Nobuo HAMADA*: Distribution of the *Ramalina siliquosa* complex (lichens) having depsidone-negative ramuli

浜田信夫*: デプシドンを含まない分枝をもつハマカラタチゴケ (地衣類) の分布

W.L. Culberson (1967) recognized six species in the *R. siliquosa* complex distributed in Europe and considered each of them to be characterized by the presence of one of the five medullary β-orcinol depsidones (salazinic, protocetraric, stictic, norstictic or hypoprotocetraric acid) or the absence of all of them. Later, Sheard (1978) considered this species complex to comprise two species which are chemically distinct (one containing either hypoprotocetraric, protocetraric or salazinic acid, and the other stictic or norstictic acid), and included depsidone-negative individuals in either one of these two species, based on their morphological and anatomical features. W.L. Culberson (1970) reported that the plants obtained from Japanese collections of the *R. siliquosa* complex contain either salazinic acid or protocetraric acid, but some plants contain no medullary depsidone. Salazinic acid has a chemical structure similar to that of protocetraric acid (Fig. 1), and is produced from protocetraric acid by oxidation (Sheard 1978).

In my previous papers (Hamada 1982b, 1984), the content of salazinic acid in *R. siliquosa* was shown to vary with the temperature of the habitat. In my another paper (Hamada 1983), it was also mentioned that salazinic acid usually contained in the apothecia of *R. subbreviuscula* was absent in some apothecia collected at cold locations. Thus the presence or absence of salazinic acid was considered to be determined by some environmental factors, probably temperature.

It is probable that the presence or absence of protocetraric acid which is another taxonomic marker of *R. siliquosa* seems to be also controlled by environmental factors. Because the content of protocetraric acid is very low, the content of protocetraric acid is not quantitatively analyzable, but the pres-

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ence or absence of protocetraric acid was qualitatively detectable by TLC method. In the following experiments, therefore, I examined the distribution of depsidone-positive and -negative thalli of *R. siliquosa* in Japan, and analyzed the factors which may cause the occurrence of depsidone-negative plants. The content of salazinic acid was also examined in relation to the frequency of the occurrence of depsidone-negative thalli.

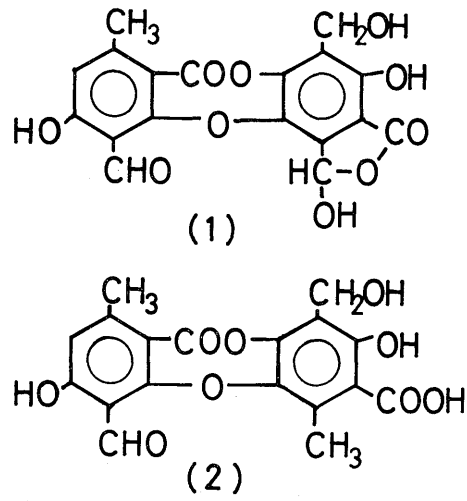


Fig. 1. (1) Salazinic acid, (2) protocetraric acid.

Material and methods Plant materials. Plants of the *R. siliquosa* complex were collected at 46 sites shown in Figs. 2 and 3, and Table 3 and located between the latitude of 39°10′N, Kinkazan Isl., Miyagi Pref. (northern limit of the distribution of this complex in Japan) and 32°20′N, Shibushi-cho, Kagoshima Pref. in 1978–1981. At 46 sites examined, either plants containing salazinic acid or ones containing protocetraric acid were found, and there was no site where only depsidone-negative plants were collected. However, both plants containing salazinic acid and ones containing protocetraric acid were found at some sites in Izu Penin., Shizuoka Pref. and Inubo Promontory, Chiba Pref. It was impossible to determine whether depsidone-negative plants are salazinic acid-negative or protocetraric acid-negative in plants collected at these sites. Therefore, these sites and ones near these sites (within 1 km) were excluded in the present study.

An unbroken ramulus from each of 30–50 lichen thalli collected at each location, free of dust and soil, having few branches, no apothecia, and 4–8 mg in dry weight, was selected and used for the chemical analysis. At each of the six sites shown in Tables 2 and 3, however, 19–35 thalli, each with 10 or more ramuli and each heavier than 4 mg in dry weight, were collected and examined for the presence or absence of depsidone in each ramulus.

