Studies on the Chemistry of the Lichen Genus *Umbilicaria* Hoffm.

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The aromatic compounds of more than 400 specimens of 33 species of the lichen genus *Umbilicaria* have been investigated by HPLC. Additionally to gyrophoric, hiascic, lecanoric, ovoic and umbilicaric acids, atranorin, norstictic and stictic acids, the new depside crustinic acid has been found. Structure elucidation of crustinic acid was made by means of HPLC cochromatography, NMR, UV and mass spectrometry. Now the secondary product patterns of *Umbilicaria* species are discussed and their significance for systematic purposes is shown.

Introduction

The lichen genus *Umbilicaria* causes a series of taxonomic problems. Neither the investigations of anatomy and morphology of the thallus [1–4] nor of the thalloconidia [5, 6] give sufficient results serving as a basis to separate many species reliably. The discussions on the separation of the species *U. nylanderiana* and *U. polyphylla* are a good example to illustrate these difficulties [4, 7].

However, secondary product chemistry of the genus *Umbilicaria* has been overlooked up to now and thought not to be of any significance for the solution of taxonomic problems [3, 4, 8–10] (Table I). Former investigations [11, 12] of a few *Umbilicaria* species have shown that characteristic product patterns can contribute to the clarification of taxonomic problems. Therefore the secondary product patterns of 33 *Umbilicaria* species are presented in this paper and their contribution to the solution of taxonomic problems is discussed.

Results and Discussion

Gyrophoric and lecanoric acids

The characteristic lichen substance of *Umbilicaria* species is gyrophoric acid which could be demonstrated in 31 of 33 investigated species. The biosynthetically closely related depside lecanoric acid always occurs as a satellite compound of this tridepside. The concentration of lecanoric acid is much smaller than that of gyrophoric acid. The ratio can be given as 90:1. The combined occurrence of gyrophoric and lecanoric acids could indicate that lecanoric acid may be a hydrolysis product of gyrophoric acid as suggested by Leuckert [13]. In case of the occurrence of this reaction orsellinic acid must be detected as a second product of hydrolysis in the same molar proportion as lecanoric acid. However, orsellinic acid can never be proven by means of HPLC when the extracts are analyzed immediately and the extractions are carried out at a temperature of +6 °C. Orsellinic acid is detected only after 1 h of extraction. The molar proportion of orsellinic and lecanoric acids amounts to 1:4. The applied method shows that the suggested hydrolysis of gyrophoric acid must be rejected. Probably, lecanoric acid must be accepted as a native lichen compound and as a link of a biosynthetic pathway. However, lecanoric acid is not of any importance for chemosystematic purposes.

Crustinic acid

Crustinic acid was classified as a depside by its UV spectrum in methanol with maxima at *ca.* 260 and 306 nm. The mass spectrum (Ch. Leuckert) shows a molecular weight peak at m/z 484, which made likely a hydroxy-gyrophoric acid. This is confirmed by the ^{1}H NMR spectrum (250 MHz, acetone-d_6) of crustinic acid: three singulets at

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Table I. Lichen substances of *Umbilicaria* species described in the literature so far. gyr = gyrophoric acid, umb = umbilicaric acid, ovo = ovoic acid, lec = lecanoric acid, atr = atranorin, nor = norstictic acid, stic = stictic acid, us = unknown substances. *U. haplocarpa, U. josiae, U. koidzumii, U. krempelhuberi, U. nepalensis, U. ruebeliana, U. thamnodes, U. trabeculata,* and *U. yunnana* have never been examined so far.

