PIGMENTS OF MARINE ANIMALS

IV.* THE ANTHRAQUINOID PIGMENTS OF THE CRINOIDS, *COMATULA PECTINATA* L. AND *C. CRATERA* A. H. CLARK

By M. D. SUTHERLAND† and J. W. WELLS†‡

[*Manuscript received September* 12, 1966]

Summary

A review of the literature describing the pigments of the crinoids (phylum Echinodermata) reveals a prevalence of indicator-type pigments which are of unknown nature, except for certain polyhydroxy-*meso*-naphthodianthrones isolated from a fossilized Jurassic stalked crinoid.

The highly coloured free-swimming crinoids, *Comatula pectinata* L. and *C. cratera* A. H. Clark, contain mixtures of indicator-type pigments which have been separated by adsorption chromatography on magnesium carbonate to yield three principal constituents, the 6-methyl and the 6,8-dimethyl ethers of rhodocomatulin, and a monomethyl ether of rubrocomatulin. The structure of the rhodocomatulin skeleton is shown to be 4-butyryl-1,3,6,8-tetrahydroxyanthraquinone rather than 2-butyryl-1,3,6,8-tetrahydroxyanthraquinone as previously suggested.[1]

INTRODUCTION

"Of all the animals in the sea there are none that exceed in beauty of coloration the shallow water crinoids. Flowerlike in form and almost flowerlike in the fixity of their habit, they are also flowerlike in the variety and distribution of their pigments." Despite this comment by A. H. Clark[2] in 1921 in his "Monograph of the Existing Crinoids", D. L. Fox[3] in 1953 could write that "the state of our knowledge regarding the pigments of sea lilies is very unsatisfactory . . ."

The crinoids form a class of the phylum Echinodermata which also includes the echinoids, pigmented principally by polyhydroxynaphthoquinones and melanin, the asteroids and ophiuroids, showing the wide variation in colour which can result from the protein binding of carotenoids, and the generally sombre-hued holothurians from a species of which has been isolated a polyhydroxynaphthoquinone protein complex.[4]

* Part III, *Aust. J. Chem.*, 1965, **18**, 182.

† Chemistry Department, University of Queensland, Brisbane.

‡ Present address: Poultry Research Centre, King's Buildings, Edinburgh 9, Scotland.

[1] Sutherland, M. D., and Wells, J. W., *Chemy Ind.*, 1959, 291.

[2] Clark, A. H., Bull. U.S. natn. Mus. No. 82 (1921).

[3] Fox, D. L., "Animal Biochromes and Structural Colours." (Cambridge University Press 1953.)

[4] Mukai, T., *Mem. Fac. Sci. Kyushu Univ.* C, 1958, **3**(2), 29.

The chemical investigation of crinoid pigments appears to have begun with Moseley's investigations[5] on board the *Challenger* in the East Indies in 1874–75. Moseley spectroscopically characterized three different pigments, "purple pentacrinin" and "red pentacrinin", being isolated from stalked crinoids, and the third, "antedonin" from a comatulid (stalkless free-swimming crinoid).

"Purple pentacrinin" was extracted by weakly acidified ethanol from certain specimens of a group of deep-sea species which Moseley referred to as *Pentacrinus* but which have since been identified as *Hypalocrinus naresianus*, *Endoxocrinus alternicirris*, and several species of *Metacrinus*.[2] The animals varied in colour from very dark purple to light pinkish red, to almost colourless, to white with orange crowns. The intensely pink solutions of "purple pentacrinin" turned bluish green with a slight red fluorescence when made alkaline with ammonia, the colour change being repeatedly reversible. Evaporation of an acidic solution of "purple pentacrinin" gave a dark violet amorphous powder which redissolved freely in ethanol only after the addition of a little hydrochloric acid. Addition of excess ammonia to a strong aqueous solution of pigment resulted in a green flocculent precipitate. Some almost colourless crinoids, when placed in ethanol, afforded a deep green solution which gave the "purple pentacrinin" spectral bands on acidification.

Moseley used a spectroscope to examine his solutions, and from his descriptions and diagrams, we have estimated the approximate wavelengths of the reported spectral absorption maxima. The acid form of "purple pentacrinin" showed maxima at *c.* 495, 565, and 600 mμ compared with those at *c.* 490, 600, and 695 mμ displayed by the green alkaline form.

"Red pentacrinin" was extracted into "weak spirit" from an apparently atypical light pinkish red specimen of *H. naresianus* to give a light red solution (maxima at 400 and 500 mμ), the colour of which was discharged rather than changed to green by ammonia.

"Antedonin" was extracted by ethanol from a dark purple comatulid which was dredged in large numbers off Cape York and which was referred to by Moseley as an *Antedon*. In 1921 A. H. Clark concluded that this species was "probably *Comatula rotolaria*" for which the current designation is *Validia rotolaria* H. L. Clark.[6] The "antedonin" extract was an intense fuchsin-coloured solution the colour of which was changed to orange (maxima at 485, 500, and 540 mμ) by hydrochloric acid or to deep violet by ammonia. Excess ammonia precipitated an amorphous purple powder which was insoluble in ethanol unless acidified.

In 1882, Krukenberg[7] extracted the Mediterranean crinoid, "*Antedon rosaceus*" (now *A. adriatica*) with ethanol to obtain a red solution of a pigment "comatulin" contaminated with some chlorophyll. Acidification of the extract yielded an orange solution from which some colouring matter precipitated. Addition of ammonia to the red solution caused precipitation without colour change. "Comatulin" did not show an absorption maximum in the visible.

[5] Moseley, H. N., *Q. Jl microsc. Sci.*, 1877, **17**, 1.

[6] Clark, H. L., "The Echinoderm Fauna of Australia. Its Composition and its Origin." Carnegie Inst. Publ. No. 566. (Carnegie Inst.: Washington 1946.)

[7] Krukenberg, C., *Vergl. physiol. Studien*, 1882, **2**, 88.

MacMunn[8] in 1890 examined extracts of "*Antedon rosacea*" (now *A. bifida*), but could find chlorophyll present only in the stomach contents. Ethanol or water yielded rose-red extracts which turned yellow on acidification. Addition of ammonia discharged the colour to a faint reddish tint. MacMunn concluded that the pigment differed from "antedonin", and that a yellow lipochrome (carotenoid?) was also present. The "antedonin of *Antedon macronema*" was also described by MacMunn, being obtained from an extract of what was probably *Ptilometra australis* Wilton (see Part VI of this series) rather than *P. macronema*.[6] The red residue from an ethanolic extract gave an orange-red solution in acidified ethanol with one absorption band at 580–589 mμ and another from 523 to 549 mμ, which was probably two bands, the darker part being at 523–532 mμ. Addition of ammonia to the ethanol solution resulted in a darker red colour and slight precipitation. This red residue was freely soluble in ether, chloroform, and benzene.

MacMunn also examined extracts of various crinoids obtained by the *Challenger* expedition some 15 years previously. An extract of *Validia rotolaria* H. L. Clark from Cape York was examined under the name *Actinometra paucicirra* assigned to it by P. H. Carpenter. Evaporation of the extract left a red residue, soluble in chloroform and partially soluble in ether, which in ethanolic solution gave a faint "antedonin" spectrum. Hydrochloric acid changed the red colour to yellow whereas ammonia or sodium hydroxide intensified the red colour. MacMunn examined a "large *Actinometra* from Banda", supposedly the source of Moseley's "antedonin", and concluded that it was "evident that antedonin, somewhat altered by time, was present". Various extracts of other crinoids were examined with rather similar results, except that *Actinometra stelligera* (now *Comatella stelligera*) may be mentioned as yielding a yellowish brown residue, soluble in ethanol to give a yellow solution virtually devoid of indicator properties.

In 1926 Abeloos and Teissier[9] found evidence for two pigments in *Antedon bifida* Pennant. Red specimens yielded a red pigment to water or alcohol but not to ether. Addition of acids changed the colour to yellow, whereas alkalis changed the red colour to violet and caused precipitation. Yellow specimens gave a yellow pigment of similar solubility but without indicator properties. Treatment with boiling alkali transformed the yellow pigment to a red substance, apparently identical with the pigment from red specimens.

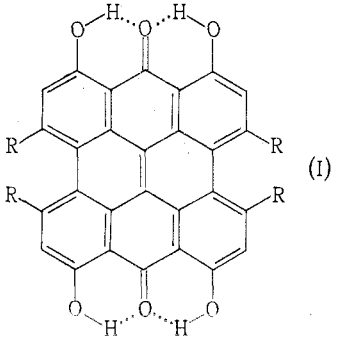
In 1931 Lönnberg,[10] surveying the carotenoid content of marine animals, observed that *Antedon pentasus* yielded a strong brown-red colour to methanol. This solution showed indicator properties, changing to yellow (maxima at 425 and 456 mμ) with acids and to red with alkalis. In 1935 Karrer and Solmssen[11] found no appreciable quantity of carotenoids in *Antedon rosacea*.

