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NMR ASSIGNMENTS OF DEPSIDES AND TRIDEPSIDES OF THE LICHEN FAMILY UMBILICARIACEAE

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Key Word Index—Umbilicariaceae; chemotaxonomy; crustinic acid; deliseic acid; hiascic acid; lasallic acid; ovoic acid; papulosic acid; umbilicaric acid; NMR.

Abstract—NMR spectral analysis provides important information for the identification of secondary products of chemotaxonomic significance in the lichen genera *Umbilicaria* and *Lasallia* (Umbilicariaceae). Two depsides (evernic and lecanoric acids) and eight tridepsides (crustinic, gyrophoric, hiascic, lasallic, 4-*O*-methylgyrophoric, ovoic and umbilicaric acids and tenuiorin) were isolated from various unrelated lichens. Seven of the ten compounds are important taxonomic characters in the family Umbilicariaceae. ^{1}H and ^{13}C NMR spectral signals were assigned for the compounds and for methyl esters of lecanoric, evernic and gyrophoric acids. Using these NMR data and mass spectrometry, chemical structures were elucidated for two new compounds, papulosic acid (2,6-dihydroxy-3-carboxy-4-methylphenyl orsellinate) from the umbilicariaceous *Lasallia papulosa* and deliseic acid (lecanoryl 3-acetoxy-4,6-dihydroxy-2-methylbenzoate) from the parmeliaceous *Cetrariella delisei*.

INTRODUCTION

A recent chemotaxonomic study of the Umbilicariaceae [1] identified the secondary metabolites of representatives of 56 species by TLC and HPLC. Most samples contained small amounts of lecanoric acid (**1**), with large amounts of gyrophoric acid (**5**). Although the proportion of **5** is often very high, ca 80% of the samples tested also contained one or two of five tridepsides, hiascic (**6**), ovoic (**7**), umbilicaric (**8**), crustinic (**9**) [2] and lasallic (**10**) [3] acids. These additional tridepsides are the secondary products most useful for the chemotaxonomy of this family. Compounds **6**–**8** are widely distributed in *Umbilicaria*, whereas *Lasallia* lacks **7** and **8**. About half of the *Lasallia* species contain **10** and the remainder contain **6** instead. Although species of *Lasallia* with compound **10** may have this substance in high concentration, the closely related tridepside **9** seems to be restricted to *Umbilicaria*. Compounds **9** and **10** show strong generic correlations within the family, differing chemically only in the position of the ester linkage to the C-ring. Furthermore, these two compounds may have a higher-level taxonomic significance in that they are known only in the Umbilicariaceae and are also the only tridepsides possessing both *para*- and *meta*-depside linkages.

Most chemotaxonomic surveys of lichens have relied heavily on TLC and HPLC, where standardized data for lichen products are readily available. On the other hand, chemists searching for new and potentially useful natural products generally prefer to isolate pure samples suitable for study by modern spectroscopic methods. These techniques are sufficiently powerful to indicate the structures of new compounds while also confirming known compounds tentatively identified by TLC and HPLC. However, many of the available NMR data recently summarized [4] were not obtained under comparable conditions. The aim of the present study was to provide critical and comparable NMR data that can be used to confirm the identification of depsides and tridepsides related to lecanoric and gyrophoric acids using the amounts (ca 5 mg) of compounds that can be isolated by preparative HPLC from small samples of accurately identified lichen thalli. The methods described and the data presented can confirm the identification of known (but difficult to distinguish) depsides and tridepsides, such as those in *Umbilicaria* and *Lasallia*, and can help to

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Table 1. ^{1}H NMR spectral data of compounds **1–16**

