Ludlow form

BRITISH LICHEN SOCIETY BULLETIN

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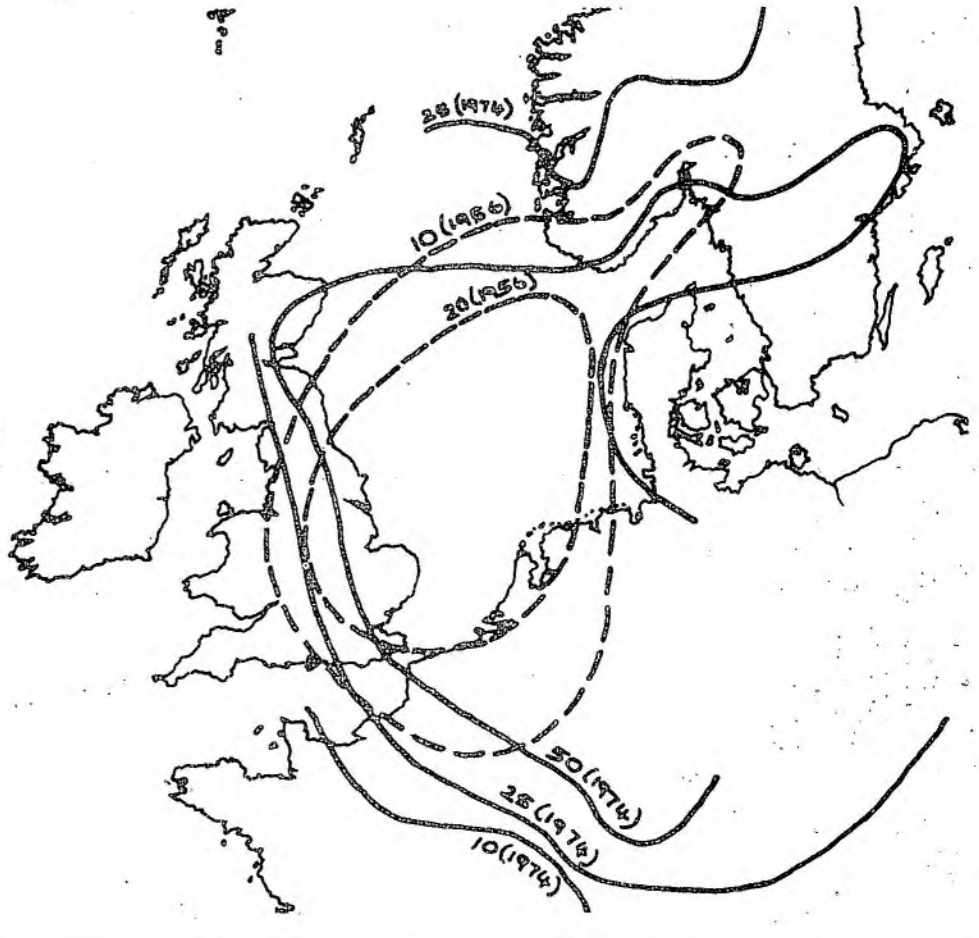
President: F. Rose, Ph.D.

Acid rain threat to lichens?

Measurements of the acidity of rain and snow have revealed that in parts of western Europe and eastern North America precipitation has changed from a nearly neutral solution 200 years ago to a dilute solution of sulphuric acid today. In the most extreme example yet recorded, a storm at Pitlochry, Scotland, 10 April 1974, the rain was pH 2.4. The main reason for this trend is the rise in consumption of fossil fuels which release sulphur and nitrogen oxides into the atmosphere; if these are emitted from tall stacks they may travel great distances before being deposited. Levels of emission in the U.K. currently c. 6 million metric tonnes SO_2 per year, 40-50% of which is released from tall stacks, may rise further under the present policy of turning increasingly to coal as a source of energy. Also further giant stack development is planned; for example the London Brick Company is proposing to build four 140 m chimneys in Bedfordshire. European emissions are currently thought to be running at a staggering 75 million metric tonnes a year.

Rain is normally slightly acid due to dissolved carbon dioxide which gives it a pH around 5.6, though in coastal areas sea-spray can shift the pH to more alkaline, and dust contamination in regions with calcareous soils may render the pH of precipitation well above 6. Acid rain is when the pH is below 5.6 - the lowest value expected for unpolluted areas. In large areas of Europe the annual average pH of



Acid precipitation in north-west Europe compared for 1956 and 1974. Acidity is shown by the numbers on the curving isolines; the larger the number, the higher the concentration of acid.

precipitation ranges from 4.0 - 4.5, in the U.K. it is 4.2, while the lowest annual pH yet recorded appears to be 3.78 in 1967 at De Bilt in the Netherlands. The windward flanks of mountains may be particularly affected as they enhance precipitation which continuously scavenges pollutants from passing masses of air; the first part of a rainstorm tends to be the most acid.

The effects of this increased acidity on the environment have not been fully assessed though it is believed that habitats with a low buffering capacity are particularly sensitive; these include many tree barks, highly siliceous rocks and some freshwater systems. The importance for lichens is that much of the sulphur deposited in the rain has been oxidised to sulphate which is many times less phytotoxic than sulphite and pH per se, i.e. the concentration of hydrogen ions may have a toxic effect on certain lichens. So beyond the range at which monitoring instruments are picking up significant levels of SO_2 a zone of enhanced acidity caused by sulphate ions may be influencing the composition of lichen communities.

A certain amount of field evidence appears to support this conjecture. In Britain even in areas remote from sources of air pollution there is a marked restriction of the Lobarion community to ash and elm trees which have a more highly buffered bark than oaks which in the past were a major habitat for the alliance. Also many old valley woodlands at the southern edge of the Scottish Highlands have a much poorer and more acidophilous lichen flora than would be expected considering their remoteness from sources of air pollution.

Dr F. Rose has recorded this kind of effect as widespread on the east side of the Cowal Peninsula in Scotland and it is also noticeable in parts of the northern Pennines and the Lake District. In such areas Lecanora conizaeoides is rare, Usnea species abundant and the general lichen cover luxuriant but strongly calcifuge. The Lobarion is very rare and only present as apparently relict populations on the oldest ash trees.

Further reading:

Likens, G.E., Wright, R.F., Galloway, J.N. and Butler, T. J. (1979) Acid rain. Scientific American, 241 (4) (October): 39-47 (from which the figure was taken).

Sage, B. (1980) Acid drops from fossil fuels. New Scientist, 85, (97) (6 March 1980): 743-745.

Hutchinson, T.C. and Havas, M. (1980) Effects of acid precipitation on terrestrial ecosystems. NATO Conference, Series 1: Ecology. Plenum Press, New York and London.

Autumn field meeting, LUDLOW, Shropshire, 24 - 27 October 1980

Ludlow is a particularly attractive and well-preserved old town, tucked between the confluence of the rivers Corve and Teme and dominated jointly by the ruined Norman Castle and the splendid St. Lawrence's Church. It is also a busy and friendly place with a quite remarkably high density of pubs. The surrounding countryside is typical of the Welsh Marches with its richly varied landscape closely reflecting the underlying geology. Very little has been published on the lichens of the area since the days of Rev. T. Salway (1791-1877). Work in Herefordshire however has shown that superb epiphytic communities still exist in the region. We hope to visit a wide range of different habitats - ancient deer park woodland relics, Silurian limestones, igneous rocks, ravines, Old Red Sandstone, etc. A room had been booked in the museum for evening work.

The Blue Boar Inn, which is included in the CAMRA Good Beer Guide, has been chosen as the headquarters daily starts will be made from here at 9.30 a.m. There should be ample room in the short-stay car park in Castle Square, 80 m to the north if there is no space outside the inn. It is advisable to bring a packed lunch each day. Members should book their own accommodation in Ludlow and are advised to do so as soon as possible. It is hoped to start the meeting with a short talk on the area on Friday evening and to disband around teatime on the Monday. Members intending to

be present at the meeting should please inform the leader on the form at the end of this Bulletin who will send further details nearer the time.

Accommodation (price for bed and breakfast only approximate)
The Blue Boar Hotel, Mill St., Ludlow. Tel. 2429 £6
The Cliff Hotel, Dinham. Ludlow, (ten mins. walk from headquarters) Tel. 2063 £8.50
The Portcullis Inn, Upper Galdeford, Ludlow. Tel. 2630 £6
Cecil Guest House, Sheet Rd., Ludlow. Tel. 2442 £6
The Croft, Dinham, Ludlow. Tel. 2076 £5.50
Mrs. Claydon, Castle Dene, Dinham, Ludlow, Tel. 2762
Youth Hostel, Ludford Lodge, Ludford, Ludlow. Tel. 2472

The leaders have additional addresses.

O.L. GILBERT and P.W. LAMBLEY

Report on annual general, lecture and exhibition meetings, 5 January 1980

The Annual General Meeting held on the Saturday morning attracted 38 members. Dr F. Rose was elected President and Dr Pauline Topham Vice-president, the first time a woman has held this post. The most important business was a general discussion on field meetings, the attendance at which has been declining. Unattractive venues, the frequent lack of laboratory facilities, too many and the high cost of accommodation were advanced as possible reasons. No immediate solution was forthcoming but it was decided to introduce "workshop" style meetings where the emphasis will be on identifying material in the laboratory. A further innovation was the appointment of a Field Meetings Officer to be responsible for co-ordinating meetings each year, a job Pauline Topham volunteered to undertake. She will be given a space in each Bulletin. The meeting closed by the President (F.H. Brightman) making a presentation to J.R. Laundon on his retirement as editor of the Bulletin after sixteen years; fortunately he has agreed to serve as secretary for a seventeenth year.

