Journal of Chromatography, 646 (1993) 417–427
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 350

Short Communication

Identification of lichen substances by a standardized high-performance liquid chromatographic method

G.B. Feige* and H.T. Lumbsch

Botanical Institute, University of Essen, P.O. Box 103 764, 45117 Essen (Germany)

S. Huneck

Institute of Plant Biochemistry, Weinberg 3, 06018 Halle (Germany)

J.A. Elix

Department of Chemistry, The Faculties, Australian National University, Canberra, ACT 0200 (Australia)

(First received March 9th, 1993; revised manuscript received May 24th, 1993)

ABSTRACT

A method for the identification of secondary aromatic lichen substances using high-performance liquid chromatography (HPLC) with reversed-phase columns, gradient elution and benzoic and solorinic acids as standards has been developed. A retention index (I), calculated from the elution time of the appropriate peak with reference to the standards, is used in identification. I values are recorded for 331 compounds chromatographed in this standard system.

INTRODUCTION

Lichens are well known for the diversity of secondary metabolites that they produce, and the identification of these substances has become an integral part of modern taxonomic investigations of these organisms [1]. Out of a total of *ca.* 550 lichen compounds so far reported, over 450 are aromatic derivatives.

Standardized methodology and further refinement of routine analytical TLC procedures for detecting and comparing lichen metabolites have been reported by Culberson and co-workers [2–6]. Further, two-dimensional TLC has considerably improved rate of flow values (R_F) discrimination of structurally similar compounds and has enabled the identification of minor constituents present in complex mixtures [7].

Recently, high-performance liquid chromatography (HPLC) has become more widely used as an effective analytical tool for the separation and identification of lichen substances. Early attempts to apply this method to lichen chemotaxonomy were made using normal-phase silica columns [8,9], but better results are obtained with bonded reversed-phase columns. While Culberson and Culberson [10] used a methanol–water–acetic acid solvent system, Lumbsch and

* Corresponding author.

0021-9673/93/$06.00

Elix [11] used a mobile phase consisting of methanol, water and orthophosphoric acid.

Although these isocratic methods produced excellent results, gradient elution is an even more effective method for HPLC analyses of lichen extracts, since such extracts frequently contain metabolites of wide-ranging hydrophobicity. Methods using gradient elution for various analyses in the genera *Cladina*, *Cladonia*, *Placopsis*, *Rhizocarpon* and the Roccellaceae and other groups of lichenized ascomycetes have been reported [12–18]. Here we propose a new standardized method of gradient elution for the identification of lichen products in chemotaxonomic investigations. The new solvent gradient devised in this study maximizes the resolution of a large number of the compounds that are found in lichens and has already been used successfully in a number of lichen genera belonging to different families, namely the Agyriaceae [19], Lecanoraceae [20,21], Pertusariaceae [22] and Roccellaceae [23].

MATERIALS AND METHODS

Sources of compounds

In general, authentic samples of lichen substances were used when available, and each compound was injected separately to determine its retention index (*I*). Otherwise, herbarium specimens with established chemistry were used as standards. In these cases 10 mg of lichen material were extracted in 1 ml of cool acetone for 60 min.

Equipment, solvent systems and internal standards

A Kontron HPLC system connected to a Data System 450 instrument with a 430 UV detector and a 360 autosampler was used. A Spherisorb 5 ODS 2 column (Kontron), 5 μm, 250 × 4.6 mm, at room temperature was used.

Two solvent systems were employed. Solvent system A was Aqua bidest containing 1% orthophosphoric acid and solvent system B was 100% methanol (Baker). The solvents were degassed for 30 min in an ultrasonic vibrator prior to use.

The substances were dissolved in acetone to which the two standards were added (20 mg of benzoic acid and 20 mg of solorinic acid per 1000 ml of acetone). Benzoic acid elutes very rapidly and solorinic acid elutes slowly. Only orsellinic acid, consalazinic acid and the phthalides elute more rapidly than benzoic acid and the *I* values (detailed later) of these compounds are given as negative numbers. Only one lichen metabolite (hierridin) eluted more slowly than solorinic acid in our gradient system.