Proton No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
3	6.22 (*d*, *J* = 2.14 Hz)	6.22 (*d*, *J* = 2.02 Hz)	6.35 (*d*, *J* = 2.14 Hz)	6.35 (*d*, *J* = 2.44 Hz)	6.24 (*d*, *J* = 2.14 Hz)	6.31 (*s*)	6.25 (*d*, *J* = 1.98 Hz)	6.37 (*d*, *J* = 2.14 Hz)
5	6.21 (*d*, *J* = 2.14 Hz)	6.21 (*d*, *J* = 2.02 Hz)	6.39 (*d*, *J* = 2.14 Hz)	6.39 (*d*, *J* = 2.44 Hz)	6.24 (*d*, *J* = 2.14 Hz)		6.25 (*d*, *J* = 1.98 Hz)	6.31 (*d*, *J* = 2.14 Hz)
8	2.34 (*s*)	2.33 (*s*)	2.37 (*s*)	2.37 (*s*)	2.37 (*s*)	2.22 (*s*)	2.42 (*s*)	2.28 (*s*)
3′	6.59 (*d*, *J* = 2.14 Hz)	6.60 (*d*, *J* = 1.53 Hz)	6.62 (*d*, *J* = 2.14 Hz)	6.61 (*d*, *J* = 1.83 Hz)	6.70 (*d*, *J* = 1.68 Hz)	6.66 (*d*, *J* = 1.98 Hz)	6.94 (*d*, *J* = 1.83 Hz)	6.66 (*d*, *J* = 1.98 Hz)
5′	6.57 (*d*, *J* = 2.14 Hz)	6.58 (*d*, *J* = 1.53 Hz)	6.59 (*d*, *J* = 2.14 Hz)	6.59 (*d*, *J* = 1.83 Hz)	6.67 (*d*, *J* = 1.68 Hz)	6.64 (*d*, *J* = 1.98 Hz)	6.84 (*d*, *J* = 1.83 Hz)	6.62 (*d*, *J* = 1.98 Hz)
8′	2.37 (*s*)	2.22 (*s*)	2.36 (*s*)	2.22 (*s*)	2.38 (*s*)	2.36 (*s*)	2.38 (*s*)	2.38 (*s*)
3″					6.65 (*d*, *J* = 1.98 Hz)	6.62 (*d*, *J* = 1.98 Hz)	6.62 (*d*, *J* = 1.98 Hz)	6.65 (*d*, *J* = 2.24 Hz)
5″					6.62 (*d*, *J* = 1.98 Hz)	6.59 (*d*, *J* = 1.98 Hz)	6.59 (*d*, *J* = 1.98 Hz)	6.61 (*d*, *J* = 2.24 Hz)
8″					2.40 (*s*)	2.37 (*s*)	2.40 (*s*)	2.40 (*s*)
2-OCH_3								3.79 (*s*)
4-OCH_3			3.75 (*s*)	3.75 (*s*)				
2′-OCH_3							3.87 (*s*)	
7′-OCH_3		3.80 (*s*)		3.80 (*s*)				
7″-OCH_3								
2-OH	10.31 (*s*)	10.29 (*s*)	10.38 (*s*)		10.29 (*s*)	9.57 (*s*)	10.38 (*s*)	
4-OH	10.00 (*s*)	9.99 (*s*)						
5-OH						7.90 (*s*)		
2′-OH		10.26 (*s*)			10.42 (*s*)	10.45 (*s*)		10.33 (*s*)
2″-OH					9.96 (*br s*)	9.87 (*s*)	10.04 (*br s*)	9.81 (*br s*)
5-OAc								

elucidate the chemical structures of new compounds separated by preparative HPLC.

In the present study, ^{1}H and ^{13}C NMR signals (Tables 1 and 2) were assigned to 13 reference depsides and tridepsides (**1–13**) by a series of DEPT, ^{1}H–^{13}C COSY (HMQC), NOESY and HMBC experiments. Ten of the 13 reference compounds came from various lichens in the Umbilicariaceae and from unrelated species: compounds **8** and **9** from *Umbilicaria polyphylla* (L.) Baumg. and *U. cinereorufescens* (Schaer.) Frey, respectively; **5** and **10** from *Lasallia papulosa* (Ach.) Llano; evernic acid (**3**), 4-*O*-methylgyrophoric acid (**12**) and compound **7** from *Evernia prunastri* (L.) Ach., *Lobaria* sp. and *Melanelia tominii* (Oksner) Essl. (= *Parmelia substygia* Räs.), respectively; tenuiorin (**13**) from *Peltigera aphthosa* (L.) Willd.; and compounds **1** and **6** from *Cetrariella delisei* (Bory ex Schaer.) Karnefelt & Thell (= *Cetraria delisei* (Bory ex Schaer.) Th. Fr.). Additionally, three methyl esters—methyl lecanorate (**2**), methyl evernate (**4**) and methyl gyrophorate (**11**)—were prepared from the corresponding free acids. During isolation of the known depsides and tridepsides, the two new lichen products papulosic acid (**14**) and deliseic acid (**15**), were obtained from *Lasallia papulosa* and *Cetrariella delisei*, respectively. In the course of elucidation of the structure of **15**, another compound **16** was obtained. Reference NMR data obtained for the 13 known depsides and tridepsides were used in large part to elucidate the chemical structures of these new compounds.

RESULTS AND DISCUSSION

The ^{1}H NMR (500 MHz, DMSO-d_6) spectral data for **1–16** are shown in Table 1. (The numbering of the carbons follows the standard of common names of depsides and depsidones where the numbers for biogenetically equivalent positions remain the same, and C-7 and C-8 refer to the carbons of the carboxylic acid and the methyl group, respectively [5]). ^{13}C NMR (125 MHz, DMSO-d_6) spectral data of **1–16** with the results of DEPT (90° and 135°) and ^{1}H–^{13}C COSY experiments are shown in Table 2. NOESY, HMBC and HMQC correlations were all reasonable for in the structures **1–16** (Scheme 1).

