Plagiochila porelloides (Plagiochilaceae, Hepaticae) from Changbai Mountain, new to China, with chemical characterization and chromosome measurements†

Lars Söderström, David S. Rycroft, W. John Cole & Sha Wei

Söderström, L., Department of Botany, Norwegian University of Science and Technology, N-7034 Trondheim, Norway
Rycroft, D. S., Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.
Cole, W. J., Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.
Wei S., Department of Biology, Qiqihar Teachers' College, Qiqihar University, Qiqihar, 161006, China

The hepatic *Plagiochila porelloides* (Nees) Lindenb. is reported for the first time from China. It was collected on Changbai Mt., Jilin Province, at four localities from 1 600–2 200 m a.s.l. on boulders, alpine heathland and decaying logs. Chromosome counts shows n = 9 (8+m). It has been characterized chemically using NMR fingerprinting and GC-MS. The lipophilic chemical profile is dominated by β-barbatene, but 2,3-secoaromadendranes are also present. The Chinese plants therefore belong to a similar chemotype as *P. porelloides* from Europe. The main 2,3-secoaromadendrane has been isolated, characterized and found to be new; a structure for this compound, plagiochiline V, is proposed on the basis of its NMR parameters.

Key words: chemistry, China, chromosomes, *Plagiochila porelloides*, taxonomy

INTRODUCTION

During the excursion to Changbai Mountain, Jilin Province, north-east China, in connection with the IAB Conference in Beijing, 1997, a *Plagiochila* species of the *P. asplenioides* group was found with perianths and antheridia. This turned out to be *P. porelloides* (Nees) Lindenb., a species not reported previously from China. In this paper the relation to other regional species, distribution, chemical characterization and chromosomes, are described. Distribution and relationships were studied mainly by Söderström, chemical characterization by Rycroft and Cole, and chromosome measurements by Sha Wei.

Plagiochila porelloides was collected at four different localities.

1. Ca. 5 km N of Tianchi (Sky Lake; 41°30'N 128°10'E), at the road junction, upper oroboreal *Betula ermanii* forest, alt. ca. 1 800 m, on

† Part 6 in the series "NMR Fingerprinting of Liverworts". For part 5, see Rycroft *et al.* (1999)

the side of a big boulder. 3. VI. 1997, *Söderström no. 97/69*

2. Ca. 2 km N of Tianchi (Sky Lake; 41°30'N 128°10'E), alpine heath, alt. ca. 2 200 m, 3. VI. 1997, *Söderström no. 97/70*; on a N-facing cliff, 3. VI. 1997, *Söderström no. 97/80.*
3. Yin Huan Hu ("Silver Ring Lake", formerly Xiao Tian Chi "Small Sky Lake"; 41°30'N 128°10'E), upper oroboreal *Betula ermanii* forest, alt. ca. 1 700 m, on a boulder, 4. VI. 1997, *Söderström no. 97/89.*
4. Gu Di Sen Lin (41°30'N 128°10'E), middle oroboreal *Abies nephrolepis-Picea jezoensis* forest, alt. ca 1 600 m, along the stream margin, 4. VI. 1997, *Söderström & Rycroft LS no. 97/116*, ca. fr. and antheridia; *Rycroft no. 97059a*, c. fr.; 5. VI. 1997, *Rycroft no. 97066*, with antheridia.

DISTRIBUTION AND RELATION TO OTHER REGIONAL SPECIES

The *Plagiochila asplenioides* complex includes four taxa in northeastern Asia: *P. porelloides, P. asplenioides* (L.) Dum., *P. satoi* Hatt. and *P. ovalifolia* Mitt.

Plagiochila porelloides is a boreal and arctic circumpolar species and is common in Europe, North America and arctic and boreal Siberia. In Asia it has been reported from northern Siberia (Konstantinova & Potemkin 1996), the Khanbarovsk region (Koponen *et al.* 1978), the Sayan Mts. (Konstantinova & Vasiljev 1994) and the Altai Mountains (Váňa & Ignatov 1995).