The programmed run

The run started with 30% B and continued isocratically for 1 min at a flow-rate of 0.7 ml/min. After 1 min, 20 μl were injected and solvent system B was increased to 70% within 14 min, then up to 100% in 30 min, and then isocratically in 100% B for a further 18 min. At the end of the run the solvent system B was decreased to 30% within 1 min and the column flushed with 30% B for 16 min before a new run was started.

The compounds were detected at $\lambda = 245$ nm and the UV spectra ($\lambda = 200$–400 nm) of each peak eluted were recorded automatically.

The retention index value

Benzoic and solorinic acids were used as internal standards by their addition to the extraction liquid (acetone). Benzoic acid was utilized previously by Huovinen *et al.* [17] as a substance that elutes more rapidly than most but not all lichen compounds. Bis-(2-ethylhexyl)-phthalide was employed as a second internal standard [17]. However, this compound elutes at the same time as some chlorolichexanthones and is therefore not recommended as a standard for the identification of lichen extracts containing chloroxanthones or long-chain depsides. We therefore sought a more hydrophobic compound and found solorinic acid to be ideal. This compound is present in large quantities in the Arctic–alpine lichen *Solorina crocea* and can be readily isolated because of its hydrophobicity. The *I* value of an unknown peak is calculated as follows:

$$I = \frac{t_R \text{ of peak} - t_R \text{ of benzoic acid}}{t_R \text{ of solorinic acid}} \times 100$$

The *I* values as defined here are very stable over the lifetime of a column.

TABLE I

RETENTION INDICES OF THE EXAMINED LICHEN SUBSTANCES AND THEIR SUBSTANCE CLASS

I	Examined substance	Substance class
−3	5,7-Dihydroxy-6-methylphthalide	Monocyclic phenol derivatives
−3	6-Formyl-5,7-dihydroxyphthalide	Monocyclic phenol derivatives
−2	Orsellinic acid	Monocyclic phenol derivatives
−1	Consalazinic acid	Depsidones
1	Constictic acid	Depsidones
2	Montagnetol	Monocyclic phenol derivatives
3	Connorstictic acid	Depsidones
3	Conporphyrilic acid	Dibenzofuranes
3	Variolaric acid	Depsidones
4	Canarione	
4	Cryptostictic acid	Depsidones
5	6-Hydroxymethyleugenitin	Chromones
5	Galapagin	Chromones
5	Menegazziaic acid	Depsidones
5	Methyl orsellinate	Monocyclic phenol derivatives
5	Salazinic acid	Depsidones
6	Pannaric acid	Dibenzofuranes
6	Pulvinic acid	Pulvinic acid derivatives
6	Stictic acid	Depsidones
7	Decarboxythamnolic acid	Depsides
7	Erythrin	Depsides
7	Hypoconstictic acid	Depsidones
7	Porphyrilic acid	Dibenzofuranes
7	Strepsilin	Dibenzofuranes
8	Eugenitol	Chromones
8	Hyposalazinic acid	Depsidones
8	Methyl 4-O-methylorsellinate	Monocyclic phenol derivatives
8	Roccellin	Chromones
9	Galbinic acid	Depsidones
9	Virensic acid	Depsidones
10	Barbatolin	Benzyl ester
10	Diploschistesic acid	Depsides
10	Lepraric acid	Chromones
10	Methyl β-orsellinate	Monocyclic phenol derivatives
10	Siphulellic acid	Depsidones
10	Succinprotocetraric acid	Depsidones
11	4-O-Demethylnotatic acid	Depsidones
11	Chiodectonic acid	Anthraquinones
11	Norlobariol	Diphenyl ether
11	Pannaric acid 6-methyl ester	Dibenzofuranes
11	Protosiphulin	Chromones
12	4-O-Demethylglomellic acid	Depsides
12	Norstictic acid	Depsidones
13	2'-O-Demethylpsoromic acid	Depsidones
13	2-O-Methylsquamatic acid	Depsides
13	Alectorialin	Benzyl ester
13	Epiphorellic acid 2	Diphenyl esters
13	Hypostictic acid	Depsidones
13	Pannaric acid 2-methyl ester	Dibenzofuranes
13	Sordidone	Chromones

