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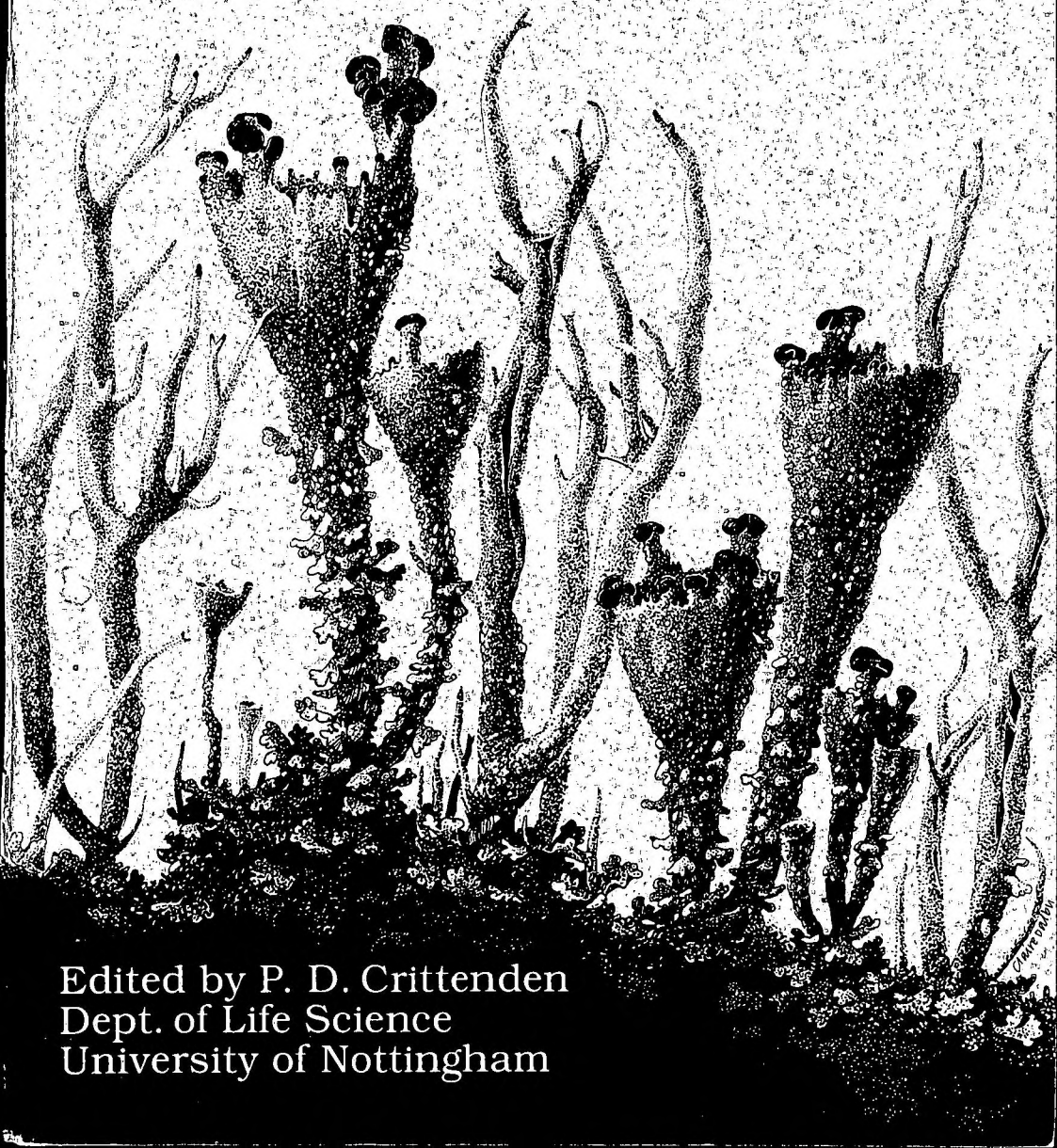

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THE BRITISH SPECIES OF *LEPRARIA* AND *LEPROLOMA*: CHEMISTRY AND IDENTIFICATION

Most lichenologists do not accept that chemical characters should be used as the basis for separation of species, unless supported by morphological differences. However, chemical characters are given considerable weight in *Lepraria*, as this is regarded as a form-genus comprising morphologically reduced, perhaps unrelated taxa which have lost the capacity for sexual reproduction. While many of the species in *Lepraria* can be recognised in the field with a good degree of accuracy by characters such as thallus colour and the form of the granules, thin-layer chromatography (TLC) is usually necessary for reliable identification.

