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A CORRELATION BETWEEN β-ORCINOL PARADEPSIDE ATRANORIN AND ORCINOL DEPSIDE LECANORIC ACID IN *PARMOTREMA TINCTORUM*

Eine Korrelation zwischen ß-Orcinolparadepsid Atranorin und Orcinoldepsid Lecanorsäure in *Parmotrema tinctorum*

by

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Key words: Chemotaxonomy, *Parmotrema tinctorum*, chemical substance, HPLC, ratio of contents, synthetic pathway.

Schlagwörter: Chemotaxonomie, *Parmotrema tinctorum*, Chemische Substanz, HPLC, Menge der Inhaltstoffe, Syntheseweg.

Summary: The content of the chemical substances in *Parmotrema tinctorum* (NYL.) HALE from Japan, Fiji and others were measured by using HPLC (High performance liquid chromatography). We found a significant relationship between the content ratio of lecanoric acid/ atranorin (16-20), although the contents of lecanoric acid and atranorin greatly varied among specimens. Moreover, the ratio in the content of two substances was very similar among even unrelated collection sites.

Zusammenfassung: Der Gehalt an chemischen Inhaltstoffen wurde bei *Parmotrema tinctorum* (NYL.) HALE mit HPLC gemessen. Die Proben stammen unter anderem aus Japan und Fiji. Wir fanden eine signifikante Korrelation zwischen dem Gehalt an Lecanorsäure und dem Gehalt an Atranorin (16-20), obwohl der Lecanorsäure/Atranorin Gehalt bei den einzelnen Proben stark variierte. Darüber hinaus war die Korrelation im Gehalt dieser beiden Substanzen auch bei Proben ganz verschiedener Fundorte gegeben.

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Introduction

The lichen substances are usually used as an important taxonomic character. Chemotaxonomy plays a important role for identifying species, genus, and family of lichens. But contents of lichen substances often vary greatly with the individual (LAMB 1964; HILL & WOOLHOUSE 1966). On the other hand, some lichen taxonomists detected the very small amount of chemical substance such as atranorin by using HPLC (LUMBSCH 1995; LUMBSCH et al.1995), and used them as the important taxonomic character.

Recognizing the quantitative stability of chemical substances is inevitable study for lichen taxonomist. Thus, the comparison of chemical characters among individuals from different sites is essential for emphasizing the validity of chemotaxonomy in lichens, although the quantitative variation among the population was studied within same collection sites (CULBERSON et al. 1983; HAMADA 1988).

Parmotrema tinctorum examined in this study is a very popular lichen around the tropical to temperate regions of Pan Pacific. In this study, we mainly compared the quantitative variation in samples from Japan and Fiji, using the method of HPLC (YOSHIMURA et al. 1991).

Materials and Methods

Twenty-seven and 10 specimens are collected in Japan (36-39N, 130-136E) and Fiji (18S, 178E) respectively. Moreover, one or two samples were collected in China (23N, 105E), Vanuatu (18S, 168E) and Uruguay (33S, 55W), too (Fig. 1). The specimens are kept in the herbarium of Saga University.

The well dried lobes, free of dust and soil, were collected and examined for their contents of lecanoric acid and atranorin. The acids in the fragments of marginal lobe, 10 mg were extracted with 3 ml of 35° C acetone. These treatment were carried out for about 5 min for avoiding the methylation of lecanoric acid.

This species contains lecanoric acid, atranorin and chloroatranorin as main substances. But content of chloroatranorin is sometimes too small (less than one-fifth of atranorin) to be detectable even by HPLC (Fig.2).

The concentrations of lecanoric acid and atranorin were measured by HPLC (Jasco 880 -LC liquid chromatography UV detector 254 nm) at 35° C conditions, using methanol-water-acetic acid solvent (80:20:1) at flow rate of 1 ml/min on a column: Finepak SIL C18 (7.2 mm x 250 mm). Retention times were 4.5 min for lecanoric acid, 15.7 min for atranorin and 20.0 min for chloroatranorin with 2.5 min (Fig. 2). The content of each sample was calculated from the relative height of the chromatographic peaks, comparison with the authentic samples; lecanoric acid from Dr. H. MATSUBARA, atranorin from Dr. K. TAKAHASHI and chloroatranorin from Sigma Co., Ltd. The lobe width are measured in wet condition.

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Results

The contents of lecanoric acid and atranorin examined varied greatly depending on the specimens. The content of lecanoric acid ranges from 0.155 μg to 15.398 μg, and that of atranorin ranges from 0.075 μg to 0.587 μg in 10 mg thallus (lobe). No correlation was found between contents of either lichen substances and the width of the lobe (Figs. 3 and 4). The ratio in the content of lecanoric acid and atranorin in each lobe was constant regardless of their concentration (Figs. 5 and 6). Especially the ratio of lecanoric acid to atranorin was 16.8 in Japan, and 20.0 in extra Japanese specimens (Fiji, Vanuatu, China and Uruguay).