Over eighty people attended the afternoon Lecture Meeting on Lichen Conservation, the contributions were of a high standard. Jack Laundon identified promotors of tidiness and formality, and church redundancies as major threats to churchyard conservation. He reminded listeners that churchyards are the sole habitat of our only gypsophilous lichen, the endemic Lecanactis hemisphaerica. Francis Rose, ever more convincing, propounded his theory that open woodland containing large trees, a type now almost confined to parkland, is closer in structure to primeval broad-leaved forest than most other types of existing woodland. David Hill gave many people their first view of the yellow, brown and purple dye colours which can be produced from lichens and suggested that a policy of offering helpful advice to dyers was more likely to benefit lichen conservation than being negative. Peter James, reviewing British atlantic lichen vegetation, emphasised the importance of documentation if remote, little known, sites were to be saved. He also warned of the harmful effects of agricultural fertilisers applied on windy days. Finally, aided by a very small map, he explained the amazing richness of the Isle of Mull by identifying it as an axial point where many phytogeographical elements met.

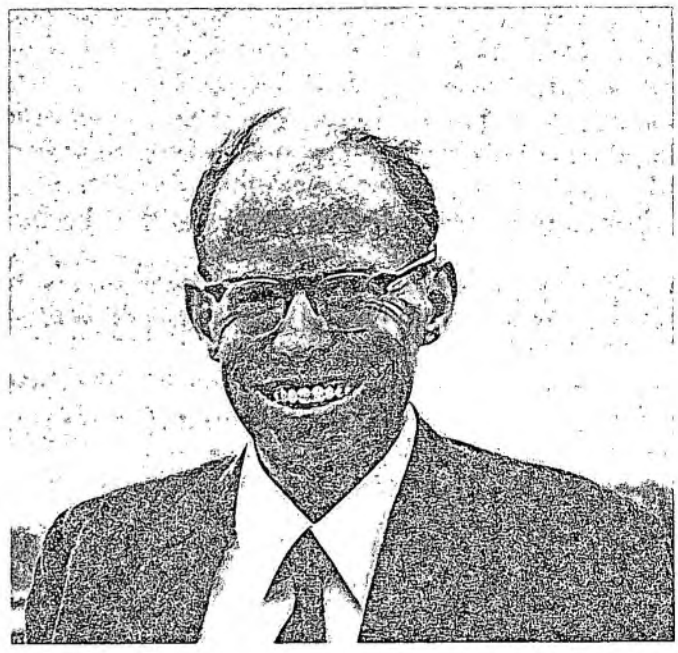
The Exhibition Meeting was better supported than last year though only four of the eleven exhibits actually featured lichen material.

BEETHAM, P.A. & KNABE, W. Epiphyte survey of Dusseldorf.
BYLINSKA, E.A. & SEAWARD, M.R.D. Nature protection in Poland.
DOBSON, F.S. Colour photographs of lichens.
HITCH, C.J.B. Lichen Flora of Suffolk.
GILBERT, O.L. Verrucaria psammophila discovered in Derbyshire.
RICHMOND PUBLISHING COMPANY. Bookstall.
ROLF, N. Some North American corticolous lichens.
ROSE, F. Proofs of a forthcoming wild flower book.
SEAWARD, M.R.D. Register of deceased British and Irish lichenologists.

SWINSCOW, T.D.V. A species pair in Usnea.
WALKER, F. Joy Lichenologist reach new heights (colour photographs).

End of an era.

At the end of 1979 Jack Laundon retired as editor of the Bulletin having held the post continuously since 1963. When he took over the job, as part of the secretary's duties, the Bulletin functioned as a two-page newssheet which kept the 271 members informed about field meetings, subscription rates (£1), requests and other domestic matters. During the next three years J.R. Laundon gradually increased the number of pages and introduced short articles on matters of general lichenological interest. Already his deep concern with the effects of air pollution, the conservation of sarsen stones and particularly the value of churchyards as major lichen habitats was becoming evident. Innovations continued, in 1967 under the heading "Extra time" the first humerous article appeared and many members must still recall the pleasure with which they opened Bulletin no. 31 (Dec. 1972) to discover the introduction of a lead article and photograph which was to become the standard format, ten years ahead of its time.



Over the years J.R. Laundon has built the Bulletin into a publication of major significance and delight, so that today it is read as avidly as the journal and on several occasions it has been extensively quoted in the National Papers. Throughout his period as editor Jack Laundon was pursuing ecological research into the lichens of the London area and taxonomic research into difficult groups such as sterile crusts, particularly Lepraria. It was the increasing demands of this work which eventually forced his resignation though fortunately he will continue to compile the long running series 'Literature on lichens'. Probably never again will the Bulletin be to such an extent the product of one man's vision.

Grapevine

Good news last year for Yorkshire lichenologists was that during his stay in this country last summer Dr Teuvo Ahti of Helsinki would be able to pay the county a brief visit. As a result they had the opportunity of seeing Dr Ahti's rare lichenological acumen at work on sites at Grass Wood, at Yorke's Folly and in some typical outcrop landscapes. Dr Ahti's understanding and appreciation of morphological plasticity exemplified the intrinsic humility of his spirit of scientific inquiry and made a lasting impression on the other members of the small party. It was an added pleasure to learn later of his appointment to professorial status. Grapevine offers him its warmest congratulations.

Bad news last year for lichenologists in Yorkshire and further afield was that Peter Earland-Bennett was relinquishing his position as Keeper of Natural History at Bankfield Museum, Halifax, and abandoning professional lichenology. Grapevine notes his departure sadly, not least because of the geological expertise he brought to the study of substrate ecology in both lab and field. A slight palliative comes in the information that Peter intends to continue with occasional work in fields not unrelated to his favourite stomping-grounds of old --- one thinks in this context of his work on Lepraria zonata, on Lecanora subaurea and L. epanora and on the green Candelariella species, C. flavovirella and C. heidelbergensis. Running a newly established antiques business in the Piece Hall, Halifax, albeit with his wife at his side, will, however, of necessity leave little time for what will

always be one of his first loves. Lichenologists are not thick enough on the ground in Britain for the loss of such an accomplished and devoted field-worker to go unregarded. The mapping-scheme alone can ill afford to lose such an arm to its bow.

On 9 April this year Su Ingle of BBC1's "Wildtrack" did some uncanvassed proselytising for the B.L.S. by exploring "and eerie graveyard" where "the tombs are more alive than they seem —— botanically speaking". Although lichens have figured more or less (mainly less!) in several radio or television programmes over the year, the "Wildtrack" programme was an invigorating foray into the subject well-geared to the needs of its youngster audience.

Janet Marsh's Nature Diary (Michael Joseph, London, £9.50) deserves to catch the eye of any lichenologist who has not already come upon it, for the delicacy of its watercolour treatment of a few lichens. It seems ironic that a non-lichenologist should offer her readers lichen representations superior in informative quality to many of those included in strictly lichenological literature. As illustrations these latter all too often fail miserably to live up to the name, frequently being models of instruction in cryptography rather than cryptogamy.

Lichens growing on azaleas had better watch out if Christopher Fairweather's "Rhododendrons and Azaleas for your garden"(1979) achieves much of a circulation. "Lichens", we learn, "are often troublesome on Azaleas ..... Deciduous varieties can be sprayed on the bark with lime-sulphur in the dormant season. Of course, the evergreen types cannot be sprayed, and the only cure is to scrub off the lichen with a nail brush, soap and water." Such anti-lichen laundry is, Grapevine feels, a fitting occupation for Lewis Carroll's gardeners whenever they finish painting the roses in the garden red!

VINIFERA

Field Meeting Secretary and questionnaire

It has been decided to create a new post of Field Meeting Secretary. The job entails submitting a programme of field meetings for approval by the membership at each A.G.M. Not all recent field meetings have been well attended and one had to be cancelled for lack of support, so members are asked to fill in and return the separate blue coloured questionnaire which is included with the Bulletin. This should help me to maximise the attractiveness of future programmes; I am particularly interested to receive views on workshop style meetings covering a specialised topic e.g. pyrenocarps, Cladonia, seashore lichens, T.L.C., etc.

Currently only a few people seem prepared to act as leaders. To lead a meeting requires only a working knowledge of lichens. If you want a meeting in your area offer to act as local secretary and make the arrangements, the experts who come along will be prepared to make the determinations.

PAULINE TOPHAM

Field meetings in the seventies

During the seventies most of the thirty-five residential field meetings were based on two or three star hotels. This luxury arose from the need for headquarters large enough to accommodate the expected number of participants and gracious hotels were often the only ones of sufficient size particularly at the more remote venues. A few members patronised bed and breakfast accommodation, camped or slept in vehicles.

Assembling in the morning parties were frequently rendered conspicuous by the preference of many participants for using ex-army canvas gas-mask satchels as field bags. These (which can still be purchased for under £2) might have been designed with lichenologists in mind, being conveniently divided inside to provide three large pockets and a small extra one suitable for holding reagent bottles. Prompt starts were in order (leading to complaints that for and entire week it was

impossible to visit a bank) and often considerable distances were travelled to the localities chosen for investigation. On all meetings the entire lichen flora, including sterile crusts, was studied over 90% of the taxa being named in the field. There was much shouting of names to be crossed off the mapping card and an occasional "I'd like to see that" for the rarer species. Only the meet leader(s) normally collected extensively, usually into tobacco tins or packets of stiff brown paper. Only twice can I remember a rare species being overcollected and only once have I heard of a habitat (a bridge) being rendered unsightly. Early in the decade woodlands received a lot of attention with the odd churchyard thrown in to boost the total for the grid square. Later, as woodland in lowland Britain became better known, attention turned increasingly to natural saxicolous and terricolous habitats.

A highly valued group of participants, modestly self-styled 'perpetual beginners', regularly attended at least one field meeting a year. They combined an enthusiasm for lichens with a love of exploring new countryside and helped to ensure that everyone enjoyed themselves, suggesting afternoon teas, swims, visits to islands, and so on. Sometimes the planned excursions were punctuated by hospitality from land owners.

