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LASALLIC ACID, A TRIDEPSIDE FROM THE LICHEN, *LASALLIA ASIAE-ORIENTALIS*

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Key Word Index—*Lasallia asiae-orientalis*; Umbilicariaceae; lasallic acid; tridepside; lichen substance.

Abstract—The structure of lasallic acid, extracted from the lichen *Lasallia asiae-orientalis*, is elucidated as 2,6-dihydroxy-3-carboxy-4-methylphenyl lecanorate by comparison of NMR data with those for the tridepsides, gyrophoric and crustinic acids.

INTRODUCTION

The lichen family Umbilicariaceae, which comprises the two genera, *Lasallia* Mérat and *Umbilicaria* Hoffm., is thought to be phylogenetically isolated from other lichen-forming fungi. A recent chemotaxonomic survey of nine species of *Lasallia* and 46 of *Umbilicaria* effectively combined a standardized three-solvent system TLC method with linear-gradient HPLC optimized for the detection and separation of tridepsides [1]. It found the new tridepside lasallic acid (**1**) in four species—*L. asiae-orientalis* (Asah.) Sato, *L. mayebarae* (Sato) Asah., *L. papulosa* (Ach.) Llano and *L. sinorientalis* Wei.

Other recent phytochemical surveys [2–6], relying primarily on HPLC, discovered previously unsuspected variation in both genera and the novel tridepside, crustinic acid (**2**). Compound **2** was shown to be the first tridepside with both *para-* and *meta*-depside linkages [7]. The distribution of **1** and **2** in the umbilicariaceous lichens shows strong taxonomic correlations. Compound **2** is restricted to *Umbilicaria*, compound **1** more characteristic of *Lasallia*, being only tentatively identified as a trace satellite accompanying a high concentration of **2** in one species of *Umbilicaria* [1].

The study reported here elucidates the chemical structure of **1**.

RESULTS AND DISCUSSION

Although the discovery of **2** owed much to the progress of gradient HPLC techniques, the discovery of **1** depended upon TLC, these two compounds being insufficiently resolved by any reverse-phase HPLC methods used so far in the analysis of these lichens. The chromatographic similarity of **1** and **2** reflects their structural similarity; **1** is shown here to differ from **2** only in the position of one C-ring hydroxylation and the B- to C-ring esterification. Evidence for the structure of **1** comes from spectral data, including comparisons with the known tridepsides, **2** and gyrophoric acid (**3**).

In the FAB mass spectrum, **3** gave a $[M+H]^+$ peak at *m/z* 469 (1.4%) corresponding to the formula $C_{24}H_{20}O_{10}$ (468) and fragments at *m/z* 301 (2.2%) and 151 (27.3%), confirming the presence of two orsellinic acid moieties in the molecule. Similarly, both **1** and **2** showed $[M+H]^+$ peaks at *m/z* 485 (2.2 and 1.8%, respectively), corresponding to the formula $C_{24}H_{20}O_{11}$ (484) and fragment peaks at *m/z* 301 (4.1 and 2.3%, respectively) and 151 (44.9 and 37.5%, respectively). These data clearly indicate that the M_r of **1** is the same as that of **2** and that these tridepsides have the same partial structure in their A- and B-rings. Therefore, **1** should be an isomer of **2**, differing only in the constitution of the C-ring.

The ^{13}C NMR (100 MHz, DMSO-d_6) spectral data for **1–3**, with the results of DEPT (90° and 135°) and ^1H–^{13}C COSY experiments, are shown in Table 1. The correlations between their NOESY and long-range ^1H–^{13}COSY NMR (COLOC: correlation spectroscopy of long-range coupling) are shown in structures **1–3** (Scheme 1).

The ^1H NMR (400 MHz, DMSO-d_6) spectral data for

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Table 1. ^{13}C NMR (100 MHz, DMSO-d_6) of lasallic acid (**1**), crustinic acid (**2**) and gyrophoric acid (**3**); chemical shifts in ppm relative to solvent peak

Carbon no.	1	2	3
1	108.0	108.2	108.4
2	160.2	160.1	160.0
3	100.5	100.5	100.5
4	161.1	161.1	161.1
5	109.9	109.9	109.8
6	140.3*	140.2‡	140.2
7	167.0†	167.1§	167.1‖
8	21.2	21.2	21.2
1′	116.2	117.9	118.0
2′	157.8	156.5	156.3
3′	107.4	107.0	107.2
4′	152.5	151.9	152.1
5′	114.6	114.3	114.2
6′	139.7*	138.7‡	138.0
7′	165.1†	165.6§	165.6‖
8′	20.3	19.5	19.3
1″	105.2	106.5	117.1
2″	156.5	159.7	158.7
3″	123.9	101.1	107.2
4″	153.5	153.4	152.1
5″	110.3	130.4	114.4
6″	139.4*	133.1‡	139.5
7″	173.0	171.9	170.4
8″	23.1	14.4	20.8

*, †, ‡, §, ‖ Assignments may be interchanged.

