

A STANDARDIZED METHOD FOR THE IDENTIFICATION OF LICHEN PRODUCTS

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SUMMARY

A procedure for the routine identification of the products of lichen-forming fungi by thin-layer chromatography is described. Microextracts of plant fragments are chromatographed in three solvent systems. The spots of unknowns are assigned to R_F classes defined by the R_F values of marker controls of two lichen substances (atranorin and norstictic acid) chromatographed on every plate. The unknowns are tentatively identified by sorting (by R_F classes) punched cards summarizing microchemical data for all compounds previously studied. The preliminary identification is then confirmed by additional microchemical tests. The open-ended system can incorporate new and unknown compounds as well as information from other chromatographic systems. Data obtained by the standardized procedure are given for 104 products.

INTRODUCTION

The natural products of the lichen-forming fungi have become intimately involved in systematics and classification¹. Some 300 specific substances have been reported from about 2,000 of the 18,000 described species of lichens². Approximately 100 of these compounds are aromatic products that have been frequently mentioned in the taxonomic literature.

Today the lichen systematist faces the problem of learning to identify these natural products without a set procedure to guide him. An important paper by SANTESSON³ records R_F values for spots of some 80 compounds on thin-layer chromatograms run in a variety of solvent systems. The compounds are grouped by chemical type and by certain color reactions and, R_F data are presented for chromatograms run in solvent systems best suited to each group. Several other papers devoted to the thin-layer chromatography (TLC) of lichen substances also treat specific groups of compounds such as the aliphatic acids⁴ and aromatic aldehydes⁵. Several chromatographic solvents have been suggested in chemotaxonomic studies where a particular system was found to be convenient for distinguishing a limited number of specific compounds. While all of this information is extremely valuable for confirmatory identifications,

the worker surveying previously unstudied lichens requires R_F data for all lichen compounds in the same solvent systems. For example, if the lichen thallus produces coloration with *p*-phenylenediamine (PD), one may choose a solvent system recommended for PD-positive substances only to find that there are also PD-negative constituents present in the same plant and for which there are no R_F data for that solvent. It is then necessary to select other solvent systems, hoping by trial and error to find a suitable one. In spite of the intensive interest in lichen chemistry, no standardized system has been presented. The method described in this report attempts to overcome (1) the hit-and-miss aspects of microchemical identifications, (2) the difficulties due to varying R_F values, and (3) the problem of incorporating and retrieving microchemical information. The system described was developed for use by the lichenologists at Duke University. While the specific solvent systems and TLC plates used have been most successful in our laboratory, the general plan of the method can be modified and applied to any solvents used with any media.

In essence the system involves the following. Chromatographic data from three standard solvent systems for all compounds previously studied are stored on punched cards. The cards are keyed not to specific R_F values in the three solutions but rather to R_F classes the limits of which are determined by the R_F values of a control mixture consistently used as a marker on every chromatogram. Unknowns are identified by determining their R_F classes in the three solvent systems and then by sorting the punched cards for the known compounds belonging to all the same R_F classes.

These possibilities are then narrowed down by careful comparison of (a) actual R_F values with respect to the R_F values of the control marker spots, (b) color reactions, (c) appearance of the spot in short- and longwave UV light, (d) solubility, and any other pertinent data available. The final identification of the compound is achieved by appropriate confirmatory tests. Punched cards made for compounds that cannot be identified are added to the deck to permit the recognition of the same substances in species studied in the future.

MATERIALS AND METHODS

Sources of lichen products

Standards consisted of pure substances when these were available and of microextracts of herbarium specimens elsewhere.

Chromatographic materials and solvent systems

Chromatograms were developed in Brinkman tanks to a height of 10 cm on Merck Silica Gel F₂₅₄ thin-layer plates that had been stored in a desiccator over CaCl₂ NaOH but not activated. The solvent systems are: solvent A: benzene-dioxane-acetic acid (90:25:4, 238 ml); solvent B: hexane-ethyl ether-formic acid (5:4:1, 200 ml); solvent C: toluene-acetic acid (85:15, 240 ml).

