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# SOME CONSTITUENTS OF THE LICHEN *RAMALINA SILIQUOSA*

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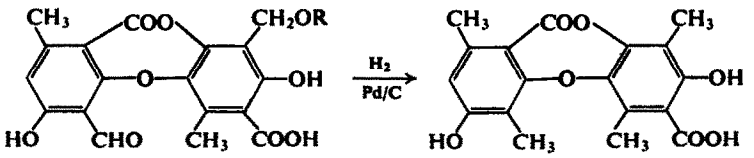
**Abstract**—Extraction of *Ramalina siliquosa* gave, in addition to (+)-usnic acid and D-(+)-arabitol, hypoprotocetraric acid, a depsidone previously known only in the laboratory. A trace of a mixture tentatively identified as atranorin and chloroatranorin was also isolated as well as a crude fraction resembling tetrahydroxy fatty acid mixtures known from other lichens. A microchemical survey of the principal phenolic constituents of 57 herbarium specimens revealed six distinct chemical races in the collective species *R. siliquosa*.

## INTRODUCTION

A MICROCHEMICAL examination of a sample of *Ramalina siliquosa* (Huds.) A. L. Sm. from France showed the presence of a new lichen acid. The major product subsequently isolated by extraction from this large collection was identified as hypoprotocetraric acid (II). Although *R. siliquosa* is the first known natural source of this compound, it has been previously synthesized. It was prepared[1] by catalytic reduction of cetraric acid (Ia) synthesized from the naturally occurring lichen depsidone fumarprotocetraric acid (Ib). The chemical structure of hypoprotocetraric acid was established by potassium hydroxide fusion,[1] by base hydrolysis of the free acid II[2] and by methanolysis and methylation of its trimethyl derivative IV to give the highly substituted diphenyl ether V.[3] Chemically hypoprotocetraric acid could be considered the parent compound of the $\beta$-orcinol-type lichen depsidones and catalytic reduction

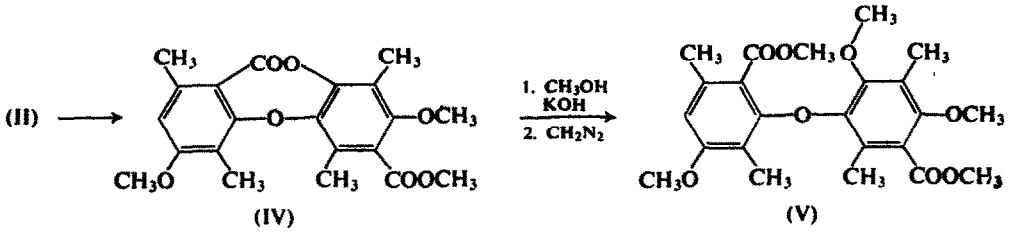



(I) (II)


(III)

*a* R = —$C_2H_5$
*b* R = —COCH=CHCOOH (*trans*)
*c* R = —H
*d* R = —$COCH_3$

[1] Y. ASAHINA and Y. TANASE, *Chem. Ber.* **66**, 700 (1933).
[2] Y. ASAHINA and J. ASANO, *Chem. Ber.* **66**, 893 (1933).
[3] Y. ASAHINA and Y. TANASE, *Chem. Ber.* **67**, 766 (1934).

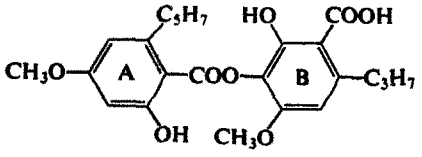



(IV) (V)

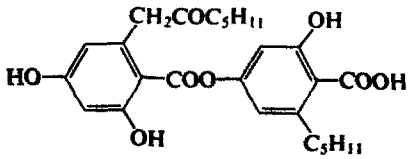
of fumarprotocetraric acid (Ib),[4] protocetraric acid (Ic),[3,5] physodalic acid (Id)[4] and virensic acid (III)[6] to hypoprotocetraric acid has established the common basic structure of these compounds.

## RESULTS AND DISCUSSION

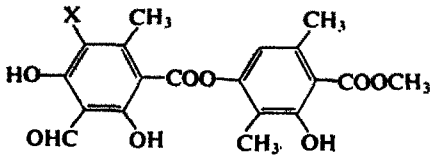
Production of hypoprotocetraric acid by *Ramalina siliquosa* fits well into the general pattern of compound types already reported from the genus *Ramalina*. A number of these substances are either unknown in other plants or occur only rarely in certain other lichens. For example, orcinol-derived depsides like sekikaic acid (VI), where the aryl ester linkage combines an acid group in ring A to a position *meta* to the acid group in ring B, are nearly restricted to *Ramalina*. On the other hand, orcinol depsides such as olivetoric acid (VII), having a partially oxidized side chain *ortho* to the aryl ester carbonyl, are very common in several other large genera of lichens but are completely unknown in *Ramalina*. Depsidones which might be derived from such depsides are also lacking. β-Orcinol depsidones related to hypoprotocetraric acid are common in the genus, although none of the β-orcinol depsides which would be considered biosynthetic precursors to these depsidones has previously been reported. There is some evidence in the present study that a β-orcinol depside, atranorin (VIIIa) and its chloro derivative (VIIIb) may be present in *R. siliquosa* in small concentration.



