Biochemistry in the Taxonomy of Lichens

The application of biochemical methods to plant taxonomy was first made in the lichens, in 1867, when potassium hydroxide and calcium hypochlorite were found to give characteristic colour reactions when applied to the thallus of certain species. Most present-day lichenologists employ these reactions as an important character in the description of species. In a large number of lichen species the exact constitution of the lichen acids causing colour reactions is known, mainly owing to the researches of Zopf[1] and Asahina[2,3]. The latter has originated a method of microchemical analysis whereby the lichen acids can be determined by the form of the crystals which they produce under the microscope when recrystallized from various solutions[4], this method requiring the extraction of only a small portion of the thallus with acetone.

Use has been made of biochemical methods as an aid to taxonomy also in the flowering plants, and it has been found that many of the larger groupings, such as families, sections and genera, originally delimited on a purely morphological basis, are characterized also by biochemical differences[5]. The application of chemical methods in Phanerogams, however, has not yet reached the point at which species are distinguished on chemical characters. Penfold, Morrison and Smith-White, for example, class individuals of the Australian tree *Leptospermum citratium* containing different essential oils as "variety A" and "variety B" respectively[6]. In lichens, the tendency has been to regard chemically distinct individuals as distinct species, irrespective of whether morphological differences are present or not. This view, if carried to its logical conclusion, means that in many cases related species could be determined only by chemical analysis. This state of affairs has already been reached in certain lichen genera, for example, *Cladonia*. In the lichens the matter is further complicated by the fact that they are not simple organisms, but a fungus–alga consortium, and in most cases it is not known with certainty to what extent the formation of the characteristic lichen acids depends on the symbiotic union. In a few cases the fungal component has proved capable of producing the characteristic substance in pure culture without Algæ[7]. It is not unlikely that the same lichen fungus may produce distinct acids in symbiosis with different Algæ. The differentiation of 'chemical species' in the lichens therefore rests on a doubly insecure foundation.

The problem has already become acute in the taxonomy of lichens to the extent of forming a serious obstacle to the work of classification; for example, in one genus now under monographic study, it would be necessary to describe more than forty 'new species' on chemical grounds alone, without supporting morphological characters, if the present conventional treatment were adhered to. The increasing interest shown by taxonomists in other plant groups in the use of biochemical methods makes it necessary, if possible, to forestall difficulties of a similar nature which will undoubtedly arise as further use is made of biochemical criteria applied to taxonomy.

It is now suggested that morphologically indistinguishable individuals of plants (other than bacteria) which differ in their chemical characters should be included in the same species as distinct 'chemical strains', the nature of the compound present being indicated in brackets, together with quotation of the taxonomic epithet previously used (if any) to designate the strain in question. The following are some examples of the treatment applied to lichen species:

Stereocaulon tomentosum Fr., Ch. str. I (stictic acid or typical strain).
" " " Ch. str. II (lobaric acid or 'sasakii'-strain) (previously described as a distinct species, *St. Sasakii*).
Cladonia chlorophæa (Flk.) Spreng., Ch. str. I (fumarprotocetraric acid or typical strain).
" " " " Ch. str. II (grayaninic acid or 'grayi'-strain).
" " " " Ch. str. III (cryptochlorophæic acid or 'cryptochlorophæa'-strain).
" " " " Ch. str. IV (merochlorophæic acid or 'merochlorophæa'-strain).
" " " " Ch. str. V (novochlorophæic acid strain).
" " " " Ch. str. VI (usnic acid strain).

The strains containing grayaninic, cryptochlorophæic and merochlorophæic acids had been previously designated as distinct species with the names *grayi*, *cryptochlorophœa* and *merochloroplœa* respectively.

The application of this method registers the chemical differences while keeping them outside the framework of formal taxonomy, and thus avoids burdening the latter with a mass of unnecessary epithets. It also enables the field-worker to determine his species on a morphological basis. Continued attention should, however, be paid to the constitution and distribution of the distinct chemical strains, for they have been found, at any rate in some of the lichens, to be significant for distribution, occurring in separate parts of the species' geographical area.

I. Mackenzie Lamb

National Herbarium,
National Museum of Canada,
Ottawa.
Feb. 1.

[1] Zopf, W., "Die Flechtenstoffe in chemischer, botanischer, pharmakologischer und technischer Beziehung" (Jena, 1907).
[2] Asahina, Y., *Acta Phytochim.*, **8**, 47 (1934).
[3] Asahina, Y., *Bot. Mag., Tokyo*, **51**, 759 (1937).
[4] Asahina, Y., *J. Jap. Bot.*, **12**, 516, 859 (1936); **13**, 529, 855 (1937); **14**, 39, 244, 318, 650, 767 (1938); **15**, 465 (1939); **16**, 185 (1940).
[5] Gibbs, R. D., *Trans. Roy. Soc. Canada*, Sect. 5, **39**, 71 (1945).
[6] Penfold, A. R., Morrison, F. R., and Smith-White, S., *J. and Proc. Roy. Soc. N.S. Wales*, **76**, 93 (1942).
[7] Thomas, E. A., *Ber. schweiz. bot. Ges.*, **45**, 191 (1936).

Permanent Preparations of Fungi Growing on Agar

The methods to be described here have been used in making preparations of predacious fungi from agar cultures, where it is important that hyphæ should be retained *in situ*; but they also appear to be generally applicable.

For making rapid preparations from living material, a modification of the chlorazol black method of Armitage[1] has been found satisfactory. From material growing in culture, agar blocks about 4 mm. square are cut out with a scalpel, and the surface of the agar bearing the fungus is carefully sliced off with a razor blade to a uniform thickness of about 200 μ. The treatment is then as follows: fix in absolute alcohol, 1 min.; stain in a saturated methyl alcohol solution of chlorazol black E, 1 sec.; dehydrate in absolute alcohol, 10 min.; mount in 'Euparal' in a cavity slide, and harden by gentle heat.