Species		gyr	umb	ovo	lec	atr	nor	stic	us	References
angulata		+	+							[20]
arctica		+					+			[20, 21, 30–33]
caroliniana		+			+				+	[11, 20, 21, 34]
cinereorufescens		+							+	[3, 20, 21, 24, 30, 33, 34]
crustulosa	a	+								[11, 24, 33, 34]
	b	+							+	
	c	+							+	
	d	+							+	
cylindrica	a			no lichen substances						[11, 17, 20, 21, 24, 33–35]
	b						+			
deusta	a	+								[16–21, 33, 34, 37–42]
	b	+	+							
	c	+				+				
esculenta		+			+					[19]
freyi		+								[15]
grisea		+	+							[34]
hirsuta	a	+							+	[16, 18, 20, 24, 30, 33, 35, 36, 43]
	b	+							+	
hyperborea		+	+							[16–18, 20, 24, 30, 33, 35, 36, 43]
mammulata		+								[20, 21, 30]
mühlenbergii		+								[20, 21, 30]
nylanderiana		+	+							[24, 33, 34]
polyphylla		+	+		+					[3, 16, 17, 20, 21, 24, 30, 33, 34, 36–38, 44]
polyrrhiza	a	+								[16, 30, 33, 34, 36, 37]
	b	+	+		+					
proboscidea	a	+					+			[11, 16, 19–21, 24, 30, 34, 36–38, 41, 42, 45]
	b	+								
	c	+	+				+			
soralifera		+								[3]
spodochroa		+	+		+					[11, 33, 34]
torrefacta	a	+		+					+	[11, 20, 30, 33, 34, 46, 47]
	b	+	+							
	c	+					+	+	+	
	d						+	+	+	
umbilicarioides				no lichen substances						[3]
vellea		+	+							[3, 19–21, 24, 33, 34]
virginis	a						+			[20, 21, 30, 33]
	b	+					+			

2.51 (3H), 2.62 (3H) and 2.71 ppm (3H) correspond to 3 methyl groups, two pairs of dublets at 6.37 (2H, J = 2.4 Hz) and 6.90 ppm (2H, J = 2.2 Hz) correspond to two aromatic protons in meta-position and a singulet (1H) at 6.47 ppm corresponds to another aromatic proton. Of the 6 theoretically possible hydroxy-gyrophoric acids only the 5-isomer, hiascic acid, was isolated from lichens. The comparison of crustinic acid with hiascic acid by means of HPLC indicates that this depside is not identical with hiascic acid. For this reason the hydroxy group must be in another position. The signals of the methyl groups appear in the ^{13}C NMR spectrum (62.76 MHz, acetone-d_6) of crustinic acid at 15.68, 23.11 and 24.31 ppm. The chemical shift of the signal at 15.68 ppm indicates a methyl group flanked by a hydroxy group. Therefore the structures A and B can be proposed for crustinic acid (Fig. 1). A decision between A and B should be made a) after isolation of a bigger

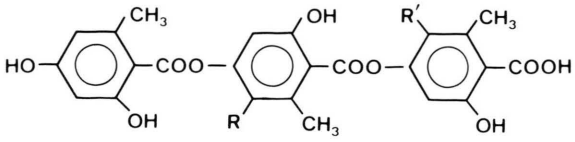

Fig. 1. Possible structures of crustinic acid.
A: R = OH, R′ = H
B: R = H, R′ = OH

amount of crustinic acid and hydrolysis of the methylesters and identification of the hydrolysis products and b) after synthesis of both isomers.

Chemotaxonomy

Of chemosystematic importance for the genus *Umbilicaria* is the presence or absence of the depsides crustinic acid, hiascic acid, ovoic acid and umbilicaric acid which always occur together with gyrophoric acid. All depsides are accompanied by satellite compounds which could not be investigated further due to lack of material.

The presence or absence of the depsidones stictic acid and norstictic acid is also of systematic importance. Accessory metabolites, *e.g.* connorstictic acid, α-methylethersalazinic acid, PCR-1 [11] and cryptostictic acid commonly occur with the main depsidones (Table II).

In *U. crustulosa* and *U. spodochroa* the depside atranorin could be found.

All investigated thalli contained the non-aromatic substance ergosterol in rather equal amounts as gyrophoric acid. The occurrence of ergosterol has been proved additionally by means of mass spectrometry of *Lasallia hispanica* (C. Leuckert, pers. comm.).

The secondary product patterns of the species studied

The secondary product patterns of 26 of 33 investigated species of the genus *Umbilicaria* have turned out to be unique so far. Chemical races are well known, especially in species of the section Anthracinae [12] and could be found additionally in 3 species during this study. A geographic separation of the chemical races could not be detected. Chemical races are often being found during the examination of different thalli of one herbarium sheet.

Chemical races show a significantly distinguishable variable chemical composition with simple replacement of one or a few substances. However, in 4 *Umbilicaria* species (*U. crustulosa, U. krempelhuberi, U. spodochroa* and *U. vellea*) sequences of biosynthetically closely related secondary product patterns have been detected. These sequences could not be classified as chemical races because chemical intermediates were common. According to Elix [14] the sequences could be defined as chemosyndromic variations. Extreme forms of chemosyndromic variation are discussed as chemotypes here.