(Continued on p. 420)

TABLE I *(continued)*

I	Examined substance	Substance class
14	4-O-Demethylloxodellic acid	Depsides
14	Lecanoric acid	Depsides
14	Protocetraric acid	Depsidones
15	Confumarprotocetraric acid	Depsidones
15	Hypothamnolic acid	Depsides
15	Norlichexanthone	Xanthones
16	2,4′-Di-O-methylnorsekikaic acid	Depsides
15	Subpicrolichenic acid	Depsones
16	3-Hydroxyumbilicaric acid	Depsides
16	Barbatolic acid	Benzyl ester
16	Echinocarpic acid	Unknown
16	Methyl evernate	Depsides
16	Norlobariol methyl ester	Diphenyl ether
16	Schizopeltic acid	Dibenzofuranes
17	Arthonin	
17	2-O-Methylhiascic acid	Depsides
17	3′-Dechlorolecideiodin	Depsidones
17	4-O-Demethyldiffractaic acid	Depsides
17	Conorlobaridone	Depsidones
17	Fumarprotocetraric acid	Depsidones
17	Malonprotocetraric acid	Depsidones
17	Oxyphysodic acid	Depsidones
17	Paludosic acid	Depsides
18	Alectorialic acid	Benzyl ester
18	Arthoniaic acid	Depsides
18	Contortin	Biphenyl
18	Glomellic acid	Depsides
18	Glomellonic acid	Depsidones
18	Methyl lecanorate	Depsides
18	Physodalic acid	Depsidones
18	Pulvinic acid	Pulvinic acid derivatives
18	Thamnolic acid	Depsides
19	5-Chloronorlichexanthone	Xanthones
19	Crustinic acid	Depsides
19	Eugenitin	Chromones
19	Hiascic acid	Depsides
19	Hypoprotocetraric acid	Depsidones
19	Leprolomin	Diphenyl ether
19	Notatic acid	Depsidones
19	Submerochlorophaeic acid	Depsides
19	Vittatolic acid	Depsidones
20	2-Chloronorlichexanthone	Xanthones
20	4′-O-Methylnorsekikaic acid	Depsides
20	4-O-Demethylglomelliferic acid	Depsides
20	Conloxodin	Depsidones
20	Hypothallin	Amino acid derivatives
20	Isonorlobaridone	Diphenyl ether
20	Lividic acid	Depsidones
20	Orcinyl lecanorate	Depsides
21	2′-O-Methylphysodic acid	Depsidones
21	3-Methoxyumbilicaric acid	Depsides
21	4′-O-Demethylsekikaic acid	Depsides
21	4-Chloronorlichexanthone	Xanthones