All proton and skeletal-carbon NMR signals of depside and tridepsides important for the chemotaxonomy of Umbilicariaceae are assigned by the experiments reported here. These assignments will find application in the identification and chemotaxonomy of umbilicariaceous species, as well as lichens in other families that have species characterized by the production of these widely distributed classes of orsellinic acid-derived depsides and tridepsides. A study of the new compounds **14** and **15** demonstrates the application of the reference NMR data to the identification of two new compounds, a depside from *Lasallia* (Umbilicariaceae) and a tridepside from *Cetrariella* (Parmeliaceae).

Compound **14** was obtained from the acetone extract of *Lasallia papulosa* during preparative-HPLC separation of **1**, **5** and **10**. Compound **14** was also detected by analytical HPLC in the three additional species *L. asiae-orientalis* (Asah.) Sato, *L. mayebarae* (Sato) Asah., and *L. sinorientalis* Wei, all of which contain compound **10**. The consistent occurrence of a low proportion of **14** in all species containing high to moderate concentrations of **10**, coupled with the significantly lower HPLC R_t of **14** compared with **10**, suggested that this new compound might be the *meta-*

(chemical shifts (ppm) in DMSO-d_6 at 500 MHz)

9	10	11	12	13	14	15	16
6.23 (*s*)	6.25 (*d*, J = 1.82 Hz)	6.23 (*d*, J = 2.14 Hz)	6.38 (*d*, J = 1.99 Hz)	6.36 (*d*, J = 2.42 Hz)	6.21 (*d*, J = 1.83 Hz)	6.41 (*s*)	6.53 (*s*)
6.23 (*s*)	6.24 (*d*, J = 1.82 Hz)	6.22 (*d*, J = 2.14 Hz)	6.41 (*d*, J = 1.99 Hz)	6.40 (*d*, J = 2.42 Hz)	6.27 (*d*, J = 1.83 Hz)		
2.38 (*s*)	2.38 (*s*)	2.35 (*s*)	2.41 (*s*)	2.38 (*s*)	2.49 (*s*)	2.10 (*s*)	2.14 (*s*)
6.68 (*d*, J = 1.68 Hz)	6.70 (*d*, J = 1.98 Hz)	6.67 (*d*, J = 1.83 Hz)	6.71 (*d*, J = 1.68 Hz)	6.68 (*d*, J = 2.02 Hz)		6.67 (*d*, J = 0.92 Hz)	6.69 (*d*, J = 1.83 Hz)
6.66 (*d*, J = 1.68 Hz)	6.69 (*d*, J = 1.98 Hz)	6.66 (*d*, J = 1.83 Hz)	6.68 (*d*, J = 1.68 Hz)	6.67 (*d*, J = 2.02 Hz)	6.33 (*s*)	6.65 (*d*, J = 0.92 Hz)	6.67 (*d*, J = 1.83 Hz)
2.44 (*s*)	2.51 (*s*)	2.35 (*s*)	2.39 (*s*)	2.36 (*s*)	2.45 (*s*)	2.35 (*s*)	2.36 (*s*)
6.35 (*s*)		6.64 (*d*, J = 1.83 Hz)	6.63 (*d*, J = 1.99 Hz)	6.63 (*d*, J = 1.92 Hz)		6.60 (*d*, J = 1.83 Hz)	6.63 (*d*, J = 1.68 Hz)
	6.39 (*s*)	6.61 (*d*, J = 1.83 Hz)	6.60 (*d*, J = 1.99 Hz)	6.61 (*d*, J = 1.92 Hz)		6.58 (*d*, J = 1.83 Hz)	6.61 (*d*, J = 1.68 Hz)
2.38 (*s*)	2.46 (*s*)	2.24 (*s*)	2.41 (*s*)	2.24 (*s*)		2.37 (*s*)	2.24 (*s*)
			3.77 (*s*)	3.75 (*s*)			3.76 (*s*)
		3.81 (*s*)		3.81 (*s*)			3.81 (*s*)
10.28 (*s*)	10.30 (*s*)		10.31 (*s*)	10.39 (*br s*)	10.26 (*br s*)	10.15 (*s*)	10.34 (*s*)
10.40 (*s*)	10.45 (*s*)		10.38 (*s*)	10.47 (*br s*)	10.09 (*s*)	10.56 (*s*)	10.49 (*s*)
9.93 (*br s*)	9.96 (*br s*)	10.27 (*br s*)		10.28 (*br s*)		10.22 (*br s*)	10.29 (*s*)
						2.26 (*s*)	2.28 (*s*)

depside combining the B- and C-rings of the tridepside **10**. Such a pattern of joint occurrence parallels that observed for the depside-tridepside pair **1** and **5**, presumably reflecting a precursor-product relationship for the compounds and probably also some decomposition of the tridepside **5** to the depside **1** over the long time (many years) that these compounds reside within the thallus of the lichen.

