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THE STRUCTURE OF SCROBICULIN, A NEW LICHEN DEPSIDE IN *LOBARIA SCROBICULATA* AND *LOBARIA AMPLISSIMA**

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Abstract—A new depside, scrobiculin, was isolated from *Lobaria scrobiculata*, which also produces (+)-usnic acid, stictic acid, and norstictic acid, and from *Lobaria amplissima*. Scrobiculin is proven to be methyl 4′-demethylsekikaiate. The monomethyl derivative formed by reaction with diazomethane is identical to methyl sekikaiate.

INTRODUCTION

IN 1947, Asahina[1] reported the presence of stictic acid (V) and an unidentified substance in *Lobaria verrucosa* Hoffm. (=*L. scrobiculata* (Scop.) DC.) on the basis of microcrystal tests. Later, Hale[2, 3] found this substance in *L. amplissima* (Scop.) Forss. by paper chromatographic comparison. He also noted a red colour reaction with base (10% KOH) followed by aqueous $Ca(OCl)_2$ and suggested that the new compound might be a depsidone similar to alectoronic acid. The purpose of the present report is to describe the extraction of *L. scrobiculata* and *L. amplissima* and the proof of structure of a new substance isolated from these species.

RESULTS AND DISCUSSION

Four collections of *Lobaria scrobiculata*, two from Scotland and two from Ireland, were extracted. All of the collections yielded the new depside scrobiculin (II), (1·11–1·63%), a mixture (0·30–0·78%) of stictic acid (V) and norstictic acid (VI), and the yellow pigment (+)-usnic acid (VII) (0·33–0·74%). The new compound was obtained in lesser yield (0·76%) from a French collection of *L. amplissima* from which other substances could not be isolated. Plants of *Polychidium umhausense* (Auersw.) Henss. (= *Dendriscocaulon umhausense* (Auersw.) Degel.), a lichen species almost exclusively found as an epiphyte on *L. amplissima*, were removed and extracted separately, but neither scrobiculin nor any other known lichen substances could be detected in the extracts.

Scrobiculin (II) gives an u.v. absorption spectrum similar to a typical lichen depside. An elemental analysis agrees with the formula $C_{22}H_{26}O_8$. The NMR spectrum (Fig. 1) shows two aryl-substituted methylenes, two terminal methyls, and two intermediate methylene groups, suggesting a pair of aryl-substituted *n*-propyl groups. The spectrum also indicates one phenolic *O*-methyl group, one ester *O*-methyl group, and three aromatic protons. The

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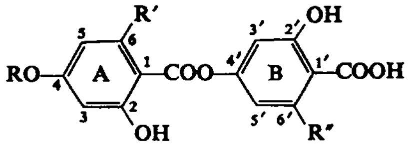
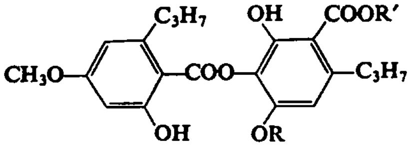
[1] Y. ASAHINA, *J. Japan. Botany* **21**, 83 (1947).

[2] M. E. HALE, *Bryologist* **60**, 35 (1957).

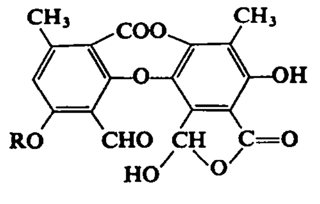
[3] M. E. HALE, *Lichenologist* **1**, 266 (1961).



