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Two New Fumarprotocetraric Acid Lactones Identified and Characterized by UHPLC-PDA/ESI/ORBITRAP/MS/MS from the Antarctic Lichen *Cladonia metacorallifera*

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Abstract: Lichens are symbiotic organisms between algae and fungi, which are makers of secondary compounds named as lichen substances. Hyphenated techniques have significantly helped natural product chemistry, especially UHPLC/ESI/MS/MS in the identification, separation, and tentative characterization of secondary metabolites from natural sources. Twenty-five compounds were detected from the Antarctic lichen *Cladonia metacorallifera* for the first time using UHPLC-PDA/ESI/Orbitrap/MS/MS. Compounds **5** and **7** are reported as new compounds, based on their MS/MS fragmentation routes, and considered as fumarprotocetraric acid derivatives. Besides, ten known phenolic identified as orsellinic acid, ethyl 4-carboxyorsellinate, psoromic acid isomer, succinprotocetraric acid, siphulellic acid, connorstictic acid, cryptostictic acid, lecanoric acid, lobaric acid and gyrophoric acid are noticed for the first time in the *Cladonia* genus.

Keywords: Antarctica; *Cladonia*; depsides; depsidones; Fumarprotocetraric acid; lichens; UHPLC; ESI/MS/MS

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Citation: Sepúlveda, B.; Cornejo, A.; Bárcenas-Pérez, D.; Cheel, J.; Areche, C. Two New Fumarprotocetraric Acid Lactones Identified and Characterized by UHPLC-PDA/ESI/ORBITRAP/MS/MS from the Antarctic Lichen *Cladonia metacorallifera*. *Separations* **2022**, *9*, 41. https://doi.org/10.3390/separations9020041

Academic Editor: Carmela Dell'Aversano

Received: 29 December 2021
Accepted: 28 January 2022
Published: 1 February 2022

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1. Introduction

Lichens are symbiotic organisms between algae or cyanobacteria and fungi. Lichens are organisms that can grow everywhere on soil, on and within rocks, on tree barks, as well as on any inanimate object. Lichens grow in tropical rainforests, deserts, and polar zones, including the Arctic and Antarctic regions [1–3]. From lichens, depsides, depsidones, dibenzofurans, depsones, anthraquinones, lactones and pulvinic acid derivatives have been isolated [4,5]. They have displayed multiple biological activities; for instance: antiulcer, gastroprotective, antibiotic, antiviral, antitumor, allergenic, plant growth inhibitory, antiherbivore, antileishmanial, anti-inflammatory, antioxidants, anti-trypanosoma and enzyme inhibitory activities [5–7].

Analyses of crude extracts were performed using chromatographic methods such as high-performance liquid chromatography (HPLC) in combination with various detection spectroscopic methods [8–12]. However, HPLC coupled to UV spectroscopy provide limited structural information when compounds are unknown [13]. Mass spectrometry (MS) provides rapid identification of unknown compounds while electro-spray ionization (ESI) is the most successful interface used in LC-MS coupled to time of flight (ToF), quadrupole or orbitrap [14]. Currently, tandem mass spectrometers operate within 1–5 ppm mass accuracy with high resolution and the quality of MS/MS spectra depend on the parameters such as precursor ion isolation width, intensity threshold, collision energy, total acquisition speed,

accumulation time on MS/MS spectrum, and others [14]. Therefore, LC-MS is a dominant analytical technique to identify secondary metabolites, and unknown constituents, in plant extracts giving information on elemental composition and structural fragmentation patterns, but are unable to distinguish positional isomers [14]. The hyphenated Q-exactive instrument with a high-resolution collision cell has significantly contributed in the area of lichens chemistry for the identification of unknown compounds based on structural characterization by MS/MS [8,12,15].

Herein, we describe the chromatographic fingerprinting of the Antarctic lichen *Cladonia metacorallifera* by UHPLC/PDA/ESI/MS/MS, which revealed the presence of two new metabolites.

2. Materials and Methods

2.1. Collection and Identification of Lichen

A 5 g aliquot of *C. metacorallifera* was collected in "Peninsula Fildes", King George Island, Antarctica during March, 2020. Vouchers specimens (reference numbers: CM-010420) were deposited at the Extreme natural product laboratory, Universidad de Chile.

2.2. UHPLC-Q/Orbitrap/ESI/MS/MS

2.2.1. Sample Preparation

A 1.0 g aliquot of *C. metacorallifera* was extracted with methanol (3 times, 10 mL each time, using a sonicator 30 min). The organic solutions were evaporated to obtain 18.0 mg of dark green gummy extract.

2.2.2. Instrument

The Thermo Scientific Dionex Ultimate 3000 UHPLC system, hyphenated with a Thermo Q exactive focus, was already reported [8,12]. For the analysis, 2 mg of each lichen extracts were first dissolved in 2 mL of methanol, then filtered (PTFE filter) and, finally, 10 µL were injected in the instrument, with all specifications set as previously reported [8].

2.2.3. LC Parameters

A UHPLC C18 column (150 mm × 4.6 mm ID, Thermo Fisher Scientific, Bremen, Germany) at 25 °C in an oven was used. Four detection UV systems were performed at 254, 280, 320 and 440 nm, and PDA from 180 to 800 nm. Mobile phases were 1% formic aqueous solution (A) and acetonitrile (B). The gradient (time (min), % B) was: (0.00, 5); (5.00, 5); (10.00, 30); (15.00, 30); (20.00, 70); (25.00, 70); (35.00, 5) and 12 min for column equilibration before each injection. The flow rate was setup at 1.00 mL min^{-1}, and the injection volume by 10 µL. Usnic acid and gyrophoric acid were use as standards of spiking experiments to perform the qualitative analysis. Standards and lichen extracts were kept at 10 °C during storage in the auto sampler.

