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Crystal gazing: How the early-19th-century discovery of lichen secondary metabolites influenced physiological and taxonomic inquiry

M. E. Mitchell

Abstract

The fungal components of most lichens synthesize carbon-based compounds that occur as crystals on the hyphal walls. In the 1840s some of those compounds were found to produce distinctive colors when treated with certain alkalis. The debate occasioned 20 years later when those reactions were recruited as taxonomic criteria is documented here. The enduring question of why lichens produce their characteristic compounds is also addressed.

Early in the 19th century several chemists focussed their attention on the constituents of two lichens then in everyday demand: *Cetraria islandica* (L.) Ach. (Fig. 1) and *Roccella tinctoria* DC. (Fig. 2). The first—known as “Iceland Moss” and its equivalent in mainland European vernaculars—was prescribed in the treatment of pulmonary complaints while the second—marketed as “Canary Weed”—was the source of orchil, a prized dyestuff. *Cetraria islandica* owed its repute to a high content of easily digested carbohydrate but was not marketable until soaked free of another, particularly bitter, compound. An investigation of the latter by Christoph Pfaff (1773–1852), professor of chemistry at the University of Kiel, convinced him that it was an undescribed acid, which, as the only one of its kind then known, he named simply “*acidum lichenicum*” (1826, p. 482).¹ Also in the 1820s Friedrich Heeren (1803–1885), professor of chemistry at Hanover Polytechnic, began to

study the constituents of *R. tinctoria*. Using ether as a solvent, Heeren (1830, p. 348) had obtained “very delicate, snow-white, silvery crystals that, under the microscope, appeared in the form of rectangular platelets”;² this description of what he named “roccellic acid” (“Roccellsäure [*sic*],” p. 315) appears to be the earliest reported microscopical observation of lichen crystals.³

Work on isolating further secondary metabolites slowly gathered pace with occasional mention being made of colors produced by treating lichen extracts with various alkalis, including potash (KOH; Schunck 1842, p. 497). John Stenhouse (1809–1880), lecturer in chemistry at St. Bartholemew’s Hospital, London, promoted the use of another reagent in his report on a study involving *R. tinctoria* (1848): with reference to a compound that he had isolated and named “orsellic [lecanoric] acid,” Stenhouse commented “[i]ts most characteristic reaction, by which its presence can be very reliably detected, is the deep blood-red colour it instantly strikes when it is brought in contact with a solution of hypochlorite of lime [Ca(ClO)₂]” (p. 65).

Five years later William Lindsay (1829–1880)—a fellow Scot then working as assistant physician at the Crichton Royal Institution in Dumfries—named that procedure “Stenhouse’s Test” (Anonymous 1853, p. 906). A further report by Lindsay (1854) on the dyeing properties of lichens included five detailed tables. The second of

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Figure 1. *Cetraria islandica*. (From Zorn 1779–1784, 2: pl. 138.)

these described the colors obtained when four lichen species were treated with an extensive list of alkalis, including KOH and $\text{Ca}(\text{ClO})_2$, and the third table listed 44 taxa “the Alcoholic Solution of which gives a red reaction with

Solution of Chloride of Lime” (p. 238). Lindsay subsequently emphasized that “the *red* or *purple* colouring matters ... are the result of chemical action on crystalline principles which were previously quite devoid of colour” (1855, p. 62).⁴



Figure 2. *Rocella tinctoria*. (From Wiesner 1900, 1:664.)

Most of the lichenologists active in the 1850s—a small band at best—would have felt, quite reasonably, that developments involving acids, crystals and reagents were no concern of theirs; they were principally occupied with the compilation of regional inventories and the description of new species. By the following decade, however, a chemical procedure had been introduced that revolutionized lichen systematics.