The following notes derive from material prepared for a workshop on TLC, held at the National Museum of Wales, Cardiff, in November 1994, which used *Lepraria* and *Leproloma* as experimental material. It is hoped that the table and chromatograms will be useful to those with access to TLC; in addition a provisional key to the taxa which does not use TLC is presented.

Taxonomy

Keys to the species are provided by Laundon (1989, 1992) and Tønsberg (1992). Four species additional to those mentioned by Laundon are now accepted as British:

Lepraria crassissima: pale grey with a bluish tinge; contains divaricatic acid, and also substantial amounts of nordivaricatic acid which gives a distinctive C + red reaction; uncommon on rock.
Lepraria elobata: a blue-grey species which contains stictic acid complex, zeorin, atranorin, and often rangiformic acid; probably widespread on bark and rock.
Lepraria jackii: contains roccellic acid and atranorin, often also with rangiformic acid and other fatty acids; locally abundant on bark, less frequently on rock.
Lepraria rigidula: pale grey, contains the fatty acid 'rigidula-unknown', granules with distinctive long projecting hyphae; frequent on rock and bark.

Note: *Lepraria jackii* and *L. rigidula* were included in *Leproloma cacuminum* by Laundon (1992), but they are sufficiently distinct in chemistry, ecology and (at least in the case of *L. rigidula*) morphology to be treated as separate species for the present.

Morphological characters in *Lepraria* and *Leproloma* are provided by colour, presence or absence of lobes, thallus stratified or not, size range of granules, and the degree to which hyphae project from the granule surface.

Chemistry

The chemistry of the British species is summarised in Table 1. Chromatograms which include the major substances are shown in Figure 1. All the British taxa can be distinguished using either solvent system A or G, except *Lepraria jackii* and *L. rigidula* which are best separated in G. Separation of the fatty acids in *L. jackii* is best carried out by running each sample in both G and C (not shown here). Some species of *Lepraria* and *Leproloma* contain angardianic acid, but this fatty acid is indistinguishable from roccellic acid by TLC. Details of solvent systems and techniques are given in White & James (1985).

Figure 1. Chromatograms in solvent systems A (above) and G (below). Dotted lines indicate fatty acids. Rf classes are indicated on the right.

Species: 1, 2. *Lepraria caesioalba*, 3. controls, 4. *Lepraria nivalis*, 5. *Lepraria eburnea*, 6. *Lepraria frigida*, 7. *Lecanora sp.*, 8. *Lepraria lobificans*, 9. *Lepraria incana*, 10. *Lepraria crassissima*, 11. *Lepraria umbricola*, 12. *Lepraria lesdainii*, 13. *Lepraria jackii*, 14. *Lepraria rigidula*, 15. *Leproloma cacuminum*, 16. *Leproloma membranaceum*, 17. controls, 18. *Leproloma vouauxii*, 19. *Leproloma diffusum*.

Compounds: 1. atranorin, 2. fumarprotocetraric acid, 3. protocetraric acid, 4. norstictic acid, 5. alectorialic acid, 6. usnic acid, 7. zeorin, 8. stictic acid, 9. constictic acid, 10. divaricatic acid, 11. nordivaricatic acid, 12. thamnolic acid, 13. lesdainin, 14. roccellic acid, 15. unidentified fatty acid, 16. rigidula- unknown, 17. rangiformic acid, 18. norrangiformic acid, 19. porphyrilic acid, 20. pannaric acid, 21. pannaric acid-2-methylester, 22. unknown, 23. oxypannaric acid-2-methylester, 24. satellite to alectorialic acid, 25. psoromic acid, 26. 2'-0-demethylpsoromic acid, 27. satellite of stictic acid.

