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THE STRUCTURES OF CRYPTOCHLOROPHAEIC ACID AND MEROCHLOROPHAEIC ACID

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Abstract—The structures of cryptochlorophaeic acid from *Cladonia cryptochlorophaea* Asahina and merochlorophaeic acid from *Cladonia merochlorophaea* Asahina were established as being the depsides of sekikaic acid-type formulated as (I) and (VII), respectively.

ON THE basis of microchemical investigation, Asahina[1] proposed to classify the lichens of *Cladonia chlorophaea* group into four species according to their special chemical constituents:

Cl. chlorophaea (Flk.) Spreng.	Fumarprotocetraric acid.
Cl. grayi Merrill	Fumarprotocetraric acid;* grayanic acid.
Cl. cryptochlorophaea Asahina	Fumarprotocetraric acid;* cryptochlorophaeic acid.
Cl. merochlorophaea Asahina	Fumarprotocetraric acid;* merochlorophaeic acid.

Mackenzie Lamb,[2] on the other hand, suggested that these differences were better explained as being due to chemical strains of *Cl. chlorophaea*, and denoted them strains I, II, III and IV.

The present study is concerned with the chemical structures of *cryptochlorophaeic* and *merochlorophaeic acids*, and with the chemotaxonomical aspect of this series of lichens.

Cryptochlorophaeic Acid

Cryptochlorophaeic acid ($C_{25}H_{32}O_8$) gives a violet ferric reaction, and dissolves in bicarbonate solution showing it is a phenolic carboxylic acid. Its i.r. spectrum showed the presence of a chelated carboxyl (1650 cm^{-1} (Nujol and tetrahydrofuran)), and a depside linkage (1735 cm^{-1} (Nujol), 1765 cm^{-1} (tetrahydrofuran)). The homofluorescein reaction is negative, but cryptochlorophaeic acid shows a positive (purple to red) bleaching powder reaction, and dissolves in 10% KOH to give first a colourless and then a wine-red solution. All this evidence suggested that cryptochlorophaeic acid is a depside, though the u.v. absorption curve (Fig. 1) does not agree with either a typical type of orcinol- or that of β-orcinol depsides.[3]

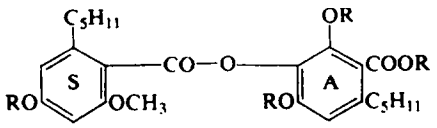
The presence of a non-chelated lactonic carbonyl in cryptochlorophaeic acid suggested that a methoxyl is present in the *ortho* position to the depside linkage in the S-ring (see (I)). Such a structure may give an abnormal u.v. curve. Two *meta*-orientated phenolic hydroxyls in the A-ring of cryptochlorophaeic acid were revealed by the positive bleaching powder

* Occurrence of fumarprotocetraric acid in these lichens is variable.

[1] Y. ASAHINA, *Japan. J. Botany* **16**, 719 (1940).
[2] I. MACKENZIE LAMB, *Can. J. Botany* **29**, 522 (1951); *Nature* **168**, 38 (1951).
[3] M. E. HALE, *Science* **123**, 671 (1956).

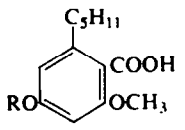
reaction.[4] Although ramalinolic acid was described by earlier workers[5] as giving no bleaching powder reaction, a renewed examination has showed that it also exhibits a positive red coloration. Such a structure cannot be ruled out for this type of lichen depside.

On hydrolytic degradation of the depside linkage with concentrated sulphuric acid, cryptochlorophaeic acid afforded olivetol carboxylic acid 2-methyl ether (III) which was identified by a mixed melting point with the synthetic sample. The A-portion was not obtained in a crystalline form by this reaction, and the proof for the structure of the A-portion was obtained when fully methylated cryptochlorophaeic acid (II) was cleaved to afford olivetol carboxylic acid dimethyl ether and a methyl ester, m.p. 73–75°.