Chemical warfare

The Finnish lichenologist William Nylander (1822–1899) was the first to recognize the taxonomic potential of the colors produced by many thalli when treated with reagents. His declaration that “iodine-induced and other chemical reactions, having proved of the

utmost value and assistance, are indispensable to present-day lichenologists” (1866a, p. 181),⁵ was quickly followed by two papers developing that view. The first (1866b) stressed the significance of $\text{Ca}(\text{ClO})_2$ as an aid to species identification with particular reference to the genera *Parmelia* and *Rocella* while the second (1866c) concerned the use of KOH to segregate species of *Lecanora*.⁶

One of Nylander’s long-standing correspondents, the Anglican clergyman William Leighton (1805–1889), quickly endorsed the new tests whose “usefulness” he declared “is at once demonstrated and enhanced by the fact that the very smallest frustule is sufficient to determine the lichen submitted to them... [the reagents] are the hypochlorite of lime and the hydrate of potash; and the details of their reaction will

be found in [Nylander's 1866b and 1866c contributions to] the 'Flora' of May 12 and 13 [*recte* 31], 1866, and more at large in an elaborate paper in the Journal of the Linnaean [*sic*] Society of London" (1866a, pp. 169–170); the "elaborate" reference is to a translation by Leighton of those May 1866 papers (Nylander 1867). A draft of that translation was submitted to Nylander, who took the opportunity to remove the names of several species from his original lists and make other alterations, one of which was a refinement of his procedure for demonstrating thalline reactions. That adjustment resulted from his having observed that when a portion of a *R. tinctoria* thallus is rubbed between finger and thumb "the whitish powder thus obtained, liquified by a solution of hypochlorite of lime, instantaneously assumes a red colour. The same colour immediately appears if we apply this reactive to the surface of the lichen in question ... A glass rod (*agitateur* [stirrer]) dipped in the liquid, and applied to the thallus ... immediately exhibits the beautiful erythrinic action." The amended translation ends with Nylander concerned "to point out the invaluable aid afforded by chemical reactions in the study of lichens" (1867, p. 359).

Leighton had promptly availed himself of the "invaluable aid" by applying the KOH test to the many *Cladonia* specimens in his personal herbarium (1866b, p. 405).⁷ He then went on to examine material of the same genus in the herbarium of the Royal Botanic Gardens, Kew. There, using both reagents, he "accidentally discovered that if the hypochlorite of lime be immediately applied to specimens of *Cladoniae* already moistened with hydrate of potash, some very remarkable reactions are produced" (1867, p. 99). The importance of Nylander's color tests was also recognized by Ernst Stizenberger (1827–1895), a German physician and able lichenologist based in Constanz. In a lecture to the Swiss Natural History Society

in August 1868, Stizenberger described "the reactions recently brought into service as all the more valuable because being independent of substratum, climate and other conditions, they relate only to the species" (Anonymous 1868, p. 99).⁸

Not all, however, were equally enthused. Edward Tuckerman (1816–1886), North America's foremost lichenologist of the 19th century, moved quickly to determine the reagents' effect on a wide range of material: "I have gone through a large part of my North American and exotic Lichens in the light afforded by these experiments, and found the facts, if sometimes suggestive of more than is stated, generally clear; much clearer than the value attributed to them. Is it not indeed safe to say at once that Species are not determinable, in Botany, by such tests?" (1868, p. 105). Though Tuckerman continued to publish until the mid-1880s, he "never changed his opinion ... with regard to use of chemical tests in distinguishing species of lichens. He did not consider that species could be distinguished in that way" (Merrill 1906, p. 68).

This view was shared by William Lindsay, who, in a paper read at a meeting of the Linnean Society of London in November 1868, drew on his knowledge of lichen chemistry to question the validity of Leighton and Nylander's results. Lindsay's opening remarks were somewhat high-handed: "[d]uring the last few years several Lichenologists* of established reputation have introduced what they are pleased to call "new criteria" or new "chemical tests" in the study of Lichens—chemical characters, in short, for the differential diagnosis of *species*. They have done so, moreover, in language so sanguine and with assertions so strong, that, if their observations could be substantiated as facts, their generalizations could not fail to be of the utmost importance in systematic Lichenology" (1869, p. 36).⁹ In an attempt to show that

Leighton and Nylander's observations could not be substantiated, Lindsay reported at length (pp. 47–61) on what he termed their “pseudo-system” (p. 61). His judgement was “on the whole, *negative*, so far as concerns my ability to confirm the confident assertions of Nylander and Leighton anent the value of chemical reaction as an absolute or corroborative ‘character’ in botanical diagnosis” (p. 62); he concluded by advising his readers not to expect any early “assistance *that can be relied upon* from colour reaction in the determination of *species!*” (p. 63).