1, **2** and **3** were measured in succession. Compound **1** gave singlets at δ 2.38 (3H), 2.46 (3H) and 2.50 (3H, overlapped by the solvent signal) for protons attached to C-8, C-8″ and C-8′, respectively. For confirmation of three methyl groups, the ^1H NMR (400 MHz, acetone-d_6) of **1** was measured, giving singlet signals at δ 2.58, 2.62 and 2.73 (3H each). Other ^1H NMR (400 MHz, DMSO-d_6) signals were a singlet at δ 6.24 (2H) for two A-ring aromatic protons, a pair of doublets at δ 6.67 and 6.69 (1H each, J = 1.71 Hz) for two B-ring aromatic protons and a singlet at δ 6.35 (1H) for one C-ring proton. Low-field singlets at δ 9.95 (1H, *br*), 10.29 (1H) and 10.38 (1H, *br*) can be assigned to three hydroxyl groups substituted at positions 2″, 2 and 2′, respectively. (Note: the numbering of the carbons follows a standard convention used in common names of depsides and depsidones where the numbers for biogenetically equivalent positions remain the same and where C-7 and C-8 refer to the carbons of the carboxylic acid and the methyl group, respectively [8].)

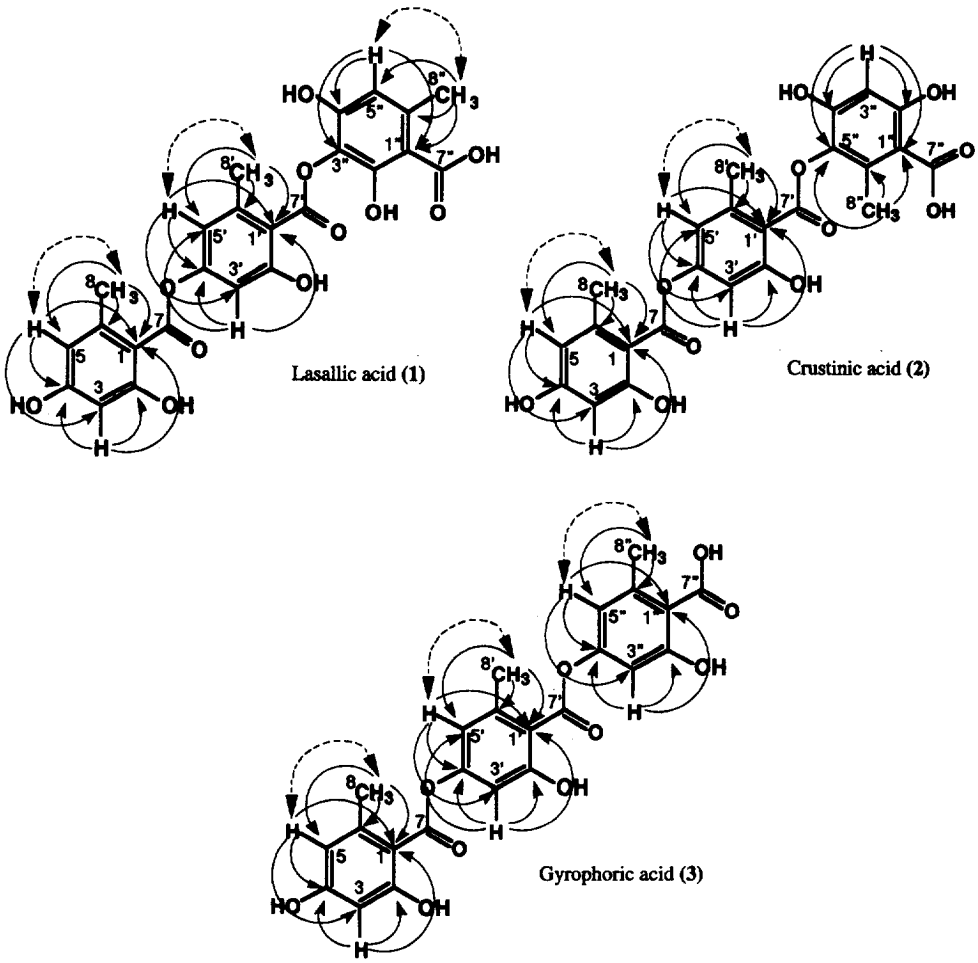
The chemical shifts and ^1H–^{13}C COSY signals for the skeletal carbons of the A- and B-rings of **1** were identical to those of **2** and **3**. Compound **3** showed three C-ring ^1H–^{13}C COSY signals due to the 3″-CH, the 5″-CH and the 8″-CH_3. On the other hand, **2** showed two C-ring ^1H–^{13}C COSY signals, for the 3″-CH and the 8″-CH_3. Compound **1** also showed two ^1H–^{13}C COSY signals, one for the 8″-CH_3 and the other for a C-ring CH, different from the one found for **2**. Compound **2** gave two NOESY from the A- and B-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. Finally **1** showed three NOESY, like those found for **3**, and one additional NOESY due to the C-ring.

