Notes on chemical races in *Sulcaria sulcata* from southeastern Tibet and adjacent regions

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Abstract: More than 200 thalli of *Sulcaria sulcata* (lichenized Ascomycotina) from southeastern Tibet and adjacent regions have been analyzed chemically. Six major chemical races were detected, characterized by the occurrence of the following major compounds: psoromic acid (chemical race 1a), 2-methoxypsoromic acid (2a), 2-hydroxyvirensic acid (3a; often in combination with 2-hydroxyconvirensic acid), virensic acid (3b), and vulpinic acid, the latter in combination with either psoromic acid (1b) or 2-methoxypsoromic acid (2b).

Introduction

The lichen genus *Sulcaria* was established by BYSTREK (1971) mainly because of the long furrow-like (=sulcate) pseudocyphellae which, in older parts of the thallus, burst and expose the partly hollow medulla (see Fig. 3). The author included two species, the shrubby erect (or slightly pendant) *Sulcaria sulcata*, which is whitish-grey (to brownish purple) in colour, and the elongate, pendant *S. virens* with a green-yellow to yellow-grey thallus due to the vulpinic acid in the cortex and medulla.

Subsequently, two further species, *Sulcaria badia* and *S. isidiifera*, have been included in the genus (see BRODO & HAWKSWORTH 1977: 146; BRODO 1986: 115). These two rare taxa are confined to the west coast of North America (for a distribution map of *S. badia* see BRODO et al. 2001: 673), whereas *S. virens* and *S. sulcata* are widely distributed in the Himalayas and adjacent southeast Asian regions.

*Sulcaria sulcata*, the subject of the present paper, has been reported from several provinces in India (AWASTHI & AWASTHI 1985; SINGH & SINHA 1994: 316), Tibet (WEI & JIANG 1986: 65), Nepal (AWASTHI 1960; BYSTREK 1969; AWASTHI & AWASTHI 1985; POELT 1990), Taiwan and the Chinese provinces Shaanxi, Sichuan, Yunnan, Zhejiang, and Hubei (WEI 1991: 240), as well as from Japan (e.g. OKAMOTO 1995; ANONYMUS 2002).

The following known secondary metabolites have been reported in *Sulcaria* species: atranorin (in *Sulcaria badia* and *S. sulcata*), protocetraric acid (in *S. isidiifera* and traces in *S. virens*), psoromic acid (in *S. sulcata*), vulpinic acid (in *S.
virens and p.p. in *S. sulcata*), virensic acid (in *S. virens* and p.p. in *S. sulcata* and
*S. isidiifera*), convirensic acid (in *S. virens*) and pulvinic acid (p.p. in *S. virens*). Two further "unknowns" were reported as occurring in *S. badia* and *S. isidiifera*.

Three chemically based entities (previously treated as "forma") have been recognized for *Sulcaria sulcata*. In addition to major amounts of atranorin, on taxon contains psoromic acid (forma *sulcata*), one with additional vulpinic acid (forma *vulpinodes* (Zahlbr.) D. Hawksw.) and a third unnamed taxon with virensic acid (see BRODO & HAWKSWORTH 1977: 147). The latter two taxa are known only from Tibet and/or Yunnan (see WEI & JIANG 1986: 65; WEI 1991: 240; WU & WANG 1992: 42; BRODO & HAWKSWORTH 1977: 147).

In the process of preparing an exsiccata of *Sulcaria sulcata* from a collection made by the first author in southeastern Tibet in 1994, it became evident that the material was chemically more diverse than had hitherto been reported. Three new depsidones have since been isolated and characterized, 2-methoxypsoromic acid (Eux et al. 1999), 2-hydroxyvirensic acid (ELIX et al. 2000), and 2-hydroxy-convirensic acid (ELIX et al. 2003).

The aim of the present paper is to provide an overview of all chemical races of *Sulcaria sulcata* and to present some new distribution data for these races in the Tibetan region.