TABLE I *(continued)*

I	Examined substance	Substance class
21	Gangaleoidin	Depsidones
21	Normiriquidisäure	Depsides
21	Psoromic acid	Depsidones
22	2′-O-Methylanziaic acid	Depsides
22	2-O-Methylobtusatic acid	Depsides
22	Aspicilin	Macrocyclic lactone
22	Loxodellic acid	Depsides
22	Nordivaricatic acid	Depsides
22	Norgangaleoidin	Depsidones
22	Parietinic acid	Anthraquinones
22	Picrolichenic acid	Depsones
22	Siphulin	Chromones
22	Squamatic acid	Depsides
23	3-O-Demethylscenscidin	Depsidones
23	4-O-Demethylmicrophyllinic acid	Depsides
23	Buellolide	Diphenyl ether
23	Cryptochlorophaeic acid	Depsides
23	Ovoic acid	Depsides
24	4′-O-Methylnorhomosekikaic acid	Depsides
24	4-O-Demethylbarbatic acid	Depsides
24	4-O-Demethylplanaic acid	Depsides
24	5-O-Methylhiascic acid	Depsides
24	7-Chloronorlichexanthone	Xanthones
24	Epiphorellic acid 1	Diphenyl esters
24	Isonotatic acid	Depsidones
24	Lecideoidin	Depsidones
24	Methyl 3,5-dichlorolecanorate	Depsides
24	Teloschistin	Anthraquinones
25	2′-O-Methylmicrophyllinic acid	Depsides
25	2-O-Methylsekikaic acid	Depsides
25	Gyrophoric acid	Depsides
25	Methyl 2,2′-di-O-methyleriodermate	Depsides
25	Physodic acid	Depsidones
25	Pseudoplacodiolic acid	Usnic acids
25	Umbilicaric acid	Depsides
25	Vulpinic acid	Pulvinic acid derivatives
26	2-O-Methylnorstenosporic acid	Depsides
26	3-Methoxy-2,4-di-O-methylgyrophoric acid	Depsides
26	4′-O-Methylcryptochlorophaeic acid	Depsides
26	4,5-Dichloronorlichexanthone	Xanthones
26	Alectoronic acid	Depsidones
26	Evernic acid	Depsides
26	Glomelliferonic acid	Depsidones
26	Haemothamnolic acid	Depsides
26	Hirtifructic acid	Unknown
26	Merochlorophaeic acid	Depsides
26	Norlobaridone	Depsidones
26	α-Collatolic acid	Depsidones
27	2,5-Dichloronorlichexanthone	Xanthones
27	Euplectin	Anthraquinones
27	Glomelliferic acid	Depsides
27	Methyl 3′-methyllecanorate	Depsides

(Continued on p. 422)

TABLE I *(continued)*

I	Examined substance	Substance class
27	Olivetoric acid	Depsides
27	Placodiolic acid	Usnic acids
28	2,7-Dichloronorlichexanthone	Xanthones
28	2-O-Methylconfluentic acid	Depsides
28	2-O-Methyldivaricatic	Depsides
28	4-O-Demethylstenosporic acid	Depsides
28	4-O-Methylhypoprotocetraric acid	Depsidones
28	Baeomycesic acid	Depsides
28	Chlorolecideoidin	Depsidones
28	Leprapinic acid	Pulvinic acid derivatives
28	Loxodin	Depsidones
28	Oxostenosporic acid	Depsides
28	Sekikaic acid	Depsides
28	Subdidymic acid	Dibenzofuranes
29	2′-O-Methylanziaic acid	Depsides
29	2,4-Dichloronorlichexanthone	Xanthones
29	2,4-Di-O-Methylgyrophoric acid	Depsides
29	4-O-Demethylimbricaric acid	Depsides
29	Confluentic acid	Depsides
29	Methyl sekikaiate	Depsides
29	Pinastric acid	Pulvinic acid derivatives
29	Wrightiin	Depsides
29	*m*-Scrobiculin	Depsides
30	4,7-Dichloronorlichexanthone	Xanthones
30	4-O-Methylisocryptochlorophaeic acid	Depsides
30	5,7-Dichloronorlichexanthone	Xanthones
30	Diffractaic acid	Depsides
30	Glaucophaeic acid	Depsides
30	Isoobtusatic acid	Depsides
30	Lobaric acid	Depsidones
30	Methyl 2′-O-methyleriodermate	Depsides
30	Methyl gyrophorate	Depsides
30	Miriquidic acid	Depsides
30	Pseudocyphellarin B	Depsides
31	2′,2″-Di-O-metyltenuiorin	Depsides
31	2-Chloro-6-O-methylnorlichexanthone	Xanthones
31	4,5-Di-O-methylhiascic acid	Depsides
31	4-O-Methylnorlobaridon	Depsidones
31	Boninic acid	Depsides
31	Methyl 2-O-methyleriodermate	Depsides
31	Methyl 4-O-demethylbarbatate	Depsides
31	Skyrin	Anthraquinones
31	*p*-Scrobiculin	Depsides
32	2′-O-Methylhyperphyllinic acid	Depsides
32	3′-Methylevernic acid	Depsides
32	3,5-Dichloro-2′-O-methylanziaic acid	Depsides
32	3-Chlorodivaricatic acid	Depsides
32	4-O-Methylcryptochlorophaeic acid	Depsides
32	5-Chloro-6-O-methylnorlichexanthone	Xanthones
32	Emodin	Anthraquinones
32	Methyl 5-chloronorobtusatate	Depsides
32	Obtusatic acid	Depsides
32	Secalonic acid B	Anthraquinones