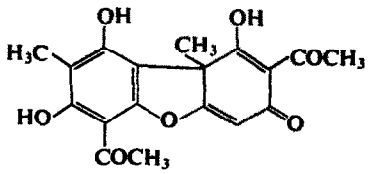
(VI)



(VII)



(VIIIa) X = H
(VIIIb) X = Cl

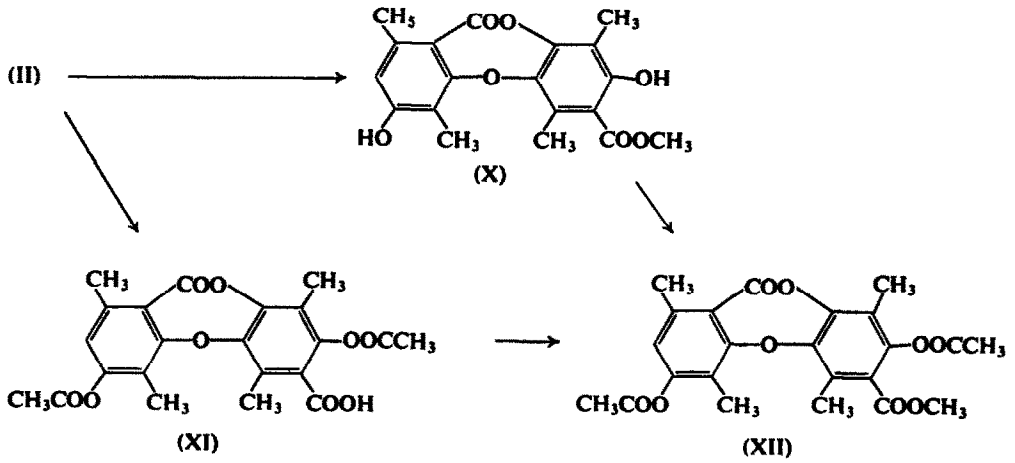


(IX)

The major product obtained from *Ramalina siliquosa* and identified as hypoprotocetraric acid could not be reduced catalytically and did not form an oxime or a 2,4-dinitrophenyl-

[4] Y. ASHINA and Y. TANASE, *Chem. Ber.* **67**, 411 (1934).
[5] Y. ASAHINA and M. YANAGITA, *Chem. Ber.* **66**, 1217 (1933).
[6] K. AGORAMURTHY, K. G. SARMA and T. R. SESHADRI, *Tetrahedron* **12**, 173 (1961).

hydrazone. The compound yielded the monomethyl ester X under controlled conditions, and this derivative could be converted to the diacetate XII. The same diacetate methyl ester XII was obtained by methylation of hypoprotocetraric acid diacetate (XI) with diazomethane.




Hypoprotocetraric acid crystallized as a monohydrate from aqueous solvents. The hydrate loses its one mole of water only after 12 hr at 110° and atmospheric pressure. The product takes on a creamy color and appears to undergo some oxidation when heated in the presence of air for longer times. The anhydrous compound is readily obtained from the hydrate by warming the powdered solid in glacial acetic acid and filtering.

Methyl hypoprotocetrarate dimethyl ether (IV) was obtained in two crystal forms showing quite different i.r. absorption in mull preparations but giving identical spectra in chloroform solution. The needle form softens or melts at 144–145° if it has not been thoroughly crushed, while a powdered sample shrinks at that temperature and melts sharply at 170–171°. The second form melts slightly higher at 172–173° without previous softening at a lower temperature. Methanolysis and methylation of the permethyl derivative of hypoprotocetraric acid gives the expected diphenyl ether V.

The identity of hypoprotocetraric acid isolated from *Ramalina siliquosa* was confirmed by comparison with authentic samples obtained by reducing fumarprotocetraric acid (from *Cladonia subtenuis*), protocetraric acid (from *Parmelia caperata*) and physodalic acid (from *Hypogymnia physodes*). Since the permethyl compound is the only simple derivative of hypoprotocetraric acid reported in the literature and since the preparation of this derivative may be complicated by crystal modifications, three other derivatives prepared in this study are described in the present report.

It has been suggested[7] that all *Ramalina* species probably contain at least some usnic acid (IX). At present there is no known exception and in all but one report of macroextractions of *Ramalina* species, the dextrorotary isomer was obtained. As expected, *Ramalina siliquosa* also yielded (+)-usnic acid and this sample of usnic acid was identified by comparison with a known sample from *Evernia prunastri*.

