Some Constituents of the Lichen *Parmelia cryptochlorophaea*

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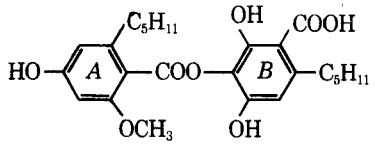
Extraction of the lichen *Parmelia cryptochlorophaea* Hale gave cryptochlorophaeic acid (2.5 per cent), chloroatranorin (0.16 per cent), and caperatic acid (1.7 per cent).

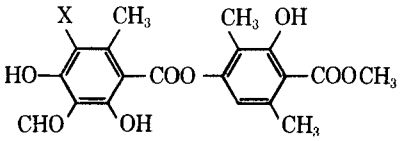
THE ANTIBIOTIC properties shown by extracts from many species of lichens, dual organisms resulting from the symbiotic life of a fungus and an alga, have stimulated research on these peculiar natural products. As a result, one of the commonest lichen substances, usnic acid, is now used abroad as the basis for several commercial antibiotic preparations. In lichens, the accumulation of certain aromatic esters, often in rather high concentration, is so characteristic that the identity of the major constituents can often be used in the systematic classification of these plants. Widespread taxonomic applications of chemical data resulted primarily after the development of microcrystal and chromatographic tests (1) for the detection of these compounds. To some extent at least, the chemical tests routinely performed by lichen taxonomists compromise reliability for the sake of simplicity of method and materials. Nevertheless, the large number of excellent identifications which have been accomplished by various workers using accurately identified contaminant-free lichen samples (usually fragments of herbarium specimens) more than justifies the few errors which might be avoided by long and elaborate procedures. Hopefully, interesting and unusual findings first reported from microchemical studies will be verified by extraction when sufficient material of the species in question becomes available. Such a verification is the subject of the present report.

In 1959 Hale (2) described a new species, *Parmelia cryptochlorophaea*, the specific epithet reflecting microchemical evidence for the presence of cryptochlorophaeic acid. At that time this compound was known only from *Cladonia cryptochlorophaea*, a species in a completely unrelated genus. Microchemical tests of the new *Parmelia* also indicated the presence of atranorin and of an unidentified fatty substance.

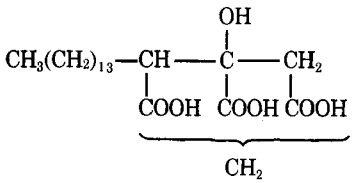
Extraction of *P. cryptochlorophaea* gave cryptochlorophaeic acid (I), chloroatranorin (III), and caperatic acid (IV). Cryptochlorophaeic acid was definitely proved by comparison with an authentic sample kindly supplied by Dr. S. Shibata. Shibata and Chiang (3) recently determined the structure of cryptochlorophaeic acid isolated from Japanese material of *C. cryptochlorophaea*. Cryptochlorophaeic acid is structurally interesting because the depside linkage *para* to the alkyl group in ring *B* is less common among lichen substances, and cryptochlorophaeic acid is the only example of such a structure among the numerous depsides yet reported from the genus *Parmelia*. Microchemical comparisons have indicated six other species of *Parmelia* containing cryptochlorophaeic acid (4), suggesting that this substance may have a much wider distribution in lichens than was previously supposed.



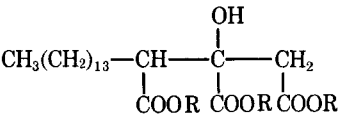
I



II, X = H
III, X = Cl



IV[1]



V, R = CH_3
VI, R = H

Judging from the ease of purification, the sample of chloroatranorin from *P. cryptochlorophaea* seemed to contain little or no atranorin (II), although this compound is probably the immediate precursor of chloroatranorin and is usually found admixed in lichens containing the chloro derivative. Caperatic acid was identified by its melting point and by preparation of two known derivatives, the trimethyl ester (V) and the demethylated compound, norcaperatic acid (VI).

EXPERIMENTAL[2]

Plant Material.—The sample of *P. cryptochlorophaea* was supplied by Dr. Hale from a larger collection made by H. A. Allard in October 1947, near Santo Domingo, Dominican Republic.

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[1] The position of the methyl ester has not been determined.
[2] Melting points were taken with a Thomas-Hoover capillary melting point apparatus and are corrected. The I.R. spectra were obtained with a Perkin-Elmer Infracord and the U.V. spectra were determined with a Bausch & Lomb Spectronic 505. The elemental analysis was performed by Clark Microanalytical Laboratory, Urbana, Ill.

