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ALKALOIDS OF SOME ASIAN SEDUM SPECIES

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Abstract—The leafy parts of 16 Asian species belonging to the three sections of Sedum were investigated for the presence of alkaloids. Only in seven species of Sedum sect. Sedum were alkaloids found. Sedum bulbiferum, S. japonicum, S. lepidopodium, S. morrisonensis, S. oryzifolium, S. polytrichoides and S. sarmentosum contain 14 pyrrolidine and piperidine alkaloids. Sedum oryzifolium differs significantly from the other species. It contains the typical S. acre alkaloids, sedamine, allosedamine and sedinone, as well as a series of monosubstituted piperidines and pyrrolidines. In addition, two new pyrrolidine alkaloids were detected in S. oryzifolium. i.e. both diastereoisomers of 1-phenyl-2-(2-N-methylpyrrolidyl)-ethanol for which the names pyrrolsedamine and pyrrolallosedamine are proposed (to express their structural relationship with sedamine and allosedamine). In five species of Sedum sect. Aizoon and four species of S. sect. Telephium, no alkaloids could be detected. The results support the hypothesis that in the family Crassulaceae, alkaloids are restricted to species of the 'Acre' lineage.

INTRODUCTION

Most reports of alkaloids in the Crassulaceae (ca. 1500 species) relate to species of Sedum L. Of the odd 60 Sedum species that have been investigated for alkaloids about two-thirds were found to be positive [1-13]. Contradictory results have been reported, however, for Sedum aizoon L., S. album L., S. crassipes Wallich. (= S. asiaticum Clarcke). S. floriferum Praeg., S. hybridum L., S. kamtschaticum Fisch. (= S. pallidum Hort.), S. lydium Boiss., S. middendorffianum Maxim., S. rupestre L., S. spurium Bieb., and S. telephium L. s.l. [4, 7-12].

Of all Sedum species investigated for the presence of alkaloids, S. acre L. has received most attention and some 25 piperidine alkaloids have been reported from this species alone [5, 6, 12]. The major alkaloids of S. acre are the two 2-monosubstituted piperidine alkaloids, sedridine and sedamine, and the three 2,6-disubstitued N-methyl piperidines, sedacrine, sednine and sedinone. In addition, a number of minor alkaloids, 2-monosubstituted as well as 2,6-disubstituted, has been recorded. The alkaloids reported for the other species of Sedum include some 23 compounds, i.e. pelletierine, sedridine, 2-(piperidyl)-butan-2-one/ol and 2-(piperidyl)-pentan-2one, as well as their N-methyl derivatives, (nor)hygrine, (nor)hygroline, 2-(piperidyl)-pentan-2-ol, (nor)sedaminon, (nor)sedamine, sedacrine, sedinine, sedinone and nicotine, some of which have also been reported for S. acre [2, 8-13].

In a recent survey of the distribution of alkaloids in 35 Sedoideae, the presence of alkaloids proved to be restricted to species of Sedum sect. Sedum, which belong to the 'Acre' lineage in a phylogeny of the Crassulaceae based on chloroplast DNA restriction site analysis [12–15]. Morphologically, the species of this lineage are characterized by a reticulate or reticulo-papillose testa, except for S. litoreum Guss, which has a bipapillate testa [14-16]. Not surprisingly, all controversial alkaloid reports relate to species with costate seeds, which do not belong to the 'Acre' lineage. They belong to Sedum sect. Aizoon Koch (S. aizoon, S. floriferum, S. hybridum, S. kamtschaticum, S. middendorffianum), S. sect. Rhodiola (S. crassipes), S. sect. Spathulata (Boriss.) H. Ohba (S. spurium), S. sect. Telephium S.F. Gray (S. telephium s.l.), and S. sect. Sedum (S. album, S. lydium, S. rupestre). The latter three species, however, appeared to be completely devoid of alkaloids on re-examination [12, 15].

