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THERMALLY INDUCED CHEMICAL ARTIFACTS IN LICHENS

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Key Word Index—*Hypotrachyna*; Parmeliaceae; lichens; thermal decomposition; chemical evolution; depsides; anziaic acid; atranorin.

Abstract—Unsuspected thermal degradation of secondary products in lichens prior to chemical study, although probably uncommon, can lead to mistaken conclusions and taxonomic error. Thermally induced artifacts were found in the chemistries of *Hypotrachyna partita* from a site of volcanic activity in Costa Rica and in the type of *H. prolongata* from Haiti that had been dried for the herbarium with heat. In the Southern Appalachian Mountains normal material of *H. prolongata* has been considered a chemically different species. Thermal stability of secondary products may be an important factor in the chemical evolution of lichens inhabiting extreme environments.

INTRODUCTION

Like many flavonoids of higher plants, the secondary products of lichens are sufficiently stable under normal field and herbarium conditions to allow even very old museum specimens to be used in chemotaxonomic research [1]. A few lichen compounds, however, are known to undergo partial decomposition in the herbarium. The red coloration assumed by some herbarium specimens containing salazinic or norstictic acids doubtless results from the partial decomposition of these compounds. Thamnolic acid is so easily decarboxylated that the traces of decarboxythamnolic acid accompanying thamnolic acid and detected by TLC are generally regarded as artifacts rather than products of an enzyme-mediated reaction. Benzyl esters are so easily hydrolyzed that small concentrations of unesterified benzyl alcohols found with esters such as fumarprotocetraric, physodalic, galbinic, barbatolic, and alectorialic acids could also represent artifacts of storage, extraction, or analytical procedures. But these well-known examples currently give no problems in chemotaxonomic studies. The present report deals with specimens of two species that normally produce a typical orcinol-type depside, anziaic acid, as the principal medullary constituent but in which we found thermal degradation products resulting from volcanic activity near the collection site in one instance and from heat applied during the pressing of the specimens for the herbarum in the second. As chemistry has become increasingly important for systematics, it is essential to understand chemical artifacts and their origins, including those that might arise prior to any chemical analysis of the plant material. The present paper gives the first report of thermal decomposition of natural products in lichen thalli and of an erroneous taxonomy based upon an altered chemistry.

RESULTS AND DISCUSSION

Re-examination of apparently normal material of *Hypotrachyna partita* Hale from Volcán Irazú (Costa Rica), collected two years after the last eruption of that volcano, showed compounds not identical to any known naturally occurring lichen products. This collection had been previously reported to be chemically aberrant in showing a trace of perlatolic acid in addition to atranorin and anziaic acid, the normal constituents of the species [2]. When the sample was analyzed by a standardized two-dimensional TLC method [3], ten distinct compounds separated (Fig. 1). In addition to atranorin (**1**), anziaic acid (**3**), and a trace of perlatolic acid (**4**), the collection contained substantial amounts of anziol (**5**, a new depside proven here to be decarboxylated anziaic acid), olivetolcarboxylic acid (**7**), and olivetol (**9**) in addition to traces of 4-*O*-methylolivetolcarboxylic acid (**8**) and

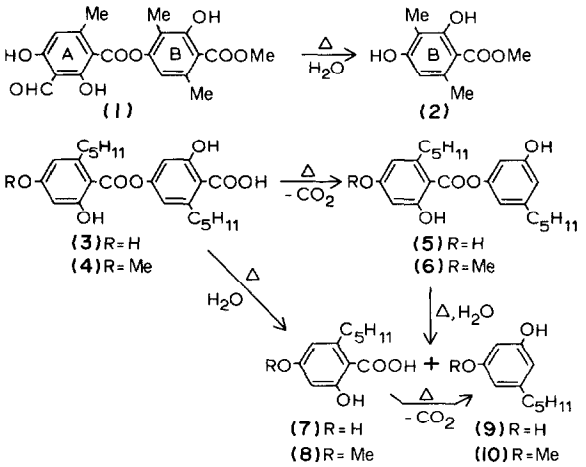

Fig. 1. Products detected by TLC after thermal decomposition of atranorin (**1**), anziaic acid (**3**), and perlatolic acid (**4**) in lichen thalli. The A ring of atranorin was not found, and decarboxylated perlatolic acid (**6**) was tentatively identified in low concentration.