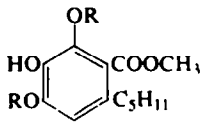
(I) R = H

(II) R = CH_3

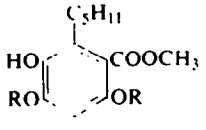


(III) R = H

(IV) R = CH_3



(V)



(VI)

R CH_3

The NMR spectrum of the methyl ester (in $CDCl_3$) showed a terminal methyl signal at τ:9·12, a broad—$(CH_2)_3$—signal at τ:8·63, and a signal of CH_2 attached to aromatic ring at τ:7·55. The signals of 1 methyl ester and 2 methoxyls appeared overlapping at τ:6·12, and a phenolic hydroxyl and a benzene ring proton gave the signals at τ:4·55 and 3·52, respectively. The chemical shifts and the magnitudes of these signals agreed alternative structures (V) and (VI) as the ester. Finally it was established that the methyl ester is identical with methyl 6-amyl-pyrogallol-1-carboxylate 2,4-dimethyl ether (V) by mixed melting point and comparison of the i.r. spectra.

Thus cryptochlorophaeic acid has been represented by the formula (I), which belongs to the sekikaic acid-type of lichen depsides.

Merochlorophaeic Acid

Merochlorophaeic acid analyses for $C_{24}H_{30}O_8$ gave the similar colour reactions and an u.v. absorption curve almost superimposable with that given by cryptochlorophaeic acid. This suggested that merochlorophaeic and cryptochlorophaeic acids possessed the same basic chemical structure.

The i.r. spectrum of merochlorophaeic acid showed the presence of chelated carboxyl (1650 cm^{-1} in KBr and tetrahydrofuran) and non-bonded depside linkage (1750 cm^{-1} in

[4] Y. ASAHINA and S. SHIBATA, *Chemistry of Lichen Substances*, p. 54, Japan Society for the Promotion of Science, Tokyo (1954).

[5] Y. ASAHINA and T. KUSAKA, *Ber. Dtsch. Chem. Ges.* **69**, 450, 1896 (1936).

KBr; 1765 cm^{-1} in tetrahydrofuran). The presence of 2 methoxyls was proved by the NMR spectrum giving the signals at τ: 5·95 and 6·06.

The structure proposed for merochlorophaeic acid is fundamentally the same as that of cryptochlorophaeic acid where the alkyl groups in the S- and A-rings could be one of the

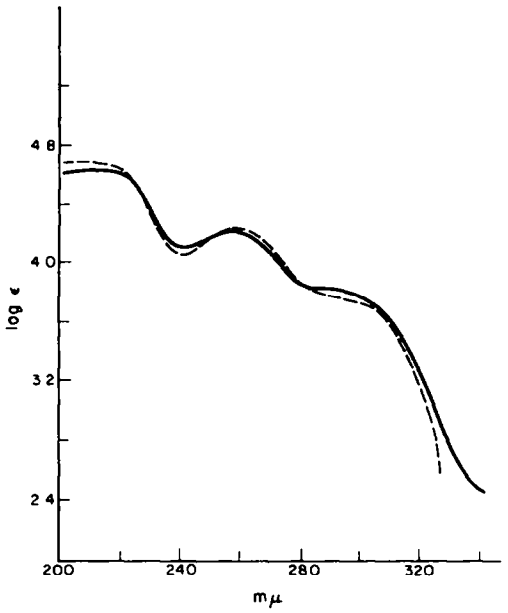
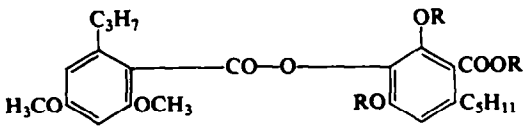


FIG. 1. THE U.V. SPECTRA OF CRYPTOCHLOROPHAEIC ACID (——) AND MEROCHLOROPHAEIC ACID (- - - - -).

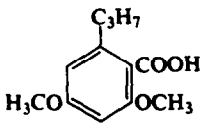
following combinations: (*a*) C_3H_7 and C_5H_{11} or (*b*) CH_3 and C_7H_{15}. The absence of the singlet of methyl in the NMR spectrum of merochlorophaeic acid, however, excludes the last possibility.