2.2.4. MS Parameters

The HESI parameters were setup as follows: gas flow rate 75 units; capillary temperature at 400 °C; auxiliary gas unit flow rate at 20 unit (N_2); auxiliary gas heater temperature 500 °C; spray voltage 2500 V (for ESI^-); and S lens RF level 30. For the compounds of interest, a scan range of *m/z* 100–1000 was chosen; the automatic gain control (AGC) was set at 3×10^6 and the injection time was set to 200 ms. Collision energy was setup at 30 kV. Detection was performed based on calculated exact mass and on retention time. The mass tolerance window was set to 5 ppm for the two analysis modes.

3. Results and Discussion

The identification of unknown secondary metabolites in metabolomics is the main bottleneck on the structural interpretation based on MS/MS spectra, making the identification an arduous time-consuming task. As a strategy, many researches are limited to library matching, software, databases, algoritms and matching learning. Among the most

known, both publicly and commercially, MS/MS software tools and databases including Mass Frontier, SmileMS, Mass^{++}, XCMS^2, NIST, METLIN, MassBank, MoNA, Wiley MS-forID, CFM-ID, and MS-DIAL. For the generation of molecular structures of unknown compounds, it is important to follow the MS fragmentation ruler analysing the main small fragments. The biosynthetic pathway of secondary metabolite should also be considered if it applies [13,14].

During our study, twenty-five compounds were characterized for the first time in the methanolic extract of *C. metacorallifera* (Table 1) using UHPLC/PDA/ESI/MS/MS in negative ion mode.

Among the identified compounds, two fumarprotocetraric acid lactones (peaks **5** and **7**) are reported here for the first time. Their structures (Figure 1) are proposed based on biosynthetic pathways, UV data and MS/MS fragmentations.

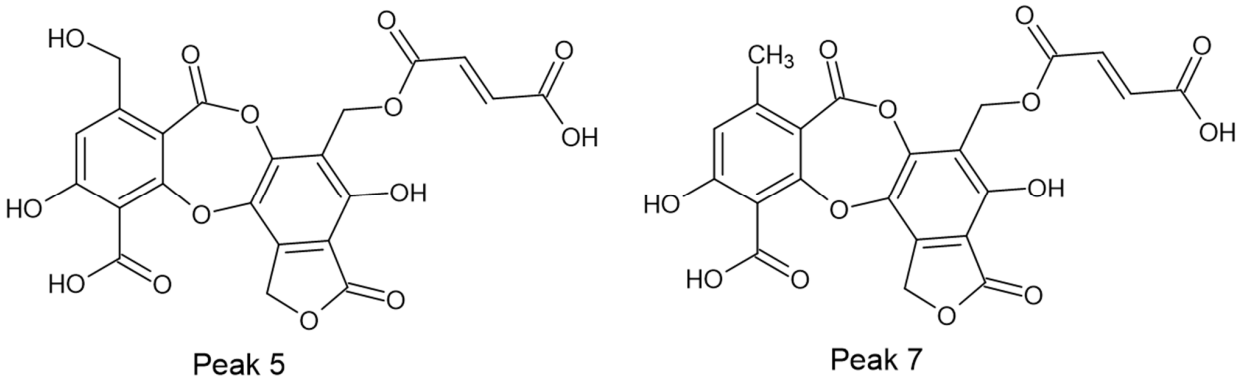

Figure 1. Chemical structures of new compounds (**5** and **7**) identified by UHPLC/ESI/MS/MS from *C. metacorallifera*. It proposes interconversion mediated by natural oxidation from peak **13** (fumarprotocetraric acid) to peak **7** and after to peak **5,** as supported by mass spectrometry and UV spectroscopy.

Peak **1** was identified as orsellinic acid ($C_8H_8O_4$). The electrospray TOF mass fragmentation of this metabolite has been previously reported [10,15]. In the present study, orsellinic acid is identified, for the first time, in *Cladonia* genus. Orsellinic acid showed antioxidant activity in the β-carotene-linoleate model, and in the nitric oxide radical scavenging assay [6]. Peak 2 with an ion at m/z 239.0546 showed UV absorbance at λmax 249, 308 nm, which is a similar to the one showed by ethyl orsellinate (λmax 219, 265, 302 nm). Their fragmentation yielded a diagnostic MS ion at m/z 195.0649 and 149.0229 (see Supplementary Material). Considering that peak **2** displayed diagnostic losses of CO_2 and C_2H_6O, we identified peak **2** as ethyl 4-carboxyorsellinate. From *Picea schrenkiana* has isolated this compound, and it is reported for the first time from lichens [16].

Peak **3** was characterized as squamatic acid (molecular anion at m/z 389.0860), whose fragmentation indicated diagnostic MS ions at m/z 209.0442 and 181.0490 [9,15]. This depside has been identified in *Cladonia uncialis*, *C. crispata*, *C. bellidiflora*, *C. squamosa*, and *C. cenotea* from Poland samples [17,18]. So far, squamatic acid has shown to be inactive against *Staphylococcus aureus*, *Escherichia coli* and *Candida albican* [19]. Peak **4** with a $[M–H]^-$ ion at m/z 357.0598 was characterized as a psoromic acid isomer. The fragmentation of this peak produced ions at m/z 313.0701 and 179.0335. This compound showed UV absorbance at λmax 249, 382 and 319 nm, which is similar to the one of psoromic acid (λmax 240, 271, 317 nm).