These spectral data reduce to three the number of possible structures of the C-ring of **1** by requiring that the –CH_3 and one of three –OH substituents be *ortho* to the –COOH, i.e. the C-ring must have the partial structure 1″-COOH, 2″-OH, 6″-CH_3, as in **3** and **2**. Furthermore, the ester linkage between the B- and C-rings cannot involve the 2″-OH, but must link instead to one of the two remaining hydroxyls, located at positions 3″,4″-, 4″,5″- or 3″,5″-. Esterification of a lecanoric acid moiety to either of these remaining C-ring hydroxyls gives six theoretically possible tridepsides: two *para-para*-tridepsides (esterified to a 4″-hydroxyl and having an additional hydroxyl at either the 3″- or 5″-position) and four *para–meta*-tridepsides (either 3″-esterified with a free hydroxyl at either the 4″- or the 5″-position or 5″-esterified with a free hydroxyl at either the 4″- or 3″-position). The *para–meta* tridepside having a free 4″-OH and the ester linkage to a 5″-OH is (**2**). Of the remaining five tridepsides, two would have a free 5″-OH and a third would have a *meta*-depside linkage from the B-ring carboxyl to a 5″-OH. For these three structures, the ^{13}C NMR (DMSO-d_6) signal for C-ring methyl (C-8″) should be at *ca* 15 ppm, but the signal for **1** was at 23.05 ppm, indicating no free or esterified hydroxyl at C-5″. Furthermore, only the two remaining structures would also have a proton at C-5″ and be expected to give the C-ring NOESY observed for **1**. The COLOC of **1** cannot distinguish between these two tridepsides, both of which should have reasonable correlations between the 5″-CH and the 8″-CH_3. Therefore, two possible structures were inferred for **1**: one the *para–para*-tridepside 3″-hydroxygyrophoric acid and the other the isomeric *para–meta*-tridepside (**1**) having a free 4″-OH and the ester linkage joining at the 3″-OH.

Compound **1** (15 mg) was treated with trimethylsilyldiazomethane for two days to methylate the free carboxylic acid on the C-ring and all free phenolic hydroxyls (i.e. excluding the relatively unreactive 2-,2′- and 2″-hydroxyls *ortho* to a carbonyl). The final proof of the structure of **1** came from the strong NOE observed in this methylation product between the 4″-methoxyl, the C-6″ methyl and the C-5″ proton. Irradiation of the C-5″ proton NOE from the methyl (8.3%) substituted at C-6″ and the C-4″ methoxyl (10.6%). Irradiation of the methyl substituted at C-6″ or the methoxyl substituted at C-4″ gave NOE (10.3 and 4.6%, respectively) in either case. From these observations, the structure 3″-hydroxygyrophoric acid could be excluded, because there was no NOE between a C-3″-methoxyl and the proton at C-5″. Hence, the structure of **1** was established as 2,6-dihydroxy-3-carboxy-4-methylphenyl lecanorate.

EXPERIMENTAL

Lichen materials. Voucher specimens for the following extracted lichens are in the Meiji College of



Scheme 1. Correlations between long-range COSY NMR spectrum (DMSO-d_6) (———) and NOESY spectrum (DMSO-d_6) (– – – – –).

