acid-treated AcOEt extracts were concentrated to yield 4.4 g of residue in all. A portion (0.7 g) of the residue was chromatographed in acetone on $MgCO_3$ (12 g, 2.3×5 cm). Elution with 5% and 8% H_2O in acetone yielded red and reddish-purple solution, respectively. These solutions were concentrated, acidified with dil. HCl and extracted with AcOEt. Evaporation of the solvent left yellowish-orange residues, 8.4 mg and 13.1 mg, respectively. The pigment eluted with 5% H_2O in acetone showed absorption maxima in EtOH at 281, 447.5 nm and *Rf* 0.82 (Silica gel G, AcOEt:MeOH:H_2O=5:2:1), and the pigment eluted with 8% H_2O in acetone, 254, 264, 295, 317, 365, and 460 nm and *Rf* 0.75. All attempt including preparative TLC to purify these crude pigments into homogeneous state was unsuccessful.

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Coloring Substances of a Lichen *Cetraria ornata*

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A depsidone fumarprotocetraric acid (I)[2] is an only constituent hitherto elucidated in a lichen *Cetraria ornata* Müll. Arg., although the presence of an unidentified yellow pigment in the same lichen has been well mentioned.[3] As a continuation of the study on the lichen phenolics,[4] we have examined the coloring substances of the lichen and have clarified that the major yellow component of the pigments is secalonic acid C (II)[5] accompanied by a small quantity of endocrocin (III)[6] as described in the present paper.

Repeated chromatographic separation of the $CHCl_3$ soluble portion prepared from the ether extract of air dried lichen afforded a yellow pigment in 0.8% yield. The substance, mp 154—160°, $[\alpha]_D$ +25° ($CHCl_3$); +0.7°±0.3° (pyridine), was colored brown by aq. $FeCl_3$ solution. It showed the quite resembled coloration on thin-layer chromatogram (TLC) (fluorescence on ultraviolet (UV) irradiation; coloration with 1% $Ce(SO_4)_2$–10% H_2SO_4 upon heating or with aq. $FeCl_3$ solution) to that of secalonic acid A (IV) which is a major ergot pigment[5] and was isolated previously from a lichen *Parmelia entotheiochroa* Hue for the first time by us,[7] although the *Rf* values do not coincide with each other.

Comparison of the physical properties of the substance with those of secalonic acid A (IV)[5,7] has disclosed that the former should be an isomer of the latter. Among these data, the proton magnetic resonance (PMR) spectrum gives the direct evidence of isomeric cor-

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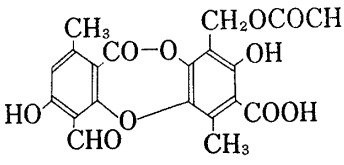
1) Location: *Toneyama, Toyonaka, Osaka.*
2) *a*) Y. Asahina and S. Shibata, "Chemistry of Lichen Substances," Japan Society for the Promotion of Science, Tokyo, Japan, 1954, p. 140; *b*) Y. Asahina, *Bot. Mag.* (Tokyo), **51**, 759 (1937).
3) Y. Asahina, *Journ. Jap. Bot.*, **11**, 10 (1935).
4) The preceding paper on the subjects: I. Yosioka, K. Morimoto, K. Murata, H. Yamauchi, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 2420 (1971).
5) *a*) B. Franck, E.-M. Gottschalk, U. Ohnsorge, and F. Hüper, *Chem. Ber.*, **99**, 3842 (1966); *b*) J.W. Hooper, W. Marlow, W.B. Whalley, A.D. Borthwick, and R. Bowden, *Chem. Comm.*, **1971**, 111.
6) ref. 2 *a*), p. 153.
7) I. Yosioka, T. Nakanishi, S. Izumi, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 2090 (1968).

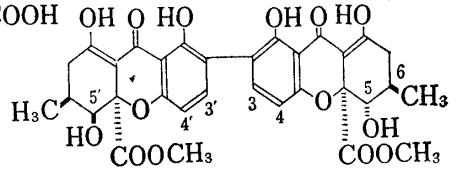
relation of both especially by the signals attributable to the protons at $C_{(3')}$, $C_{(4')}$, and $C_{(5')}$ (see experimental section), and consequently the identity of the substance with secalonic acid C (II) has been assumed. The direct comparion (mixed mp, TLC, and infrared (IR) spectra (KBr) substantiated the assumption.

Furthermore, since Franck and his co-workers have elucidated that the ergochromes in ergot are biosynthesized *via* the acetate-malonate pathway through the anthraquinone derivatives such as endocrocin (III) or emodin (V),[8] we have sought after the precursory or biogenetically related substance of secalonic acid C (II) in the lichen.

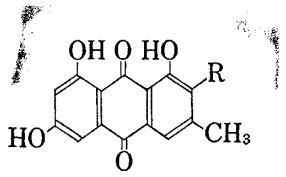
Careful TLC examination of the total extract has revealed the presence of a minor yellowish component which was colored orange-pink by ethanolic $Mg(OAc)_2$ solution.[9] The amount of pigment was so tiny and accompanied by the other minor ergochromes of close polarity that its isolation was only realized by the independent separation procedure as given in the experimental section. The orange substance, mp >300°, thus obtained in 0.005% yield was identified with endocrocin (III) by TLC and IR (KBr).