The sample of *Lasallia papulosa* used to isolate **14** contained **10** in high concentration (estimated from TLC spot sizes). In addition, peak-area proportions of **5**:**1** and **10**:**14** were very similar, and the chromatographic behaviors (retentions by normal-phase TLC and reverse-phase HPLC) were consistent with two depside-tridepside pairs, in which each depside differed from its associated tridepside by lacking one orsellinic acid unit. FAB mass spectrometry of **14** gave an $[M+H]^+$ m/z 335 indicating M_r 334. HR FAB mass spectrometry of **14** gave the formula $C_{16}H_{14}O_8$. Similarly, compound **1** showed an $[M+H]^+$ at m/z 319 indicating M_r 318. HR FAB mass spectrometry of **1** gave the formula $C_{16}H_{14}O_7$. Both **1** and **14** showed a fragment peak at m/z 151 indicating one orsellinic acid moiety in the molecule. ^1H NMR of **14** showed singlets at δ 2.49 (3H) and 2.45 (3H) for protons attached to C-8 and C-8′, respectively. Other ^1H NMR signals of **14** were a pair of doublets at δ 6.21 and 6.27 (1H each, J = 1.83 Hz) for two A-ring aromatic protons and a singlet at δ 6.33 (1H) for one B-ring proton. Low-field singlets of compound **14** at δ 10.26 (1H, *br*) and 10.09 (1H) can be assigned to two hydroxyl groups substituted at positions 2 and 2′, respectively.

The chemical shifts and ^1H–^{13}C COSY signals for the skeletal carbons of the A-ring of **14** were identical to those of **1**. Compound **1** showed three B-ring ^1H–^{13}C COSY signals due to 3′-CH, 5′CH and 8′CH_3. On the other hand, **14** showed two B-ring ^1H–^{13}C COSY signals, one for the 8′-CH_3 and the other for the 5′-CH, as in the C-ring ^1H–^{13}C COSY signals of compound **10**. Like **1**, compound **14** showed two NOESY from the A- and B-rings (δ 2.49–6.27 and 2.45–6.33, respectively), which can be assigned to those between the methyl (8-CH_3 and 8′-CH_3) and the methine (5-CH and 5′-CH) groups of each ring. A high-field signal for the 8′-CH_3 of **14** was at δ 22.50 indicating no free or esterified hydroxyl at C-5′. Finally, the chemical shifts and ^1H–^{13}C COSY signals for the skeletal carbons of the B-ring of **14** were identical to those of **10**. Consequently, **14** should have the same *meta*-depside B-C ring structure shown for compound **10**. All NMR data of **14** agree with the structure proposed. Hence, the structure of **14** is established as 2,6-dihydroxy-3-carboxy-4-methylphenyl orsellinate.

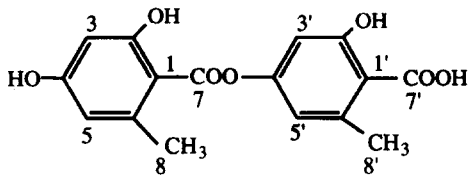
Compound **15** was found in the acetone extract of *Cetrariella delisei* during the isolation of compounds **1**, **5** and **6** by HPLC. FAB mass spectrometry of **15** gave $[M+H]^+$ at m/z 527 and $[M-H]^-$ at m/z 525, indicating M_r 526 and the formula $C_{26}H_{23}O_{12}$. Fragment HR FAB mass spectrometry showed peaks at m/z 359.07693 for $C_{18}H_{15}O_8$, and m/z 209.04503 for $C_{10}H_9O_5$. This means that A-ring of **15** must have an acetyl group.

Compound **6** and **15** showed similarities in TLC spot colours before and after visualization. In addition, on unsprayed plates exposed to the air, the pinkish-beige spot of **15** turned to a characteristic reddish pink within 2–3 h, as previously observed for compound **6**. This colour change appears to be characteristic of orcinol-type tridepsides oxidized at position 5 of the A-ring.

