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NEW DEPSIDONES FROM *LOBARIA OREGANA*

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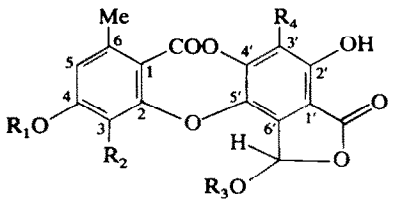
Key Word Index—*Lobaria oregana*; lichen metabolites; methylstictic acid; crypto-stictic acid; cryptostictinolide.

Abstract—From the lichen, *Lobaria oregana*, two new minor metabolites, methylstictic acid (**4**) and cryptostictic acid (**5**) were isolated. The structures of **4** and **5** were elucidated by ^{1}H NMR and MS analysis.

The metabolites of the lichen, *Lobaria oregana* (Tuck.) Müll. Ar. were studied earlier by Asahina *et al.* [1] and Culberson [2] to report the occurrence of (+)-usnic acid, norstictic acid (**1**), stictic acid (**2**) and constictic acid (**3**). The present study concerns minor constituents of *L. oregana* collected in British Columbia, Canada.

The lichen material was first extracted with C_6H_6 to isolate (+)-usnic acid and ergosterol peroxide, then extracted at room temperature sucessively with $CHCl_3$ and Me_2CO.

The Me_2CO extracts were chromatographed over a 0.5 N oxalic acid-impregnated Si gel column using C_6H_6–Me_2CO as the solvent. TLC of the eluate developed with C_6H_6–dioxan–HOAc (90:25:4) gave more than 7 spots among which norstictic acid (**1**), stictic acid (**2**) and constictic acid (**3**) were identified, while the occurrence of two new compounds named methylstictic acid (**4**) and cryptostictic acid (**5**) was observed along with some artifacts.



	R_1	R_2	R_3	R_4	
1	H	CHO	H	Me	Norstictic acid
2	Me	CHO	H	Me	Stictic acid
3	Me	CHO	H	CH_2OH	Constictic acid
4	Me	CHO	Me	Me	Methylstictic acid
5	Me	CH_2OH	H	Me	Cryptostictic acid
6	Me	Me	H	Me	Hypostictic acid

Methylstictic acid (**4**), $C_{20}H_{16}O_9$, colourless needles, mp 250 ~ 251°, gave a purple colour with $FeCl_3$ similar to stictic acid (**2**), and also showed very similar absorptions of UV and IR to those of **2**. The ^{1}H NMR in DMSO-d_6 of **4** gave signals for two methyls (δ 2.48, 2.22), one each for methoxyl (δ 3.91) and aldehyde (δ 10.39) attached to aromatic rings at almost the same chemical shifts given by **2**, whereas an additional methoxyl signal at δ 3.44 was given by **4** in place of a signal for a lactol OH at δ 8.1 observed for **2**. The MS of **4** showed a M^+ at *m/e* 400 and a peak *m/e* 368 (M^+-MeOH, while **2** gave M^+ *m/e* 386 and M^+-H_2O at *m/e* 368 [3] (Scheme 1). On these results, methylstictic acid has been represented by the structure (**4**).

Cryptostictic acid (**5**), $C_{19}H_{16}O_9$, colourless needles, mp 242 ~ 244° (decomp.) showed a very similar ^{1}H NMR spectrum to that of stictic acid (**2**), the only difference being the absence of the signal for CHO and the appearance of one for CH_2OH at δ 4.6 (1H *br. d* J = 10 Hz) and δ 4.72 (1H *br. d* J = 10 Hz). On acetylation **5** afforded a triacetate to confirm the presence of a CH_2OH group. The location of the CH_2OH on the A ring was proved by the MS fragmentation giving a peak *m/e* 177 (41.9%) (Scheme 2).