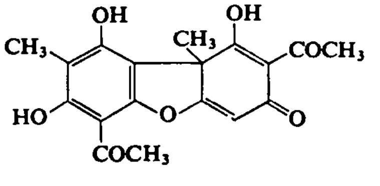
(I)



(II) R=H, R'=CH$_3$
(III) R=CH$_3$, R'=H
(IV) R=R'=CH$_3$



(V) R=CH$_3$
(VI) R=H



(VII)

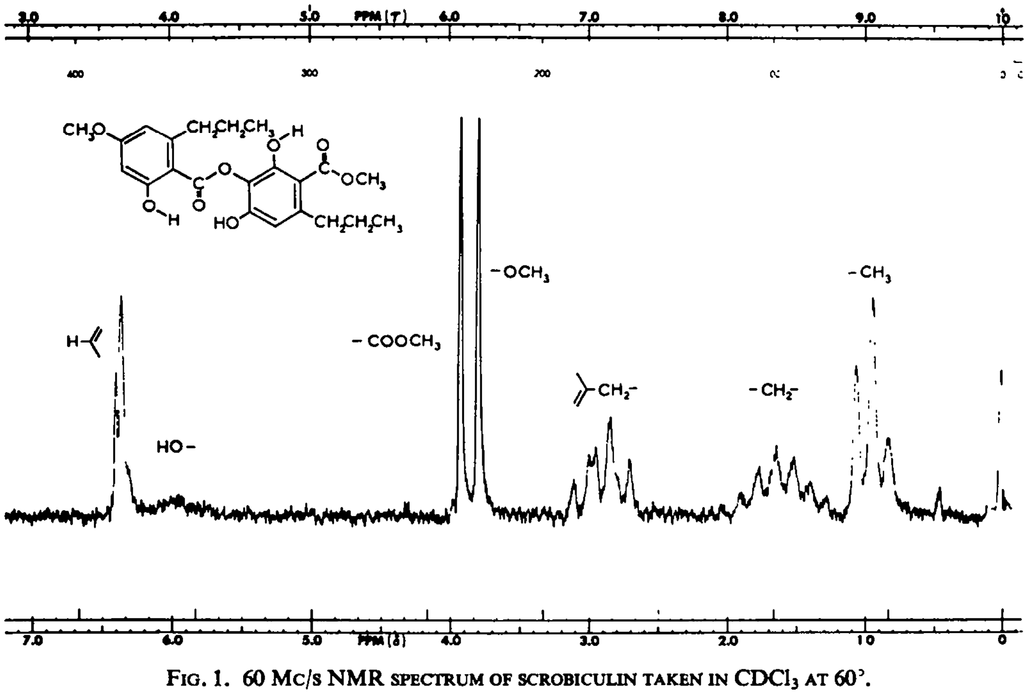
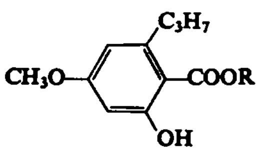
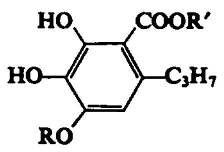


FIG. 1. 60 Mc/s NMR SPECTRUM OF SCROBICULIN TAKEN IN CDCl$_3$ AT 60°.

commonest orcinol-type lichen depsides are esterified at the 4'-hydroxyl (*para* depsides (I)) and have four aromatic protons, but a few lichen depsides, linked at the 3' position (*meta* depsides), have just three aromatic protons. The NMR spectrum indicates that scrobiculin may be such a *meta* depside with two —C$_3$H$_7$ substituents similar to the known lichen substance sekikaic acid (III), and formation of a triacetate of scrobiculin confirms the presence of three free hydroxyl groups. These data and the i.r. spectrum indicate that scrobiculin may be the methyl ester of 4-demethyl or 4′-demethyl sekikaic acid, since the hydroxyl peak at 3420 cm^{-1}, missing in the spectrum of sekikaic acid, is due to a phenolic group which cannot undergo intramolecular hydrogen bonding in a six-membered ring bridging the phenolic oxygen and the carbonyl oxygen. The presence of a methoxyl substituent in ring A was confirmed by hydrolysis of scrobiculin to divaricatinic acid (VIII), which was also obtained by hydrolysis of sekikaic acid (III), and to an ester fraction which should be methyl 2,3,4-trihydroxy-6-propylbenzoate (X). Finally, a sample of scrobiculin reacted with a slightly more than one equivalent of diazomethane yielding a product identical in every respect to methyl sekikaiate (IV). Therefore scrobiculin must be methyl 4′-*O*-demethyl-sekikaiate (II).