TABLE I *(continued)*

I	Examined substance	Substance class
33	2″-O-Methyltenuiorin	Depsides
33	2-O-Methyltenuiorin	Depsides
33	4-O-Demethylsphaerophorin	Depsides
33	4-O-Methylolivetoric acid	Depsides
33	5-Chlorovirensic acid	Depsidones
33	Divaricatic acid	Depsides
33	Secalonic acid C	Anthraquinones
34	3,5-Dichloro-4-O-demethylplanaic acid	Depsides
34	4′-O-Methylnorcryptochlorophaeic acid	Depsides
34	4-Chloro-6-O-methylnorlichexanthone	Xanthones
34	4-O-Methylgyrophoric acid	Depsides
34	7-Chloro-1,6-di-O-methylemodin	Anthraquinones
34	Imbricaric acid	Depsides
34	Nephroarctin	Depsides
34	Planaic acid	Depsides
34	Rhizocarpic acid	Pulvinic acid derivatives
34	Secalonic acid A	Anthraquinones
34	Superpicrolichenic acid	Depsones
35	2-O-Methylstenosporic acid	Depsides
35	7-Chloro-1-O-methylemodin	Anthraquinones
35	Anziaic acid	Depsides
35	Arthothelin	Xanthones
35	Didymic acid	Dibenzofuranes
35	Eriodermin	Depsidones
35	Homosekikaic acid	Depsides
35	Hyperconfluentic acid	Depsides
35	Isovicanicin	Depsidones
35	Stenosporonic acid	Depsidones
35	Vinetorin	Xanthones
36	2′-O-Methylimbricaric acid	Depsides
36	2′-O-Methylstenosporic acid	Depsides
36	2,5-Dichloro-6-O-methylnorlichexanthone	Xanthones
36	Demethylchodatin	Xanthones
36	Epanorin	Pulvinic acid derivatives
36	Isoarthothelin	Xanthones
36	Leoidin	Depsidones
36	Usnic acid	Usnic acids
37	1′-Chlornephroarctin	Depsides
37	2′-O-Methyltenuiorin	Depsides
37	2,4,7-Trichloronorlichexanthone	Xanthones
37	3-Dechlorodiploicin	Depsidones
37	4,5-Dichloro-6-O-methylnorlichexanthone	Xanthones
37	Asemone	Xanthones
37	Barbatic acid	Depsides
37	Congrayanic acid	Diphenyl ether
37	Grayanic acid	Depsidones
37	Methyl 4-O-methyleriodermate	Depsidones
37	Pannarin	Depsidones
37	Vicanicin	Depsidones
38	4,5-Dichloro-3-O-methylnorlichexanthone	Xanthones
38	7-Chloroemodin	Anthraquinones
38	Atranorin	Depsides

(Continued on p. 424)