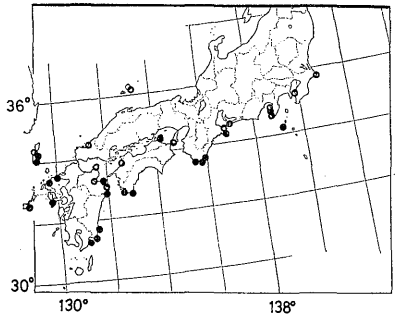
As has been shown before (Hamada 1982b), the salazinic acid content of the lichens growing on dark-colored rocks was always higher than that of the lichens growing on light-colored rocks in the same locality. This was attributed to the difference between the temperatures on or near the surface of the light- and dark-colored rocks (Hamada 1982b). Therefore, the color of the rocks from which the lichens had been collected was noted. In the present experiment, yellow and pale-colored sedimentary rocks were regarded as light-colored rocks, but the rocks with intermediate color were excluded from the comparison between light- and dark-colored rocks.

Methods of the chemical analysis. The standardized thin-layer chromatography (TLC) method of Culberson & Kristinsson (1970) as modified by C. F. Culberson (1972) has been used for detecting the presence of depsidones, salazinic and protocetraric acids. Each ramulus to be examined was extracted with 2 ml acetone and 25 μl of the extract was spotted on 0.3 mm thick TLC plate and developed with three kinds of Culberson's solvent, A (benzene-dioxane-acetic acid=130 : 45 : 5), B (hexane-diethyl ether-formic acid=130 : 80 : 20) and C (toluene-acetic acid=200 : 30). Depsidones were detected by UV light (254 nm).

For the ramuli in which salazinic acid was detected, the salazinic acid content was measured by the method described before (Hamada 1982a). On the other hand, for the ramuli in which neither salazinic nor protocetraric acid was detected, further analysis was made. That is, all of the remaining extract was condensed and spotted on the TLC plate and developed by solvent C which is the best solvent to detect a very small amount of salazinic or protocetraric acid. Only the ramuli in which neither salazinic nor protocetraric acid was detected by these procedures were regarded as depsidone-negative ramuli.

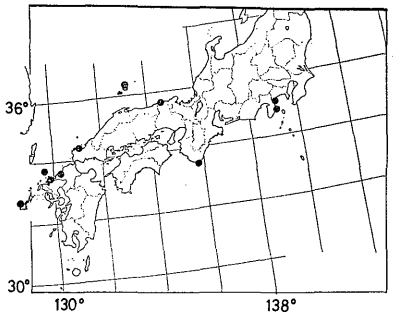
Results and Discussion The site where plants with salazinic acid-negative ramuli were found are shown in Figs. 2 and 3, Fig. 2 for light-colored rocks and Fig. 3 for dark-colored ones. At all sites where I found salazinic acid-negative ramuli, salazinic acid-positive ramuli were also found at various ratios. For both light-colored and dark-colored rocks, sites where plants with salazinic acid-negative ramuli were found are distributed in a more northern area than that where only plants with salazinic acid-positive ramuli were found are distributed. This suggests that the plants having salazinic acid-negative ramuli are found more frequently in colder areas than in warmer areas.

Table 1 which shows the relationship between the occurrence of plants



Light colored rock

Fig. 2. The sites where *R. siliquosa* were collected from light-colored rocks. ●: All plants collected contained depsidone (salazinic acid). ○: Some of the plants collected did not contain depsidone.



Dark colored rock

Fig. 3. The sites where *R. siliquosa* were collected from dark-colored rocks. ●: All plants collected contained depsidone (salazinic acid). ○: Some of the plants collected did not contain depsidone.

Table 1. Number of sites, where plants with salazinic acid-negative and only salazinic acid-positive ramuli were found, at different classes of annual mean temperature.

Color of rocks	Salazinic acid	13.1-14.0°	14.1-15.0°	15.1-16.0°	16.1-17.0°	17.1°-
Light	negative	1	2	11	—	—
	positive	—	1	8	8	3
Dark	negative	1	1	—	—	—
	positive	1	—	3	4	—

with salazinic acid-negative ramuli and the annual mean temperature supports this idea. Plants with salazinic acid-negative ramuli were found only at the locations where the annual mean temperature was lower than 16°C on light-colored rocks and lower than 15°C on dark-colored rocks. The slight difference in the temperature range which allowed the appearance of plants with depsidone-negative ramuli may be caused by the difference in temperature on or near the surface of light-colored and dark-colored rocks (Hamada 1982b).