Although β-orcinol depsides have never been reported from the genus *Ramalina*, the β-orcinol depsidones, such as hypoprotocetraric acid, presumed to be formed from such

[7] Y. ASAHINA, *J. Japan Botany* **14**, 721 (1938).

depsides are not uncommon. But up to now the most widespread lichen depside, atranarin, has not been found in this genus. A small fraction of a substance obtained in the present study seems to be a mixture of atranorin and chloroatranorin. A rigorous proof of the identity of this material has not been made, but the product gives all the microchemical tests expected for atranorin, showing good crystals by the microcrystal method of Asahina and has the expected $R_f$ values on chromatography.[8] But the i.r. spectrum shows some deviation from that of a pure sample of atranorin extracted from *Cladonia evansii* and a Beilstein test for halogen was positive. Chloroatranorin commonly occurs with atranorin, although as yet no satisfactory method has been devised to identify this compound in micro-mixtures. The i.r. spectrum of the mixed product was very similar to that of a mixture of atranorin and chloroatranorin from *Evernia prunastri*.

A sugar alcohol isolated during this study was identified as D-(+)-arabitol by comparison with an authentic sample and by preparation of the pentacetate derivative. D-(+)-Arabitol has been reported from many other species of *Ramalina*. Arabitol was identified along with mannitol in every species tested in the Gymnocarpeae in a chromatographic survey of low molecular weight carbohydrates in lichens of that group.[9]

A fraction of a slightly soluble material was isolated after prolonged acetone extraction of the lichen previously extracted with ether. The product, probably a mixture, showed i.r. absorption virtually identical to that published by Solberg[10] for mixtures of tetrahydroxy fatty acids extracted from several species of lichens. As in Solberg's report, the material was only slightly soluble in organic solvents but could be recrystallized from glacial acetic acid. Two recrystallizations of the present sample did not give a pure product, however, the resulting solid being slightly tan and softening at a temperature 30° below that at which it melted (217–218°). This melting point is much higher than any reported by Solberg but corresponds closely to that of Zellner's[11,12] hypogymnole II, a material considered by Solberg to have been a tetrahydroxy fatty acid. But after a third recrystallization from glacial acetic acid and washing with several solvents, the melting point dropped to 191–192° which is within the range reported by Solberg. This material, available in only small supply, was not considered pure and was not investigated further.

Fifty-seven herbarium samples of *Ramalina siliquosa* sen. lat. from throughout the range of the species in western Europe were tested microchemically to determine their principal phenolic constituents. The range of morphologic variation in these specimens would include the putative species *R. armorica* Nyl., *R. curnowii* Cromb., *R. kullensis* Zopf, *R. scopulorum* (Retz.) Ach., and *R. siliquosa* (Huds.) A. L. Sm. (including var. *cuspidata* (Ach.) Magn.) all of which are very poorly understood botanically. The literature reports on the chemical constituents of particular species in this group should therefore be regarded with caution and may be more applicable to the group in the broad taxonomic sense used here than to segregate species recognized in some botanical treatments. Actually, it seems now that many early extractions reported previously can be explained only as having been made upon mixtures of different chemical races or of different species.

The microchemical tests were performed on fragments removed from specimens in the Duke University Herbarium and from three collections supplied by Dr. O. Almborn. The

[8] S. SHIBATA, *Modern Meth. Plant Anal.* **6**, (1963).
[9] B. LINDBERG, A. MISIORNY and C. A. WACHTMEISTER, *Acta Chem. Scand.* **7**, 591 (1953).
[10] Y. J. SOLBERG, *Acta Chem. Scand.* **14**, 2152 (1960).
[11] J. ZELLNER, *Monatsh.* **64**, 6 (1934).
[12] J. ZELLNER, *Monatsh.* **66**, 81 (1935).

compounds were identified by microcrystal tests and by paper chromatography. Table 1 shows the variation observed and the number of specimens of each chemical type which were found among the samples tested.

TABLE 1. MAJOR CONSTITUENTS OF SAMPLES FROM THE EUROPEAN POPULATION OF *Ramalina siliquosa*

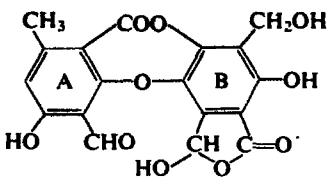
| Chemical constituents* | Origin | Number of samples tested |
|---|---|---|
| Hypoprotocetraric acid, usnic acid (6) | England, Wales, France, Portugal | 15 |
| Norstictic acid, stictic acid, usnic acid (1) | Sweden, England, Scotland, France, Portugal | 9 |
| Norstictic acid, usnic acid (1) | England, Wales, France | 10 |
| Protocetraric acid, usnic acid (4) | France | 8 |
| Salazinic acid, usnic acid (1) | Sweden, Wales, France, Germany | 10 |
| Usnic acid only | Scotland, Wales, France | 5 |

* The data in parentheses indicate the number of specimens out of those in the group with atranorin.