The society often remained in the field till late, their return being further delayed by the inability of certain members to pass any roadside tree above a certain dimension without a quick look. Supper at the headquarters hotel or at a cheaper restaurant in town was invariably followed by passing the mapping cards round over a pint. The practice of renting a school room for evening work practically died out after the summer of 1970. Following the gastronomic excesses of certain meetings, culminating in caviare and grouse at Goathland, it would have been inappropriate to retire anywhere other than the bar.

Memorable visions of the decade include a leader drawing himself up, sniffing the air, peering around the landscape and declaring we'll find Lobaria over there (which of course they did). On another occasion a member engaged a householder in conversation at his back door while a particularly puzzling specimen was collected off his front wall. My favourite recollection is of the leader who announced (to me) that as this was such a good site he was going to loose the party for the next hour so he could get down to some serious lichenology.

Throughout the 1970's the majority of participants were self-financed amateurs who expected their lichenology to be mixed with visits to attractive places and wanted to have a bit of a holiday as well. In the early 1970's as an experiment papers were read at several autumn meetings but this fell out of favour as audiences were small and often a day in the field had to be sacrificed. Later the introduction of long (3-4 day) weekend meetings proved a more successful innovation and these are likely to continue. Meetings over the last decade should perhaps be seen largely as mapping meetings, venues being chosen not for their outstanding flora but to fill a blank on the map.

It is to be regretted that during such an active phase of the Society more of the meetings, particularly in Wales and Scotland, were not written up.

Canterbury field meeting 19-21 October 1979

The headquarters hotel was just outside the city walls, though well within the present day built up area. It was comfortable, and a room was provided for our use in the evening. The meeting began on Friday afternoon with a visit to a churchyard within the walls, an examination of the flora of the walls themselves, and a visit to another churchyard, reputedly the oldest in England, originally outside the city but now well within the suburbs. The contrast between the two churchyards had been accentuated by the removal of many of the tombstones from the city one. It was difficult to find 20 species, although these did include Lecanora polytropa on iron and a scrap of Parmelia sulcata on an otherwise bare tree trunk. The city walls had been so heavily restored that they were not of great interest. On the other hand it

was easy to find more than 50 species in the ancient churchyard.

Saturday was largely spent in exploring old parkland. First we visited Denton Court, by kind permission of the owner, who met us and showed us round his garden. A number of old trees surrounded by brushwood stood amongst pasture, in marked contrast to the land on the other side of the valley where the remaining trees were isolated in the midst of hundreds of acres of arable. The trees in the pasture, others in the garden, walls, and an adjacent small churchyard, enabled us to list 75 species. Later we travelled south, pausing briefly to examine some old trees on the outskirts of Acrise Park, which supported well-developed Parmelia acetabulum but not a great deal else of especial interest. We took lunch at the Cat and Custard Pot Inn at Paddlesworth. We visited the churchyard here; the church is reputedly the smallest and at the highest altitude in Kent. We recorded over 40 species. In the afternoon we visited, with permission, the extensive park of Godington Hall, which contains some very large and ancient trees of oak, sycamore and field maple. Again we recorded more than 40 species, including ten Parmelia spp. and eight Pertusaria spp. On Sunday we travelled further south towards the coast. Our first stop was by the Royal Military Canal on the edge of Romney Marsh. Some of the small-leaved elm trees still survive there and support an interesting lichen flora, including Anaptychia ciliaris and Caloplaca luteoalba. Next we visited a ruined church in the marsh near Eastbridge, where we found Ramalina duriaei, R. farinacea and 30 other species. Finally we visited Dungeness. Although this area has deteriorated greatly in recent years largely due to a falling water table resulting from extensive gravel extraction, it is still of outstanding lichenological interest. Some patches of blackthorn scrub survive, though greatly diminished, and support an interesting lichen flora. Much of the shingle is inherently very stable, although it is suffering increasing human disturbance; from it we recorded 18 species of Cladonia and also Pseudevernia furfuracea growing on the ground.

Although Kent has been well-worked lichenologically and no great rarities can be expected, it is still full of interest for a lichen weekend.

F.H. BRIGHTMAN

Green spots spread alarm

A strange green fungus-like growth in Chiswick, London has started to worry local residents. The "fungus" was first noticed about two years ago, but at that time it had only appeared on a few paving stones. Now it has started to grow on several streets and also on roofs and residents fear that it may be dangerous to health, or destroy property. They are also concerned that it may be spreading towards Hounslow. Said Grove Park Group secretary Denzil Webber, "When we first noticed it we didn't bother to do anything about it. But at a recent meeting many people said they'd seen it and were worried." The growth, which appears in round patches, is not washed away by the rain and so far all attempts to get rid of it have failed. A sample has been taken by biologists who are trying to discover what it is and how it is caused. "One interesting aspect of this fungus", said Mr. Webber, "is that it only grows on dark paving stones, not on light ones. We want to know why, and whether it is harmful to people or buildings, or indeed to the atmosphere". One theory is that the growth is caused by chemicals put into American jet engine fuel to reduce the risk of lead pollution. Last week Mr Webber wrote to Hounslow's environmental health department, but so far they have not come up with any ideas about the green spots.

Brentford & Chiswick Times, 11 January 1980

The following week a reassuring letter appeared in the paper pointing out that the worrying growth was the harmless lichen Lecanora muralis.

J.L. GILBERT

Secretary's report for 1979

The membership situation is quite healthy with the numbers reaching 548 at the end of 1979 in comparison with 516 at the beginning of the year. The number of new members joining the Society was 63, the highest yearly total since 1966.

Field meetings were held at Penrith, Swaledale, Crieff and Canterbury, and there were day excursions to the New Forest, Darent valley in Kent and Harfield. Mr. F.H. Brightman, Mr B.J. Coppins, Dr D.L. Hawksworth, Dr F. Rose and Dr Pauline B. Topham are thanked for arranging and leading these excursions. Some of these were rather poorly attended and the proposed Yelverton field meeting was cancelled for this reason.

The Lichenologist expanded to three issues a year. Two parts of the Bulletin were published. Three Council meetings were held. Much work was carried out on the proposed new checklist and atlas, both due for publication in 1980. I wish to conclude by thanking all officers, referees and members for their help and co-operation during the year.

J.R. LAUNDON
Honorary Secretary

(This report was presented at the Annual General Meeting on 5 January 1980)

New members

The following joined the Society between November 1979 and April 1980.
FM = family member.

Mr J. S. Adams, 18 Peasland Road, Watcombe Park, TORQUAY, Devon.
Mr K. D. Bennett, Peterhouse College, CAMBRIDGE, CB2 1RD.
Mr J.W. Berry, 48 Longdale Lane, Ravenshead, NOTTINGHAM.
Mr F.M. Blighe, The Poplars, off Burnetts Lane, West End, SOUTHAMPTON, Hampshire, SO3 3HH.

Miss S. Bonny, 22 College Grove, MALVERN, Worcestershire.
Mr T.E. Chapman, 37 Cleveland Road, Crumpsall, MANCHESTER, M8 69T.

Mr J.C. David, Upfolds, Balls Cross, PETWORTH, Sussex, GU28 9JP.
Mr P.M. David, Upfolds, Balls Cross, PETWORTH, Sussex, GU28 9JP.
Mr I.P. Day, Raughton Cottage, Raughton, Dalston CARLISLE, Cumbria.
Dr J. Hafellner, Institute of Botany, University of Graz, Holteigasse 6, A-8010 GRAZ, Austria.
Dr C. Halls, Room 313, Department of Geology, Royal School of Mines, Prince Consort Road, South Kensington, LONDON, SW7 2BP.
Mr J.T. Hedderwick, Little Croft, Harvel, Meopham, GRAVESEND, Kent, DA13 0DE.
Mr L. Kok, Kwadijk 51, 1471 CC KWADIJK, Netherlands.
Mr C. J. Miles, Sibly Hall, Redhatch Drive, Earley, READING, Berkshire.
Mr J. A. Mills, 85 Ridge Road, Winchmore Hill, LONDON, N21 3EL.
Mr P. Mitchell, 11 Jasper Close, RADCLIFFE-ON-TRENT, Nottinghamshire.
Mr A. J. Moore, 21 Sandringham Drive, Heacham, KING'S LYNN, Norfolk.
Mr C. R. Parsons, 2 Rew Lea Cottages, ASHBURTON, South Devon.
Dr Martha A. Sherwood, c/o Commonwealth Mycological Institute, Ferry Lane, Kew, RICHMOND, Surrey.
Mr J. C. Workman, 24 Grant Road, Wealdstone, HARROW, Middlesex.
Mrs K. Wyatt, 8 Wilton Grove, Wimbledon, LONDON, SW19 3QX. (FM).
Mr T.E. Wyatt, 8 Wilton Grove, Wimbledon, LONDON, SW19 3QX.

The Societys collection of colour slides

In addition to the extensive collection of reprints, the British Lichen Society Library holds a number of 35mm colour transparencies. The nucleus of this collection

is 160 slides of herbarium specimens taken by J.H.G. Peterken and 138 views of habitats and lichenologists taken by Nancy Wallace on various field meetings. The Librarian is keen to obtain further donations of (fully labelled) slides of lichens, habitats and lichenologists to increase the value of the collections. In the future photographers might like to take duplicate pictures and let the Society have one, especially if rare or unusual specimens are being photographed.

At any one time up to 36 slides may be borrowed from the collection for 50p (postage included) per order. A catalogue of the lichen slides is available from the Librarian (Dr D.H. Brown, Department of Botany, The University, Bristol, BS8 1UG) for two 8p stamps.