**Material and methods**

If not otherwise stated, all the material studied is housed in GZU and collected in the main by the first author in the southeast Tibetan region during expeditions in 1994 and 2000. Chemical analyses were carried out by using standard methods of thin layer chromatography (CULBERSON & AMMANN 1979; ELIX et al. 1987) and some selected specimens by high performance liquid chromatography (ELIX & WARDLAW 2000). For a more effective separation of psoromic acid and 2-methoxypsoromic acid TLC-plates were run to a height of 15 cm. Microchemical tests with the crystallizing reagent G.E. (glycerin and acetic acid, 1:3) were performed according to the methods compiled by HALE (1974). Microcrystallization images were prepared using a Leica Confocal Laserscanning Microscope (Leica TSC SP). Images of the thalli were generated with a Hewlett Packard ScanJet 4c/T.

**The chemical races of Sulcaria sulcata** (Lev. apud Jacquem.) Bystrek ex Brodo & D.Hawksw.

**Chemical race 1a**

**Chemistry:** Atranorin (major / rarely present in traces), psoromic acid (major), methyl psoromate (rarely major / minor / traces or not detected), 2'-O-demethyl-psoromic acid (traces or not detected), 2-methoxypsoromic acid (minor [only in one specimen from Japan] / traces or not detected), unknowns (minor / traces or not detected)

**Notes:** Methyl psoromate occurred as an accessory compound in some of the specimens, sometimes in significant amounts (e.g. Obermayer 06462/066).
Methoxypsoromic acid was either not detectable or present only in traces, except in one exsiccata specimen from Japan (11 thalli analyzed). Most thalli of this Gyelnik-Exsiccata also contained significant amounts of 2-methoxypsoromic acid (with concentrations almost equal to that of psoromic acid) and several other unknowns. Whether this should be regarded as a further chemical race may be elucidated by further studies.

In southeastern Tibet the psoromic acid race is as common as the 2-methoxy-psoromic acid race. Whether or not the psoromic acid and 2-methoxypsoromic acid races should be regarded as separate see the discussion below under chemical race 2a.

Specimens examined (92 thalli):–CHINA. SE Tibet, prov. Xizang: Nyanqentanglha Shan, 360 km E of Lhasa near the bend of the river Tsangpo, N-side of Gyala Peri, 9 km S of Tongjug village, 29°54-55’N/94°52-53’, 3200 - 3500 m alt., Rhododendron-Abies forest, on Prunus, 20.VIII.1994, W. Obermayer (08665-1, 3, 4; 06462-001, 004, 008, 012 → 016, 019, 023, 027, 031, 035 → 037, 042, 044 → 046, 052, 057, 058, 060 → 067, 069, 080, 081, 083, 086, 087, 089, 092, 093, 095, 098, 101 → 104, 114 → 118, 121, 122, 124, 127 → 129, 131); 5 km S of Tongjug village, 29°56’N/94°54’E, 3350 m alt., on dead Salix, 18.VIII.1994, W. Obermayer (08664); 370 km E of Lhasa, 55 km NNE of Nyingchi, river valley at the west side of Gyala Peri, 29°59’N/94°53’E, 2500 m alt., deciduous trees along the river-bank, on Salix, 17.VIII.1994, W. Obermayer (08659b); near the bend of the river Tsangpo, N-side of the mountain Gyala Peri, between the villages Tongjug and Tangmai, 30°01’N/94°58’E, deciduous trees along the river, on Salix, 23.VIII.1994, W. Obermayer (08672-1). SE-Tibetan fringe mountains (=Hengduan Shan). prov. Sichuan: Daxue Shan, 57 km S of Kangding, Gongga Shan, Hailougou glacier and forest park, NW of Hailougou Station, 29°34’05-40”N/101°59’35-55”E, 2950 - 3050 m alt., Abies fabri forest, on Populus spec., 30.VII.2000, W. Obermayer (08656a,b; 08657; 08658a,b); on Salix spec., 29.VII.2000, W. Obermayer (08653a,b,d→j, 08653h only with traces of atranorin); on Rhododendron, 28.VII. 2001, W. Obermayer (08627b, d → f).–NEPAL: above Sauwala Khola, on shrubs on ridge top, talt. 10,500 ft., 13 September 1954, Stainton, Sykes & Williams (4379). Langtang Area, slopes N above Syarpagaon, S-exposed, Quercus semecarpifolia forest, pasture with low rocks, 3000 m, 31.8.1986, J. Poelt (N86-L439; N86-L1018). Langtang Area, above Sherpa, 2970 m alt., lichen on Quercus semecarpifolia forest, WNW facing, 6.5.1986, G.&S. Miehe (1491). –TAIWAN [=Formosa], Hwalien C.: Mt. Hohuan-shan-syan, Hsiulin-gun, on trunks of Abies spec., 3100 m alt., July 31, 1985, H. Shibuichi (7964), K. Yoshida & M. Mineta, Lichenes Rariores et Critici Exsiccati 653. –JAPAN. In montis Fuji, ad cort. Fagi, 30.X.1935, M.M. Sató, V.Köfaragó-Gyelnik, Lichenotheca 100 [11 thalli analyzed].