Discussion

As the width of lobe is thought to reflect the age of individual, the contents of lecanoric acid and atranorin seem to be accumulated regardless the age (Figs. 3 and 4). The other factors than aging, for example environmental ones, seem to control their contents, as described by RUNDEL (1969) and HAMADA (1991).

We want to emphasize the ratio of contents among lecanoric acid and atranorin is highly constant unrelated to collection sites, even if across the equator (Figs. 5 and 6), although the ratio of some compounds was reported to be very constant in the same collection site (CULBERSON et at. 1983; HAMADA 1988). In *P. tinctorum,* the productive ratio of two substances in pathway seems to be genetically controlled, different from those quantity. The variation in the contents of lecanoric acid and atranorin is considered to be caused by the variation of some factors regulating the reactions common to the synthesis or accumulation of these depsides, probably with-pathway compounds.

Three dibenzofuran derivatives in *Cladonia cristatella* (CULBERSON et al. 1983) and three sekikaic acid derivatives in *Ramalina subcomplanata* (HAMADA 1988) is very close in the pathway. However, lecanoric acid is orcinol depside and atranorin is β-orcinol depside. Thus, that pathway of both substances is not so close as the sekikaic derivatives and the dibenzofuran derivatives. HAMADA (1988) found a negative correlation between the content of β-orcinol depsidone, salazinic acid and that of orc inol depside, sekikaic acid derivatives and pointed out the reason that salazinic acid has a different synthetic pathway from sekikaic derivatives. On the contrary, YAMAZAKI et al. (1965) indicate both lecanoric acid and atranorin are produced simultaneously and resonantly in *P. tinctorum*. This result supports the reports of YAMAZAKI et al. (1965), and seems to be interesting from the point of biosynthetic pathway, and the stability in the ratio of two compounds seems to be useful in lichen taxonomy.

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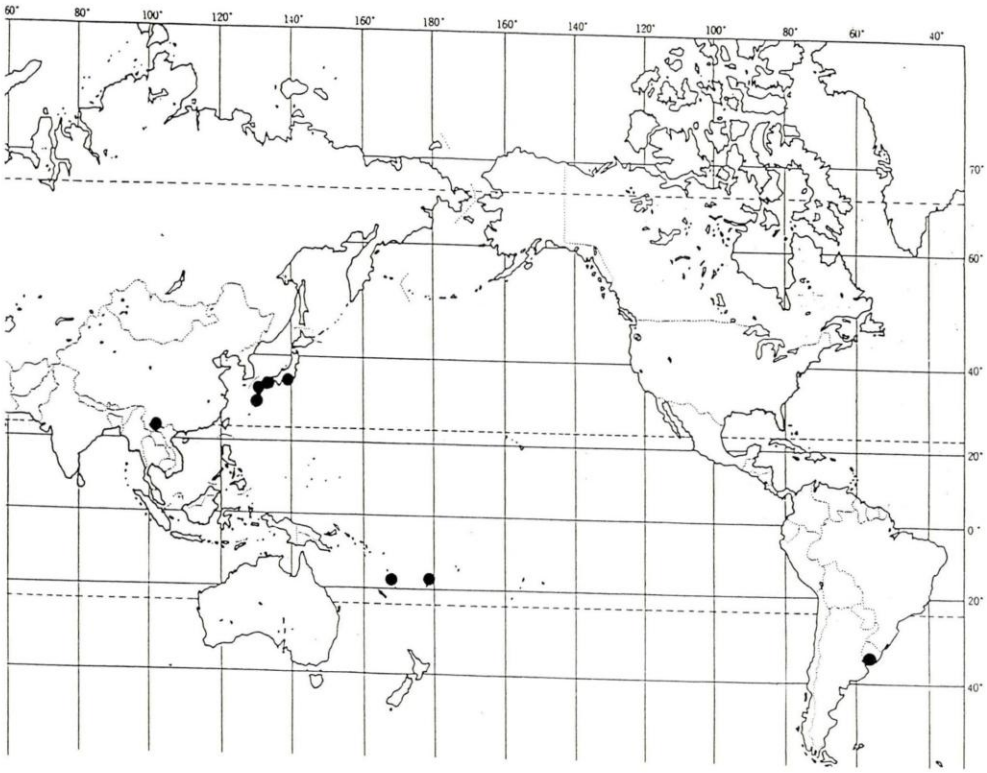