The secondary product patterns of species which have appeared as unique so far are summarized below. Species showing chemical races or chemotypes are summarized subsequently (Table II).

1. *Umbilicaria umbilicarioides* (B. Stein) Krog and Swinscow

According to Krog and Swinscow [3] no lichen products were found in *U. umbilicarioides*. Here *U. bolusiana* Frey is reduced to a synonym of *U. umbilicarioides*.

2. *U. hirsuta* (Sw. ex Westr.) Ach and *U. soralifera* (Frey) Krog and Swinscow

Both species produce gyrophoric acid as the main lichen substance (4–6.5% dw) which is always accompanied by lecanoric acid (0.5% dw). Additionally an unknown depside, RT 13, was detected in small amounts (% dw < 0.1). Because of lack of material RT 13 could not be investigated further. The unknown depside occurs only when gyrophoric acid is present in concentrations above 6% dw.

In herbaria *U. hirsuta* is often confused with *U. grisea*. However, beside morphological characters, *e.g.* the presence of rhizinomorphs and the dainty areolated under side [15], *U. hirsuta* can be clearly distinguished from *U. grisea* by its very characteristic secondary product pattern. In addition to lecanoric and gyrophoric acids, *U. grisea* always contains umbilicaric acid. *U. grisea* can also be identified by its coarsely areolated under side and the absence of rhizinomorphs.

3. *U. angulata* Tuck., *U. deusta* (L.) Baumg., *U. esculenta* (Miy.) Minks, *U. koidzumii* Yas. Sato, *U. mammulata* (Ach.) Tuck., *U. polyrrhiza* (L.) Ach., *U. thamnodes* Hue, *U. trabeculata* Frey and Poelt, *U. yunnana* (Nyl.) Hue

The main lichen substance is gyrophoric acid. The concentration of this depside varies from 2 to 5% dw. As minor compounds lecanoric acid (0.2 to 0.5% dw) and hiascic acid (0.1 to 0.4% dw) were detected.

In *U. deusta,* a lichen commonly distributed in Europe, the depside umbilicaric acid co-occurring with gyrophoric acid is described by various authors [16–21]. In contrast to this, umbilicaric acid

Table II. Aromatic lichen substances of *Umbilicaria* species. M = major substance, m = minor substance. a, b, c = different chemotypes, gyr = gyrophoric acid, lec = lecanoric acid, umb = umbilicaric acid, hia = hiascic acid, ovo = ovoic acid, cru = crustinic acid, atr = atranorin, = nor = norstictic acid complex including connorstictic acid, stic = stictic acid complex including α-methylethersalazinic acid, cryptostictic acid and PCR-1, us = unknown depsides (for further informations see text).

Species		gyr	lec	hia	umb	ovo	cru	atr	nor	stic	us
angulata		M	m	m							
arctica		M	m		m						
caroliniana		M	m	m		m					
cinereorufescens		m	m				M				m
crustulosa	a	M	m	m							
	b	M	m	m			m				
	c	M	m	m			m	m			
cylindrica	a	no lichen substances									
	b								M		
deusta		M	m	m							
esculenta		M	m	m							
freyi		M	m		m						
grisea		M	m		m						
haplocarpa		M	m		m						
hirsuta		M	m								m
hyperborea		M	m		m						
josiae		M	m		m						
koidzumii		M	m	m							
krempelhuberi	a	M	m								
	b	M	m	m			m				
mammulata		M	m	m							
mühlenbergii		M	m		m						
nepalensis		M	m		m						
nylanderiana		M	m		m						
polyphylla		M	m		M						m
polyrrhiza		M	m	m							
proboscidea	a	M	m	m		m					
	b	M	m	m		m			m		
ruebeliana		M	m		M						m
soralifera		M	m								m
spodochroa	a	M	m	m			m				
	b	M	m	m			m	m			
	c	M	m	m	m						
	d	M	m	m	m			m			
thamnodes		M	m	m							
torrefacta	a									N	m
	b	M	m	m		N				N	m
trabeculata		M	m	m							
umbilicarioides		no lichen substances									
vellea	a	M	N								
	b	M	N	m							
	c	M	N	m	m						
virginis		M	N						N		
yunnana		M	N	m							

could never be found in thalli of *U. deusta* during this study. An original sample of umbilicaric acid isolated by Zopf (Zopf 584 from *U. deusta*) was identified by HPLC as gyrophoric acid. It is believed that the authors have confused umbilicaric acid with gyrophoric acid because of great difficulties in separating both substances with TLC methods commonly used for the analyses of lichen products [22].