The genus Sedum s.l. in Eastern Asia comprises ca. 200 species which are generally arranged in four sections, i.e. S. sect. Rhodiola (L.) Scop. (\equiv Rhodiola L.), S. sect. Telephium (\equiv Hylotelephium H. Ohba), S. sect. Aizoon Koch and S. sect. Sedum [17]. The species of S. sect. Rhodiola, S. sect. Telephium and S. sect. Aizoon are characterized by costate or bipapillate seeds, but many species of S. sect. Sedum have a reticulate or a reticulopapillose testa [16, 18-20]. Two species of the latter group, S. morrisonensis and S. sarmentosum, were shown

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to belong to the 'Acre' lineage [14] and, furthermore, for S. sarmentosum the alkaloids, N-methylpelletierine and N-methylallosedridine, have been reported [2, 21].

In the present study, we compared the alkaloid composition of 16 Sedum species representing three of the East Asian sections of the genus, i.e. five species of S. sect. Aizoon, four species of S. sect, Telephium and seven species with a reticulo-papillose testa of S. sect. Sedum, to test the validity of our hypothesis on alkaloid distribution in the family Crassulaceae [15].

RESULTS

In our survey of East Asian species of Sedum (Table 1), all seven species of Sedum sect. Sedum were found to contain alkaloids, whereas the five species of S. sect. Aizoon and the four species of S. sect. Telephium alkaloids could not be detected. A total of 14 piperidine and pyrrolidine alkaloids from species of S. sect. Sedum were found (Table 2) and identified by TLC, GC and GC-EI-mass spectrometry comparison with reference compounds (Fig. 1). The 2-monosubstituted piperidine alkaloids, pelletierine, N-methylpelletierine, N-methylsedridine and Nmethylallosedridine, are the major alkaloids of most species of S. sect. Sedum included in the survey. In addition, S. oryzifolium contains significant amounts of the typical S. acre alkaloids, sedamine, allosedamine and sedinone.

A new alkaloid was detected in S. oryzifolium by GC-EI-mass spectrometry. The compound showed a mass spectrum virtually identical to that of norsedamine and its diastereoisomer ($[M]^+$ at m/z 205 and a base peak at m/z 84 representing the heterocyclic ring) but it had a significantly lower retention time than the latter piperidines. As the 2-monosubstituted N-methylpyrrolidines elute earlier from an apolar GC column than their piperidine isomers [12, 13], the unknown alkaloid was tentatively identified as the pyrrolidine analogue of (allo)sedamine, viz., 1-phenyl-2-(2-N-methylpyrrolidyl)ethanol. The structure was confirmed by GC-comparison of the extracts with synthetic 1-phenyl-2-(2-N-methylpyrrolidyl)-ethanol prepared from pyrrolidine and benzoyl acetate following the general procedure for the synthesis of 2-monosubstituted piperidines [22].

The synthetic pyrrolidine was separated by column chromatography on silica gel into its diastereoisomeric

