41. Monji Mitsuno: Paper Chromatography of Lichen Substances. I.

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In microdetection of lichens, caustic alkali and bleaching powder have frequently been used as the coloring reagents. These reactions, however, are not specific to the individual components of lichens.

As already reported by Asahina and the present author[1], the observation of crystals extracted from lichen by a micro-technique under the polarizing microscope could give some valuable evidences for characterization of lichens. There occurs, however, some difficulties in distinguishing by this method when some very analogous compounds coexist in a certain lichen or when the formation of crystalline derivatives of the lichen substance is inhibited by the accompanying products.

For example the following couples of lichen acids could hardly be distinguished: Alectoronic and α-collatolic acids; evernic and obtusatic acids; salazinic and norstictic acids; protocetraric and fumar-protocetraric acids.

Better results in the micro-detection of lichen or lichen substance has now been brought about by the application of paper chromatographic method. During the course of this study, immediately before presenting this manuscript, report of a Swedish worker[2] on the paper chromatography of lichen substances came to the notice of the author.

In the present paper, a systematic study on the relationship between the structures of lichen substances and their degradation products, and their Rf-values are discussed.

Experimental

Paper chromatography of depsides, depsidones, and usnic acid—The most widely distributed lichen substances, depsides, depsidones, and usnic acid were determined by the following procedure:

The chromatography was carried out by the ascending system using a filter paper (40 cm. × 5 cm., Toyo-Roshi No. 50).

As the developing solvent, conc. NH_4OH-saturated butanol or butanol-acetone-water (5:1:2) was employed.

The following coloring reagents were used for detection: (i) 1% Alcoholic solution of $FeCl_3$ for depsides and depsidones giving purple or reddish brown coloration; (ii) 1% alcoholic solution of *p*-phenylenediamine for the lichen substances having aldehyde grouping such as thamnolic, baeomycesic, psoromic, salazinic, stictic, norstictic, protocetraric, and fumarprotocetraric acids which give orange coloration; (iii) 5% chloramine-T alcoholic solution (CAT) for usnic acid giving a yellow coloration.

Five to 10 mg. of crystalline sample dissolved in 0.5 cc. of acetone or 0.1 g. of lichen thallus extracted with 0.5 cc. of acetone was used for detection. The results are shown in Table I.

Paper chromatography of phenol-carboxylic acids which constitute lichen depsides and depsidones—Some depsides, such as obtusatic and evernic acids, could hardly be distinguished by the usual micromethod or by the paper chromatographic technique. However, the paper chromatogarphic separation of the phenol-carboxylic acids which constitute the above depsides is available for the determination.

On hydrolysis with conc. H_2SO_4, evernic acid gives everninic and orsellic acids, while obtusatic acid yields rhizonic and orsellic acids. 0.2 to 0.3 g. of lichen thallus was extracted with acetone in a small test tube and the solvent was evaporated. The residue was dissolved by the addition of 1 drop of conc. H_2SO_4. After standing for a few minutes, the reaction mixture was diluted with water and then shaken with 0.5 cc. of ether. The ethereal layer was used for the test solution of paper chromatography. Ammonia-saturated butanol was employed as the developing solvent and 1% alcoholic solution of $FeCl_3$ as the coloring reagent. The results are shown in Table III.

* Okuda, Toyama (三ツ野岡治).

1) Y. Asahina, M. Mitsuno: J. Jap. Bot., 12, 516, 859 (1937); *ibid.*, 13, 529, 855 (1937); *ibid.*, 14, 39, 244, 318, 650, 767 (1938); *ibid.*, 15, 465 (1939); *ibid.*, 15, 465 (1940); *ibid.*, 16, 185 (1941).

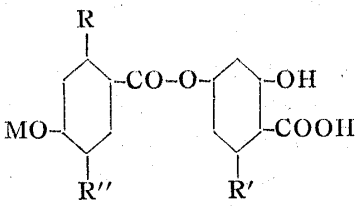
2) C.A. Wachmeister: Acta Chim. Scand., 6, 818 (1952), C.A., 47, 694 (1953).

