (30 g) with $CHCl_3$–MeOH as eluent with increasing MeOH content. The residue (126 mg) obtained from the eluate with MeOH–$CHCl_3$ (1:9) was further purified by passing through a charcoal (1.5 g) column to give sweroside (**25**) (109 mg) as an amorphous powder [identified by ^{1}H NMR], which on acetylation gave sweroside tetraacetate, mp 166–170°, $[\alpha]_D^{25}$ −175° ($CHCl_3$; *c* 1.32). [identified by mmp, IR, ^{1}H NMR].

Isolation of asperuloside (**1**) *and monotropein* (**16**) *from* Morinda umbellata. Dried leaves (570 g) were extracted with hot MeOH (7.5 l × 3), the methanolic extract was concd *in vacuo* and the residue was taken in H_2O (total 600 ml). After washing with AcOEt (250 ml × 3), the aq. extract was chromatographed on charcoal (100 g) with H_2O–EtOH as eluent. The eluate (3.27 g) with H_2O–EtOH (4:1) was further chromatographed on Amberlite IRA-410 (OH type, 500 ml) with H_2O–AcOH as eluent. The fractions eluted with 0.6 M AcOH gave monotropein (**16**) (1.15 g), mp 172–174°, $[\alpha]_D^{29}$ −108.1° (H_2O, *c* 1.15) [identified by mmp, IR, ^{1}H NMR]. The H_2O–EtOH (1:1) eluate (4.3 g) from the above charcoal column was chromatographed on silica gel (60 g) with $CHCl_3$–MeOH (9:1) to give asperuloside (**1**) (1.42 g), mp 129–130°, $[\alpha]_D^{29}$ −179.4° (MeOH; *c* 1.09) [identified by mmp, IR, ^{1}H NMR].

Isolation of asperuloside (**1**) *and deacetylasperulosidic acid* (**2**) *from* Morinda citrifolia. Work-up of fresh fruits (300 g) in the same way as above gave rise to deacetylasperulosidic acid (**2**) (100 mg), mp 153–154°, $[\alpha]_D^{29}$ +28.9° (MeOH; *c* 1.09) and asperuloside (**1**) (144 mg). In the same way, dried leaves (700 g) afforded asperuloside (**1**) (1.74 g).

Isolation of asperuloside (**1**) *and monotropein* (**16**) *from* Damnacanthus major. Work-up of dried leaves (200 g) in the same way as above afforded asperuloside (**1**) (316 mg) and monotropein (**16**) (316 mg).

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