Table 1. Summary of chemistry of British species of *Lepraria* and *Leproloma*

	Leproloma vouauxii	Leproloma membranaceum	Leproloma diffusum	Leproloma cacuminum	Lepraria umbricola	Lepraria rigidula	Lepraria nivalis	Lepraria neglecta	Lepraria lobificans	Lepraria lesdainii	Lepraria jackii	Lepraria incana	Lepraria frigida	Lepraria elobata	Lepraria eburnea	Lepraria crassissima	Lepraria caesioalba
alectorialic acid								+					+		+		
angardianic acid				+				+									
atranorin		±		+		+	+		+		+			+	(±)		±
barbatolic acid													+		+		
constictic acid									+					+			
2'-0-demethylpsoromic acid																	±
divaricatic acid												+				+	
fumarprotocetraric acid																	±
gyrophoric acid							(±)					±tr					±
lesdainin										+							
nordivaricatic acid												tr				+	
norrangiformic acid								(±)			±						±
oxypannaric acid-2-methylester			+														
pannaric acid	±tr	+	±tr														
pannaric acid-6-methylester	+																
parietin												±					
porphyrilic acid				+													
protocetraric acid							+								+		±
psoromic acid				(±)													±
rangiformic acid				±			±	(±)			±			±			±
rigidula-unknown						+											
roccellic acid		+		±			±				+						±
stictic acid									+					+			
thamnolic acid					+							±					
zeorin		(±)							+			+		+	(±)	+	