Register of deceased British and Irish lichenologists

Over the past few years there has been a renewed interest in the history of lichenology. It is surprising how little biographical information there is available (including photographs) for many British and Irish lichenologists.

Members are therefore invited to take part in a biographical survey; further details and questionnaire(s) can be obtained from: Dr M.R.D. Seaward, School of Environmental Science, University of Bradford, Bradford, BD7 1DP.

A register will be compiled in the first instance for use by the British Lichen Society, and it is hoped to publish a select (comprehensive, if possible) biographical index in due course. Authors of all contributions will be specifically acknowledged.

Joint excursion with the British Mycological Society 5 October 1980

Ruislip Wood, Middx., Sunday 5 October 1980.

Assemble: 2.15 p.m. at the Ruislip Lido bus stop (O.S. map sheet 176; grid ref. 51(TQ)/086.892); Metropolitan Line tubes run to Ruislip Station, from which there is a bus service (or $1\frac{1}{2}$ mile walk).

Leader: Dr D.L. Hawksworth. The excursion will take in the old willow carr of interest for the improvements now taking place in the lichen flora of this area (see D.L. Hawksworth & C.G. Rose J. Ruislip Distr. nat. Hist. Soc. 22 : 23-29, 1979).

Bargain

For sale: photocopy of The Lichenologist, 2, (3), 205-294, £2.14 (post free). Also The Lichenologist, 3, (1), 1-94 and 154-174, only the New check-list being omitted, £2.35 (post free). Reproduced by a 3M copier, diagrams particularly clear. First come first served, postal order or cheque to J.B. Fildes, 5 Jaquet's Court, North Grey Road, Bexley, Kent, DA5 3NF.

Literature on lichens - 34

AHMADJIAN, V. 1979. Lichens: clean air sentinels. Garden, New York 3 (6): 18 - 24. [Review. Photographs.]

APLIN, P. S. & HILL, D. J. 1979. Growth analysis of circular lichen thalli. J. theor. Biol. 78: 347 - 363. [New model.]

BUSCHARDT, A. 1979. Zur Flechtenflora der inneralpinen Trockentäler. [Biblthca lichenologica 10] Cramer, Vaduz. [Lichen flora of the inner dry valleys of the Alps. Keys, maps, etc.]

CHRISTIANSEN, M. S., RAMKAER, K., ROSE, F. & SØCHTING, U. 1979. Additions to the Danish lichen flora. Bot. Tidsskr. 74: 89 - 115. [30 species.]

COPPINS, B. J. & GILBERT, O. L. 1979. George Johnston's lichen herbarium at the R.B.G., Edinburgh. Notes R. bot. Gdn Edinb. 37: 381 - 385.

CULBERSON, W. L. & CULBERSON, C. F. 1979 ["1978"]. Cetrelia cetrarioides and C. monachorum (Parmeliaceae) in the New World. Bryologist 81: 517 - 523. [Includes record of the chemotype C. monachorum (Zahlbr.)W.Culb. & C.Culb. from Scotland.]

DERUELLE, S., LALLEMANT, R. & ROUX, C. 1979. La vegetation lichenique de la basilique Notre-Dame de l'Epine (Marne). Docums phytosociol. II, 4: 217 - 234e. [39 species and several communities from the walls of l'Epine church in northern France.]

DOBSON, F. S. 1979. Common British Lichens. Jarrold, Norwich. [£0.60. Book, with colour photographs, for beginners.]

EARLAND-BENNETT, P. M. 1979. Lichens. In BELL, R. (Editor) A Sketchbook of the Natural History of the Country Round Wakefield. Lion & Unicorn, London. [Fold-out chart of the plant life. 67 lichen taxa.]

ERKAMO, M. V. 1974. Raidan bakteeriäkämistä (Agrobacterium tumefaciens) ja nüden levinneisyydestä Suomessa. Mem. Soc. Fauna Flora fenn. 50: 31 - 43. [Crown galls on Salix caprea in Finland are considered to be biological indicators of polluted areas, in contrast to most lichens. Photographs, maps.]

EVANS, I. M. (Editor) 1979. North-east Leicestershire Coalfield. Report of a Biological Survey, 1978. Leicestershire Museums, Leicester. [Survey of Coal Board's proposed three mining sites at Ashfordby, Hose and Saltby in Vale of Belvoir. Detailed account of the lichen flora by A. Fletcher. Includes 12 species new to Leicestershire.]

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A REVISED GUIDE TO MICROCHEMICAL TECHNIQUES FOR THE IDENTIFICATION OF LICHEN PRODUCTS

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British Museum (Natural History)

INTRODUCTION

Lichens are now known to produce over 500 chemical compounds, many of which do not occur in other groups of plants. A large number of these substances have now been named and their structures elucidated; a compendium of most of this information is to be found in the works of Culberson (see Culberson 1969; 1970; Culberson & Johnson 1977). A proportion are weak phenolic acids derived from orcinol and β-orcinol derivatives, whilst pigments, terpenoids and fatty acids are also represented. Lichen substances are accumulated on the surface of the fungal hyphae and may be widely dispersed or confined to specific tissues within the thallus. Atranorin as well as many pigments, including usnic acid, are often confined to the cortex, whilst most colourless substances (depsides and depsidones) tend to be concentrated within the medulla and algal layer. The general chemical composition of the thallus remains the same throughout the life of an individual specimen, but on maturity apothecia, and sometimes soralia and pycnidia, may possess a different or additional chemistry from the parent vegetative thallus. The length of storage of herbarium material does not seem to have an effect on the chemical constituents. However, in a few species there is a deposition of numerous minute, pale blue-grey crystals (probably sterols) on the lichen surface after two or three years' storage.

The chemical composition of many species is more or less uniform; some substances are constantly present, whilst others, usually referred to as accessory substances, may or may not occur, and are indicated in the literature by a ± sign. There are instances where a seemingly uniform morphological entity may occur as two or more chemically different races. The taxonomic status of each chemical strain may be determined by other factors such as distribution, ecology, and anatomy. At present there is considerable variance in the interpretation of chemical races, and students are advised to consult Hawksworth (1976) for a consensus of current opinion on this aspect. However, even though the morphology of a species may be variable, the chemistry is often relatively uniform, so that the latter can often be used as a reliable indicator in the determination of sterile, difficult or fragmentary material.

Early scientific studies on lichen substances generally required large quantities of material in order to obtain pure samples. The work of Asahina and others, initiated in the 1930s, has resulted in the development and refinement of simple microchemical techniques which enable substances to be detected and identified from small thallus fragments. Sophisticated techniques, e.g. spectrophotometry, have also been adopted for the identification of substances and the elucidation of chemical formulae. These require much more specialised equipment and equivalent skills which are normally only available to the analytical chemist with special laboratory facilities; these are excluded from this article, but have been adequately summarised by Nourish and Oliver (1974). The tests described below range from simple spot tests to microcrystal tests and thin-layer chromatography. Where doubt exists of the identity of a particular substance it may be advisable to use several techniques and compare the resulting data using known controls.

Great care should be taken handling herbarium material when undertaking any of the microchemical analyses outlined below. Specimens are often very fragile or fragmentary, and if testing is essential, only the smallest fragment should be used. All thallus spot tests should be undertaken on a fragment removed from the main thallus. Care must be taken not to deface the specimen nor to allow any chemical reagents to come into contact with the material or herbarium sheets, etc. Specimens in national herbaria are irreplaceable and these, and in particular type specimens, must always be handled with extreme care. Types should only be examined when a

serious taxonomic revision is being undertaken and not for routine studies. Full details of the results obtained, along with the method of analysis, should always be attached to the specimen on a suitable label written in permanent ink. Adopting this procedure avoids unnecessary duplication of work and a further depletion of the critical material.

This article combines and updates earlier guides to microchemical techniques published in the Bulletin of the British Lichen Society (e.g. Menlove 1974), and includes methods in current use at the British Museum (Natural History).

THALLUS COLOUR TESTS

Spot tests for colour reaction of thalli are universally used as a rapid, albeit crude and nonspecific, means for detecting the presence of certain unspecified lichen substances. An important feature of these tests is that they are convenient and are simple to perform, even under field conditions. However, it is important that they are regarded as only a preliminary step in the process of identification of the lichen or its substances. In order to identify a particular lichen substance it will always be necessary to use more sensitive tests, e.g. thin-layer chromatography, described below. As a precaution it should be noted that there have been many instances where misinterpretation of spot tests have led to errors in determination. Examples of particular pitfalls and possible reasons for the more frequent misinterpretations are given at the end of this section.

Method

Ideally tests should be carried out on a separated fragment of thallus which should be discarded after treatment. The reagent should be applied with either a small paint brush, sharpened match-stick or thin glass rod. Direct testing, particularly if herbarium material is being studied, should involve the smallest amount of fluid to avoid unsightly stains on the specimen and must be carried out under a hand lens (x10) or, preferably, a binocular microscope. For tests involving examination of the medulla a small area of the overlying cortex should first be removed with a razor blade.

Test Solutions

C. A fresh undiluted solution of a commercial brand of household bleach, (e.g. "Parazone") is normally used. Proprietary brands with additive which increases their viscosity (e.g. "Domestos") are unsatisfactory, as they are not quickly absorbed by the thallus. Storage in a refrigerator when not in use helps to maintain the strength of the solution. Alternatively, bleaching powder (calcium hypochlorite) may be used according to the manufacturer's instructions, although a new solution will need to be prepared for each session of identification. Positive colour reactions include: red (e.g. anziaic acid, lecanoric acid, erythrin); orange-rose (e.g. gyrophoric acid); or, exceptionally, emerald-green (strepsilin). Examples of testing controls for the strength of the reagent include the surface of Ochrolechia tartarea or O. androgyna (orange-red) or the medulla of Parmelia glabratula (red).