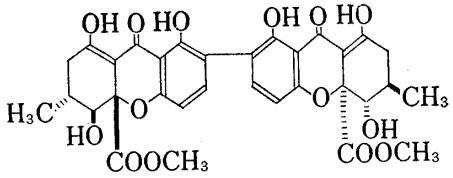
fumarprotocetraric acid (I)



secalonic acid C (II)



R=COOH : endocrocin (III)
R=H : emodin (V)



secalonic acid A (IV)

The present finding offers the first instance of co-occurrence of ergochrome and anthraquinone pigments in the lichen family and is of much interest from the biogenetic view-point, since it has been assumed that there would be probable chemotaxonomical correlation between ergot and a symbiotic fungus of the present lichen. This paper also constitutes the second example of isolation of ergochrome from the lichen.[7]

Experimental[10]

Isolation of Secalonic Acid C(II)——Successive ether extraction of the air dried lichen (*Cetraria ornata* MÜLL. ARG., kindly provided by Prof. F. Fuzikawa, 570 g) furnished a yellow-brown extract (27.1 g), which was treated with $CHCl_3$ to remove the insoluble material. The $CHCl_3$ soluble portion was then subjected to preparative TLC developing with $CHCl_3$–MeOH (24:1) to collect the major yellow component. The TLC plates were made with silica gel Camag D-5 using aq. 0.1N $(COOH)_2$ solution instead of water. The yellow substance was further purified by preparative TLC again using the same adsorbent and developing with benzene–AcOEt (2:1). Repeated preparative TLC altogether afforded the pure pigment in a yield of *ca.* 0.8% from the lichen. The purified yellow pigment thus obtained was then crystallized from benzene–

8) *a*) B. Franck, F. Hüper, D. Gröger, and D. Erge, *Chem. Ber.*, **101**, 1954 (1968); *b*) D. Gröger, D. Erge, B. Franck, U. Ohnsorge, H. Flasch, and F. Hüper, *Chem. Ber.*, **101**, 1970 (1968).
9) S. Shibata, M. Takido, and O. Tanaka, *J. Am. Chem. Soc.*, **72**, 2789 (1950).
10) The following instruments were used for the physical data. Specific rotation: the Rex Photoelectric Polarimeter, UV Spectra: the Shimadzu Recording Spectrometer MPS-50L, IR Spectra: the Hitachi IR Spectrometer EPI-S2, PMR Spectra: the Vavian A-60 NMR Spectrometer, and Mass Spectra: the Hitachi RMU-6D Spectrometer.

cyclohexane. mp 154—160°. $[\alpha]_D$ +25° (*c*, 0.84, $CHCl_3$); +0.7° ±0.3° (c, 3.0, pyridine) (lit.[5]: secalonic acid C (II): $[\alpha]_D$ +25° ($CHCl_3$); −10.3° (pyridine)). UV λ_{max}^{EtOH} nm (log ε): 240 (4.25, infl.), 266 (4.20, infl.), 339 (4.56). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 1740 ($COOCH_3$), 1610 (chelated CO), 1589, 1564 (aromatic ring). Mass Spectrum *m/e* (%): 638 (M^+, $C_{32}H_{30}O_{14}$, 21.4), 620 (M^+-H_2O, 1.8), 579 ($M^+-COOCH_3$, 100), 260 ($M^+/2-COOCH_3$, 17.6). PMR ($CDCl_3$, τ): 8.83 (6H, d, J=5.5 Hz, >CH-C<u>H</u>$_3$×2), 7.1—8.1 (6H, m, >C<u>H</u>-CH_3×2, -C<u>H</u>$_2$- ×2), 6.29 (6H, s, -COOC<u>H</u>$_3$×2), *ca.* 6.0—6.25 (1H, unclear signal due to the overlapping, >$C_{(5)}$<u>H</u>-OH), 5.85 (1H, br. s, >$C_{(5')}$<u>H</u>-OH), 3.45, 2.58 (2H), 3.40, 2.57 (2H) (ABq each, J=8.5 Hz, four aromatic protons). The substance obtained here was identified (mixed mp, TLC, and IR (KBr)) with authentic secalonic acid C (II) kindly provided by Prof. B. Franck.

Isolation of Endocrocin (III)——The $CHCl_3$ soluble portion obtained as above was passed through a polyamide column (Wako Pure Chem. Ind., C-300) with the aid of $CHCl_3$–MeOH mixture. After eluting out all the yellow pigment completely, the column was next eluted with MeOH–water–conc. NH_4OH (10:8:2) mixture. The eluted compound was then crystallized from aq. acetone to give the orange crystals (*ca.* 0.005% yield from the lichen), mp >300°, Mg $(OAc)_2$: orange-pink, conc. H_2SO_4: red. The crystals were identified with endocrocin (III) by TLC and IR (KBr).

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