O-methylolivetol (**10**). None of these newly identified compounds are of known natural occurrence in lichens, and all could form by hydrolysis and/or decarboxylation of anziaic and perlatolic acids.

That heat alone can account for all of the unusual compounds found in high concentration in *H. partita* from Irazú is shown by the experimentally induced thermal decomposition of anziaic acid in a related species, *H. rachista* (Hale) Hale. In a specimen of *H. rachista* heated at 85° for 16.5 hr anziaic acid (**3**) was completely degraded to anziol (**5**), olivetol (**9**), and a small amount of olivetolcarboxylic acid (**7**). A pure sample of anziol was separated by TLC, and acid hydrolysis of this product yielded nearly equal amounts of olivetolcarboxylic acid (**7**) and olivetol (**9**). Therefore, anziol must be the decarboxylation product of anziaic acid. Table 1 gives representative R_f values for the compounds chromatographed by the standardized TLC method.

The secondary products in thalli of *H. rachista* heated in an oven for increasing periods of time at 43°, 85°, and 105° showed varying degrees of decomposition (Table 2). At 43° no decomposition products were detected after 18 hr. Both atranorin (**1**) and anziaic acid (**3**) may have been slightly decomposed after 45 hr, however, as judged by trace spots with the correct R_f values for anziol (**5**, from anziaic acid) and methyl β-orcinolcarboxylate (**2**, the B ring of atranorin). Atranorin and anziaic acid were extensively degraded after 22 hr at 85° and after only 15 min at 105°. On the basis of spot sizes, the relative concentrations of anziaic acid, anziol, olivetolcarboxylic acid, and olivetol in the fragment heated at 105° for 15 min were remarkably similar to those of the Irazú speciments of *H. partita*.

The sample of *H. partita* from Irazú did not show methyl β-orcinolcarboxylate expected from the thermal degradation of atranorin. Atranorin accumulates in the upper cortex, and its low-melting degradation products could have been lost by slow volatilization over a long period of time. A high R_f substance may be decarboxylated perlatolic acid, but the identification could not be confirmed because the compound was in low concentration and poorly resolved from other trace constituents. One-dimensional chromatographic comparisons of fragments from different parts of the thallus showed that a few small lobules contained relatively more anziaic acid than the mature lobes, and one showed none of the decomposition products. Lobules with the normal chemistry of the species probably grew during the two-year period between the end of the eruptive cycle (1963–65) of Irazú and the time of the collection (1967).

Hypotrachyna rachista was recently described [4] to accommodate an epiphyte abundant at the highest elevations of the Southern Appalachian Mountains. By producing anziaic acid it was considered to be distinct

Table 1. Representative R_f data for the depsides and thermal degradation products detected in *Hypotrachyna*.

	R_f Classes			R_f Values		
	A	B	C	A	B	C
Anziaic acid (**3**)	3–4	5–6	5	40/41, 79	55/29, 74	35/28, 85
Anziol (**5**)	5	5–6	4	50/41, 79	46/28, 73	29/28, 85
O-Methylolivetol (**10**)	5–6	6	5–6	65/42, 80	62/29, 70	59/28, 85
4-*O*-Methylolivetolcarboxylic acid (**8**)	5	7–8	6	59/41, 80	75/30, 74	65/28, 83
Methyl β-orcinolcarboxylate (**2**)	6	6	5	63/41, 79	62/29, 74	53/28, 85
Olivetol (**9**)	4	5	3	43/41, 79	43/30, 74	21/28, 85
Olivetolcarboxylic acid (**7**)	4	5–6	5	43/42, 79	53/30, 74	35/29, 86
Perlatolic acid (**4**)	5	7–8	5–6	46/42, 80	72/30, 74	64/29, 87

R_f values following the solidus (/) refer to the control substances norstictic acid and atranorin respectively. Solvent A (toluene–dioxane–HOAc 36:9:1; solvent B (hexane–Et_2O–HCO_2H 12:9:2; solvent C (toluene–HOAc 20:3).