Peak **5** provided an $[M–H]^-$ ion at m/z 501.0284 ($C_{22}H_{13}O_{14}$). This fragmentation indicated that peak **5** is a new compound related to fumarprotocetraric acid (peak **13**). The key for this proposal implied the presence of the residue butendioic acid ($C_4H_3O_4^-$; 115.0023) along with the residue $C_{17}H_9O_8^-$ (341.0303). Considering that peak **5** showed UV absorbance at λmax 215, 248 and 315 nm, which is similar to the one of peak **13** (λmax

212, 238, 314 nm), and according to biosynthetic considerations, we tentatively identified peak **5** as hydroxy fumarprotocetraric acid lactone (Figure 2). Finally, the presence of fumarprotocetraric acid in *Cladonia* species supported this idea to be a derivative [17,18].

Table 1. UHPLC/Orbitrap/ESI/MS/MS data for Antarctic lichen *C. metacorallifera*.

Peak	Tentative Identification	[M-H]$^-$	Retention Time (min.)	Theoretical Mass (*m*/*z*)	Measured Mass (*m*/*z*)	Accuracy (ppm)	Metabolite Type	MS Ions (ppm)
1	Orsellinic acid	$C_8H_7O_4$	10.95	167.0344	167.0336	5.5	A	123.0441
2	Ethyl 4-carboxyorsellinate	$C_{11}H_{11}O_6$	14.77	239.0556	239.0546	6.5	A	195.0649; 149.0229
3	Squamatic acid	$C_{19}H_{17}O_9$	15.18	389.0873	389.0860	3.3	d	209.0442; 181.0490
4	Psoromic acid isomer	$C_{18}H_{13}O_8$	15.58	357.0610	357.0598	3.4	d	313.0701; 179.0335
5	Hydroxy fumarprotocetraric acid lactone	$C_{22}H_{13}O_{14}$	15.84	501.0311	501.0284	5.4	D	341.0285; 297.0389; 253.0491; 225.0542; 115.0023
6	Connorstictic acid	$C_{18}H_{13}O_9$	18.57	373.0565	373.0547	4.8	D	329.0666; 181.0555
7	Fumarprotocetraric acid lactone	$C_{22}H_{13}O_{13}$	18.81	485.0362	485.0336	5.4	D	369.0232; 297.0386; 253.0489; 225.0540; 167.0334; 115.0022
8	Siphulellic acid	$C_{19}H_{13}O_{10}$	19.20	401.0509	401.0495	3.5	D	253.0505; 149.0238; 123.0444
9	Lecanoric acid	$C_{16}H_{13}O_7$	19.44	317.0667	317.0651	5.0	d	167.0343; 149.0237; 123.0444
10	Succinprotocetraric acid	$C_{22}H_{17}O_{12}$	19.62	473.0725	473.0701	5.1	D	355.0441; 311.0544; 117.0179
11	Pentahydroxytetracosanoic acid	$C_{24}H_{47}O_7$	19.71	447.3327	447.3305	4.9	L	-
12	tetrahydroxydocosanoic acid	$C_{22}H_{43}O_6$	19.85	403.3065	403.3040	6.2	L	-
13	Fumarprotocetraric acid	$C_{22}H_{15}O_{12}$	20.11	471.0569	471.0544	5.3	D	355.0441; 311.0545; 115.0023
14	tetrahydroxytricosanoic acid	$C_{23}H_{45}O_6$	20.35	417.3222	417.3201	5.0	L	-
15	Cryptostictic acid	$C_{19}H_{15}O_9$	20.57	387.0716	387.0726	2.6	D	343.0826; 311.0566; 267.0661
16	6-ethyl-6-n-pentylpentadecan-4,5,7,8,15-pentol-15-acetate	$C_{24}H_{47}O_6$	20.83	431.3373	431.3358	3.5	L	-
17	Thamnolic acid	$C_{20}H_{19}O_{10}$	20.99	419.0978	419.0963	3.6	d	375.0722; 211.0238; 167.0345
18	Gyrophoric acid *	$C_{24}H_{19}O_{10}$	21.30	467.0978	467.0959	4.1	d	317.0667; 167.0345; 149.0238; 123.0443
19	Psoromic acid	$C_{18}H_{13}O_8$	21.58	357.0610	357.0599	3.1	d	269.0791; 177.0181
20	Dihydroxyoxodocosanoic acid	$C_{22}H_{41}O_5$	23.94	385.	385.2940	2.4	d	-
21	Lobaric acid	$C_{25}H_{27}O_8$	24.81	455.1711	455.1709	0.4	D	411.1815; 367.1909; 352.1681; 296.1048
22	Ethyl-4-O-methylolivetolcarboxylate	$C_{15}H_{21}O_4$	25.34	265.1445	265.1465	7.5	A	-
23	Usnic acid *	$C_{18}H_{15}O_7$	26.15	343.0818	343.0807	3.2	DBF	328.0591; 259.0609; 231.0661
24	Atranorin	$C_{19}H_{17}O_8$	26.48	373.0923	373.0911	3.2	d	177.0187; 163.0394
25	Dihydroxyheptadecatrienoic acid	$C_{17}H_{27}O_4$	27.46	295.1915	295.1916	0.3	L	-

* Identified by spiking experiments. A = Aromatic; L = Lipid; D = depsidone; d = depside; DBF = dibenzofuran.