Pharmacy Herbarium and Duke University: (1) *Lasallia asiae-orientalis* (1.05 g, Japan, Shikoku, Tokushima Pref. (Prov. Awa), Mt Tengudake, at the summit area on quartzite rocks, *ca* 1800 m, *I. Yoshimura*, 25 August 1960; CFC11130), and (2) *Umbilicaria cinereorufescens* (Schaer.) Frey (1.02 g, Sweden, Opland, Dovre, S. of Gamleseter, on a boulder in the alpine region, *ca* 1090 m, *S. Ahlner*, 27 June 1948; CFC1372). The upper and lower surfaces of both lichens were cleaned under a dissecting microscope to remove contaminants.

Extraction. Cleaned thalli were pulverized and extracted for 20 min at 40° with Me_2CO (100 ml). The filtered extract was evapd *in vacuo* to dryness (Me_2CO extract of *L. asiae-orientalis*: 129 mg, 12.3%; *U. cinereorufescens*: 50 mg, 4.9%).

Preparative HPLC. The Me_2CO extracts containing lichen metabolites were sepd by HPLC using a UV detector ($\lambda = 254$ nm) and a Cosmosil ODS 5C18 (250 × 10 mm) column. 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: 2.0 ml min^{-1}. Analysis time: 50 min.

TLC. The TLC method used three solvent systems and control lanes of norstictic acid and atranorin [9]. Data for **1–3** are available elsewhere [1].

Lasallic acid (**1**). The Me_2CO extract of *L. asiae-orientalis* was sepd by HPLC using the conditions described above. Two depsides were isolated: **1** (39.3 mg) and **3** (27.1 mg). Compound **1** recrystallized ×2 from Me_2CO–H_2O to give microcrystals, mps: uncorr. 181–183°. NaOCl + red. HR FABMS (*m/z*): $[M-H]^-$ 483.0941. $[M-H]^-$ calc. 483.0927 for $C_{24}H_{20}O_{11}$. ^1H NMR (400 MHz, DMSO-d_6): δ 2.38 (3H, *s*, H-8), 2.46 (3H, *s*, H-8″), 2.50 (3H, *s*, H-8′), 6.24 (2H, *s*, H-3), 6.35 (1H, *s*, H-5″), 6.67 (1H *d*, $J = 1.71$ Hz, H-5′),* 6.69 (1H, *d*, $J = 1.71$ Hz, H-3′),* 9.95 (1H, *br s*, OH-2″), 10.29 (1H, *s*, OH-2), 10.38 (1H, *br s*, OH-2′); ^1H NMR (400 MHz, Me_2CO-d_6): δ 2.58 (3H, *s*, H-8″), 2.62 (3H, *s*, H-8′), 2.73 (3H, *s*, H-8), 6.31 (1H, *d*, $J = 2.44$ Hz, H-3′), 6.40 (1H, *d*, $J = 2.44$ Hz, H-5′), 6.51 (1H, *s*, H-5″), 6.89 (2H, *s*, H-3,5).

Crustinic acid (**2**). Compound **2** (34.8 mg) was obtained from the Me_2CO extract of *U. cinereorufescens* as described for **1**. ^1H NMR (400 MHz, DMSO-

d_6): δ 2.37 (3H, *s*, H-8″), 2.38 (3H, *s*, H-8), 2.44 (3H, *s*, H-8′), 6.24 (2H, *s*, H-3,5), 6.36 (1H, *s*, H-3″), 6.66 (1H, *s*, H-5′),* 6.68 (1H, *s*, H-3),* 9.97 (1H, *br s*, OH-2″), 10.29 (1H, *s*, OH-2), 10.46 (1H, *br s*, OH-2′). (*Assignments may be interchanged.)

Gyrophoric acid (**3**). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.36 (3H, *s*, H-8), 2.37 (3H, *s*, H-8′), 2.38 (3H, *s*, H-8″), 6.23 (1H, *d*, $J = 2.01$ Hz, H-5),* 6.24 (1H, *d*, $J = 2.01$ Hz, H-3),* 6.62 (1H, *d*, $J = 2.01$ Hz, H-5″),$^+$ 6.64 (1H, *d*, $J = 2.01$ Hz, H-3″),$^+$ 6.67 (1H, *s*, H-5′),‡ 6.68 (1H, *s*, H-3′),‡ 10.03 (1H, *s*, OH-2″), 10.33 (1H, *s*, OH-2), 10.51 (1H, *s*, OH-2′). (*, +, ‡ Assignments may be interchanged.)

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