The two fragments obtained by the acid cleavage of merochlorophaeic acid were identified by thin-layer chromatography as being identical with the A-portion of cryptochlorophaeic acid and divaric acid dimethyl ether, respectively. Methylation with

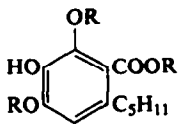


(VII) R = H

(VIII) R = CH_3



(IX)



(X) R = H

(V) R = CH_3

diazomethane yielded (VIII), the NMR spectrum of which showed 18 protons of the alkyl side-chains, 5 methoxyls and 3 protons of the benzene rings.

The ester fragment obtained by the hydrolytic degradation of (VIII) was proved to be identical with the ester fragment (V) of cryptochlorophaeic acid permethylate, while the acid fragment was identified with divaric acid dimethyl ether (IX) which was also obtained directly from merochlorophaeic acid by hydrolysis.

Fully methylated merochlorophaeic acid (VIII), m.p. 76–78°, has been proved to be identical with methyl boninate methyl ether, m.p. 76–78°, by a mixed melting point and comparison of i.r. spectrum. Thus the structure (VII) has finally been established to represent merochlorophaeic acid.

Biogenetical Consideration on the Principles of Cladonia chlorophaea *Group*

Cryptochlorophaeic (I) and merochlorophaeic acids (VII) have now been proved to belong to the sekikaic acid-type of lichen depside. Previously we have established the structure of grayanic acid isolated from *Cl. grayi* as being (XV).[6]

A postulated biogenetical scheme of these depsides and depsidone of *Cl. chlorophaea* group lichens is illustrated in Fig. 2.

In the biogenetical pathway forming cryptochlorophaeic and merochlorophaeic acids, hydroxylation of olivetol carboxylic acid would occur prior to the depside linkage formation. In *Cl. grayi* the hydroxylation of sphaerophorol carboxylic acid does not occur and the intermediate depside (XIV) is subjected to the intramolecular oxidative coupling at the *para* position of free hydroxyl in the A-ring to form a depsidone linkage by the scheme as suggested by Barton *et al.*,[7] and Erdtman *et al.*[8]

It is suggested that cryptochlorophaeic and merochlorophaeic acids are formed via a mutual biogenetical scheme, though they differ in the length of alkyl side-chains of the S-ring. It is, therefore, interesting that *Cl. cryptochlorophaea* and *Cl. merochlorophaea* are scarcely distinguished morphologically. An entirely different biogenetical scheme is suggested to occur in *Cl. grayi* to form grayanic acid, and a slight morphological difference between this and the former lichens can be observed. In regard to the biogenetical standpoint, it therefore seems reasonable to accept Asahina's classification of the *Cl. chloropnaea* group of lichens as consisting of four independent species.

EXPERIMENTAL

Isolation of Cryptochlorophaeic Acid

Cladonia cryptochlorophaea Asahina and *Cl. cryptochlorophaea* Asahina f. inactiva Asahina (25 g) collected at Nodayama, Kanazawa and Senjogahara, Nagano pref., Japan, were extracted with ether, and the extracted material recrystallized from benzene to obtain cryptochlorophaeic acid—colourless needles, m.p. 182–184°; the yield was 32 mg (0·16 per cent of dried material). It gave a single spot on the thin-layer chromatogram developed with hexane:ether:formic acid (5:4:1). Found: C, 65·37; H, 6·72. Calc. for $C_{25}H_{32}O_8$: C, 65·20; H, 7·00%.)

Cryptochlorophaeic acid gave a violet coloration with 1% $FeCl_3$ in alcohol, and a yellow colour with diazotized sulphanilic acid while it showed a bluish fluorescence under u.v. light.

[6] S. SHIBATA and HSÜCH-CHING CHIANG, *Chem. Pharm. Bull., Tokyo* **11**, 926 (1963).
[7] D. H. R. BARTON and T. COHEN, *Festschrift*, p. 130, Prof. Dr. A. Stoll, Birkhäuser, Basel (1957)
[8] H. ERDTMAN and C. A. WACHTMEISTER, *Festschrift*, p. 160, Prof. Dr. A. Stoll, Birkhäuser, Basel (1957).