Table 2. Major (M) and minor (m) compounds in fragments of *Hypotrachyna rachista* heated at three temperatures for various times

Temperature	Time (hr)	Anziaic acid	Anziol	Olivetol–carboxylic acid	Olivetol	Atranorin	Methyl β-orcinol-carboxylate
43°	18	M	—	—	—	M	—
	45	M	?	—	—	M	?
85°	1	M	—	—	—	M	—
	4	M	?	?	?	M	—
	22	m	M	M	M	M	m
105°	0.25	m	M	m	M	M	m
	1	?	M	m	M	—	m
	2.5	?	m	m	M	—	—
	3	—	m	?	M	—	—
	16	—	?	?	M	—	—

from *H. prolongata* (Kurok.) Hale, known only from the type collection from Haiti and containing an unidentified substance [2]. We tested several specimens, including the holotype, of *H. prolongata*. One-dimensional TLC analysis of all specimens showed atranorin and anziol as the major constituents with traces of olivetolcarboxylic acid and olivetol. The ratio of the concentrations of anziol to anziaic acid, present only in traces, is much higher in these specimens than in *H. partita* from Irazú. A two-dimensional chromatogram of *H. prolongata* showed several trace constituents that may have included methyl β-orcinolcarboxylate, but the concentrations were too small to permit further study. No perlatolic acid or 4-*O*-methylolivetolcarboxylic acid could be detected.

The type locality of *H. prolongata* is non-volcanic and had apparently not been affected by fire before the original material was collected. The specimens, however, were processed in a plant dryer with artificial heat for an unknown period of time (Imshaug and Wetmore, personal communication). The upper surface of the specimens shows the pale pinkish discoloration noted by Kurokawa in his original description [5]. An acetone-soluble pink pigment, considered to be an additional chemical character separating *H. prolongata* from *H. rachista* [4], formed readily when we dried normal specimens of *H. rachista* with high heat. Specimens of *H. rachista*, hydrated and put in a press in the dryer used to process flowering-plant specimens in the herbarium of Duke University, were altered to the chemistry of *H. prolongata*. The correct name for the widespread epiphyte of the Southern Appalachians studied here is consequently the older one, *H. prolongata*, and *H. rachista* is its synonym. It should be clear from this report that lichens should not be subjected to the temperatures routinely used in drying vascular plants for the herbarium. (At Duke University, plant presses of lichens are dried at room temperature with an electric fan.)

Many depsides and depsidones are rapidly hydrolyzed under mild conditions. Alkaline hydrolysis is even so rapid that it forms the basis of a thallus spot-test (the lichenologist's so-called "KC test") useful for some groups of compounds. Most depsides and depsidones are also thermally labile, a property that makes them difficult to analyze by GLC and MS. At the same time these compounds are noteworthy for their stability under all normal field conditions. It is significant that the secondary products of lichens are produced during the short periods of hydration and of high metabolic activity but reside in the thallus for the entire lifetime of the lichen, which may be many years. Undoubtedly some attrition occurs by chelation and leaching, and a certain degree of mobility in the thallus is suspected for some compounds [6]. Nevertheless, decomposition products of the major secondary compounds of lichens are rarely encountered, and the qualitative secondary-product chemistries of older (but not moribund) and younger parts are usually identical.

Thermal stability of the lichen's secondary products may be significant in the chemical evolution of these organisms. Thermal stability can be related to melting points for homologous series of simple orcinol-type compounds because decarboxylation accompanies melting in these compounds. A graph of melting points for the two homologous series with reduced side-chains that include perlatolic and anziaic acids gives a nearly straight-line relationship to total side-chain length. Extrapolated melting points of the (unknown) depsides that would combine two seven-carbon side chains (the next biogenetic step in side-chain length) fall from 75° to 95°. This approximates the temperature range in which desert lichens show the first evidence of thermal damage after 30 min [7]. Anziaic acid is not a common lichen substance, and most species producing it inhabit cool environments in high mountains. The 4-*O*-methylated derivative of anziaic acid is the common lichen depside perlatolic acid, and it may be significant that 4-*O*-methylated derivatives decompose more slowly (even though their melting points are slightly lower). The colorful lichen species typically seen on rocks in deserts—such as yellow *Acarosporae* and orange *Caloplacae*—have thermally stable pigments. Of all compounds commonly found in the upper cortex, the only thermally labile depside is atranorin, a substance accumulated by few desert lichens.