K. An aqueous solution of potassium hydroxide is used. Students may find different concentrations given in the literature. However, a solution produced by dissolving 70 g potassium hydroxide in 200 ml water is recommended for standard testing. The resulting solution should not be too viscous since this impairs rapid penetration into the thallus. Care should be taken to avoid recording a misleading K+ yellow reaction of the cortex in certain lichens which can be produced 5 to 10 seconds after application of this reagent. This is due to the clearing effect K solution has on the normally opaque cortex thus allowing the colour of the underlying algae to predominate. Most frequent positive colour reactions include:

yellow-orange (e.g. stictic and thamnolic acids); yellow changing to red accompanied by blood-red needle-shaped crystals in microscope preparations (norstictic acid); yellow changing to dull brownish-red (e.g. fumarprotocetraric acid); purple (parietin complex); or purple-red (e.g. rhodocladonic acid or haemoventosin).

Examples of controls for this reagent include: Cladonia polydactyla (yellow-orange); Pertusaria pseudocorallina or Phlyctis argena (yellow changing to red); Xanthoria and Caloplaca species (purple).

KC. This test involves application of K quickly followed by C; either directly onto the thallus or by using the filter paper technique (see Special Cases below). This test may sometimes be used to enhance a weak or suspected C reaction. Positive colour reactions include: red or rose (e.g. alectoronic acid and physodic acid), or violet (picrolichenic acid). Examples of controls for this test are the medulla of Hypogymnia physodes or Cetrelia olivetorum (rose-red) or the soralia of Pertusaria amara (violet).

CK. This is the reverse of a KC test and is not commonly included as a standard method. It is used in special cases where it produces an orange colouration (e.g. barbatic or diffractaic acids). The test can be demonstrated on the podetia of Cladonia floerkeana.

PD. Always use a freshly prepared solution for this test made from dissolving two or three crystals of p-phenylenediamine in one or two drops of ethanol in a watch glass. Application should be made by means of either a pointed matchstick or a needle. The residue remaining in the watchglass after evaporation can be reused with the addition of further drops of ethanol when required, but it should always be discarded immediately after a completed session of identification. Steiner's Stable PD solution can be used as an alternative which can be stored for up to three months. The formula for this reagent is: 1 g PD : 10 g sodium sulphite : c. 0.5 ml detergent : 100 ml water. This solution is recommended for filamentous genera, e.g. Alectoria (see Special Cases below). Positive colour reactions include: red or yellow changing to red (e.g. fumarprotocetraric acid); yellow (e.g. psoromic acid); yellow changing to orange (e.g. thamnolic acid). Examples of controls for this reagent include: Cladonia pyxidata (red); Schismatomma niveum (orange); medulla of Parmelia saxatilis or P. sulcata (yellow to red).
CAUTION: It is important that PD should not be allowed to come in contact with cloth, paper, etc., as it causes an unsightly brown staining. It must also be handled with great care and not allowed to come in contact with the skin or inhaled as it is under suspicion as a carcinogen. Students are advised to take note of the health warnings and follow the recommendations given in the following articles:

Cleden, J. Bull. Brit. Lichen Soc. 40 : 12 (May 1977)
Laundon, J.R. Bull. Brit. Lichen Soc. 26 : 5 (May 1970)
Santesson, J. Lichenologist 3 : 215 - 217 (1966)
Swinscow, T.D.V. Lichenologist 1 : 120 (1959)

Problems

Failure to obtain or observe an anticipated positive result may be due to one of the following causes:

1. Ageing solutions

Reagents often deteriorate during storage. C, in particular, quickly weakens in its effect, often within a few days especially if kept in a warm room. Solutions must therefore be checked at frequent intervals by using test lichens of known chemical constitution which will give the correct colour change and intensity to ascertain that the reagents are still capable of producing clear, rapid and satisfactory results.

K solution is stable for many months but should be replaced as soon as it shows signs of becoming clouded. Alcoholic PD solution recrystallises and in a short time oxidises to a deep brown colour in which condition it is then of no value.

Steiner's solution is therefore recommended as it is more stable and easier to use once prepared.

2. Low concentration of lichen substances

Sometimes the concentration of the lichen substance is not sufficient to produce a convincing colour reaction. In such instances more sensitive methods, e.g. thin-layer chromatography, need to be used.

3. Reaction time

Reactions with C and KC are nearly always fleeting, taking place within a second of application of reagent and very soon fading, and consequently often missed. The thallus should be carefully observed under a lens or, if possible, a binocular microscope immediately after application of the reagent. Conversely PD may take up to 30 seconds or more to react and the colour produced may change or intensify after a short time lapse of about one minute. It may be necessary to confirm certain reactions by means of a microscope squash preparation; these techniques are described below under Special Cases.

4. Localisation of lichen substances

General tests involving non-specific areas of the thallus should be carried out on young, actively growing parts, such as lobe and branch tips. However, substances are frequently localised within specific areas of the thallus and in certain species a particular substance may only be present in either the cortex, medulla, soralia, pycnidia, apothecial margin or disc. Care must be taken when using identification keys to ensure that the correct organ or area is tested.

5. Colour of thallus

Colour reactions can often be missed or overlooked in the case of dark thalli. To overcome this difficulty special techniques must be used, e.g. filter paper or microscope squash preparations (see Special Cases below).

Special Cases

1. Use of filter paper

(i) With dark thalli the colour changes are best observed by absorption of the applied reagent from the thallus using a small piece of filter paper or tissue. An example is the specialised technique developed by Brodo and Hawksworth (1977) for testing Alectoria filaments with Steiner's Stable PD solution. Filaments are placed on a square of filter paper on a glass slide and flooded with 2 or 3 drops of the reagent. Colour produced will diffuse out of the thallus and spread onto the paper. Excessive quantities of reagent should be avoided at all times since this causes over dilution and thus produces unsatisfactory results. This method is also used in Alectoria to distinguish which part of the thallus, e.g. soralia or corticate parts, is reacting. Steiner's solution renders the filament transparent so that any positive reaction of the inner cortex or medulla can be registered through the cortex and will not diffuse out onto the filter paper.

(ii) For KC (and CK) tests. Apply K to the thallus and absorb the reagent onto the corner of a piece of filter paper. Add a corresponding spot of C onto the filter paper which will produce a colouration; note that this reaction is nearly always fugitive and short-lived.

2. Use of microscope preparations

CAUTION: Chemical tests involving microscope preparations should always be carried out under low power since the reagents, particularly K, may damage the objective of the microscope. On no account should the objective come into contact with the testing solutions.

(i) Thallus tests

If the reaction produced during a spot test is at all uncertain it is advisable to make a squash or thin section of the tissue (e.g. medulla), or soralia, pycnidia etc. on a microscope slide in a minimum amount of water and run in the appropriate reagent under a coverslip. Any colour produced may become visible by

placing the slide against a white background (e.g. medulla colouration) but it is preferable to observe the flow of reagent through the preparation using the low power objective of a microscope. Some tests (e.g. iodine) can ony be successfully performed using a microscope preparation.

K. The formation of characteristic numerous blood-red, needle-shaped crystals in microscope squash preparations indicates the presence of norstictic acid.

Iodine. All iodine tests must be carried out with a microscope. A satisfactory solution is achieved by disolving 0.5 g iodine in 1.5 g potassium iodide and 200 ml distilled water. The medulla of certain lichen species, e.g. Huilia tuberculosa, contains isolichenin which produces a blue or mauve colouration with iodine. The reaction is often rather patchy and the test is best performed on a thin squash of tissue mounted in a small amount of water. This should then be observed under the microscope (low power) whilst the reagent is diffusing through the tissue. This may be done by placing a small drop of iodine solution at the edge of the coverslip and drawing it through the mount by means of filter paper at the opposite side. Alternatively Meltzer's reagent may be used for this test (see below).

(ii) Fruiting bodies

(a) Lirellate species

Meltzer's Reagent. Besides the clearing of spores in pyrenocarpous species this reagent is used for detecting amylin in the spores of the genera Graphis, Graphina and Phaeographis, the presence of which is indicated by a purplish colouration of mature spores in preparations in this reagent. The solution is prepared by dissolving 0.5 g iodine in 1 g potassium iodide and mixing with 20 ml distilled water and 20 g chloral hydrate.

K. Species of Opegrapha may be divided into two groups according to the reaction of the hypothecium with K solution which produces either a greenish or brownish colouration. The colour change produced must be carefully compared with a preparation mounted in water. Some species produce a reaction in the carbonaceous part of the fruit in K, the colour diffusing into the adjacent solution, e.g. Opegrapha ochrocheila.

(b) Discocarpous species

K. This solution is most effective in squash or slide preparations, as it tends to clear the tissues enabling details of the spores and apothecial anatomy to be seen more clearly. In many cases the pigmented part of the discocarp (e.g. epithecium, hypothecium, exciple) also changes in colour in preparations with K. These changes should be carefully noted and compared with mounts in water to determine their intensity and colour variation. In those fruits containing norstictic acid numerous small needle-shaped blood-red crystals are produced about 20 seconds after preparation. Other colour changes are yellow, red or violet and are often diagnostic for the species concerned. A special change occurs in Bacidia absistens, Catillaria pulverea, Mycoblastus fucatus and Schaereria tenebrosa where the epithecium changes from purple-violet to aeruginose-green. Many species contain granules and/or oil droplets in various parts of the fruit and it is important to record whether these dissolve in the K solution and what colour changes result from its application. Certain pigments may also undergo a colour change with K, e.g. in Caloplaca spp. the disc (epithecium) produces a red, violet or purple colour; it should be noted that the orange-yellow pigment of Candelariella remains unchanged in K.