4. *U. arctica* (Ach.) Nyl., *U. freyi* Codogno, Poelt and Puntillo, *U. grisea* Hoffm., *U. haplocarpa* Nyl., *U. hyperborea* (Ach.) Hoffm., *U. josiae* Frey, *U. mühlenbergii* (Ach.) Schol., *U. nepalensis* Poelt, *U. nylanderiana* (Zahlbr.) H. Magn., *U. polyphylla* (L.) Baumg., *U. ruebeliana* Frey

Gyrophoric acid (2 to 6% dw), lecanoric acid (0.2 to 0.4% dw) and umbilicaric acid were detected in all species. The concentration of umbilicaric acid varies extremely. The depside occurs as a minor compound (0.1 to 0.7% dw) in all species mentioned above with the exception of *U. polyphylla* and *U. ruebeliana*. In these species umbilicaric acid (*ca.* 6% dw) is the major lichen substance and is always accompanied by an unknown depside, RT 14, which is expected to be closely related to umbilicaric acid in the biosynthetic sequence.

The secondary product pattern does not help to classify the species *U. arctica, U. hyperborea* and *U. nylanderiana,* whose separation is widely discussed [2, 21, 23–25]. The separation of *U. polyphylla* and *U. nylanderiana,* which are expected by Schade [7] to be only taxonomically unimportant variations of growth is also not possible. The secondary product pattern does not help to clear the taxonomic status of *U. freyi* and *U. grisea*.

5. *U. caroliniana* Tuck

U. caroliniana contains gyrophoric acid (6% dw), lecanoric acid (0.25% dw), hiascic acid (0.2% dw) and ovoic acid (0.1% dw). This secondary product pattern can be found in *U. proboscidea* and *U. torrefacta,* too, and will be discussed there.

6. *U. cinereorufescens* (Schaer.) Frey

HPLC analyses confirm the presence of crustinic acid (5.6% dw) as a main compound of this species [2]. As minor compounds gyrophoric acid (1% dw), lecanoric acid (0.1% dw) and the unknown depsides RT 12 and RT 16 (0.1% dw each) were detected.

The very characteristic secondary product pattern distinguishes *U. cinereorufescens* clearly from *U. vellea* (Fig. 2). Both species are often mixed up in herbarium specimens. In addition to chemical features *U. vellea* and *U. cinereorufescens* are characterized by their different shaped rhizinomorphs. In *U. cinereorufescens* long slender often branched rhizinomorphs never occur. Long slender rhizinomorphs are typical for *U. vellea* and can be found at least sporadically on all thalli. This characteristic was reported by Frey in detail [1].

The secondary product pattern helps to clear the taxonomic status of *U. trabeculata* and *U. cinereorufescens* which are also characterized by their different shaped rhizinomorphs [4].

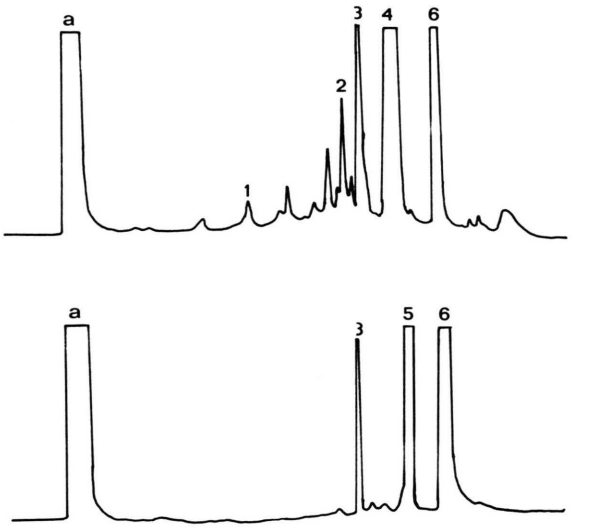

Fig. 2. Secondary product patterns of *U. cinereorufescens* (Frey 420) and *U. vellea* (Poelt 4663). a = acetone, 1 = unknown depside RT 12, 2 = unknown depside RT 16, 3 = lecanoric acid, 4 = crustinic acid, 5 = umbilicaric acid, 6 = gyrophoric acid. Chromatographic conditions: HPLC (Kontron): programmer 200, 2 pumps LC 410, integrator HP 3390A Rheodyne valve 7125 with 20 µl loop, UV detection at 260 nm (UVIKON 720LC). Column: Nucleosil RP-18 5 µ (Macherey & Nagel), 250 × 4 mm, gradient from 50% methanol to 90% in 30 min, from 90% to 100% in 1 min subsequently, 10 min constant at 100% methanol. Flow rate 0.7 ml/min, eluents are methanol and H_3PO_4 (1%).