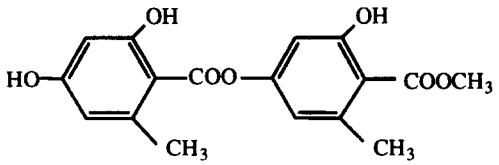
Table 2. ^{13}C NMR spectral data of compounds **1**–**16** (chemical shifts (ppm) in DMSO-d_6 at 125 MHz)

Carbon No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	108.23	108.37	110.54	110.68	108.23	108.84	107.67	112.82	108.15	108.19	108.42	110.74	110.74	105.49	110.11	111.04
2	160.15	159.99	159.25	159.13	160.08	150.97‡	160.89	158.44	160.54	160.15	159.91	159.41	159.06	162.52	155.14‖	155.17
3	100.47	100.46	98.98	98.98	100.49	100.79	100.70	97.01	100.73	100.52	100.47	99.25	98.99	100.47	101.55	98.30
4	161.11	161.04	162.14	162.09	161.08	149.46‡	161.54	159.98	161.37	161.12	161.04	162.35	162.08	162.00	156.29‖	153.54
5	109.86	109.80	108.10	108.04	109.85	136.20	110.31	109.04	110.17	109.90	109.78	108.31	108.00	110.87	130.28	130.55
6	140.31	140.18	139.72	139.60	140.21	123.97	141.95	137.77	140.54	140.30	140.14	139.83	139.57	142.33	130.57	129.95
7	167.12*	167.14	166.68†	166.72	167.07	167.00	167.47	165.36	167.33	167.04	167.07**	166.80	166.67††	166.47§§	167.13¶¶	165.76***
8	21.33	21.24	20.91	20.83	21.22	13.56	21.78	19.20	21.49	21.26	21.19	20.96	20.80	22.88	13.58	13.29
1′	116.52	118.63	116.70	118.74	117.88	117.94	119.91	117.78	117.92	116.39	118.04	118.16	118.14	105.42	118.27	118.25
2′	159.22	156.16	158.75	156.14	156.30	156.25	157.52	156.30	156.82	157.78	156.21	156.51	156.21‡‡	156.36	156.37	156.21
3′	107.36	107.05	107.32	107.01	107.17	107.13	104.01	106.96	107.22	107.34	107.17	107.30	107.13	123.75	107.18	107.06
4′	152.19	151.66	152.21	151.64	152.18	152.25	152.46	152.23	152.18	152.52	152.07	152.26	152.05	153.20	152.20	152.04
5′	114.61	114.09	114.67	114.02	114.17	114.18	115.73	113.92	114.51	114.72	114.17	114.29	114.12	110.03	114.24	114.06
6′	139.62	137.80	139.47	137.82	137.95	137.94	137.38	137.96	138.99	139.75	137.87	138.17	137.91	139.09	138.16	137.96
7′	170.62*	168.04	170.53†	168.02	165.52	165.62§	165.19‖	165.42¶	165.85	165.06	165.67**	165.66	165.66††	172.70§§	165.79¶¶	165.65***
8′	21.08	19.44	20.91	19.42	19.26	19.28	18.85	19.20	19.77	20.37	19.21	19.43	19.23	22.50	19.40	19.22
1″					116.80	117.17	117.27	116.33	106.54	105.07	118.99	116.89	119.01		117.45	119.04
2″					158.77	158.78	152.11	158.95	160.12	156.35	156.11	159.54	156.10‡‡		158.89	156.09
3″					107.14	107.13	107.19	107.09	101.36	123.86	106.85	107.32	106.85		107.21	106.86
4″					152.08	152.07	159.29	152.31	153.63	153.64	151.60	152.33	151.59		152.14	151.52
5″					114.43	114.32	114.30	114.40	130.61	110.50	113.83	114.41	113.84		114.42	113.84
6″					139.56	139.51	139.86	139.67	133.37	139.46	137.87	139.97	137.88		139.67	137.89
7″					170.37	170.39§	170.47‖	170.34	172.20	173.22	167.93	170.56	167.94		170.50	167.94
8″					20.84	20.87	21.03	20.87	14.61	23.17	19.34	21.16	19.36		20.98	19.36
2-OCH_3								55.71								
4-OCH_3			55.19	55.19								55.33	55.19			55.84
2′-OCH_3							56.58									
7′-OCH_3		51.93		51.92												
7″-OCH_3											51.92					51.95
5-OAc(C=O)													51.93		168.95	168.95
5-OAc(CH_3)															20.34	20.36

*,†,‡,§,‖,¶,**,††,‡‡,§§,‖‖,¶¶,*** assignment may be interchanged.



lecanoric acid (1)



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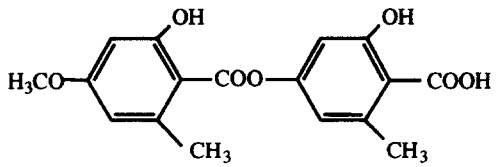

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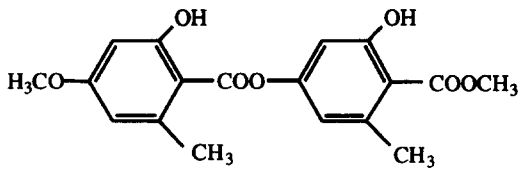

