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# ALECTOSARMENTIN, A NEW ANTIMICROBIAL DIBENZOFURANOID LACTOL FROM THE LICHEN, *ALECTORIA SARMENTOSA*

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ABSTRACT.—From the alcoholic extract of the lichen *Alectoria sarmentosa*, four compounds showing antimicrobial activity were isolated. Of these, (−)-usnic acid and physodic acid are well known lichen products, 8′-*O*-ethyl-β-alectoronic acid [**2**] is believed to be an artifact formed during isolation and fractionation, and alectosarmentin [**1**] is a new natural product whose structure was shown, by spectroscopy and chemical transformations, to be that of a dibenzofuranoid lactol. The antimicrobial activity of these substances accounts for the activity of the lichen.

Certain lichens and lichen products have a long-established reputation in folk medicine for antimicrobial activity (1). A number of recent studies have justified this by the isolation and characterization of individual active constituents (2). Recently, we had occasion to screen *Alectoria sarmentosa* Ach. (Usneaceae) extracts and found them to be active against Gram-positive, acid-fast, and fungal microorganisms. Detailed experiments, described herein, were subsequently performed, which revealed the identity of the active agents.

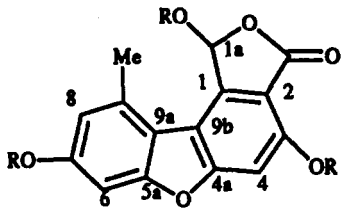
The results of previous studies of the chemical constituents of *Alectoria sarmentosa* have indicated the presence of antitumor active polysaccharides (3), arabitol and mannitol (4), usnic acid and alectoronic acids (5), and lipids (6).

## RESULTS AND DISCUSSION

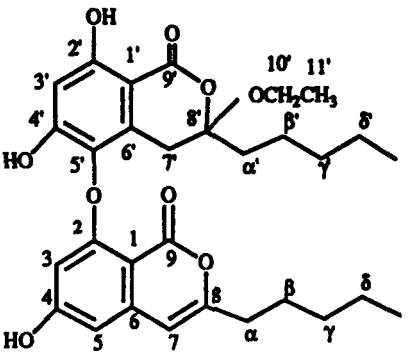
The fractionation scheme that we customarily employ uses acid and base treatments in bulk transfer operations in the early stages (7). This was found to destroy the activity in this extract and to effect changes in the constituents. Consequently, a more delicate sequence of steps was employed. Fortunately, the purification of the constituents proved to be comparatively simple. Following cold EtOH extraction, a substantial quantity of (−)-usnic acid was crystallized directly from the extract. Subsequent Si gel chromatography of the mother liquors led to the isolation in pure form of three additional active constituents. Biological assays were performed according to our usual protocol (26).

(−)-Usnic acid, physodic acid, and 8′-*O*-ethyl-β-alectoronic acid [**2**] were readily identified from their physical and spectroscopic properties. Usnic acid is one of the most common lichen substances and is occasionally reported in the (+) as well as in the (−) form (8–10). Physodic acid (11–13) is a typical depsidone, a relatively large class of lichen metabolites (14). Compound **2**, 8′-*O*-ethyl-β-alectoronic acid, is a phenoxyisocoumarin derivative that, from its structure and history, is most likely to be an artifact formed from β-alectoronic acid during the treatment with EtOH. Such artifacts have been encountered with some frequency in working with lichen substances (15). β-Alectoronic acid itself has been isolated from the lichens of the Parmeliaceae and is a well-known substance (16). The position of attachment of the *O*-ethyl group was made by analogy and must be considered provisional.

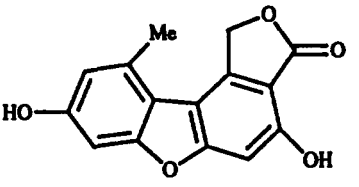
Alectosarmentin [**1**] analyzed for $C_{15}H_{10}O_6$. Three of the oxygens were shown to be hydroxyl groups from the $^1$H-nmr spectrum (signals at δ 8.05, 9.90, and 10.81; $D_2O$ exchangeable) and by the formation of a triacetate ester [**4**] and a trimethyl ether [**3**]. Interestingly, the least acidic of these hydroxyls gave a doublet coupled with a strongly