Results and Discussions

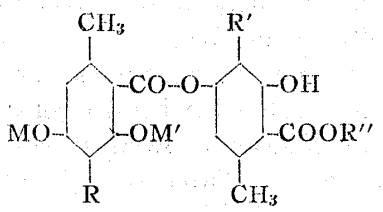
TABLE I

Compounds	Rf BuOH-NH_4OH	Rf BuOH-Acetone-H_2O	Lichens
Lecanoric acid (I)	0.33	—	*Parmelia tinctorum* Despr.
Evernic acid (II)	0.61	—	*Ramalina commixta* Asahina
Divaricatic acid (III)	0.83	—	*Evernia mesomorpha* Müll. Arg.
Sphaerophorin (IV)	0.88	—	*Sphaerophorus fragilis* Pers.
Perlatolic acid (V)	0.93	—	*Cladonia pscudoevansii* Asahina
Obtusatic acid (VI)	0.60	—	*Ramalina pollinaria* Ach.
Barbatic acid (VII)	0.65	0.87	*Cladonia bacillaris* Nyl.
Diffractaic acid (VIII)	0.80	0.83	*Usnea diffracta* Wain.
Baeomycesic acid (IX)	0.50	0.64	*Thamnolia subvermicularis* Asahina
Squamatic acid (X)	0.28	0.28	*Cladonia uncialis* (L.) Web.
Atranorin (XI)	0.63	—	*Cetraria* sp.
Olivetoric acid (XII)	0.74	—	*Alectoria divergens* Nyl.
Microphyllic acid (XIII)	0.91	—	*Cetraria japonica* Zahl.
Thamnolic acid (XIV)	—	0.37	*Thamnolia vermicularis* (SW.) Schaer.
Lobaric acid (XV)	0.54	—	*Stereocaulon paschale* Ach.
α-Collatolic acid (XVI)	0.66	—	*Cetraria collata* Müll. Arg.
Alectoronic acid (XVII)	0.45	—	*Parmelia diffugiens* Zahl.
Salazinic acid (XVIII)	—	0.50	*Parmelia conspersa* Ach.
Stictic acid (XIX)	—	0.52	*Stereocaulon japonicum* Th. Fr.
Norstictic acid (XX)	—	0.62	*Usnea japonica* Wain.
Protocetraric acid (XXI)	—	0.45	*Parmelia* sp.
Fumarprotocetraric acid (XXII)	—	0.38	*Cetraria islandica* Ach.
Physodalic acid (XXIII)	—	0.68	*Parmelia physodes* Ach.
Psoromic acid (XXIV)	—	0.62	*Alectoria sulcata* Nyl.
Usnic acid (XXV)	0.92	—	*Usnea* sp.

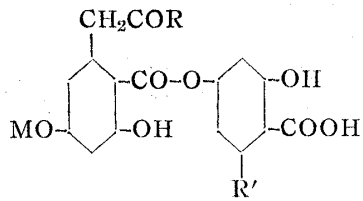
* —: The solvent is not suitable for determination of this sample giving Rf 1.00.



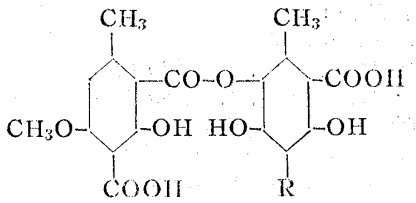
(I) R=R'=CH_3, R''=H, M=H
(II) R=R'=CH_3, R''=H, M=CH_3
(III) R=R'=C_3H_7, R''=H, M=CH_3
(IV) R=CH_3, R'=C_7H_{15}, R''=H, M=CH_3
(V) R=R'=C_5H_{11}, R''=H, M=CH_3
(VI) R=R'=R''=CH_3, M=CH_3



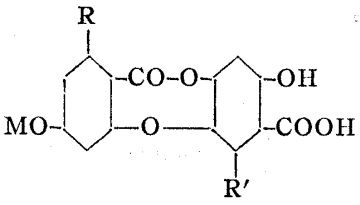
(VII) R=R'=CH_3, R''=H, M=CH_3, M'=H
(VIII) R=R'=CH_3, R''=H, M=M'=CH_3
(IX) R=CHO, R'=CH_3, R''=H, M=CH_3, M'=H
(X) R=COOH, R'=CH_3, R''=H, M=CH_3 M'=H
(XI) R=CHO, R'=R''=CH_3, M=CH_3, M'=H



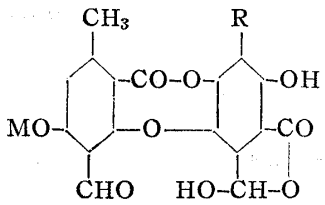
(XII) R=R'=C_5H_{11}, M=H
(XIII) R=C_5H_{11}, R'=$CH_2COC_5H_{11}$, M=H



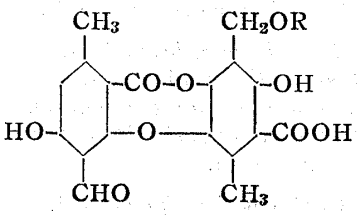
(XIV) R=CHO



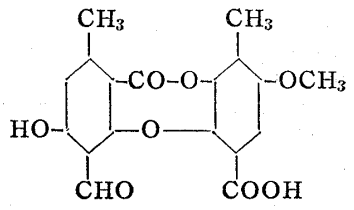
(XV) R=COC_4H_9, R'=C_5H_{11}, M=CH_3
(XVI) R=R'=$CH_2COC_5H_{11}$, M=CH_3
(XVII) R=R'=$CH_2COC_5H_{11}$, M=H



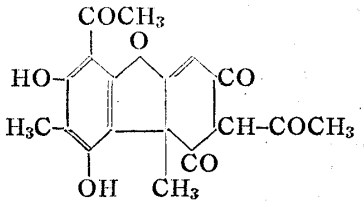
(XVIII) R=CH_2OH, M=H
(XIX) R=CH_3, M=CH_3
(XX) R=CH_3, M=H



(XXI) R=H
(XXII) R=COCH:CH-COOH
(XXIII) R=$COCH_3$



(XXIV)



(XXV)

α-Collatolic acid and alectoronic acid which are frequently contained in the same lichen could hardly be determined by the former microchemical method. On the other hand, usnic acid and atranorin, when they occur together, would be difficult to prove microchemically.