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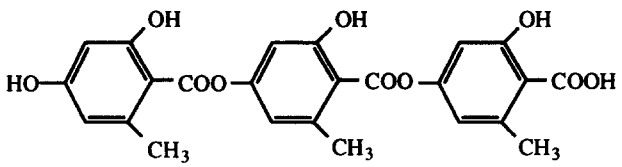

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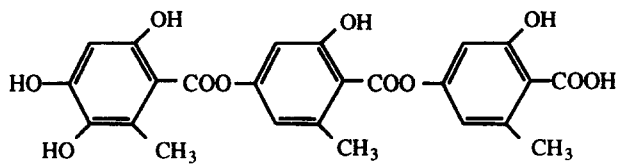
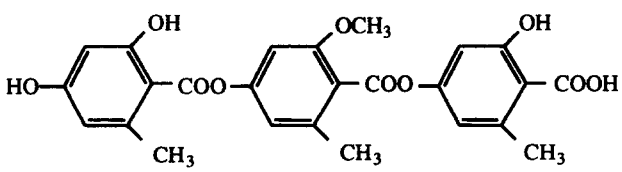
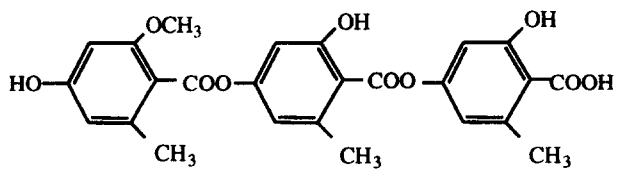


hiascic acid (6)

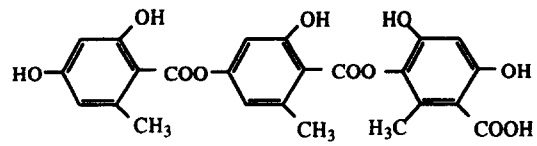


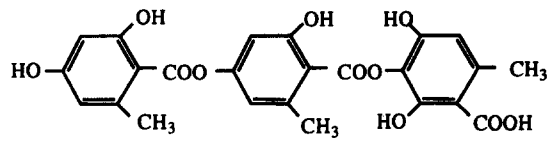
ovoic acid (7)

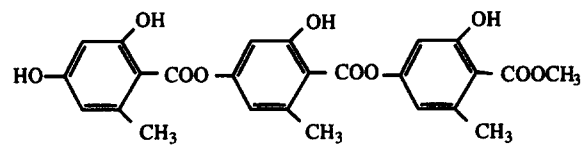


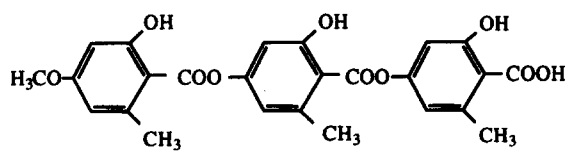
umbilicaric acid (8)

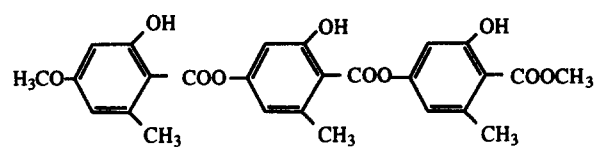
Scheme 1.

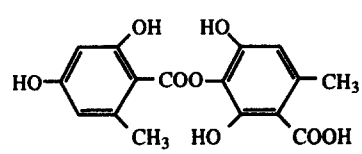

crustinic acid (9)

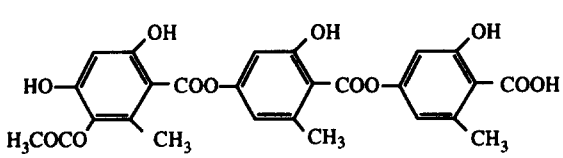

lasallic acid (10)

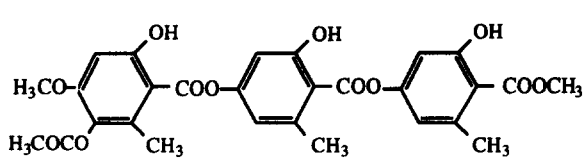

methyl gyrophorate (11)


4-O-methylgyrophoric acid (12)


tenuiorin (13)


papulosic acid (14)


deliseic acid (15)


methyl 4-O methyldeliseate (16)

Scheme 1—*Continued.*

Compound **15** showed singlet methyl groups at δ 2.10 (3H), 2.35 (3H) and 2.37 (3H) for protons attached to C-8, C-8′ and C-8″, respectively, and gave another singlet methyl at δ 2.26 (3H) attached to a carbonyl group. Other ^{1}H NMR signals were a singlet at δ 6.41 (1H) for one A-ring aromatic proton, a pair of doublets at δ 6.65 and 6.67 (1H each, J = 0.92 Hz) for two B-ring aromatic protons and a pair of doublets at δ 6.58 and 6.60 (1H each, J = 1.83 Hz) for two C-ring aromatic protons. Low-field singlets at δ 10.15

(1H), 10.22 (1H, *br*) and 10.56 (1H) can be assigned to three hydroxyl groups substituted at positions 2, 2″ and 2′, respectively.