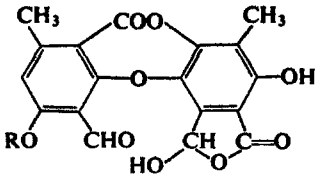
Although there have been several studies on certain of these lichens, the present microchemical survey considerably extends knowledge of the total chemical variation within the group. Zopf[13] extracted "scopuloric acid", later shown to be identical to stictic acid (XIVb),[14] from *Ramalina scopulorum*. But Asahina[15] found salazinic acid (XIII) in Japanese samples identified as this species and suggested that perhaps Curd and Robertson had studied *R. armorica*.[7,16] Japanese material also yielded D-arabitol and usnic acid.[17] *R. siliquosa* var. *cuspidata* gave usnic acid and protocetraric acid.[16] The earliest report on this lichen (as *R. cuspidata*) is by Hesse[18] who described "cuspidatic acid". This substance has properties which do not correspond to any of the substances found in the present study and Hesse's compound was probably not pure. Zopf[13] also obtained a compound ("kullensic acid") which he could not identify from *R. kullensis*, but this substance may have been only impure protocetraric acid.[19] Similarly, compounds of intermediate properties extracted by Ryan and O'Riordan[20] from several species of the *R. siliquosa* species complex could have been mixtures. Indeed, considering the difficulties which would be involved in the purification by recrystallization of a mixture of these slightly soluble β-orcinol depsidones, and the way in which the chemical types of the plants themselves often grow intermixed in nature, problems of these sorts would be expected.

Comparison of the structures of atranorin (VIIIa), hypoprotocetraric acid (II), protocetraric acid (Ic), norstictic acid (XIVa), salazinic acid (XIII) and stictic acid (XIVb), shows the principal cause of the variation observed to be related to changing oxidation state of the $C_1$ substituents of the aromatic rings. Similar variation has been noted in many other lichen

[13] W. ZOPF, *Ann.* **352**, 1 (1907).
[14] F. H. CURD and A. ROBERTSON, *J. Chem. Soc.* 1379 (1935).
[15] Y. ASAHINA, *Botan. Mag. (Tokyo)* **51**, 759 (1937).
[16] Y. ASAHINA, *J. Japan Botany* **23**, 1 (1949).
[17] Y. ASAHINA and M. YANAGITA, *Chem. Ber.* **67**, 799 (1934).
[18] O. HESSE, *J. prakt. Chem.* **62**, 430 (1900).
[19] W. THIES, in *Handbuch der Pflanzenanalyse*, Bd. 3. *Spezielle Analyse*. Teil 2. *Organische Stoffe* (Edited by G. KLEIN), p. 429, Julius Springer, Vienna (1932).
[20] H. RYAN and W. M. O'RIORDAN, *Proc. Roy. Irish Acad. (Dublin)* **33B**, 91 (1917).




(XIII)




(XIVa) R = H
(XIVb) R = $CH_3$—

groups producing β-orcinol type depsides and depsidones. It was pointed out recently[21] that all the known β-orcinol type lichen depsidones can be considered as reduced forms of salazinic acid (XIII). This would also apply to hypoprotocetraric acid. But it was suggested that substituents on ring A are never modified, except by methylation of the free phenolic group. In the case of hypoprotocetraric acid, when considered as a reduced form of salazinic acid, $C_1$ substituents in both rings are reduced.

## EXPERIMENTAL

All melting points are corrected and were determined with a Hoover Capillary Melting Point Apparatus (Arthur Thomas Co.). Ultraviolet spectra were obtained with a Bausch & Lomb Spectronic 505. Infrared spectra were determined with a Perkin Elmer Infracord. Microanalyses were performed by Weiler and Strauss, Oxford, and by Clark Microanalytical Laboratory, Urbana, Illinois.

*Mass Extractions of* Ramalina siliquosa

A large sample of *R. siliquosa* was collected in June, 1962, at Carnac (Morbihan), France, where it grows abundantly on prehistoric stone monuments erected by the Druids. The coarsely crushed, air-dried lichen (350 g; 90% dry wt.) was extracted by five soakings in warm, anhydrous, peroxide-free ethyl ether. Concentration of the total extract (7·5 l.) to a smaller volume (about 200 ml) gave several crops of yellow solid which were combined and washed with chloroform (100 ml) and with acetone (25 ml). The resulting tannish solid (19·7 g; 5·6% of the air dried lichen) had an i.r. spectrum identical to a fraction from a crude acetone extract after elution from a column of silicic acid using a benzene–ethyl ether gradient. For the reactions described below, the solid was purified by two recrystallizations from anhydrous acetone and trituration with anhydrous benzene or with glacial acetic acid which yields a nearly colorless product. For analysis a small sample was recrystallized from acetone–petroleum ether (Found: C, 62·80; H, 4·67. Calc. for $C_{18}H_{16}O_7$: C, 62·79; H, 4·68%), m.p. turns pink near 230° and melts at 242–243°, decomposing to a foaming red liquid (reported for hypoprotocetraric acid, m.p. 241° *d* with coloring at 220°;[3] 240–241° *d* with coloring at 230°;[4] 240°[6]).