Plagiochila asplenioides is also common in northern Europe. Additionally it occurs in Turkey (Gökler & Öztürk 1991) and the Caucasus (Manakyan 1995). It is lacking from eastern North America but occurs in the west from Colorado and Washington to Alaska (Schuster 1980) and is found also in the nearby Chukotka Peninsula (Abramova *et al.* 1987). In China it has been reported from Hebei and Zhejiang provinces (Piippo 1990). However, all specimens from China should be re-examined to determine whether they are true *P. asplenioides* or if they belong to *P. porelloides* or any other related taxon.

Plagiochila satoi has a circumpacific distribution. In North America it occurs from Oregon to Alaska (Hong 1987). It is known in Asia from the Russian Far East (Konstantinova *et al.* 1992), Japan (H. Inoue 1958), Korea (Yamada & Choe 1997) and in China from Heilongjiang, Jilin and Hubei provinces (Piippo 1990).

Plagiochila ovalifolia is an Asiatic endemic known from China (Tibet and Fujian provinces; Piippo 1990), Taiwan (Piippo 1990), Japan (Mizutani 1984) and Korea (Yamada & Choe 1997).

Plagiochila porelloides is closely related to *P. asplenioides* and often regarded as a subspecies of it (e.g. Schuster 1980). It differs in the smaller size (3–6 vs. 6–10 cm long and 3–6 vs. 6–8 mm wide) and cells (median cells 25–32 µm vs. 30–36 µm). *Plagiochila satoi* has even smaller cells (21–30 × 25–34 µm) and more dentate leaves (*P. porelloides* is sometimes nearly edentate, as in the specimens from Changbai Mt.). *P. ovalifolia* has larger cells and the perianth mouth is oblique *versus* straight in *P. porelloides.*

The following key is provided to distinguish the regional taxa.

1. Plants with large cells (median cells 32–36 × 35–40 µm, marginal cells 25–32 µm wide) 2
1. Plants with smaller cells (median cells 21–32 µm or smaller, marginal cells 17–25 µm wide) 3
2. Plants large (6–10 cm long and 3–6 mm wide). Perianth mouth straight. Leaves edentate to dentate with small teeth .... *P. asplenioides*
2. Plants smaller (to 6 cm long and 4.5 mm wide). Perianth mouth oblique, dentate. Leaves coarsely dentate .... *P. ovalifolia*
3. Median cells 21–25 × 22–26 µm, marginal cells 10–20 µm wide, leaves coarsely dentate (very rarely with reduced teeth) .... *P. satoi*
3. Median cells 25–32 × 30–36 µm, marginal cells 21–25 µm wide. Leaves dentate to entire .... *P. porelloides*

CHEMICAL CHARACTERIZATION

Members of the liverwort genus *Plagiochila* have been classified into eight chemotypes (Asakawa 1995); a ninth chemotype, to which *P. spinulosa* (Dicks.) Dum., *P. punctata* Tayl., *P. killarniensis* and some Neotropical *Plagiochilae* belong, is characterized by the presence of 9,10-

dihydrophenanthrenes (Anton *et al.* 1997, Rycroft 1998a, Rycroft *et al.* 1999). The largest chemotype group is characterized by the presence of 2,3-secoaromadendranes; plagiochilines A–S are among the known members of this class of compound (Asakawa 1995, Valcic *et al.* 1997). *Plagiochila porelloides* from Europe belongs to this chemotype as previous studies have reported the presence of plagiochilines A, C, D and H (Asakawa *et al.* 1980) as well as 2,3-secoaromadendrane esters of fatty acids (Toyota *et al.* 1994).

Two specimens, one female and one male, of *Plagiochila porelloides* from China have been studied using the complementary techniques of NMR fingerprinting (Rycroft 1996, 1998) and GC-MS. GC, GC-MS and ^{1}H NMR (360 or 400 MHz, $CDCl_3$) were performed as described previously (Rycroft *et al.* 1998b). Extracts were prepared by triturating dried plant material with sufficient $CDCl_3$ to produce 0.6–0.7 mL of a filtered solution.