The structure of **5** has also been chemically proved by comparison with the established structure of **2**. On catalytic reduction of **2** with Pd–C in a mixture of EtOAc and HOAc (4:1) **5** was afforded, which was identified by mmp and comparisons of TLC and IR with the naturally isolated sample. Asahina *et al.* [1] reported that catalytic reduction of **2** with Pd–C in

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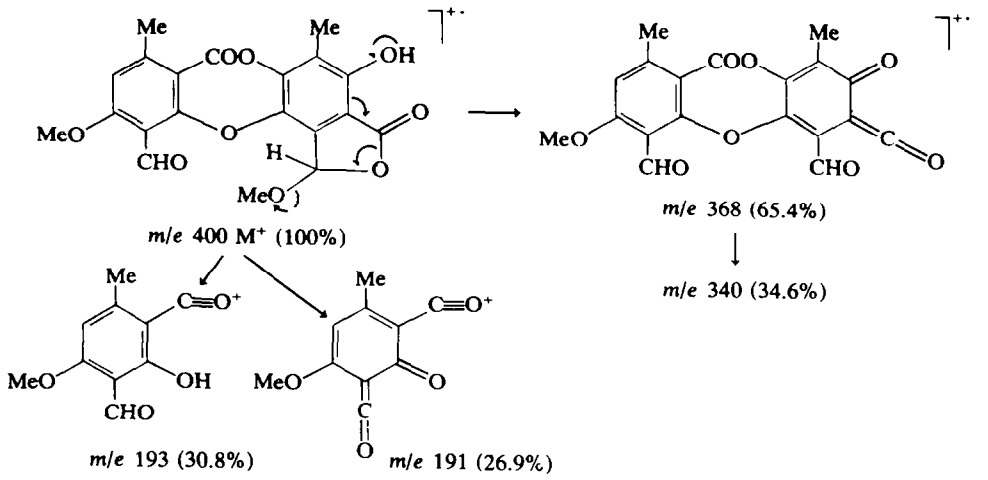
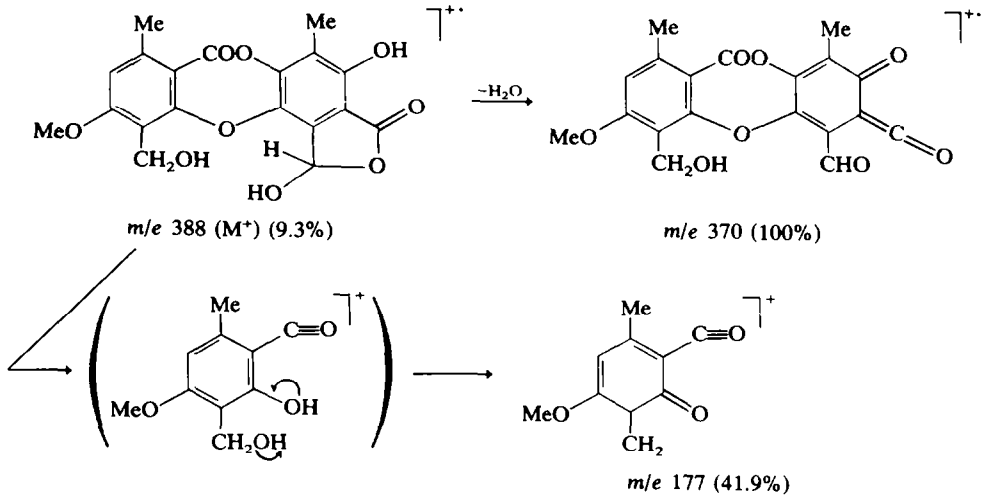


Scheme 1.



Scheme 2.