Jean Müller (1828–1896), professor of botany at the University of Geneva, was another dissenter. A distinguished flowering plant monographer, Müller had broadened his research interests by the 1860s to include lichen taxonomy, on which he also became an authority. When the Swedish botanist Theodor Fries (1832–1913) published the first volume of his *Lichenographia Scandinavica* (1871), Müller (1872) reviewed it in largely favorable terms. He took exception, however, to Fries' use of chemical tests, insisting (p. 88) that “no specific delimitation can be based on these alone.”¹⁰ Despite these objections from botanists of standing, the fact that color tests facilitated lichen identification ensured their gradual acceptance. As Krempelhuber (1867–1872, 3:35–36) observed, “in general, these reagents will be of the utmost service for the identification of problematic species (especially those with foliose or fruticose thalli), and it will be understood that instances of a well-known species reacting anomalously to the reagent often depends either on the specimen having been misidentified or... on flawed reagents having been used.”¹¹

Nylander ignored Müller and Tuckerman's criticisms but did respond to Lindsay's. In a characteristically acerbic letter to the editor of the *Journal of Botany*, Nylander (1869b) pointed out that “evidence is not to be denied,

and to anyone able to see... the reactions here in question are as evident as they are easy to produce... Dr. Lindsay speaks of the numerous cases he has observed of inconstancy in chemical character; but is he quite sure the Lichens he had to deal with are correctly named? Is it not probable, rather, that where he fancied he discovered variability in the reactions (the result, according to him, being sometimes positive and sometimes negative) he had to do with different species? He himself allows he is unacquainted with the common *Parmelia olivetorum!* *Ab uno disce omnes.*”

A wariness as to the reliability of color tests nevertheless prevailed during the 1870s when, it would appear, reagents were employed only by Arnold (1870), Fries (1871–1874), Leighton (1871, 1872, 1879), Nylander (1870) and Richard (1878). That meagre deployment of the new technique was commented on by Frank Schwartz (1867–1928) while working at the University of Graz. In the introduction to a concise account of lichen chemistry, Schwarz (1880, p. 249) wrote “it is quite understandable that lichenologists have so far responded so divergently to the efforts by Nylander, Leighton, Th. Fries and others to distinguish between certain lichen species on the basis of chemical reactions. Those authors restricted themselves essentially to the use of calcium hypochlorite and potassium hydroxide as reagents, and were satisfied with the red or yellow color these produced, or failed to produce; they paid no attention to the individual lichen acids responsible for those reactions.”¹²

Schwarz addressed that deficiency by supplying detailed procedures for the extraction of lichen compounds, all of which he regarded as “in fact, products excreted by the growing lichen. Indeed their occurrence in crystalline form on the outer surface of the hyphae supports this” (1880, p. 264)¹³; some examples of those products are illustrated

here in Figure 3. Apart from his contribution to lichen chemistry, Schwarz deserves to be remembered for having been the first to ask “what purpose do these lichen products serve? Do they perhaps have an antiseptic effect, which would explain lichens’ durability? In that regard, they may belong with derivatives of the benzol series, such as the well-known antiseptics phenol, cresol, salicylic acid and benzoic acid” (p. 265).¹⁴ Schwarz did not return to the subject of lichenology—he subsequently became professor of botany at the Forestry Academy in Eberswalde, northeast of Berlin—and no further attempt to assign a function to lichen crystals was made until 1890.

Why are they there?