In the present study, the relationship between the concentration of salazinic acid in the plants and the frequency of occurrence of plants with salazinic acid-

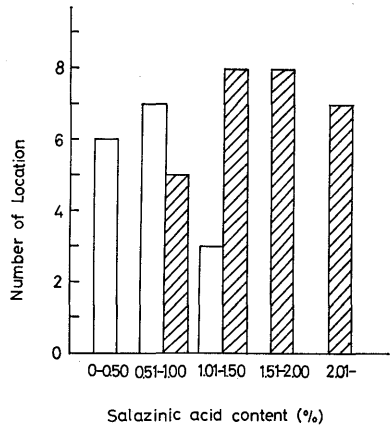


Fig. 4. Comparison of number of sites, where plants with salazinic acid-negative (open columns) and only salazinic acid-positive ramuli (shaded columns) were found, at different classes of average contents of salazinic acid.

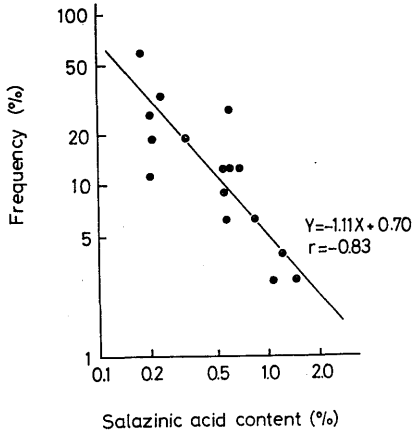


Fig. 5. The relationship between the frequency of the occurrence of salazinic acid-negative plants and the salazinic acid content.

negative ramuli is also examined (Fig. 4). At the sites where the average content of salazinic acid in plants was greater than 1.5%, no plant with salazinic acid-negative ramuli were detected. On the contrary, at all 6 sites where the average salazinic acid content of all plants examined was lower than 0.5%, plants with salazinic acid-negative ramuli were found together with those with positive ramuli. Only in 3 out of 11 sites where the average salazinic acid content of the plants examined was 1.01-1.50%, and in 7 out of 12 sites where the average salazinic acid content of the plants examined were 0.51-1.00%, plants with salazinic acid-negative ramuli were detected.

Fig. 5 shows the relationship between the ratio of plants with salazinic acid-negative ramuli to all plants examined and the average content of salazinic acid at each site, where salazinic acid-negative plants were found. Negative correlation was observed between these values. That is, the lower the salazinic acid content, the higher the percentage of plants with salazinic acid-negative ramuli.

Because the content of salazinic acid has been shown to be higher, the higher the annual mean temperature of the habitat (Hamada 1982b), the results shown in Figs. 2 and 3 again support the idea that the occurrence of salaziinc acid-negative plants is caused by low temperature.

Table 2. Number of thalli at different classes of ratios (%) of salazinic acid containing-ramuli to all ramuli of the thallus at four sites.

Site	100	99–80	79–60	59–40	39–20	19–1	0(%)	Total	Annual mean temp.
Inuki (Shimane)[a]	18	6	1	2	1	1	1	30	13.6°C
Igo (Shimane)[b]	21	4	2	—	—	1	2	30	13.6°C
Inubo Prom. (Chiba)[a]	15	5	4	3	1	—	5	33	15.2°C
Gogo Isl. (Ehime)[a]	16	5	2	1	1	—	—	25	15.7°C

[a]—light-colored rocks [b]—dark-colored rocks

Table 3. Number of thalli at different classes of ratios (%) of protocetraric acid containing-ramuli to all ramuli of the thallus on light-colored rocks at two sites.

Site	100	99–80	79–60	59–40	39–20	19–1	0(%)	Total	Annual mean temp.
Kinkazan Isl. (Miyagi)	2	2	—	3	2	4	22	35	11.2°C
Hokkawa (Shizuoka)	19	—	—	—	—	—	—	19	16.1°C

Even in the same thallus, on the other hand, I found both depsidone-positive and depsidone-negative ramuli. Tables 2 and 3 show the number of thalli which have depsidone-positive ramuli at various percentages. This seems to show that the presence or absence of depsidone may be determined physiologically. The difference in microclimate (e.g. temperature) surrounding each ramulus seems to cause such difference.

