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Pigments of Sea-Lilies. I. Quinonoids of *Tropiometra afra macrodiscus* (HARA) and *Comanthus japonica* (MÜLLER)

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Several reports on the pigments of Crinoidea animals (sea-lilies; feather-stars) have been published,[2,3] and the chemical constitution of anthraquinone derivatives occurring in this class of marine animals were elucidated by Australian workers.[2a–e] However, any chemical data on crinoids of Japanese coast does not seem to have been available. This paper describes the result of an investigation of the quinonoid pigment of Japanese crinoids, *Tropiometra afra macrodiscus* (HARA) (Japanese name: Oh-umishida, family: Tropiometridae) and *Comanthus japonica* (MÜLLER) (Japanese name: Nippon-umishida, family: Comasteridae).

T. afra macrodiscus (HARA)[4] is a ten-armed comatulid, and easily discriminated from other species because of its relatively longer arm-length (*ca.* 20 cm). The appearance in colour is usually dark reddish-purple, but less frequently yellow specimens are found.

The dark reddish-purple specimens fresh from the sea at Kashiwajima, Kochi-ken, were immediately immersed in acetone, and the acetone solution was treated as described in the Experimental section. In the extraction procedure, the mother liquor of the exhaustive extraction of neutral concentrate with ethyl acetate was acidified and again extracted with the same solvent. Both ethyl acetate solutions thus obtained were found to contain the same pigment on thin-layer chromatographic (TLC) examination, and it was noticed that the neutral mother liquor contained the pigment in a water-soluble form which on acidification liberated the pigment extractable into the organic solvent.

On TLC examination, the extract contained an orange-coloured pigment (**1**), which changed its colour to red on spraying magnesium acetate solution. A purification on a neutral alumina column was applied, and the pigment (**1**) was obtained as red needles from the acid washings of strongly adsorbed purple coloured zone.

These chromatographic behaviour including colour reaction with magnesium acetate suggested that the pigment should be of hydroxyanthraquinone character. Ultraviolet (UV) and visible (VIS) spectra of (**1**) in ethanol showed absorption maxima at 227, 278, and 448 nm, and the infrared (IR) spectrum (Nujol) showed bands at 1675 (weak), and 1625 (strong) cm^{-1} attributable[5] to quinone carbonyl with *peri*-oriented hydroxyl group(s), and another strong band at 1710 cm^{-1}. The IR absorption band at 1710 cm^{-1} was assumed to be due to

1) Location: *Misasagi, Yamashina, Higashiyama-ku, Kyoto, 607, Japan.*

2) *a*) M.D. Sutherland and J.W. Wells, *Chem. Ind.* (London), **1959**, 291; *b*) T.F. Low, R.J. Park, M.D. Sutherland, and I. Vessey, *Australian J. Chem.*, **18**, 182 (1965); *c*) M.D. Sutherland and J.W. Wells, *ibid.*, **20**, 515 (1967); *d*) V.H. Powell, M.D. Sutherland, and J.W. Wells, *ibid.*, **20**, 535 (1967); *e*) V.H. Powell, and M.D. Sutherland, *ibid.*, **20**, 541 (1967); *f*) R.A. Kent, I.R. Smith, and M.D. Sutherland, *ibid.*, **23**, 2325 (1970); *g*) I.R. Smith, and M.D. Sutherland, *ibid.*, **24**, 1487 (1971).

3) *a*) E.J. Dimelow, *Nature*, **182**, 812 (1958); *b*) H. Singh, R.E. Moore, and P.J. Scheuer, *Experientia*, **23**, 624 (1967).

4) S. Nishimura and K. Suzuki, "Common Seashore Animals of Japan in Color," Supervised by H. Utinomi, Hoikusha, Osaka, 1971, p. 148.

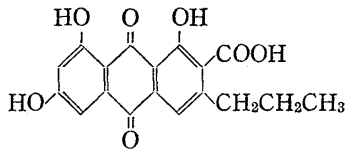
5) K. Nakanishi, "Infrared Absorption Spectroscopy, Practical," Holden-Day, San Francisco, 1962, p. 43.

carboxylic carbonyl, and the spectra were compared with those of endocrocin (**2**),[6] a lichen pigment possessing the hydroxyanthraquinone carboxylic acid structure. The UV and VIS spectra were found to be superimposable and the functional group region in the IR spectra were quite similar and it was evidenced that the both pigments have a common chromophoric arrangement in their molecules.