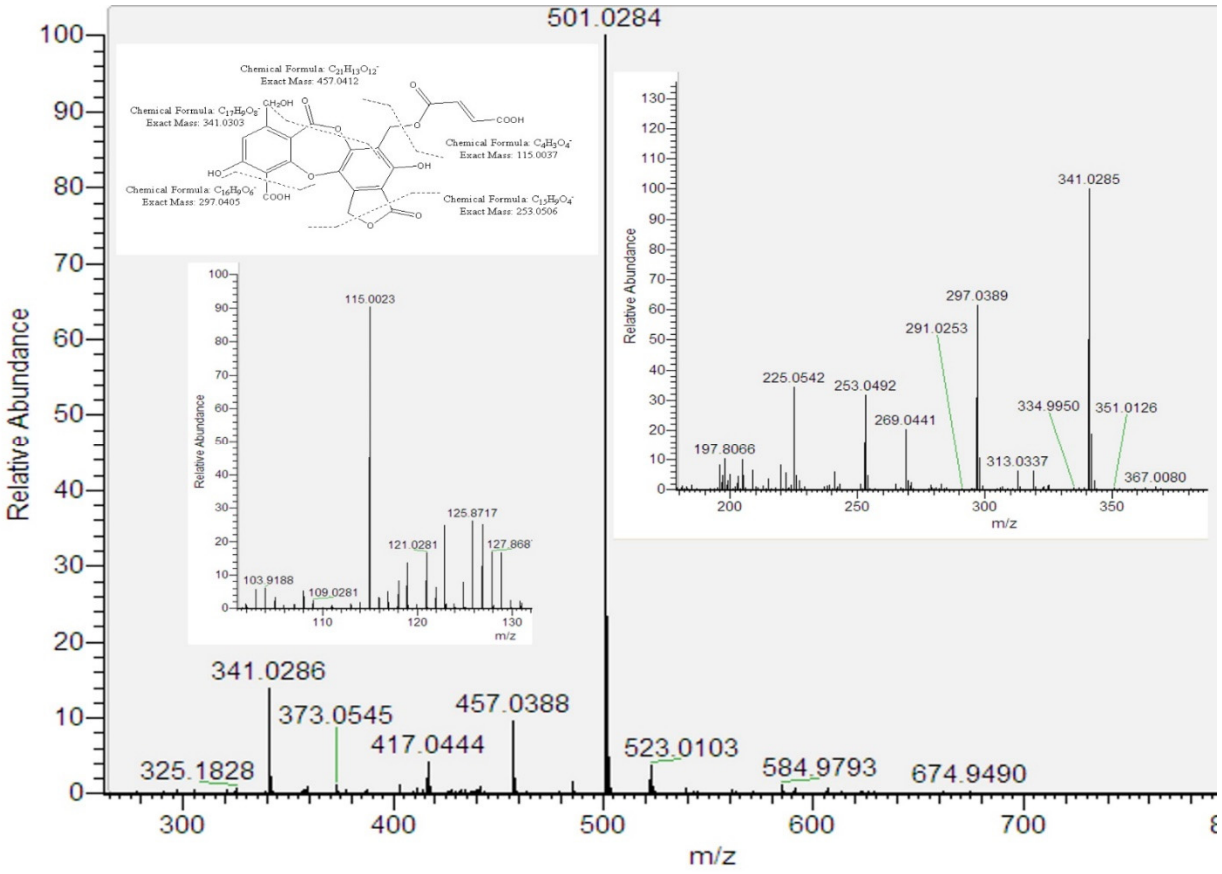

Figure 2. ESI/MS/MS spectra of peak **5** and its proposed fragmentation pathway.

Peak **6** was identified as the depsidone connorstictic acid (*m*/*z* 373.0547) and its fragmentation produced ions at 329.0666 and 181.0555 [9,10,15]. To the best of our knowledge, there is no paper of connorstictic acid being present *Cladonia* genus. To support this fact, the presence of a related compound norstictic acid has been reported in the *Cladonia* genus [17,18]. No biological activity has been reported so far.

Peak **7** showed an $[M–H]^-$ ion at *m*/*z* 485.0336 ($C_{22}H_{13}O_{13}$). According to their fragmentation pathway indicated that peak **7** is a new depsidone related to fumarprotocetraric acid (peak **13**). The proposal implied the presence of three daughter fragments at *m*/*z* 369.0252 ($C_{18}H_9O_9^-$), *m*/*z* 115.0023 (butendioic acid; $C_4H_3O_4^-$) and the residue $C_{17}H_9O_7^-$ (325.0354). Besides, peak 7 showed UV absorbance at λmax 248 and 311 nm, which is similar to peak 13. Considering this evidence and based on the biosynthesis of lichen metabolites, we tentatively identified peak **7** as a fumarprotocetraric acid lactone (Figure 3). It is well known that depsides as fumarprotocetraric acid are synthesized by the acetate-polymalonate pathway, which are formed by the bonding of two β-orcinol-type phenolic unit with ether, ester and C-C link [20]. In this context, both peak **5** and peak **7** derived from fumarprotocetraric acid are probably formed by lactonisation for peak **7** and oxidation and lactonisation for peak **5**.