Comparison of the geographical distributions of salazinic acid-containing and protocetraric acid-containing plants is difficult because protocetraric acid-containing plants were found only at two sites (see Table 3). At Hokkawa, all ramuli examined contained protocetraric acid, and I could not find any protocetraric acid-negative ramuli. By contrast, at Kinkazan Isl., many of the ramuli had neither protocetraric nor salazinic acid, and only a small number of thalli had protocetraric acid-containing ramuli. Among these plants, both protocetraric acid-containing and -negative ramuli were found in the thallus suggesting that

the occurrence of protocetraric acid-negative plants is caused by physiological factors. The ratio of protocetraric acid-negative ramuli to all ramuli examined in each thallus are shown in Table 3. Annual mean temperature at Hokkawa is 16.1°C, and that at Kinkazan Isl. is 11.5°C. The occurrence of protocetraric acid-negative plants may be also considered to be caused by low temperature.

In conclusion, the content of depsidone (salazinic and protocetraric acids) in the thallus of *R. siliquosa* seems to be influenced by temperature; the depsidone-negative ramulus may be formed under low temperature. Thus, depsidone-positive and -negative plants of *R. siliquosa* can not be separated taxonomically without any difference in other characters.

The author wishes to express his deepest appreciation to Prof. A. Takimoto, Kyoto Univ. for his critical reading of the manuscript and valuable suggestions.

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日本産ハマカラタチゴケ（地衣類）には，髄層成分としてデプシドンであるサラチン酸，あるいはプロトセトラール酸を含むものと，デプシドンを全く含まないものがある。デプシドンを含まないものは，暖かい地域より寒い地域でより多く見られ，1個の地衣

体を構成する分枝の間でも，デプシドンを含む分枝と含まない分枝が見られる。さらに，普通種であるサラチン酸の方を含むものについて着目すると，サラチン酸の平均含量の高い生育地（暖かい所）では，デプシドンを含まない分枝は見られないのに対して，サラチン酸の平均含量の低い生育地（寒い所）では，デプシドンを含まない分枝はしばしば見られる。また，より平均含量の低い生育地ほど高頻度でデプシドンを含まない分枝が見られる。ゆえに，ハマカラタチゴケにおいては，デプシドンを含まないものは，低温の影響によって生ずると考えられる。

○*Parmelia diffractaica* はブラジルにも産する（黒川 道） Syo KUROKAWA: *Parmelia diffractaica* (Parmeliaceae, Lichenes) new to Brazil

1971年にブラジルの Parana 州で採集したウメノキゴケ属の1標本は，量的にも充分とは云えず，また後述するように，従来知られている分布とあまりにもかけ離れているために，最終的な同定を保留してきた。最近この標本を精査する機会があり，標本はやや小さいが，その特徴を充分にそなえていることから，*Parmelia diffractaica* Essl. と同定したのでここに報告する。

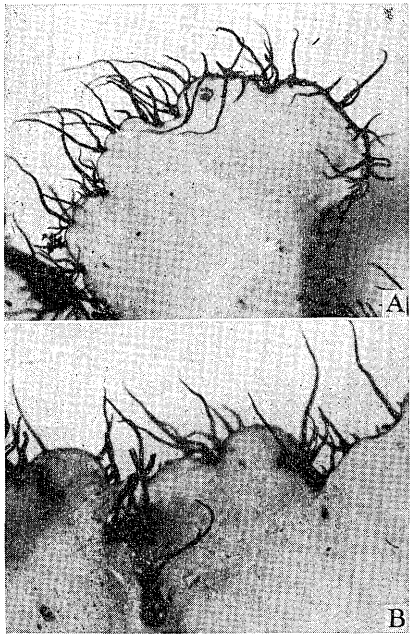

Fig. 1. Cilia of *Parmelia diffractaica* (A; S. Kurokawa 8384) and *P. spinibarbis* (B; S. Kurokawa 8353). ×9.

Parana 州の標本からはアトラノリンとジフラクタ酸が検出され，地衣体は粉芽をつけ，地衣体表面にかすかに網目状の白斑があり，裂片の縁に分枝したシリアをつける。このシリアは非常に特色があって，大部分はその根元のところで分枝し，分枝の1本は上方に，他の1-2(まれに3) 本は下方に向っている (Fig. 1A)。これらの点は，北米東部に特産であるとして記載された *Parmelia diffractaica* と全く一致する。なお，ここに述べたようなシリアの分類学的な意義については今後さらに追及する必要があるが，著者がさきに記載した *P. spinibarbis* (Kurokawa, S. in Bull. Natn. Sci. Mus. Tokyo 17: 299. 1974) でも同様のシリアが見られる (Fig. 1B)。*P. spinibarbis* はブラジルから記載された種であるから，