One further brief report on the pigments of existing comatulids appeared after the present investigations had been commenced. Dimelow[12] in 1958 recognized

[8] MacMunn, C. A., *Q. Jl microsc. Sci.*, 1890, **30**, 51.
[9] Abeloos, M., and Teissier, G., *Bull. Soc. zool. Fr.*, 1926, **51**, 145.
[10] Lönnberg, E., *Ark. Zool.* A, 1931, **22**, 12.
[11] Karrer, P., and Solmssen, V., *Helv. chim. Acta*, 1935, **18**, 915.
[12] Dimelow, E. J., *Nature*, 1958, **182**, 812.

β-carotene, astaxanthin in free and esterified form, and xanthophyll in extracts of the arms and pinnules of *Antedon bifida*. Also present was an indicator pigment, which gave a green ferric chloride coloration. The pigment was reduced to a colourless product by sodium dithionite solution, the colour being restored by air. From this behaviour and from the absorption peaks at 262, 337, and 460 mμ, the pigment was considered comparable with the hydroxynaphthoquinone (probably echinochrome A) of the echinoid *Diadema antillarum*. Cromatie[13] found that some 500 specimens of *A. bifida* yielded 3 mg only of the echinochrome-like pigment.

The persistence of polyhydroxyquinonoid pigments in a fossil stalked crinoid, *Apiocrinus* (*Millericrinus*) sp., from certain Swiss Upper Jurassic beds has been demonstrated by Blumer.[14] Several pigments (fringelites A to F) have been isolated in crystalline form and are formulated as polyhydroxy-*meso*-naphthodianthrones of



(I)

structure (I) (R = H, OH, or alkyl).[15,16] The spectra of the red acid form and the green alkali salts of Moseley's "purple pentacrinin" suggest that the pigments of present day stalked crinoids may well have a closer relationship with the fossil *Apiocrinus* pigments than with present day comatulid pigments.

The Pigments of Comatula pectinata

The 10-armed comatulid, *Comatula pectinata* L., which had not been chemically examined previous to our work, is a member of the Comasteridae family[6] and is therefore related to *Comatella stelligera* and to the *Actinometra* sp. from Banda which were examined by MacMunn, and to *Validia rotolaria* examined both by MacMunn and by Moseley.

The species *C. pectinata* now includes a colour variant formerly designated *C. purpurea*. As H. L. Clark[6] remarks, "the diversity of colour is amazing and is apparently in no way associated with habits or habitat. Shades of red and purple are most frequent and unicolour specimens are more common than varied ones". In seawater with added magnesium sulphate "the colour comes out rapidly and copiously, usually being of a brownish-red shade".

[13] Cromatie, R. T., personal communication to M. D. Sutherland, 1958.
[14] Blumer, M., *Mikrochem. mikrochim. Acta*, 1951, **36–37**, 1048.
[15] Blumer, M., *Nature*, 1960, **188**, 1100; *Geochim. cosmochim. Acta*, 1962, **26**, 225.
[16] Blumer, M., *Science*, 1965, **149**, 722.

The animals used in the present investigation were collected off Myora in Moreton Bay, Queensland, typically from the underside of coral boulders just below low tide level on the edge of the Rainbow Channel and were of uniform bright to dark red colour.

Placing the animals in acetone led to an immediate liberation of a strong red colour, extraction being completed by repeatedly soaking the whole animals in fresh acetone at room temperature over a period of weeks. The colouring matter is readily extractable by a variety of organic solvents and also passes from wounded, moribund, or dead animals into seawater. Dialysis of freshly ground tissue against seawater (pH 8) gave a violet-red diffusate, the colour of which would only partly extract into ether unless the pH was lowered, when the colour changed to orange-red.

TABLE 1

SEPARATION OF C. PECTINATA PIGMENTS BY COLUMN CHROMATOGRAPHY

Chromatography of 1070 mg of crude pigment on a column (33 cm by 4 cm) of magnesium carbonate (B.D.H. chromatography grade). Yield percentages are of recovered pigment after removal of lipid and other impurities as far as possible. (Slight losses, colourless products, carotenoids, brown wax, and other material amounted to 18% of the crude pigment.) R_F and spot colour in the n-butanol/concentrated ammonia system on paper chromatography.

Zone	Colour on $MgCO_3$	Eluent	Yield (%)	R_F and Spot Colour
Brown wax and carotenoids	—	acetone	—	—
Initial zone	orange	2% water in acetone	1	0·52; orange
Rhodocomatulin 6,8-dimethyl ether	red-orange	4% water in acetone	36	0·66; red
Intermediate zones	(*a*) violet-red	4% water in acetone	1	0·35; violet
	(*b*) red			0·17; violet
Rhodocomatulin 6-methyl ether	violet-red	6–8% water in acetone	30	0·72; violet-red
Rubrocomatulin monomethyl ether	violet	8–30% water in acetone	11	0·55; violet
Upper zones	(*a*) red-violet	recovered by extrusion	3	0·19; blue-violet
				0·17; blue
	(*b*) red-violet			0·12; brown
				0·03; blue
				0·00; brown

These observations suggest that the pigments are not bound as stable complexes with protein analogous to the caroteno-proteins displayed particularly by the asteroids[3] or to the hydroxynaphthoquinone–protein complex isolated by Mukai[4] from a holothurian. The residual tissue after acetone extraction has a grey colour and yields only small amounts of pigment on digestion with dilute acid in the presence of ether. This contrasts with the echinoids which commonly incorporate a large proportion of their hydroxynaphthoquinones as salts in their calcareous spines and tests.

The acetone-soluble material from evaporated extracts of *C. pectinata* was next partitioned between hexane and 90% methanol whereby the neutral lipid material, unlike the pigment, passed into the epiphase. The partially crystalline hypophasic

residue, here termed crude pigment, usually amounted to 0·6–0·7 g per animal and represented 4–5% of the dry weight of the animal.

Fractionation of the crude pigment could be achieved either by paper chromatography using a butanol/ammonia system or more effectively by adsorption chromatography on magnesium carbonate columns using as eluting solvents first acetone and then progressively richer acetone/water mixtures. Concentration and acidification of the eluates yielded the separated pigments in filterable form. Table 1 shows in slightly simplified form a typical separation involving the column chromatography of 1070 mg of crude pigment.

An attempt was made to compare the pigment yields from several bright red and dark red animals. The bright red specimens had a marginally greater crude pigment content associated with a lower proportion of rhodocomatulin dimethyl ether and distinctly higher proportion of the more strongly adsorbed (heavily hydroxylated) quinones.

The Pigments of Comatula cratera

Specimens of the less spectacularly coloured *C. cratera*[6] were taken from the nets of prawn trawlers operating at 25–30 fathoms off the south Queensland coast between Jumpinpin and Tweed Heads. The yield of crude pigment from this species averaged *c.* 0·7% of dry weight or *c.* 90 mg per animal, and was more variable than for *C. pectinata*. The pigment mixture on chromatography revealed the same principal constituents as found in *C. pectinata* with rubrocomatulin monomethyl ether and the other strongly adsorbed pigments present in greater proportion:

Rhodocomatulin 6,8-dimethyl ether	25%
Intermediate zone pigments	17%
Rhodocomatulin 6-methyl ether	14%
Rubrocomatulin monomethyl ether	28%
Strongly adsorbed pigments	16%

Characterization of the Principal Pigments

The major pigment, rhodocomatulin 6,8-dimethyl ether, eluted from magnesium carbonate columns with 4% aqueous acetone, crystallized from acetone as yellow orange needles, m.p. 208·5–209° or 229·5–230·5°, and analysed as $C_{20}H_{18}O_7$, including two methoxy groups and one *C*-methyl group. It afforded a bright yellow diacetate, $C_{18}H_{10}O_3(OCH_3)_2(OCOCH_3)_2$, m.p. 199·5–201°, a pale orange monobromide $C_{20}H_{17}BrO_7$, m.p. 222·5–223·5°, a yellow-orange dimethanesulphonyl ester $C_{18}H_{10}O_3(OCH_3)_2(OSO_2CH_3)_2$, m.p. 248–250° (dec.), and the yellow rhodocomatulin tetramethyl ether $C_{18}H_{10}O_3(OCH_3)_4$, m.p. 203·5–204° or 211–212°.

The second pigment, rhodocomatulin 6-monomethyl ether, $C_{19}H_{16}O_7$ (including one methoxyl and one *C*-methyl group), eluted with 6–8% aqueous acetone and crystallized as orange-red prisms, m.p. 250–252° (dec.), from ethanol. This substance yielded a pale yellow triacetate, $C_{18}H_{10}O_3(OCH_3)(OCOCH_3)_3$, m.p. 194–196°. The formation of the same tetramethyl ether as yielded by the rhodocomatulin dimethyl ether described above established that these two pigments differed only in the number of *O*-methyl groups.

