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### Lichen Acids of the *Stereocaulon ramulosum* Group in Central East Africa

**Abstract.** *Chemical investigations have been carried out on lichen specimens of the Stereocaulon ramulosum group from Central East Africa (Zaire/Kivu, Rwanda and Burundi). Thin-layer chromatography and UV spectrum studies have shown the existence of atranorin and perlatolic acid in both Stereocaulon ramulosum (Sw.) Räsusch. and Stereocaulon meyeri Stein; specimens identified as S. meyeri subsp. atlanticum Lamb contain atranorin, perlatolic, stictic and norstictic acids.*

A large amount of *Stereocaulon* has been recently collected in the mountains of Central East Africa (Zaire/Kivu, Rwanda and Burundi). The specimens belonging to the *S. ramulosum* group turned out to be difficult to identify without chemical data. This paper deals with that chemical investigation.

#### MATERIALS AND METHODS

The collection is preserved at the Department of Botany in the University of Liège (LG). The studied specimens belonging to the *Stereocaulon ramulosum* group are numbered "Lambinon 71/1343, 72/252, 72/263, 72/264, 72/265, 72/328, 72/363, 72/690, 72/692, 72/787, 72/806, 72/811t, 72/827, 72/828, 72/900, 72/901, 74/1545, 74/1546."

Dried and pulverized material was successively extracted in the following phases: light petroleum ether ( $d = 0.60$ ), peroxide free anhydrous ethyl ether and anhydrous acetone. Two thin-



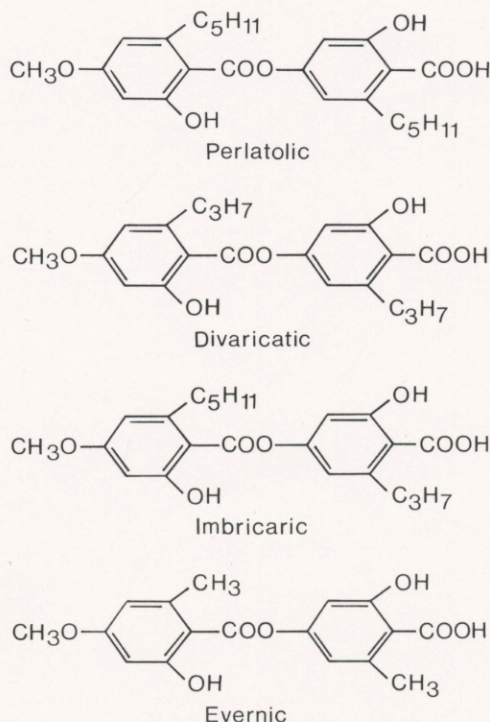


FIGURE 1. Acid formulae.

layer chromatographies were carried out: on Kieselgel Merck 60 F<sub>254</sub> (art. 5554) plates with the phases A, B, C proposed by Culberson and Kristinsson (1970) as developing solvents and on Kieselgel Merck HF<sub>254</sub> (type 50, art. 7739) plates prepared in a solution 0.2 M of NaAc with Pastuska solution as developing solvent (method suggested by Ramaut, 1967). Examination under UV light ( $\lambda = 254$  nm), color tests (alcoholic p-phenylenediamine solution, aqueous solution of E. B. reagent), determination of  $R_f$  values and comparison with known compounds have been used for identification. Spots obtained by chromatography were eluted in ether for registration of UV spectra. Eluates were filtered, evaporated and dried under vacuum and the precipitates were dissolved in 95% ethanol. UV spectra were registered using a Cary 17 R spectrophotometer.

TABLE 1.  $R_f$  data for the substances detected in lichens from the *Stereocaulon ramulosum* group (including values for reference atranorin and norstictic acid).

Lichen acids	$R_f$			HF <sub>254</sub> NaAc Plates Pastuska solution
	Solvent System (standardized method)			
	A	B	C	
Atranorin <sup>a</sup>	.92	.45	.50	.96
Norstictic <sup>b</sup>	.65	.17	.17	.56
Perlatolic	.73	.55	.40	.46
Unknown		.28-.29	.35-.32	.40

<sup>a</sup> from lichens or reference.

<sup>b</sup> reference.