TABLE I *(continued)*

I	Examined substance	Substance class
38	Hyperhomosekikaic acid	Depsides
39	2'-O-Methylperlatolic acid	Depsides
39	3-Chlorostenosporic acid	Depsides
39	Caloploicin	Depsidones
39	Evernine	Depsides
39	Methyl eriodermate	Depsides
39	Tenuiorin	Depsides
40	7-Chloro-6-O-methylnorlichexanthone	Xanthones
40	Melacarpic acid	Dibenzofuranes
40	Methyl 5-chloro-4-O-demethylbarbatate	Depsides
40	Stenosporic acid	Depsides
41	4-O-Demethylgrayanic acid	Depsidones
41	Colensoic acid	Depsidones
41	Isousnic acid	Usnic acids
41	Superconfluentic acid	Depsides
41	Thiophaninic acid	Xanthones
42	2-O-Methylperlatolic acid	Depsides
42	4-O-Methylsuperolivetoric acid	Depsides
42	Chloroatranorin	Depsides
43	8-O-Methylthiomelin	Xanthones
43	Argopsin	Depsidones
43	Diploicin	Depsidones
43	Pulvinic dilactone	Pulvinic acid derivatives
44	2'-O-Methylisohyperlatolic acid	Depsides
44	Pseudocyphellarin A	Depsides
44	Thiophanic acid	Xanthones
44	Valsarin	Anthraquinones
45	3-Chloroperlatolic acid	Depsides
45	Thuringione	Xanthones
46	2,7-Dichloro-6-O-methylnorlichexanthone	Xanthones
46	4-Dechlorothiomelin	Xanthones
46	5,7-Dichloro-3-O-methylnorlichexanthone	Xanthones
46	Lichexanthone	Xanthones
46	Norsolorinic acid	Anthraquinones
46	Sphaerophorin	Depsides
47	2,5,7-Trichloro-3-O-methylnorlichexanthone	Xanthones
47	2-Chlorolichexanthone	Xanthones
47	Calycin	Pulvinic acid derivatives
47	Methyl barbatate	Depsides
47	Perlatolic acid	Depsides
48	1,8-Dihydroxy-3,6-dimethoxy-9-H-xanthen-9-one	Xanthones
48	2'-O-Methylsuperlatolic acid	Depsides
48	2,7-Dichloro-3-O-methylnorlichexanthone	Xanthones
48	5-Chlorolichexanthone	Xanthones
48	6-O-Methylarthothelin	Xanthones
49	6-O-Methylasemone	Xanthones
49	Chodatin	Xanthones
50	2,5-Dichlorolichexanthone	Xanthones
50	4-Chlorolichexanthone	Xanthones
50	Fragilin	Anthraquinones
50	Parietin	Anthraquinones
51	3-O-Methylasemone	Xanthones
51	6-O-Methylthiophanic acid	Xanthones

TABLE I *(continued)*

I	Examined substance	Substance class
52	7-Chlorolichexanthone	Xanthones
53	2,7-Dichlorolichexanthone	Xanthones
53	3-O-Methylthiophanic acid	Xanthones
53	Thiomelin	Xanthones
54	2,5,7-Trichlorolichexanthone	Xanthones
54	Hyperlatolic acid	Depsides
55	2,4-Dichlorolichexanthone	Xanthones
55	4,5-Dichlorolichexanthone	Xanthones
55	5,7-Dichlorolichexanthone	Xanthones
55	Isohyperlatolic acid	Depsides
56	1,3,6-Tri-O-methylarthothelin	Xanthones
56	6-O-Methylthiophanic acid	Xanthones
57	6-O-Methylaverythrin	Anthraquinones
59	4,4'-Disolorinic acid	Anthraquinones
59	Superlatolic acid	Depsides
62	2,4,5-Trichlorolichexanthone	Xanthones
75	Solorinic acid	Anthraquinones
84	Hierridin	Monocyclic phenol derivatives

RESULTS AND DISCUSSION

The aromatic lichen substances can be identified by comparison of the internal *I* values. The *I* values of 331 compounds are listed in Table I. These data have also been included in a computerized database with the TLC R_F values and further information, such as mass spectrum data, substance class, etc. [24].

The substance classes can be distinguished by their UV spectra and to some extent by their *I* value. Most monocyclic phenol derivatives elute very rapidly and have *I* values between −3 and +5, whereas the depsidones with highly variable hydrophobicities have *I* values which range between −1 and +43. The chromones also elute quite rapidly (with *I* values between 5 and 22). Whereas depsides with short alkyl side-chains elute rapidly, those with longer alkyl side-chains elute more slowly. In the extreme case of superlatolic acid ($I = 59$) with two C_7 side-chains, only the standard solorinic acid and 2,4,5-trichlorolichexanthone elute more slowly. Norlichexanthones and lichexanthones can be readily separated by gradient HPLC. The norlichexanthones have *I* values between 15 and 44 depending on the degree of chlorination, while the more hydrophobic lichexanthones have *I* values between 46 and 52. Similarly, the depsones have *I* values between 15 and 34 (depending on the length of the side-chains), while the usnic acids have *I* values between 25 and 36.