The chemical shifts and ^{1}H–^{13}C COSY signals for the skeletal carbons of the B-and C-rings of compound **15** were identical to those of **5** and **6**. Compound **5** showed three A-ring ^{1}H–^{13}C COSY signals due to the 3-CH, the 5-CH and the 8-CH_3. On the other hand, compound **6** showed two A-ring ^{1}H–^{13}C COSY signals due to the 3-CH and the 8-CH_3. **15** also showed two ^{1}H–^{13}C COSY signals, one for the 8-CH_3 and the other for an A-ring CH. Like **5**, compound **15** showed two NOESY from the B- and C-rings, which can be assigned to those between the methyl (8′-CH_3 and 8″-CH_3) and the methine (5′-CH and 5″CH) groups of each ring. The ^{13}C NMR (DMSO-d_6) signal for the A-ring methyl (C-8) was at δ 13.6 ppm. This value was near ca 15 ppm, indicating a free or esterified hydroxyl at C-5. Comparison between the values for the A-ring skeletal carbons of the A-ring for compounds **15** and **5**, showed that in compound **15**, positions *ortho* and *meta* to C-5 exhibited equal acetylation shifts. The same acetylation shifts were observed for **15** and **6**. These results indicate an acetylated hydroxyl at position 5 of the A-ring.

Compound **15** was treated with trimethylsilyl-diazomethane to methylate the free carboxylic acid on the C-ring and a free phenolic hydroxyl on the A-ring. The final proof of the structure of **15** came from the strong NOE observed in this methylation product between the 4-methoxyl and the C-3 proton. Irradiation of the C-3 proton showed NOE from the C-4 methoxyl (13.9%). Irradiation of the methoxyl substituted at C-4 gave NOE (16.4%). From these observations, the structure of **15** was established as lecanoryl 3-acetoxy-4,6-dihydroxy-2-methylbenzoate.

EXPERIMENTAL

Lichen materials

Voucher specimens of lichens used are in the herbaria of the Meiji College of Pharmacy and Duke University: (1) *Peltigera aphthosa* (9.6 g, Canada, Alberta, Grande Cache Highway, 53° 24'N, 118° 01'W, on soil, *T. Narui*, 29 July 1995), (2) *Cetrariella delisei* (2.02 g, Japan, Hokkaido, Prov. Ishikari, south slope of Mt Akadake, Daisetsu Mts, on soil, ca 2000 m, *S. Kurokawa* 71132, 18 September 1971; as *Kurok.*, *Lich. Rarr. Crit. Exs.* 204), (3) *Cetrariella delisei* (69.70 g, Iceland, N.-Mul.: Jokuldalsheioi, near Armotasel, on soil, ca 550 m, *H. Kristinsson* 17625, 7 August 1968), (4) *Umbilicaria polyphylla* (2.32 g, France, Basses-Pyrenees, near Col du Pourtalet., on rocks in alpine meadow, *W. L. Culberson* 14008 and *C. F. Culberson*, 11 June 1964), (5) *Lasallia papulosa* (2.1 g, U.S.A., North Carolina, Stokes County, Hanging Rock State Park, near Window Falls, on exposed rock face, *W. L. Culberson* 22284 and *C. F. Culberson*, 18 September 1994) and (6) *Lasallia papulosa* (2.12 g, U.S.A., Tennessee, Carter County, Doe River, rocks along the river, *J. P. Dey*, 7 August 1994). Both surfaces of all lichens were cleaned under a dissecting microscope to remove contaminants.

Extraction

Cleaned thalli were pulverized and extracted for 20 min at 40°C with Me_2CO (100–200 ml) and ultrasound. Filtered extracts were evapd *in vacuo* to dryness (Me_2CO extract of *P. aphthosa*: 502 mg, 5.2%; *C. delisei*: 176 mg, 8.7%; *U. polyphylla*: 204 mg, 8.8%; *L. papulosa*: 329 mg, 15.7%; *L. papulosa*: 283 mg, 13.3%).

Preparative HPLC

Me_2CO extracts containing lichen secondary metabolites were separated by HPLC using a Beckman Ultrasphere ODS-5 μ (10 × 250 mm) column with UV (254 nm) detection. Solvents: A, 30% MeOH containing 1% H_3PO_4; B, MeOH. Gradient: linear from 20 to 85% B (40 min), holding 85% B (20 min), linear from 85 to 95% B (5 min), holding 95% B (5 min). Flow rate: 2 ml min^{-1}. Analysis time: 90 min. Isolation of each compound by HPLC was continued until 10–90 mg of each compound had been isolated.