The results (Table 1) show that the lipophilic chemical profiles are dominated by β-barbatene (peak 4, concentration ca. 7 mM in extract 96059a from comparison with the intensity of the 0.2% residual $CHCl_3$ signal in the ^{1}H NMR spectrum). β-barbatene (Fig. 1) is a common liverwort constituent (Toyota *et al.* 1996) along with bicyclogermacrene, octenyl acetate, α- and β-chamigrene. Several plagiochiline-type compounds are also present (the proton NMR signals of H-2 and H-3 of many 2,3-secoaromadendranes are readily visible in a characteristic region of the spectrum). The only positive identification to date of known plagiochilines in the extracts is of plagiochiline C (Fig. 1).

The dominant plagiochiline in the ^{1}H NMR spectra (concentration ca. 1 mM in extract 96059a) gives rise to a set of signals, self-consistent between the two samples, that is comprised of at least a prominent methoxyl signal at δ 3.73, a doublet (J = 10.6 Hz) at δ 6.39 and a singlet at δ 7.40, and does not correspond to any of the known plagiochilines (we have also observed similar signals in the $CDCl_3$ extract of a sample of *Plagiochila porelloides* from Scotland, but not in *P. asplenioides*). This compound was isolated (ca. 0.1 mg) using TLC and found to correspond to peak 16 in Table 1. H-3 in 2,3-secoaromadendranes normally resonates between δ 6 and δ 7, but deshielding of H-3 to δ 7.40 can arise from the presence of a carbonyl substituent at C-4, and the present data have some features reminiscent of plagiochiline M (Fig. 1) (Hashimoto *et al.* 1995). The ^{1}H NMR data (including connectivity based on the spin-spin coupling constants and a 2D COSY experiment) are shown in Fig. 2. The structure proposed for this compound, plagiochiline V, is shown in Fig. 1 (we have already applied the names plagiochiline T and U to two new 2,3-secoaromadendranes isolated from *P. carringtonii* (Balf.) Grolle; Lamont 1998, Rycroft, *et al.*, to be published). The stereochemical assignments are based on the coupling constants (including the zero vicinal coupling constants from H-9α to H-8 and H-10, and from H-10 to H-14) and the constraints imposed by the ether link from C-8 to C-14. This last feature has not

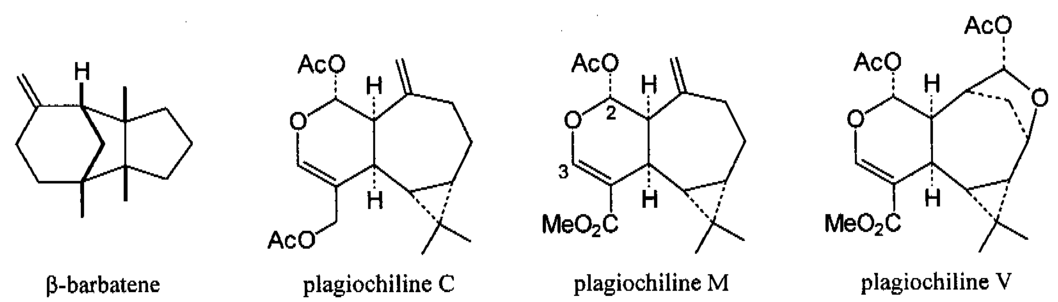

Fig. 1. Chemical structures.

Table 1. GC-MS analysis of $CDCl_3$ extracts of two specimens of *Plagiochila porelloides* from China.

Peak No. 3)	Assignment 4)	R_i	M+ 5)		Base peak	Sample 1 1) Relative abundance 6)	Sample 2 2)
1	octenyl acetate	1091	[128]	(9)	48	2	1
2	(α-barbatene)	1387	204	(22)	93	2	+
3		1406	[179]	(11)	135	2	2
4	β-barbatene	1416	204	(4)	93	100	100
5	β-chamigrene	1453	204	(38)	189	2	+
6	bicyclogermacrene	1475	204	(21)	121	9	10
7	α-chamigrene	1480	204	(21)	136	1	+
8		1507	204	(16)	69	3	-
9		1541	[205]	(51)	43	5	-
10		1782	234	(1)	95	2	2
11		1837	270	(1)	68	4	4
12		1879	278	(16)	43	2	+
13		1913	260	(4)	79	2	-
14	fusicoccadiene	1962	272	(21)	135	3	+
15	plagiochiline C	2085	[274]	(1)	43	2	1
16	plagiochiline V	2183	[292]	(2)	43	2	+
17		2196	304	(4)	43	2	2
18		2380	[336]	(19)	43	-	1
19	plagiochiline-type	2557	[335]	(1)	43	1	3

1) Collection *Rycroft 97059a*, at the stream crossing the path to the Underground Forest (Gu di sen lin), on a rotten log in the middle of the stream, 4.IV.1997, with sporophytes, 277 mg extracted 5.V.1998.