These two pigments dissolved in aqueous sodium carbonate, but not in sodium bicarbonate solution, giving red and violet-red solutions, stable to air. Similar colorations developed in methanolic solutions containing magnesium acetate.

Further development of the magnesium carbonate column with 10–30% aqueous acetone eluted the third major constituent, rubrocomatulin monomethyl ether, $C_{19}H_{16}O_8$, which formed scarlet prisms, m.p. 298–299° (dec.) from acetic acid. In acetic acid solution, it displayed a pink fluorescence, and in aqueous sodium hydroxide it formed a violet-red solution which faded slowly on exposure to air. The pigment was characterized by a yellow tetraacetate, $C_{18}H_9O_3(OCH_3)(OCOCH_3)_4$, m.p. 203–205° (dec.), and a pentamethyl ether, $C_{18}H_9O_3(OCH_3)_5$, yellow needles, m.p. 152–153·5° or 214–215°. The chemistry and structure of this quinone is reviewed in Part V of this series.

Carbon Skeleton and Hydroxylation Pattern of the Rhodocomatulins

The electronic spectrum of the pale yellow fluorescent leucotetraacetate $C_{18}H_{10}O(OCH_3)_2(OCOCH_3)_4$, m.p. 231–232° (dec.), of rhodocomatulin dimethyl ether showed maxima in the near visible region which are characteristically those of the anthracene nucleus.[17] Furthermore, the spectra of the di- and tri-acetates referred to above show bands at 280 and 274 mμ respectively, such as commonly exhibited by 9,10-anthraquinones.[18] The solubility of both pigments in sodium carbonate solution may then be attributed to the presence of β-hydroxyl groups, while the magnesium acetate colorations resemble those given by anthraquinones with α-hydroxy groups.

The hydroxylation pattern of the nucleus was determined by examination of the product obtainable by acidic hydrolysis of either pigment. Prolonged refluxing with a hydrobromic–acetic acid mixture afforded quantitatively a red quinone, $C_{14}H_8O_6$, m.p. >360°, with four phenolic hydroxyl groups as shown by the formation of a pale yellow tetraacetate, $C_{14}H_4O_2(OCOCH_3)_4$ and (using dimethyl sulphate and potassium carbonate in refluxing acetone) a tetramethyl ether, $C_{14}H_4O_2(OCH_3)_4$, m.p. 220–221·5°. Its behaviour as a poor mordant dye and its stability in alkali eliminated the possibility of a 1,2-, a 1,2,3-, or a 1,2,4-hydroxyl distribution. The visible spectrum (λ_{max} 452 mμ) and the lack of fluorescence in acetic acid excluded a 1,4-dihydroxy pattern. The two bands at 1669 and 1624 cm^{-1} ($\Delta\nu$ 45 cm^{-1}) in the infrared spectrum are typical of those attributed in 1,8-dihydroxyanthraquinones[19] to free and hydrogen-bonded carbonyl groups. The only known quinone with appropriate structure appeared to be 1,3,6,8-tetrahydroxyanthraquinone, m.p. >360°, originally described by Koller and Russ[20] as a degradation product of the lichen pigment, solorinic acid.

A major discrepancy existed in the description[20] of the tetramethyl ether of the quinone as orange-red needles, m.p. 241–242°. Methylation of the rhodocomatulin-derived quinone, using the method of Koller and Russ (dimethyl sulphate

[17] Brockmann, H., and Budde, G., *Chem. Ber.*, 1953, **86**, 342.
[18] Spruit, C. J. P., *Recl Trav. chim. Pays-Bas Belg.*, 1949, **68**, 325.
[19] Bloom, H., Briggs, L. H., and Cleverley, B., *J. chem. Soc.*, 1959, 178.
[20] Koller, G., and Russ, H., *Mh. Chem.*, 1937, **70**, 54.

and sodium hydroxide solution at 40–50°) afforded after several recrystallizations orange crystals, m.p. 242–244°, which were chromatographically heterogeneous. Purification on a magnesium carbonate column and recrystallization yielded yellow orange needles, m.p. 251·5–252·5°, analysing as $C_{14}H_5O_3(OCH_3)_3$. This derivative, which was sparingly soluble in cold sodium hydroxide solution, showed carbonyl bands at 1650 and 1625 cm^{-1}, and was further methylated (methyl sulphate/potassium carbonate) to give the tetramethoxyanthraquinone of m.p. 220–221·5°. Hence the "tetramethyl ether" described by Koller and Russ was apparently an impure specimen of 1-hydroxy-3,6,8-trimethoxyanthraquinone. Shibata[21] prepared 1,3,6,8-tetrahydroxyanthraquinone from emodic acid and described the tetraacetate as yellow needles, m.p. 195°, comparable with the melting point 197–197·5° reported by Koller and Russ. Our chromatographically pure tetrahydroxyquinone yielded the tetraacetate as pale yellow needles of significantly higher melting point, 204–205°. A recently reported unambiguous synthesis[22] of 1,3,6,8-tetramethoxyanthraquinone provided yellow needles, m.p. 220–221°, which did not depress the melting point of the product from the rhodocomatulin derivatives.

Subsequently Bullock *et al.*[23] obtained from Farbenfabriken Bayer A. G. a sample of synthetic 1,3,6,8-tetrahydroxyanthraquinone, prepared by an undisclosed route, and described properties for the quinone and its tetramethyl ether which approximate those described here.

By following the course of hydrobromic–acetic acid hydrolysis of rhodocomatulin dimethyl ether with the aid of paper chromatography, it was revealed that rhodocomatulin monomethyl ether identical with the natural pigment, and another quinone, were present after 2 min refluxing. However, after ½ hr, only the latter quinone and 1,3,6,8-tetrahydroxyanthraquinone were present. Although more conveniently prepared by another reaction (see below), the new quinone was isolated by chromatography on calcium sulphate or by preparative paper chromatography (butanol/ammonia system) as dark red needles, $C_{14}H_7O_5(OCH_3)$, m.p. 292–294°. The insolubility in aqueous sodium bicarbonate, the absorption maximum at 447 mμ, the carbonyl bands at 1658 and 1609 cm^{-1}, and the resistance to demethylation of this quinone confirmed its structure as 1,3,8-trihydroxy-6-methoxyanthraquinone, previously isolated only as an impure byproduct in the synthesis of 1,3,8-trihydroxyanthraquinone.[24]

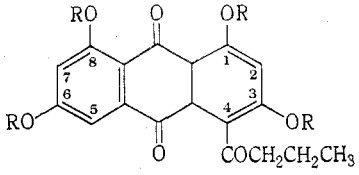
Another product of the hydrolysis proved to be n-butyric acid, identified by both gas and paper chromatography. Butyric acid was formed by heating rhodocomatulin monomethyl ether with water at 250–260°, 1,3,6,8-tetrahydroxyanthraquinone and its 6-methyl ether being also formed. The identification by paper chromatography of the steam-volatile acids from *C*-methyl determinations of the natural quinones as a mixture of butyric, propionic, and acetic acids confirmed the unbranched character of the side-chain.

Although mull spectra of the rhodocomatulin dimethyl and monomethyl ethers showed only two carbonyl stretching bands, both apparently of the anthra-

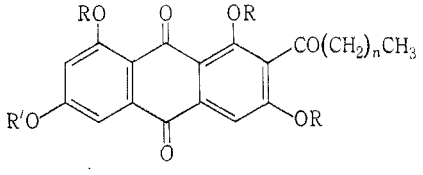
[21] Shibata, S., *J. pharm. Soc. Japan*, 1941, **61**, 103.
[22] Low, T. F., Park, R. J., Sutherland, M. D., and Vessey, I., *Aust. J. Chem.*, 1965, **18**, 182.
[23] Bullock, E., Kirkaldy, D., Roberts, J. C., and Underwood, J. G., *J. chem. Soc.*, 1963, 829.
[24] Eder, R., and Hanser, F., *Helv. chim. Acta*, 1925, **8**, 126.

quinone carbonyl type, the spectra of their respective di- and tri-acetates showed strong bands at 1695 and 1701 cm^{-1} lying outside the quinone and acetate carbonyl regions. Similar bands were found at 1695 cm^{-1} in the spectrum of rhodocomatulin tetramethyl ether and at 1693 cm^{-1} for the leucoacetate of rhodocomatulin dimethyl ether. These spectral data and the formation of n-butyric acid and 1,3,6,8-tetrahydroxyanthraquinone by acid hydrolysis are consistent with the presence of a *C*-butyryl side-chain. The preparation of a monooxime $C_{18}H_{12}O_4(OCH_3)_2(NOH)$, m.p. 225°, from rhodocomatulin dimethyl ether under conditions which usually exclude oximation of quinonoid groups supported this conclusion. Other ketone derivatives could not be obtained.

Rhodocomatulin tetramethyl ether was therefore formulated as (II; R = CH_3) or (III; R = R′ = CH_3, $n = 2$).