The benzene, hexane, and toluene were dried (CaCl₂), redistilled, and stored over sodium. The dioxane and anhydrous ethyl ether were passed through columns of alumina to remove peroxides. The ether was stored over sodium. The same solvent systems prepared without these purifications gave results sufficiently similar to those reported here to indicate that these precautions are not required for routine analyses.

The level of the solutions in the tanks was maintained constant, and the solutions

were replaced when the R_F values of the controls deviated significantly from their usual values. Atranorin and norstictic acid were selected for marker controls because of their ready availability and because of their behavior in the particular solvent systems used. A mixture of these two compounds is available in a single extract of many common species, for example *Parmelia perforata* (Jacq.) Ach. in North America and *P. acetabulum* (Neck.) Duby in Europe.

Eighteen spots were applied to the plates 2 cm from the bottom and 9 mm apart. On every plate the first, ninth, and eighteenth positions were used for controls. The controls on a plate were spotted from the same solution to eliminate R_F variation due to concentration.

Determination of R_F classes

The developed plates were air-dried, examined under UV light (254 nm and 366 nm), sprayed with 10% H_2SO_4 , and heated at about 110° until colors developed. The colors of the spots were recorded as soon as the plates were taken from the oven.

In order to identify R_F classes, first the centers of the atranorin spots at positions 1 and 9 and at positions 9 and 18 are connected by a ruled line in pencil. The same is done for the norstictic acid spots. (Usually spots are slightly lower towards the center of the plate, the effect being more pronounced at lower R_F values, but sometimes the direction of variation is reversed.)

The distance between the origin and the solvent front was divided arbitrarily into eight regions measured with respect to the R_F values of the controls. The limits of the eight R_F classes are shown in Fig. 1. All R_F values for spots were recorded and

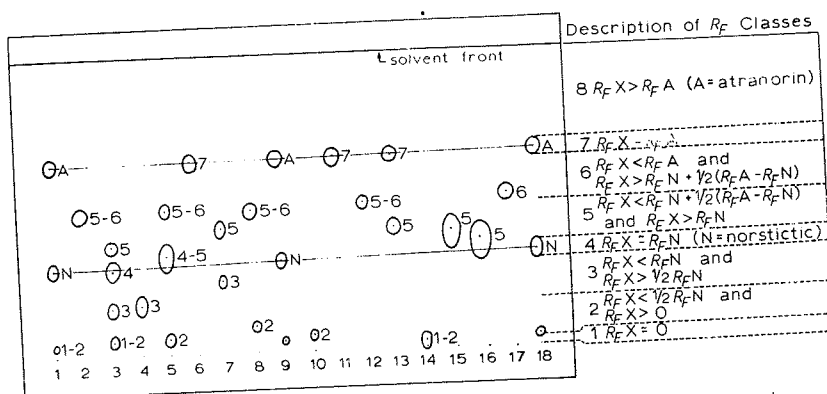


Fig. 1. A copy of a plate run in solvent C showing the controls of atranorin and norstictic acid at positions 1, 9, and 18, the horizontal penciled lines connecting the control spots, and the R_F classes of the spots of the principal compounds chromatographed. The number (or numbers) associated with each spot indicate its R_F class and the diagram to the right of the plate summarizes the R_F classes. The identities of the principal spots at each position on the plate are: (1) atranorin (A), norstictic acid (N), and an unknown substance, an acetone extract of *Parmelia perforata*; (2) acetylportentol; (3) alecatorialic acid (class 4) and barbatolic acid (class 3); (4) alecatorialic acid (class 4); (5) anisic acid (classes 4-5); (6) atranorin; (7) baecomycetic acid (class 5) and squamatic acid (class 5); (8) barbatolic acid (classes 5-6); (9) atranorin (A) and norstictic acid (N), the control; (10) fumiprotocetraric acid and protocetraric acid (running together as a single spot); (11) chloroatranorin; (12) confluent acid; (13) atranorin (class 7) and α -collatolic acid (class 5); (14) constictic acid; (15) cryptochlorophaeic acid; (16) 4-O-demethylbarbatolic acid; (17) didymic acid; and (18) atranorin (A) and norstictic acid (N), the control.