1 R=H
3 R=H
4 R=Ac




2




5

shifted methine proton. The explanation for this became clear when a lactol ring was identified (see below). There is some precedent for this in that such signals are often described as "broad" (17), but in our case a sharp doublet was present. The chemical shift of one of the acetate methyls (δ 2.18) and one of the ether methyls (δ 3.59) was distinctly different from those of the others (δ 2.36, 2.45; δ 3.89, 4.06, respectively) indicating attachment of the different hydroxyl to a non-aromatic carbon. A methine proton signal at δ 6.92 is rather more downfield than usual. These signals underwent characteristic shifts following acetylation and methylation. Taken in connection with an absorption in the ir spectrum at 1740 $cm^{-1}$ (18), a $^{13}C$-nmr signal at δ 96.42, and cross-peak from an HMBC nmr spectrum in which the carbonyl carbon (δ 167.13) correlated with the methine proton (19), these findings lead to the assignment of a lactol ring. In the ether [**3**] and acetate [**4**] derivatives, similar correlations are observed. These observations closely correspond to those found for other lactols (20,21). The presence of a lactol ring accounts for two more oxygen atoms. Lactol rings are relatively rare among lichen products but are not without precedent in depsidones (18,21,22). The dibenzofuran ring system contains the remaining oxygen and was assigned originally based on the lack of correspondence of the spectra to the more common classes of lichen substances and was encouraged by the close correspondence of the nmr spectra of alectosarmentin with those of other dibenzofurans, most particularly with the closely related strepsilin [**5**] and its methyl ether (23,24). The orientation of the functional groups was assigned from compatible signals in the nmr spectra. The orientation of the lactol ring of alectosarmentin was not clear from these measurements but was established from nOe experiments in which the irradiation of the *C*-methyl group (δ 2.73) in the methyl ether led to enhancement in integral intensity of the C-1a proton and methoxyl group signals along with that of H-8. The spatial relationship of the C-9 methyl group and the C-7 hydroxyl group is also confirmed by the HMBC experiment in which the C-8 proton, the C-7 and C-9 methyl protons, and C-8 showed the required correlations. These findings require that alectosarmentin has structure **1**. Absolute confirmation of these inferences followed the reductive conversion ($NaBH_4$) of alectosarmentin to strepsilin [**5**].

The antimicrobial activities of the *Alectoria sarmentosa* constituents are listed in Table 1 (26). The activity is confined to *Staphylococcus aureus* (Gram-positive), *Mycobacterium smegmatis* (acid fast), and *Candida albicans* (yeast), and is sufficiently weak that these agents are unlikely to have a therapeutic future other than as novel structural leads for exploitation. The bioactivities revealed for the various constituents are sufficient to account for the activity of the lichen. They provide additional confirmation of the presence of potentially useful antimicrobial activity in lichen substances and alectosarmentin is added to the increasing roster of antimicrobially active secondary constituents found in plants. Once again the power of bioassay-directed methodology to reveal novel constituents in previously well-studied sources is demonstrated.

TABLE 1. Antimicrobial Potency of *Alectoria sarmentosa* Components In Vitro (26).

| Compound | Microorganism[a] (μg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **a** | **b** | **c** | **d** | **e** | **f** | **g** |
| Crude extract | 100 | i[b] | i | i | 100 | 1000 | i |
| (−)-Usnic acid | 25 | i | i | i | 6.25 | 50 | i |
| Physodic acid | 25 | i | i | i | 100 | i | i |
| 8′-*O*-Ethyl-β-alectoronic acid [**2**] | 50 | i | i | i | 50 | 100 | i |
| Alectosarmentin [**1**] | 25 | i | i | i | 25 | i | i |
| Streptomycin sulfate | 5 | 5 | 50 | 2.5 | 1.25 | i | i |

[a]Microorganism **a**, *Staphylococcus aureus* ATCC 13709; **b**, *Escherichia coli* ATCC 9637; **c**, *Salmonella gallinarum* ATCC 9184; **d**, *Klebsiella pneumoniae* ATCC 10031; **e**, *Mycobacterium smegmatis* ATCC 607; **f**, *Candida albicans* ATCC 10231; **g**, *Pseudomonas aeruginosa* ATCC 27853.