Table	1.	Origin	and	accession	number	of	Sedum	plants	studied
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Taxon	Origin	Accession number
Sedum sect. Sedum		
S. bulbiferum Makino	Korea: Taejeon; Yooseong	32768
	Korea: prov. Cheonnam; Mt. Paegyang	32769
	Korea Jeju Isl., Seongsan	32795
S. japonicum Siebold	Japan: Honshu, Fukui pref.; Tohjibo	31504
S. lepidopodium Nakai	Korea: Gojae Isl. Haeogumgang	32775
S. morrisonensis Hayata	Taiwan: Mt. Yu Shan (3800 m)	26400
	Taiwan: Mt. Yu Shan (3080 n)	26402
	Taiwan: distr. Nantou; Kum Yang	31048
	Taiwan: distr. Nantou; Kum Yang	32838
S. oryzifolium Makino	Korea: Gojae Isl., Haeogumgang	32771
	Korea: Gojae Isl., Haeogumgang	32772
	Korea: Jeju Isl., Seongsan	32796
	Korea: Doek Isl.	32901
S. polytrichoides Hemsl.	Korea: prov. Chungbook: Mt. Songni	32761
	Korea: Jeju Isl., Mt. Halla, 1100 m	32797
S. sarmentosum Bunge	China: prov. Zhejiang; Hangzhou	30671
-	Korea: prov. Chungbook; Mt. Songni	32762
	Korea: prov. Cheonnam; Damyang-gun	32770
	Korea: Gojae Isl., Haegumgang	32773
Sedum sect. Aizoon		
S. ellacombianum Praeger	China: prov. Xinjiang; Tianchi	30672
S. kamtschaticum Fisch.	Korea: prov. Kyeongbook; Mt. Chuwang	32765
	Korea: Gojae Isl., Haegumgang	32774
S. selskianum Regel & Maack.	Russia: exact prov. unknown	25461
S. takesimense Nakai	Korea: Ullung Isl., Jeodong	32902
S. zokuriense Nakai	Korea: prov. Chungbook; Mt. Songni	32763
Sedum sect. Telephium		
S. duckbongii Chung & Kim	Korea: prov. Kyeongbook; Mt. Chuwang	32764
S. taquetii Praeger	Korea: Jeju Isl., Mt. Halla, 1100 m	32798
S. telephium L.	Turkey: prov Bolu; Yedigoller	30867
	France: dep. Vienne; Charroux	31028
S. viviparum Maxim.	Korea: prov. Kyeongbook; Mt. Chuwang	32766

Table 2. Alkaloid profiles of some Asian Sedum species

	Call. no.	Alkaloid pattern*													
		Pyrrolidines					Piperidinest								
Species		1	2	3	4	5	6	7	8	9	10	11	12	13	14
S. oryzifolium	32771	4	_	-	_	2	16	18	~	_	_	8	3	9	39
S. oryzifolium	32772	3	-	-	-	2	18	21		-		8	4	10	34
S. oryzifolium	32796	4	‡	1	4	1	2	9	2	12	-		40	12	13
S. oryzifolium	32901	7	2	2		9	4	12	8	16	-	_	6	20	14
S. japonicum	31504	35	_	_	-	_	20	40		5	-				
S. lepidopodium	32775		_	-	-	-	13	~	76		10	-	_		-
S. morrisonensis	26400	20		-		_	30	-	50	-				-	
S. morrisonensis	26402		_	-			-	-		_	-	-	-	-	
S. morrisonensis	31048	-	-	-	-		10	-	87	-	3		_	_	
S. morrisonensis	32838	ţ.	_	-	-	-	67	4	24	-	5	-	-	-	
S. polytrichoides	32761	-	_	_	-	_	70	30	~	-	_	_	-	_	
S. polytrichoides	32797	6	4	‡	_	-	3	-	29	44	13	-	-	-	
S. sarmentosum	30671	_		-	-	-	7		49	-	44	-	-	-	
S. sarmentosum	32762	_	-		_	-	‡	-	21	_	78	-		-	-
S. sarmentosum	32770	-	-	_	-		6	~	36	_	58		_	-	
S. sarmentosum	32773	_	_	_	-	-	3	-	31	-	66	_	-	~	
S. bulbiferum	32768	_	_	-	-	-	100			-	-	-		-	
S. bulbiferum	32769	_	-	-		-	100		-	-	-	-	-	-	
S. bulbiferum	32795	-	-	-		-	100	-	-	-	-			-	_

*Abundance in percent of total alkaloids determined by GC. Signs: - = not detected, $\ddagger =$ trace amount (< 1% of total alkaloids). †For key to alkaloid numbers see Fig. 1.



