

The Lichen Genus *Usnea* at Meifeng, Central Taiwan

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Abstract. Eleven species of the lichen genus *Usnea* have been recorded at Meifeng, central Taiwan. Among them, the most common 3 species are *U. himalayana*, *U. bismolliuscula*, and *U. orientalis*. A morphological investigation as well as chemical analysis including color tests and thin-layer chromatography were employed in this study. Morphological characteristics and the lichen substances of each taxon are described. A key to the species is also provided.

Key words: chemotaxonomy, lichen substances, taxonomy, *Usnea*.

INTRODUCTION

The lichen genus *Usnea* Adans. (Ascomycetes: Lecanorales: Usneaceae,) is a typical group of fruticose lichens (Hale, 1983; Dobson, 1992). There are about 600 species of the *Usnea* in the world. In Taiwan, most species grow in virgin forests of middle to high elevations of 1000~3000 m, especially in fir forests. The thalli of this genus are usually corticolous, while those of some species are terricolous or specifically saxicolous or otherwise (Awasthi, 1986).

The thalli of *Usnea* are erect, procumbent or pendulous, and attach to the substratum by a basal holdfast. Moreover, the erect types tend to be procumbent, and procumbent ones pendulous if there is better growth in the thallus. The pendulous forms usually develop attachment points for support here and there on the substrate (Awasthi, 1986).

There are three branching types of *Usnea*: (1) the excurrent type, in which a main stem called a sympodium runs up to the apex of the thallus and is generally present in *Usnea*; (2) the deliquescent type, in which the identity of the main stem gets lost due to repeated dichotomous branching; and (3) the filamentous type, in which a short main stem is divided subsymmetrically into primary branches and these are further divided more or less dichotomously into branches which possess characteristic more or less perpendicular

branchlets of usually limited growth (Asahina, 1956).

The fresh thalli of *Usnea* are usually yellowish-green to grayish-green, and change to yellowish-brown, dark brown, or black after drying. Moreover, fresh collections cannot be identified by the use of such color differentiation (Awasthi, 1986).

The cortex in *Usnea* has been reported to be prosoplectenchymatous, composed of a shortly septate hyphae disposed at right angles to the longitudinal axis. The algal stratum, which is usually more or less discontinuous, is situated below the cortex and varies in thickness. The hyphae of the medulla are dense, lax, or arachnoid (Awasthi, 1986).

The surface of the thallus in several taxa of *Usnea* has minute protuberances (also called papillae), which are hemispherical, conical, or cylindrical and consist solely of cortical tissue. Tubercles are usually larger than papillae and are connected with the medulla of branches by its own medullary tissue (Swinscow and Krog, 1975, 1976, 1977, 1979). Sometimes the apices of tubercles are broken or revealing a whitish medulla and the appearance of as pseudocyphellae (Awasthi, 1986). Pseudocyphellae are usually minute, punctiform to elongate, orbicular or effigurate, plane or raised white breaks in the cortex.

A few species are truly soresdiate from the

beginning and continue to produce soredia. Such soralia are white, orbicular or oblong and excavate. In some cases, the pseudocyphellae later begin to produce soredia and can then be referred to as soraliate. In others the soralia may later become isidiate (Awasthi, 1986).

Isidia are found in several species of *Usnea*. Their preponderance in the genus *Usnea* is indicative of their usefulness as vegetative propagules. They are filiform, white or whitish, and may develop primarily on the cortex or secondarily on the pseudocyphellae or soralia. Sometimes isidia are dark-tipped.

Among species at Meifeng, central Taiwan, soredia or isidia are present in *U. aciculifera*, *U. angulata*, *U. bismolliuscula*, *U. dasaea*, *U. longissima*, *U. nidifica*, *U. pangiana*, and *U. rubescens*.