TABLE I

TLC DATA FOR 104 LICHEN PRODUCTS CHROMATOGRAPHED IN THREE SOLVENT SYSTEMS

<i>R_F</i> classes			<i>Compounds</i>			<i>Example R_F × 100 values</i> (<i>R_F</i> of X <i>R_F</i> of N, <i>R_F</i> of A*)			<i>Spot coloration with H₂SO₄</i> <i>and heat, (*), pigmentation,</i> <i>color reactions, etc.^b</i>		
A	B	C		A	B	C					
1	1	1	Rhodocladonic	0	0	0			Red pigment; K+		
1	2-3	2	Protocetraric	3/40, 68 ^a	17/27, 54	5/31, 66			Dark gray*; PD+		
1	3	2	Fumarprotocetraric	1-2/40, 68	24/28, 54	5/32, 67			Dark gray*; PD+		
1	3	2	Thamnolic	2/42, 70	19/26, 53	12/32, 69			Brownish*; PD+		
2	1	1-2	Constrictic	8/42, 71	2/27, 58	3/31, 65			Orange-brown*; PD+		
2	1-2	1	Erythrin	6-7/41, 71	5/27, 58	2/31, 67			Yellow*; C+		
2	2	2	Salazinic	10/41, 71	6/27, 58	6/32, 66			Yellow*; K+, PD+		
2	3	2	Polyporic	0-22/40, 68	0-14/23, 55	0-6/31, 67			Brownish pigment; pale*; K+		
2	3	2	Unknown with stictic ^c	17/42, 67	15/27, 55	9/30, 67			Reddish gray*		
2	3	3	Hypothamnolic	5/40, 68	21-22/26, 53	21-22/33, 68			Greenish*; K+, C+		
2	3	3	Squamatic	9-10/40, 67	24/27, 59	25-26/31, 67-68			Yellow*; UV++		
2	3	2	Endocrocin	10/42-43, 70	26/25, 55	7/32, 67-68			Orange pigment; K+		
2	4	2	Diploschistesic	17/43, 69	27/26, 53	11/31, 67			C+ blue		
2	4	2	Siphulin	17/40, 67	25/24, 57	5-6/34, 70			C+ red		
2	4-5	3	Physodalic	11/41, 71	30/26, 58	20/31, 66			Dark gray*; PD+		
2	4-5	3	Haemathamnolic	16/41, 71	31/27, 58	29/33, 68			Yellow*; PD+		
2	5	2	Pulvinic	13/42, 69	33/25, 56	7/32, 67			Yellow pigment; UV+ orange		
2	5	2	Unknown with psoromic ^d	20/44, 70	32/26, 57	11/30, 67			Greenish*		
2	5	2-3	Barbatolic	7/40, 69	35/25, 57	17/30, 65			Yellow*; PD+, K+		
2-3	4-5	3	Umbilicatic	23/43, 68	27-28/25, 56	21/29, 65			Yellow*		
3	2	2	Unknown with stictic ^c	23/42, 67	9/27, 55	11/29, 66			Yellow*		
3	2	2	Variolatic	23/43, 68	8/25, 56	11/34, 69			Yellow or gray*		
3	2	3	Stictic	31/37, 69	8/25, 54	22/31, 68			Orange-yellow*; PD+		
3	2-3	3	Echinocarpic	26/40, 68	12/26, 53	18/31, 67			Reddish gray*; PD+		
3	3	3	Galbinic	28/38, 65	16/27, 58	21/32, 67			Deep yellow*; PD+		
3	3	5-6	Unknown triterpene(?) ^e	33/42, 62	26/29, 60	37/31, 67			Brownish*; UV-		
3	4-5	3	Alectoronic	32/43, 71	30/27, 54	16/29, 64			Pale*; KC+, UV++		
3	5	3	Hypoprotocetraric	26/42, 70	37/27, 53	18/30, 69			Greenish brown or gray*		
3	5	3	Physodic	28/43, 71	34/27, 57	21/31, 66			Greenish*; KC+		
3	5	3	Gyrophoric	28/43, 71	35/27, 57	21/29, 66			Yellow or gray*; C+		
3	5	3	Lecanoric	29/42, 71	37/27, 57	21/30, 64			Yellow*; C+		
3	5	4-5	Lividic	33/42, 71	30/26, 57	3/30, 64			Greenish or green-brown*		
3	5	5	Lobaric	27/41, 71	37/26, 57	35/30, 64			Pale green*; KC+		
3	5	5	Virensic	24/37, 65	42/24, 57	35/29, 61			Gray black*; PD+, K+		
3	5	5	Baeomycesic	36/40, 66	33/27, 54	44/31, 67			Yellow*; PD+		
3	5	5	Psoromic	35/43, 69	37/26, 56	42/31, 66			Dull yellow or brown*; PD+		
3	5	5	Picrolichenic	34/42, 71	55-56/20, 57	35/31, 66			Dull yellow or green*; KC+		
3	5	5	4-O-Methylphysodic	37/42, 71	37/26, 57	42/31, 64			Gray green*; KC+		
3	5-6	5	4-O-Methylgyrophoric	34/43, 69	41/26, 57	42/30, 65			Yellow*		
3	6	5	Evernic	33/42, 71	45-46/27, 57	45/32, 67			Yellow*		
3						45/32, 67			Brownish*		

