

## Notes

Monji Mitsuno : Paper Chromatography of Lichen Substances. II.<sup>1)</sup>

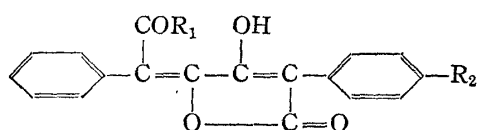
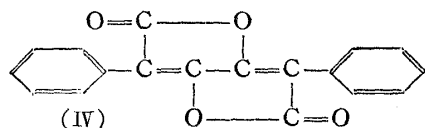
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Paper chromatography of depsides and depsidones among the lichen substances, and the phenol-carboxylic acids constituting them was carried out and reported in the previous paper.<sup>1)</sup> In the present series of experiments, paper chromatography was carried out on pigments of the pulvic acid derivatives and the results are described herein.

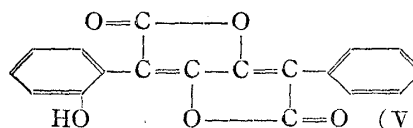
Of these pigments, vulpinic acid (I), pinastric acid (II), pulvic acid (III), pulvic acid anhydride (IV), calycin (V), epanorin (VI), and rhizocarpic acid (VII) (cf. Table I) are known. Zopf<sup>2)</sup> maintains that *Sticta aurata* Ach. contains stictaurin which is said to be a molecular compound composed of two moles of pulvic acid anhydride and one mole of calycin. This lichen was used in the present paper chromatography and the componental pulvic acid anhydride and calycin as well as pulvic acid were detected but not stictaurin itself. This raised the necessity of confirming whether or not molecular compounds are separated into each componental part by paper chromatography and this point was also investigated.

Analyses of organic molecular compounds by adsorption chromatography has already been studied by H. Kondo<sup>3)</sup> who showed that covalent bonds in higher molecular compounds, such as Kompral, Pyramidon-Veronal, Hypnal, anthracene picrate, and phenanthrene picrate, were isolated into each componental part by adsorption through alumina. Several molecular compounds were prepared and submitted to paper chromatography. Considering the importance of the effect of the reaction of developing solvents, several kinds of each of neutral, acid, and alkaline media were used. As a result, it has been concluded that in all cases, molecular compounds are also separated into each componental part by paper partition chromatography.

TABLE I.

(I)  $R_1 = \text{OCH}_3$ ,  $R_2 = \text{H}$  (II)  $R_1 = R_2 = \text{OCH}_3$ (III)  $R_1 = \text{OH}$ ,  $R_2 = \text{H}$ (IV)  $R_1 = \text{NH}-\text{CH}-\text{COOCH}_3$ ,  $R_2 = \text{H}$   
 $\quad \quad \quad \text{CH}_2-\text{CH}(\text{CH}_3)_2$ (VII)  $R_1 = \text{NH}-\text{CH}-\text{COOCH}_3$ ,  $R_2 = \text{H}$   
 $\quad \quad \quad \text{CH}_2-\text{C}_6\text{H}_5$ 

(IV)



(V)

The writer expresses his deep gratitude to Prof. Emeritus Y. Asahina and Prof. Shoji Shibata of the University of Tokyo for their unfailing guidance. A part of the expenses was defrayed by a Grant in Aid for Scientific Research from the Ministry of Education (Representative of the General Research Unit : Prof. Kariyone), for which the writer is also grateful.

## Experimental

## Paper Chromatography of Pulvic Acid Derivatives—Using Toyo Roshi No. 50 (2×40 cm.),

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1) Part I : This Bulletin, 1, 170(1953).

2) W. Zopf : "Flechtenstoffe," 86(1907).

3) H. Kondo : J. Pharm. Soc. Japan, 57, 832(1937).

acetone solution containing about 50% of the sample was submitted to paper chromatography by the one-dimensional, ascending method. Various solvents were tried as the developer for this series and good results were obtained with the use of four kinds of alkaline medium, as shown in Table II. The detection was made by fluorescence seen under the ultraviolet rays, in which calycin appears reddish brown, and others show bright yellow fluorescence. Standards for the samples were the specimens in the Pharmacognosy Laboratory of the University of Tokyo, and the lichen samples used were the specimens appraised by Prof. Asahina. Specimen of rhizocarpic acid could not be obtained and its study was deferred to a later date.