Ewald Bachmann (1850–1937), a highschool teacher at Plauen, south of Jena, broadened Schwarz’ suggestion. Having published two papers on color reactions (1886, 1887), Bachmann was familiar with a range of thalline products and now believed he could assign them a function: “Arnold [the lichenologist Ferdinand Arnold (1828–1901)] has seen snails nibble at *Peltigera* thalli and also lichens epiphytic on beech. The relatively soft, acid-free thalli of most *Peltigera* species have no defense against hungry snails. Other lichens are, in contrast, well protected, especially by chemical means; these include ... crystalline substances, some of which, known for their intense bitterness or fiery taste ... can no doubt provide ... protection against snails” (1890, p. 17).¹⁵ Though Bachmann indicated that he had undertaken experimental work on the ingestion of lichen products by snails, he did not again discuss the topic in print.

The first published investigation of lichen herbivory was made by the Austrian botanist Hugo Zukal (1845–1900). A lecturer at the Viennese Institute for Female Teacher Training, Zukal (1895, p. 1318) placed snails in a flask containing pieces of what appear

to have been *Cetraria islandica* thalli as their only sustenance; these they refused to touch and so expired. Zukal found that when the lichen’s bitter constituent was removed with alcohol, snails readily consumed the result. He concluded, therefore, that crystalline deposits in lichens “must be seen as constituting a defense against herbivory” (p. 1320).¹⁶ Zukal’s conclusion was of particular interest to Wilhelm Zopf (1845–1909), professor of botany at Münster. Zopf had, for several years, logged the damage caused to thalli of 16 lichen species by browsing insects. In a report on those observations he noted that the lichens in question contain a total of 14 acids, none of which, he claimed, serve a protective function. Zopf also detailed an experiment he had carried out to establish whether other lichens may be toxic to snails. This involved recording their reaction when fed slices of raw potato, each coated with the crystals of one of seven lichen acids. Since all were consumed save that baited with vulpinic acid, Zopf dismissed “Zukal’s recent and strongly emphasized pronouncement that lichen acids are effective and extensive inhibitors of herbivory as completely wrong and consequently unsustainable” (1896, p. 604).¹⁷

Zopf’s pronouncement was, in turn, of more than passing interest to the erstwhile lichenologist Georg Stahl (1848–1919). He had come to prominence as the first to achieve the spore-to-spore synthesis of a lichen, but later—as professor of botany at the University of Jena—had worked mainly with angiosperms. Stahl’s interests included plants’ defense strategies against browsing by snails, though his observations on that subject (1888) did not then extend to lichens; a reading of Zopf’s paper led him, however, to remedy that omission. Stahl’s subsequent findings convinced him that Zopf was mistaken. When Stahl (1904, p. 365) placed specimens of the snail *Cepaea hortensis* (Müller) (as *Helix hortensis*) in contact with thallus