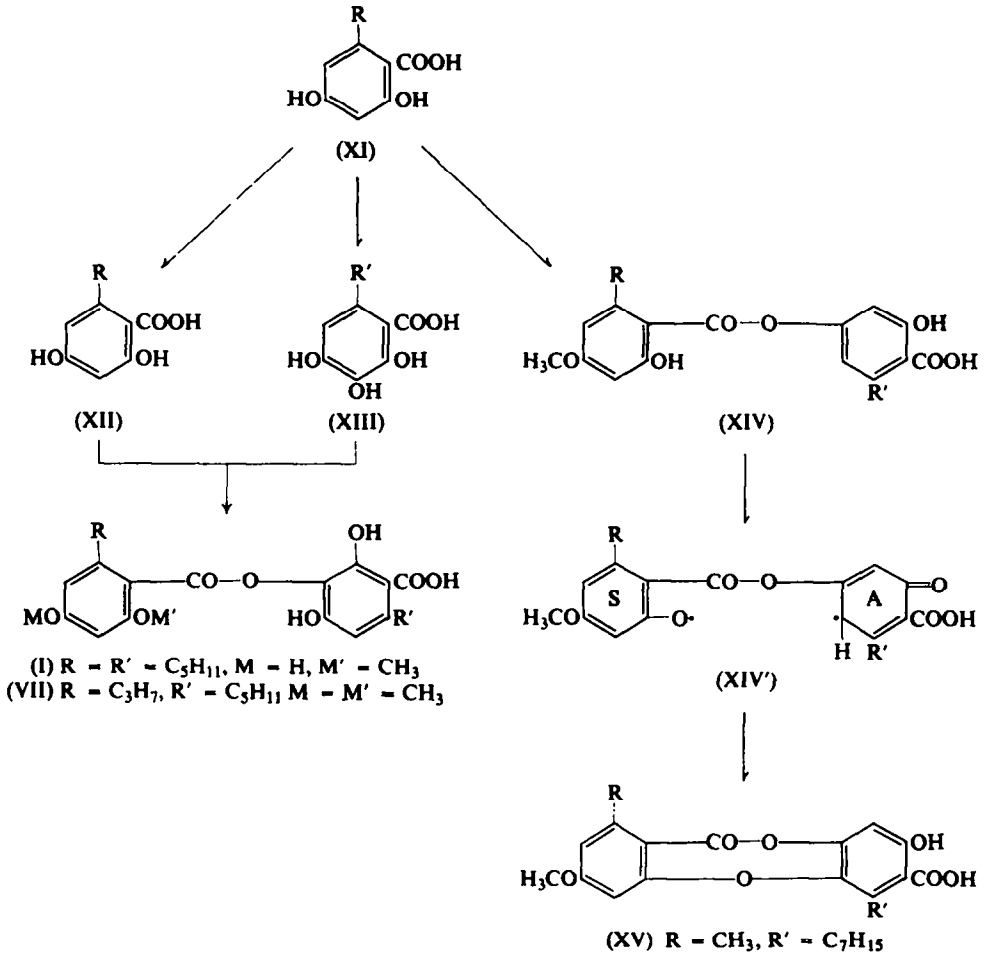


FIG. 2. SUGGESTED BIOGENETICAL SCHEME OF DEPSIDES AND DEPSIDONES IN *Cl. chlorophaea.*

With bleaching powder, it exhibited first a purple colour which turned immediately into red which became colourless on standing. It dissolves in 5% $NaHCO_3$, and 10% KOH to form a colourless solution which turns yellow and then to wine red. It gives no positive homofluorescein reaction with KOH and chloroform. It dissolves readily in ether, acetone, alcohol, tetrahydrofuran, and ethyl acetate, and sparingly soluble in chloroform, carbon tetrachloride, and insoluble in petroleum ether and benzene. I.r $\nu_{max.}^{Nujol}$ (cm^{-1}): 3400 (bonded OH), 1735 (depside C:O), 1650 (bonded COOH), 1620, 1580 (benzenoid).