EXPERIMENTAL

Chromatograms were prepared by standardized one-dimensional [8,9] and two-dimensional [3] TLC methods previously described.

Phenolic constituents of Hypotrachyna partita *from Volcán Irazú, Costa Rica*. Lichen was collected in March 1967 from *Quercus* sp. on the middle slopes (*ca* 2800 m) of Volcán Irazú above Cartago, Costa Rica (*Culberson* 12,395, DUKE). Thalli were heavily infiltrated with ash from the last eruption of the volcano 2 yr earlier. A fragment of the specimen was extracted first with toluene and then with warm Me_2CO. 1-D chromatograms were difficult to interpret because the components were poorly resolved, and some changed relative R_f values in the 3 solvent systems of the standardized method. Three 2-D chromatograms were prepared using (1) solvent C in the first direction and solvent B in the second direction, (2) solvent C in the first direction and solvent A in the second direction, and (3) solvent A in the first direction and solvent C in the second direction. The chemistry of *H. partita* from Irazú was compared to that of four specimens of the species from Venezuela. Three (*Hale 42,144, 42,646B, 43,167*, US) contained only atranorin and anziaic acid. One (*Loveless 1599*, DUKE) showed additional trace spots possibly resulting from small amounts of anziol, olivetol, and olivetolcarboxylic acid.

Phenolic constituents of Hypotrachyna prolongata. All of the specimens (*Imshaug 23210*, MSC, US; *Wetmore 3233*, holotype, MSC; *Wetmore 3286*, MSC) of *H. prolongata* were collected on 27 July 1958 near (*ca* 2100 m) the summit of Morne Macaya, Massif de la Hotte, Département du Sud, Haiti. They were analyzed by the standardized 1-D TLC method. One (*Imshaug 23210*, US) was also analyzed by the 2-D method using solvent A for the first direction and solvent C for the second.

Thermal decomposition products experimentally induced in Hypotrachyna rachista. The material of *H. rachista* (*Dey 2480*, DUKE) was collected in 1972 from *Abies* on Clingmans Dome (*ca* 1980 m) near the boundary between North Carolina and Tennessee. A fragment was heated at 85° for 16.5 hr, and part (37 mg) of it was extracted several times with Et_2O. The extract was washed with H_2O, conc at room temp, and diluted with Me_2CO. Most of the extract was streaked onto a TLC plate and compacted to a narrow band by developing the plate in Me_2CO to a height of 4 mm from the origin. The chromatogram was then developed in solvent C to a height of 13.3 cm. The band of the unknown substance, subsequently identified as decarboxylated anziaic acid and named here as anziol, was located under UV light (254 nm), removed to a small sintered-glass funnel, and washed 4 times with small volumes of Me_2CO. Approximately half the residue from the evaporation of the Me_2CO filtrate was dissolved in 6 drops of conc H_2SO_4, transferred to a

stoppered vial, and treated by the usual procedure for the acid hydrolysis of lichen depsides [8]. Standardized 1-D TLC plates were prepared to compare (1) an extract of unheated *H. rachista*, (2) the extract of the heated sample of *H. rachista*, (3) unhydrolyzed anziol separated by TLC as described above, (4) acid-hydrolyzed anziol, and (5) acid-hydrolyzed perlatolic acid. Fragments of a specimen of *H. rachista* (*Dey 5910*, DUKE) collected in 1973 from *Abies* on Celo Knob, Yancey County, North Carolina, were heated at different temperatures (43°, 85°, and 105°) for increasing time intervals. Acetone extracts of the fragments were compared chromatographically.

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