2) Collection *Rycroft 97066*, same place as *97059a*, at the stream margin, 5.VI.1997, with antheridia, 53 mg extracted 25th June 1997.

3) All components with an abundance in either extract greater than 1% of the most abundant component (β-barbatene) are included.

4) Based on comparison with authentic materials or literature retention indices and MS (Adams, 1995); assignments in parentheses are tentative.

5) Figures in square brackets indicate *m/z* of the heaviest ion observed rather than M^+ (figures in parentheses show the % abundance relative to the base peak).

6) From GC-MS TIC integration (these figures are affected by different detection sensitivities and, in the absence of standards, do not quantitatively reflect the amounts present); + indicates that the compound was detectable in low amount.

been observed previously in plagiochilines, but can arise from cyclization of a β-hydroxyl substituent at C-8 (as occurs in plagiochilines O and P; Valcic *et al.* 1997), to an aldehyde function at C-14 (as occurs in other 2,3-secoaromadendranes; Asakawa 1995).

Although the new 2,3-secoaromadendrane, plagiochiline V, has been isolated and characterized, further work is in progress to obtain supporting evidence for the proposed structure and, in particular, to confirm the relative stereochemistry. Full details will be reported elsewhere.

CHROMOSOME MEASUREMENTS

For the chromosome number and karyological observations, stem tips of the male gametophytes were pre-treated in 1:1 mixture of saturated of *p*-dichlorobenzene and 1 % α-brohronaphtalene for 3-6 hours at 10° C before they were fixed in a 3:1 mixture of absolute alcohol and glacial acetic acid. They were then hydrolysed in a 1:1 mixture of hydrochloric acid and 95% alcohol for 15-20 minutes. After being washed with water, they were stained with car-

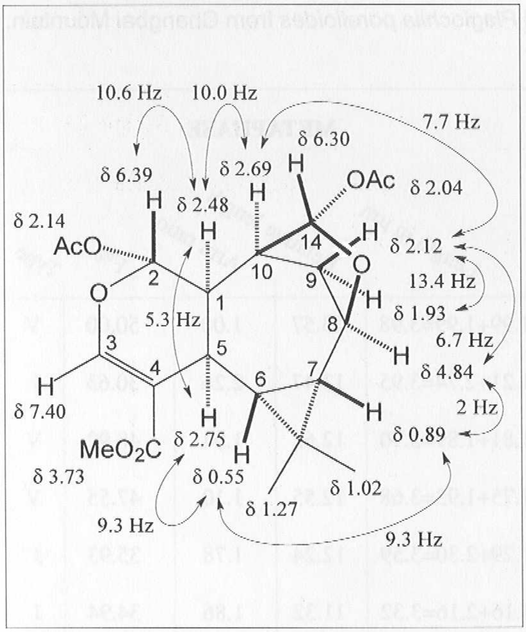

Fig. 2. NMR data of plagiochiline V.

bol fuchsin before slide preparations were made. Many cells with well-dispersed chromosomes were observed, from which 5 cells were chosen for karyological analysis.

The chromosome number of the male gametophytes was nine. This number agrees with other species of the genus and the number given by other authors for *Plagiochila porelloides* (H. Inoue 1967, 1968, 1975, 1977, S. Inoue *et al.* 1985, Segawa 1965a, b).

