CHEMOTAXONOMIC STUDIES OF LICHENS FROM SAYAP-KINABALU, SABAH: CONSTITUENTS OF *PSEUDOCYPHELL*ARIA, LOBARIA AND *PELTIGERA*

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An investigation of the chemical constituents present in the lichens Pseudocyphellaria, Lobaria and Peltigera collected from Sayap, Mount Kinabalu, Sabah, revealed the taxonomic identity of the lichens respectively. Seven lichens have been identified i. e P. sulphurea variant I, P.sulphurea variant II,P.sulphurea variant III, Lobaria insularis, Peltigera polydactyl, Pseudocyphellaria cf. neglecta and P.cf argycea.

INTRODUCTION

The first chemical test conducted on lichen thalli for taxonomic purposes was carried out by Nylander in the 1860s (Nylander, 1866). He detected the presence of various lichen substances by spotting chemical reagents directly onto the lichen thallus (spot tests) to produce characteristic colour changes. Nylander utilised the characteristic medullary and cortical reactions as a specific character but the origin of these characteristic colour reactions remained unknown.

The first serious chemical investigations were conducted by Zopf, in his publication of Die Fiechtens toffe in 1907 (Zopf, 1907) with the description of over 150 lichen compounds. However the ultimate structural elucidation of many lichen metabolites was due to the work of Asahina and co-workers in the 1930's (Asahina & Shibata, 1954). This laid the foundation for further research on the lichenic compounds in recent time.

Subsequently the techniques of paper chromatography and particularly thin layer chromatography (TLC) have thereby improved the speed and certainty of recognition of lichen substances by means which are simple to use and relatively inexpensive. Standardized methodology and further refinement of analytical TLC procedures for detecting and comparing lichen metabolite have been reported by Culberson and colleagues (Culberson, 1972, Culberson & Ammann, 1979; Culberson & Johnson, 1982).

In an effort to make most of literature information on standardised TLC, RF values and spot colour characteristic more readily accessible, and to keep such a library of information current as more and more lichen metabolites are identified and characterised (Elix, *et al*., 1987) have prepared a data bank suitable for storing such information.

In this paper we report some of our findings during a chemotaxonomic survey of various lichens collected from Sayap, Mount Kinabalu, Sabah. Lichen samples were collected around the base camp in Sayap, Kinabalu Park in June 1992 and Herbarium specimens are deposited in Jabatan Kimia, Universiti Kebangsaan Malaysia.
The dry lichen fragments were freed as far as possible from obvious organic substrate materials and extracted with warm acetone for thin layer chromatography analysis (TLC). Compounds were identified by TLC using methods standardized for lichen products (Culberson & Ammann, 1979; Culberson & Johnson 1976, 1982, Elix, et al., 1987)

RESULTS AND DISCUSSION

A total of 13 known substances were identified in the specimens examined. These included the pulvinic acid group of yellow pigments pulvinic dilactone (1); the triterpenes, 7-β-acetoxyhopan-22-ol (2) and hopane 15, 22- diol (3); tridepsides, gyrophoric acid (4), ethyl gyrophorate (5), 4-0-methyl gyrophoric acid (6) and tenuiorin (7); orcinol para depsides, methyl lecanorate (8) and depsidones norstictic acid (9), salazinic acid (10), stictic acid (11), cryptostictic acid(12) and constictic acid(13). The standardized chromatographic data for these compounds are listed in Table 1.

Lobaria insularis Vain was the only Lobaria species collected on the bark of dipterocarp trees. The specimen examined by TLC showed the presence of only one compound i.e. gyrophoric acid. On the big rock in the Sayap River, Peltigera polydactyl was found growing together with mosses.

Table 2 shows the chemical constituents of the lichens collected from Sayap, Mount Kinabalu~Sabah. Pseudocyphellaria sulphurea (Schaerer) D. Galloway [P.quercifolia (T) Vain.] is a Polynesian species ranging from the Philippines to Eastern Australia (Maass, 1975). From the collection, three chemical variants have been encountered. In the chemical variant I (Say 86) only gyrophoric acid as methyl gyrophorate accumulated. Chemical variant II (Say 30) shows 4-0-methyl gyrophoric acid as the main constituent but also a trace amount of gyrophoric acid, as methyl ester. In chemical variant III (Say 42) gyrophoric acid is the predominant phenolic constituent. However, it also shows minor amounts of 4-0-methyl gyrophoric acid, methyl gyrophorate and tenuiorin. Such alterations of the chemical complements are not uncommon in Pseudocyphellaria (Maass, 1969).

Table 1
Standardised Thin Layer Chromatographic Data for Lichen Metabolites from Sayap Kinabalu, Sabah.