(VIII) R=H
(IX) R=CH_3



(X) R=H, R′=CH_3
(XI) R=CH_3, R′=H

Other known *meta* depsides with free hydroxyls in the B ring, such as merochlorophaeic acid, cryptochlorophaeic acid, and ramalinolic acid, show a red colour reaction with aqueous base.[4] But scrobiculin gives a bright yellow reaction with this reagent. The positive red reaction with aqueous $Ca(OCl)_2$ indicates *meta* dihydroxy phenols, although the reaction produced by free hydroxyls in ring B of a *meta* depside is less definite than that observed when the free hydroxyls are in the A ring of a normal *para* depside (i.e. in lecanoric acid, anziaic acid, and olivetoric acid).

A mass spectrum (at 150°) of scrobiculin showed major peaks at masses 192 and 226, but no peak for a molecular ion (M = 418). The peaks at masses 192 and 226 correspond to the masses of the A and B ring fragments formed by rupture of the depside linkage with hydrogen transfer. The mass 192 fragment (from ring A) may loose CO to form a mass 164 fragment and a metastable peak is also observed at mass 140. A peak at mass 194 could arise by loss of methyl alcohol from the *o*-hydroxy methyl ester function of the mass 226 fragment[5] (from ring B). Further loss of CO from the mass 194 fragment may produce the mass 166 ion observed. A peak at mass 384 was absent in a spectrum run at lower temperature (115°). An isotopic abundance analysis of this peak indicates the formula $C_{22}H_{24}O_6$ suggesting the loss of two hydroxyls by a process involving thermal decomposition.

No substances closely related to scrobiculin have previously been reported from *Lobaria* although stictic acid and norstictic acid are common in the genus. Gyrophoric acid, a tridepside derived from three molecules of orcellinic acid, occurs with stictic acid and norstictic acid in *L. pulmonaria* and a similar tridepside, tenuiorin, is known from a related species. (−)-Usnic acid was reported from one *Lobaria* species, but (+)-usnic acid has not previously been identified in this genus. Scrobiculin is the first *meta* depside known from *Lobaria*, and it is the first substance identified in the genus that has alkyl side-chains of more than one carbon.

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[4] S. Shibata and H.-C. Chiang, *Phytochem.* 4, 133 (1965).

[5] H. Budzikiewicz, C. Djerassi and D. H. Williams, *Interpretation of Mass Spectra of Organic Compounds.* Holden-Day, San Francisco (1964).

It is thought that *meta* depsides may arise by hydroxylation of the aromatic ring followed by esterification or by internal rearrangement of a *para* depside. But there is also some possibility that these compounds may result from direct attack of a carboxylate anion or radical at the *meta* position when the *para* hydroxyl is blocked by methoxylation. By such a mechanism, sekikaic acid would be formed directly from two molecules of divaricatinic acid. Scrobiculin might then be derived from sekikaic acid by demethylation at the 4′-hydroxyl and esterification.

EXPERIMENTAL

All melting points are corrected and were determined with a Hoover Capillary Melting Point Apparatus. Ultra-violet spectra were obtained with a Bausch & Lomb Spectronic 505. Infrared spectra were determined with a Perkin Elmer Infracord. Mass spectra are by Morgan -Schaffer Corp., Montreal, Canada, and microanalyses are by the Clark Microanalytical Laboratory, Urbana, Illinois, and the Schwarzkopf Microanalytical Laboratory, Woodside, New York.