The karyotype of *Plagiochila porelloides* is k(n) = 9 = 5V+3J+v. At the metaphase each chromosome in the complement was distinguishable in size and shape (Fig. 3d, f). Measurement of the length of the chromosomes (Table 2) shows that at the metaphase the constriction in chromosomes 3, 4, 7, 8 and 9 is submedian and in 2, 5 and 6 is subterminal. At the prometaphase the chromosomes are longer than

Fig. 3. *Chromosomes of Plagiochila porelloides.* – 1, 2: Resting nuclei. – 3, 4: Prometaphase. – 5, 6: Metaphase (x 2300).

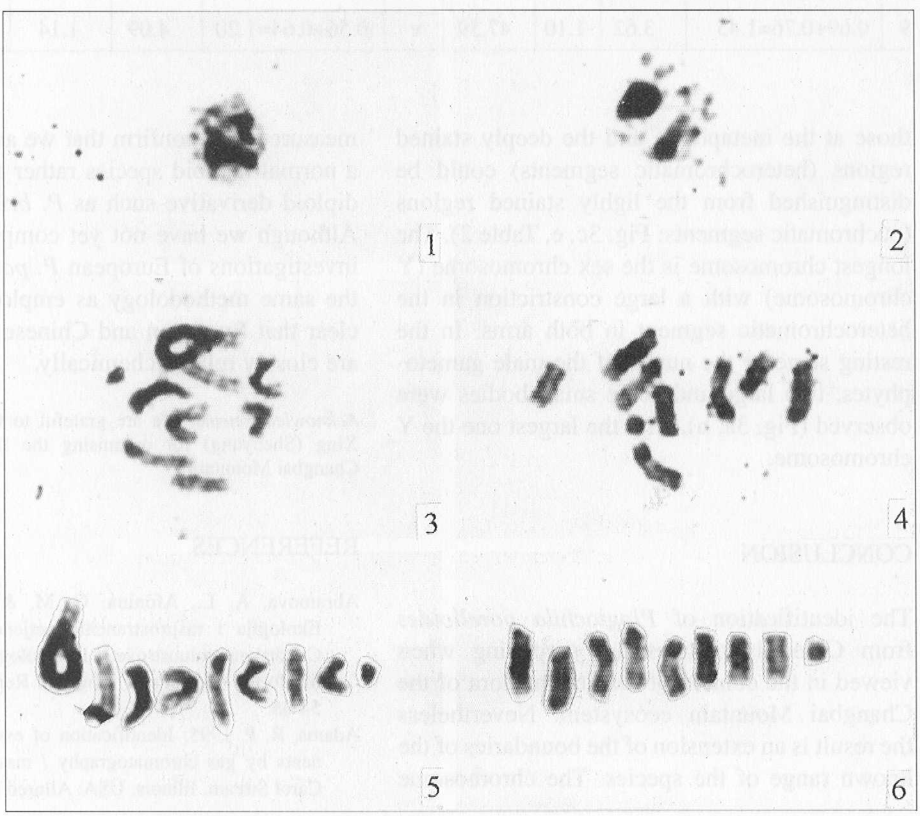

Table 2. Measurements of somatic chromosomes of *Plagiochila porelloides* from Changbai Mountain, Jilin Province, China.

PROMETAPHASE						METAPHASE				
Chromosome No.	Length in µm	Relative length	Arm ratio	F%	Type	Length in µm	Relative length	Arm ratio	F%	Type
1	5.02+5.04=10.06	25.14	1.00	49.90	V	1.99+1.99=3.98	13.57	1.00	50.00	V
2	1.67+2.96=4.63	11.57	1.77	36.07	J	1.21+2.74=3.95	13.47	2.26	30.63	J
3	1.77+2.76=4.53	11.32	1.56	39.07	V	1.81+1.89=3.70	12.62	1.04	48.92	V
4	1.99+2.39=4.38	10.95	1.20	45.43	V	1.75+1.93=3.68	12.55	1.10	47.55	V
5	1.47+2.85=4.32	10.80	1.94	34.03	J	1.29+2.30=3.59	12.24	1.78	35.93	J
6	1.30+2.48=3.78	9.45	1.91	34.39	J	1.16+2.16=3.32	11.32	1.86	34.94	J
7	1.53+1.93=3.46	8.65	1.26	44.22	V	1.25+1.71=2.96	10.10	1.37	42.23	V
8	1.54+1.86=3.40	8.50	1.21	45.29	V	1.25+1.69=2.94	10.03	1.35	42.52	V
9	0.69+0.76=1.45	3.62	1.10	47.59	v	0.56+0.64=1.20	4.09	1.14	46.67	v