No.	Compound R_F	A	B*	C	G
1.	Pulvinic dilactone	80	82	90	-
2.	7-β-acetoxyhopan-22-ol	63	52	46	61
3.	hopane-15,22-diol	40	39	36	42
4.	gyrophoric acid	24	42	24	-
5.	methyl gyrophorate	52	42	24	-
6.	4-0-methyl gyrophoric acid	37	42	45	-
7.	tenuiorin	76	55	76	-
8.	methyl lecanorate	52	48	39	-
9.	norstictic acid	40	32	30	57
10.	salazinic acid	10	7	4	26
11.	stictic acid	32	9	18	34
12.	cryptostictic acid	14	10	10	27
13.	constictic acid	7	1	2	9
14.	usnic acid (standard)	70	65	71	88
15.	physadillic acid(standard)	10	33	19	16
16.	notqatic acid(standard)	24	44	18	55

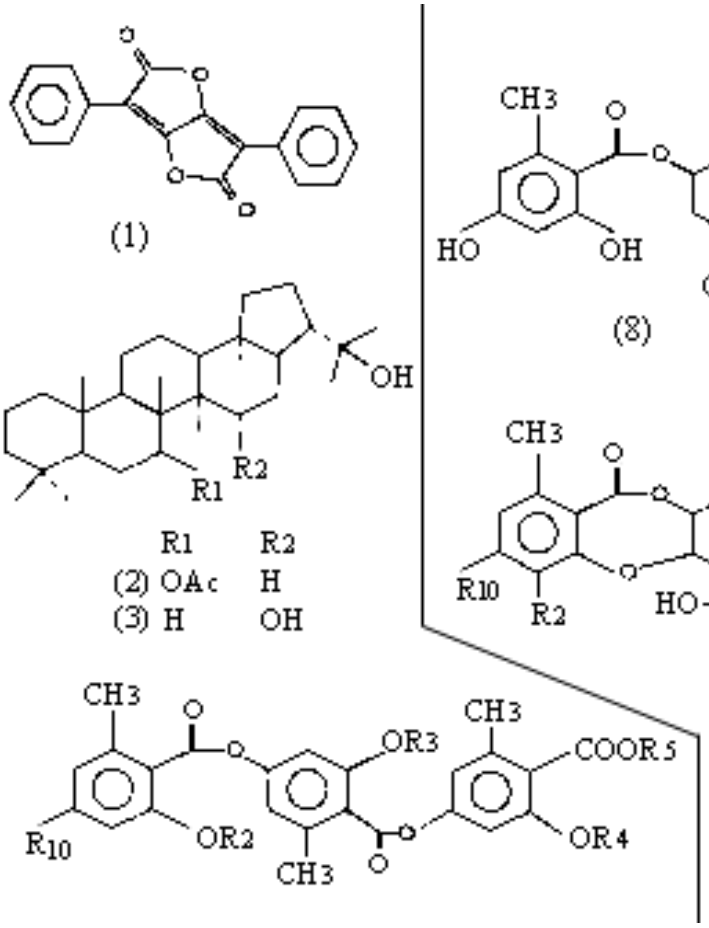
Notes: Standard R_F Values (x100) were determined in four independent t.1.c. *Solvents system*: (A) toluene/dioxane/acetic acid (180:45:5); (B*) hexane/t-butylmethyl ether/ formic acid (140:72:18); (C) toluene/acetic acid (170:30); (G) toluene/ethyl acetate/formic acid (l39:83:8)

Table 2
Chemical constituents of the Lichens collected from Sayap, Mount Kinabalu, Sabah.

Lichens	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Pseudocyphellaria sulphurea (Say86)		*	*	*	*									*	
Pseudocyphellaria sulphurea (Say36)		*	*	*	*	*	*								
Pseudocyphellaria sulphurea (Say 42)		*	*	*	*		*								
Lobaria insularis				*											
Peltigera polydactyl (Say84)	*			*		*									
Peltigera cf.argyracea (Say96)	*	*													*
Peltigera cf.neglecta (Say54)	*	*	*	*		*	*	*	*	*	*	*			

Note: No. 14: Traces of triterpenoids
No 15: Phenolic compounds (Rf(c) 45 & 48(Green)

Two other species of *Pseudocyphellaria,* were collected from Sayap. One of these showed the presence of 12 compounds as shown in Table 2. The chemotaxonomy of this species corresponded to P cf. neglecta although the morphology are not identical compared to the herbarium (Elix, 1993 Private communication) sample. The other species was identified as P.cf.argyracea based on the morphology, but the chemical constituents different. P.cf.argyracea contain compounds (2), (3) and in addition, 2 unknown compounds which phenolic in nature (Rf(C) 45 & 48). The compound with Rf(C) 45 & 48 gave a green colour after spraying with 10% H2SO4.



	R_1	R_2	R_3	R_4	R_5
(4)	H	H	H	H	H
(5)	H	H	H	H	Me
(6)	Me	H	H	H	H
(7)	Me	H	H	H	Me

	R_1	R_2	R_3
(9)	H	CHO	CH_3
(10)	H	CHO	CH_2OH
(11)	CH_3	CHO	CH_3
(12)	CH_3	CH_2OH	CH_3
(13)	CH_3	CHO	CH_2OH

CONCLUSION

Members of Lobariaceae contribute a distinctive and well developed epiphytic community of lichens and bryophytes associated with forests in temperate and tropical regions. This alliances forms a species rich epiphytic community with an unusually high number of faithful species, especially in the families Lobariaceae, Pannariaceae, Collemataceae and Peltigeraceae (Johns *et al*., 1977). This forest (Sayap Forest) and their associated epiphytic vegetation represent a climate vegetation that has survived major changes in the earth's climate since the early century. Older forms of forest management, such as wood-texture and a sustainable slash and burn technique have allowed ecological continuity for the many component species of these communities. However, recent changes in forest management and in agricultural practices are threatening to destroy this habitat. The collection from Sayap shows an interesting phenomenon whereby from a relatively small collection area, several species of *Pseudocyphel*laria and three chemical variants of P.sulphurea are in existence. In the light of these findings, the *Pseudocyphellaria* rich colony in Sayap is unique, unlike other montane areas of Peninsular Malaysia which we have studied.

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