In order to obtain some definite evidence for the distribution of the above four compounds in the lichens of *Parmeliaceae*, the paper chromatographic method was applied successfully (Table II).

CAT-reagent gave a sufficient result for the determination of α-collatolic and alectoronic acids giving yellowish brown color for the former and reddish brown color for the latter, while $FeCl_3$ solution showed a reddish brown coloration for both compounds.

As shown in Table II, it is interesting to note that α-collatolic acid always occurs in nature accompanied by alectoronic acid (norcollatolic acid).

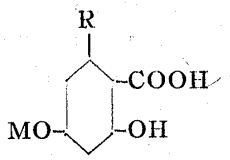
TABLE II

Lichens	Usnic acid	Atranorin	Alectoronic acid	α-Collatolic acid
Parmelia diffugiens Zahlbr.	+++	+++	+++	−
P. centrifuga (L.) Ach.	+++	+++	+++	−
P. incruva (Pers.) Fr.	+++	−	+++	−
P. Arnoldii Du Rietz	−	+++	+++	+++
Cetraria japonica Zahlbr.	−	+++	−	−
C. collata Müll. Arg. f. *isidiata* Asahina	−	+++	+	+++
C. collata Müll. Arg. f. *nuda* Hue	−	+++	+	+++
C. Kurodakensis Asahina	−	+++	+	+++
C. Wallichiana (Nyl.) Müll. Arg.	−	−	+++	−
C. pseudocomplicata Asahina	−	−	+++	−
C. chrysanta Tuck.	+++	+++	+++	−
C. ciliaris Ach.	−	−	+	+++
C. rugosa (Asahina) Sato	+++	−	−	−

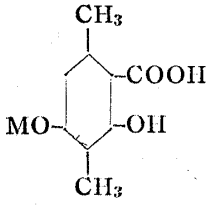
As mentioned above, the determination of the Rf-values of orcinol- and β-orcinol-carboxylic acid derivatives would give a remarkable contribution to the study of the lichen depsides and depsidones. The results are summarized in Table III.

TABLE III

Compounds	Rf-values ($BuOH-NH_4OH$)
Orsellic acid (XXVI)	0.15
Everninic acid (XXVII)	0.53
Divaric acid (XXVIII)	0.41
Divaricatinic acid (XXIX)	0.77
Olivetol-carboxylic acid (XXX)	0.55
Sphaerophorol-carboxylic acid (XXXI)	0.80
Methyl orsellate (XXXII)	0.62
β-Oricinol-carboxylic acid (XXXIII)	0.33
Rhizonic acid (XXXIV)	0.64
β-Oricinol-carboxylic acid dimethyl ether (XXXV)	0.66
Orcinol-dicarboxylic acid monomethyl ether (XXXVI)	0.04



(XXVI) $R=CH_3$, $M=H$
(XXVII) $R=CH_3$, $M=CH_3$
(XXVIII) $R=C_3H_7$, $M=H$
(XXIX) $R=C_3H_7$, $M=CH_3$
(XXX) $R=C_5H_{11}$, $M=H$
(XXXI) $R=C_7H_{15}$, $M=H$



(XXXIII) $M=H$
(XXXIV) $M=CH_3$

On the basis of the present experimental results, it would be possible to present some conclusions on the relationship between the chemical structures of lichen substances and their Rf-values.

i) Methylation of hydroxyl group of depsides, depsidones, and their components, phenol carboxylic acids, causes the increase of the Rf-value:

Lecanoric acid (0.33)< evernic acid (0.61); barbatic acid (0.65)< diffractaic acid (0.80); alectoronic acid (0.45)< α-collatolic acid (0.66); orsellic acid (0.15)< everninic acid (0.53); divaric acid (0.41)< divaricatinic acid (0.77).

ii) Increase in the number of carbon atoms in alkyl side-chain of orcinol derivatives causes increase of the Rf-value:

Evernic acid (0.61)< divaricatic acid (0.82)< sphaerophorin (0.88)< perlatolic acid (0.93).

Orsellic acid (0.15)< divaric acid (0.41)< olivetol-carboxylic acid (0.55)< sphaerophorol-carboxylic acid (0.80).

iii) Degrees of oxidation of the side chain of β-orcinol derivatives affects the Rf-values:

Barbatic acid (0.65) >baeomycesic acid (0.50) >squamatic acid (0.28).

This work was carried out in the Pharmaceutical Institute, University of Tokyo, using the samples stored there. The author wishes to thank Dr. Y. Asahina, Prof. Emeritus, and Prof. S. Shibata of the University of Tokyo for their kind advices. The expenses for this study were supported by the Scientific Research Fund provided by the Ministry of Education for which the author's thanks are due.

Summary

As a microchemical method for determining lichens and their products, a paper chromatographic study was carried out. The relationship between chemical structures of lichen depsides, depsidones, and their components, phenolcarboxylic acids, and the Rf-values was discussed.

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