those at the metaphase, and the deeply stained regions (heterochromatic segments) could be distinguished from the lighly stained regions (euchromatic segments; Fig. 3c, e, Table 2). The longest chromosome is the sex chromosome (Y chromosome) with a large constriction in the heterochromatic segment in both arms. In the resting stage of the nuclei of the male gametophytes, two large and some small bodies were observed (Fig. 3a, b), with the largest one the Y chromosome.

CONCLUSION

The identification of *Plagiochila porelloides* from China is perhaps not surprising when viewed in the context of the known flora of the Changbai Mountain ecosystem. Nevertheless the result is an extension of the boundaries of the known range of the species. The chromosome measurements confirm that we are dealing with a normal haploid species rather than a putative diploid derivative such as *P. britannica* Paton. Although we have not yet completed chemical investigations of European *P. porelloides* using the same methodology as employed here, it is clear that European and Chinese *P. porelloides* are closely related chemically.

Acknowledgements. We are grateful to Cao Tong and Fu Xing (Shenyang) for organising the field excursion to Changbai Mountain.

REFERENCES

Abramova, A. L., Afonina, O. M. & Duda, J. 1987: Ekologija i rasprostranenic petjenotjikh mkhov na Cukotskom poluostrove. – In: Ecology, distribution and life-forms of plants of Magadan Region, Vladivostok: 54-65.

Adams, R. P. 1995: Identification of essential oil components by gas chromatography / mass spectrometry. – Carol Stream, Illinois, USA: Allured Publ. Corp., Carol