Peak **8** with an ion at *m*/*z* 401.0514 was identified as siphulellic acid, which showed diagnostic daughter ions at *m*/*z* 123.0444, 149.0238, and 253.0505. To the best of our knowledge, this is the first information of its presence in the *Cladonia* genus [17,18]. No biological activity has been reported according to Scopus so far. Peak **9** was detected as lecanoric acid, which showed an $[M–H]^-$ ion at *m*/*z* 317.0668 according to published data [9–11]. Therefore, this is the first report of the presence of lecanoric acid in the *Cladonia* genus [17,18]. Lecanoric acid has displayed antioxidant, antibacterial and anticancer activities [5–7]. Peak **10** showed an $[M–H]^-$ ion at *m*/*z* 473.0701 (λmax 250 and 311 nm). Their fragmentation produced daughter ions at *m*/*z* 355.0441, 311.0545 and 117.0179 indicating that peak 10

is not confumarprotocetraric acid [17]. Considering these fragments, we identified as succinprotocetraric acid [21] (see Supplementary Material).

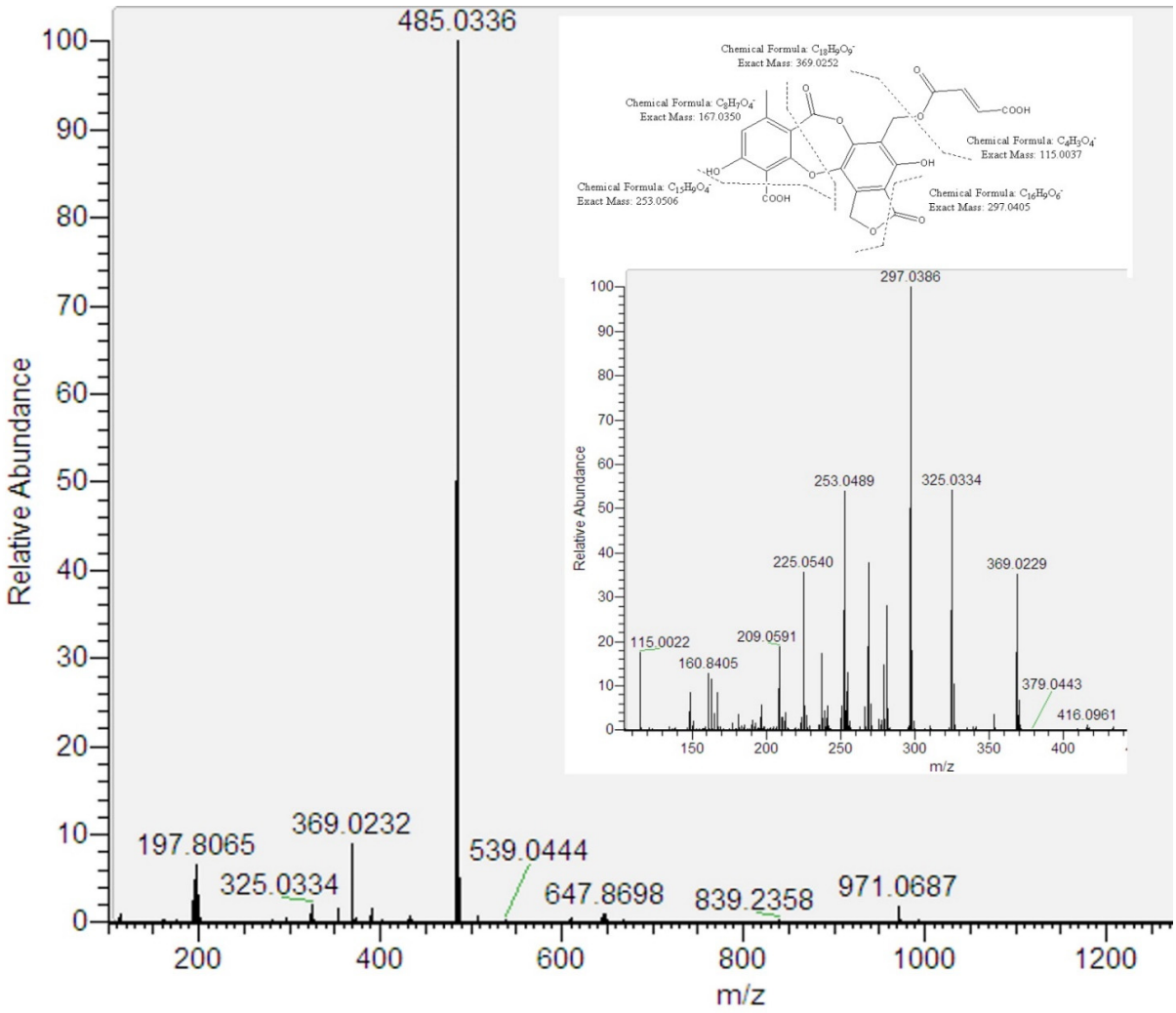

Figure 3. ESI/MS/MS spectra of $[M–H]^-$ of peak **7** and its proposed fragmentation pathway.

Peaks **11**, **12**, **14**, **20** and **25** showed an $[M–H]^-$ ion at *m*/*z* 447.3305, 403.3040, 417.3201, 385.2940 and 295.1916, respectively. They were tentatively characterized as the fatty acids pentahydroxytetracosanoic acid ($C_{24}H_{48}O_7$), tetrahydroxydocosanoic acid ($C_{22}H_{44}O_6$), tetrahydroxytricosanoic acid ($C_{23}H_{46}O_6$), dihydroxyoxodocosanoic acid ($C_{22}H_{42}O_5$), and dihydroxyheptadecatrienoic acid ($C_{17}H_{28}O_4$), respectively. Compounds related to these peaks have also been reported [17].