Given the large number of minor lichen substances still to be characterized and identified, it should be emphasized that compounds should not be identified solely on the basis of their *I* value using this HPLC method. Often substances that elute at the same time belong to different substance classes and can readily be distinguished by their UV spectra, though even then independent confirmation of their identity should be sought. Complementary analytical methods include standardized TLC [2–6], two-dimensional TLC [7], lichen mass spectrometry [25] or comparative chromatography with authentic lichen compounds. The resolution attainable by our HPLC method is illustrated in Fig. 1, which shows the chromatogram of a mixture of 30 lichen compounds and the two standards, and Fig. 2, which shows the chromatogram of an extract from *Lecanora leprosa*. The potency of this system can readily be appreciated when one considers that in practice relatively few peaks are encountered in the HPLC analyses of crude

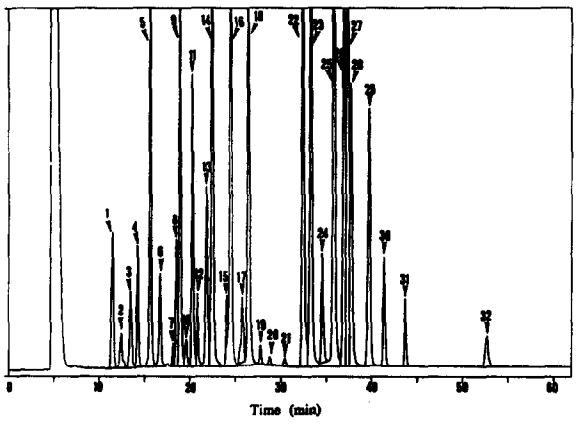

Fig. 1. HPLC of a mixture of 30 lichen substances. Peaks: 1 = orsellinic acid; 2 = benzoic acid, internal standard; 3 = constictic acid; 4 = variolaric acid; 5 = salazinic acid; 6 = stictic acid; 7 = diploschistesic acid; 8 = chiodectonic acid; 9 = norstictic acid; 10 = hypostictic acid; 11 = lecanoric acid; 12 = subpicrolichenic acid; 13 = fumarprotocetraric acid; 14 = thamnolic acid; 15 = gangaleoidin; 16 = picrolichenic acid; 17 = gyrophoric acid; 18 = placodiolic acid; 19 = confluentic acid; 20 = pseudocyphellarin B; 21 = divaricatic acid; 22 = usnic acid; 23 = atranorin; 24 = stenosporic acid; 25 = chloroatranorin; 26 = pseudocyphellarin A; 27 = lichexanthone; 28 = 2-chlorolichexanthone; 29 = 2,5-dichlorolichexanthone; 30 = isohyperlatolic acid; 31 = superlatolic acid; 32 = solorinic acid, internal standard.

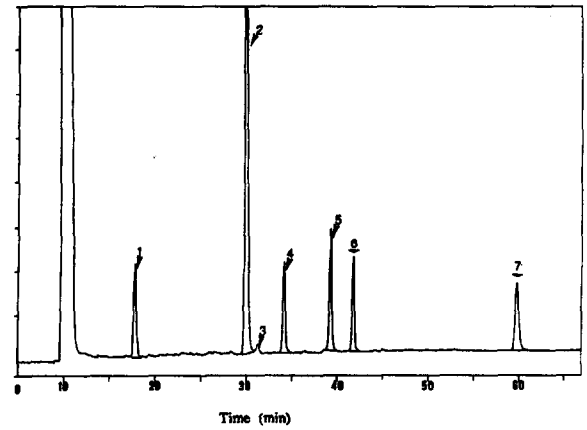

Fig. 2. HPLC of *Lecanora leprosa* (BRI-491270). Peaks: 1 = benzoic acid, internal standard; 2 = gangaleoidin; 3 = norgangaleoidin; 4 = chlorolecideoidin; 5 = atranorin; 6 = chloroatranorin; 7 = soloronic acid, internal standard.

lichen extracts. However, a few lichens are exceptional. In *Haematomma pachycarpum*, for example, twelve substances were detected [26], but this assemblage of secondary products was reported to be the most complex known in any one lichen species.