Stream.
Anton, H., Kraut, L., Mues, R. & Morales-Z, M. I. 1997: Phenanthrenes and bibenzyls from a Plagiochila species. – Phytochemistry 46: 1069–1075.
Asakawa, Y. 1995: Chemical constituents of the bryophytes. – In: Herz, W., Kirby, G. W., Moore, R. E., Steglich, W. & Tamm, Ch. (eds), Progress in the chemistry of organic natural products 65: 1-618. Springer Verlag, Vienna.
Asakawa, Y., Inoue, H., Toyota, M. & Takemoto, T .1980: Sesquiterpenoids of fourteen Plagiochila species. – Phytochemistry 19: 2623–2626.
Gökler, I. & Öztürk, M. 1991: Liverworts of Turkey and their position in South-West Asia. – Candollea 46: 359-366.
Hashimoto, T., Nakamura, I., Tori, M., Takaoka, S. & Asakawa, Y. 1995: Epi-Neoverrucosane- and *ent*-clerodane-type diterpenoids and *ent*-2,3-secoaromadendrane- and calamenene-type sesquiterpenoids from the liverwort Heteroscyphus planus. – Phytochemistry 38: 119–127.
Hong, W. S. 1987: The distribution of Western North American Hepaticae. Endemic taxa and taxa with a North Pacific arc distribution. – Bryologist 69: 344-361.
Inoue, H. 1958: The family Plagiochilaceae of Japan and Formosa. I. – J. Hattori Bot. Lab. 19: 25-59.
Inoue, H. 1967: Chromosome studies on some Japanese liverworts. – Bot. Mag. Tokyo 80: 172–175.
Inoue, H. 1968: Chromosome numbers of some Malayan and Taiwan liverworts. – Bull. Nat. Sci. Mus, Tokyo 11: 397–403.
Inoue, H. 1975: Chromosome studies in some Arctic Hepaticae. – Bull. Nat. Sci. Mus., Ser. B (Bot) 2: 39–46.
Inoue, H. 1977: Chromosome numbers of some Japanese Hepaticae (2). – Bull. Nat. Sci. Mus., Ser. B (Bot.) 3: 55–61.
Inoue, S., Watanabe, I. & Mizutani, M. 1985: Karyological studies on some Japanese liverworts. – Hikobia 9: 235–241.
Konstantinova, N. A. & Potemkin, A. D. 1996: Liverworts of the Russian Arctic: an annotated check-list and bibliography. – Arctoa 6: 125-150.
Konstantinova, N. A. & Vasiljev, A. N. 1994: On the hepatic flora of Sayan Mountains (south Siberia). – Arctoa 3: 123-132.
Konstantinova, N. A., Potemkin, A. D. & Schljakov, R. N. 1992: Checklist of the Hepaticae and Anthocerotae of the former USSR. – Arctoa 1: 87-127.
Koponen, T., Järvinen, I. & Isoviita, P. 1978: Bryophytes from the Soviet far east, mainly the Khabarovsk territory. – Ann. Bot. Fennici 15: 107-121.
Lamont, Y. M. 1998. Comparative chemical study of the liverwort genus Plagiochila: new 2,3-secoaromadendranes from Plagiochila carringtonii. – B.Sc. Thesis, Univ. Glasgow.
Manakyan, V. A. 1995: Results of bryological studies in Armenia. – Arctoa 5: 15-33.
Mizutani, M. 1984: Checklist of Japanese Hepaticae and Anthocerotae, 1983. – Proc. Bryol. Soc. Japan 3: 155-163.
Piippo, S. 1990: Annotated catalogue of Chinese Hepaticae and Anthocerotae. – J. Hattori Bot. Lab. 68: 1-192.
Piippo, S., He, X.-L., Koponen, T., Redfearn, P. & Li, X.-J. 1998: Hepatics from Yunnan, China, with a checklist of Yunnan Hepaticae and Anthocerotae. – J. Hattori Bot. Lab. 84: 135-158.
Rycroft, D. S. 1996: Fingerprinting of plant extracts using NMR spectroscopy: application to small samples of liverworts. – Chemical Communications: 2187–2188.
Rycroft, D. S. 1998a: Plagiochila atlantica F. Rose newly identified in England: chemotype classification. – J. Bryol. 20: 240–242.
Rycroft, D. S. 1998b: Chemical comparison of liverworts using NMR spectroscopy. – J. Hattori Bot. Lab. 84: 105-111.
Rycroft, D. S., Cole, W. J., Aslam, N. & Lamont, Y. 1999: Killarniensolide, methyl orsellinates and 9,10-dihydrophenanthrenes from the liverwort Plagiochila killarniensis from Scotland and the Azores. – Phytochemistry (in press).
Rycroft, D. S., Cole, W. J. & Rong, S. 1998: Highly oxygenated naphthalenes and acetophenones from the liverwort Adelanthus decipiens from the British Isles and South America. – Phytochemistry 48: 1351-1356.
Schuster, R. M. 1980: The Hepaticae and Anthocerotae of North America. Vol. 4. – Columbia Univ. Press, New York. 1334 pp.
Segawa, M. 1965a: Karyological studies in liverworts sex chromosome I. – J. Sci. Hiroshima Univ., Ser. B, Div. 2, 10: 69–80.
Segawa, M. 1965b: Karyological studies in liverworts sex chromosome II. – J. Sci. Hiroshima Univ., Ser. B, Div. 2 10: 81–148.
Toyota, M., Nakamura, I., Huneck, S. & Asakawa, Y. 1994: Sesquiterpene esters from the liverwort Plagiochila porelloides. – Phytochemistry 37: 1091–1093.
Toyota, M., Koyama, H., Mitzutani, M. & Asakawa, Y. 1996: (–)-*ent*-Spathulenol isolated from liverworts is an artifact. – Phytochemistry 41: 1347–1350.
Valcic, S., Zapp, J. & Becker, H. 1997: Plagiochilines and other sesquiterpenoids from Plagiochila (Hepaticae). – Phytochemistry 44: 89–99.
Váňa, J. & Ignatov, M. S. 1995: Bryophytes of Altai Mountains. V. Preliminary list of the Altaian hepatics. – Arctoa 5: 1-13.
Yamada, K. & Choe, D.-M. 1997: A checklist of Hepaticae and Anthocerotae in the Korean peninsula. – J. Hattori Bot. Lab. 81: 281-306.