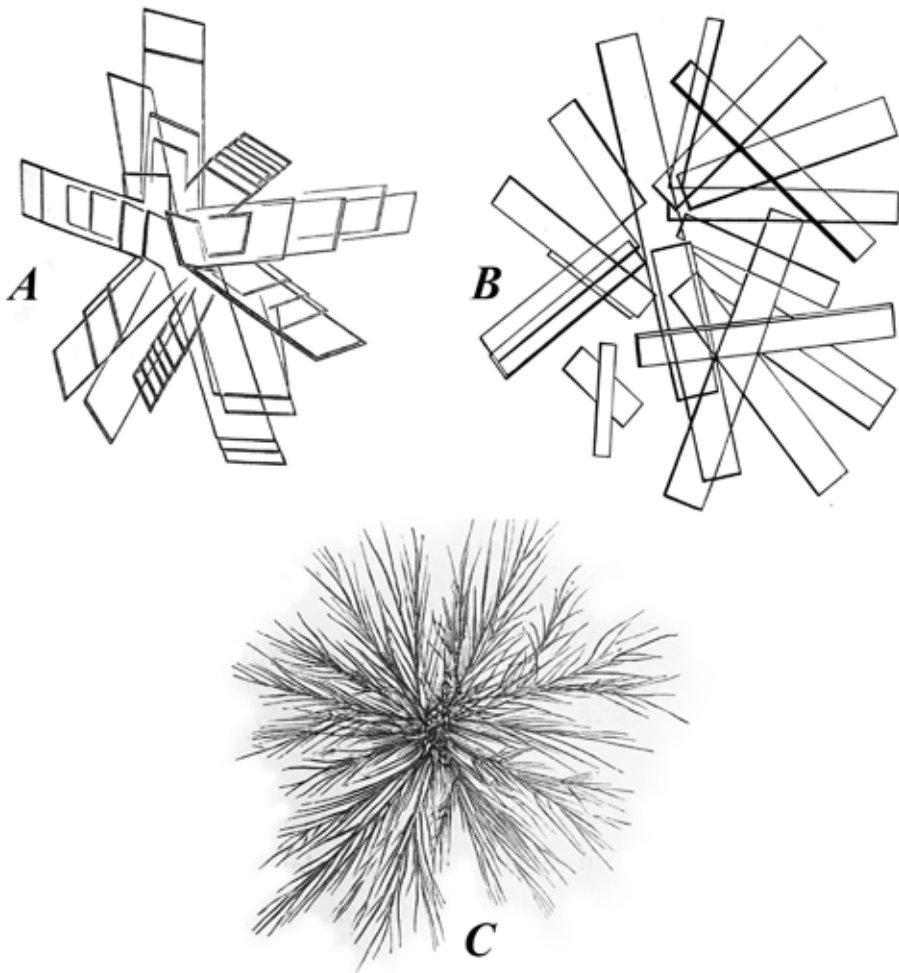


Figure 3. A. Roccellic acid. (From Zopf 1897, p. 264.) B. Strepsilin. (From Zopf 1903, p. 333.) C. Squamatic (as sphaerophoric) acid. (From Zopf 1905, p. 281.)

fragments—some washed in an aqueous solution of sodium carbonate, the others untreated—of *Evernia prunastri* (L.) Ach., *Flavoparmelia caperata* (L.) Hale (as *Imbricaria caperata*), *Icmadophila ericetorum* (L.) Zahlbr. (as *I. aeruginosum*), *Pseudevernia furfuracea* (L.) Zopf (as *Evernia furfuracea*) and *Rhizocarpon geographicum* (L.) DC., he found that the washed material was eaten while the rest went almost untouched. Stahl therefore concluded that “the lichens employed as fodder [in his investigation] contain substances soluble in

dilute soda solution that protect them against the named animals” (p. 365).¹⁸

Zopf made a brief return to the subject of chemical defense in his classic survey of lichen products (1907); having re-examined the evidence—without, however, devoting much attention to Stahl’s results—and, having found no reason to change his opinion, he concluded that “lichen acids, in general, lack the potential to deter herbivores” (p. 372).¹⁹ In the same work Zopf commented on the venerable perception of lichens as pioneer

colonizers of rock surfaces. That hypothesis visualized the hyphae of saxicolous species as actively penetrating and fragmenting their substrata, an image that had led a Victorian clergyman to style such species the “humble sappers and miners of the vegetable kingdom” (Macmillan 1861, p. 87). Zopf, however, aired the possibility that erosion is effected by secondary products and that—because such products were then believed insoluble in water—some of these could be cleaved by alkalis into soluble and insoluble units through a naturally occurring process. Zopf therefore considered it “very possible that such cleavage can occur through the action of atmospheric and soil ammonia, and that the liberated compound serves to decompose mineral constituents” (1907, p. 341).²⁰ Neither he nor any of his contemporaries pursued that possibility, and almost 50 years elapsed before lichens again came to be investigated as agents of chemical erosion.

Other functions were attributed to lichen products in the two decades following Zopf’s suggestion: Senft (1913, p. 60) surmised that cortical crystals shield photobionts from high light intensities, and Goebel (1926, p. 160) ascribed a water-repellant role to medullary crystals. These speculations also failed to attract attention at the time—indeed no further work involving lichen crystals appears to have been undertaken in the 20 years up to and including World War II.

Multi-purpose molecules?