[b]Crude preparations not active at 1000 μg/ml and pure substances not active at 100 μg/ml are scored as i (inactive).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Unimelt apparatus and are uncorrected. Nmr spectra were recorded with a Bruker 500 spectrometer in $CDCl_3$ unless otherwise mentioned; δ are in ppm downfield to TMS as internal standard, *J* in Hz. The $^{13}C$-nmr assignments were made by analogy with values for known substances and, in some instances, by resorting to HMBC measurements. In the latter, the field delay in the HMBC sequence was set at 8.3 Hz. Cc was performed on Merck Si gel 60, and tlc on Merck Si gel plates (0.25 mm); detection: uv lamp and 10% $H_2SO_4$ reagent. Ir spectra were recorded with a Perkin-Elmer 1420. Uv spectra were recorded with a Hewlett-Packard 8450 A.

PLANT MATERIAL.—*Alectoria sarmentosa* Ach. (Usneaceae), was purchased from Carolina Biological Supply Co., Burlington, North Carolina, from whom verification specimens can be obtained.

EXTRACTION AND ISOLATION.—The powdered whole lichen, *Alectoria sarmentosa* (187 g), was extracted at room temperature with 95% EtOH (12×200 ml), which gave a yellowish green colored residue (14 g, 7.5%) after solvent evaporation under reduced pressure. On direct crystallization of the crude extract, (−)-usnic acid (4.37 g, 2.3%) was obtained as yellow crystals from $Me_2CO$. The remaining marc was extracted in a Soxhlet apparatus with 95% EtOH. The extract was concentrated to a residue under reduced pressure at 40° bath temperature. The residue obtained (7.6 g, 4.0%) was combined with the mother liquors from the direct crystallization of usnic acid and subjected to cc on Si gel (250 g Si gel). A total of 127 fractions (100 ml each) were obtained with a polarity gradient solvent system using $CH_2Cl_2$ and EtOAc. Fractions 82–92 [eluted with $CH_2Cl_2$-EtOAc (19:1)] yielded 12.9 g (6.9%) of residue that on crystallization with EtOAc/$C_6H_6$, yielded 0.86 g (0.46%) of pure physodic acid. The mother liquors (12 g) were chromatographed two more times in sequence with the same system to produce an additional 0.56 g (0.3%) of physodic acid. A portion of the filtrate from the second lot of physodic acid was evaporated to a residue (1 g) and was subjected to repeated prep. tlc using $C_6H_6$-EtOAc (1:1). This produced, after crystallization from $Et_2O$-hexane, 0.154 g (0.08%) of 8′-*O*-ethyl-β-alectoronic acid [**2**] as colorless crystals. Fractions 104–127 from the original large-scale column were eluted with $CH_2Cl_2$-EtOAc (3:2). Evaporation produced 0.75 g (0.4%)

of residue which, upon repeated crystallization from EtOH/$C_6H_6$, afforded colorless needles of alectosarmentin [**1**] (0.082 g, 0.044%).

*Usnic acid.*—Yellow crystals: mp 198° ($Me_2CO$) [lit. (8) 203° ($CHCl_3$-EtOH)]; $[\alpha]^{25}D$ −576° ($c$=0.234, $CHCl_3$); spectral data were in good agreement with those reported (8).

*Physodic acid.*—Crystals: mp 189–192° (EtOAc-$C_6H_6$) [lit. (8) 205° (MeOH-$H_2O$)]; spectral data were in good agreement with those reported (13,25).