Extraction of Lobaria scrobiculata *(Scop.) DC.*

The lichen was collected from a shaded stone wall 4 miles east of Salen, Ardnamurchan Peninsula, Argyllshire, Scotland, on July 30, 1964. A sample (No. 11,814) is deposited in Duke Herbarium. Adhering bryophytes were removed and 15·5 g of air-dry lichen was extracted in a Soxhlet extractor with 600 ml of anhydrous, peroxide-free ethyl ether for 9 hr. The ether solution was evaporated to dryness, and the solid residue was washed with small portions of ethyl ether (35 ml total volume) to remove scrobiculin and with chloroform to remove usnic acid, and finally taken up in acetone from which a mixture of stictic acid (V) and norstictic acid (VI) crystallized. The colourless precipitate from acetone consisted of 121 mg (0·78 %) of crude stictic acid (identified by microcrystal tests in the *o*-T and An solutions[6]) containing a trace of norstictic acid (detected in the K_2CO_3-KOH solution[6]). Thin-layer chromatography on silica gel-G plates developed with benzene–dioxane–acetic acid (90:25:4) and sprayed with Steiner's stable *p*-phenylenediamine solution,[7] showed stictic acid at R_f 0·37 but no norstictic acid (R_f 0·46).

The chloroform solution of usnic acid was concentrated and then diluted with 95% ethanol which precipitated 52 mg of (+)-usnic acid (VII), m.p. 203–204° (reported[8] m.p. 203–204°), $[\alpha]_D^{33\cdot6}+480°$ ($c.=0\cdot130$ g/100 ml in chloroform), (reported[8] $[\alpha]_D^{17°}+492\cdot2°$).

The ether solution containing *scrobiculin* (II) was diluted with ether and extracted with small portions of 10% $NaHCO_3$ (totalling 75 ml), washed with water, and dried (Na_2SO_4). Evaporation of the ether and recrystallization of the residue from benzene–light petroleum yielded 0·252 g (1·63 %) of crude scrobiculin, m.p. 134·5–135°. A small sample was purified by repeated recrystallizations from benzene–light petroleum, m.p. 135·5–136°. The compound gave a single spot (R_f 0·53) by thin-layer chromatography on silica gel-G using hexane–ether–formic acid (5:4:1 v/v) as a solvent and turned dark-brown when sprayed with 10% H_2SO_4 and heated at 70° for several hours. Found: C, 63·08; H, 6·22. $C_{22}H_{26}O_8$ required:

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[6] S. SHIBATA, In *Modern Methods of Plant Analysis* (Edited by H. F. LINSKENS and M. V. TRACEY), Vol. 6, p. 155. Springer, Berlin (1963).

[7] J. SANTESSON, *Acta Chem. Scand.* **19**, 2254 (1965).

[8] Y. ASAHINA and S. SHIBATA, *The Chemistry of Lichen Substances*. Japan Society for the Promotion of Science, Tokyo (1954).

C, 63·15; H, 6·26%; M.W. 418. A mass spectrum showed major peaks at 384 (M—2 OH?), 226 (ring B), and 192 (ring A).

Scrobiculin (II) gives a blue colour with 1% alcoholic $FeCl_3$ solution. It dissolves in 10% aqueous KOH with a bright yellow colour which turns red upon addition of saturated aqueous $Ca(OCl)_2$. A momentary red colour is observed when a drop of aqueous $Ca(OCl)_2$ is added to an alcoholic solution of scrobiculin. The compound is easily soluble in alcohol, ether, acetone, and benzene and slightly soluble in light petroleum. I.r. (nujol), ν cm^{-1}: 3420 (phenolic OH), 1660 and 1650 (bonded C=O of ester groups), 1630 and 1590 (benzenoid). U.v. (95% ethanol); λ_{max}, nm (log ϵ): 217·5 (4·69), 265 (4·51), 302·5 (4·13). λ_{min}, nm (log ϵ): 239 (3·95), 286·5 (4·03). NMR ($CDCl_3$), δ p.p.m.: 0·95 (terminal methyls of alkyl side chains, six protons), 1·65 (intermediate methylenes of alkyl side-chains, four protons), 2·93 (methylenes attached to benzene rings and to methylene, four protons), 3·81 (phenolic *O*-methyl, three protons), 3·93 (ester *O*-methyl, three protons), 6·38–6·49 (three aromatic protons).