(II)



(III)

From initial studies of the methylation of the pigments and by analogy with the lichen pigment, solorinic acid (III; R = H, R′ = CH_3, $n = 4$), structure (III; $n = 2$) was tentatively preferred for the basic skeleton of the pigments. This incorrect structure must now be replaced by (II) (R = H) for rhodocomatulin on the basis of conclusive chemical evidence and the proton magnetic resonance spectrum.

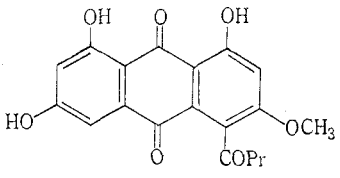
Oxidation of rhodocomatulin tetramethyl ether with alkaline permanganate yielded a tetramethoxyanthraquinone carboxylic acid, $C_{15}H_4O_4(OCH_3)_4$, m.p. 315–316° (dec.) (ν_{max} 1719, 1664, and 1638 cm^{-1}), which formed a methyl ester, $C_{14}H_3O_2(OCH_3)_4COOCH_3$, m.p. 283–284° ($\nu_{max}$ 1723 and 1652 cm^{-1}). The structure of this methyl ester has now been established by comparison with a synthetic specimen of 1,3,6,8-tetramethoxy-4-methoxycarbonylanthraquinone, m.p. 283–284°, prepared by Low *et al.*[22] The isomeric 2-methoxycarbonyl ester, m.p. 204°, has been synthesized by Matsuura and Ohta[25] and found identical with the oxidation product derived from fully methylated solorinic acid (III; R = R′ = CH_3, $n = 4$).[26]

The p.m.r. spectrum of rhodocomatulin tetramethyl ether (II; R = CH_3) exhibited the following features: (i) a triplet (J 7 c/s) centred on 8·95 τ and assigned to the methyl protons of the butyryl group, (ii) a broad sextet at 8·20 τ due to the β-methylene protons, (iii) a triplet (J 7·5 c/s) at 7·20 τ due to the α-methylene protons of the side-chain, (iv) three singlets at 6·01 (3H), 6·08 (3H), and 6·12 τ (6H) from the four methoxy groups, (v) an AB system of doublets (J 3 c/s) at 2·76 and 3·25 τ assigned to the *meta*-orientated α- and β-protons at C5 and C7, and (vi) a singlet (1H) at 3·20 τ which must be assigned to a β-proton. The field position of

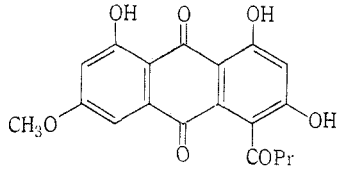
[25] Matsuura, S., and Ohta, K., *J. pharm. Soc. Japan*, 1962, **82**, 959.

[26] Anderson, H. A., Smith, J., Thomson, R. H., and Wells, J. W., *Bull. natn. Inst. Sci. India*, 1965, **28**, 46.

the latter is comparable with the singlets in the spectra of 1,3-dimethoxy-4-methoxycarbonylanthraquinone (3·20 τ) and 1,3,6,8-tetramethoxy-4-methoxycarbonylanthraquinone (3·30 τ), and is well supported by published data for similar protons. The uncoupled α-proton of solorinic acid trimethyl ether resonates at 2·56 τ.

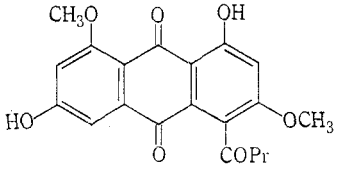


(IV)

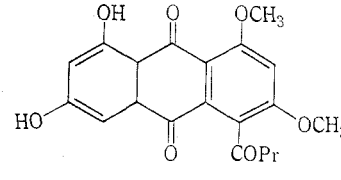


(V)

The alternatives (IV) and (V) for the natural rhodocomatulin monomethyl ether follow from the isolation of 1,3,8-trihydroxy-6-methoxyanthraquinone as a product of side-chain extrusion by pyrolysis, by hydrolysis with hydrobromic–acetic acid, or by alkaline dithionite treatment of the pigment followed by aerial oxidation of the resulting anthrahydroquinone (see below). In the choice between (IV) and (V), the apparent absence in Nujol mulls of β-hydroxyl bands between 3150 and 3650 cm^{-1} and of any side-chain carbonyl band near 1700 cm^{-1} and the presence of such bands at 3630 and 1698 cm^{-1} and at 3515 and 1702 cm^{-1} in tetrachloroethane and dioxan solutions respectively is significant. Since these solvents are stated to dissociate intermolecular, but not intramolecular, bonds[27] (V) appears to be excluded. The natural rhodocomatulin monomethyl ether would then be (IV) and the natural dimethyl ether would be (VI), since the latter yields the natural monomethyl ether as the first product of demethylation, and structure (VII) can be excluded because of the positive dithionite extrusion reaction shown by the natural dimethyl ether but not by the tetramethyl ether.



(VI)



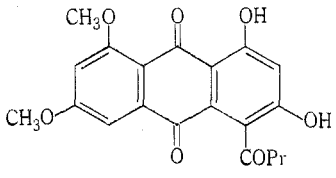
(VII)

However, the p.m.r. spectra of the acetates of the natural pigments are inconsistent with the acceptance of structures (IV) and (VI). The dimethyl ether diacetate shows an AB system (J 3 c/s) at 2·75 and 3·29 τ, comparable with that observed in rhodocomatulin tetramethyl ether (2·76 and 3·25 τ) and numerous other 1,3-dimethoxyanthraquinones. The di- and the tri-acetate show aromatic one-proton singlet resonances at 2·69 and 2·65 τ, these field positions being rather too low for β-protons flanked by one acetoxy and one methoxy group (*c.* 3·12 τ). The AB system of the monomethyl ether triacetate (2·47 and 3·13 τ) is closely modelled by that of the C2–H and the C4–H of 1-acetoxy-3,6,8-trimethoxyanthraquinone

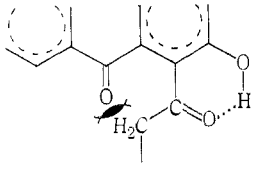
[27] Åkermark, B., *Acta chim. scand.*, 1961, **15**, 985.

(2·36 and 3·12 τ), whereas a 1,3-diacetoxy system would result in α- and β-proton resonances at about 2·05 and 2·75 τ (see Part VI of this series). Only by formulating natural rhodocomatulin monomethyl ether as (V) and the natural dimethyl ether as (VIII) can the observed p.m.r. spectra be rationalized.

This conclusion was confirmed chemically by oxidizing the natural dimethyl ether with alkaline permanganate to yield 3,5-dimethoxyphthalic acid, identified by its chromatographic behaviour and by a mixed melting point test of the derived anhydride with an authentic specimen.



(VIII)



(IX)

The appearance of the side-chain carbonyl and of β-hydroxy infrared bands in tetrachloroethane and dioxan solutions of (V) must be attributed to the presence in these solutions of a proportion at least of (V) in an unbonded state. This seems likely as a consequence of the interference between the α-methylene and the quinone oxygen (as in (IX)), forcing the side-chain carbonyl out of the plane of the aromatic ring and away from the hydroxy proton. There is thus no essential conflict between the infrared and the p.m.r. spectra as to the correctness of structures (V) and (VIII).

An attempt to reduce the side-chain keto group using a method which has proved successful with other phenolic ketones[28] led to the discovery of the useful dithionite extrusion reaction referred to above. Reduction of rhodocomatulin monomethyl ether by stirring with Raney nickel alloy in alkaline solution, and oxidation of the product with air, yielded 1,3,8-trihydroxy-6-methoxyanthraquinone, identical with the product obtained by a limited acid hydrolysis of either natural pigment. Reduction by excess alkaline dithionite solution in the absence of air and subsequent oxidation of the resulting anthrahydroquinone without isolation by the passage of an air stream, was found to give higher yields and proved convenient for the removal of the butyryl group without loss of *O*-methyl groups. The requirement of at least one phenolic group in the butyryl-substituted ring is evident from the failure of the tetramethyl ether and the 1,3,6-trimethyl ether to yield isolatable dithionite extrusion products.