Chemical race 1b

Chemistry: Atranorin (major), psoromic acid (major), methyl psoromate (traces or not detected), vulpinic acid (major), unknowns (traces or not detected).

Notes: One single thallus was found to contain vulpinic acid in addition to psoromic acid. Only a few branches of this thallus show visible high concentrations of the pigment whilst other branches are not yellow coloured (see notes under "chemical race 2b").

35
Specimen examined (1 thallus): -CHINA. SE-Tibetan fringe mountains (=Hengduan Shan), prov. Sichuan: Daxue Shan, 57 km S of Kangding, Gongga Shan, Hailougou glacier and forest park, NW of Hailougou Station, 29°34’35"N / 101°59’50"E, 3000 m alt., Abies fabri forest, on Rhododendron, 28.VII.2001, W. Obermayer (08627c)

Chemical race 2a

Chemistry: Atranorin (major / rarely present in traces or not detected), 2-methoxypsoromic acid (major), 2-hydroxypsoromic acid (traces or not detected), unknowns (traces or not detected)

Notes: The mobility of 2-methoxypsoromic acid (in solvents systems A, B' and C) is rather similar to that of psoromic acid but slightly less than of psoromic acid (in all three solvent systems; see Fig. 1). If the solvent front is permitted to run only to 10 cm these two substances are very difficult to distinguish even when run side by side. Furthermore, the lichen-spot test for 2-methoxypsoromic acid resembles those of psoromic acid (Pd+ yellow). However, the colour of 2-methoxypsoromic acid under UV$_{366}$ before spraying is not whitish like in psoromic acid, but yellowish(-red). After spraying, 2-methoxypsoromic acid develops a dark greyish-brown colour whilst psoromic acid is reddish-brown. When it is present in high concentrations, the former substance can be seen in daylight before spraying (but only in solvent A). Microcrystal-tests with G.E. reveal fascicles of long straight colourless needles or plates from which curved lateral branches arise (see Fig. 2A), whereas psoromic acid produces fascicles of bushy dendroid-branched needles (see Fig. 2B and HALE 1974: 124, plate 14L).

ASAHINA & HAYASHI (1928) were the first to report secondary metabolites from Sulcaria sulcata, namely atranorin and "sulcatic acid". This paper was followed by three further publications solely on "psoromic acid" (ASAHINA & HAYASHI 1933; 1937; ASAHINA & SHIBATA 1939), which the authors then regarded to be the older, established name for "sulcatic acid". Some years later, ASAHINA & SHIBATA (1951: 227) proposed a new formula for psoromic acid. In summary, ASAHINA & SHIBATA (1954) gave a brief history of the various investigations on psoromic acid the structure of which was established unambiguously by HUNECK & SARGENT (1976). It is possible that the original authors had difficulties in establishing the formula of psoromic acid because they were dealing with two different compounds or a mixture of both.

The difficulties experienced in distinguishing these two chemical products, and the fact, that 2-methoxypsoromic acid has also been reported from other lichen genera (e.g. ARCHER 2001), lead one to suspect that psoromic acid 2-methoxypsoromic acid may have been confused in other lichen species.

In southeastern Tibet, thalli of Sulcaria sulcata, producing 2-methoxypsoromic acid as a major compound were almost as frequently as those producing psoromic acid.