The product shown here to be hypoprotocetraric acid (II) contained no methoxyl, gave a blue color with alcoholic $FeCl_3$, and a negative Gibbs reaction[22] unless first treated with base (10% KOH). Attempts to prepare a semicarbazone and an oxime failed. The compound dissolved in 95% EtOH did not absorb $H_2$ at atmospheric pressure in the presence of 10% Pd on carbon. Hypoprotocetraric acid (II) chromatographs well in n-butanol saturated with conc. ammonium hydroxide ($R_f$ 0·43, on Whatman No. 1 paper), showing a deep blue

[21] F. M. DEAN, *Naturally Occurring Oxygen Ring Compounds*, p. 574. Butterworth, London (1963).
[22] C. A. WACHTMEISTER, *Botan. Notiser* **109**, 313 (1956).

fluorescence in u.v. light (366 mμ) and staining yellow with tetrazotized benzidene and blue with 0·01% $FeCl_3$ in *n*-butanol. The i.r. spectrum (nujol mull) has maxima at 3490 (m), 1685 (s), 1650 (m), 1620 (s), 1580 (m), 1495 (m), 1415 (m), 1395 (m), 1263 (s), 1190 (s), 1138 (s), 1073 (m), 1020 (m), 854 (m), 787 (w), 757 (w), 737 (w), 716 (w) and 701 (w) $cm^{-1}$. An u.v. spectrum (95% EtOH) shows $\lambda_{max}$ = 216 mμ ($\log \epsilon$ 4·59) and 262 mμ ($\log \epsilon$ 4·09) and $\lambda_{min}$ at 246 mμ ($\log \epsilon$ 4·03).

Hypoprotocetraric acid (II) gives colorless needles of a *monohydrate* from dilute acetone, ethanol and methanol. This product has the same m.p. as the original material, and loses water only slowly after extended heating above 110°. Anhydrous hypoprotocetraric acid (II) is most easily prepared from the hydrate by gently warming in glacial acetic acid. The monohydrate is not a product of hydrolysis of the ester linkage since the Gibbs test[22] is still negative except after prior treatment with base. A sample of the hydrate from dilute acetone had m.p. 215° turning pink and 241–242·5° melting to a red liquid with gas evolution, (Found: C, 59·65; H, 4·91; loss of wt. on drying, 4·98. $C_{18}H_{16}O_7 \cdot H_2O$ required: C, 59·67; H, 5·01; loss of wt. on drying 4·98%). The i.r. spectrum (nujol mull) shows maxima at 3590 (w), 3240 (m), 2340–2580 (w), 1680 (s), 1625 (m), 1590 (s), 1310 (m), 1265 (s), 1210 (m), 1130 (s), 1075 (m), 1015 (w), 1002 (m), 848 (w), 802 (m), 783(m), 741 (m) and 707 (m) $cm^{-1}$. The u.v. spectrum (in 95% EtOH) shows inflections at the same wave lengths as anhydrous product.

The chloroform and acetone washings of crude hypoprotocetraric acid and the ethyl ether mother liquor from the extraction were each evaporated to dryness under diminished pressure. The residues were washed thoroughly with small volumes of chloroform and the washings were combined (about 100 ml). This yellow solution was concentrated at diminished pressure. Addition of excess ether precipitated a crop of usnic acid (IX) which was recrystallized again from chloroform–ethyl ether. The mother liquors were concentrated further and treated with excess ether. A precipitate which formed was a mixture of yellow needles and colorless prisms. The components of this mixture were provisionally identified as usnic acid (IX) and atranorin (VIII) by chromatographic and microcrystal comparisons with authentic samples of these compounds. The compounds were separated by fractional recrystallization from benzene, a process involving considerable loss of the smaller atranorin fraction. The combined crops (0·41 g, 0·12%) of usnic acid (IX), m.p. 199–200°, were recrystallized again from benzene (Found: C, 62·61; H, 4·76. Calc. for $C_{18}H_{16}O_7$: C, 62·79; H, 4·68%), m.p. 201·5–202·5° (reported[23] m.p. 203–204°), $[\alpha]_D^{26°}$ +495·6° ($c$ = 1·2 in $CHCl_3$) (reported[23] $[\alpha]_D^{17°}$ +492·2°). The i.r. spectrum of this product was identical to that of (+)-usnic acid isolated from *Evernia mesomorpha* ($[\alpha]_D^{20°}$ +484·5°, $c$ = 0·834 in $CHCl_3$) and no depression of the melting point was observed on admixture.