	3	5	5	5	5	30/40, 50 35/43, 69	33/27, 54 37/26, 56	44/31, 67 42/31, 66	Yellow*: PD + Dull yellow or brown*: KC + Dull yellow or green*: KC + Gray green*: KC + Yellow* Yellow* Brownish* Yellow*: C + Brown*: C +, KC +, PD + Pigment; pale streak* Pale*: C + green Yellow*: PD +, K + Yellow*: C + Brownish*: K +, KC +, (C +) Pale*: KC + Yellow*: KC + Brownish purple* Yellow*: C + Yellow*: C + Yellow* Yellow* Brown* Yellow-brown* Pinkish gray*: C + blue-green Yellow* Yellow* Yellow* Yellow* Yellow* Pale*: KC + Brownish*: C + Pigment; greenish*: K + Greenish*: KC + Brown*: (K +), (C +), KC + Yellow* Yellow* Greenish* Purple*: K +, KC +, (C +) Yellow pigment Pink or pale brown*: UV - Brown* Bright lavender*: UV - Yellow pigment; K +: UV + + +
Picloric	3	5	5	5	5	34/42, 71	35-30/20, 57	30/31, 60	
4-O-Methylphysodic	3	5	5	5	5	37/42, 71	37/26, 57	42/31, 64	
4-O-Methylglyphoric	3	5-6	5	5	5	34/43, 69	41/26, 57	42/30, 65	
Evermic	3	6	5	5	5	33/42, 71	45-46/27, 57	45/32, 67	
Grayanic	3	6	5	5	5	35/43, 71	46-47/27, 57	44/33, 67	
Olivetoric	3-4	5	3	3	3	36-37/43, 71	36/28, 61	23-24/31, 64	
Alectoralic	3-4	5-6	4	4	4	38-39/42, 71	42/27, 58	28/29, 64	
Entothecin	3-6	1-3	1-4	1-4	1-4	30-44/38, 66	0-15/23, 54	0-35/30, 66	
Strepsilin	4	3	3	3	3	40/49, 61	19/25, 57	24/30, 66	
Norstictic	4	4	4	4	4				
Orsellinic	4	4	4	4	4	39/40, 67	39-40/26, 57	25/31, 64	
Paludosic	4	5	4-5	4-5	4-5	38-39/40, 64-65	38/26, 57	30/28, 64	
α -Collatolic	4	5	5	5	5	39/40, 65	33/26, 54	40/31, 65	
Glomelliferic	4	5	5	5	5	39/40, 66	38/25, 53	43/29, 65	
Boninic	4	5	5-6	5-6	5-6	43/42, 69	38/27, 57	45-46/27, 61	