Peak **13** was detected as fumarprotocetraric acid ($C_{22}H_{15}O_{12}$), which displayed an $[M–H]^-$ ion at *m*/*z* 471.0544. The fragmentation of this peak produced ions at *m*/*z* 355.0441, 311.0545 and 115.0023 according to reported [21]. This depside has been identified in *Cladonia verticillata, C. cariosa, C. phyllophora, C. merochlorophaea, C. trassii, C. symphycarpia, C. subulata, C. pyxidata, C. fimbriata, C. chlorophaea and C. stricta* from Poland samples [18]. Fumarprotocetraric acid has considerably shown antimicrobial, antioxidant, anticarcinogenic and immunoestimulatory activities [4–7].

Peak **15** was identified as the depsidone cryptostictic acid ($[M–H]^-$ ion at *m*/*z* 387.0726) and its fragmentation showed ions at *m*/*z* 343.0826, 311.0566 and 267.0661. These fragments have been reported [22]. This is the first report of the presence of cryptostictic acid in the *Cladonia* genus. No significant antioxidant activity has been found for this compound [6]. Peak **16** was tentatively identified as 6-ethyl-6-n-pentylpentadecan-4,5,7,8,15-pentol-15-

acetate ($C_{24}H_{48}O_6$) [17]. Peak **17** with a $[M–H]^-$ ion at m/z 419.0963 was identified as thamnolic acid, which showed daughter ions at m/z 375.0722, 211.0238 and 167.0345 [11]. The presence of thamnolic acid in *Cladonia* species has been previously informed. No important biological activity has been reported so far. Peak **18** was identified as gyrophoric acid showing a molecular anion at m/z 467.0959. Its fragmentation informed ions at m/z 317.0647 $[M–H^-, C_8H_6O_3]^-$, 167.0336 $[M–H^-, C_{16}H_{12}O_6]^-$, 151.0387 $[M–H^-, C_{16}H_{12}O_7]^-$, and 123.0438 $[M–H^-, C_{17}H_{12}O_8]^-$ confirming this tridepside [9–11]. This is the first report of the presence of gyrophoric acid in the *Cladonia* genus. Gyrophoric acid demostrated to be cytotoxic, anticarcinogenic, antibacterial, and antiproliferative [4–6]. Peak **19** was identified as psoromic acid according to data reported previously [15]. This depside has been identified in *Cladonia symphycarpia, and C. macrophyllodes* from Poland samples [18]. Psoromic acid has shown antioxidant, cardioprotective, cytotoxic and apoptotic activities [4–6]. Peak **21** was assigned as lobaric acid (m/z 455.1712). The fragmentation produced ions at m/z 411.1808 $[M–H^-\ CO_2]^-$, 367.1909 $[M–H^-, 2CO_2]^-$, 352.1675 $[M–H^-, 2CO_2–CH_3]^-$, and 296.1049 $[M–H^-, 2CO_2–C_5H_{11}]^-$. Such fragmentations have been reported previously [8–10]. This is the first report of the presence of lobaric acid in *Cladonia* genus. Lobaric acid has shown antibacterial, antioxidant, and anticarcinogenic activities. Also, the inhibition of arachidonate-5-lipooxygenase, cyclooxygenase, DNA synthesis, and tubulin polymerization has also been reported [4–7]. An aromatic compound was identified as ethyl-4-O-methylolivetolcarboxylate at the peak **22**, which was reported from *C. macaronesica* previously [17]. No important enzymatic activity has been reported as 5-lipooxygenase inhibitor [23]. Peak **23** was identified as usnic acid according to the studies [4,8,10]. This dibenzofuran has been identified and isolated in many *Cladonia* samples [17,18]. Usnic acid has been the most evaluated metabolite in lichens regarding biological activity. Among them, we can mention anti-inflammatory, antioxidant, antimicrobial, immunoestimulatory, gastroprotective, cytoprotective, cardioprotective and anticarcinogenic activities [4–7,24]. Peak **24** was assigned to atranorin, and their major diagnostic daughter ions were at m/z 177.0187 and 163.0394 a.m.u. These findings are in good agreement with previous reported studies [10,11]. This depside has been identified and isolated in many *Cladonia* samples [17,18]. Atranorin showed interesting biological activities such as antioxidant, antimicrobial, cytoprotective, pro-oxidant, cytotoxicity, pro-apoptotic, and anticarcinogenic activities [4–7].

Some reports from the genus Cladonia including *C. metacorallifera* var. *reagens* KoLRI002260 as a rare lichen from Korean. They have been studied as mycobiont producing red naphthoquinonic pigments as cristazarin, and 6-methylcristazarin in culture media with 1% fructose and/or light [25]. Besides, these two compounds are produced by *Cladonia cristatella* mycobiont, but not in lichen thalli [26]. From *Cladonia cariosa* fumarprotocetraric acid was isolated, which showed 50 μM decrease on the β sheet content, demonstrated through Thioflavin T assays. In addition, the oligomers formed in that study with fumarprotocetraric acid were not toxic in N2a neuroblastoma cells [27]. A study based on UHPLC/QTOF/HRMS/MS of Algerian lichen *Cladonia rangiformis* detected the presence of the following thirteen metabolites highlighting ethyl orsellinate, squamatic acid, atranorin, evernic acid, usnic acid, roccellic acid, jackinic acid, norrangiformic acid, isorangiformic acid, and rangiformic acid [28]. In this context, our study detected twenty five metabolites using UHPLC/Q/Orbitrap/MS/MS.

According to our search on SciFinder, the compounds identified with peaks **5** and **7** are reported here for the first time. As mentioned above, fumarprotocetraric acid display considerable pharmacological activity. Therefore, these two compounds could be isolated to evaluate their potential as pharmacological agent.