The post-war resumption of lichen research included several Russian studies devoted to rock weathering. In a commentary on the corresponding publications, Jacks (1953, p. 304) cited one author’s conclusion that “acid excretions from the [lichen] hyphae had eaten into the solid rock.” This judgement was quickly challenged by Schatz et al. (1954). These authors insisted that “[t]he

chemical breakdown of minerals is not attributable to acidity” because “ordinary acid production by rock lichens appears to be inconsequential. But lichens contain unusually large amounts of a wide variety of complex organic compounds ... Many of these are powerful chelators by means of which lichens weather rock material” (p. 48).²¹ Despite the confident tone of that declaration, it had little impact because of the then prevailing belief that secondary metabolites were insoluble in water. Some years later, however, Schatz (1963) reported that certain of those metabolites were in fact slightly soluble, and he provided “results [to] show that low solubility ... does not prevent these substances from actively weathering rocks and minerals via chelation”; he also claimed that since the metabolites “are extracellular constituents which crystalize out on hyphae ... they can act directly on rock substrates” (p. 116). Schatz further believed that “[t]he nutritional poverty of plain rock surfaces makes production of such extracellular substances in large quantities an anabolic luxury that saxicolous lichens can ill afford unless the acids are of some use to them. The most logical, indeed the only function which can be attributed to lichen acids is that of supplying the symbionts with needed minerals. Since these have literally to be extracted from rocks, saxicolous lichens must be efficient weathering agents, and lichen acids must be the means by which they accomplish this action” (p. 117). The German lichenologist Gerhard Follmann (1930–) was not convinced by that sweeping statement. He suggested that secondary metabolites have an allelopathic effect on bryophytes and pteridophytes (Follmann 1964) and also raised the possibility of their facilitating nutrient exchange between the symbionts (Follmann and Villagrán 1965).

There was now no shortage of explanations on offer for the occurrence of lichen crystals, and Follmann decided it was time for an assessment of their relevance. At a colloquium

organized by the Société Botanique de France in November 1967, he expressed the opinion that just four of the current “Hypothesen” (1969, p. 162) could be considered plausible: chelation, reduction of light intensity and his own two proposals. For Culberson (1969, p. 24), however, the apparent potential of cortical crystals to shield photobionts of some species from high irradiation “probably cannot be called upon to explain the known production of characteristic lichen compounds in hundreds of species from a wide variety of habitats.” That scepticism was more forcefully put by Henssen and Jahns (1973, p. 179): “though lichen substances have been studied for more than one hundred years, and though thalli contain substantial amounts, nothing is actually known of their function.”²²

Follmann’s survey was succeeded by many others in reports published over the following half century, e.g., Rundel (1978), Lawrey (1986, 2009), Fahselt (1994), Rikkinen (1995), Huneck (1999), Gauslaa (2009), Molnár and Farkas (2010), and Calcott et al. (2018). Many of the results detailed in those publications relate to laboratory studies—the extent to which inferences drawn from such studies actually apply in nature has yet to be definitively established. At present, the only function that lichen crystals can with certainty be said to serve is that expediently assigned them by taxonomists.