Although morphologically similar, several species of *Usnea* chemically differ. The lichen substances of *Usnea* have been studied by many lichenologists and chemists (Asahina, 1956, 1961, 1968, 1971; Carlin and Swahn, 1977; Clerc, 1987; Swinscow and Krog, 1975; White and James, 1985). The known lichen substances of this genus alectorialic acid, atranorin, barbatic acid, barbactolic acid, caperatic acid, diffractaic acid, evernic acid, fumarprotocetraric acid, galbinic acid, norstictic acid, protocetraric acid, psoromic acid, salazinic acid, squamatic acid, stictic acid, stictic acid complex, thamnolic acid, usnic acid, and undetermined substances (Awasthi, 1986).

MATERIALS AND METHODS

Materials

All of the examined specimens in the present study were collected at Meifeng (Nantou County, central Taiwan) by the author and R.C. Chang (張蓉茜), and are stored at the herbarium of the National Museum of Natural Science (TNM), Taichung, Taiwan.

Methods

Morphological observations and chemical analysis of lichen substances were employed in the present study.

(A) Morphological study

The outer morphology and interior structure of the lichens were studied with the aid of a dissecting microscope magnifier, and optic microscope. Five morphological features were especially emphasized:

- (1) the type of branching;
- (2) the presence or absence of protuberances (papillae) on the surface of the thallus;
- (3) the presence or absence of soredia and isidia on the surface of the thallus;
- (4) the presence or absence of annular cracks on the surface of the thallus;
- (5) the color of the cortex and medulla.

(B) Chemical analyses

(1) Color tests

The lichen thallus was crushed on a piece of glass and then combined with a few drops of acetone. A few drops of reagent were added and the color observed was recorded. The reagents and their abbreviations are listed as follows:

- (a) K, a 1% KOH aqueous solution;
- (b) C, Clorox;
- (c) KC, C added immediately after the addition of the KOH aqueous solution; and
- (d) P, a 2% ethanolic solution of $C_6H_8N_2$.

Color reactions of the lichen genus *Usnea* at Meifeng are listed in Table 1.

(2) Thin-layer chromatography

The lichen substances were extracted with acetone and then spotted onto a silica gel TLC-plate. The spotted TLC plate was developed with a solvent system in a glass cabinet. The developed TLC plate was air-dried, then observed under short-wavelength (253~254 nm) UV light, and the spots were recorded. The R_f class and R_f value (Culberson and Kristinsson, 1970; Culberson, 1972a) of each spot were calculated and recorded.

(a) Solvent systems

Solvent system A- was comprised of toluol-dioxane-acetic acid (180- 45- 5).

Solvent system B- was comprised of hexane-ethyl ether-formic acid (5- 4-1).

(b) TLC plates

Merck silica gel 60 F254 TLC plates were used.

(c) Marker control

Norstictic acid (extracted from specimens of *Lobaria orientalis* (Asah.) Yoshim.) was used.

RESULTS AND DISCUSSION

The lichen substances found in this study included barbatic acid, diffractaic acid, norstictic acid, salazinic acid, stictic acid, and usnic acid. By analyzing these six lichen substances, we could

allocate the members of the lichen genus *Usnea* at Meifeng into separate categories. The TLC characteristics of these lichen substances are

shown in Table 2. Table 3 is a list of lichen substances found in taxa of *Usnea* at Meifeng.

Table 1. Color reactions of members of the lichen genus *Usnea* at Meifeng, Taiwan

Species	Color reaction			
	K	C	KC	P
<i>U. aciculifera</i>	+	—	+	+
<i>U. angulata</i>	+	+	+	+
<i>U. bismolliuscula</i>	+	—	+	+
<i>U. dasaea</i>	+	—	+	+
<i>U. diffracta</i>	—	—	+	—
<i>U. himalaya</i>	+	—	+	—
<i>U. longissima</i>	—	—	—	—
<i>U. nidifica</i>	+	—	+	+
<i>U. orientalis</i>	—	—	—	—
<i>U. pangiana</i>	+	—	—	+
<i>U. rubrotincta</i>	+	—	+	+

Table 2. TLC data of lichen substances in two solvent systems of the genus *Usnea* at Meifeng, Taiwan