Extraction.—An air-dried sample of *P. cryptochlorophaea* (9.3 Gm.) was extracted (Soxhlet) with anhydrous peroxide-free ether (300 ml.) for 14 hr., and the resulting yellow solution was diluted to 400 ml., washed with cold 10% sodium bicarbonate (six 5-ml. portions), and then with distilled water (five 10-ml. portions) until the washings were neutral. The dried (anhydrous sodium sulfate) ether layer, concentrated at reduced pressure, deposited crystals slowly at room temperature.

Cryptochlorophaeic Acid.—The precipitate from the ether solution consisted of fine needles and stout prisms. A small volume of ether, which was added to the precipitate, dissolved the needles. The less soluble prisms of chloroatranorin were separated by filtration and washed once with a few drops of ether. Benzene was added to the combined ether mother liquor and washing. A solid, which crystallized slowly, was collected by filtration and dissolved in hot benzene. The solution, decolorized with activated charcoal, deposited colorless needles of cryptochlorophaeic acid[3] (236 mg., 2.5%), m.p. 183–184°. [Lit. m.p. 182–184° (3).] $\nu_{max.}^{Nujol}$ 3470 (OH), 1740 (depside C=O), 1720, 1650 (bonded COOH), 1625, 1590 cm.$^{-1}$ (aryl C=C); $\lambda_{max.}^{EtOH}$ (ϵ) 216 (46,800), 256 (15,200); $\lambda_{min.}^{EtOH}$ 239 (11,500); λ_{sh}^{EtOH} 285 mμ (6040). A mixed melting point of this solid with an authentic sample of cryptochlorphaeic acid was not depressed, and the samples showed identical absorption in the infrared.

Anal.—Calcd. for $C_{25}H_{32}O_8$: C, 65.20; H, 7.00. Found: C, 65.41; H, 6.99.

Chloroatranorin.—The chloroatranorin crystals described above were recrystallized from acetone–ethyl ether yielding 15 mg. (0.16%) of very pale yellow prisms, m.p. 207–208°. [Lit. m.p. 208–208.5° (5).] $\nu_{max.}^{Nujol}$ 1665 (bonded C=O), 1600 (aryl C=C), 1282, 1270 (typical of chloroatranorin), and no absorption at 1300 cm.$^{-1}$ (typical of atranorin).

This product gave all of the microchemical tests (1) typical of atranorin (II) and chloroatranorin (III) including microcrystal tests, color reactions, and chromatographic R_f values. Microrecrystallization from a glycerin–acetic acid solution (1) gives well-known prisms with straight extinction, and characteristic yellow condensation products are formed in microcrystal test solutions (1) containing aniline or *o*-toluidine. Also a Beilstein test for halogen is positive, and the melting point is very close to that reported for chloroatranorin. The compound was identical in all respects to a sample of chloroatranorin from the lichen *Physcia aegialita*, and the melting point of a mixture of the two samples was not depressed.

Caperatic Acid.—The sodium bicarbonate-soluble washing of the original lichen extract was cooled in an ice bath and acidified by dropwise addition of cold 1 *N* H_2SO_4 to the stirred solution. The suspension was transferred to a separator, extracted with ether (four 100-ml. portions), and the ether solution was washed with distilled water until the washings were neutral. The ether layer was dried over anhydrous sodium sulfate and a solid, obtained by evaporation of the solvent under diminished pressure, was recrystallized from methanol–water yielding 160 mg. (1.7%) of caperatic acid (IV), m.p. 131.5–132°. [Lit. m.p. 132–133.5° (6), 131–132° (7).] $\nu_{max.}^{Nujol}$ 3600 (OH), 1750 ($COOCH_3$), 1720 and 1690 cm.$^{-1}$ (COOH).

A methanol solution (5 ml.) of this compound (39 mg.) was treated with diazomethane in ether yielding the neutral trimethyl ester (V) (38 mg., 92%), m.p. 56–56.5° (from methanol–water). [Lit. m.p. 56.5–57.5° (6), 57–58° (7).] $\nu_{max.}^{Nujol}$ 3620 (OH), 1760 and 1740 cm.$^{-1}$ ($COOCH_3$); $\nu_{max.}^{CHCl_3}$ 3620 (OH), 1750 cm.$^{-1}$ ($COOCH_3$).

A solution of caperatic acid (41 mg.) in 5% potassium hydroxide (10 ml.) was refluxed for 1.5 hr., cooled, and acidified (1 *N* H_2SO_4). The sticky precipitate crystallized from ethanol–water yielding norcaperatic acid (VI) (23 mg., 57%), m.p. 139.5–140°. [Lit. m.p. 137–138° (6).] $\nu_{max.}^{Nujol}$ 3600 (OH), 1725 and 1700 cm.$^{-1}$ (COOH).

[3] Although cryptochlorophaeic acid is acidic, in three separate extractions it was isolated in the ether layer which had been washed with dilute aqueous sodium bicarbonate.

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