*8′-O-Ethyl-β-alectoronic acid* [**2**].—Colorless crystals: mp 182°; uv λ max (MeOH) (log ε) 215 (4.27), 245 (4.76), 259 sh (4.37), 269 sh (4.29), 290 (3.87), 318 (4.10) nm; ir ν max (KBr) 3280, 1690, 1675, 1658, 1595, 1480, 1460, 1355, 1256, 1160, 1105, 1070, 1035, and 930 $cm^{-1}$; $^1H$ nmr δ 0.84 (3H, t, $J$=5.9 Hz, Me), 0.89 (3H, t, $J$=6.9 Hz, Me), 1.07 (3H, t, $J$=6.9 Hz, Me-11′), 1.20 (2H, m, $CH_2$), 1.24–1.64 (12H, m, $CH_2$), 1.33 (2H, m, $CH_2$), 1.63 (2H, m, $CH_2$), 1.90 (2H, m, $CH_2$-α′), 2.44 (2H, t, $J$=7.5 Hz, $CH_2$-α), 2.90 (1H, br d, $J$=15.8 Hz, $H_a$-7′), 3.10 (1H, br d, $J$=15.8 Hz, $H_b$-7′), 3.57 (1H, m, $H_a$-10′), 3.63 (1H, m, $H_b$-10′), 6.13 (1H, s, H-7), 6.32 (1H, br s, H-3), 6.40 (1H, s, H-3′), 6.46 (1H, d, $J$=1.5 Hz, H-5), 8.98 (1H, br s, OH), 11.0 (1H, br s, OH); $^{13}C$ nmr (125.7 MHz) δ 13.9 (2×Me), 15.2 (C-11′), 22.3 (C-δ), 22.4, (C-δ′), 23.1 (C-β′), 26.4 (C-β), 31.1 (C-γ), 31.2 (C-γ′), 31.6 (C-α′), 33.1 (C-α), 35.5 (C-7′), 58.1 (C-10′), 99.9 (C-1′), 102.5 (C-1), 103.2 (C-7), 103.4 (C-3, C-3′), 105.6 (C-5), 107.7 (C-8′), 131.7 (C-6′), 133.3 (C-5′), 142.2 (C-6), 156.9 (C-4′), 159.1 (C-8), 160.8 (C-9), 161.8 (C-2), 162.8 (C-4), 163.8 (C-2′), 169.1 (C-9′); eims $m/z$ [M−OEt]$^+$ 494 ($C_{28}H_{32}O_8$) (2.4), 476 (1.0), 279 (10), 167 (30.6), 150 (11.6), 149 (100); fabms $m/z$ [M+H]$^+$ 541.

*Alectosarmentin* [**1**].—Colorless needles, mp 291°; uv λ max (MeOH) (log ε) 229 sh (4.50), 235 (4.51), 260 (4.45), 295 (4.11), 305 (4.17), 329 (3.88) nm; (MeOH+HCl) 227 (4.48), 238 (4.49), 260 (4.45), 296 (4.07), 305 (4.16), 331 (3.87) nm; (MeOH+NaOH) 236 (4.53), 274 (4.31), 319 (4.13), 357 (3.90) nm; ir ν max (KBr) 3400, 1740, 1695, 1620, 1590, 1465, 1425, 1335, 1285, 1205, 1165, 1145 $cm^{-1}$; $^1H$ nmr (DMSO-$d_6$) δ 2.74 (3H, s, Me), 6.64 (1H, d, $J$=1.9 Hz, H-8), 6.82 (1H, br d, $J$=1.9 Hz, H-6), 6.92 (1H, d, $J$=7.0 Hz, $H_a$-1), 7.11 (1H, s, H-4), 8.05 (1H, d, $J$=7.0 Hz, OH, $D_2O$ exchangeable), 9.90 (1H, br s, OH, $D_2O$ exchangeable), 10.81 (1H, s, OH, $D_2O$ exchangeable); $^{13}C$ nmr (125.7 MHz, DMSO-$d_6$) δ 20.7 (Me-9), 95.5 (C-6), 96.4 (C-1a), 99.8 (C-4), 107.8 (C-2), 112.2 (C-9b), 112.9 (C-9a), 113.8 (C-8), 133.6 (C-9), 141.3 (C-1), 155.0 (C-4a or C-3), 157.4 (C-7), 157.9 (C-5a), 161.0 (C-3 or C-4a), 167.1 (C=O); eims $m/z$ [M]$^+$ 286 ($C_{15}H_{10}O_6$) (62), 240 (26), 212 (100), 184 (22), 155 (22), 149 (21), 128 (23), 77 (24), 69 (40), 44 (52).