Fig. 1. Alkaloids from some Asian Sedum species.

alcohols, 4 and 5, and characterized by ¹H NMR comparison with sedamine, norsedamine and their diastereoisomers. Without exception, the sedamine-type alkaloids show -CH(OH)- signals at higher field than the allosedamine-type compounds [23; Dr C. Hootelé, pers. commun.]. Thus, for the sedamine-type pyrrolidine alkaloid (H-7 at 4.82 ppm) we propose the name 'pyrrolsedamine' and for the allosedamine-type pyrrolidine, 'pyrrolallosedamine' (H-7 at 5.07 ppm). The diastereoisomeric alcohols were not resolved under the GC conditions employed, but they could be (partially) separated as their trimethylsilyl ethers which allowed us to detect either of the two diastereoisomers in complex alkaloid mixtures. To this end, alkaloid extracts of S. oryzifolium were treated with (bis)-trimethylysilyl acetamide and examined by GC; mixtures of the extracts with pyrrolsedamine and pyrrolallosedamine were also chromatographed after trimethylsilylation. Pyrrolallosedamine was found in all plants of S. oryzifolium and, in addition, one plant of S. oryzifolium contained pyrrolsedamine. TLC proved to be of no help in identifying the natural pyrrolidine alkaloid because pyrrolsedamine and pyrrolallosedamine are masked by their piperidine analogues on silica gel plates.

DISCUSSION

Alkaloid patterns in Asian Sedum species agree with the hypothesis on the distribution of alkaloids in Crassulaceae proposed by Stevens and coworkers who stated that the occurrence of alkaloids is restricted to species of the 'Acre' lineage which are characterized by a reticulate testa [14, 15]. Of the 16 Asian Sedum species investigated, only the seven species with reticulate or reticulo-papillate seeds contained alkaloids. The five species of Sedum sect. Aizoon contained no alkaloids and our results corroborate earlier negative reports for species of this section [11].

The alkaloid report for S. kamtschaticum could not be confirmed [10] and positive reports for other species of this section, viz., S. aizoon, S. floriferum, S. hybridum and S. middendorffianum [4, 9, 11], need to be checked, especially the reports of methylisopelletierine, sedamine and sedinine in S. aizoon and S. hybridum [9]. The four species of Sedum sect. Telephium also proved to be devoid of alkaloids and our findings support the negative reports for species of this section [11]. The positive records for S. telephium s.l. (= S. maximum Suter, = S. purpureum Link), including reports of the occurrence of methylisopelletierine, sedamine, sedinine and sedridine in this species [8,9], could not be confirmed and positive reports for other species of this section, i.e. S. ewersii Ladeb. and S. populifolium Pallas [9, 10], should also be checked carefully. The Asian Sedum species with a reticulate or reticulo-papillose testa of Sedum sect. Sedum, on the other hand, contained a wide variety of alkaloids. The occurrence of N-methylpelletierine and N-methylallosedridine in S. sarmentosum [2, 21], the only species of this group previously studied, could be fully confirmed (Table 1). Although, as yet only ca. 6% of the species of the Crassulaceae have been examined for alkaloids and ca 12% of the species of Sedum, the present results strengthen our hypothesis that in Crassulaceae, alkaloids are restricted to the 'Acre' lineage.