Lichen substances	Rf x 100 values(Rf of X/Rf of N)		Spot color H ₂ SO ₄
	A	B	
Barbatic acid	44/42	69/27	-
Diffractaic acid	44/42	64/32	Yellow
Norstictic acid			
Salazinic acid	10/39	7/30	Orange
Stictic acid	32/42	9/27	Orange-brown
Usnic acid	62/40	48/26	Greenish

A= solvent system A; B= solvent system B; N= norstictic acid; X= lichen substance

Table 3. List of lichen substances of the genus *Usnea* at Meifeng, Taiwan

Species	Color reaction					
	bar	dif	nor	sal	sti	usn
<i>U. aciculifera</i>			+		+	+
<i>U. angulata</i>				+		+
<i>U. bismolliuscula</i>						+
<i>U. dasaea</i>	+			+		
<i>U. diffracta</i>						+
<i>U. himalayana</i>				+		+
<i>U. longissima</i>	+	+				+
<i>U. nidifica</i>			+	+		+
<i>U. orientalis</i>				+		+
<i>U. pangiana</i>				+		+
<i>U. rubrotincta</i>			+	+	+	+

bar= barbatic acid; dif= diffractaic acid; nor= norstictic acid; sal= salazinic acid; sti= stictic acid; usn= usnic acid.

According to Wei (1991), the genus *Usnea* is classified into 35 species, 4 subspecies, 2 varieties, and 2 forms in Taiwan; however, 11 species were recognized in the results of the present study.

Key to the species of *Usnea* at Meifeng, Taiwan

1. Cortex or medulla red
 2. Cortex white, medulla red*U. angulata*
 2. Cortex red, medulla white*U. rubrotincta*
1. Cortex and medulla white
 2. Branching the filamentous type
.....*U. longissima*
 2. Branching not the filamentous type
 3. Thalli without annular crack
 4. Thalli without soredia *U. orientalis*
 4. Thalli with soredia
 5. Soredia spinal-like *U. aciculifera*
 5. Soredia not spinal-like
 6. Thalli with isidia *U. dasaea*
 6. Thalli without isidia *U. pangiana*
 3. Thalli with annular crack
 4. Branching the deliquescent type (dichotomous branching) *U. diffracta*
 4. Branching not the deliquescent type
 5. Thalli lacking both soredia and papillae
..... *U. himalayana*
 5. Thalli with either soredia or papillae
 6. Norstictic acid absent
..... *U. bismolliuscula*
 6. Norstictic acid present *U. nidifica*

Taxa of *Usnea* at Meifeng, Taiwan

(1) *Usnea aciculifera* Vain., Bot. Mag. Tokyo 1921; 35: 45.

Thallus corticolous, decumbent to pendulous, yellowish-brown to brown, blackish, and flattened near base; branching sub-sympodial, without annular cracks on surface, but cortex transversely cracked; surface smooth to verrucose; cortex and medulla white; soredia spinal-like; isidia white, filiform, solitary or in groups.

TLC: norstictic acid, stictic acid, and usnic acid

Specimens examined: Nantou Co.: Meifeng, fallen on forest floor, Lin373 (TNM L0373); Lin376 (TNM L0376), Nov. 30, 1990. On dead trunk, Chang2 (TNM L0848), Oct. 18, 1992; Chang28 (TNM L0849), Oct. 18, 1992. On living tree, Chang12 (TNM L0847), Oct. 18, 1992.

(2) *Usnea angulata* Ach., Synops. Lich. 1814; 307.

Thallus corticolous, pendulous, grayish-yellow when collected; branching filamentous, and with irregular annular cracks on surface; cortex white;

medulla red; isidia white, solitary or in groups.

TLC: norstictic acid and usnic acid

Specimens examined: Nantou Co.: Meifeng, on living tree, Chang14 (TNM L0850), Oct. 18, 1992; Chang37 (TNM L0851), Oct. 18, 1992.

(3) *Usnea bismolliuscula* Zahlbr., Cat. Lich. Univ. 1923; 6: 542.

Thallus corticolous, decumbent to pendulous, grayish-green to light brown, sometimes basal part blackish; branching sympodial; branches articulate and inflated; surface annularly cracked; papillate; dense papillae on branches; cortex and medulla white; sorediate; soredia white, spine-like, always in groups.