Fig. 1: Sampling sites of *Parmotrema tinctorum* (NYL.) Hale

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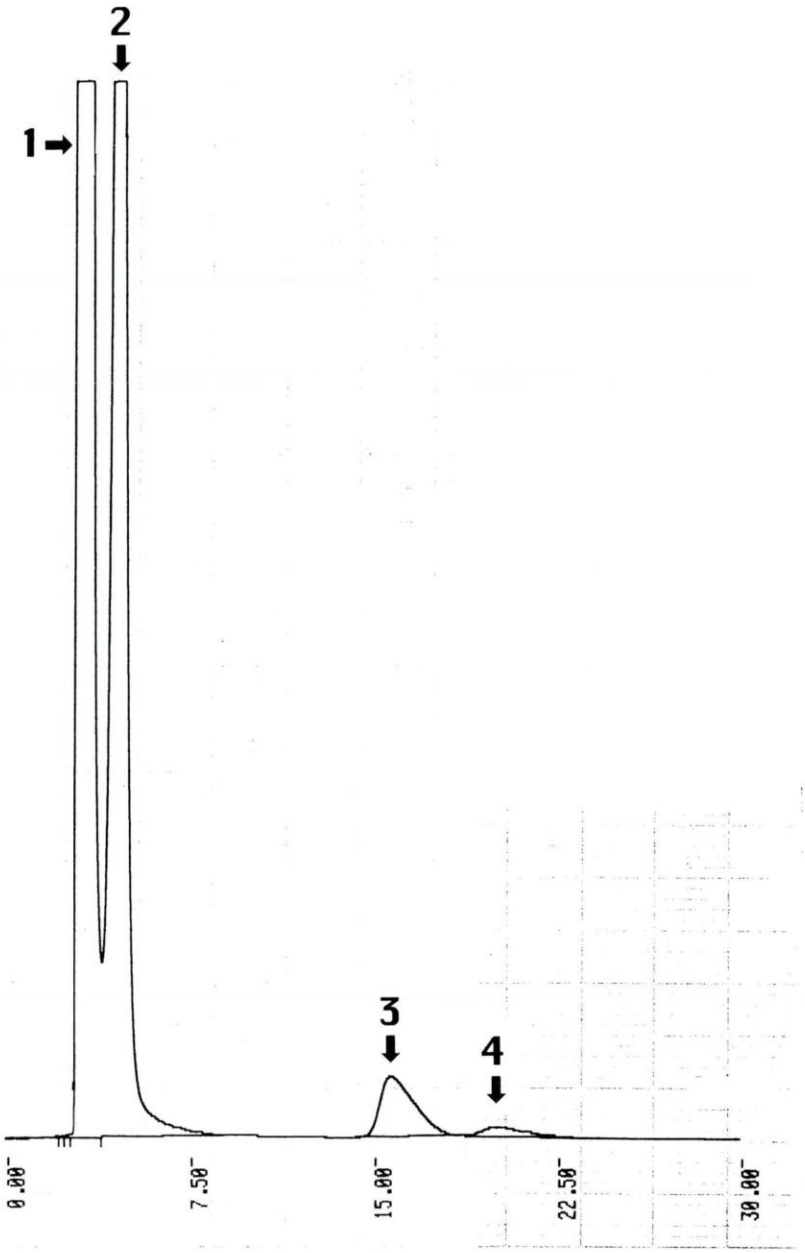
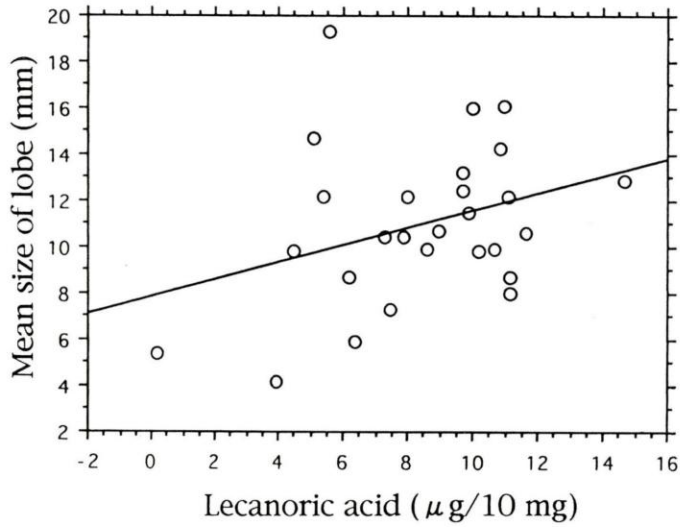