Alkaloid composition varies considerably among the Asian species of Sedum sect. Sedum. Tentatively four groups can be distinguished: 1) The four plants of S. oryzifolium which contain a wide spectrum of alkaloids, most notably the typical S. acre alkaloids, sedamine and sedinone, as well as the pyrrolidines, hygrine, norhygrine and hygroline. As lysine, ornithine, and phenylalanine are the biogenetic precursors of these alkaloids [24, 25], the additional presence of the newly discovered pyrrol(allo)sedamine in S. oryzifolium may indicate a pathway in which phenylalanine is coupled with Δ^1 -pyrrolideine, the cyclic intermediate produced from ornithine. In this respect, they differ markedly from the closely related S. japonicum which Ohba [26] included in S. uniflorum Hook.f. & Arnott, together with S. oryzifolium 2) The eight plants of S. japonicum, S. morrisonensis, S. lepidopodium and S. polytrichoides, in which N-methylpelletierine and pelletierine are the main alkaloids. In addition, these species contain varying amounts of the pyrrolidines, sedridine and N-methyl(allo)sedridine. 3) The four plants of S. sarmentosum in which the principal alkaloids are N-methylallosedridine and Nmethylpelletierine. 4) The three plants of S. bulbiferum which contain only pelletierine, Of the seven species studied here, Berger [17] recognized only S. japonicum, and S. sarmentosum, which he classified in S. series Orientalia Berger and S. series Chinensia Berger, respectively. Fröderström [18] included six of the seven species in his study of Sedum (except S. lepidopodium) and placed these all in S. series Japonica (Max.) Fröd. Distribution patterns of alkaloids in Eurasian Sedum species appeared to be significantly correlated with an infrageneric classification based on biosystematic characters and chloroplast DNA RFLPs [12-14, 27]. Especially, the presence of

pyrrolidines and the 2,6-disubstituted piperidines proved to be highly significant from a systematic point of view. Our results, therefore, suggest that systematic and evolutionary relationships among Asian Sedum species are much more intricate than present infrageneric classifications indicate. Most notably, the presence in S. oryzifolium of the 2,6-disubstituted piperidine (sedinone), which so far is only known from S. acre [12], is a valuable lead for further studies of evolutionary and systematic relationships between Asian and Eurasian species of Sedum sect. Sedum.

Infraspecific variation in the alkaloid fraction of the Asian Sedum species varies considerably. Sedum bulbiferum and S. sarmentosum show no or little variation, though accessions of the latter species originate from geographically very distant regions. The accessions of S. morrisonensis, S. oryzifolium and S. polytrichoides, on the other hand, show much more variation. In general, the variation seems to be correlated with the origin of the plant material, rather than with morphological variation. For example, the composition of the alkaloid fractions of two plants of S. oryzifolium from Gojae island are identical, but differ from the other two accessions. Also, the plants of S. morrisonensis from Kum Yang and Mt. Yu Shan differ. In this species, however, there is considerable variation among plants from the same region, most notably the complete lack of alkaloids in one of the two plants from Mt. Yu Shan. A similar variation has been observed in the European S. sexangulare which, like S. morrisonensis, is highly polyploid [13]. The composition of the alkaloid fractions of the two plants of S. polytrichoides differ markedly, in particular, in the presence of several pyrrolidines in the plant from Jeju island. It also differs considerably from the closely related S. lepidopodium. The variation in the alkaloid fraction of our accessions of S. polytrichoides parallels the extreme morphological and cytological variation reported for the S. polytrichoides species complex which comprises two continuous series of gametic chromosome numbers, n = 11-16 and n = 20-27, respectively, and the number n = 35 [28].

EXPERIMENTAL

Plant material. Living plants were collected in the wild and, for further study, cultivated under uniform conditions in the experimental garden at Utrecht. Origins of plants are given in Table 1. Voucher specimens are deposited in the Botanical Institute at Utrecht. The vegetative (leafy) parts of the plants were either used fresh or kept at -18° prior to phytochemical analysis.

Extraction of alkaloids. Fresh (frozen) vegetative parts of plant material (20 g) were immersed in CHCl₃ for 15 sec to remove leaf waxes and then homogenized in 40 ml of MeOH with a food processor for 2 min. After addition of conc HCl (2.4 ml), the mixt. was left standing overnight and then centrifuged. The supernatant was concd to ca. 20 ml in vacuo at 60°. The aq. soln (plant material contained ca 85% moisture) was extracted with CHCl₃ (1 × 20 ml, 2 × 10 ml; the comb. organic layers were discarded) and, after basification with 10 N NaOH to pH *ca* 11, extracted with CHCl₃ (1 × 20 ml, 2 × 10 ml) again. Separation of the layers was achieved by centrifugation if necessary. The comb. organic layers were dried (Na₂SO₄) and evapd *in vacuo* at 40°. The residue was dissolved in 1 ml of CHCl₃ and stored at 4° prior to chromatography.