TABLE II. Rf Values of Pulvic Acid Derivatives

Substance	Solvent*				Lichen
	(A)	(B)	(C)	(D)	
Pulvic acid	0.70	0.70	0.44	0.19	<i>Sticta aurata</i> Ach.
Pulvic acid anhydride	0.40	0.40	0.06	0	<i>Sticta aurata</i> Ach.
Vulpinic acid	0.92	0.91	0.80	0.50	{ <i>Evernia vulpina</i> L. <i>Cetraria pinastri</i> (Scop) Rohl
Pinastric acid	0.90	0.88	0.73	0.40	{ <i>Cetraria pinastri</i> (Scop) Rohl <i>Cetraria juniperina</i> (L.) Ach.
Calycin	0.64	0.63	0.26	0.06	<i>Sticta aurata</i> Ach.
Epanorin	1.00	1.00	1.00	1.00	<i>Sticta crocata</i> Ach.

\* Solvent : (A) BuOH : 28% NH<sub>4</sub>OH (4:1) (B) BuOH : EtOAc : 28% NH<sub>4</sub>OH (2:2:1)  
(C) BuOH : EtOAc : 28% NH<sub>4</sub>OH (1:8:1) (D) EtOAc : 28% NH<sub>4</sub>OH (saturated)

**Paper Chromatography of Molecular Compounds**—(1) Materials: Pyramidon-Veronal, Hypnal, homosulfamine-sulfathiazole, picrates of alkaloids and amino acids, and four kinds of quinhydrone-type compounds were prepared for use. Inorganic salts of alkaloids were also used as a reference,

(2) Methods: Toyo Roshi No. 50 (2×40 cm.) was used, in one-dimensional, ascending method.

(3) Solvent: It would have been desirable to use as many kinds of solvents as possible but the following four were used considering the reaction of the solution. (A) BuOH saturated water; (B) BuOH : HOAc : water (4:1:2); (C) EtOH : 30% aq. urea solution (3:1); (D) BuOH : 10% NH<sub>4</sub>OH (4:3). Temperature: 12–17°. Time: 30 hrs., developing to around 30 cm.

(4) Coloration: (a) Homosulfamine: After spraying NaOH solution, freshly prepared 0.5% potassium naphthoquinonesulfonate solution is sprayed by which orange color appears. (b) Sulfathiazole: 5% Methanolic solution of dimethylaminobenzaldehyde, containing 1/3 volume of conc. HCl (Ehrlich reagent) is sprayed. (c) Veronal: Violet color appears by spraying a solution of 4 cc. of 10% CuSO<sub>4</sub> solution containing 1 cc. of pyridine and 5 cc. of water. (d) Naphthol and Resorcinol: Pauly reagent is used. A solution of 0.05 g. of sulfanilamide dissolved in 5 cc. of 10% HCl and 5 cc. of 5% NaNO<sub>2</sub> are placed in a 50-cc. glass-stoppered measuring cylinder, mixed by shaking for 1 min., BuOH is added to the 50 cc. mark, and this BuOH layer is used. This BuOH solution is sprayed on the paper, dried, and Na<sub>2</sub>CO<sub>3</sub> solution is sprayed by which yellow to pale orange color appears. (e) Amino Acids: 0.2% Ethanolic solution of ninhydrin. (f) Picric Acid, Quinine, and Berberine: Detected by fluorescence under ultraviolet rays. The results are shown in Table III, from which it may be seen that almost all the molecular compounds form two spots and each spot is identical with that of the original component compound.