4. Conclusions

Our results demonstrate that the identification for lichen metabolites using UHPLC-Q/Orbitrap/ESI/MS/MS is a fast and accurate methodology. Based on this hyphenated technique, we identified twenty-five compounds; two of them being reported for the first

time, based on careful high resolution mass spectrometry analysis and biosynthetic considerations (peak **5** and peak **7**). Besides, ten phenolic compounds known as orsellinic acid (peak **1**), ethyl 4-carboxyorsellinate (peak **2**), psoromic acid isomer (peak **4**), connorstictic acid (peak **6**), siphulellic acid (peak **8**), lecanoric acid, (peak **9**), succinprotocetraric (peak **10**), cryptostictic acid (peak **15**), gyrophoric acid (peak **18**), and lobaric acid (peak **21**) are reported for the first time in the *Cladonia* genus. Finally, six lipids were tentatively identified as peaks **11**, **12**, **14**, **16**, **20** and **25**. Further prospection in metabolomics based on LC/MS/MS should be considered from Antarctic lichens.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/separations9020041/s1, Figure S1: UHPLC-ESI-MS-MS Chromatogram of methanolic extract of C. metacorallifera.

Author Contributions: Conceptualization, C.A.; methodology, C.A., B.S. and A.C.; formal analysis, D.B.-P. and J.C.; investigation, C.A., B.S. and A.C.; writing—original draft preparation, C.A.; writing—review and editing, D.B.-P. and J.C.; supervision, C.A.; funding acquisition, C.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by INACH RT 18-19; INACH RT 13-13 and Fondecyt Regular 1190314. D.B.-P. and J.C. thank the support of Technology Agency of the Czech Republic (NCK grant TN010000048/03, J.C. and D.B.-P.) and the National Programme of Sustainability I of the Ministry of Education, Youth and Sports of the Czech Republic (ID: LO1416, J.C.).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Daniela Bárcenas-Pérez gratefully acknowledges the research supervision of José Cheel (Centre Algatech–Czech Academy of Sciences) as well as the University of South Bohemia for a doctoral scholarship and the grant GAJU 017/2019/P.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Boustie, J.; Grube, M. Lichens—A promising source of bioactive secondary metabolites. *Plant Genet. Resour.* **2005**, *3*, 273–287. [CrossRef]
2. Shukla, V.; Joshi, G.P.; Rawat, M.S.M. Lichens as a potential natural source of bioactive compounds: A review. *Phytochem. Rev.* **2010**, *9*, 303–314. [CrossRef]
3. Molnár, K.; Farkas, E. Current Results on Biological Activities of Lichen Secondary Metabolites: A Review. *Z. Naturforsch. C. J. Biosci.* **2010**, *65*, 157–173. [CrossRef] [PubMed]
4. Boustie, J.; Tomasi, S.; Grube, M. Bioactive lichen metabolites: Alpine habitats as an untapped source. *Phytochem. Rev.* **2011**, *10*, 287–307. [CrossRef]
5. Shrestha, G.; Clair, L.L.S. Lichens: A promising source of antibiotic and anticancer drugs. *Phytochem. Rev.* **2013**, *12*, 229–244. [CrossRef]
6. White, P.A.S.; Oliveira, R.C.M.; Oliveira, A.P.; Serafini, M.R.; Araújo, A.A.S.; Gelain, D.P.; Moreira, J.C.F.; Almeida, J.R.G.S.; Quintans, J.; Quintans-Junior, L.J.; et al. Antioxidant Activity and Mechanisms of Action of Natural Compounds Isolated from Lichens: A Systematic Review. *Molecules* **2014**, *19*, 14496–14527. [CrossRef]
7. Zambare, V.P.; Christopher, L.P. Biopharmaceutical potential of lichens. *Pharm. Biol.* **2012**, *50*, 778–798. [CrossRef]
8. Calla-Quispe, E.; Robles, J.; Areche, C.; Sepulveda, B. Are Ionic Liquids Better Extracting Agents Than Toxic Volatile Organic Solvents? A Combination of Ionic Liquids, Microwave and LC/MS/MS, Applied to the Lichen *Stereocaulon glareosum*. *Front. Chem.* **2020**, *8*, 450. [CrossRef]
9. Parrot, D.; Jan, S.; Baert, N.; Guyot, S.; Tomasi, S. Comparative metabolite profiling and chemical study of *Ramalina siliquosa* complex using LC–ESI-MS/MS approach. *Phytochemistry* **2013**, *89*, 114–124. [CrossRef]
10. Musharraf, S.G.; Kanwal, N.; Thadhani, V.M.; Choudhary, M.I. Rapid identification of lichen compounds based on the structure–fragmentation relationship using ESI-MS/MS analysis. *Anal. Methods* **2015**, *7*, 6066–6076. [CrossRef]
11. Le Pogam, P.; Schinkovitz, A.; Legouin, B.; Le Lamer, A.-C.; Boustie, J.; Richomme, P. Matrix-Free UV-Laser Desorption Ionization Mass Spectrometry as a Versatile Approach for Accelerating Dereplication Studies on Lichens. *Anal. Chem.* **2015**, *87*, 10421–10428. [CrossRef] [PubMed]
12. Cornejo, A.; Salgado, F.; Caballero, J.; Vargas, R.; Simirgiotis, M.; Areche, C. Secondary Metabolites in *Ramalina terebrata* Detected by UHPLC/ESI/MS/MS and Identification of Parietin as Tau Protein Inhibitor. *Int. J. Mol. Sci.* **2016**, *17*, 1303. [CrossRef] [PubMed]