Three additional samples of *L. scrobiculata* were extracted. A collection (28·3 g) from oak trunks, 10 miles west of Salen, Argyllshire, Scotland (Sample No. 12,089), gave 1·11% scrobiculin, 0·30% of crude stictic acid containing a trace of norstictic acid, and 0·40% of (+)-usnic acid. A sample (7·8 g) from branches of oaks at Glengarriff, Co. Cork, Ireland, yielded 1·58% scrobiculin, 0·69% crude stictic acid containing a trace of norstictic acid, and 0·48% (+)-usnic acid. A sample (36·0 g) from branches of oaks on Torc Mountain, Killarney, Co. Kerry, Ireland, gave 1·44% scrobiculin, 0·66% crude stictic acid containing a trace of norstictic acid, and 0·74% (+)-usnic acid.

Extraction of Lobaria amplissima *(Scop.) Forss.*

The lichen was collected from the bark of oaks near Col d'Osquich (Basses-Pyrénées), France, in the summer of 1964 (Sample No. 12,165). A second lichen, *Polychidium umhausense* (Auersw.) Henss., growing on the upper surface was removed, in part, for separate examination. The lichen was otherwise free from bark, mosses, and other lichens. 30 g of air-dry plant material were extracted in a Soxhlet with anhydrous, peroxide-free ethyl ether (700 ml) for 13 hr. The ether solution was washed with small portions of 10% $NaHCO_3$ (totalling 100 ml) and with water. Evaporation of the dried (Na_2SO_4) ether layer gave a residue which was washed with light petroleum and recrystallized from benzene–light petroleum yielding 0·198 g (1·52%) of scrobiculin (II), m.p. 133·5–134°. The melting point was not depressed by admixture with scrobiculin from *L. scrobiculata*, and the i.r. spectra were identical.

The $NaHCO_3$ extract of the original ether solution was acidified (1 N H_2SO_4) and extracted with ether. The washed and dried (Na_2SO_4) ether solution gave only a slight residue which did not contain any of the acidic substances found in *L. scrobiculata* and was not studied further. An acetone extraction of the ether extracted lichen also failed to give crystalline material.

The air-dried sample (3·1 g) of *P. umhausense* was extracted with ether and then with acetone. Neither extract contained scrobiculin and no crystalline product could be isolated.

Scrobiculin Triacetate

A solution of scrobiculin (100 mg) in acetic anhydride (5 ml) containing conc. H_2SO_4 (1 drop) was heated gently on a steam bath for 9 hr and held at 70° for 2 days. The solution, poured over ice with stirring, yielded the triacetate (94 mg, 42%), m.p. 130·5–131°, after one recrystallization from methanol–water. Found: C, 61·52; H, 5·91. $C_{28}H_{32}O_{11}$ required:

C, 61·73; H, 5·92%. I.r. (nujol), v cm^{-1}: 1775 and 1740 (ester C=O), 1620 and 1580 (benzenoid).

Methyl Sekikaiate (IV)

(A) *From sekikaic acid (III).* Sekikaic acid (100 mg) in ethyl ether (10 ml), treated dropwise at ice temperature with one equivalent of CH_2N_2 in ether yielded the methyl ester (44 mg, 84%), m.p. 122·0–122·5°, after one recrystallization from benzene–petroleum ether. The product was recrystallized again from absolute methanol, m.p. 122·5–123° (reported[9] m.p. 124°). Found: C, 63·85; H, 6·54. Calc. for $C_{23}H_{28}O_8$: C, 63·88; H, 6·53%. I.r. (nujol), v cm^{-1}: 1670 (bonded ester C=O), 1625 and 1580 (benzenoid).