TLC

TLC was by a standardized method using three solvent systems (A–C) and control lanes of atranorin and norstictic acid [6].

Methylation

In separate reactions, compounds **1**, **3** and **5** (20–30 mg each) were treated with trimethylsilyl-diazomethane for 10 min to methylate their C-ring carboxylic acids. The reaction mixts were poured into ice-H_2O and the solns extracted with methyl *tert*-butyl ether. Residues on evapn of solvent were purified by prep. HPLC, yielding the Me esters **2**, **4** and **11**.

Lecanoric acid (**1**). The Me_2CO extract of *C. delisei* was separated by HPLC under the conditions described above. Two depsides were isolated, **1** (26 mg) and **6** (21 mg). Compounds **1** and **6** were purified further by repeated HPLC (2×). TLC: R_f Classes A3B5C3.

Methyl lecanorate (**2**). Compound **1** (18.4 mg) was methylated with trimethylsilyldiazomethane for 10 min as described above. Compound **2** was separated from the reaction mixt. and further purified by HPLC. TLC: R_f Classes A5B5/5-6C5.

Evernic acid (**3**). Compound **3** was an authentic sample isolated earlier from *Evernia prunastri* [7]. TLC: R_f Classes A4B6/5-6C5.

Methyl evernate (**4**). Compound **3** (23 mg) was

methylated and the product was purified by HPLC as described above. TLC: R_f Classes A7B6C7.

Gyrophoric acid (**5**). Compound **5** (60 mg) was separated and purified by HPLC (as described for **1**) using an Me_2CO extract of a sample of *L. papulosa* that contained a significant proportion of **10**. TLC: R_f Classes A3B5C3.

Hiascic acid (**6**). Compound **6** (21 mg) was prepared from *C. delisei* as described above. TLC: R_f Classes A2B4/5C2; spot colour pinkish beige, turning yellow after visualization with 10% H_2SO_4 and heat.

Ovoic acid (**7**). Compound **7** (5.6 mg) was an authentic sample isolated earlier from *Melanelia tominii* [8] and kindly supplied by Siegfried Huneck. TLC: R_f Classes A3B4C3/2–3.

Umbilicaric acid (**8**). The Me_2CO extract of *U. polyphylla* was separated by HPLC, and compound **8** (90 mg) was purified by HPLC (2×). TLC: R_f Classes A3B4C3.

Crustinic acid (**9**). Compound **9** (34 mg) was obtained from the Me_2CO extract of *U. cinereorufescens* and purified as described for **1**. TLC: R_f Classes A2B4C2; spot colour beige, turning to blackish brown after visualization with 10% H_2SO_4.

Lasallic acid (**10**). Compound **10** (40 mg), obtained from the Me_2CO extract of *L. papulosa* along with **5** and **14**, was purified as described for **1**. TLC: R_f Classes A2B4/5C2; spot colour yellow, turning to yellowish brown after visualization with 10% H_2SO_4 and heat.

Methyl gyrophorate (**11**). Compound **5** (30.8 mg) was methylated with trimethylsilyldiazomethane as described above. Compound **11** (5.8 mg) was separated from the reaction mixture by HPLC under the same conditions used for **1**. TLC: R_f Classes A5B5C5.

4-O-*Methylgyrophoric acid* (**12**). Compound **12** (5.8 mg) was an authentic sample isolated from *Lobaria* sp. [9]. TLC: R_f Classes A4/3BC7/6.

Tenuiorin (**13**). Compound **13** (10 mg) was obtained from the Me_2CO extract of *Peltigera aphthosa* and purified as described for **1**. TLC: R_f Classes A6B6C6.

Papulosic acid (**14**). Compound **14** (9.7 mg), obtained from *L. papulosa* along with compounds **5** and **10**, was purified as described for **1**. TLC: R_f Classes A2B4C2.

Deliseic acid (**15**). Compound **15** (31 mg) was obtained from the Me_2CO extract of *C. delisei* along with compounds **1** and **6**, and purified as described for **1**. Colourless microcrystals from Me_2CO mp (uncorr) 191–192.5°, softening from 188°. TLC: R_f Classes A2B4C2–3.

Methyl 4-O-*methyldeliseate* (**16**). Compound **16** was obtained by methylation of **15** (17.8 mg) with trimethylsilyldiazomethane for 2 h at room temp. The reaction mixt. contained a small amount of Me deliseate and **16**. Compound **16** (9 mg) was separated and purified by repeated HPLC (2×).

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