Specimens examined (80 thalli): -CHINA. Tibet, prov. Xizang: Nyanqentanglha Shan, 360 km E of Lhasa near the bend of the river Tsangpo, N-side of Gyala Peri, 9 km S of Tongjug village, 29°54-55’N / 94°52-53’, 3200 - 3500 m alt., Rhododendron-Abies forest, on Prunus, 20.VIII.1994, W. Obermayer (06462-003, 009, 010, 017, 018, 020, 021, 022, 024, 026, 030, 032, 038, 039, 040, 041, 047, 048, 050, 051, 053, 055, 056, 068, 072, 073, 075, 077, 079, 084, 085, 088, 090,
091, 094, 100, 105 → 108, 110 → 112, 119, 120, 126, 130, 132; 08665-2; 5 km S of Tongjug village, 29°56'N/94°54'E, 3350 m alt., on dead Salix, 18.VIII.1994, W. Obermayer (08663; 06840, part of the same thallus only with traces of atranorin); 5 km S of Tongjug village, 29°58'N/94°54'E, 2700 m alt., on Berberis, 18.VIII.1994, W. Obermayer (08661); 6 km S of Tongjug village, 29°56-58'N/94°53-54'E, 3200 m alt., on Populus, 21.VIII.1994, W. Obermayer (08666; 08667-1,2; 08668; 08669; 08670-1,2; 08671-1,2,3); between the villages Tongjug and Tangmai, 30°01'N/94°58'E, deciduous trees along the river, on Salix, 23.VIII.1994, W. Obermayer (06850; 08672-2,3,4; 08673); 370 km E of Lhasa, 55 km NNE of Nyingchi, river valley at the west side of Gyala Peri, 29°59'N/94°53'E, 2500 m alt., deciduous trees along the river-bank, on dead Salix, 16.-17.VIII.1994, leg. W. Obermayer (06331; 06390; 06412; 08659a). SE-Tibetan fringe mountains (=Hengduan Shan), prov. Sichuan: Daxue Shan, 57 km S of Kangding, Gongga Shan, Hailougu glacier and forest park, path from Hailougu Station to the glacier-viewpoint, 29°34'09"N/101°58'54"E, 3050 - 3250 m alt., Abies fabri forest, on Rhododendron, 28.VII.2000, W. Obermayer (08207); NW of Hailougu Station, 29°34'05-40"N/101°59'35-55"E, 2950 - 3050 m alt., Abies fabri forest, on Rhododendron, 28.VII.2001, W. Obermayer (08627a); on Salix spec., 29.VII.2000, W. Obermayer (08653c); on Populus spec., 30.VII.2000, W. Obermayer (08654; 08655). Prov. Yunnan: in montis Yulong-Schan, prope urbem Lidjiang, regione frigide temperata, 3600 m.s.m., H.Handel-Mazzetti, Kryptog.Exsicc. 2766; Montes Yulong Shan, 30 km ad septentriones versus ab oppido Likiang [=Lijiang], alt. 4000 m s.m., in ramulis arborum, 25.VII.1990, J.Soják, A. Vězda, Lich.Sel.Exs. 2452. –BHUTAN, Wangdi District: Phobji Valley, Khebeythang, mixed coniferous forest, 27°22,9"N/90°11,4"E, 2620 m alt., on bark, 14 April 1998, U. Sochting (US 8081, dupl.).

Chemical race 2b

Chemistry: Atranorin (major / rarely present in traces or not detected), 2-methoxypsoromic acid (major / minor), vulpinic acid (major / minor), unknowns (traces or not detected)

Notes: Here the higher concentration of vulpinic acid can be seen under the stereo-microscope in the medulla (through the open sulcae), in the apical parts of the branches and as a pruina on the borders of the furrows. Sometimes young lateral branches (spreading from near the base of the main branch) contain visible amounts of the pigment, whiles other parts of the same thallus show no trace of yellow colouration (see also above under chemical race 1b). The apothecia and neighbouring regions seem to lack vulpinic acid. One thallus investigated contained only vulpinic acid together with minor traces of atranorin. Vulpinic acid races were collected between 2300 and 3500 m altitude and are relatively rare (8 thalli) . Wu & Wang (1992) have found this chemical race between 3200 m and 3450 m in Mt. Yu Long where it is said to be very common; but these authors did not distinguish between 2-methoxypsoromic acid and psoromic acid.
Fig. 1: Chromatograms of the chemical races of *Sulcaria sulcata*.