+ = constant
± = not constant
( ) = rare
tr = trace

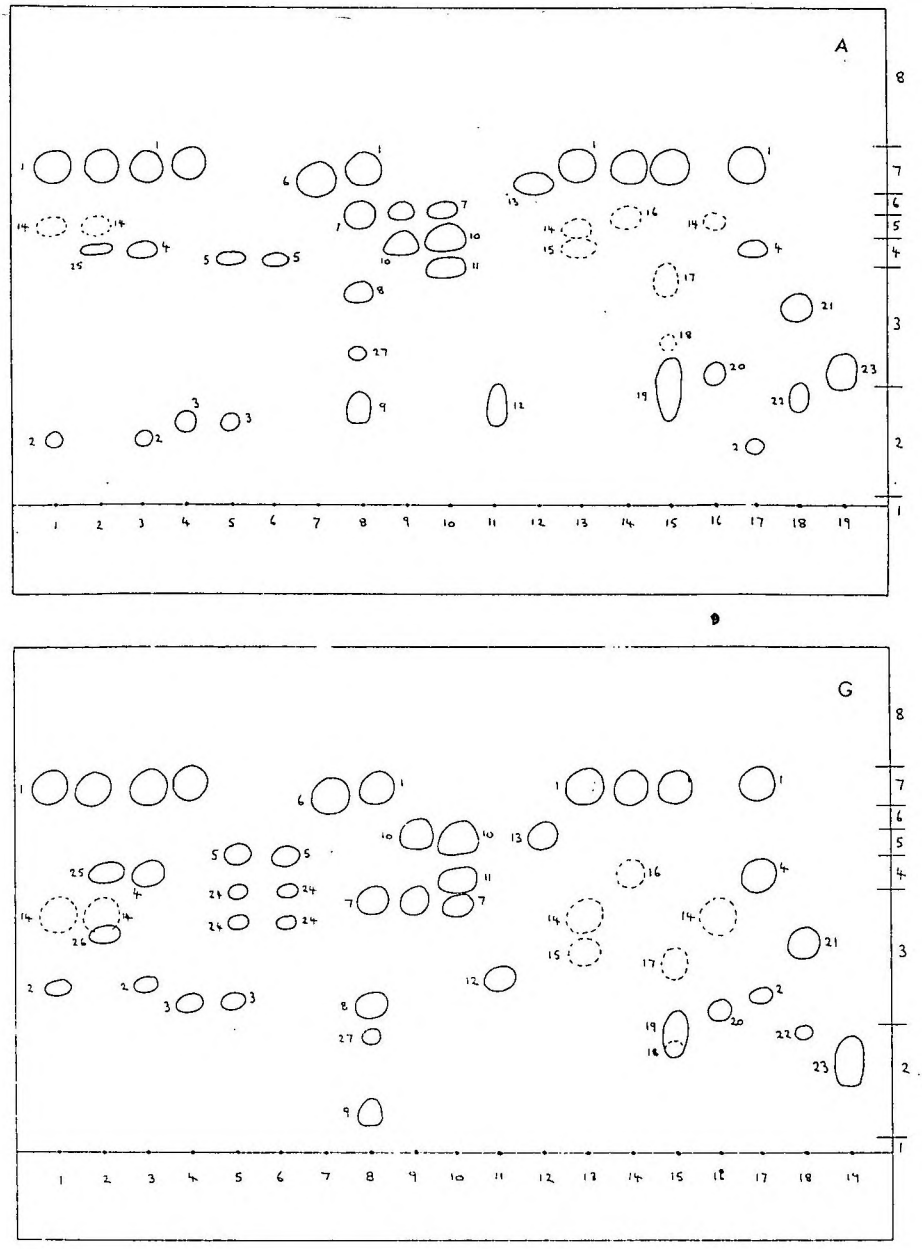

Figure 1.

Provisional key to *Lepraria* and *Leproloma*, not using data from TLC.

The following key is a first attempt at identification using only morphology, spot tests, and microcrystal tests. Construction of such a key is difficult, as microcrystal tests are often not particularly distinctive in these genera, because some of the species have more than one compound present at once, and because the occasional presence of accessory compounds may give atypical reactions. It may never be possible to determine accurately all the taxa without TLC, but the author would be pleased to hear of any corrections or improvements to this key.

The key relies heavily on spot tests which are best observed under a dissecting microscope. They should be carried out on acetone extracts of the thallus spotted onto filter paper (many are difficult to see on the intact thallus). For the KC reaction, add a small amount of K, then a small amount of C on top of it; a positive reaction appears as a fleeting 'blush' of a pinkish or orange-pink colour.

A 1% solution of ferric chloride ($FeCl_3$) in water gives results which may be useful, though further specimens need to be studied. A positive reaction has been obtained with all those species containing aromatic lichen substances except for those with only atranorin and/or porphyrilic acid. Atranorin has been reported to give a reddish-violet reaction but it has not been possible to confirm this, and the apparent lack of reaction with atranorin has been used in the key. The following results have been observed: 1. no reaction or at most a faint brownish colour (*L. jackii, L. lesdainii, L. rigidula, Leproloma cacuminum*), 2. dull pink (most species), 3. dull pinkish-violet to dull violet (*L. umbricola*), 4. dull grey brown with pinkish tinge (*L. diffusum*), 5. grey (*Leproloma membranaceum, L. vouauxii*). In practice, reactions 2, 3 and 4 may not be distinguishable from each other, but reaction 5 appears to be useful in indicating *L. vouauxii*.

GE = microcrystal reagent of glycerol: glacial acetic acid 1:3; $Ba(OH)_2$ = saturated solution of barium hydroxide in water. (Used for thamnolic acid.)

1. Negative with all four reagents; thallus green, soft; growing on calcareous rocks; in GE forming moss-like branching patterns of minute crystals **L. lesdainii**

– Nearly always positive with one of the reagents; if negative, then thallus not green and not on calcareous rock 2

2. Reactions C– to + pink or red, KC + orange-pink to red, K– to + yellow (the KC reaction is different to that obtained by K alone). 3

– Reactions C– to + yellow to orange-brown, KC– to + orange brown, K– to + yellow to yellow-brown or orange (if there is a KC reaction it is not pinkish and it is similar to that obtained by K alone). 5

3. Reactions PD - (C + red, K– , KC–) **Lepraria crassissima**

– Reactions PD + yellow to orange (C– to + orange-pink, K– to + yellow), specimens turning herbarium packets pink after a few years (alectorialic acid) 4

4. Thallus forming rosettes in habitats not sheltered from rain, granules usually without projecting hyphae **Lepraria neglecta**

– Thallus in habitats sheltered from rain, granules with shortly projecting hyphae **Lepraria eburnea** and **Lepraria frigida**

5. $FeCl_3$ - (at most faintly brownish), K and PD + yellow (sometimes faint) 6

– $FeCl_3$ + pink, violet, grey or grey-brown, often PD + orange 10

6. Thallus forming rosettes in habitats not sheltered from rain, granules usually without projecting hyphae **Leproloma cacuminum**

– Thallus growing in habitats sheltered from rain, granules with shortly projecting hyphae 7

7. Acetone extracts with a distinct yellow pigment (K– or masked by pigment, KC + yellow, PD + yellow); granules fine; often forming extensive pale yellowish colonies in deeply sheltered rock overhangs **Lecanora** sp.

