Studies on the Chemistry of Lichens

23.* Thin Layer Chromatography of Pulvic Acid Derivatives

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The identification of pulvic acid derivatives, of frequent occurrence in lichens, by paper chromatography has been studied by several authors.[1-3] In the present paper, a method of separating compounds of this class by thin layer chromatography is presented. The results are summarized in Table 1.

In three of the solvent systems, the separation of vulpinic acid and pinastric acid is easily achieved. The separation of rhizocarpic acid and epanorin is achieved in at least one of the solvent systems.

In an attempt to isolate the pulvic acid derivative "coniocybic acid", described by Zopf,[4] the crustaceous lichen *Coniocybe furfuracea* (L.) Ach. was investigated using this method.

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However, two yellow spots, chromatographically indistinguishable from vulpinic acid and rhizocarpic acid, were obtained. These two acids were isolated by preparative thin layer chromatography and identified by comparison with authentic samples.

Hale[5] reported vulpinic acid from *Coniocybe furfuracea*, but no experimental data were given. Rhizocarpic acid has so far not been reported from this lichen.

Experimental. The thin layer chromatography was carried out according to Stahl.[7] Silica gel G was used as adsorbant. Pulvic dilactone, pulvic acid, and vulpinic acid were synthesized as described by Volhard.[8] Calycin[9] was obtained from *Pseudocyphellaria aurata* (Ach.) Vain.[10] and *Chrysotrix noli-tangere* (Mont.) Mont.[11]

Pinastric acid was isolated from *Cetraria juniperina* (L.) Ach.[10] Rhizocarpic acid was obtained from *Acarospora chlorophana*.[6] Epanorin was prepared as described by Frank *et al.*[12]

Coniocybe furfuracea, collected in Fiby, 20 km W Uppsala, Sweden, (0.16 g) was extracted with chloroform (2 × 4 ml) and the combined and concentrated extracts (0.2 ml) were chromatographed as bands in solvent system A (see Table 1). After developing, the zones containing the yellow bands with R_F = 0.10–0.24 and 0.65–0.75, respectively, were removed and extracted with chloroform. Evaporation yielded yellow crystals (5 mg), identical with vulpinic acid (mixed m.p., IR) and yellow crystals (1 mg),

Table 1.

Compound	R_F × 100 in solvent system:				Colour of the spot in:	
	A	B	C	D	visible light	UV 365 mμ
Pulvic acid	03–04	16–18	01	00	yellow	yellow
Pulvic dilactone	79–82	75–77	70–73	84–85	»	»
Calycin	30–33	59–60	19–20	16–19	orange red	dark brown red
Vulpinic acid	12–14	32–33	12–14	12–13	yellow	orange yellow
Pinastric acid	18–20	33–35	22–24	18–20	»	orange
Rhizocarpic acid *	66–69	69–71	58–60	60–62	»	»
Epanorin *	70–72	73–76	61–64	55–56	»	orange (yellow)

* Rhizocarpic acid and epanorin are best separated in solvent system D. Only small amounts of substance should be chromatographed.

A: acetone-chloroform 1:1 (v/v).
B: acetone-chloroform-ethanol (99.5 %) 4:4:1 (v/v/v).
C: acetone-chloroform 1:3 (v/v).
D: chloroform-pyridine 24:1 (v/v).

identical with rhizocarpic acid (mixed m.p., IR).

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1. Wachtmeister, C. A. *Botaniska Notiser* **109** (1956) 313.
2. Mitsuno, M. *Pharm. Bull. (Tokyo)* **3** (1955) 60.
3. Hess, D. *Planta* **52** (1958) 65.
4. Zopf, W. *Beiträge z. Physiol. u. Morphol. niederer Org.* **5** (1895) 45.
5. Hale, M. E., Jr. *Lichen Handbook*, Washington D. C. 1961, p. 56.
6. Zopf, W. *Beiträge z. Physiol. u. Morphol. niederer Org.* **1** (1892) 41.
7. Stahl, E. *Dünnschicht-Chromatographie*, Berlin 1962.
8. Volhard, J. *Ann.* **282** (1894) 1.
9. Åkermark, B. *Acta Chem. Scand.* **15** (1961) 1695.
10. Asahina, Y. and Shibata, S. *Chemistry of Lichen Substances*, Tokyo 1954.
11. Senft, E. *Ber. Deut. Botan. Ges.* **34** (1916) 592.
12. Frank, R. L., Cohen, S. M. and Coker, J. N. *J. Am. Chem. Soc.* **72** (1950) 4454.

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Isolation of Intrinsic Factors from Human Gastric Juice

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Many attempts have been made to isolate intrinsic factor (IF), mostly utilizing hog stomach as the starting material. However, the most active preparations have been dissimilar,[1,2] for instance in respect to the sedimentation constants (5.4—4.0). In 1962, we published a method [3] for purifying IF from human gastric juice. The method is based on a series of chromatographic and gel filtration steps and utilizes the well established fact [4,5] that vitamin B_{12} is bound to and stabilizes IF. From gastric juice, neutralized outside the stomach and to which radioactive cyanocobalamin had been added, three vitamin B_{12} complexes were isolated, the IF-active complexes S and I and the non-IF-active complex R. Complexes S and I have later been found to be antigenically identical.[6] R has been shown to be ubiquitous in the body fluids [6] and to be produced by leucocytes.[7] Complex S possessed IF activity in the Schilling test in a dose of about 40 μg of protein bound to 1 μg of vitamin B_{12} [3] (protein determined by the Lowry method using Clinton standard bovine serum as the reference).

Owing to the low content of IF in the starting material, very large quantities of the latter are needed to prepare sufficient amounts of the active substance for molecular characterization. For this purpose we now collected a pool of 40 litres of neutralized gastric juice derived from 376 persons subjected to Histalog® stimulation. To the pool was added an excess of cyanocobalamin containing a trace of ^{57}Co-labelled vitamin. Two-litre batches of the pool were subjected to treatment with CM-cellulose and DEAE-cellulose chromatography, whereupon the pools were combined and rechromatographed on DEAE-cellulose, followed by DEAE-Sephadex and CM-Sephadex. Complex S was thereafter run through Sephadex G-200, whereas complex I was rechromatographed on DEAE-Sephadex before being filtered through Sephadex. The yields, as dry weight of protein, were 9.4 mg of complex S and 4.8 mg of complex I.

Complex S was found to be homogeneous in the ultracentrifuge and to have a concentration-dependent sedimentation constant, the extrapolated S_{20w} being 5.75 ± 0.08. The diffusion constant of the complex was determined in the ultracentrifuge by the synthetic boundary method and found to be $D_{20} = 4.4 \pm 0.2$ (uncorrected for concentration). It was also homogeneous in polyacrylamide disc electrophoresis [8] over a wide concentration range. Its nitrogen content was 12.1 %. Depending on the partial specific volume, the molecular weight can be assumed to be in the range of 100 000—120 000. The vitamin B_{12} content of complex S was 25 μg per mg, indicating that 2 moles of cyanocobalamin were bound per mole of IF. Compared with the vitamin B_{12}