**Specimens examined (8 thalli):** – CHINA. Tibet, prov. Xizang: Nyainqêntanglha Shan, 370 km E of Lhasa, 55 km NNE of Nyingchi, river valley at the west side of Gyala Peri, 29°59'N/94°53'E, 2500 m alt., deciduous trees along the river-bank, on dead *Salix*, 16-17.VIII.1994, leg. W. Obermayer (06339; 06843; 08659d); between the villages Tongjug and Tangmai, 30°0'N / 94°58'E, 2300 - 2400 m alt., deciduous trees along the river, on *Salix*, 23.VIII. 1994, W. Obermayer (06844;
Chemical race 3a

Chemistry: Atranorin (major, rarely present in traces), 2-hydroxyvirensic acid (major), 2-hydroxyconvirensic acid (major / minor / traces or not detected), virensic acid (minor / traces), unknowns (traces or not detected)

Notes: 2-Hydroxyvirensic acid and 2-hydroxyconvirensic acid are visible (if sufficiently concentrated) on TLC-plates in daylight before spraying (greenish-grey) and develop a dark-grey colour in daylight after spraying (like virensic acid) as well as a dark-violet (=quenching) colour in UV_366 after spraying (whereas virensic acid is dark-brown). When sufficiently concentrated, 2-hydroxyvirensic acid appears always as a spot with a trailing tail in solvent system A. Depending on its concentration this compound exhibits different mobility (at high concentration it is closer to norstictic acid while at low concentration it is closer to the origin). Chemical race 3a can be separated from all of the other chemical races even after applying the acetone as it forms a yellow colour at the centre of the spot rather than at the margin, whilst races with vulpinic acid produce a yellow ring at the margin. Virensic acid may also be present in rather low concentrations.

The 2-hydroxyvirensic acid race of Sulcaria sulcata seems to be a typical element of the southeast Tibetan region. Further chemical investigations on material from other localities may confirm this suggestion.

Specimens examined (30 thalli): –CHINA. Tibet, prov. Xizang: Nyanqentanglha Shan, 360 km E of Lhasa near the bend of the river Tsangpo, N-side of Gyala Peri, 5 km S of Tongjug village, 29°56'N/94°54'E, 3350 m alt., on Berberis, 18.VIII.1994, W. Obermayer (08660); 9 km S of Tongjug village, 29°54-55'N / 94°52-53', 3200 - 3500 m alt., Rhododendron-Abies forest, on Prunus, 20.VIII.1994, W. Obermayer (06462-109; 113 with vulpinic acid as a major compound, and only traces of atranorin).

Chemical race 3b

Chemistry: Atranorin (major), virensic acid (major), 2-hydroxyvirensic acid (minor [in apothecia] / traces or not detected), convirensic acid (minor / traces), unknowns (traces or not detected)

Notes: Some samples with large fruiting bodies contained 2-hydroxyvirensic acid in significantly higher amounts (but with concentrations still lower than that of virensic acid), than those from the same thalline branches lacking apothecia, where only traces of this substance were present.
Fig. 2: Recrystallized lichen substances in G.E. from Sulcaaria sulcata: A, Psoromic acid (Poelt N86-L439); B, 2-methoxy-psoromic acid (Obermayer 08661).
The virensic acid race seems to be very rare in south-east Tibet (2 thalli), but probably more common at the south facing slopes of the Himalayas (seven thalli from different localities in Nepal, Sikkim and Bhutan).