ACKNOWLEDGEMENTS

We wish to thank Dr. Alan W. Archer (Sydney), Dr. Chicita F. Culberson (Duke) and Professor A.G. Gonzales (Tenerife) for sending us authentic samples of lichen substances or lichen specimens for our examination. The technical assistance of M. Neugebauer is gratefully acknowledged.

REFERENCES

1 D.L. Hawksworth, in D.H. Brown, D.L. Hawksworth and R.H. Bailey (Editors), *Lichenology: Progress and Problems*, Academic Press, London, 1976, p. 139.
2 C.F. Culberson, *J. Chromatogr.*, 72 (1972) 113.
3 C.F. Culberson and K. Ammann, *Herzogia*, 5 (1979) 1.
4 C.F. Culberson, W.L. Culberson and A. Johnson, *Bryologist*, 84 (1981) 16.
5 C.F. Culberson and A. Johnson, *J. Chromatogr.*, 238 (1982) 483.
6 C.F. Culberson and H. Kristinsson, *J. Chromatogr.*, 46 (1970) 85.
7 C.F. Culberson and A. Johnson, *J. Chromatogr.*, 128 (1976) 253.
8 C.F. Culberson, *Bryologist*, 75 (1972) 54.
9 R. Nourish and R.W.A. Olivier, in D.H. Brown, D.L. Hawksworth and R.H. Bailey (Editors), *Lichenology: Progress and Problems*, Academic Press, London, 1976, p. 185.
10 C.F. Culberson and W.L. Culberson, *Exp. Mycol.*, 2 (1978) 245.
11 H.T. Lumbsch and J.A. Elix, *Pl. Syst. Evol.*, 150 (1985) 275.
12 G.B. Feige, B. Viethen, M. Geyer and G. Follmann, *J. Hattori Bot. Lab.*, 60 (1986) 143.
13 G. Follmann and M. Geyer, *Z. Naturforsch. C: Biosci.*, 41 (1986) 1117.
14 G. Follmann, M. Schulz and S. Huneck, *Crypt. Bot.*, 2/3 (1991) 298.
15 M. Geyer, *Dissertation*, University of Essen, Essen, 1985.
16 M. Geyer, T. Feuerer and G.B. Feige, *Pl. Syst. Evol.*, 145 (1985) 41.
17 K. Huovinen, R. Hiltunen and M. von Schantz, *Acta Pharm. Fenn.*, 94 (1985) 99.
18 D. Strack, G.B. Feige and R. Kroll, *Z. Naturforsch. C: Biosci.*, 34 (1979) 695.

19 H.T. Lumbsch, H. Kashiwadani and H. Streimann, *Pl. Syst. Evol.*, 185 (1993) 285.
20 H.T. Lumbsch, *Bryologist*, 95 (1992) 430.
21 H.T. Lumbsch and G.B. Feige, *Mycotaxon*, 45 (1992) 473.
22 H.T. Lumbsch, G.B. Feige and K.E. Schmitz, *Bibl. Lichenol.*, (1993) in press.
23 G.B. Feige, H.T. Lumbsch and B. Mies, *Crypt. Bot.*, 3 (1993) 101.
24 E. Mietzsch, H.T. Lumbsch and J.A. Elix, *Wintabolites (Mactabolites for Windows) Users Manual*, University of Essen, August 1992.
25 J. Santesson, *Ark. Kemi*, 30 (1969) 363.
26 C.F. Culberson, W.L. Culberson, S. Gowan and A. Johnson, *Am. J. Bot.*, 74 (1987) 403.