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Notes

1. Similar compounds discovered over the following 150 years were routinely described as “lichen acids” despite the fact that not all are acidic; the now general use of the substitute term “secondary metabolite” has attended to that anomaly.
2. “schneeweiss, in äußerst zarten, silberglänzenden, unter dem Mikroskop in Gestalt rechtwinkelig vierseitiger Täfelchen erscheinenden, Krystallen.”
3. The diversity of crystals that Meier (1774, p. xxi) saw under his microscope in the course of a study involving *Cetraria islandica* did not come from the lichen. Maier had begun by carbonizing the material and had then infused, filtered and evaporated the residue. He did not specify the medium used for the infusion, but it was, evidently, the source of his crystals.
4. Those “principles” were further described by the Swiss botanist Simon Schwendener (1829–1919) in a study of lichen anatomy published while he was attached to the University of Munich. In connection with *Alectoria ochroleuca* (Hoffm.) Massal. (as *Bryopogon ochroleucos*), Schwendener noted (1860, p. 148) that “small granules of a yellowish pigment [usnic acid] ... are deposited among the cortical filaments” (“Zwischen die Rindfasern sind kleine Körnchen eines gelblichen ... Farbstoffes eingelagert.”) The illustration—not particularly successful—of that observation in his pl. 5, fig. 3 is the first published depiction of lichen crystals. In the caption to that figure (p. 182), Schwendener noted “[i]n thicker sections the yellow granules likewise form large or small clusters; in thin sections such as that shown, they sometimes surround the individual filaments that produce them” (“Die gelben Körperchen bilden auf dickern Schnitten ebenfalls grössere oder kleinere Anhäufungen; auf dünneren, wie der gezeichnete, umgeben sie stellenweise die einzelnen Fasern, deren Producte sie sind.”)
5. “reactiones chemicas, iodicas et alias, probatur summi esse ponderis adminiculaque practica sistere lichenologis hodiernis maxime necessaria.”
6. Nylander quickly extended the use of KOH to the genera *Lecidea* and *Cladonia*, saying “we can now safely identify young or sterile and imperfect specimens, which previously could at best remain doubtful” (“nunc tute distinguere etiam valemus specimina juvenilia aut sterilia et imperfecta, ubi antea quidem optima incerta manere potuerunt” (1866d, p. 421). Vitikainen (2000, p. 354) has fittingly emphasized that “[a]mong Nylander’s indisputable achievements is the fact that he observed the chemical differences of lichens and put those differences on an equal footing with morphological and anatomical characteristics.”
7. In the introduction to that paper, Leighton wrote “[w]here the reactive produces a yellow colour, it may be indicated by this sign, K+; where no

reaction takes place, or only a slight fuscescence, thus, K–.” When Nylander first had occasion to use the K+ symbol in print (1869a, p. 314), he added a footnote stating, “I thus described the reaction produced by potassium hydroxide in a letter, some time ago, to Revd Leighton, and where there was no reaction of note, I wrote K–. The Revd author has used those symbols in his work, but without attributing them to me” (“Sic olim in litt. revdo Leighton indicavi reactionem ope hydratis kalici effectam, et ubi nulla obvia reactio scripsi K–. Signis his rev. auctor in scriptis suis usus est, sed ea mihi attribueri neglexit.”) Nobody was going to steal Nylander’s thunder, however muted!