**Specimens examined** (9 thalli): –CHINA. Tibet, prov. Xizang: Nyainqêntanglha Shan, 370 km E of Lhasa, 55 km NNE of Nyingchi, river valley at the west side of Gyala Peri, 29°59'N/94°53', 2500 m alt., deciduous trees along the river-bank, on *Salix*, 17.VIII.1994, W. Obermayer (08659c); near the bend of the river Tsangpo, N-side of the mountain Gyala Peri, between the villages Tongjug and Tangmai, 30°01'N / 94°58', 2300 - 2400 m alt., deciduous trees along the river, on *Populus/Fraxinus*, 23.VIII.1994, W. Obermayer (06842). –NEPAL. Central Himalaya, Langtang Area, slopes toward Pang Sang Lekh, E of Khangjung, pasture, rocks, mixed *Abies* forest, 3200 - 3300 m alt., 29.8.1986, J. Poelt (N86-L1298; N86-L724); W of Sherpa, 3000 m alt., on *Quercus semicarpifolia*, 7.5.1986, G. & S. Miehe (1588); Trisuli Khola, below Sching Gompa, 3100 m, *Abies* forest, 7.4.1986, G & S. Miehe (644). –INDIA, Sikkim: Himalaya, südlich des Kanchenjunga, nahe der Grenze nach Nepal, am Weg Dzongri to Tshoka to Bakhim, 3300 m alt., 7.4. 1978, E. Albertshofer (s.n.). –BHUTAN, forest slopes above Sengor, NW of Mongar, 27°23'N / 91°01'E, 3500 m alt., 14 June 1979, A.J.C. Grierson & D.G. Long (1912a,b) (E).

**Discussion**

Although AwaSTHI & AwaSTHI (1985) have presented detailed descriptions of both the morphological and anatomical features of *Sulcaria sulcata*, they did not report the variable chemistry, mentioning only atranorin and psoromic acid. The authors also drew attention to the rather variable morphology of thalli of *Sulcaria sulcata*. They presumed that ecological adaptation may be responsible for the broad range of morphological diversity. In the past this has led to the description of some further, (infra)specific taxa including f. *ciliata* Hue, f. *indica* Gyelnik, var. *barbata* Hawksw., and *Alectoria spinosa* Tayl. ex Hook.

Aging processes may also influence the habit of the thalli. Young thalli, for example, are much more densely branched than older ones, which instead bear many more and larger fruiting bodies. There is also a wide range in the size of the sulcus, varying from a rather narrow pseudocyphellate one (as in *Bryoria*) to a wide, gaping one, exposing the medulla composed of cross beam like struts (Fig. 3C). The colour of the thalli varies from greyish-white (the whole thallus, or at least main branches) to dark, greyish-brown, frequently with a violet-brown tinge (the whole thallus, or at least tips of the lateral branches). Furthermore, the colour of the thallus-base ranges from greyish-white to almost black.

In several specimens, the holdfasts of three or four distinct thalli with different chemistry (chemical races 1a and 2a) arise from within a few millimeters of one another (e.g. Obermayer 08653a,b with psoromic acid; 08653c with 2-methoxypsoromic acid). This fact leads to the presumption that these thalli may have developed from a number of spores originating from the same ascus. If this were so, the lichen must be able to produce spores with different genes expressing different chemical compounds within one ascus (and possibly different morphotypes). In some cases, different branches of the same thallus have been tested.
Fig. 3: Sulcaria sulcata (Obermayer 08654). Lowerside with typical furrow like pseudo-cyphellae.

chemically to determine whether one thallus can produce different chemical races. However, apart from the vulpinic acid races mentioned above (chemical race 1b and 2b) the individual thallus fragments were found to be homogeneous in the chemistry as regards the major compounds. The same was also observed for branches with or without fruiting bodies (with the exception of the vulpinic acid races and the virensic acid race, where the concentration of the minor compound...
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was distinctly higher in thallus fragments with apothecia - see notes under chemical race 3b).

The many chemical and morphological characters cited above did not appear to be correlated with one another. This, together with the fact that *Sulcaria sulcata* is very commonly fertile (consistent with major variability of features within the one species), the chemically distinct taxa are treated as chemical races of a single species.

**Parasites**

One lichenicolous heterobasidiomycete, *Tremella sulcariae* Diederich & M.S. Christ. (see DIEDERICH 1996: 162 - 164) has been reported from the Chinese province of Yunnan and from Nepal. This parasite, as well as another which belongs to the genus *Lichenostigma*, occur on some of the present specimens. They are currently the subject of further investigations in collaboration with Josef Hafellner (Graz).

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**References**