Anziaic	4	6	4-5	4-5	4-5	38-39/40, 67	48/26, 56	33/30, 64	
4-O-Demethylbarbatic	4	6	5	5	5	39/41, 70	45/27, 55	36/32, 66	
Divaricatic	4	6	5-6	5-6	5-6	41/43, 70	49/27, 54	48/31, 67	
Diffractic	4	6	5-6	5-6	5-6	42-43/42, 70	48/27, 53	47-48/31, 66	
Barbatic	4	6	5-6	5-6	5-6	42/43, 67	55/26, 59	47/30, 65	
Sekikaic	4	6	6	6	6	41-42/40, 67	47/26, 57	50/31-32, 66	
Homosekikaic	4	6	6	6	6	42-43/40, 67	50/26, 57	50/28, 66	
Didymic	4	6-7	6	6	6	44/43, 67	55/27, 55	51/31, 67	
Imbricatic	4	7	6	6	6	40/39, 67	55/25, 57-58	52/33, 68	
Stenosporic	4	7	6	6	6	41/39, 67	56/25, 57-58	51/32, 67	
Perlatolic	4	7	6	6	6	41/39, 67	56/25, 57-58	51/31, 66	
Sphaerophorin	4	7	6	6	6	41/38-39, 67	56/25, 57-58	51-52/32, 67	
Microphyllinic	4-5	5	5	5	5	40/38, 68	32/25, 57	44-45/31, 64	
Unknown triterpene(?) ^e	4-5	5	5	5	5	47/43, 70	37/29, 60	53/31, 68	
Orcinol	5	5	2-3	2-3	2-3	43/38, 68	33/25, 57	16-17/31, 64	
Rhodophyscin	5	5	3	3	3	44/40, 66	30-31/24, 56	18-19/27, 63	
Norlobaridone	5	5	3	3	3	47/39, 67	33-34/26, 57	23/31, 64	
Cryptochlorophaeic	5	5	5	5	5	43/40, 67	38/26, 57	40/31, 66	
Confluent	5	5	5-6	5-6	5-6	46-47/40, 67	34/28, 60	49/31, 65	
Planatic	5	5	6	6	6	45/40, 64	35/25, 57	56/31, 66	
Unknown with usnic ^g	5	5	6	6	6	52/40, 67	37/25, 57	51-52/29, 64	
Merochlorophaeic	5	5-6	6	6	6	47/40, 67	43-44/26, 56	53/31, 64	
Thiophanic	5	6-7	5	5	5	49/40-41, 67	49/26, 56	48/33, 69	
Zeorin	5-6	6	6	6	6	52/40, 67	37/25, 57	53/34, 70	
Ergosterol	5-6	6	6	6	6	52/39, 65	47-48/25, 56-57	56-57/34, 70	
Ursolic	5-6	6	6	6	6	53/38, 67	43/25, 57	55-56/33, 69	
Emodin	5-6	6	6	6	6	53/39, 65	43/24, 57	40/30, 66	