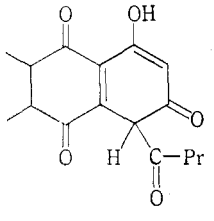
A rhodocomatulin dimethyl ether (m.p. 225–227°, ν_{max} 1698, 1670, and 1600 cm^{-1}) isomeric with the natural 6,8-dimethyl ether resulted from treatment of the 6-monomethyl ether with diazomethane in benzene or alcohol-free ether. Since α-hydroxyls are not readily methylated under these conditions, this is formulated as the 3,6-dimethyl ether. Dithionite extrusion yielded 1,8-dihydroxy-3,6-

[28] Papa, D., and Schwenck, E., *J. org. Chem.*, 1942, **1**, 587.

dimethoxyanthraquinone which was also obtained by the action of diazomethane in dry ether on 1,3,8-trihydroxy-6-methoxyanthraquinone; on the other hand, the use of diazomethane in ether/acetone solution resulted in the formation of two trimethyl ethers which were separable only with some difficulty. One trimethyl ether, m.p. 249–250° (ν_{max} 1697, 1664, and 1622 cm^{-1}), could be obtained also from the natural 6,8-dimethyl ether by the same reagent and is therefore the 3,6,8-trimethyl ether. The dithionite extrusion reaction yielded 1-hydroxy-3,6,8-trimethoxyanthraquinone described above from the methylation (methyl sulphate/sodium hydroxide) of 1,3,6,8-tetrahydroxyanthraquinone. The second trimethyl ether, m.p. 236·5–237·5° (ν_{max} 1700, 1664, and 1632 cm^{-1}), did not undergo dithionite extrusion and from the spectral data must be rhodocomatulin 1,3,6-trimethyl ether. The dithionite extrusion reaction applied to the naturally occurring rhodocomatulin 6,8-dimethyl ether yielded a new quinone which may be formulated as 1,3-dihydroxy-6,8-dimethoxyanthraquinone, m.p. 277–279° (ν_{max} 1662 and 1614 cm^{-1}).

It is interesting to compare the reactivities of rhodocomatulin 6-methyl ether (V) and solorinic acid (III; R′ = CH_3, R = H) with regard to their side-chain extrusion reactions.[26] Whereas (V) is smoothly converted into 1,3,8-trihydroxy-6-methoxyanthraquinone and thence into 1,3,6,8-tetrahydroxyanthraquinone by refluxing hydrobromic–acetic acid, solorinic acid yields no detectable 1,3,6,8-tetrahydroxyanthraquinone under these conditions. Thus presumably the side-chain, not having been eliminated, takes part in condensation reactions which lead to the observed black insoluble product. With hot hydriodic acid, solorinic acid loses the hexanoyl group probably from the anthrahydroquinone or the anthrone formed by reduction.[20]

Although the rhodocomatulin derivatives with phenolic groups at C1 readily undergo cleavage in alkaline dithionite at room temperature, solorinic acid survives and, even at 100°, a low yield only (12%) of the trihydroxymethoxyanthraquinone results.[26]



(X)

The elimination of butyric acid from rhodocomatulin derivatives under acidic conditions may result from a reversed Friedel–Crafts type of acylation reaction involving attack by a proton at C4. An alternative possibility would involve cleavage of the β-diketone resulting from ketonization at C3 and C4 as in (X).

The enhanced susceptibility of the rhodocomatulins to cleavage as compared with solorinic acid and typical acylresorcinols[28] is attributed basically to the existence of considerable steric strain in the planar hydrogen-bonded configuration (IX). This must promote the non-planar configuration which may be more susceptible to side-chain extrusion through ketonization, or may facilitate some other appropriate cleavage mechanism.

Biogenesis

The pattern of distribution of the hydroxyl groups in the rhodocomatulin pigments is typical of that displayed in other plant anthraquinones, particularly from moulds and fungi. Although the position of the n-butyryl side-chain is unusual,

the biogenesis is readily accommodated by the Birch polyketide condensation hypothesis, with subsequent oxidation and *O*-methylation. Solorinic acid provides an example of the biosynthesis of a closely related substance by a lichen.

As animal constituents, the rhodocomatulin pigments are unusual in two ways. Firstly the occurrence of *O*-methyl groups in animal metabolites has been demonstrated only recently in a few cases. Man has been shown to metabolize adrenalin to 4-hydroxy-3-methoxymandelic acid,[29] while rats and rabbits methylate the 3-hydroxyl of a number of catechol acids.[30] The important dimethoxybenzoquinone, ubiquinone, occurs widely in animal tissue. Of the marine invertebrates, the sponge, *Cryptotethia crypta*, is reported to contain the ribofuranoside of 6-amino-2-methoxypurine,[31] and a holothurian, *Polycheria rubescens*, is known to be coloured by a protein complex involving hexahydroxynaphthaquinone 6-methyl ether.[4,32] Secondly, anthraquinones occur rarely in animal tissue, the only other examples known being the Coccidae family of insects.

Three possibilities require consideration as the source of anthraquinones in crinoidal tissue. The pigments may accumulate in the tissue from the ingestion of anthraquinones in the plankton, detritus, or other food sources of the crinoids. However, there are as yet no reports of phytoplankton or marine algae containing anthraquinonoid derivatives. This hypothesis will be eliminated on other grounds in Part VI of this series. Again the presence of these pigments may result from the synthetic activities of symbiotic organisms, but these, if present, have not yet been recognized.[2] The third possibility, that the anthraquinone pigments are true metabolic products of crinoidal tissue, would simply be a further illustration of the unity of living matter.

Experimental

Melting points were determined in acid-washed Pyrex capillaries and are corrected. Electronic absorption spectra were measured with a Perkin–Elmer Spectracord 4000A, infrared spectra with a Perkin–Elmer 21 (sodium chloride optics), and p.m.r. spectra with a Varian A60 using deuterochloroform as solvent. The electronic spectra of all hydroxyanthraquinones were determined in 96% ethanol containing 1% v/v acetic acid to repress ionization. For other substances, 96% ethanol was used as solvent. Points of inflexion are shown in italics.

The colours reported below were described by reference to the useful colour charts provided with Mulliken's text.[33] The *C*-methyl determination by the procedure of Barthel and La Forge[34] using the apparatus of Clark[35] yielded acids which were identified by the paper chromatographic system of Lederer and Reid.[36]

Pigments of C. pectinata

(a) Extraction of Crude Pigment

After collection, the animals were stored in acetone. Three or four changes of solvent extracted most of the colouring matter. The combined extracts were filtered and then evaporated

[29] Axelrod, J., *Biochem. biophys. Acta*, 1951, **27**, 210.
[30] De Eds, F., Boot, A. N., and Jones, F. T., *J. biol. Chem.*, 1957, **225**, 615.
[31] Bergmann, W., and Burke, D. C., *J. org. Chem.*, 1956, **21**, 226.
[32] Mukai, T., *Bull. Soc. Chem. Japan*, 1960, **33**, 453, 1234.
[33] Mulliken, A., "A Method for the Identification of Pure Organic Compounds." 1st Edn, Vol. III. (Chapman & Hall: London 1911.)
[34] Barthel, W. F., and La Forge, F. B., *Ind. Engng Chem. analyt. Edn*, 1944, **16**, 434.
[35] Clark, E. P., *Ind. Engng Chem. analyt. Edn*, 1936, **8**, 437.
[36] Lederer, M., and Reid, R. L., *Biochem. J.*, 1951, **50**, 60.

to dryness. After drying to constant weight, the solid was extracted repeatedly with refluxing acetone, leaving a dark red sticky gum which was discarded. The acetone-soluble fraction, after removal of the solvent, was dissolved in 90% aqueous methanol which was equilibrated with hexane (2 volumes). Evaporation of the hypophase then gave "crude pigment" which consisted of dark red crystals imbedded in a sticky red-orange gum.

(*b*) *Adsorption Chromatography of Crude Pigment*

In a typical experiment (see Table 1), a solution of the crude pigment (1·07 g) in acetone (200 ml) was poured onto a magnesium carbonate column (33 cm by 4 cm). Careful development with aqueous acetone with stepwise increase in the water content to 30% over 9 days eluted five zones. Acidification of the various fractions followed by evaporation of the organic solvent gave readily filterable precipitates. The strongly adsorbed zones were recovered by extrusion and dissolution of the absorbent in dilute hydrochloric acid.

(*c*) *Comparison of Pigment Contents of Bright Red and Dark Red Specimens*

Two bright red crinoids (total dry weight 24·5 g) and two dark red crinoids (24·5 g) were separately extracted with acetone and aliquots were chromatographed on magnesium carbonate in the usual way. The ratio of pigment in bright red to dark red specimens was determined by measuring the optical density of the appropriate eluates as 0·8 for rhodocomatulin dimethyl ether, 1·1 for rhodocomatulin monomethyl ether, 1·6 for rubrocomatulin monomethyl ether, and 2·6 for the more highly adsorbed pigments. The "crude pigment" content amounted to 6·0% and 5·2% respectively.

(*d*) *Rhodocomatulin 6,8-Dimethyl Ether and Derivatives*

(i) *4-Butyryl-1,3-dihydroxy-6,8-dimethoxyanthraquinone.*—The pure *quinone* was obtained by recrystallization from acetone as large orange-yellow prisms or more usually as long interlocking needles, m.p. 208·5–209°. Heating at 150° in vacuum for 0·5 hr afforded a polymorphic form, m.p. 229·5–230·5°, which was readily converted into the lower melting form by recrystallization from acetone (Found: C, 64·4; H, 5·2; OCH_3, 16·3; *C*-CH_3, 7·3. $C_{20}H_{18}O_7$ requires C, 64·9; H, 4·9; 2×OCH_3, 16·7; 1×*C*-CH_3, 7·3%). Light absorption: λ_{max} *256*, *268*, 287, *310*, 361, 448 mμ (log ε 4·14, 4·22, 4·42, 3·95, 3·56, 3·93); ν_{max} 1668, 1621, 1588, 1548 (Nujol); 3700, 1697, 1639, 1605, 1560 (tetrachloroethane); 3519, 3450, 3150, 1700, *1666*, 1627, 1596, 1556 cm^{-1} (dioxan).