The small atranorin fraction (0·016 g, 0·0046%) was recrystallized from benzene–petroleum ether, m.p. 196–196·5° (reported[23] m.p. 196°). A Beilstein test for halogen was positive. The i.r. spectrum was very similar to one of pure atranorin (VIIIa) isolated from *Cladonia evansii* but resembled even more closely that of a sample of an atranorin chloroatranorin (VIIIa–VIIIb) mixture from *Evernia prunastri*. The product was also compared by microcrystal tests and by chromatography,[8] but the amount of compound from this extraction of *Ramalina siliquosa* was too small for further study.

A larger quantity of *Ramalina siliquosa* (563·1 g, air dry wt.) was extracted directly with hot acetone. Concentration of the combined extracts (about 4·0 l.) gave a large precipitate of hypoprotocetraric acid containing usnic acid which could be removed with chloroform.

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

The extracted lichen was then placed in a large soxhlet-type extractor modified for continuous drainage and it was extracted with acetone for about one week.

The acetone solution evaporating slowly at room temperature deposited brown spherical crystals which were separated by hand. The solid was recrystallized from methanol-acetone and decolorized with norite, yielding D-(+)-arabitol (1·35 g, 0·25%), m.p. 101–102°. A sample was purified further by two recrystallizations from methanol-acetone and three recrystallizations from 95% ethanol. There was no change in the melting point and a mixed melting point with an authentic sample of D-(+)-arabitol was not depressed, $[\alpha]_D^{29}+11{\cdot}3°$ ($c = 1{\cdot}13$, satd. $Na_2B_4O_7$ solution); the authentic sample (General Biochemicals) gave $[\alpha]_D^{29}+11{\cdot}4°$ ($c = 1{\cdot}06$, satd. $Na_2B_4O_7$ solution); (reported[24] m.p. 103°, $[\alpha]_D^{20}+7{\cdot}7°$ [$c = 9{\cdot}26$, satd. borax solution]). The pentacetate derivative was prepared and recrystallized from dilute ethanol, m.p. 75–76° (reported[23] m.p. 76°).

A sample (150 g) of *Ramalina siliquosa*, extracted with ethyl ether to remove usnic acid and hypoprotocetraric acid, was extracted with acetone for 5 days. When the solution was filtered, a solid was collected which was only slightly soluble in the common organic solvents. This material was recrystallized twice from glacial acetic acid to give a slightly tan product, m.p. 217–218°, softening at 187°. A portion of the solid was recrystallized again from glacial acetic acid and then washed with small volumes of absolute methanol, anhydrous acetone and finally petroleum ether (30–60°), yielding a white powder (Found: C, 65·49; H, 10·44%), m.p. 191–192°, becoming amorphous at 182–183°. The total yield was 95 mg (0·063%). The i.r. spectrum (nujol mull) shows maxima at 3380 (s), 3300 (s), 1625 (m), 1070 (s), 1048 (m), 1030 (m), 943 (m) and 720 (m) $cm^{-1}$, and it corresponds closely to the spectra published for tetrahydroxy fatty acids extracted from other species of lichens.[10]

*Methyl Hypoprotocetrate Dimethyl Ether (IV)*

A solution of anhydrous hypoprotocetraric acid (1·02 g, 3 m-moles) in absolute MeOH (200 ml) was treated with excess diazomethane in ether for 24 hr. Unreacted diazomethane was destroyed by adding glacial acetic acid dropwise until the yellow color of the solution just disappeared. Some MeOH was removed at diminished pressure. When the product began to precipitate, it was collected by filtration and the mother liquor was concentrated further to collect a second crop. The yield of white solid, m.p. 169–170°, was 0·635 g (56%). The permethyl derivative IV was obtained in two crystal forms using either acetone–petroleum ether (30–60°) or MeOH for recrystallization. The product as long colorless needles (Found: C, 65·31; H, 5·78. Calc. for $C_{21}H_{22}O_7$: C, 65·28; H, 5·74%), softens or melts at 144–145° depending upon the extent to which the crystals are pulverized. A well-powdered sample shrinks at this temperature but then melts sharply at 170–171° (reported m.p. 169–170°;[6] 170°[3]).

The i.r. spectrum of the needle form (nujol mull) shows absorption maxima at 1735 (s), 1618 (m), 1580 (m), 1340 (m), 1318 (m), 1275 (m), 1265 (s), 1200 (s), 1145 (m), 1085 (m), 997 (m), 852 (w), 794 (w), 784 (w) and 767 (w) $cm^{-1}$.

