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HYDROCARBON CONSTITUENTS OF THREE SPECIES OF NORWEGIAN LICHEN: *CETRARIA NIVALIS, C. CRISPA, SIPHULA CERATITES*

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Key Word Index—*Cetraria nivalis*; *C. crispa*; *Siphula ceratites*; Lichens; *n*-alkanes; anteiso-alkanes; methyl heptadecanes.

The use of hydrocarbon composition in taxonomy is an attractive possibility. The ubiquity of occurrence of hydrocarbons in organisms, their biosynthetic status as secondary metabolites and their ease of analysis recommend them as taxonomic criteria.[1] Furthermore, an analytical scheme based on solvent extraction and chromatographic analyses culminating in combined GC–MS allows for a rapid accumulation of data which may be systematised by computer classification techniques.[2] Past work has in general suggested caution in treating hydrocarbon distributions as being species specific.[1,3] While Eglinton *et al.*[4] found an encouraging constancy of wax hydrocarbon distribution for a particular species with variation in place of growth, season, age and part of plant, work by Martin-Smith *et al.*[5] and Weete *et al.*[6] has cast some doubt on species specificity as a generalization for plant hydrocarbons.

Lichens are organisms of particular interest in that they are able to populate relatively hostile environments.[7] Although they represent the symbiosis of fungi and algae, lichens have been found to contain chemical components not produced by either symbiont in isolation.[8] Analysis of a single lichen by Stransky *et al.*[9] represents the only reference in the literature to the hydrocarbons of lichens, though the aliphatic hydrocarbons of both algae[10,11] and fungi[12] have been more extensively studied.

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[1] EGLINTON, G. and HAMILTON, R. J. (1963) in *Chemical Plant Taxonomy* (SWAIN, T., ed.), Academic Press, London.

[2] SMITH, D. H. and EGLINTON, G. (1972) *Nature* **235**, 325; SMITH, D. H., GRAY, N. A. B., PILLINGER, C. T. KIMBLE, B. J. and EGLINTON, G. (1971) in *Advances in Organic Geochemistry* (VON GAERTNER, H. R. and WEHNER, H., eds.), Pergamon Press, Oxford; SMITH, D. H. (1972) *Anal. Chem.* **44**, 536.

[3] DOUGLAS, A. G. and EGLINTON, G. (1966) in *Comparative Phytochemistry* (SWAIN, T., ed.), Academic Press, London.

[4] EGLINTON, G., HAMILTON, R. J., RAPHAEL, R. A. and GONZALEZ, A. G. (1962) *Nature* **193**, 839.

[5] MARTIN-SMITH, M., SUBRAMANIAN, G. and CONNOR, H. E. (1967) *Phytochemistry* **6**, 559.

[6] WEETE, J. D., RIVERS, W. G. and WEBER, D. J. (1970) *Phytochemistry* **9**, 2041.

[7] HALE, M. E. (1967) *The Biology of Lichens*, Arnold, London.

[8] CULBERSON, C. F. (1969) *Chemical and Botanical Guide to Lichen Products*, University of North Carolina Press, Chapel Hill.

[9] STRANSKI, K., STREIBL, M. and HEROUT, V. (1967) *Coll. Czech. Chem. Commun.* **32**, 3213.

[10] GELPI, E., SCHNEIDER, H., MANN, J. and ORO, J. (1970) *Phytochemistry* **9**, 603.

[11] ORO, J., TORNABENE, T. G., NOONER, D. W. and GELPI, E. (1967) *J. Bacteriol.* **93**, 1811.

[12] WEETE, J. D. (1972) *Phytochemistry* **11**, 1201.