7. *U. virginis* Schaer.

Umbilicaria virginis produces gyrophoric acid (0.5% dw), lecanoric acid (0.1% dw) and norstictic acid (0.2% dw). The depsidone often occurs in such low concentrations that sometimes it can only be demonstrated after concentrating the extracts.

8. *U. cylindrica* (L.) Del.

Within this species two chemical races were found, one without lichen substances and one with norstictic acid as a main compound (1% dw) accompanied by the accessory metabolite connorstictic acid (0.1% dw). According to Krog and Swinscow [3] *U. propagulifera* (Vain.) Llano is reduced to synonymy with *U. cylindrica*.

9. *U. crustulosa* (Ach.) Frey

U. crustulosa is very variable species which could be assigned to three chemotypes. The most simple secondary product pattern (chemotype a) shows gyrophoric acid as the major substance (3 to 6% dw) accompanied by lecanoric acid (0.1 to 0.2% dw) and hiascic acid (0.1 to 0.2% dw).

In chemotype b gyrophoric, lecanoric and hiascic acids are present in similar concentrations as in chemotype a. Additionally crustinic acid (0.5% dw) was detected.

In addition to the lichen substances of chemotype b chemotype c contains atranorin in very low concentrations (% dw < 0.1).

Chemotypes a and b are extreme forms of a sequence of secondary product patterns. The chemotypes are not related to morphological characters. For example, varieties of *U. crustulosa* cannot be correlated with chemotypes.

10. *U. krempelhuberi* Müll.-Arg.

The species belongs to two chemotypes, one with lecanoric acid (0.1% dw) and gyrophoric acid (3.7% dw) and one with lecanoric acid (0.2% dw), gyrophoric acid (4% dw), hiascic acid (0.1% dw) and crustinic acid (0.3% dw).

11. *U. spodochroa* (Ach.) Frey

In *U. spodochroa* four chemotypes were found. Chemotype a contains gyrophoric acid (4% dw) as the major compound. Minor substances are lecanoric acid (0.3% dw), hiascic acid (0.1% dw) and crustinic acid (0.5% dw). In addition to these substances atranorin was found in chemotype b (0.1% dw).

Chemotype c produces gyrophoric acid (4% dw), lecanoric acid (0.2% dw), hiascic acid (0.1% dw) and umbilicaric acid (0.3% dw). Chemotype d resembles chemotype c. The depside atranorin was detected as well (0.1% dw). In contrast to the closely related *U. crustulosa* crustinic acid could not be proven for this species so far.

Umbilicaria spodochroa is widespread all over Europe, especially in Scandinavia, in atlantic shapes. It also occurs commonly in Sardinia and Corsica.

12. *U. proboscidea* (L.) Schrad.

U. proboscidea shows 2 chemical races which are commonly mixed up. Chemical race a contains the depsides lecanoric acid (0.3% dw), hiascic acid (0.1% dw), ovoic acid (1% dw) and gyrophoric acid (4% dw). In addition to the substances of chemical race a the depsidones norstictic acid (1% dw) and connorstictic acid (0.1% dw) were detected in chemical race b.

13. *U. torrefacta* (Lightf.) Schrad.

Three different chemical races are characteristic of *U. torrefacta:* Chemical race a contains stictic acid (1.5% dw) and the accessory substances norstictic and cryptostictic acids and PCR-1 (0.1% dw, each). Chemical race b [4] produces in addition to the stictic acid complex gyrophoric acid (1.5% dw), ovoic acid (0.5% dw), hiascic acid (0.1% dw) and lecanoric acid (0.2% dw). In chemical race c lecanoric acid, hiascic acid, ovoic acid and gyrophoric acid are present. Stictic acid could not be found.

14. *U. vellea* (L.) Ach.