TABLE III. Rf Values of Molecular Compounds (×100)

Compound	m.p. °C	Solvent*				Coloring reagent
		A	B	C	D	
Homosulfamine-Sulfathiazole	164	12	37	63	22	K Naphthoquinonesulfonate
		72	76	79	35	Ehrlich reagent
Chloral hydrate-Antipyrine	68	82	84	92	79	Resorcinol in NaOH soln.
		77	79	85	78	FeCl <sub>3</sub> soln.
Barbital-Aminopyrine	115~140	82	83	89	70	Cu-pyridine soln.
		83	74	92	84	AgNO <sub>3</sub> soln.
Berberine picrate	241	54	68	29	39	Fluorescence
		44	65	81	66	"
Quinine picrate		83	82	85	86	Ammoniacal AgNO <sub>3</sub>
		44	64	80	66	Fluorescence
Glycine picrate	200	1	23	32	1	Ninhydrin soln.
		42	63	85	68	Fluorescence
Aminopyrine picrate	181	77	74	92	83	AgNO <sub>3</sub> soln.
		42	62	82	66	Fluorescence
8-Hydroxyquinoline picrate	205	82	81	94	79	FeCl <sub>3</sub> soln.
		44	63	82	65	Fluorescence

Benzoquinone picrate	120	80 44	82 65	86 82	— —	Ammoniacal AgNO <sub>3</sub> Fluorescence
Benzoquinone- $\beta$ -Naphthol	156	80 86	82 85	86 95	— —	Ammoniacal AgNO <sub>3</sub> Pauly reagent
Benzoquinone- Phenol	71	81 95	81 100	— —	— —	Ammoniacal AgNO <sub>3</sub> Pauly reagent
Benzoquinone- Resorcinol	>300	80	82	86	—	Ammoniacal AgNO <sub>3</sub> Pauly reagent
Benzoquinone- Hydroquinone	171	79	82	88	—	Ammoniacal AgNO <sub>3</sub>

\* Solvent—A : BuOH:H<sub>2</sub>O (saturated)

B : BuOH:AcOH:H<sub>2</sub>O (4:1:2)

C : EtOH:30% Urea aq. (3:1)

D : BuOH:10% NH<sub>4</sub>OH aq. (4:3)

Quinone, resorcinol, and hydroquinone gave identical R<sub>f</sub> values by the solvents used in the present experiments that it was impossible to make any conclusive judgement but since other quinhydrone-type had clearly been isolated, these must have been separated too.

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### Kiyoshi Yoshimura and Minoru Morita : Colorimetric Determination of Homosulfamine.\*

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It has previously been reported that homosulfamine produced a yellow color with *p*-dimethylaminobenzaldehyde in diluted hydrochloric acid<sup>1)</sup> and an orange yellow color with sodium 1,2-naphthoquinone-4-sulfonate in alkaline solution.<sup>2)</sup> Momose and Yasumura<sup>3)</sup> utilized the latter for the colorimetric determination of homosulfamine and established a simple colorimetric method which is described in the J. P. VI. However, the color is not stable and it is reported that the error is about 10%.

Recently Perez and Poirier<sup>3)</sup> reported on the colorimetric determination of primary aliphatic amines such as methylamine, ethylamine, and propylamine, using diazotized *p*-nitraniline. According to Beyer,<sup>4)</sup> this coloration (red) is due to the existence of quinoid derivatives. In view of the fact that homosulfamine is one of the aliphatic amines, it was reacted with the above-mentioned diazonium reagent and it produced a red color having the absorption maximum at 520 m $\mu$  as shown in Fig. 1. Experiments were carried out to determine very small amounts of this drug by a colorimetric method in which the color is more stable. On the other hand, the color intensity was greatly influenced by various factors such as the amount of diazonium reagent, reaction temperature, length of reaction period, time elapsed after addition of all reagents, and pH during reaction, as shown in Figs. 2~5 and Table I.

TABLE I.

pH	6.3	7.0	7.7	7.85	8.0
Absorbancy	0.163	0.421	0.638	0.660	0.729

As pH increases, blank soln. becomes somewhat red.

\* Paper read at the Kinki Local Meeting of the Pharmaceutical Society of Japan, Oct. 16, 1954.

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1) W. Hoffmann, G. Willkens : Pharm. Ztg., **83**, 65~75.

2) T. Momose, *et al.* : J. Pharm. Soc. Japan, **70**, 672(1950).

3) M. Perez, P. Poirier : Bull. soc. chim. France, **1953**, 754.

4) B. H. Beyer : J. Am. Chem. Soc., **64**, 1318(1942).