(B) *From scrobiculin (II).* One equivalent of diazomethane in ethyl ether was added to a solution of scrobiculin (50 mg) in ethyl ether at room temperature. The solution was stirred until the colour disappeared. An excess (about 15%) of diazomethane was added and the solution was allowed to stand overnight. The residue after evaporation of the ether was recrystallized from benzene–petroleum ether yielding methyl sekikaiate (23 mg, 44%), which was recrystallized again from absolute methanol, m.p. 121·5–122°. The melting point was not depressed by admixture with the product from methylation of sekikaic acid. The i.r. spectra of the two materials are identical and both products show a spot at R_f 0·64 by thin-layer chromatography on silica gel-G developed with *n*-hexane–ethyl ether–formic acid (5:4:1) and sprayed with 10% H_2SO_4.

Hydrolysis of Scrobiculin (II)

Scrobiculin (114 mg) was dissolved in cold concentrated H_2SO_4 (4 ml). The solution was stirred in an ice bath 10 min and poured over crushed ice (25 g). The mixture was extracted with ether and the ether layer was re-extracted with small volumes of 5% aqueous $NaHCO_3$ (totalling 100 ml). The ether solution was washed with water, dried (Na_2SO_4), and evaporated. Recrystallization of the residue from light petroleum yielded *methyl* 2,3,4-*trihydroxy*-6-*propylbenzoate* (*X*) (43 mg. 70%), m.p. 69–70°. Found: C, 58·70; H, 6·36. $C_{11}H_{14}O_5$ required: C, 58·40; H, 6·24%. I.r. (nujol), v cm^{-1}: 3540, 3450 (—OH), 1660 (bonded C=O), 1620 (benzenoid).

The $NaHCO_3$ extract was cooled in an ice bath, neutralized (1 N H_2SO_4), and extracted with ether. The ether solution gave a crude residue which recrystallized from benzene yielding divaricatinic acid (VIII) (32 mg, 55%), m.p. 158·5–160·5°, identical to divaricatinic acid from hydrolysis of sekikaic acid.

Isolation of Sekikaic Acid

Sekikaic acid was extracted from ***Physcia aegialita*** (Ach.) Nyl. collected from the bark of ***Quercus virginiana***, 6 miles south-west of McClellanville, Charleston Co., South Carolina, U.S.A. (Sample No. 10,047), in 1960. The lichen (30 g), extracted with ether, yielded 0·74 g (0·40%) of sekikaic acid (III) recrystallized from benzene–light petroleum, m.p. 146–147° (reported[8] m.p. 147°). Found: C, 63·37; H, 6·16. Calc. for $C_{22}H_{26}O_8$: C, 63·15; H, 6·26%. I.r. (nujol), v cm^{-1}: 1660 (bonded C=O), 1630 and 1585 (benzenoid).

Methanolysis of a sample of sekikaic acid yielded hydroxy divaricatinic acid (XI) which was recrystallized from water, m.p. 165·5–168° (reported[8] m.p. 163–164°). Found: C, 58·40;

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[9] Y. ASAHINA and S. NONOMURA, *Chem. Ber.* **66**, 30 (1933).

H, 6·12. Calc. for $C_{11}H_{14}O_5$: C, 58·40; H, 6·24%. The fraction insoluble in aqueous $NaHCO_3$, methyl divaricatinate (IX), gave a yellow oil which was hydrolysed with 40% aqueous KOH and recrystallized from dilute acetic acid yielding divaricatinic acid (VIII), m.p. 160–163·5 (reported[10] m.p. 150–160°). Found: C, 63·10; H, 6·95. Calc. for $C_{11}H_{14}O_4$: C, 62·84; H, 6·71%.

Acknowledgements—I thank Dr. Peter Jeffs for help with the NMR spectrum of scrobiculin and Dr. Michael Mitchell for providing samples of *Lobaria scrobiculata* from Ireland.

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[10] A. Sonn, *Chem. Ber.* **64**, 1851 (1931).