The secondary product pattern of *U. vellea* is not homogeneous. Three closely related chemotypes must be described. As mentioned for *U. crustulosa* the chemical patterns are extreme forms of a series and therefore are labelled as chemotypes. Chemotype a contains only lecanoric acid (0.2% dw) and gyrophoric acid (2% dw). Chemotype 2 is characterized by the occurrence of lecanoric acid (0.2% dw), hiascic acid (0.1% dw) and gyrophoric acid (3.5% dw). In chemotype c leca-

noric acid (0.2% dw), gyrophoric acid (3.5% dw) and umbilicaric acid (1% dw) are present. The chemotypes cannot be correlated to their geographic distribution.

Experimental

Material

Over 400 herbarium specimens from 33 species of the lichen genus *Umbilicaria* have been investigated. Because of the multitude of material only selected samples can be listed below. The specimens are preserved by various herbaria under the following numbers:

U. angulata (4 specimens): Canada: Kurokawa: Lich. Rar. Crit. Exs. 289, *U. arctica* (16 specimens): Iceland: Kristinsson 12716, Norway: ESS 3879, Hertel 15539, Poelt 2077, Sweden: Th. M. Fries: Lich. Scand. Exs. 1859–65 (1864, BM), Poelt 405, *U. caroliniana* (2 specimens): Japan: Kurokawa: Lich. Sel. Exs. 99, Korea: Huneck 4757, *U. cinereorufescens* (32 specimens): Nepal: Poelt 13653, Austria: Türk 5035, ESS 7258, Switzerland: ESS 4392, Spain: Sancho MAF Q10 (6), Frey 399, *U. crustulosa* (27 specimens): Norway: ESS 4504, Austria: Türk 1055, ESS 360, Spain: Sancho MAF Q26A (15II), *U. cylindrica* (31 specimens): Germany: ESS 4013, Norway: ESS 4785, Austria: ESS 3460, Switzerland: ESS 4410, *U. deusta* (32 specimens): France: ESS 2932, Canada: Brodo 6894, Korea: Huneck 4763, Austria: Türk 1938, Sweden: ESS 3345, Spain: Sancho MAF Q23A (12II), *U. esculenta* (4 specimens): Korea: Huneck 1509, Japan: Kurokawa & Kashiwadani: Lich. Rar. & Crit. Exs. 422, Kurokawa: Lich. Rar. & Crit. Exs. 16, *U. freyi* (2 specimens): France: Frey 1048, Spain: Frey 6167, *U. grisea* (15 specimens): Germany: Poelt 7492, Italy: ESS 1273, Romania: Vezda: Lich. Sel. Exs. 1288, Sweden: H: Flora Suecica 5313, Spain: Sancho MAF Q34A (13), *U. haplocarpa* (8 specimens): Argentina: Lamb 5556, Lesotho: Kofler 1963, Peru: Frey 13819, South Africa: Schelpe 1284, *U. hirsuta* (42 specimens): Lesotho: Kofler 1963 (Lund 3101115), Austria: Wittmann 0943, Sweden: ESS 6070, Switzerland: ESS 4412, Spain: G. Follmann: Lich. Exs. Sel. a Museo Hist. Nat. Cass. ed. 300, Sancho 1980, South Africa: Hilliard & Burff 12576, U.S.S.R.: Huneck U.S.S.R. 1990-20, *U. hyperborea* (29 specimens): Iceland: Kristinsson 996, CANL: Lich. Canad. 13037, Norway: ESS 4510, Austria: Türk 2727, Sweden: ESS 7053, U.S.S.R.: Zhurbenko LE7, *U. josiae* (2 specimens): France: Bagneur du Luchon, 1948 (BM), *U. koidzumii* (1 specimen): Japan: Koidzumi 1921 (Berne, Isotype), *U. krempelhuberi* (6 specimens): Argentina: Lamb 5237, Spain: ESS 2668, *U. mammulata* (5 specimens): Canada: Garton 20857, U.S.A. Egan El-4226, Culberson 12353, *U. mühlenbergii* (4 specimens): Canada: Scotter 6709 (H), Korea: Huneck 1058, Mongolian Peoples Republic: Huneck MVR-83-175, *U. nepalensis* (2 specimens): Nepal: Poelt 13577, Poelt 13659 (Isotype), *U. nylanderiana* (19 specimens): Andorra: ESS 2934, Bolivia: Poelt 9115, Austria: Türk 4602, Spain: ESS 9011, Venezuela: Oberwinkler & Poelt 7561, *U. polyphylla* (17 specimens): Germany: Türk 315, France: ESS 4019, Austria: Türk 1059, ESS 3489, Spain: Sancho, MAF Q41A (22), *U. polyrrhiza* (9 specimens): Germany: Poelt 4131, Finland: Laurila 1936 (H) United Kingdom: Türk 1313, Portugal: Tavares 8/1979 (BM), Poelt 711, Sweden: ESS 7057, Spain: Sancho MAF Q43 (23), *U. proboscidea* (15 specimens): Iceland: Kristinsson 17915, Norway: Mezger 132, Sweden: Hasselrot 1949 (Lund), Poelt 406, Spain: ESS 9018, U.S.S.R.: Huneck U.S.S.R. 1990-18, *U. ruebeliana* (13 specimens): France: ESS 1107, Poelt 9639, Yugoslavia: Vezda: Lich. Sel. Exs. 1330, Austria: Türk 9238, Switzerland: M: Lich. Alpium 120, Spain: Egea MAF Q47 (25), *U. soralifera* (4 specimens): Lesotho: Lund: Lich. Austroafricani, Kofler 1963, South Africa: Schelpe 1907, *U. spodochroa* (21 specimens): Italy: ESS 5368, Norway: ESS 4866, Portugal: Poelt 710, Sweden: Arnold 1101, Bäck 1961 (H), *U. thamnodes* (2 specimens): Nepal: Poelt 13578, *U. torrefacta* (19 specimens): Germany: ESS 4012, Finland: Malmström 1932 (H), Iceland: Kristinsson 12709, Sweden: Poelt 4984, Spain: Sancho MAF Q57A, U.S.S.R.: Zhurbenko LE4, *U. trabeculata* (1 specimen): Nepal: Poelt 13625 (Isotype), *U. umbilicarioides* (9 specimens): Kenia: Frey 15510, Kongo: Schelpe 1963 (Isotype), South Africa: Esterhuysen 9302 (Cotype), *U. vellea* (33 specimens): France: ESS 3456, Norway: Poelt 13552, Austria: Türk 2796, Spain: MAF Q61 (30), South Africa: Schelpe 1972, U.S.A.: Lich. Exs. Univ. Colorado Mus., Boulder 38, *U. virginis* (15 specimens): Finland: Poelt 6931, Austria: Poelt 410, Spain: Top-