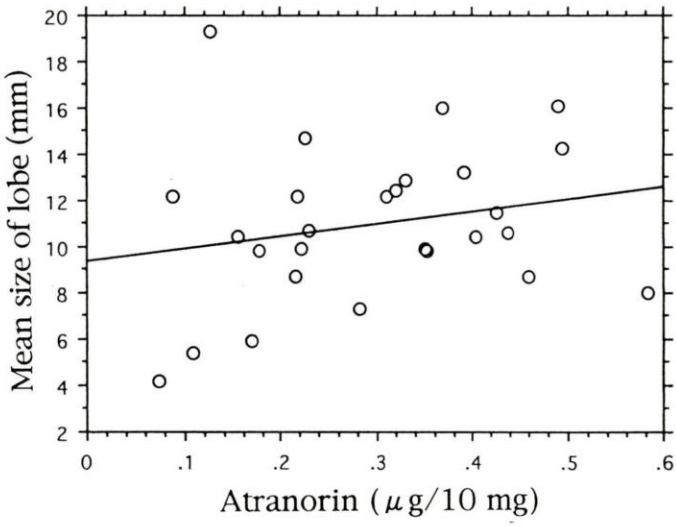
Fig. 2: HPLC of *P. tinctorum* (hm 8568 in Japan).
1 ... acetone; 2 ... lecanoric acid; 3 ... atranorin; 4 ... chloroatranorin

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$Y=0.371x+7.87 \quad R^2=0.111 \quad P=0.089$

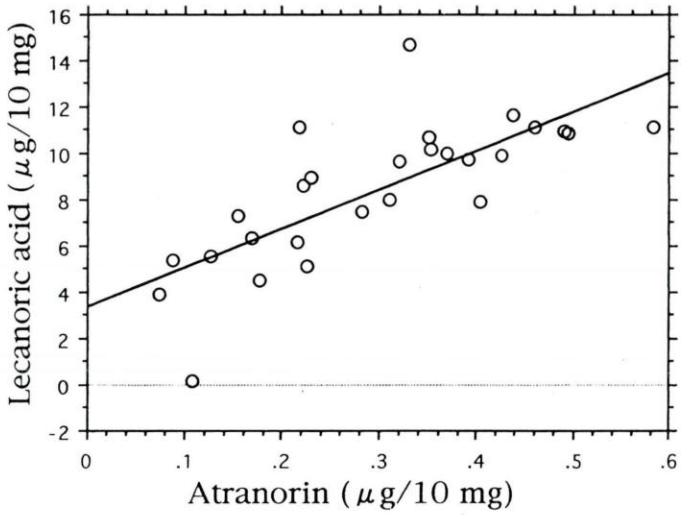
Fig. 3: The relationship between the width and the content of lecanoric acid in *P. tinctorum* from Japan.



$Y=0.547x+9.36 \quad R^2=0.048 \quad P=0.27$

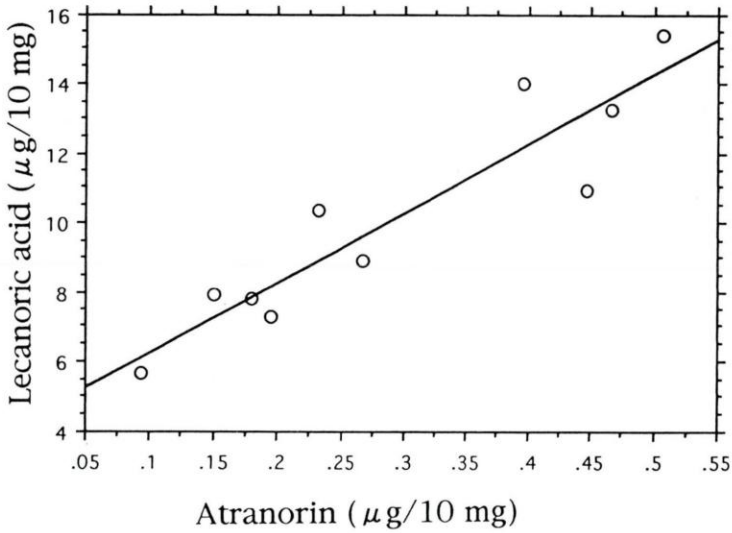
Fig. 4: The relationship between the width and the content of atranorin in *P. tinctorum* from Japan.

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$Y=16.76x+3.43 \quad R^2=0.562 \quad P<0.001$

Fig. 5: The relationship between the content of atranorin and that of lecanoric acid in *P. tinctorum* from Japan.



$Y=20.0x+4.3 \quad R^2=0.855 \quad P=0.001$

Fig. 6: The relationship between the the content of atranorin and that of lecanoric acid in *Parmotrema tinctorum* collected in China, Fiji, Vanuatu and Uruguay.

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