Nobuo HAMADA*: **Distribution pattern of two lichen substances, evernic and obtusatic acids, in the thallus of *Ramalina***

浜田信夫*: 2種の地衣成分エベルン酸とオブツザート酸の地衣体中での分布パターン

The distribution pattern in the thallus of lichen substances has been examined by many investigators (Culberson & Culberson 1958, Mirando & Fahselt 1978, Hamada 1982). Such studies are necessary not only from a taxonomical viewpoint, but also to elucidate the physiological meaning and accumulation mechanism of lichen substances. Up to the present time, however, the distribution pattern of two lichen substances co-existing in a thallus, especially of those with similar structure, has not been studied, because it was difficult to separate lichen substances from each other. But the recent high performance liquid chromatography (HPLC) enables us to separate and measure each lichen substance.

Archer (1981) reported that both merochlorophaeic and 4-*O*-methylcryptochlorophaeic acids, which are orcinol *meta*-depsides with similar structure, were different in content among various parts of the podetia, but the ratio of these two acids was relatively constant in all parts of podetia of *Cladonia merochlorophaea*. This is the only report dealing with the distribution pattern of two lichen substances with similar structure co-existing in a thallus. Here, I examined by using HPLC the distribution pattern of orcinol *para*-depsides, evernic and obtusatic acids (Fig. 1), the contents in the apothecium and various parts of ramulus of Japanese collections of three species of *Ramalina*, *R. yasudae*, *R. commixta*, and *R. subgeniculata*.

Materials and methods Experimental materials were collected at the following localities: *R. yasudae*: Mine, Tsushima Isl., Kyushu, on rock, alt, ca 5 m, June 1979, and other 5 localities during 1977-1982; *R. commixta*: Oiwake, Mt. Tsurugi, Shikoku, on bark, alt. ca 1250 m, September 1982, and other 2 localities during 1980-1982; *R. subgeniculata*: Kamegamori, Mt. Ishizuchi, Shikoku, on

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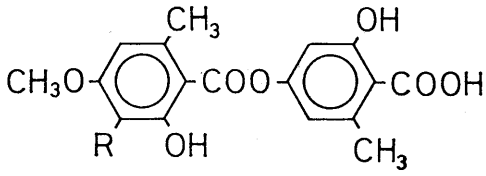

1) R=H 2) R=CH_3

Fig. 1. Structure of evernic (1) and obtusatic acids (2).

bark, alt. ca 1450 m, September 1982, and other 2 localities during 1980–1982.

More than 6 unbroken ramuli free of dust and soil, each from a separate thallus, were collected at each locality. Ramuli, each more than 10 mg in dry weight, having apical apothecia and a few branches, were used for chemical analysis. Apical apothecia were separated from the ramuli and each ramulus was cut transversally into three parts at equal intervals. Other apothecia and branches were cut off from each ramulus. The sample thus prepared was extracted with 2 m*l* absolute methanol for 48 hr at room temperature and analyzed by HPLC (Fig. 2).

Ten μl of each extract solution was chromatographed on a 15 cm×4.5 mm Zorbax ODS column (Dupont Instruments) with a water-methanol-acetic acid (20 : 80 : 2.0, v/v/v) at 1.0 m*l*/min (800 kg/cm^2). Chromatographic peaks of

Tab. 1. Concentration (percentage in dry weight) of lichen substances in the ramulus.

		Evernic	Obtusatic	Ratio (Ev/Ob)	Dry weight (mg/ramulus)
R. yasudae	apothecium	3.03±0.64	0.25±0.03	13.56±4.25	3.5±0.5
	tip	1.52±0.20	0.55±0.09	3.11±0.43	2.7±0.6
	middle	0.62±0.05	0.42±0.03	1.52±0.14	2.2±0.2
	base	0.31±0.07	0.21±0.03	1.49±0.19	1.9±0.3
R. commixta	apothecium	2.55±0.37	0.39±0.09	7.05±0.78	2.8±0.6
	tip	1.76±0.49	0.39±0.07	4.26±0.40	3.0±0.7
	middle	1.32±0.31	0.43±0.09	2.99±0.21	3.3±0.3
	base	0.89±0.19	0.32±0.05	2.76±0.44	2.7±0.4
R. subgeniculata	apothecium	2.69±0.39	0.41±0.08	7.24±0.89	3.7±0.6
	tip	1.21±0.03	0.32±0.04	3.90±0.30	2.6±0.2
	middle	1.03±0.18	0.33±0.07	3.23±0.33	2.6±0.3
	base	0.92±0.11	0.31±0.03	2.99±0.32	2.1±0.3

lichen substances were monitored by the absorption of 254 nm light, and was compared with the peak of the authentic substances whose chemical structure and purity had been confirmed by GC-MS and TLC. The height of the absorption peak and the concentration of two authentic depsides were found to show linear relationship as mentioned by Nourish & Oliver (1976). Therefore, the concentration was calculated from the height of the peak.