8. “Die erst in neuerer Zeit in Anwendung gekommenen Reactionen haben übrigens einen um so höhern Werth, als es sich erweist, dass sie von Boden, Klima- und sonstigen Verhältnissen unabhängig und nur an die Species gebunden sind.”
9. The asterisk in that quotation relates to a footnote in which the strength of Lindsay’s opposition to the use of reagents for taxonomic purposes caused him to remark, rather uncivilly, that Leighton and Nylander’s “views appear to be supported by Dr. Stenhouse, who wrote me in February 1867, ‘I quite agree that much light may yet be thrown on the *botany* of lichens by means of *chemical* reaction.’ I cannot, however, accept the testimony of a *chemist* on a question of *botanical* diagnosis.”
10. “auf sie allein keine spezifische Differenzirung zulässig sei.” In addition to rejecting color tests, Müller dismissed the contemporaneous discovery that lichens are composite organisms—as did Lindsay and Tuckerman.
11. “Im Allgemeinen wird man sich jener Reagentien zur Erkennung zweifelhafter Flechten-Exemplare (besonders solcher mit blatt- oder strauchartigem Thallus) mit dem allergrössten Nutzen bedienen und bald die Ueberzeugung gewinnen, dass das Vorkommen jener Fälle, wo ein unregelmässiges oder unbeständiges Verhalten der untersuchten Exemplare einer gut bekannten Spezies gegen das angewendete Reagens beobachtet wurde, zum öftern darauf beruht, dass entweder die betreffenden Exemplare unrichtig bestimmt waren, oder dass ... unkräftige Reagentien bei den betreffenden Untersuchungen angewendet worden sind.”
12. “Wenn die Versuche Nylander’s, Leighton’s, Th. Fries’ und Anderer, zum Erkennen gewisser Flechtenspecies chemische Reactionen heranzuziehen, bei den Lichenologen bisher nur eine sehr getheilte Anerkennung gefunden haben, so erscheint dies nicht ganz ungerechtfertigt. Jene Autoren beschränkten sich im wesentlichen auf zwei Reagentien, nämlich Chlorkalklösung und Kalilauge, und sie liessen sich an dem Roth- oder Gelbwerden der Flechten, resp. an dem Nichteintreten dieser Reactionen genügen, ohne auf die Ursache derselben—die einzelnen Flechtensäuren—Rücksicht zu nehmen.” In a discussion of chemical tests, Hawksworth (1976, p. 141) claimed that Fries had “questioned their value” in his *Lichenes Spitsbergenses* (1867) though, in fact, he made liberal use of tests in that work, e.g., when treating *Cladonia*, *Lecanora*, *Lecidea* and *Parmelia* taxa.
13. “Es sind eben Ausscheidungsprodukte beim Wachstum der Flechte. Schon das Auftreten in krystallinischer Form an der Aussenseite der Hyphen spricht dafür.”
14. “Eine Frage wäre es noch, welchen Zweck die Flechtensäuren erfüllen. Wirken sie vielleicht antiseptisch und wäre so vielleicht die lange Lebensdauer der Flechten zu erklären? Wären sie vielleicht in dieser Beziehung den zahlreichen Derivaten der Benzolreihe anzuschliessen, die wie Phenol, Cressol, Salicylsäure, Benzoesäure eminent fäulniswidrig wirken?”
15. “Schnecken sind von Arnold an Peltigera und auf Buchen an Rindenflechten fressend beobachtet worden. Die meisten Arten der Gattung Peltigera mit ihrem verhältnissmässig zarten, flechtensäurefreien Thallus besitzen keinerlei Schutzmittel, durch welche hungrige Schnecken zurückgeschreckt werden könnten. Andere Flechten dagegen sind um so besser geschützt, besonders durch chemische Schutzmittel. Unter diesen [sind] ... krystallinische Flechtenstoffe, von denen sich bekanntlich einzelne durch intensive Bitterkeit oder brennenden Geschmack ... sind wahrscheinlich ... im Stande, Schutz vor Schnecken zu gewähren.”
16. “dass sie also als Schutzmittel wider den Thierfrass aufgefasst werden müssen.”
17. “dass die neuerdings mit sehr starker Betonung vorgetragene Ansicht Zukal’s, die Flechtensäuren hätten die biologische Bedeutung eines wirksamen Schutzmittels gegen Tierfraß in solch weiter Fassung gänzlich unzutreffend und darum unhaltbar ist.”
18. “daß die zur Fütterung benutzten Flechten durch gewisse, in verdünnter Sodalösung lösliche Körper gegen die genannten Tiere geschützt sind.” Hartmann (2008, pp. 4543–4545) has provided a detailed survey of Stahl’s contributions to the study of herbivory.
19. “Die Flechtensäuren sind daher im allgemeinen nicht imstande, Schutzmittel gegen Tierfraß abzugeben.”

20. “Es ist daher sehr wohl möglich, daß diese Spaltung in der freien Natur unter dem Einfluß des Ammoniaks der Atmosphäre und des Bodens erfolgt, und daß die so frei gewordene Fumarsäure zur Aufschließung mineralischer Bestandteile dient.”
21. Chelators are compounds that can decompose others by combining with certain of their elements. The term “chelation—a derivative of “chelate” (from *L. chela*, a claw) long applied by zoologists to crustaceans’ pincers—was introduced by Morgan and Drew (1920, p. 1457, fn.)
22. “Obwohl man die Flechtensubstanzen über ein Jahrhundert studiert hat und obwohl sie in bemerkenswerter Menge im Flechtenthallus vorkommen, ist eigentlich nichts Sicheres darüber bekannt, welche Rolle sie für die Flechten spielen.”

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