Chromatography. TLCs were run on silica gel plates (Merck art. 5715) with CHCl₃-MeOH-NH₄OH (85:18:1). Spots were visualized with Dragendorff's reagent (undild) and with the *o*-toluidine reagent [29] for the specific detection of secondary amines. GC was performed on a WCOT fused silica CP Sil 5 CB column (25 m × 0.25 mm i.d., film thickness 0.12 μ m) prog. from 70° to 290° at 6° min⁻¹; injector: 175°; FID: 300°; N₂ at 1 ml min⁻¹; inj. vol. 1 μ l; split ratio 1:60. The GC-EIMS (70 eV) instrument was equipped with an identical column programmed from 50° to 290° at 6° min⁻¹; He 1 ml min⁻¹; inj. vol. 1-2 μ l.

Reference compounds. Synthesis and/or isolation of compounds 1-3. 6-8 and 12-14 is recorded in refs [12, 13]. N-methylsedridine 9 (also prepared by N-methylation of sedridine from S. acre) and N-methylal-losedridine 10 were generous gifts from Dr L. Maat. Silica gel CC of synthetic 1-phenyl-2-(2-piperidyl)-ethanol gave norallosedamine 11 and norsedamine, which were identified by comparison of their ¹H NMR data with those reported in ref. [23].

Synthesis of 1-phenyl-2-(2-N-methylpyrrolidyl)-ethanol. The intermediate product, phenacyl-2-pyrrolidine, was prepared from pyrrolidine and ethyl benzoyl acetate according to the general procedure for the synthesis of 2-monosubstituted piperidines [22]. Phenacyl-2-pyrrolidine · HCl, mp 165.5-167°. A portion of the HCl was converted to the base. IR v_{max} (film) cm⁻¹; 1680 (-CO-Ph); ¹H NMR (300 MHz, CDCl₃): δ 8.0-7.2 (phenyl protons), 3.55 (m, H-5e), 3.15 (2H, d, ,H-6), 2.38 (N-H); EIMS (probe) m/z (rel. int.): 189 [M]⁺ (72), 120 (5), 105 (78), 84 (98), 77 (100), 70 (91). N-Methylation and subsequent reduction [12] of the intermediate product vielded a mixt. of the diastereoisomeric alcohols of 1phenyl-2-(2-N-methylpyrrolidyl)-ethanol, 4 and 5. GC-EIMS m/z (rel. int.): 205 [M]⁺ (2), 84 (100), 77 (4); R_t 17.73 min (no separation by GC).

The synthetic pyrrolidine was separated into its diastereoisomeric alcohols, 4 and 5, by silica gel CC using increasing proportions of the TLC solvent (80–100%) in CHCl₃ as the eluent. Comparison of the ¹H NMR data with those of sedamine and allosedamine [22] allowed assignment of their structures, for which we propose the names pyrrolsedamine and pyrrolallosedamine. Pyrrolallosedamine. ¹H NMR (300 MHz, CDCl₃): δ 7.4–7.2 (5H, phenyl protons), 5.07 (1H, dd, J = 2.5 and 10.3 Hz, H-7), 3.13 (1H, m, H-5), 2.64 (1H, m, H-2), 2.40 (3H, s, N-Me), 2.22 (1H, m, H-6a); R_t TMSi ether, 17.66 min. Pyrrolsedamine. ¹H NMR (300 MHz, CDCl₃): δ 7.4–7.2 (5H, phenyl protons), 4.82 (1H, dd, J = 2.6 and 9.9 Hz, H-7), 3.07 (1H, m, H-5), 2.82 (1H, m, H-2), 2.39 (3H, s, N-Me), 2.01 (1H, m, H-6a); R_t TMSi ether 17.60 min. The TMSi ethers of both diastereoisomers had identical GC-EIMS: m/z 277 [M]⁺ (3), 84 (100). Trimethylsilyl ether derivatives were prepd by dissolving samples (dried with a stream of N₂) in a mixture of pyridine and (*bis*)trimethylsilyl acetamide (1:1); the reaction mixt. was kept at 70° for 60 min prior to injn on the GC column.