Results Only the data obtained by using the materials collected at three localities described in detail in "Materials and methods" are shown in Tab. 1. Similar results were obtained with the materials collected at other localities. In all of three species examined, the concentration of evernic acid was highest in the apothecium followed by the apical part of the ramulus, and decreased from the apical to the basal part of the ramulus. On the other hand, the distribution pattern of obtusatic acid differed with the species. In *R. yasudae*, the concentration was highest in the apical part of the ramulus, and decreased toward the base. In this species, the concentration of obtusatic acid in the apical apothecium was lower than that in the apical or middle part of the ramulus. On the other hand, in *R. commixta* and *R. subgeniculata*, the concentration of obtusatic acid was similar in all parts of ramulus. The ratio of the content of two depsides (evernic/obtusatic) was highest in the apothecia followed by the apical part of the ramulus, and decreased from the apical to the basal part of the ramulus in all three species examined.

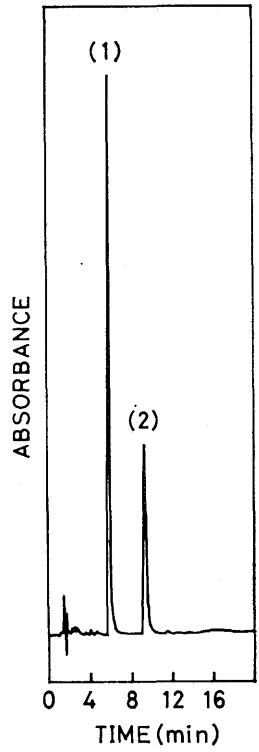

Fig. 2. HPLC chart of evernic (1) and obtusatic acids (2) extracted from *Ramalina yasudae*.

Discussion In *Ramalina*, the distribution patterns in the ramulus of two orcinol *para*-depsides, evernic and obtusatic acids, were quite different (Tab. 1). The concentration of salazinic acid in *Ramalina siliquosa* is highest at the top of ramulus decreasing

toward the base (Hamada 1982), just as that of evernic acid in three *Ramalina* species examined here, and a similar distribution pattern has been reported for many lichen substances in various lichens (Fedoseev & Yakimov 1960, Mirando & Fahselt 1978, Archer 1981). Because the ramulus of lichens shows apical growth (Kärenlampi 1971) and the apical apothecium was the youngest in the thallus, these lichen substances including evernic acid and salazinic acid are considered to be produced only in the actively growing part and may be degraded gradually with maturation of the tissue as suggested by Mirando & Fahselt (1978). One may argue that the decrease in the concentration (% in dry weight) of lichen substances from the younger to older part of the ramulus is caused by the increase with age of the dry matter relative to the amount lichen substances which may be stable. However, the dry weight of the ramulus at the basal one third is similar to, or rather smaller than that at the apical one third, and a great difference between the concentrations of evernic acid at the top and that at the base of the ramulus (Tab. 1) can not be explained by the increase in dry matter with tissue maturation.

The concentration of obtusatic acid in *R. commixta* and *R. subgeniculata* was rather uniform in all parts of the ramulus, while that of evernic acid decreased from the top (younger part) to the base (older part) of the ramulus. Since obtusatic acid is produced from evernic acid by methylation (Follmann & Huneck 1969), and evernic acid is considered to be produced only at the actively growing part, obtusatic acid may also be produced at an actively growing part. In the above two species, obtusatic acid may remain unchanged during maturation giving a uniform distribution in all parts of ramulus, while evernic acid is degraded in old tissue. Evernic acid may be stabilized by methylation.

In *R. yasudae*, on the contrary, the distribution pattern of obtusatic acid significantly different from that in the other two species. The concentration was low in apothecia, and was high at the apical part of ramulus, decreasing toward the base. What causes such a difference between the species is uncertain.

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2種類の地衣成分，エベルン酸とオブツザート酸を同時に含むカラタチゴケ属の日本産地衣類は3種知られている。従来，このような構造の類似した2種類の成分の地衣体中での分布パターンを調べるのは困難であったが，高速液体クロマトグラフィーを用いることによって可能になった。3種の地衣類ともエベルン酸は先端の方ほど量が多く，基部の方では少なかった。一方，オブツザート酸は分布パターンが種によって異なっていた。すなわち，オブツザート酸の分布パターンがエベルン酸と同じ種と，部位による差の見られない種があった。また，エベルン酸のオブツザート酸に対する量比は部位によって異なるが，この比は先端ほど3種とも高かった。このような量比の差は成分の生合成特性および安定性によるものかもしれない。