The present work involves the characterization of the hydrocarbon components of three species of lichen. Two collections of each lichen were made, several years apart but at the same locations. In the second collection, every precaution was taken to eliminate contamination, both by other species and during the analytical procedure. Except in the second sample of *S. ceratites*, the hydrocarbon fractions consisted almost exclusively of alkanes. Total hydrocarbons extracted (Table 1) for the second set represented a far lower proportion of plant dry weight than with the first set. The proportion of total alkanes in higher plants varies greatly between species, rising to greater than 50% in flower petal waxes.[1]

TABLE 1. ALKANE CONSTITUENTS OF *Cetraria nivalis*, *C. crispa* AND *Siphula ceratites*

Species	*C. nivalis*		*C. cripsa*		*S. ceratites*	
Sample collection	(1)	(2)	(1)	(2)	(1)	(2)
Total alkanes: ppm dry wt lichen material	300	24	200	75	300	6
Normal alkanes ($C_n H_{2n+2}$) distribution*						
$n = 17$					1	2
18					1	
19		1			2	2
20		1			2	2
21		2	1		3	6
22		1	2		4	8
23	3	2	1	1	10	19
24	1	2	2		5	12
25	5	4	4	1	9	24
26	1	1	2		5	12
27	10	14	14	5	12	11
28	1	4	2	1	3	1
29	31	64	30	39	16	2
30	3	1	2	1	2	
31	33	2	31	43	16	
32	2	2	1	1	1	
33	10		8	6	10	
Carbon preference index†	11·5	7·4	6·3	31·7	3·3	1·8
Branched alkanes:						
total anteiso-alkanes* (C_{22}–C_{28})						12
methyl heptadecanes*	tr		tr		2	9

* Expressed as percentage (where this exceeds 1%) of total normal alkanes.

† Carbon preference index = total abundance of odd carbon number n alkanes (C_nH_{2n+2})/total abundance of even carbon number n-alkanes (C_nH_{2n+2}).

Generally, the correspondence observed between the alkane distributions in the two sets of samples is poor (Table 1). Each distribution shows the odd:even carbon number predominance that is characteristic of biogenic alkanes,[1] though this ratio is much higher for the two *Cetraria* species than for *S. ceratites*. The alkane fractions from both *S. ceratites* samples include a component whose mass spectrum indicates it to be an equimolar mixture of 7- and 8-methyl heptadecanes.[13] This component may probably be attributed to the algal symbiont since it has been independently reported[10,13] in several species of algae. The

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[13] McCARTHY, E. D., HAN, J. and CALVIN, M. (1968) *Anal. Chem.* **40,** 1475.

same component was found in trace amounts in the first samples of the two *Cetraria* species. Also observed in the alkane fraction of the second sample of *S. ceratites* was a series of anteiso-alkanes, maximising at anteiso-C_{26} with smaller amounts of ai-C_{25}, ai-C_{24}, ai-C_{23} and ai-C_{22}.

These limited results do not permit a decision as to the possible value of hydrocarbon distributions to lichen taxonomy. Variations, within a lichen species, in triterpenoid composition have been found by Bruun,[14] where the concept of 'chemical strains' may be applicable.[15] However, the observed levels of abundance of hydrocarbons in lichens is extremely low. Clearly, therefore, survey work will require the strict elimination of contaminants both during sample collection and in the laboratory, and standardised experimental techniques.

EXPERIMENTAL

Sample collection. Cetraria nivalis and *C. crispa* samples were collected on both occasions from Ålmenberget, Oppdal, Norway; *Siphula ceratites* was collected from Kongsmoen, Norway.

Extraction. The first samples of air-dried, crushed plant material were extracted with Et_2O (Soxhlet). Later extractions of freeze-dried, crushed material employed $CHCl_3$–MeOH (3:1) and sonication.

Analysis. Hydrocarbons were isolated from the total lipids (5–12% of lichen dry wt) by eluting with hexane (or with light petrol. b.p. 40–60° for the earlier samples) from a column of alumina. Separation into alkane and alkene fractions was by TLC on 10% $AgNO_3$–silica gel, with hexane as solvent. Characterization of individual components was by GLC and GC–MS (Varian MAT CH-7 mass spectrometer coupled to a Varian Aerograph 1200 gas chromatograph by a Watson–Biemann all-glass He separator.) Care was taken throughout to minimize contamination, e.g. by the use of glassware washed in chromic acid.

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DIE BIFLAVONE VON *SEQUOIADENDRON GIGANTEUM*

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Da über die Biflavonausstattung von *Sequoiadendron giganteum* (Lindl.) Buchholz bislang nichts bekannt war, haben wir diese Pflanze in unsere Untersuchungen über die

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