TLC: usnic acid

Specimens examined: Nantou Co.: Meifeng, fallen on forest floor, Lin374 (TNM L0374), Nov. 30, 1990; Lin380 (TNM L0380), Nov. 30, 1990; Lin383 (TNM L0383), Nov. 30, 1990; Chang22 (TNM L0886), Oct. 18, 1992. On rotten log, Chang5 (TNM L0874), Oct. 18, 1992; Chang26 (TNM L0880), Oct. 18, 1992; Chang48 (TNM L0875), Dec. 13, 1992. On tree, Chang15 (TNM L0877), Oct. 18, 1992; Chang29 (TNM L0884), Oct. 18, 1992; Chang31 (TNM L0881), Oct. 18, 1992; Chang51 (TNM L0883), Dec. 13, 1992; Chang76 (TNM L0882), Dec. 13, 1992. On dead branch, Chang44 (TNM L0885), Dec. 13, 1992; Chang72 (TNM L0878), Dec. 13, 1992; Chang77 (TNM L0879), Dec. 13, 1992; Chang 79 (TNM L0876), Dec. 13, 1992.

(4) *Usnea dasaea* Stirt. Scott. Nat. 1881; 6: 104.

Thallus corticolous, erect to procumbent, greenish-gray to yellowish-green; branching sympodial, base of main branch never black; surface smooth; cortex and medulla white; sorediate without isidia, soredia in groups; apothecia minute.

TLC: salazinic acid.

Specimens examined: Nantou Co.: Meifeng, fallen on forest floor, Lin381 (TNM L0381), Nov. 30, 1990; Lin386 (TNM L0386), Nov. 30, 1990. On rotten log, Chang4 (TNM L0854), Oct. 18, 1992. On living tree, Chang18 (TNM L0852), Oct. 18, 1992. On dead trunk, Chang63 (TNM L0853), Dec. 13, 1992.

(5) *Usnea diffracta* Vain., Bot. Mag. Tokyo 1921; 35: 45.

Thallus corticolous, procumbent to pendulous,

pale green to grayish-green, basal part greenish-brown to brown; branching dichotomous to subdichotomous giving rise to filamentous branches, surface with annular cracks; cortex and medulla white; soredia and isidia absent.

TLC: barbatic acid and usnic acid

Specimens examined: Nantou Co.: Meifeng, fallen on forest floor, Chang34(TNM L0855), Oct. 18, 1992.

(6) *Usnea himalayana* C. Bab., Hook. J. Bot. 1852; 4: 243.

Thallus corticolous, pendulous, generally soft to touch, pale green to grayish-green when collected; branching dichotomous; branches articulate and inflated, lateral branchlets absent; surface with annular cracks; pseudocyphellae present, white; cortex and medulla white; soredia and isidia absent; apothecia rarely, margin ciliate.

TLC: salazinic acid and usnic acid

Examined specimens: Nantou Co.: Meifeng, fallen on forest floor, Lin367(TNM L0367), Nov. 30, 1990; Lin370(TNM L0370), Nov. 30, 1990; Lin379(TNM L0379), Nov. 30, 1990; Lin382(TNM L0382), Nov. 30, 1990; Lin388(TNM L0388), Nov. 30, 1990; Lin394(TNM L0394), Nov. 30, 1990; Chang38(TNM L0866), Oct. 18, 1992; Chang55(TNM L0863), Dec. 13, 1992; Chang59(TNM L0858), Dec. 13, 1992; Chang61(TNM L0864), Dec. 13, 1992; Chang62(TNM L0870), Dec. 13, 1992; Chang69(TNM L0861), Dec. 13, 1992; Chang70(TNM L0859), Dec. 13, 1992; Chang71(TNM L0860), Dec. 13, 1992. On dead trunk, Chang19(TNM L0872), Oct. 18, 1992; Chang30(TNM L0867), Oct. 18, 1992; Chang49(TNM L0865), Dec. 13, 1992; Chang57(TNM L0869), Dec.13, 1992; Chang68(TNM L0862), Dec.13, 1992. On rotten log, Chang36(TNM L0857), Oct. 18, 1992. On living tree, Chang45(TNM L0871), Dec. 13, 1992; Chang52(TNM L0868), Dec. 13, 1992; Chang66(TNM L0856), Dec. 13, 1992.