(continued on p. 90)

TABLE I (continued)

<i>R_F</i> classes		<i>Compounds</i>	<i>Example R_F × 100 values</i> (<i>R_F</i> of <i>X</i> / <i>R_F</i> of <i>N</i> , <i>R_F</i> of <i>A</i>)			<i>Spot coloration with H₂SO₄</i> <i>and heat, (*), pigmentation,</i> <i>color reactions, etc.^b</i>
<i>A</i>	<i>B</i>		<i>A</i>	<i>B</i>	<i>C</i>	
6	3-4	Acetylportentol	61-62/41, 67	27-38/33, 63	47/29, 64	Brown*; UV -
6	4	Unknown red pigment ^b	65/40, 70	31/31, 62	52-53/29, 66	UV + orange
6	5	Methyl gyrophorate	58/43, 70	40/30, 57	42/31, 64	Yellow*; C+
6	5	Gangaleoidin	61/41, 69	34/25, 56	57/34, 70	Deep yellow*
6	5-6	Unknown triterpene(?) ⁱ	63/42, 67	47/29, 61	54/31, 67	Pink*; UV -
6	6	Ethyl orsellinate	59/41, 69	42.5/24.5, 56	43/31, 67	Yellow*; C+
6	6	Unknown triterpene(?) ^j	61/41, 66	31/25, 57	52/30, 67	Bright lavender*; UV -
6	6	Diploicin	62/41, 69	45/27, 54	55/31, 67	Very pale or colorless*
6	6	Thiophanic	62/41, 66	49/24, 56	57/30, 67	Pigment; pale or colorless*
6	6	Usnic	62/40, 66	48/26, 54	57/29, 64	Greenish*; UV quench
6	6-7	Pannarin	62/41, 67	49-51/24, 53	60/30, 68	Dark gray*; PD+
7	5	Rhizocarpic	66-67/41, 67	34/24, 57	59/30, 67	Yellow pigment; UV +
7	5	Unknown pigment	67/41, 67	36/24, 57	59/30, 67	Yellow pigment
7	5-6	Epanorin	69/40, 68	38/23, 56	59/31, 66	Yellow pigment; UV +
7	6	Scrobiculin	61-62/39, 65	45-46/24, 56	51-52/32, 67	Brown*; (C+), KC+
7	6-7	Tenuorin	66/39, 65	45/24, 56	66-67/32, 68	Yellow*
7	6	Lichexanthone	66/39, 65	45/24, 56	64/30, 64	Pale yellow*; UV +
7	6	Vulpinic	65/39, 65	50/24-25, 56	68/32, 68	Yellow pigment
7	6	Leprapinic	69/40, 68	45/25, 56	66/31, 66	Yellow pigment
7	6	Pinastric	69-70/41, 69	44/24, 55	65/32, 67	Yellow pigment
7	7	Vicanicin	64/39, 64	60/27, 60	64/35, 70	Pale*
7	7	Parietin	68/39, 66	57/27, 58-59	67/31, 66	Yellow pigment; K+; UV + +
7	7	Atranorin				Yellow*; PD+
7	7	Chloroatranorin	62/36, 62	53/25, 53	65/31, 65	Yellow*; PD+
7-8	8	Calycin	72/40, 69	52/23, 55	74-75/30, 66	Yellow pigment
8	8	Pulvinic dilactone	73/40, 69	62/24, 56	79/30, 69	Yellow pigment; UV + + +

^a The two numbers following the virgule (/) are measurements in millimeters to the norstictic acid line (*R_F* of *N*) and the atranorin line (*R_F* of *A*). (See Fig. 1.)

^b Abbreviations for color reagents are: K = 10% aqueous KOH; PD = concentrated alcoholic *p*-phenylenediamine; C = saturated aqueous Ca(OCl)₂; KC = K followed by C.

^c With two other unknowns and stictic acid in a number of species including *Usnea rubicunda* Stirt., for example.

^d With atranorin and psoromic acid in *Argopsis* sp.

^e This unidentified substance, which may be a triterpene, occurs in *Parmelia aurulenta* Tuck.

^f This compound has been reported in lichens but not recently confirmed.

^g In *Lecanora rubina* (Vill.) Ach.

^h In *Arthonia rubroinctum* Meri.

ⁱ This unknown substance, which may be a triterpene, occurs in *Pyxine caesiopruinosa* (Nyl.) Imsh.

^j This unknown substance, which may be a triterpene, occurs in *Physcia* sp.

followed by the values measured to the norstictic acid and atranorin lines at the same horizontal position (see Table I).

Recording information on punched cards

A punched card, prepared for every compound, is keyed for the R_F classes in the three solvent systems and for an alphabetic sequence to the common chemical names. Additional data and specific R_F values, always expressed with the corresponding values of the norstictic acid and atranorin controls, are recorded directly on the cards. As an example, Fig. 2 shows the punched card for lobaric acid.