Iodine. Individual colour changes of the thecium and asci in iodine solution need careful monitoring. The most satisfactory method is to make an appropriate squash in water or K solution and draw in iodine solution (see under thallus tests above for formula) noting carefully any colour changes in the tissue being tested. A squash in K solution will intensify the result. Special attention should be paid to the differential staining of the apices of mature asci and these should be carefully recorded. It will be found that different concentrations of iodine

solution may give a range of colour changes from pale blue to deep blue or pale straw to deep brown-red according to the species. Negative reactions should be recorded as these are just as important as positive colour changes.

C. In the case of C solution it should always be remembered that any reaction that occurs will be of a transient nature, rapidly fading within seconds of application. Tissues to be tested should be squashed in a small amount of water and the C solution run in at the side of the coverslip and observed immediately. Any colour change should be observed as the reagent flows into the material. A KC test can be performed in the same way; the preparation being first mounted in a small amount of K after which a drop of C is allowed to run under the coverslip (e.g. violet colouration with soralia of Pertusaria amara).

Nitric acid. A 50% solution of nitric acid is occasionally useful for the colour changes it produces, usually blue to violet-pink or red, in pigmented tissues (particularly the epithecium) of a species of Ionaspis, Lecanora, and Lecidea. Usually blue or blue-black pigments show this colour change, whereas deep brown or reddish-brown colouration show little change. Attention is drawn to the warning at the beginning of the section as care should be taken to avoid contact of the acid with the microscope objective. However, nitric acid may occasionally be useful in clearing tissues where other solutions have proved unsatisfactory.

(c) Pyrenocarpous species

As a general rule, except for species of Porina and Strigula, Meltzer's reagent provides a more effective method than K for clearing spores in squashes or sections of pyrenocarpous lichens. For Porina and Strigula, as well as discocarp and lirellate lichens, K solution is generally more effective.

ULTRA-VIOLET FLUORESCENCE

An important number of lichen substances show a range of colour and intensity of fluorescence in UV light. In some cases the UV fluorescence is characteristic of the substance whilst still in the lichen, while in others it is only revealed after the processing of TLC plates. In the former case the substances that fluoresce are pigments in the cortex, e.g. xanthones, which fluoresce various shades of red and orange, or depsides and depsidones, which are concentrated in the medulla and are only revealed by cutting or scraping away the cortex with a razor blade. Closely related taxa are rapidly and easily separated by direct examination of thalli under UV light at 350 mμ. For example, the first of the following species pairs are UV +: Parmelia arnoldii and P. perlata (medulla); Cladonia impexa and C. mitis (surface); C. uncialis and C. amaurocraea (surface); C. squamosa subsp. squamosa and C. squamosa subsp. subsquamosa (medulla and surface); Lecanora atra and L. gangaleoides (medulla). Fuscidea cyathoides may be separated from other Fuscidea species as it is the only common species in Britain in which the medulla does no flouresce blue-white as it lacks divaricatic acid. UV fluorescence of the medulla is due to several substances, the most widespread being squamatic, alectoronic and α-collatolic acids. Amongst the pigments thiophaninic acid and arthothelin are the most important, but others, such as rhizocarpic acid and epanorin, are also diagnostic in certain genera. As several different substances are involved it is important that specific and accurate identification is carried our using TLC in the normal way.

MICROCHEMICAL TESTS

Microchemical or microcrystal tests were developed and standardized by the distinguished Japanese lichenologist Asahina in the 1930s in order that lichen substances could be identified accurately and quickly on a microscope slide. These tests are essential for the identification of substances with similar Rf values and colours on TLC plates, and are often required for satisfactory confirmation of results obtained from other techniques. The lichen substances are identified by the formation of characteristic crystals of particular compounds in the various reagents.

Reagents

The first two of the following reagents are the most frequently used.

G.E.	glycerol 1 : glacial acetic acid 3
G.A.W.	glycerol 1 : ethanol 1 : water 1
G.A. o-T	glycerol 2 : ethanol 2 : o-toluidine 1
K.K.	5% potassium hydroxide 1 : 20% potassium carbonate 1
G.A.Q.	glycerine 2 : ethanol 2 : quinoline 1
G.A.An.	glycerine 2 : ethanol 2 : aniline 1

For warnings concerning the use of toxic substances involved, and examples of lichen substances which may be crystallized from each reagent, see Hale (1974), Hawksworth (1971) and Shibata (1963). Solutions should be freshly prepared and tested on control extracts of known chemical composition.

Method

Tests are made on acetone extracts of the substance from the thallus which may be prepared in one of the following ways :

(i) Crumble fragments of the lichen onto a microscope slide placed on a slide-warming hot plate at 50-70°C. Add acetone (or alternative organic solvent as recommended) at a drop at a time, allowing each drop to evaporate before the next is added, restricting the acetone extract to within as small an area as possible. A residue containing the lichen substance will be seen to form around the fragments as a white or gummy deposit. When a required amount of residue has been obtained the slide should be allowed to dry and the lichen fragments lightly brushed away by means of a fine paint brush.

(ii) Alternatively, if required, the extract which has previously been extracted in a glass phial as a result of TLC analysis may be remoistened with a minimum amount of acetone, and a portion of the extract transferred to a slide by means of a capillary tube. This method also eliminates the problems caused by thallus fragments and sand or rock grains and more easily permits the use of the same extract for more than one solvent.

(iii) A more elaborate method involves the use of 'Asahina's Microchemical Extractor' which is a specially designed testing tube with the base extended and drawn out to form a fine side arm. Thallus fragments are placed in the extractor and the solvent added and boiled gently over a spirit lamp for two ir three minutes. The solution is then dropped out from the side-arm onto a warmed slide so that a residue is formed as in (i). This method is useful if more than one organic solvent is being used, and fractions from each are required separately.

2. Remove the slide with the dry residue from the warming plate and add a very small drop of the recrystallizing reagent; just sufficient to go under a coverslip or part of one is all that is needed. It is often advisable to concentrate the residue by scraping it together with a razor blade before the recrystallizing reagent is added, since the best crystals are formed where the concentration of the substance in the reagent is high. A small coverslip, or alternatively part of one, is then placed over the reagent and gentle pressure applied to slightly disperse the residue. Fragments of coverslips may be placed over different parts of a residue and several tests, using different recrystallizing reagents, performed simultaneously on a single slide, although it is always best to use a separate slide for each recrystallizing reagent.

3. Warm the slide over a spirit lamp or electric Bunsen until at least part of the residue has dissolved. Slides should be heated until bubbles just appear under the coverslip and part of the residue is dissolved. Heat is not necessary for some reagents, e.g. K.K., but even in such cases generally leads to the production of better crystals.

4. Allow the slide to cool at room temperature. Recrystallisation for many substances occurs within 5 - 10 minutes but some take longer so that slides should be examined at intervals up to 24 hours.

5. Crystals must be examined microscopically and use of a polarizing filter is recommended. Crystals often appear near the edge of the coverslip or in proximity to the remaining residue. There is no comprehensive work containing all known crystal photographs, but Evans (1943) and Hale (1974) give the largest range. Practice is essential on material containing known lichen substances before reliable interpretation of results is possible. The same substance will give different crystal forms in different reagents, and greater concentrations of substances may result in the formation of exaggerated crystal complexes, although the basic shape can usually be discerned.

Problems

When carrying out microchemical tests the following considerations should be taken into account which may lead to test failure.

1. Too much reagent tends to dilute the residue, thus depressing the formation of crystals. Small coverslips or parts of large ones help to overcome this difficulty, particularly when only small amounts of residue are available.
2. Overheating and vigorous bubbling not only dissolves all of the residue but dispatches it outside the perimeter of the coverslip, so that either no crystals or imperfect crystals are formed.
3. Absence of crystals sometimes indicates that the amount of residue is too small or a larger extract is required. Some substances do not form crystals in any, or more frequently some, reagents. Consequently an unidentified substance should be tested in a range of recrystallizing reagents; even in the case of known or anticipated substances recrystallization should be carried out in several reagents.
4. The presence of lichen fragments, sand grains, or rock crystals may prevent recrystallization, as an excessive amount of reagent would be needed for satisfactory production of crystals. Such preparations should be discarded and a new extract made. However, care should be taken not to mistake such debris for crystals.
5. Difficulties frequently arise if, as sometimes happens in many lichens, two or more substances are present in the same species resulting in the formation of combined crystal complexes of uncertain shape. If this is suspected then recrystalization should be repeated using a range of reagents and it should then be possible to get adequate resolution in at least one of these.
6. Care must be taken to avoid moving the coverslip after heating as this prevents crystal formation, and fragments those that have already formed. No pressure should be applied at any time to the coverslip after heating the preparation. Placing the slide on a moderately warm hot plate or radiator top sometimes speeds up the formation of crystals.
7. Crystal shapes of some substances in particular reagents are sometimes difficult to tell apart. For this reason respective crystal tests should be performed using a range of reagents, e.g. four, since the form in other reagents may be more distinguishable and thus help to separate the two substances concerned.
8. All reagents should be kept scrupulously clean and free from moisture and replaced at frequent intervals. Old solutions, or introduced impurities often arrest crystal formation or cause aberration in the crystal forms.

THIN-LAYER CHROMATOGRAPHY

This method has now largely replaced that of paper chromatography (see Hawksworth 1971 for details) as it is a quicker and more sensitive technique.

Equipment : See Appendix I.

Solvent Systems

1. Basic solvent systems All samples to be tested should be run in the following three solvents :

H.E.F.	Hexane : diethylether : formic acid; 130 : 100 : 20 ml.
T.D.A.	Toluene : dioxan : acetic acid; 180 : 60 : 8 ml.
T.A.	Toluene : acetic acid; 200 : 30 ml.

2. Additional solvent systems To be used in special cases (see below) :

E.A. diethylether : acetic acid; 200 : 2 ml.
Used for the separation of gyrophoric and lecanoric acids; must be freshly prepared.
E.H. diethylether : hexane; 3 : 1
Used for the separation of hopane triterpenoids. Plates should be run two or three times in the solvent before processing to give adequate separation of spots.