(ii) *1,3-Diacetoxy-4-butyryl-6,8-dimethoxyanthraquinone.*—A solution of the quinone (41 mg) in pyridine (0·5 ml) and acetic anhydride (1 ml) after standing overnight and pouring into water afforded a yellow precipitate (40 mg), m.p. 191–193°. Recrystallization from ethanol resulted in long bright yellow needles, m.p. 199·5–201° of the *diacetate*. Poorer yields resulted from using sodium acetate and concentrated sulphuric acid as catalysts (Found: C, 63·5; H, 5·0; OCH_3, 13·5; mol. wt., 458 (Signer). $C_{24}H_{22}O_9$ requires C, 63·4; H, 4·9; 2×OCH_3, 13·7%; mol. wt., 454). Light absorption: λ_{max} 243, 280, 334, 406 mμ (log ε 4·39, 4·34, 3·60, 3·69); ν_{max} 1773, 1695, 1657, 1596, 1557, 1542 cm^{-1} (Nujol). P.m.r. spectrum:* 2·69 (1H, s), 2·75 (1H, d, 3 c/s), 3·29 (1H, d, 3 c/s), 6·07 (3H, s), 6·11 (3H, s), 7·25 (2H, t, 7·5 c/s), 7·53 (3H, s), 7·73 (3H, s), 8·11 (2H, m) 8·92 τ (3H, t, 7 c/s).

(iii) *1,3,9,10-Tetraacetoxy-4-butyryl-6,8-dimethoxyanthracene.*—A solution of the diacetate (91·5 mg) together with sodium acetate (50 mg) and zinc dust (200 mg) in acetic anhydride (5 ml) was heated on the water-bath with occasional swirling for 7 hr and filtered while hot into water to give a pale orange-yellow precipitate (93·5 mg), m.p. 210–211° (dec.). An analytical sample of the *leucoacetate*, obtained as pale yellow rhombs from methanol, melted at 231–232° (dec.) (Found: C, 62·6; H, 5·4; OCH_3, 11·3. $C_{28}H_{28}O_{11}$ requires C, 62·2; H, 5·2; 2×OCH_3, 11·5%). Light absorption: λ_{max} 238, 273, *336*, 349, 368, *385*, 404, *425* mμ (log ε 4·44, 4·98, 3·31, 3·59, 3·76, 3·70, 3·78, 3·64); ν_{max} 1765, 1747, 1693, 1614, 1571, *1560* cm^{-1} (Nujol).

(iv) *Dimethanesulphonyl ester.*—A solution of the quinone (50 mg) and methanesulphonyl chloride (0·25 ml) in pyridine (3 ml) was allowed to stand for 3 days, then added to ethyl

* Spectra are listed as: chemical shift (proton number, multiplicity, coupling constant). s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

acetate (50 ml), and the solution washed with 0·5N sulphuric acid and with water. Evaporation of the solvent yielded a red solid (65·5 mg), which was purified by recrystallization from methanol, ethyl acetate, and finally acetic acid to yield the *methanesulphonate* as yellow-orange needles, m.p. 248–250° (dec., immersed at 220°) (Found: C, 50·4; H, 4·5; O, 33·6; S, 12·1. $C_{22}H_{22}O_{11}S_2$ requires C, 50·0; H, 4·2; O, 33·5; S, 12·2%).

(v) *2-Bromo-4-butyryl-1,3-dihydroxy-6,8-dimethoxyanthraquinone.*—Bromination[37] yielded two products as judged by chromatography on magnesium carbonate. The major *bromination product* (34 mg) from 57 mg of rhodocomatulin dimethyl ether was obtained from benzene/light petroleum as pale orange needles, m.p. 222·5–223·5° (dec.) (Found: C, 53·3; H, 4·1; Br, 17·5. $C_{20}H_{17}BrO_7$ requires C, 53·3; H, 3·8; Br, 17·8%).

(vi) *Oxime.*—This derivative was prepared following Koller and Russ[20] but purification was effected by chromatography on magnesium carbonate. The *oxime* was obtained as orange needles from methanol, m.p. 225° (dec. immersed at 162°) (Found: C, 61·8; H, 5·0; N, 3·8. $C_{20}H_{19}NO_7$ requires C, 62·4; H, 5·0; N, 3·6%).

(vii) *4-Butyryl-1,3,6,8-tetramethoxyanthraquinone.*—A solution of the quinone (31 mg) in acetone (50 ml) was refluxed with dimethyl sulphate (1 ml) and potassium carbonate (10 g) for 28 hr. The residue from the evaporation of the solution was shaken for 30 min with 1% sodium hydroxide solution (50 ml). The benzene-soluble fraction was filtered through a short magnesium oxide column from which a bright yellow zone was eluted by chloroform. Removal of the solvent gave a yellow solid (29·5 mg; 81% yield), m.p. 200–202°. Recrystallization from benzene yielded the *quinone* as yellow needles of m.p. 203·5–204°, or 211–212° if the sample had been maintained at about 150° for some minutes or if a molten sample had solidified at above 150° (Found, for a sample dried at 100°/10 mm: C, 68·5; H, 5·4; O, 25·7; OCH_3, 27·8; *C*-CH_3, 5·6. $C_{22}H_{22}O_7$,$\frac{1}{2}C_6H_6$ requires C, 68·7; H, 5·7; O, 25·6; 4×OCH_3, 28·4; 1×*C*-CH_3, 6·2%. Found, for a sample dried at 150°/10 mm: C, 66·0; H, 5·7; OCH_3, 30·7. $C_{22}H_{22}O_7$ requires C, 66·3; H, 5·6; 4×OCH_3, 31·2%). Light absorption: λ_{max} 224, 283, 342, 418 mμ ($\log \epsilon_{418}$ 3·80); ν_{max} 1695, 1662, 1647, 1597, 1559, 1555 cm^{-1} (Nujol).

(viii) *4-Butyryl-1-hydroxy-3,6,8-trimethoxyanthraquinone.*—Excess diazomethane (*c.* 150 mg) in ether was added to a solution of rhodocomatulin dimethyl ether (56·7 mg) in acetone. After standing 20 hr in the refrigerator, the solution was concentrated to yield the *quinone* as orange needles (46·5 mg), m.p. 249–250° from acetone or acetic acid (Found: C, 65·7; H, 5·4; OCH_3, 23·8. $C_{21}H_{20}O_7$ requires C, 65·6; H, 5·2; 3×OCH_3, 24·2%). Light absorption: λ_{max} 252, *270*, 288, *310*, 362, 444 mμ ($\log \epsilon$ 4·16, 4·23, 4·43, 3·95, 3·52, 3·95); ν_{max} 1697, 1664, 1622, 1594, 1549 cm^{-1} (Nujol). The same product was obtained by methylation of the quinone using diazomethane in benzene solution.

(*e*) *Rhodocomatulin 6-Methyl Ether and Derivatives*

(i) *4-Butyryl-1,3,8-trihydroxy-6-methoxyanthraquinone.*—The crude *quinone* was readily purified by recrystallization from methanol or ethanol, as small red orange prisms or needles, m.p. 250–252° (dec.) (Found: C, 64·1; H, 4·7; OCH_3, 8·6; *C*-CH_3, 6·5. $C_{19}H_{16}O_7$ requires C, 64·0; H, 4·5; 1×OCH_3, 8·7; 1×*C*-CH_3, 7·6%). Light absorption: λ_{max} 256, 263, 293, 317, 366, 456 mμ ($\log \epsilon$ 4·24, 4·25, 4·42, 3·96, 3·46, 4·05). ν_{max} 1669, 1627, 1592, 1550 (Nujol); 3630, 1698, 1638, 1605, 1560 ($C_2H_2Cl_4$); 3515, 3450, 3110, 1702, 1663, 1625, 1596, 1556 cm^{-1} (dioxan).

(ii) *1,3,8-Triacetoxy-4-butyryl-6-methoxyanthraquinone.*—The quinone (70 mg) and sodium acetate (500 mg) in acetic anhydride (5 ml) were heated on the water-bath for 4 hr. Pouring into water gave a yellow precipitate, purified by chromatography on calcium sulphate and by recrystallization from methanol to yield the *triacetate* as pale yellow needles, m.p. 194·5–196° (Found: C, 62·2; H, 4·7; OCH_3, 6·0. $C_{25}H_{22}O_{10}$ requires C, 62·2; H, 4·6; 1×OCH_3, 6·4%). Light absorption: λ_{max} 248, 274, 338, 365 mμ ($\log \epsilon$ 4·26, 4·52, 3·63, 3·61); ν_{max} 1756, 1701, *1661*, 1656, 1595, 1585, 1555 cm^{-1} (Nujol). P.m.r. spectrum: 2·47 (1H, d, 3 c/s), 2·65 (1H, s), 3·13 (1H, d, 3 c/s), 6·09 (3H, s), 7·30 (2H, t, 8 c/s), 7·59 (6H, s), 7·75 (3H, s), 8·14 (2H, m), 8·93 τ (3H, t, 7 c/s).