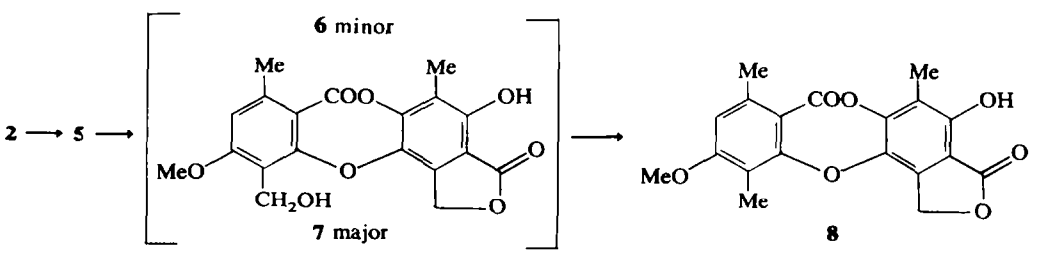
HOAc yielded hypostictic acid (**6**). In the present study **2** was reduced with PtO_2 as catalyst in a mixture of EtOAc and HOAc (4:1) for a longer time to produce cryptostictinolide (**7**) as a major product and hypostictic acid (**6**) as a minor one in an earlier stage of reaction, which were finally converted into hypostictionlide (**8**) (Scheme 3).

Thus a solvent effect has been shown in the course of catalytic reduction of the CHO group of **2**.

EXPERIMENTAL

IR spectra were measured in KBr and ^1H NMR spectra in DMSO-d_6 with TMS as an int. standard at 100 MHz.

Lobaria oregana (*Tuck.*) *Müll. Arg.* This was collected in British Columbia, Canada in 1975. 6 kg were extracted ×3 with C_6H_6 at room temp. to separate (+)-usnic acid (1.47%) and ergosterol peroxide (0.01%). Residue was extracted with $CHCl_3$ and then Me_2CO at room temp. From the



Scheme 3.

supernatant of the conc Me_2CO-extracts by 0.5 N oxalic acid-impregnated Si gel column chromatography using C_6H_6–Me_2CO as solvent, norstictic acid (**1**), stictic acid (**2**) and constictic acid (**3**) were separated as the major products (total yield: *ca* 8%) and methylstictic acid (**4**) and cryptostictic acid (**5**) as the minor ones in a yield of 0.02 and 0.03%, respectively.

Methylstictic acid (**4**). $C_{20}H_{16}O_9$, colourless needless (from Me_2CO), mp 250–251° (decomp.), $[\alpha]_D^{20} = \pm 0°$; UV λ_{max}^{EtOH} nm(log ε):238 (4.62), 312 (3.77); IR ν_{max}^{KBr} cm^{-1}: 3370, 1755, 1740, 1695; ^1H NMR (DMSO-d_6) δ ppm: 2.22 (3H, *s*, Me at C-3'), 2.48 (3H, *s*, Me at C-6), 3.44 (3H, *s*, MeO), 3.91 (3H, *s*, MeO at C-4), 6.44 (1H), 7.08 (1H, *s*, arom. H), 10.3 (1H, *s*, OH), 10.39 (1H, *s*, CHO); ^1H NMR (Py-d_5) δ ppm: 2.36 (3H, *s*, Me at C-3'), 2.58 (3H, *s*, Me at C-6), 3.68 (3H, *s*, OMe), 3.81 (3H, *s*, OMe at C-4, 6.62 (1 H), 6.85 (1 H, *s*, arom. H), 10.98 (1 H, *s*, CHO); MS: *m/e* 400 (M^+, 100), 368 (M^+ – MeOH), 340 (368 – CO, 193, 191; High resolution MS: Observed M^+: 400.0793. Calc. for $C_{20}H_{16}O_9$ M^+: 400.0793. $FeCl_3$: purple.