Hydrolytic Cleavage of Cryptochlorophaeic Acid

Cryptochlorophaeic acid (10 mg) was dissolved in conc. H_2SO_4 (0·5 ml) with ice-cooling. After 10 min the reaction mixture was poured into ice water, and extracted with ether which on evaporation gave an oily residue, this was chromatographed on a silica-gel column using a mixture of acetone:hexane:benzene (1:1:2) as the solvent to separate the two fluorescent bands. From the lower band a brownish amorphous substance was obtained, which could not be purified, and from the upper band olivetol carboxylic acid 2-methyl ether (2 mg),

m.p. 105–106°, was isolated which was identified by a mixed fusion and a comparison of i.r. spectra with a sample prepared by an unambiguous way from anziaic or perlatolic acids.

Olivetol-Carboxylic Acid 2-Methyl Ether

Methyl perlatolate dimethyl ether (70 mg), m.p. 57°, was prepared by methylation of anziaic or perlatolic acids with diazomethane, then hydrolysed with conc. H_2SO_4 (1 ml) under ice-cooling for 30 min.

The ethereal reaction mixture was shaken with aq. $NaHCO_3$ solution to separate olivetol carboxylic acid dimethyl ether (25 mg). The ethereal layer was evaporated, and the oily residue was refluxed with 10% KOH (3 ml) for 1·5 hr, and an acidic product was separated by the usual method and recrystallized from benzene to form colourless needles, m.p. 105–106°, the yield was 15 mg.

Hydrolysis of Fully Methylated Cryptochlorophaeic Acid

Cryptochlorophaeic acid (60 mg) was methylated with an excess of diazomethane in ether. After standing for 1 week, occasionally adding a few drops of methanol to complete the reaction, evaporation of the solvent produced oily, fully methylated cryptochlorophaeic acid, $\overset{Cap.}{max.}\ \nu$ (cm^{-1}): 1760 (depside C:O); 1735 (ester C:O), which was subjected to hydrolysis with conc. H_2SO_4 (2 ml) without further purification. After 30 min, the reaction mixture was treated as mentioned above. By thin-layer chromatography, using the two solvent systems, benzene:ethyl acetate:formic acid (5:4:1), and hexane:ether:formic acid (5:4:1), two components were shown to be present in the bicarbonate-soluble portion, one of which was identified as olivetol carboxylic acid dimethyl ether (IV) and the other seemed to be 6-amyl-pyrogallol-1-carboxylic acid 2,4-dimethyl ether. On silica-gel column chromatography, using chloroform as the solvent, olivetol carboxylic acid dimethyl ether was eluted first, and recrystallized from petroleum ether to give colourless needles, m.p. 52°, which were identified by mixed fusion with the authentic specimen. The second product eluted from the upper layer was identified by thin-layer chromatography with the hydrolysis product obtained from methyl 6-amyl-pyrogallol-1-carboxylate 2,4-dimethyl ether. (Solvent system: Hexane:ether:formic acid; 5:4:1) i.r. $\overset{CCl_4}{max.}\ \nu$ (cm^{-1}): 1705, 1750 (free COOH) 3600 (free OH).

From the bicarbonate-insoluble portion, an oily substance was obtained on evaporation of the solvent, which was chromatographed on silica gel using chloroform as the solvent to obtain colourless oil. The product was recrystallized from ligroin to give colourless needles, m.p. 72–73°, which showed a light yellow diazonium reaction and negative ferric reaction (yield, 8 mg). It was identified by a mixed melting point and a comparison of i.r. spectra as being methyl 6-amyl-pyrogallol-1-carboxylate 2,4-dimethyl ether. NMR spectrum (in $CDCl_3$): 9·12 (terminal methyl of C_5H_{11}); 8·63 (intermediate CH_2 of C_5H_{11}); 7·55 (end CH_2 of C_5H_{11} attached directly to the benzene ring); 6·12 ($1COOCH_3$ and $2OCH_3$); 4·55 (phenolic OH); 3·52 (benzene ring proton); i.r. $\overset{CCl_4}{max.}$ (cm^{-1}): 3600 (free OH); 1740 (ester C:O); ∂ 870 (five, substituted benzene).