ham 1976 (BM), Egea MAF Q62A (31 I), Turkey: Poelt 2088, U.S.A.: Shushan sl-5761, *U. yunnana* (3 specimens): China: Wei 2718, Nepal: Poelt L280.

Methods

The equipment and the conditions used in the HPLC analyses have been described before [12, 26–28].

Identification and quantification of the lichen substances are carried out by comparison of retention times, UV spectra in methanol, cochromatography with authentic reference substances (J. A. Elix, Canberra) and comparison of hydrolysis products obtained from heating the lichen substances [28]. Additionally the identity of gyrophoric acid and umbilicaric acid is proved by means of mass spectrometry of a sample of *U. hyperborea* (ESS 2777, C. Leuckert, pers. comm.).

Extraction solvents

It is unavoidable to use acetone as extraction solvent though acetone reacts with hiascic acid to give hiascic acid acetale [28]. Methanol, which could be used alternatively because of the good solubility of lichen substances, cannot be used here because methanol reacts with depsides during the extraction period. During this reaction methylesters of the depsides are formed. These substances can be confused easily with naturally occurring lichen substances [11, 29].

Quantification

Quantitative data are expressed as percentage of dry weight (% dw). Especially in case of unknown substances a calibration cannot be presented. The calibration curve of gyrophoric acid is used instead to obtain quantitative data of unknown depsides. Unknown depsidones are calibrated by means of the calibration curve of stictic acid.

Safe statistical data cannot be presented because the total content of lichen substances within individuals of one species living under the same conditions on one location varies extremely. The examination of 20 thalli of *Lasallia pustulata* (Wittmann 7552/1) and *Lasallia hispanica* (ESS 4252) shows deviations from the total content of lichen substances of about 25%. These deviations can be expected for all other investigated *Umbilicaria* species, too.

Isolation of crustinic acid

Umbilicaria crustulosa (80 g) was homogenized and extracted at room temperature for 2 h with diethyl ether (2 l). The extract was reduced to approx. 100 ml and the less soluble gyrophoric acid removed by filtration. The filtrate was reduced to a few milliliters, the crustinic acid separated, washed with a small amount of diethyl ether and dried at room temperature: 0.025 g (0.03%) of a brownish powder.

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