13. Kind, T.; Tsugawa, H.; Cajka, T.; Ma, Y.; Lai, Z.; Mehta, S.S.; Wohlgemuth, G.; Barupal, D.K.; Showalter, M.R.; Arita, M.; et al. Identification of small molecules using accurate mass MS/MS search. *Mass Spectrom. Rev.* **2018**, *37*, 513–532. [CrossRef] [PubMed]
14. Vaniya, A.; Fiehn, O. Using fragmentation trees and mass spectral trees for identifying unknown compounds in metabolomics. *Trends Analyt. Chem.* **2015**, *69*, 52–61. [CrossRef] [PubMed]
15. Castro, O.N.; Benites, J.; Rodilla, J.M.L.; Santiago, J.C.; Simirgiotis, M.; Sepulveda, B.; Areche, C. Metabolomic Analysis of the Lichen *Everniopsis trulla* Using Ultra High Performance Liquid Chromatography-Quadrupole-Orbitrap Mass Spectrometry (UHPLC-Q-OT-MS). *Chromatographia* **2017**, *80*, 967–973. [CrossRef]
16. Li, Z.H.; Wang, Q.; Lia, J.J.; Ruan, X.; Pan, C.D.; Jiang, D.A.; Luo, C.C. Elementary identification of potential autotoxins from *Picea schrenkiana* litters. *Chin. J. Anal. Chem.* **2009**, *6*, 888–892.
17. Huneck, S.; Yoshimura, I. Data of lichen substances. In *Identification of Lichen Substances*; Springer: Berlin/Heidelberg, Germany, 1996; Volume 3, pp. 125–446.
18. Osyczka, P. The lichen genus Cladonia (Cladoniaceae, lichenized Ascomycota) from Spitsbergen. *Pol. Polar Res.* **2006**, *27*, 207–242.
19. Studzińska-Sroka, E.; Hołderna-Kędzia, E.; Galanty, A.; Bylka, W.; Kacprzak, K.; Ćwiklińska, K. In Vitro antimicrobial activity of extracts and compounds isolated from *Cladonia uncialis*. *Nat. Prod. Res.* **2015**, *29*, 2302–2307. [CrossRef]
20. Ranković, B.; Kosanić, M. Lichens as a potential source of bioactive secondary metabolites. In *Lichen Secondary Metabolites*, 1st ed.; Ranković, B., Ed.; Springer: Cham, Switzerland, 2015; pp. 1–26.
21. Xu, M.; Heidmarsson, S.; Thorsteinsdottir, M.; Kreuzer, M.; Hawkins, J.; Omarsdottir, S.; Olafsdottir, E.S. Authentication of Iceland Moss (*Cetraria islandica*) by UPLC-QToF-MS chemical profiling and DNA barcoding. *Food Chem.* **2018**, *245*, 989–996. [CrossRef]
22. Salgado, F.; Albornoz, L.; Cortéz, C.; Stashenko, E.; Urrea-Vallejo, K.; Nagles, E.; Galicia-Virviescas, C.; Cornejo, A.; Ardiles, A.; Simirgiotis, M.; et al. Secondary Metabolite Profiling of Species of the Genus Usnea by UHPLC-ESI-OT-MS-MS. *Molecules* **2018**, *23*, 54. [CrossRef]
23. Oettl, S.K.; Gerstmeier, J.; Khan, S.Y.; Wiechmann, K.; Bauer, J.; Atanasov, A.G.; Malainer, C.; Awad, E.M.; Uhrin, P.; Heiss, E.H.; et al. Imbricaric Acid and Perlatolic Acid: Multi-Targeting Anti-Inflammatory Depsides from *Cetrelia monachorum*. *PLoS ONE* **2013**, *8*, e76929. [CrossRef] [PubMed]
24. Araújo, A.A.S.; de Melo, M.G.D.; Rabelo, T.K.; Nunes, P.S.; Santos, S.L.; Serafini, M.R.; Santos, M.R.V.; Quintans, L.; Gelain, D.P. Review of the biological properties and toxicity of usnic acid. *Nat. Prod. Res.* **2015**, *29*, 2167–2180. [CrossRef] [PubMed]
25. Jeong, M.-H.; Park, C.-H.; Kim, J.; Choi, E.; Kim, S.; Hur, J.-S.; Park, S.-Y. Production and Activity of Cristazarin in the Lichen-Forming Fungus *Cladonia metacorallifera*. *J. Fungi* **2021**, *7*, 601. [CrossRef] [PubMed]
26. Yamamoto, Y.; Matsubara, H.; Kinoshita, Y.; Kinoshita, K.; Koyama, K.; Takahashi, K.; Ahmadjiam, V.; Kurokawa, T.; Yoshimura, I. Naphthazarin derivatives from cultures of the lichen *Cladonia cristatella*. *Phytochemistry* **1996**, *43*, 1239–1242. [CrossRef]
27. González, C.; Cartagena, C.; Caballero, L.; Melo, F.; Areche, C.; Cornejo, A. The Fumarprotocetraric Acid Inhibits Tau Covalently, Avoiding Cytotoxicity of Aggregates in Cells. *Molecules* **2021**, *26*, 3760. [CrossRef]
28. Brakni, R.; Ahmed, M.A.; Burger, P.; Schwing, A.; Michel, G.; Pomares, C.; Hasseine, L.; Boyer, L.; Fernandez, X.; Landreau, A.; et al. UHPLC-HRMS/MS Based Profiling of Algerian Lichens and Their Antimicrobial Activities. *Chem. Biodivers.* **2018**, *15*, e1800031. [CrossRef]