Isolation of Merochlorophaeic Acid

Clodonia merochlorophaea Asahina and *Cl. merochlorophaea* Asahina f. inactiva Asahina (45 g) collected in Hoppo, Shiga Kogen and Senjogahara, Nagano Pref., Japan, was extracted with ether, and the extracts were recrystallized from a mixture of benzene and hexane, and then from aq. methanol to obtain merochlorophaeic acid, colourless plates, m.p. 164–166° (yield: 120 mg, 0·28 per cent of the dried material). It gave a single spot on the thin-layer

chromatogram. (Found: C, 64·78; H, 6·64. Calc. for $C_{24}H_{30}O_8$: C, 64·56; H, 6·77%.) All the chemical and physical properties, including a negative homofluorescein reaction, are very similar with those of cryptochlorophaeic acid; i.r. $\nu_{max.}^{KBr}$ (cm^{-1}): 3400 (bonded OH); 1750 (depside C:O); 1650 (bonded COOH), 1620, 1600 (benzenoid). NMR (in $CDCl_3$): τ:8·97 (terminal CH_3 of alkyl side-chains); 8·53 (intermediate CH_2 of alkyl side-chains); 7·03 (end CH_2 of alkyl side-chain attached directly to the benzene ring); 5·95, 6·06 ($2OCH_3$); 3·35 (benzene ring proton).

Hydrolytic Cleavage of Merochlorophaeic Acid (VII)

On treatment with conc. H_2SO_4 for 10 min under ice-cooling, merochlorophaeic acid was cleaved into two components which were shown on thin-layer chromatogram developed with the solvent systems, hexane:ether:formic acid (5:4:1), and chloroform:acetone: formic acid (5:4:1).

One of the spots which gave a positive diazonium reaction was identified with the A-ring of cryptochlorophaeic acid, and the other one which showed no coloration with diazonium reagent was identified with an authentic specimen of divaric acid dimethyl ether.

Methyl Merochlorophaeate Dimethyl Ether (VIII)

Merochlorophaeic acid (25 mg) was treated with an excess of diazomethane in ether for 5 days, and a few drops of methanol were added on the last day to complete the reaction. The solvent was removed, and the residue was recrystallized from aq. methanol to obtain colourless plates, m.p. 76–78° (yield, 15 mg), which were identified with methyl boninate methyl ether by a mixed fusion and the comparison of i.r. spectra, (Found: C, 66·37; H, 7·43. Calc. for $C_{27}H_{36}O_8$: C, 66·23; H, 7·32%), i.r. $\nu_{max.}^{CHCl_3}$ (cm^{-1}): 1750 (depside C:O); 1735 (ester C:O); NMR (in $CDCl_3$): τ:8·98 (terminal CH_3 of alkyl side-chains (6 protons)); 8·44 (intermediate CH_2 of alkyl side-chains (8 protons)); 7·25 (end CH_2 of alkyl side-chain attached directly to the benzene ring (4 protons)); 6·12 ($1COOCH_3$ and $4OCH_3$); 3·6 (aromatic ring proton (2 protons); 3·4 (aromatic ring proton (1 proton)).

Hydrolytic Cleavage of Methyl Merochlorophaeate Dimethyl Ether (VIII)

On treatment with conc. H_2SO_4 (0·3 ml) under ice-cooling for 30 min, methyl merochlorophaeate dimethyl ether (10 mg) afforded oily products which were chromatographed over silica gel, using chloroform as the solvent to separate an ester portion and a carboxylic acid fragment. The ester portion was recrystallized from ligroin to give crystals, m.p. 72–73° (yield, 2 mg); i.r. $\nu_{max.}^{CCl_4}$ (cm^{-1}): 3600 (free OH); 1740 (ester C:O); ∂870 (five, substituted benzene).

The i.r. spectra of the ester portion was completely superimposable on that given by the ester portion of the hydrolysate of fully methylated cryptochlorophaeic acid and the sample of methyl 6-amyl-pyrogallol-1-carboxylate 2,4-dimethyl ether. The identity of both samples was also proved by a mixed melting point and the *Rf* value of thin-layer chromatograms developed by the solvent systems: pure chloroform; chloroform:ether (2:1); chloroform:acetone (5:1); benzene:acetone (5:1).

The acid portion was recrystallized from petroleum ether to give crystals (1–2 mg) which identified with divaric acid dimethyl ether by thin-layer chromatography developed with solvent systems: benzene:ethyl acetate:formic acid (5:4:1); benzene:ethyl acetate (1:1), and a mixed fusion.

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