Cryptostictic acid (**5**). $C_{19}H_{16}O_9$, colourless needles, mp 242–244° (decomp.) (from aq. Me_2CO) UV λ_{max}^{EtOH} nm (log ε): 214 (4.54), 267 (3.98), 316 (3.51); IR ν_{max}^{KBr} cm^{-1}: 3370, 3110, 1736; ^1H NMR (in DMSO-d_6) δ ppm: 2.17 (3H, *s*, Me at C-3'), 2.42 (3H, *s*, Me at C-6), 3.84 (3H, *s*, OMe), 4.60 (1 H, *d*, J = 10 Hz, —CH_2—OH), 4.72 (1 H, *d*, J = 10 Hz —CH_2—OH), 6.92 (2 H, arom-H and O—CH—OMe), 8.20 (1 H, *s*, OH), 10.05 (1 H, *s*, OH); ^1H NMR (Py-d_5) δ ppm: 2.34 (3 H, *s*, Me at C-3'), 2.6 (3 H, *s*, Me at C-6), 3.7 (3 H, *s*, MeO), 5.39 (2 H, —CH_2OH), 6.79 (1 H, *s*, arom-H), 8.03 (1 H); MS: *m*/2 388 (M^+), 370 (M^+—H_2O (100)), 177. Anal. Calc. for $C_{19}H_{16}O_9$: C, 58.76; H, 4.15 Found: C, 58.58; H, 4.10%; $FeCl_3$: bluish-purple.

Triacetate of **5**. $C_{25}H_{22}O_{12}$, colourless needles, mp 270–271.5° (decomp.); ^1H NMR ($CDCl_3$) δ ppm: 2.07, 2.19, 2.27, 2.39, 2.50 (3 H each, *s* (2Me, 3OAc)), 3.85 (3 H, *s*, OMe), 4.79 (1 H, *d*, J = 12 Hz), 5.52 (1 H, *d*, J = 12 Hz), 6.64 (1 H, s, arom.-H), 7.48 (1 H, *s*); MS: *m/e* 514 (M^+), 472 (100).

Catalytic reduction of **5** (*formation of cryptostictinolide* (**7**)). PtO_2 (20 mg) was suspended in EtOAc (6 ml) and saturated with H_2 (1 hr), then a soln of **5** (30 mg) in a mixture of EtOAc and HOAc (4:1) (10 ml) was added and shaken for 4 hr. The product was extracted into EtOAc and the extract chromatographed on a 0.5 N oxalic acid impregnated Si gel column to isolate **7**. Cryptostictinolide (**7**), $C_{19}H_{16}O_8$, colourless needles, mp 275 ~ 276° (decomp.) (From Me_2CO). ^1H NMR (Py-d_5) δ ppm: 2.3 (3 H, *s*, Me at C-3'), 2.49 (3 H, *s*, Me at C-6), 3.6 (3 H, s, OMe), 4.91 (2H, —CH_2—O—c) 5.84 (2H, —CH_2—OH), 6.68 (1 H, *s* arom.-H); MS: *m/e* 372 (M^+), 254 (100).

Catalytic reduction of **2** (*formation of* **5**). Stictic acid (**2**) (30 mg) was dissolved in a mixture of EtOAc (20 ml) and HOAc (5 ml) and shaken with Pd–C (10%) (30 mg) under a H_2 atmosphere for 1.5 hr. The product was chromatographed on a 0.5 N oxalic acid impregnated Si gel column using C_6H_6–Me_2CO as the solvent to separate a main product, mp 242°, which was proved to be identical with **5** by mmp and comparisons of IR and TLC. Stictic acid (**2**) (50 mg) was reduced with Pt_2O in a mixture of EtOAc (6 ml) and HOAc (14 ml) for 8 hr. After removing catalyst, the reaction mixture was chromatographed on a 0.5 N oxalic acid impregnated Si gel column to separate hypostictinolide (**8**) and cryptostictinolide (**7**).

Hypostictinolide (**8**). $C_{19}H_{16}O_7$, colourless needles, mp 277 ~ 278.5° (from Me_2CO); ^1H NMR (DMSO-d_6) δ ppm: 2.14 (6H, *s*, Me at C-3' and C-3'), 2.37 (3H, *s*, Me at C-6), 3.81 (3H, *s*, OMe), 5.44 (2H, *s*), 6.84 (1H, *s*, arom.-H), 9.77 (OH); MS: *m/e* 356 (M^+) (100), 179.

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