– Acetone extracts without distinct yellow pigment; granules fine to coarse, on rock or bark 8

8. Granules relatively coarse, up to 200-300 µm wide, with ± straight, conspicuously projecting hyphae at least 60 µm long **Lepraria rigidula**

– Granules relatively fine, up to c. 100 µm wide, with only shortly projecting hyphae 9

9. With very fine, straight, hair-like crystals in GE, often in radiating clusters (porphyrilic acid) (in addition to flat, very thin plates and/or branching patterns of small crystals due to fatty acids); usually on rock (or soil) **Leproloma cacuminum**

– Without fine hair-like crystals in GE (but crystals of fatty acids are present); frequent on bark, occasional on rock **Lepraria jackii**
(The crystals of porphyrilic acid may be difficult to detect in practice, as they may be in small quantity, accompanied by more abundant crystals of fatty acids. *Haematomma ochroleucum* is a convenient control for porphyrilic acid.)

10. Thallus forming rosettes in habitats not sheltered from rain, granules usually without projecting hyphae (reactions K– or + yellow, C– , PD– to + orange, $FeCl_3$ + pink) **Lepraria caesioalba**

– Thallus diffuse or delimited, in habitats sheltered from rain 11

11. $FeCl_3$ + greyish, PD + orange 12

– $FeCl_3$ + pink to grey brown or violet 13

12. Thallus distinctly lobed (medulla not conspicuously exposed, hypothallus well-developed) **Leproloma membranaceum**

– Thallus not or only obscurely lobed (medulla usually conspicuously exposed, hypothallus poorly developed) **Leproloma vouauxii**

13. Reactions K– , C– , PD– ($FeCl_3$ + pinkish); granules fine, with shortly projecting hyphae, thallus usually tinged distinctly bluish-grey **Lepraria incana**

– Reactions K + or PD + 14

14. K + violet red (parietin), thallus often tinged dull orange **Lepraria incana**

– K– to + yellow or orange 15

15. Thallus whitish, delimited, on calcareous rock (K– or + yellow, PD + orange) **Lepraria nivalis**

– Thallus not delimited 16

16. Thallus strongly yellow (an acetone-insoluble pigment) **Leproloma diffusum** var. **chrysodetoides**

– Thallus whitish to cream, pale grey, bluish grey or green 17

17. K + bright yellow (thamnolic acid), (PD + orange, $FeCl_3$ + dull violet to dull pinkish-violet), greenish species of acidic substrata, in $Ba(OH)_2$ producing clusters of boat-shaped crystals **Lepraria umbricola**

– K + yellow (less vivid colour) 18

18. Thallus granules fine, with shortly projecting hyphae, medulla not or scarcely differentiated, thallus pale grey to bluish-grey
Lepraria elobata

– Thallus granules with short or long projecting hyphae, medulla present 19

19. Thallus often with a bluish-grey tinge ($FeCl_3$ + pinkish)
Lepraria lobificans

– Thallus white to cream ($FeCl_3$ + dirty grey brown with pinkish tinge) **Leproloma diffusum**
(These species look different in the field and it should be possible to devise microcrystal tests to separate them.)

References:

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White, F.J. & James, P.W. (1985) A new guide to microchemical techniques for the identification of lichen substances. *British Lichen Society Bulletin* **57** (suppl.).

Alan Orange

Professor Josef Poelt

Just as this Bulletin was going to press we received the sad news of the death of Josef Poelt. He was an Honorary Member of this Society and one of the most eminent figures in contemporary lichenology. A full obituary will follow in a Society publication.