Additional substances

Acetone: used for the extraction of lichen substances.
Sulphuric acid: a 10% solution for developing the plates.
Other developing agents such as solutions of C, K, KC, $FeCl_3$ and PD may be required to give specific colour reactions on the plates.
Silicone grease: Used for making the tanks airtight.

Basic Method

A. Preparation of solvent systems

The three basic solvent systems should be made up in a fume-cupboard as a protection against harmful vapours. Care should be taken that the glassware used is clean and free from any contaminating moisture. Only small quantities of solvents should be prepared at one time and replaced at frequent intervals as they tend to deteriorate in storage. The H.E.F. solution is particularly prone to evaporation, tending to give poor results after a relatively short period of storage, but separation in this solvent can sometimes be improved or resurrected by adding more diethylether. Place solvents in individual chromatography tanks, each to a depth of about 1 cm. Filled tanks and any excess solvent (stored in a brown glass bottle) should be kept in a cool place. A spare plate or sheet of filter paper should be placed at the back of each tank. This helps to achieve a uniform vapour saturation of the solvent throughout the tank assuring even running of the solvent front. Grease the top and lid of each tank with silicone grease as a seal against undue evaporation of the solvents. Tanks should be then left to stand for a period of time (e.g. two hours) to stabilise before the first plate is run.

B. Preparation of the plates

Aluminium plates are used for all routine work and can be cut according to various requirements, e.g. special cases where different developing agents are used, or for comparison of a known control substance with an unknown, or for tests with PD, C, KC, K, or other specified reagents. Glass plates are necessary for the detection of fatty acids (see below). Plastic plates are not suitable for acid and heat treatment, but can be used with alternative sprays, e.g. K. C. PD. etc. One plate is needed for each solvent system used. Many substances can be identified using only two solvent systems, H.E.F. and T.D.A., but the additional solvent system, T.A., is always essential for critical work and should be run as a matter of principle. Mark up the required number of aluminium plates as follows :
If a whole plate is used, mark a base line with a soft (2B) pencil 1.5 - 2 cm from the bottom by using a ruler with notches at 1 cm intervals. This enables 19 points to be marked on each 20 cm plate, which should then be numbered. Rule a terminating front line 11 cm above the base line. Label each plate with the solvent system to be used. Alternatively, for economy, plates cut in half may be used with a base line drawn 1.5 cm from the bottom and the solvent front will be run until the top of each plate is reached. This is a more rapid method which does not give such a good separation of Rf values, but has been found to be adequate for routine analysis.

C. Extraction of substances

1. Select control substances to be placed at points 3 and 17 or 1 and 19 on each plate. An appropriate mixture of Parmelia acetabulum and Platismatia glauca will give spots for norstictic acid (Rf value class 4) and atranorin (Rf value class 7) for each solvent. It is often useful to have a control containing fumarprotocetraric

acid (Cladonia subcervicornis) or protocetraric and usnic acids (Parmelia caperata) which may be used instead of Platismatia glauca. These markers assist in establishing the Rf classes of unknown spots on developed plates, and in theory any suitable combination of control substances can be used to fit any special requirements.

2. Number and record the lichens to be examined. This may be done on suitably annotated data sheets (see Appendix II).

3. Place a small fragment of the lichen to be tested in a small glass phial. It is extremely important to ensure that the sample is not a mixture of species, and is completely dry. Samples from crustose species should be carefully removed under a binoc. microscope to prevent the possibility of an inadvertant mixture of two or more species. Samples of foliose and fruticose species should be pushed well down into the bottom of the tube to maximise contact with the solvent.

4. To all samples add one drop of cold acetone or just a sufficient amount of liquid to moisten the fragments; excess should be avoided in all cases. This solvent will extract soluble lichen substances present and deposit them as a residue on evaporation. Glass slides may be used as an alternative, but small phials ensure that a more concentrated extract is produced which can if required be easily stored for further extractions or for other tests, e.g. microcrystal test.

5. Using a clean capillary tube for each specimen, transfer the acetone extract to the corresponding numbered point on each TLC plate. (If necessary redissolve the extract in the phial with a further drop of acetone). These initial spots on the base line should be kept as small as possible so as to minimise the likelihood of undue spreading and merging on the developed plates; three applications per spot are recommended to give adequate results, but heavier or lighter loading of the spots may be necessary in some cases, e.g. heavier for Usnea and Alectoria species and lighter for some species containing large amounts of gyrophoric, lecanoric or norstictic acids. The capillary tubes and phials may be reused only if they are thoroughly cleaned with warm acetone. (CARE: acetone is highly inflammable).

6. Place a prepared plate in each of the tanks and leave until the solvent has reached the terminating front line. The running time for a 20 cm plate is 30 - 40 minutes, depending on how recently the solvent systems have been prepared and the temperature and other atmospheric conditions prevailing at the time.

7. Remove the plates and air-dry for a few minutes in a fume-cupboard or oven fitted with an extractor fan.

D. Examination of plates

1. Examine plates by daylight for pigments which appear as coloured spots and note their colour and position.

2. Examine plates by UV light at two wavelengths. The lamp must be used in a darkened room. The user's eyes must be protected from harmful radiation by either wearing a pair of special spectacles or protection afforded by placing a sheet of clear perspex in front of the lamp. In special cases if the spots below Rf value 4 (norstictic acid) are complex or crowded the plates can be returned to the same solvent, and re-run to increase the separation. However, if this is done then the spots above Rf 4 are of no value.

(a) at 254 mμ UV light most substances are indicated by dark shadows on a fluorescent background. Mark all spots, however faint, by circling the darkened area with a soft (2B) pencil.

(b) at 350 mμ UV light note especially any special fluorescence of the outlined spots and also any darker or coloured shadows which may indicate the presence of pigments, including usnic acid. Any entirely new spots should be indicated by a dotted outline so as to distinguish them from those marked at 254 mμ in (a). The colour of fluorescence of individual spots should also be noted on the plate.

3. The spots may be developed by spraying with a 10% solution of sulphuric acid using a spray gun. This should always be done in a fume-cupboard and the plates should be well moistened with acid. Heat the plates in a pre-heated oven at 110°C for about five minutes until the various diagnostic colours are well intensified. On heating, extra unmarked spots of a purplish tone may appear; these are triterpenoids (e.g. zeorin) or substances which may sometimes occur in bark and may therefore give misleading results. Where doubt exists specimens should be run against

their relevant substrate. Additional important information may also be obtained by noting the colour of flourescence of spots under 350 mμ UV light after acid and heat treatment. It is also important to monitor any change in colour of spots (e.g. with pannaric and lepraric acids) after a few minutes and after several hours and even after two or three days.

After 24 hours or more some substances develop characteristic colours, e.g. barbatic acid can be distinguished from diffractaic acid as the yellow spot darkens to a rust brown colour; diffractaic acid turns a deep mahogany brown in about 7 days.

Alternatively the plates may be developed by spraying with C, K, KC, $FeCl_3$ or PD in a fume cupboard for certain colour reactions in these cases no heat is required. Great care should be taken when spraying with PD solution and a safer method is to paint the solution onto the plate. Plates treated with these reagents should be discarded after the colour reaction has been noted. As for spot tests, the C, KC, and K reactions are fugitive and easily missed. Once a plate has been developed it cannot be resprayed or treated with another developing agent.

4. Join up the centres of the control spots for norstictic acid and atranorin or other controls used. The Rf classes for the unknown spots can then be determined and the substance identified using the tables in Culberson and Kristinsson (1970) and Culberson (1972). An abridged version of the tables is provided (Table 1) together with examples of lichens which may be used as a comparison for the identification of the substances in question. Identification of substances can be achieved either by running the extract adjacent to an extract containing a known substance or alternatively using a pure sample of a particular lichen acid. A comparison of the properties of the unknown and known substance can effect identification. Alternatively, a microcrystal test of the original acetone extract may be required to confirm the identity, and may be the only simple means of distinguishing between perlatolic, barbatic, didymic and divaricatic acids and related compounds, all with similar Rf values.

5. The developed plates may be easily stored, although colour may fade or become uniform, and cross-referenced with the data sheets containing details of the specimens and substances detected, even if unidentifiable. Each specimen tested should be labelled to show the substances found, and also cross-referenced with the respective data sheet and stored plate.

Specialised techniques

1. Detection of fatty acids

Fatty acids may be present in lichens, particularly those in which no substances are revealed on aluminium plates treated as above. For their detection glass plates are used in the same way as aluminium plates, but an additional stage is necessary. Plates are developed in the normal way but, after acid and heat treatment (for 10 - 15 minutes), they are sprayed with water and then placed in an oven (110°C) and observed carefully as they gradually dry out. Spots not previously marked appear white against a black background and dark or opaque when illuminated from below. These spots are fatty acids, and their position is indicated by marking a dotted line across the centre with a soft pencil (2B). Plates can then be completely dried and stored. The relevant bark substances may need to be tested along side the specimens as these sometimes also contain different fatty acids.

2. Separation of gyrophoric and lecanoric acids

The solvent system E.A. is used to separate these two acids and as an alternative to crystal tests. However, in this instance it is the Rf value of the bottom of the spot that is significant, not the top. Examples of species which may be used as controls are Parmelia glabratula for lecanoric acid and Ochrolechia androgyna for gyrophoric acid.