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REFERENCES

- 1. Marion, L. (1945) Can. J. Res. 23B, 165.
- 2. Marion, L. and Chaput, M. (1949) Can. J. Res. 27B, 215.
- 3. Franck, B. (1958) Chem. Ber. 91, 2803.
- 4. Franck, B. (1958) Angew. Chem. 70, 269.
- 5. Hegnauer, R. (1964) Chemotaxonomie der Planzen Vol. 3. Birkhäuser, Basel.
- Hegnauer, R. (1989) Chemotaxonomie der Planzen Vol. 8. Birkhäuser, Basel.
- 7. Karpovich, V. N. (1961) Tr. Leningrad. Khim.-Farmatsevt. Inst. 12, 191.
- 8. Logar, S., Mesicek, N., Perpar, M. and Seles, E. (1974) Farm. Vestn. 25, 21.
- Krasnov, E. A., Petrova, L. V. and Bekker, E. F. (1977) Chim. Prir. Soedin. 4, 585.
- Gill, S., Raszeja, W. and Szynkiwecz, G. (1979) Farm. Pol. 35, 151.
- 11. Diak, J. and Kohlmünzer, S. (1981) Herba Hung. 20, 7.
- Stevens, J. F., Hart, H. 't, Hendriks, H. and Malingré, T. M. (1992) Phytochemistry. 31, 3917.
- Stevens, J. F., Hart, H. 't, Hendriks, H. and Malingré T. M. (1993) *Pl. Syst. Evol.* 185, 207.
- 14. Ham, R. C. H. J. van (1994) Ph.D. Thesis, Utrecht University, The Netherlands.
- Stevens, J. P., Hart, H. 't, Ham, R. C. H. J. van, Elema, E. T., Endt, M. M. V. X. van den, Wildeboer, M. and Zwaving, J. H. (1995) *Biochem. Syst. Ecol.* 23, 157.
- Hart, H. 't and Berendsen, W. (1980) Pl. Syst. Evol. 135, 107.
- 17. Berger, A. (1930) in *Die natürlichen Pflanzenfamilien* (Engler, A. and Prantl. K. eds), 2nd Edn, Vol. 18a. Engelmann, Leipzig.
- 18. Fröderström, H. (1931) Acta Horti. Gothob. 6 app., 1.
- 19. Kim, J. H. (1989) Ph.D. Thesis, Seoul National University, Korea.
- 20. Knapp, U. (1994) Bot. Jahrb. Syst. 116, 157.
- Beyerman, H. C., Bordes, B. S. L., Maat, L. and Warnaar, F. M. (1972) *Rec. Trav. Chim.* 91, 1441.
- 22. Halin, F., Slosse, P. and Hootelé, C. (1985) Tetrahedon 41, 2891.

- 23. Hootelé, C., Halin, F. and Thomas, S. (1985) Tetrahedon 41, 5563.
- Gupta, R. N. and Spencer, I. D. (1967) Can. J. Chem. 45, 1275.
- 25. Mann, J. (1987) Secondary metabolism, 2nd Edn. Clarendon Press, Oxford.
- Ohba, H. (1982) in Wild flowers of Japan (Satake, Y., Ohwi, J., Kitamura, S., Watari, S., and Tominari, T., eds), Vol. 2. Heibonsha, Tokyo.
- 27. Hart, H. 't (1991) Fl. Medit. 1, 31.
- 28. Uhl, C. H. and Moran, R. (1972) Cytologia 37, 59.
- 29. Reindel & Hoppe (1954) Chem. Ber. 87, 1103.