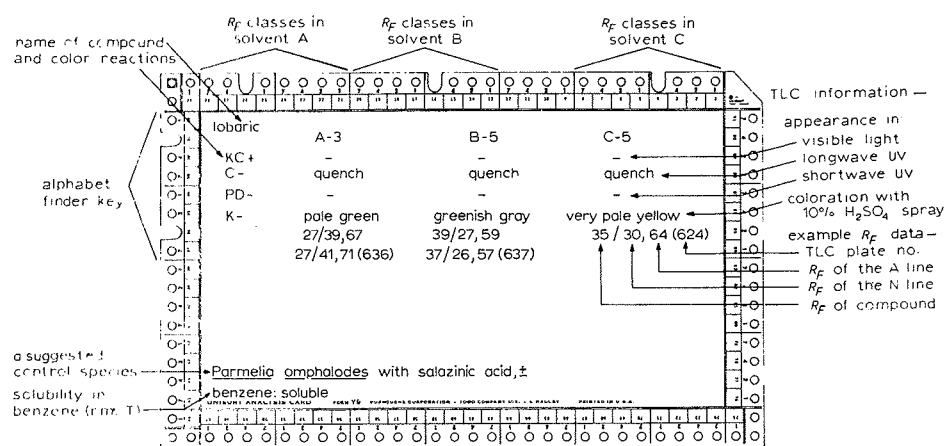


Fig. 2. An example of a punched card for a known compound showing the information stored.

Identification of compounds in crude extracts of lichens for chemotaxonomic surveys

Chemotaxonomic studies on lichens are usually based upon chemical analysis of fragments of herbarium specimens. In our laboratory the following procedure is used: cleaned plant material (1 cm² or less) is placed in a shell vial (Mercer Glass Works, Inc., N.Y.; shell vials, short style, 1/2 dr, 35 × 12 mm) numbered in India ink. The fragment is extracted three times by 5–10-min soakings in benzene at room temperature. The extracts are evaporated at about 50° on a microscope slide. The processes are repeated using acetone to extract the sample at elevated temperature (50°), and the acetone extracts are evaporated on a second slide. Usually many specimens are analyzed simultaneously, and the benzene and acetone extracts of each sample are chromatographed side-by-side on the thin-layer plate. As usual, at positions 1, 9, and 18, the controls of atranorin and norstictic acid are included.

To spot the chromatograms, a drop or two of acetone is flowed over the residue on the slide and collected in a micropipette. Three plates may be spotted simultaneously. The R_F values of the spots are recorded and the R_F classes determined. The punched cards make possible the identification of all previously studied compounds with the same R_F classes. Of the 104 compounds listed in Table I the maximum number of compounds with the same R_F classes is six. The additional information on the cards (Fig. 2) reduces the number of possibilities still further and suggests the best solution or solutions in which to rechromatograph the unknown with appropriate

standards. If the compound is not identifiable, a punched card for it is prepared and added to the deck.

RESULTS AND DISCUSSION

Table I summarizes the data obtained for the most common aromatic and triterpenoid products of lichens. The compounds are arranged according to increasing R_F classes so that those with the most similar R_F values in the three solvent systems are grouped together. It is unlikely that these R_F values could be reproduced exactly in another laboratory, since over a period of some months the R_F values of the controls of atranorin and norstictic acid change slightly. But it is expected that the R_F classes which depend only on the relative order of spots will be much more constant.

In the identification of unknowns from crude extracts of lichens containing several compounds it may be difficult to distinguish which spots on the chromatograms run in different solutions are due to the same compounds. Sometimes, one or more spots will be concealed by other spots in some solvents. Colorations with different spray reagents can sometimes help. Elution of a band run in one solvent and rechromatography of the eluted material in the three standard solvents usually solve the most difficult problems. And finally, the double extraction of the sample, first with benzene and then with acetone, often allows the identification of compounds that would be obscured in a simple acetone extract.

The system of punched cards described in this report can be varied in many ways and it can be expanded by adding new solvent systems and by the continual addition of cards for new compounds and unknowns encountered in surveys. Using this method, new students of lichenology in our laboratory have been able to identify many of the common aromatic lichen substances from their first chromatographic plates. The method gives meaning to the designation "unidentified substance" and assures that any particular unidentified substance will be recognized again if it is encountered in other species, allowing all such records to be cleared up when the substance is finally characterized.

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