3. Triterpenoids

Triterpenoids are characteristic or abundant in certain groups of lichens, e.g. the genera Dirinaria, Pyxine, Pseudocyphellaria, Nephroma, Peltigera and Anaptychia. The triterpenoids occuring in a particular genus are closely related and

lie on the same biosynthetic pathway. Particular care is needed in separating the various elements of these complexes and aluminium TLC plates should be run in three basic solvent systems and also in E.A. Plates should be run two or three times in E.H. to give adequate separation of these spots. Triterpenoids also occur in bark, peat, vegetable detritus and some mosses but the majority are different from those which are found in lichens. Consequently it is very important to differentiate between the lichen itself and its substrate, and if in doubt assays of the substrate should be run beside the lichen extract in question. Triterpenoids can easily be distinguished on developed plates as they appear as a salmon pink line or oval spot under UV light of wavelength 350 mμ.

4. Two-dimensional chromatography

This method is very valuable where a considerable number of substances are present in a single species. Aluminium plates may be cut to give four 10 x 10 cm squares. Two base lines are drawn 1.5 cm in from the margin of the plate and at right angles to each other, where the two lines cross marks the point where the particular acetone extract of the lichen is to be spotted. The prepared plate is then run up the full distance of the plate in H.E.F. solution, allowed to dry, and then run the same distance in T.D.A solution using the second base line as the starting point.

The solvent run in H.E.F. separates the substances as in a normal H.E.F. chromatogram and in this instance spreads them along the second baseline used for T.D.A. The T.D.A. solvent spreads those spots resulting from the H.E.F. separation according to their Rf values in T.D.A., thus producing a two-way spread of spots. Sometimes what appears as a single spot on one-dimensional plates may break down into several different components when subjected to two-way resolution. For examples of application of this method see Wilkins and James (1979) and Maass (1975).

TABLE 1. Identification of common lichen products by TLC.

Adapted from Culberson (1972) and Culberson & Kristinsson (1970) and based on experimental data.

Rf class A TDA	Rf class B HEF	Rf class C TA	Compound	Spot colouration with sulphuric acid and heat.	Examples of control species
1	3	2	fumarprotocetraric	slate grey	Cladonia fimbriata, C. pocillum, Fuscidea cyathoides
1	2-3	2	protocetraric	slate grey	Parmelia caperata, Ramalina farinacea (race)
1	3	2	thamnolic	green-brown A, yellow-orange BC	Cladonia macilenta, C. polydactyla, Haematomma elatinum, Usnea subfloridana (race), U. florida
1-2	1-2	1	erythrin	grey-orange A, pale orange BC	Dirina repanda f. stenhammarii, Roccella phycopsis
1-2	4	1-2	caperatic	(fatty acid)	Parmelia caperata, Platismatia glauca
2	2	1-2	porphyrilic	pale	Cladonia luteoalba, Haematomma ochroleucum
2	2	2	salazinic	pale yellow-orange	Parmelia sulcata, P. omphalodes
2	2	2	variolaric	yellow-grey (UV+)	Ochrolechia turneri, Pertusaria multipuncta
2	3	3	squamatic	blue A, orange BC (UV+++)	Cladonia crispata, C. glauca, C. squamosa
2	3-4	2	pannaric	pale or grey	Lepraria membranacea
2	4-5	2	conpsoromic	olive-straw	Lecanora varia, Sclerophyton circumscriptum
2	4-5	3-4	physodalic	black	Hypogymnia physodes
2	5	2-3	barbatolic	pale yellow	Bryoria capillaris, B. nadvornikiana
3	2	3	lepraric	grey, rapidly to rose-pink	Lecanactis subabietina, Roccella fuciformis
3	2	3	stictic	orange-brown	Parmelia conspersa, Lobaria scrobiculata
3	4	3	alectoronic	pale or pale pink-grey (UV+++)	Parmelia arnoldii, P. incurva
3	4-5	3	physodic	grey-brown	Hypogymnia physodes, H. tubulosa
3	5	3	gyrophoric **	grey A, yellow BC	Ochrolechia androgyna, Parmelia revoluta, Umbilicaria pustulata
3	5	3	hypoprotocetraric	olive A, yellow BC	Ramalina siliquosa (race), R. subfarinacea (race)
3	5	3	lecanoric **	grey A, yellow BC	Parmelia glabratula, Lecanactis abietina
3	5	4-5	lobaric	pale green-grey	Lecanora badia, Parmelia omphalodes, Stereocaulon evolutum
3	5	5	protolichesterinic	(fatty acid)	Cetraria chlorophylla
3	5	5	psoromic	dark brown	Schismatomma niveum, Sclerophyton circumscriptum, Squamarina cartilaginea (race)
3	5-6	4	alectorialic	pink-brown	Alectoria nigricans, Buellia pulverea, Usnea florida
3-4	4-5	3	olivetoric *	pale yellow-orange	Cetrelia olivetorum, Pseudevernia furfuracea var. ceratea
3-4	5	5	baeomycesic	orange-yellow	Baeomyces roseus
3-4	5	5	picrolichenic	pale or colourless	Pertusaria amara
3-4	6	4-5	anziaic	yellow-orange	Cetrelia sanguinea (foreign)
3-4	6	5	evernic *	yellow	Evernia prunastri, Ramalina fastigiata
4	3	5	schizopeltic	grey then lilac-mauve	Lecanactis abietina

TABLE 1 contd.....

4	4	4	norstictic	bright yellow	Parmelia acetabulum, Phlyctis argena
4	4	4-5	arthothelin	orange then brown-orange	Lecanora straminea, Ochrolechia inversa
4	5	5	∝-collatolic	colourless (UV+++)	Lecanora atra, Parmelia arnoldii
4	5-6	5	cryptochlorophaeic	dusky orange then deep orange	Cladonia chlorophaea (race)
4	5	5-6	miriquidic	peacock blue then emerald green	Aspicilia superiuscula, Lecidea leucophaea
4	5-6	5-6	sekikaic	pinkish-orange then dull orange	Ramalina calicaris
4	6	5-6	barbatic *	yellow then rust	Cladonia floerkeana, Usnea subfloridana (race)
4	6	5-6	divaricatic *	yellow (UV+)	Fuscidea kochiana, F. lightfootii, Haematomma ventosum
4	6	5-6	diffractaic *	chrome yellow then mahogany brn.	Usnea ceratina
4	6	5-6	grayanic	dull straw	Cladonia fragillissima, C. grayii
4	6-7	5-6	didymic *	pale yellow	Cladonia didyma
4-5	6	5-6	homosekikaic	pale yellow-pink then pink brown	Cladonia rei
4-5	7	5-6	perlatolic *	bright yellow	Cladonia impexa
4-5	7	5-6	sphaerophorin	yellow-ochre	Sphaerophorus spp.
5	5	5-6	confluentic	pale straw then brown (often + UV++ ice-blue accessory after charring)	Enterographa crassa, Huilia tuberculosa
5	5	6	planaic	yellow (+ grey ring) then brown	Lecidea plana
5	5-6	5-6	merochlorophaeic	dusky pink then deep crimson	Lethariella intricata (foreign), Cladonia chlorophaea (race)
5	5	5	zeorin	violet (after charring)	Cladonia coccifera, C. luteoalba, Lecanora epanora
6	5	6	gangaleoidin	deep orange-yellow	Lecanora gangaleoides, L. jamesii
6	5	6	rhizocarpic	citrine yellow	Psilolechia lucida, Rhizocarpon geographicum
6	6	6	thiophaninic	pale	Pertusaria flavicans
6	6	6	scrobiculin	light brown	Lobaria scrobiculata
6	6	6	usnic	pale green-grey (UV quench)	Cladonia impexa, C. coccifera, Haematomma ochroleucum var. ochroleucum, Usnea spp.
6-7	5	5	epanorin	citrine yellow	Lecanora epanora
7	6	7	pannarin	brown then violet (rapid)	Pannaria rubiginosa
7	6	7	pinastric	lemon yellow	Cetraria juniperina, C. pinastri
7	6	7	tenuiorin	yellow	Lobaria linita, Peltigera horizontalis, P. polydactyla
7	7	7	atranorin	yellow-orange	Platismatia glauca, Stereocaulon spp.
7	6	7-8	vulpinic	citrine yellow	Alectoria tortuosa, Letharia vulpina (foreign)
7	7	7-8	parietin	yellow	Xanthoria parietina

* crystal test required for confirmation
** separate using E.A. solvent or crystal test

Appendix 1 — Suppliers of TLC equipment

TLC plates	Anderman & Co. Ltd., Central Avenue, East Molesey, Surrey, KT8 0Q2 Merck silica gel 60 F_{254} pre-coated aluminium, glass or plastic plates. 20 x 20 cm. Sold in packs of 25.
TLC tanks	Griffin & George Ltd., 285 Ealing Road, Wembley, Middlesex, HA0 1HJ. Various sizes are available, see supplier's catalogue.
Capillary tubes	A.R. Horwell Ltd., 2 Grangeway, Kilburn High Road, London, N.W.6. 1 mm. or 0.5 mm. bore available.
Glass phials	Washington Research Products Ltd., Victoria Industrial Estate, Victoria Road West, Hebburn, Tyne and Wear, NE31 1UB. 55 x 10.5 mm. soda glass test tubes.
Test tube racks for phials	Washington Research Products Ltd. (see above) Plastic-coated wire racks available with 20 or 60 spaces.
Ultra-Violet lamp	Griffin & George Ltd., (see above). Camag Universal U.V. lamp, wavelengths 254 mμ and 350 mμ.
Laboratory Spray Gun	Scientific Supplies Co. Ltd., Vine Hill, London, E.C.1. Replaceable power units are also available.

Appendix II
Lichen Thin-Layer Chromatography Data Sheet

Solvents: Spray: Plate No .....

T.D.A..... H_2SO_4..... Date .....

H.E.F. ..... Pd ..... Operator .....

T.A. ..... H_2O .....

Other ..... Other .....

SPECIMEN	LICHEN SUBSTANCES DETECTED			
1.				
2.				
3. Control Sample	atranorin	norstictic		
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				
13.				
14.				
15.				
16.				
17. Control Sample	atranorin	norstictic		
18.				
19.				

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