As a pigment of marine origin having the same chromophoric arrangement with endocrocin, Sutherland, *et al.* isolated ptilometric acid (**1**) from Australian crinoids, *Ptilometra australis* Wilton and *Tropiometra afra* Hartlaub and elucidated the structure (**1**) of the compound.

On the basis of the zoological relationship[7] in *T. afra* species and of the spectral data stated above, the pigment was supposed to be ptilometric acid. Direct comparison of the pigment with the authentic ptilometric acid kindly donated by Dr. Sutherland revealed that both were identical in every respect (UV, VIS, IR, TLC, and colour reactions), and the occurrence of ptilometric acid in *T. afra macrodiscus* (Hara) was proved.

The rest of the crude pigment extract was purified according to Sutherland's procedure[2e] using magnesium carbonate column with aqueous acetone as the solvent. After this purification, dark reddish-purple animals were found to contain an average of 7.3 mg of ptilometric acid (**1**) per animal, while yellow-coloured specimens collected at Kashiwajima contained 0.7 mg of the acid (**1**) per animal.



1

2: $-CH_3$ instead of $-CH_2CH_2CH_3$

Occurrence of ptilometric acid in dark reddish-purple specimens of *T. afra macrodiscus* collected at Kiki, Tokushima-ken, and Goza, Mie-ken, was also confirmed by TLC comparisons.

Comanthus japonica (Müller) is a crinoid with about forty arms and most frequently seen in waters of Japanese coast. Dark orange-brown coloured specimens of the crinoid collected at Kiki were extracted by the same procedure as above. Magnesium carbonate column chromatography afforded a very minute amount of unidentified quinonoids, but further studies could not be conducted because of the scarcity of the sample obtained. Ptilometric acid (**1**) was not detected in the extractives.

However, on acidification the quinonoid content of *C. japonica* showed a similar behaviour to that of *T. afra macrodiscus*. When the mother liquor of exhaustive extraction with ethyl acetate was acidified and again extracted, a "liberated" pigment came into organic layer, and further, in case of *C. japonica*, this "liberated" form was found to be generated also from the extract of neutral concentrate on treatment with dilute hydrochloric acid. These findings seem to suggest that the quinonoid pigments in crinoids should be present in "bound" forms in the living cells of animals.

Experimental

(a) Extraction of Dark Reddish-Purple Specimens of *Tropiometra afra macrodiscus* (Hara)——The animals (54 specimens) fresh from the sea at Kashiwajima, Kochi-ken, Nov. 1970, were treated as shown in Chart 1. The animals were immediately immersed in acetone to yield deep red solution, and the extraction was practically completed by soaking the whole animals in acetone at room temperature. Repeated extraction with acetone gave almost colourless solution. The acetone solution showed two orange spots on TLC (*Rf* values: 0.50 and 0.30, Silica gel G, AcOEt: MeOH: H_2O=5:2:1), and the orange colour changed

6) Y. Asahina and F. Fuzikawa, *Yakugaku Zasshi*, **55**, 1122 (1935).
7) Australian species, *T. afra afra* (Hartlaub) is the nominate subspecies of *T. afra macrodiscus* (Hara). *cf.* ref. 4).