(7) *Usnea longissima* Ach., Lich. Univ. 1810; 626.

Thallus corticolous, pendulous, pale yellow, grayish-green, to brownish, sometimes intermittently black; branching filamentous; lateral branchlets dense, greatly variable in length and branching; with annular cracks near base of branching; cortex and medulla white; soraliolate; soredia white, rarely isidia also present.

TLC: barbatic acid, diffractaic acid, and usnic acid

Specimens examined: Nantou Co.: Meifeng, fallen on forest floor, Lin375(TNM L0375), Nov. 30, 1990; Lin377(TNM L0377), Nov. 30, 1990. On dead wood, Chang 27(TNM L0890), Oct. 18, 1992; Chang80(TNM L0887), Dec. 13, 1992. On tree, Chang53(TNM L0889), Dec. 13, 1992; Chang73(TNM L0888), Dec. 13, 1992.

(8) *Usnea nidifica* Taylor, London J. Bot. 1847; 6: 191.

Thallus corticolous, pendulous, yellowish-green; branching subsympodia with dense branchlets; surface with annular cracks; papillate; dense papillae on branchlets; cortex and medulla white; soredia present; isidia absent.

TLC: norstictic acid, salazinic acid, and usnic acid

Specimens examined: Nantou Co.: Meifeng, on dead trunk, Chang7(TNM L0873), Oct. 18, 1992.

(9) *Usnea orientalis* Motyka, Lich. Gen. *Usnea* Stud. Monogr. Pars Syst. 1936-38; 2: 547.

Thallus corticolous, erect, greenish-gray, deep yellow to yellowish-brown; branching sympodial, branches somewhat irregularly swollen, sometimes with annular cracks; surface slightly waxy in appearance, papillate; papillae dense; cortex and medulla white; soredia and isidia absent. Apothecia large, margin ciliate.

TLC: salazinic acid and usnic acid

Specimens examined: Nantou Co.: Meifeng, fallen on forest floor, Lin369(TNM L0369), Nov. 30, 1990; Lin384(TNM L0384), Nov. 30, 1990; Lin387(TNM L0387), Nov. 30, 1990; Lin390(TNM L0390), Nov. 30, 1990; Lin392(TNM L0392), Nov. 30, 1990; Chang74(TNM L0893), Dec. 13, 1992. On dead tree, Chang1(TNM L0897), Oct. 18, 1992; Chang9(TNM L0892), Oct. 18, 1992; Chang25(TNM L0900), Oct. 18, 1992; Chang32(TNM L0899), Oct. 18, 1992; Chang40(TNM L0894), Dec. 13, 1992; Chang41(TNM L0898), Dec. 13, 1992; Chang56(TNM L0895), Dec. 13, 1992; Chang67(TNM L0891), Dec. 18, 1992. On tree, Chang6(TNM L0896), Oct. 18, 1992. On leaves of forest floor, Chang10(TNM L0901), Oct. 18, 1992.

(10) *Usnea pangiana* Stirt. Scott. Nat. 1883; 7: 77.

Thallus corticolous, erect to pendulous, rigid, pale yellowish-brown to grayish-brown; branching sympodial, surface with annular cracks near base of branchlets; cortex and medulla white; sorediate

without isidia, soredia in groups.

TLC: salazinic acid and usnic acid

Specimens examined: Nantou Co.: Meifeng, on dead trunk, Chang11 (TNM L0902), Oct. 18, 1992; Chang46 (TNM L0903), Dec. 13, 1992. Fallen on forest floor, Chang43 (TNM L0904), Dec. 13, 1992.

(11) *Usnea rubrotincta* Stirt., Scott. Nat. 1881; 6: 103.