The other crystal form is obtained as colorless rhombic plates, m.p. 172–173° (Found: C, 65·32; H, 5·84. Calc. for $C_{21}H_{22}O_7$: C, 65·28; H, 5·74%). A mixed melting point with the needle form was not depressed. The i.r. spectrum (nujol mull) shows absorption maxima at 1735 (s), 1620 (s), 1572 (m), 1330 (s), 1320 (s), 1280 (s), 1268 (s), 1232 (s), 1190 (m), 1140 (s), 1095 (m), 1085 (m), 1055 (s), 1030 (m), 990 (s), 943 (w), 902 (w), 865 (w), 798 (w), 787 (w), 766 (w) and 758 (w) $cm^{-1}$.

[24] O. RUFF, *Chem. Ber.* **32**, 550 (1899).

While the i.r. spectra of the two crystal forms are markedly different in nujol mull, they are identical in chloroform solution, showing maxima at 2970 (w), 1735 (s), 1620 (m), 1572 (m), 1470 (m), 1415 (sh.), 1340 (m), 1320 (m), 1275 (s), 1140 (s), 1120 (m), 1085 (w), 1020–1050 (w), 993 (m) and 960 (w) $cm^{-1}$. In the u.v. (95% EtOH) $\lambda_{max} = 208{\cdot}5$ and 271 m$\mu$ and $\lambda_{min} = 245$ m$\mu$.

*Methyl Hypoprotocetrarate (X)*

Hypoprotocetraric acid (220 mg, 640 $\mu$moles) dissolved in a minimum volume of acetone was cooled in an ice bath and a solution of diazomethane in ether was added slowly with stirring. The addition was continued until the yellow color of diazomethane no longer faded rapidly. Addition of petroleum ether gave clumps of colorless crystals which were recrystallized once from acetone–petroleum ether yielding the *methyl ester X* (165 mg, 72%) (Found: C, 63·61; H, 5·11. $C_{19}H_{18}O_7$ required: C, 63·68; H, 5·06%), m.p. 257–260° decomposing to a red melt without effervescence. The i.r. spectrum shows maxima at 3430 (m), 1705 (s), 1670 (m), 1618 (m), 1590 (m), 1265 (s), 1200 (m), 1150 (s), 1080 (w), 852 (w), 800 (w) and 786 (w) $cm^{-1}$. In the u.v., $\lambda_{max} = 218$, 262 and 320 m$\mu$ and $\lambda_{min} = 243{\cdot}5$ and 314 m$\mu$.

*Hypoprotocetraric Acid Diacetate (XI)*

Hypoprotocetraric acid (317 mg, 920 $\mu$moles) in acetic anhydride (30 ml) containing a trace of conc. $H_2SO_4$ (4 drops) was allowed to stand at 40° for 4 days. The cooled solution was poured slowly into ice water (200 ml) being stirred vigorously with a magnetic stirrer. The oily precipitate which solidified slowly was separated by filtration, m.p. 164–180°, and recrystallized once from NaOH yielding the *diacetate derivative XI* (0·226 g, 57%), m.p. 189–190·5°. A sample recrystallized from MeOH and dried overnight at 110° over $P_2O_5$ had m.p. 190·5–191·5° (Found: C, 61·69; H, 4·64. $C_{22}H_{20}O_9$ required: C, 61·68; H, 4·71%). The product gave no color with alcoholic $FeCl_3$ but turned bright blue with the Gibbs reagent after previous treatment with base. The i.r. spectrum (nujol mull) shows maxima at 1170 (s), 1755 (s), 1705 (s), 1270 (m), 1195 (s), 1135 (s), 1065 (m) and 904 (w) $cm^{-1}$. In the u.v. (95% ethanol) $\lambda_{max} = 209$ m$\mu$ with shoulders at 245 and 285 m$\mu$.

*Hydrolysis of Methyl Hypoprotocetrarate Dimethyl Ether (IV)*

Methyl hypoprotocetrarate dimethyl ether (354 mg, 1 m-mole) in 2% methanolic KOH was stirred for 3 min and then poured into water (100 ml) and acidified immediately with 1·0 N $H_2SO_4$. The soft white precipitate collected by filtration gave only an oil upon attempted recrystallization from a number of solvents. The thick, pale yellow oil (324 mg) was taken up in MeOH (10 ml) and treated with excess diazomethane at room temperature for 2 days. When the solution was evaporated, the amorphous product solidified on standing. The solid ether V recrystallized from acetone–petroleum ether (0·204 mg, 46%) and from MeOH (Found: C, 64·04; H, 6·39. Calc. for $C_{23}H_{28}O_8$: C, 63·88; H, 6·53%), m.p. 115–116° (reported[3] m.p. 115–116°). The i.r. spectrum (nujol mull) shows maxima at 1750 (s), 1735 (s), 1625 (m), 1578 (w), 1285 (s), 1270 (s), 1220 (s), 1145 (s), 1060 (m), 997 (w), 835 (w), 802 (w), 763 (w), and 745 (w) $cm^{-1}$. In the u.v. (in 95% ethanol) $\lambda_{max} = 211$ m$\mu$ with a shoulder at 280 m$\mu$.