[37] Fries, K., and Schurmann, G., *Ber. dt. chem. Ges.*, 1919, **52**, 2185.

(iii) *4-Butyryl-1,3,6,8-tetramethoxyanthraquinone.*—Methylation by dimethyl sulphate/potassium carbonate in acetone gave a nearly quantitative yield of rhodocomatulin tetramethyl ether of identical m.p., mixed m.p., and infrared spectrum with the product described under (*d*)(vii).

(iv) *4-Butyryl-1,8-dihydroxy-3,6-dimethoxyanthraquinone.*—A suspension of rhodocomatulin monomethyl ether (76 mg) in benzene was treated with an excess of diazomethane (*c.* 200 mg) in sodium dried ether for 45 hr at room temperature. The residue from evaporation of the orange solution was chromatographed in chloroform on a magnesium carbonate column (13 by 2·3 cm). After two minor zones had been eluted, the major pink zone was extruded and dissolved in dilute acid to yield an orange precipitate (69 mg), m.p. 216–218°. The analytical sample of the *quinone*, purified by crystallization from methanol, ethyl acetate, and finally acetic acid, melted at 225–227° (Found: C, 64·8; H, 4·9; OCH_3, 16·3. $C_{20}H_{18}O_7$ requires C, 64·9; H, 4·9; $2\times OCH_3$, 16·7%). Light absorption: λ_{max} 256, 264, 294, *313*, 365, 448 mμ (log ε 4·25, 4·26, 4·38, 3·97, 3·45, 4·06); ν_{max} 1698, 1670, 1600, 1560 cm^{-1} (Nujol).

(v) *4-Butyryl-8-hydroxy-1,3,6-trimethoxyanthraquinone.*—A solution of rhodocomatulin monomethyl ether (87 mg) in acetone, treated with excess diazomethane (*c.* 150 mg) in ether, had deposited a yellow precipitate after standing for 24 hr. The evaporated reaction mixture was then chromatographed in benzene on a magnesium carbonate column. A major orange zone was eluted by benzene/chloroform (3 : 1), leaving a minor pink zone, probably of rhodocomatulin 3,6-dimethyl ether, at the top of the column. The resulting orange solid (84 mg), m.p. 214–218°, on repeated crystallization from methanol, afforded the *quinone* as yellow-orange needles (16 mg), m.p. 236·5–237·5°, which depressed the melting point of 4-butyryl-1-hydroxy-3,6,8-trimethoxyanthraquinone to 217–219° (Found: C, 65·7; H, 5·2; OCH_3, 24·2. $C_{21}H_{20}O_7$ requires C, 65·6; H, 5·4; $3\times OCH_3$, 24·2%). Light absorption: λ_{max} 251, *275*, 288, *310*, 365, 441 mμ (log ε 4·12, 4·31, 4·44, 3·95, 3·54, 3·98); ν_{max} 1700, 1664, 1632, 1582, 1540 cm^{-1} (Nujol).

From the mother liquor, 4-butyryl-1-hydroxy-3,6,8-trimethoxyanthraquinone (9·5 mg; m.p. 239–241°) was obtained. Admixture with an authentic sample raised the m.p. to 241–242·5° and comparison of the i.r. spectra confirmed the identity.

(*f*) *Acid Hydrolyses*

(i) Rhodocomatulin 6,8-dimethyl ether (22·8 mg) was suspended in constant-boiling hydrobromic acid (7 ml) in an ampoule which had been nitrogen-flushed and evacuated before sealing. After heating at 130–132° for 25 hr, cooling resulted in the deposition of bright red needles (16·5 mg). Chromatography on calcium sulphate separated 1,3,6,8-tetrahydroxyanthraquinone (9·5 mg; 57% yield), m.p. >360°, and its 6-methyl ether (5·5 mg; 31%), m.p. and mixed m.p. 290–292°. Steam distillation of the filtrate, after the addition of sodium hydroxide (2·4 g), gave a yield of 91·5% butyric acid (estimated by titration with standard barium hydroxide solution). The dried barium salts were esterified with n-butanol (55 mg) and *p*-toluenesulphonic acid. Gas chromatography of the resulting oil showed two peaks corresponding to butanol and to butyl butyrate, and distinct from butyl isobutyrate.

(ii) A solution of rhodocomatulin 6,8-dimethyl ether (143 mg) in a mixture of acetic acid (10 ml) and hydrobromic acid (10 ml; 48%) was refluxed for 16 hr. On cooling, the solution deposited dark red needles (89 mg), m.p. >360°. Chromatography on magnesium carbonate and elution with 10% water in acetone separated the major violet-red zone from a more strongly adsorbed black zone. Acidification and evaporation of the eluate afforded a bright red precipitate (81 mg), m.p. >360°. The analytical sample obtained by recrystallization from acetic acid formed fluffy red needles, m.p. >360° (Found: C, 61·2; H, 3·0; O, 35·0; OCH_3, 0. Calc. for $C_{14}H_8O_6$: C, 61·8; H, 3·0; O, 35·3%). Light absorption: λ_{max} 253, 262, *286*, 291, 318, 372, 452 mμ (log ε 4·17, 4·17, 4·43, 4·48, 3·96, 3·47, 4·01). ν_{max} 3210, 1669, 1624, 1608, 1583 cm^{-1} (Nujol).

(iii) A sealed ampoule containing a suspension of rhodocomatulin 6-methyl ether (22·7 mg) in freshly boiled distilled water (7 ml) was heated at 252–260° for 2·5 hr. After cooling, the resulting fine brown precipitate was removed by filtration. Distillation of the filtrate following the usual *O*-acetyl procedure indicated a 67% yield of butyric acid. Paper chromatography on

Whatman No. 1 paper using butanol saturated with 1·5N ammonium hydroxide gave only one spot at R_F 0·24, identical with that of butyric acid.

The acetone-soluble fraction from the brown solid, on paper chromatography using butanol saturated with concentrated ammonium hydroxide, yielded 1,3,6,8-tetrahydroxyanthraquinone, m.p. >360° (9·5 mg), and its 6-methyl ether, m.p. and mixed m.p. 291–293° (5·5 mg) (see (*g*)(vi)).

(*g*) *Derivatives of 1,3,6,8-Tetrahydroxyanthraquinone*

(i) *1,3,6,8-Tetraacetoxyanthraquinone.*—Acetylation with acetic anhydride–pyridine mixture yielded fine pale yellow needles, m.p. 204–205° from methanol and from isopropyl ether (Found: C, 59·7; H, 3·8; *O*-acetyl, 51·9. Calc. for $C_{22}H_{16}O_{10}$: C, 60·0; H, 3·7; 4×*O*-acetyl, 53·3%).

(ii) *1,3,6,8-Tetramethoxyanthraquinone.*—Reaction of the tetrahydroxyquinone (32 mg) with excess of dimethyl sulphate and potassium carbonate in refluxing acetone for 8 hr with subsequent chromatography on magnesium oxide yielded a yellow solid (30 mg), insoluble in boiling 10% sodium hydroxide solution. Recrystallization from methanol gave fine yellow needles, m.p. 220–221·5°. A similar treatment of the trimethyl ether (see (*g*)(iii)) led to the formation of the same product, m.p. and mixed m.p. 220–221·5° (Found: C, 65·9; H, 5·1; OCH_3, 36·7. Calc. for $C_{18}H_{16}O_6$: C, 65·8; H, 4·9; 4×OCH_3, 37·8%). Light absorption: λ_{max} 223, 280, *300*, 342, 411 mμ (log ε 4·60, 4·44, 3·97, 3·56, 3·73); ν_{max} 1651, 1596, 1559 cm^{-1} (Nujol). P.m.r. spectrum: 2·65 (2H, d, 2·5 c/s), 3·19 (2H, d, 2·5 c/s), and 6·05 τ (4×3H, s).

(iii) *1-Hydroxy-3,6,8-trimethoxyanthraquinone.*—(1) Dimethyl sulphate (18 ml) was added portionwise over 8·25 hr to a vigorously shaken solution of the tetrahydroxyanthraquinone (61 mg) in 10% sodium hydroxide (65 ml) at 40–60°. Acidification of the resulting red solution deposited an orange solid (78 mg), m.p. 222–225° (sintering at 188°). Recrystallization from various solvents yielded material of m.p. 242–244°. This material was then chromatographed on magnesium carbonate; elution with chloroform separated a faint yellow zone which was not examined further, a major orange zone which gave orange crystals (59 mg) and left two other zones coloured red and violet-red still adsorbed.