Thallus corticolous, sometimes saxicolous, procumbent to pendulous, greenish, yellowish, to brownish-red; branching subsympodial; cortex red; medulla white; surface pseudocyphellate; pseudocyphellae white; soredia absent; isidia solitary or in groups.

(Race I) TLC: norstictic acid, salazinic acid, and usnic acid.

Specimen examined: Nantou Co.: Meifeng, fallen on forest floor, Chang47(TNM L0905), Dec. 13, 1992.

(Race II) TLC: norstictic acid, stictic acid, and usnic acid.

Specimen examined: Nantou Co.: Meifeng, fallen on forest floor, Chang39(TNM L0906), Dec. 13, 1992.

REFERENCES

- Asahina, Y. 1956. Nomenclature of Japanese species of *Lobaria*, sect. *Ricasolia*. *Mise. Bryol. Lichenol.* 1: 1-3.
- Asahina, Y. 1961. *Lichenologisch Notizen*, 180-181. *J. Jpn. Bot.* 36: 225-228.
- Asahina, Y. 1968. *Lichenologisch Notizen*, 209. *J. Jpn. Bot.* 43: 97-101.
- Asahina, Y. 1971. *Lichenologisch Notizen*, 245-247. *J. Jpn. Bot.* 46(9): 257-262.
- Awasthi, G. 1986. Lichen genus *Usnea* in India. *Hattori Bot. Lab.* 61: 333-421.
- Carlin, G. and U. Swahn. 1977. De svenska *Usnea*-arterna. *Svensk Bot. Tidskr.* 71: 89-100.
- Clerc, P. 1987. Systematics of the *Usnea fragilesens* aggregate and its distribution in Scandinavia. *Nord. J. Bot.* 7: 479-495.
- Culberson, C.F. and H. Kristinsson. 1970. A standardized method for the identification of lichen products. *J. Chromatog.* 46: 85-93.
- Culberson, C.F. 1972. Improved conditions and new data for thin-layer chromatographic method. *J. Chromatog.* 72: 113-125.
- Dobson, F. S. 1992. *Lichens*, 3rd ed. Richmond Publishing, UK.
- Hale, M.E., Jr. 1983. *The biology of lichens*, 3rd ed. Edward Arnold, London.
- Ohmura, Y. 2001. Taxonomic study of the genus *Usnea* (lichenized ascomycetes) in Japan and Taiwan. *J. Hattori Bot. Lab.* 90: 1-96.
- Solberg, Y.L. 1970. Studies on the chemistry of lichens VIII. *Lichenologist* 4: 271-282.
- Swinscow, T.D.V. and H. Krog, 1975. The *Usnea undulata* aggregate in East Africa. *Lichenologist* 7: 121-138.
- Swinscow, T.D.V. and H. Krog, 1976. The *Usnea bornmuelleri* aggregate in East Africa. *Norw. J. Bot.* 23: 23-31.
- Swinscow, T.D.V. and H. Krog, 1977. Pendulous species of *Usnea* in East Africa. *Norw. J. Bot.* 25: 221-241.
- Swinscow, T.D.V. and H. Krog, 1979. The fruticose species of *Usnea* subgenus *Usnea* in East Africa. *Lichenologist* 11(3): 207-252.
- Wei, J.C. 1991. An enumeration of lichens in China. International Academic Publishers, Beijing.
- White, F.J. and P.W. James. 1985. A new guide to microchemical techniques for the identification of lichen substances. *Bull. Br. Lichen Soc.* 57: 1-41.

臺灣梅峰地區菘蘿屬地衣的研究

林仲剛

國立自然科學博物館植物學組

本研究乃應用地衣外部形態特徵的比較，以及其所含地衣物質的化學分析(包括顏色試驗與薄層色層分析等)，來探討臺灣梅峰地區(南投縣)所產菘蘿屬的種類。共發現11種，其中的*U. himalayana*、*U. bismolliuscula*，以及*U. orientalis*為數量較多的種。

文中對梅峰所產每一種菘蘿均附有特徵描述，以及所含地衣物質的種類等資料，並備有種的檢索表。

關鍵詞：化學分類學，地衣物質，分類學，菘蘿屬。