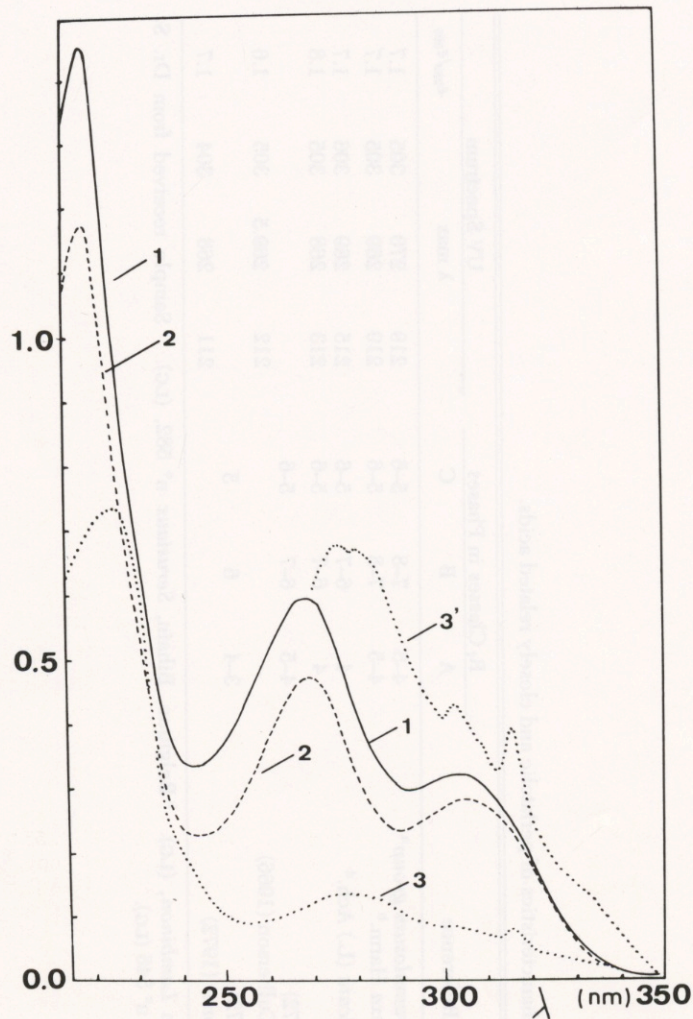


FIGURE 2. UV spectrum of perlatolic acid extracted from *Cladonia impexa* Harm. (1), and from *Stereocaulon ramulosum* (Sw.) Räscher. (2) and of an unidentified substance found in low concentrations with perlatolic acid in *Stereocaulon ramulosum* (Sw.) Räscher. (at two different concentrations) (3, 3').

Acid hydrolysis was performed by adding five to six drops of concentrated sulfuric acid to a small amount (less than 1 mg) of the substance. After 10 min at room temperature, crushed ice was added, and the solution was extracted with diethylether. The ether layer was chromatographed using TLC with Kurokawa phase as developing solvent: n-hexane, diethyl-ether, formic acid (5:4:1) (Kurokawa & Jinzenji, 1965) and paper chromatography with Whatman No. 1 paper and ammonium hydroxide-saturated n-butanol as developing solvent.

#### RESULTS AND CONCLUSIONS

The determination of the chemical constitution of lichens from the *Stereocaulon ramulosum* group by paper and thin-layer chromatography shows the existence of



TABLE 2.  $R_f$  values and UV spectrum characteristics of perlatic and closely related acids.

Acid	Source or Reference	$R_f$ Classes in Phases			UV Spectrum		$\epsilon_{269}/\epsilon_{305}$
		A	B	C	$\lambda$ max		
Perlatic	<i>Stereocaulon ramulosum</i> group <sup>a</sup>	4-5	7-8	5-6	219	270	1.7
	<i>Cladonia impexa</i> Harm. <sup>b</sup>	4-5	7-8	5-6	219	269	1.7
Divaricatic Pure <sup>c</sup>	<i>Evermia dicaricata</i> (L.) Ach. <sup>d</sup>	4	6-7	5-6	215	269	1.7
		4	6-7	5-6	213	268	1.8
Imbricatic	Culberson (1972)	4-5	6-7	5-6			
	Culberson & Culberson (1966)				212	269.5	1.6
Evermic	Culberson (1972)	3-4	6	5			
	Ramaut & Thonar (1972)				211	268	1.7

<sup>a</sup> From Central East Africa (Collection *Lambinon*, (LG). <sup>b</sup> Belgique, Bihain, *Serustiaux* n° 582, (LG). <sup>c</sup> Sample received from Dr. S. Huneck. <sup>d</sup> France *Ve zda* Lich. Sel. Exsicc. n° 546 (LG).