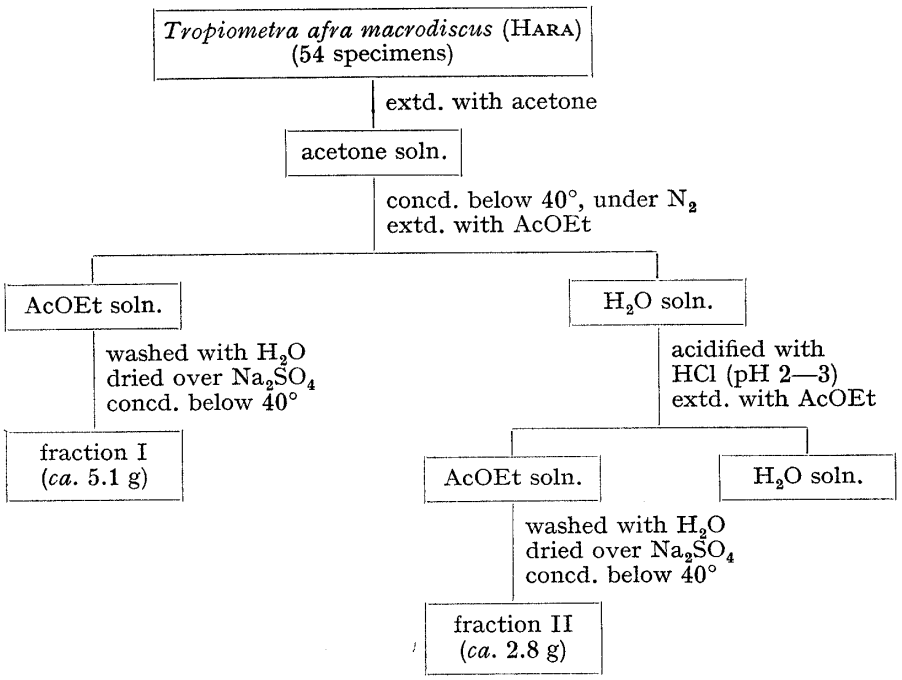


Chart 1

to red on spraying $Mg(AcO)_2$ solution (0.5% in MeOH). The extract was concentrated and exhaustively extracted with AcOEt. The pigment which showed larger *Rf* was extractable into AcOEt and the pigment with smaller *Rf* remained in mother liquor. The mother liquor was acidified with HCl and again extracted with AcOEt. On acidification, the pigment which had shown smaller *Rf* became undetectable, but the one extractable into the solvent was found to have the same *Rf* value as the pigment with larger *Rf* value. The AcOEt solutions were washed with water, dried over anhyd. Na_2SO_4 and concentrated separately to give 5.1 g (fraction I) and 2.8 g (fraction II) of dark brown residue, respectively. These two fractions were found to contain the same pigment on TLC. A portion (1 g) of fraction I was purified using a column (2.5×12 cm) of Al_2O_3 (Merck, neutral, Grade 1, 50 g). After elution of neutral impurities, strongly adsorbed purple zone was taken out, pigment was dissolved in methanolic HCl, and the resulted solution was freed of MeOH *in vacuo*, then extracted with AcOEt. The AcOEt solution was washed with water and dried over Na_2SO_4, and the solvent was evaporated off. Trituration of the residue with benzene–ether afforded 10 mg of pigment (**1**) as red needles. A portion (2 g) of fraction I was chromatographed on $MgCO_3$ (BDH Chromatographic Adsorption Analysis Grade, 65 g, 3.8×9 cm) using aqueous acetone as eluting solvent. The water-content of the solvent was progressively increased up to 30—50% until the main band was eluted. Red-coloured eluate was concentrated, acidified with dil. HCl, and extracted with AcOEt. Removal of the solvent yielded 54 mg of **1**. Total yield of **1** from fractions I and II was 137.7 mg and 254.8 mg, respectively.

The animals collected at Kiki, Tokushima-ken, Aug. 1970, and at Goza, Mie-ken, Apr. and July 1971, were extracted by the same procedure. These extracts showed the same patterns on TLC with that of Kashiwajima specimens and the main extractable pigment was found to behave identically with **1** on TLC.

(b) **Identification of Ptilometric Acid (1,6,8-Trihydroxy-3-propylanthraquinone-2-carboxylic Acid)**——The pigment was recrystallized from acetic acid as red needles, mp >280°. The yellow methanolic solution turns red on addition of $Mg(AcO)_2$ solution (0.5% in MeOH). The UV, VIS, and IR spectra were superimposable with those of a sample of ptilometric acid.[2e] Both samples behaved identically on TLC (solvent systems, AcOEt:MeOH:H_2O=5:2:1, toluene:HCOOEt:HCOOH=5:4:1).

(c) **Extraction of Yellow Specimens of *T. afra macrodiscus* (HARA)**——Four animals collected at Kashiwajima, Kochi-ken, Nov. 1970, were extracted as in (a) and purified on $MgCO_3$ column to give 2.8 mg of **1**.

(d) **Extraction of *Comanthus japonica* (MÜLLER)**——Fourteen animals collected at Kiki, Tokushima-ken, Aug. 1970, were treated by the same procedure as Chart 1. On TLC examination, the AcOEt extract of neutral concentrate showed different chromatograms from that of acidified mother liquor. No spot corresponding to ptilometric acid (**1**) was detected in either extract. Treatment of the extract of neutral concentrate with dil. HCl yielded the pigment which showed similar TLC chromatograms to those of the extract of acidified mother liquor.

Another group of specimens (20 animals) collected at Kiki, Aug. 1970, were extracted with EtOH and the EtOH extract was treated with the same procedure. The AcOEt solutions thus obtained behaved in the same way as with the case of acetone-treated 14 specimens on TLC before and after acidification. The

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).
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6) ref. 2 *a*), p. 153.
7) I. Yosioka, T. Nakanishi, S. Izumi, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 2090 (1968).