*Methyl Hypoprotocetrarate Diacetate (XII)*

(*a*) *From hypoprotocetraric acid diacetate (XI).* Hypoprotocetraric acid diacetate (85 mg, 200 $\mu$moles) in acetone (2 ml) was cooled in an ice bath and treated with diazomethane in

ether. When the solution had warmed to room temperature, the solvents were evaporated and the residue was recrystallized from absolute methanol yielding the *diacetate methyl ester XII* (75 mg, 85%), m.p. 180·5–181·0°. A sample for analysis was recrystallized from dilute acetone, m.p. 181–182° (Found: C, 62·50; H, 5·01. $C_{23}H_{22}O_9$ required: C, 62·44; H, 5·01%). The i.r. spectrum (nujol mull) shows maxima at 1770 (s), 1750 (s), 1620 (w), 1575 (w), 1240 (m), 1270 (m), 1200 (s), 1135 (s), 1065 (m), 904 (m), 863 (w), 790 (w), 787 (w) and 764 (w) $cm^{-1}$. In the u.v., $\lambda_{max}$ (in 95% ethanol) = 208·5 m$\mu$ with shoulders at 244 m$\mu$ and 283 m$\mu$.

(*b*) *From hypoprotocetraric acid methyl ester* (*X*). A solution of methyl hypoprotocetrarate (72 mg, 200 $\mu$moles) in acetic anhydride (10 ml) containing one drop of $H_2SO_4$, was allowed to stand at room temperature overnight. When the solution was poured slowly into ice water (100 ml) with rapid stirring, an oil precipitated which solidified. The product collected by filtration was air dried and recrystallized from benzene–petroleum ether yielding the diacetate methyl ester XII (74 mg, 85%), m.p. 180·5–181·0°. A mixed melting point with the product from (*a*) was not depressed and the i.r. spectra are identical.

*Catalytic Reduction of Protocetraric Acid* (*Ic*), *Fumarprotocetraric Acid* (*Id*) *and Physodalic Acid* (*Ib*)

Protocetraric acid (108 mg from *Parmelia caperata*) in 95% EtOH (30 ml) absorbed an equivalent of 3 moles of hydrogen per mole by reduction in 95% EtOH (30 ml) over 10% Pd on carbon at room temperature and atmospheric pressure. The catalyst was removed by filtration and the solution was concentrated somewhat at diminished pressure before it was diluted to the cloudy point with water. Hypoprotocetraric acid monohydrate (74 mg, 74%) precipitated which crystallized as anhydrous product from glacial acetic acid, m.p. 243–244° *d*, turning pink near 225°. The i.r. spectrum was identical to that of hypoprotocetraric acid from *Ramalina siliquosa* and a mixed melting point of the two products was not depressed. A small sample of the monohydrate was converted to the permethyl derivative, which crystallized from methanol as needles, m.p. 171–172°, shrinking near 144°. The i.r. spectrum (in solution in chloroform) was identical to that of permethylated hypoprotocetraric acid from *R. siliquosa*.

Fumarprotocetraric acid (45 mg, 95 $\mu$moles) from *Cladonia subtenuis* in 95% ethanol (30 ml) absorbed 4 moles of hydrogen per mole of starting material using 10% Pd on carbon catalyst. The product recrystallized from dilute ethanol (16 mg, 50%) showed i.r. absorption identical to hypoprotocetraric acid monohydrate from *Ramalina siliquosa* and from protocetraric acid (m.p. 243–244·5° *d*, mixed m.p. undepressed) and gave anhydrous product with glacial acetic acid.

A small sample of physodalic acid from *Hypogymnia physodes* reduced in the same way also yielded hypoprotocetraric acid.

*Microchemical Survey of* Ramalina siliquosa

Samples (50–200 mg) of each herbarium specimen were extracted by the usual method[8] with benzene at room temperature and then with warm acetone. Each extract was chromatographed (Whatman No. 1 paper) in ammonium hydroxide-saturated *n*-butanol and the acetone extracts were also chromatographed in pyridine–ethyl acetate–water (1:2:1). The first chromatogram was sprayed with diazotized benzidene and the second with a *p*-phenylenediamine solution. The benzene extracts were tested for usnic acid and for atranorin by the microcrystal method of Asahina.[8] All the acetone extracts were checked in four of the stan-

dard microcrystal solutions used in lichen chemistry (GE, GAW, o-T, and KK, summarized recently by Shibata[8]) to confirm the results obtained chromatographically.

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