*Alectosarmentin trimethyl ether* [**3**].—To the $Me_2CO$ solution of alectosarmentin [**1**] (20 mg), dimethyl sulphate (1.0 ml) was added. The mixture was refluxed over anhydrous $K_2CO_3$ for 3 h. The usual workup of this mixture yielded **3**, which was crystallized as colorless needles from $Et_2O$/hexane mixture (23 mg); mp 236°; $^1H$ nmr δ 2.75 (3H, br s, Me), 3.59 (3H, s, OMe-1a), 3.89 (3H, s, OMe-3), 4.06 (3H, s, OMe-7), 6.54 (1H, s, $H_a$-1), 6.77 (1H, dd, $J$=2.3 and 0.7 Hz, H-8), 6.92 (1H, d, $J$=2.3 Hz, H-6), 7.15 (1H, s, H-4); $^{13}C$ nmr (125.7 MHz) δ 20.9 (Me-9), 55.5 (OMe-1a), 55.7 (OMe-3), 56.5 (OMe-7), 93.9 (C-6), 96.8 (C-4), 101.2 (C-1a), 109.8 (C-2), 113.4 (C-9b), 113.6 (C-8), 114.5 (C-9a), 133.5 (C-9), 139.3 (C-1), 157.1 (C-3), 158.6 (C-5a), 159.8 (C-7), 162.2 (C-4a), 166.9 (C=O); eims $m/z$ [M]$^+$ 328 ($C_{18}H_{16}O_6$) (40), 297 (38), 268 (20), 149 (17), 139 (12), 69 (15), 44 (100).

*Alectosarmentin triacetyl ester* [**4**].—The mixture of alectosarmentin [**1**] (10 mg) in $Ac_2O$ (0.5 ml) and pyridine (0.5 ml) was kept at room temperature for 12 h. The usual workup gave 12 mg of **4**, which was crystallized from $Me_2CO$/MeOH mixture. Mp 210°; $^1H$ nmr δ 2.18 (3H, s, OAc), 2.36 (3H, s, OAc), 2.47 (3H, s, OAc), 2.65 (3H, br s, Me), 6.98 (1H, dd, $J$=2.0 and 0.7 Hz, H-8), 7.29 (1H, d, $J$=2.0 Hz, H-6), 7.52 (1H, s, H-4), 7.74 (1H, s, $H_a$-1); $^{13}C$ nmr (125.7 MHz) δ 20.7 ($OCOCH_3$), 21.0 ($OCOCH_3$), 21.0 ($OCOCH_3$), 21.1 (Me), 92.0 (C-1a), 103.8 (C-6), 109.3 (C-4), 114.3 (C-9b), 117.7 (C-2), 118.6 (C-9a), 119.8 (C-8), 133.6 (C-9), 138.3 (C-1), 147.5 (C-3), 150.8 (C-7), 157.9 (C-5a), 161.3 (C-4a), 165.0 (C=O), 168.8 ($OCOCH_3$), 169.0 ($OCOCH_3$), 169.2 ($OCOCH_3$).

*Conversion of alectosarmentin* [**1**] *to strepsilin* [**5**].—Alectosarmentin [**1**] (14 mg) was dissolved in 10 ml of absolute EtOH to which 5 mg of $NaBH_4$ in 3 ml of EtOH was added slowly while stirring. The reaction mixture was kept overnight and the solvent was removed *in vacuo*. To the residue was added 15 ml of 5% $H_2SO_4$ and this was extracted with EtOAc (4×15 ml). The extract was dried over anhydrous $Na_2SO_4$ and the solvent filtered and evaporated. The residue showed the presence of **1** and a major new spot. The new substance, strepsilin [**5**], was separated by prep. tlc using $CH_2Cl_2$-MeOH (9:1). Mp 290° (dec) [lit. (24) 324° (AcOH)]; ir ν max (KBr) 3430, 1730, 1700, 1615, 1590, 1440, 1335, 1140 $cm^{-1}$; $^1H$ nmr (DMSO-$d_6$) δ 2.67 (3H, s, Me), 5.83 (2H, s, $CH_2$-1a), 6.63 (1H, d, $J$=2.0 Hz, H-8), 6.83 (1H, d, $J$=2.0 Hz, H-6), 7.06 (1H, s, H-4); $^{13}C$ nmr (125.7 MHz, DMSO-$d_6$) δ 20.4 (Me-9), 69.4 (C-1a), 95.6 (C-6), 98.2 (C-4), 107.3 (C-2), 110.9 (C-9b), 113.4 (C-9a), 113.6 (C-8), 131.9 (C-9), 141.5 (C-1), 155.4 (C-3 or 4a), 157.1 (C-4a

or C-3), 157.5 (C-7 or C-5a), 160.5 (C-4a or C-3), 168.8 (C=O); eims *m/z* $[M]^+$ 270 (100), 241 (83), 185 (11), 128 (10), 119 (7), 115 (9), 91 (10).

*Antimicrobial Assays.*—Antimicrobial assays were performed by our published agar-dilution streak methodology using streptomycin sulfate as a positive control (26).

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