TABLE 3. Published data on the chemical constitution of *Stereocaulon ramulosum* (Sw.) Räsch.

Authors	Origin of Material	Chemical Constitution	Assumed Chemical Strain
Lamb (1951)	?	Atranorin	I
	?	Atranorin, norstictic acid	II
Huneck & Follmann (1967)	Chile	Atranorin	I
Cambie (1968)	New Zealand	Atranorin, anziaic and dimethyl-etheranziaic acids	IV
Fox & Huneck (1970)	New Zealand	Atranorin, perlatolic acid	III
	Jamaica	Atranorin, perlatolic acid	III
Fox, Follmann & Huneck (1971)	Costa Rica	Atranorin, perlatolic acid	III
	Argentina	Atranorin, perlatolic acid	III
Duvigneaud (1942)	Campbell Island	Atranorin, divaricatic acid, "ramulosin"	see text

atranorin and perlatolic acid in both *Stereocaulon ramulosum* (Sw.) Räsch. and *S. meyeri* Stein and of atranorin, stictic, norstictic and perlatolic acids in *S. meyeri* subsp. *atlanticum* Lamb.  $R_f$  data for the substances detected in lichens from the *Stereocaulon ramulosum* group are given in Table 1 for plates prepared in the solvent systems A, B and C of the standardized method of Culberson and Kristinsson (including values for reference atranorin and norstictic acid) and for Kieselgel Merck HF<sub>254</sub> plates prepared in 0.2 M NaAc solution with Pastuska solution as developing solvent. In the last conditions a spot with  $R_f$  value slightly lower than perlatolic acid was detected. It was eluted and the UV spectrum (in ethanol 95%) was registered (Fig. 2, curves 3 and 3'). When on Kieselgel NaAc plates, this compound was the only one to be detected at  $R_f$  values lower than perlatolic acid; nevertheless, it possibly corresponds to a mixture. Indeed, as shown in Table 1, TLC of the same extracts using the standardized method of Culberson and Kristinsson revealed in solvent systems B and C the occurrence of two spots at  $R_f$  values lower than perlatolic acid. These spots were not detected in solvent system A; they correspond to very low concentrated lichen substances and it was not possible to measure the UV absorption spectrum. UV spectroscopy (Table 2, Fig. 2) and analysis of hydrolysis products have been used to check identification of perlatolic acid. As a matter of fact, this acid is close to divaricatic, imbricatic and evernic acids (Fig. 1). The  $R_f$  values of these compounds increase slightly as the total length of the side-chains on the ring increases. As shown by Culberson (1972) and by Culberson and Culberson (1966), UV spectra and chromatography of hydrolysis products can be most helpful in that matter. We have compared hydrolysis products of perlatolic acid (extracted from *Cladonia impexa* Harm. and from our *Stereocaulon ramulosum* group) of imbricatic acid (pure sample received from Dr. S. Huneck) and of divaricatic acid (extracted from *Evernia divaricata* (L.) Ach.)

Published records on the chemical constitution of *Stereocaulon ramulosum* are presented in Table 3. Data published by Duvigneaud (1942) are not accepted as they are based on Asahina's method of microcrystallizations: divaricatic acid might have been confused with closely related acids, e.g. perlatolic acid, and the exact identity of "ramulosin" is naturally unknown. Four chemical strains are recognized for that



species: strain III (atranorin and perlatolic acid) is the most common and the most widespread.

Stictic acid usually in conjunction with atranorin has been found in *S. meyeri* by Lamb (1951) (described as chemical strain II, and no ch. str. I can be traced in the paper). The latter is assumed to contain atranorin only (Frey, 1967). Duvigneaud (1956) studying material from the same area as ours, described three new species (*S. karisimbiense*, *S. penicillium* and *S. haumanianum*) that are synonyms of *S. meyeri*. Chemical constitution of those species is said to be atranorin or atranorin and "ramulosin." Since this information is based on Asahina's method, it can not be accepted.

According to the original description, based on material from Açores Islands (Poelt, 1969), *S. meyeri* subsp. *atlanticum* contains atranorin and stictic acid. The material examined contains a large amount of atranorin and stictic acid, and norstictic and perlatolic acids as traces. It might constitute a more active chemical phase (sensu Lamb & Ward, 1974).

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