Recrystallization of the major component from ethyl acetate, ethanol/chloroform, and finally acetic acid afforded the *hydroxyquinone* as fine yellow orange crystals, m.p. 251·5–252·5° (see (*b*)) (Found: C, 64·9; H, 4·7; OCH_3, 30·3. $C_{17}H_{14}O_6$ requires C, 65·0; H, 4·5; 3×OCH_3, 29·6%). Light absorption: λ_{max} 249, 284, *305*, *370*, 438 mμ (log ε 4·12, 4·42, 3·98, 3·49, 3·91); ν_{max} 1650, 1625, 1594, 1553 cm^{-1} (Nujol).

(2) A suspension of rhodocomatulin 3,6,8-trimethyl ether (14 mg) in 1% sodium hydroxide solution (15 ml) was heated on the water-bath with excess sodium dithionite (68 mg) for 1 hr. Aerial oxidation and acidification yielded a brown solid. Chromatography of the benzene-soluble fraction on magnesium carbonate with subsequent development using 10% chloroform in benzene eluted an orange zone. Recrystallization of the product from methanol afforded yellow-orange crystals (6 mg), m.p. 256–258°. The mixed melting point of this material and that from (*g*)(iii)(1) was 256–257° (sintering 255°). The identity was confirmed by the close correspondence of the infrared spectra of Nujol mulls which showed no band in the 1700 cm^{-1} region.

(iv) *1,3-Dihydroxy-6,8-dimethoxyanthraquinone.*—Treatment of rhodocomatulin 6,8-dimethyl ether (67 mg) as in (*g*)(iii)(2) yielded an orange-red precipitate (57 mg), m.p. 274–276°. Recrystallization from methanol and from acetone gave the *dihydroxyquinone* as transparent dark red plates, m.p. 277–279° (Found: C, 63·9; H, 4·1; OCH_3, 20·5. $C_{16}H_{12}O_6$ requires C, 64·0; H, 4·0; 2×OCH_3, 20·7%). Light absorption: λ_{max} 249, *261*, 285, 303, 359, 440 mμ (log ε 4·14, 4·16, 4·45, 4·02, 3·56, 3·92); ν_{max} 3260, 1662, 1614, 1595, 1572 cm^{-1} (Nujol).

(v) *1,8-Dihydroxy-3,6-dimethoxyanthraquinone.*—(1) A suspension of 1,3,8-dihydroxy-6-methoxyanthraquinone (57 mg) in benzene was treated with excess diazomethane in alcohol-free ether at 5° for 24 hr. The resulting solution was treated with a few drops of acetic acid and then washed successively with 5% aqueous sodium carbonate and with 1N sodium hydroxide solution. The violet-red sodium hydroxide extract was then acidified and extracted with ethyl acetate, evaporation of which yielded orange crystals (39 mg), m.p. 234–236°. Recrystallization from

ethanol and from acetic acid afforded orange rhombs of *1,8-dihydroxy-3,6-dimethoxyanthraquinone*, m.p. 246–247° (Found: C, 64·0; H, 4·2; OCH_3, 20·8. $C_{16}H_{12}O_6$ requires C, 64·0; H, 4·0; $2\times OCH_3$, 20·7%). Light absorption: λ_{max} 252, 262, 288, *310*, *370*, 443 mμ; ν_{max} 1672, 1598, 1555 cm^{-1} (Nujol).

(2) A solution of rhodocomatulin 3,6-dimethyl ether (20 mg) and sodium dithionite (60 mg) in 10% aqueous sodium hydroxide was allowed to stand under hydrogen at room temperature for 45 hr. On bubbling air through the orange solution, the colour changed to violet. The product recovered by acidification and extraction was purified by chromatography on calcium sulphate, yielding orange crystals (13 mg), m.p. 236–238°. Recrystallization from methanol afforded orange needles, m.p. 240–242°. On admixture with 1,8-dihydroxy-3,6-dimethoxyanthraquinone (m.p. 246–247°), the melting point was raised to 242–244°.

(vi) *1,3,8-Trihydroxy-6-methoxyanthraquinone.*—A solution of rhodocomatulin 6-methyl ether (117 mg) in 1% aqueous sodium hydroxide was heated on the water-bath under an atmosphere of hydrogen in the presence of sodium dithionite (83 mg) for 15 min, the colour of the solution changing from violet-red through blood red to deep orange. After cooling, air was bubbled through the solution for 30 min, the colour becoming violet red. Acidification and boiling afforded an orange precipitate (91 mg), m.p. 276–278°. The analytical sample was recrystallized from methanol and from acetic acid to yield the *quinone* as dark red needles, m.p. 292–294° (Found: C, 62·7; H, 3·7; OCH_3, 11·5. $C_{15}H_{10}O_9$ requires C, 62·9; H, 3·5; $1\times OCH_3$, 10·8%). Light absorption: λ_{max} 252, 261, 290, *310*, 364, 447 mμ ($\log\epsilon$ 4·19, 4·20, 4·44, 3·97, 3·44, 4·03); ν_{max} 3310, 1658, 1609, 1564, *1539* cm^{-1} (Nujol).

Methylation with dimethyl sulphate/potassium carbonate in refluxing acetone yielded 1,3,6,8-tetramethoxyanthraquinone, m.p. and mixed m.p. 220–221·5°.

(*h*) *Oxidation of Rhodocomatulin Tetramethyl Ether*

A solution of potassium permanganate (317 mg) in water (30 ml) was added dropwise over 25 min to a vigorously stirred suspension of rhodocomatulin tetramethyl ether (203 mg) in 2N sodium hydroxide solution at 90°. Stirring was continued for a further 10 min after which the reaction mixture was cooled and saturated with sulphur dioxide.

Filtration through kieselguhr removed a fine yellow suspension but the filtrate required several extractions with pentanol to remove the remaining yellow colour. The precipitate partly dissolved in aqueous sodium bicarbonate and careful acidification of the hot solution yielded a readily filterable yellow precipitate (44 mg), m.p. 300–301°. Evaporation of the pentanol extracts gave a less pure sample of the acid (22 mg). Extraction of the kieselguhr pad with chloroform recovered unchanged starting material (65 mg).

The *acid* was extremely insoluble in most organic solvents, but the analytical sample was recrystallized from acetic acid as yellow needles, m.p. 315–316° (dec.) (Found: C, 61·4; H, 4·4; OCH_3, 31·8. $C_{19}H_{16}O_8$ requires C, 61·4; H, 4·3; $4\times OCH_3$, 33·3%). Light absorption: λ_{max} 412 mμ in acidified ethanol, 430 mμ in ethanolic sodium hydroxide; ν_{max} 1719, 1664, 1638, 1598, 1584, 1550 cm^{-1} (Nujol). Treatment with diazomethane gave the methyl ester as bright yellow needles, m.p. 286–288° (Found: C, 62·5; H, 4·8; OCH_3, 37·6. Calc. for $C_{20}H_{18}O_8$: C, 62·2; H, 4·7; $5\times OCH_3$, 40·2%). Light absorption: ν_{max} 1723, 1652, 1588, 1557 cm^{-1} (Nujol). P.m.r. spectrum: 2·76 (1H, d, 2·5 c/s), 3·30 (1H, s), 3·33 (1H, d, 2·5 c/s), 6·00 (6H, s), 6·08 (3H, s), 6·09 (3H, s), and 6·12 τ (3H, s). This ester showed no depression of melting point when mixed with synthetic 1,3,6,8-tetramethoxy-4-methoxycarbonylanthraquinone.[22]

(*i*) *Oxidation of Rhodocomatulin 6,8-Dimethyl Ether*

The quinone (82 mg) oxidized by the procedure of Mahmoodian and Stickings,[38] yielded no substituted phthalic acids. The neutral products however were further oxidized on the water-bath for 1 hr with an excess of permanganate in 1N sodium hydroxide. Recovery of the acidic products after the addition of excess bisulphite yielded a colourless solid (20 mg) with the blue fluorescence (u.v.) and the chromatographic mobility of 3,5-dimethoxyphthalic acid in butanol/

[38] Mahmoodian, A., and Stickings, C. E., *Biochem. J.*, 1964, **92**, 369.

ammonia on paper and in benzene/ethyl acetate/formic acid (15 : 30 : 0·45) on silica gel HF 254/360. Sublimation (140–150°/5 mm) of the crude product yielded a pale yellow crystalline solid, m.p. 150–151°, which did not depress the melting point of authentic 3,5-dimethoxyphthalic anhydride and showed an identical infrared spectrum.

(*j*) *Rubrocomatulin Monomethyl Ether*

This *quinone* crystallized as scarlet prisms, m.p. 298–299° (dec.) from acetic acid (Found: C, 61·2; H, 4·5; O, 34·2; OCH_3, 8·0. $C_{19}H_{16}O_8$ requires C, 61·3; H, 4·3; O, 34·4; $1 \times OCH_3$, 8·3%). Light absorption: λ_{max} 237, 261, 275, 292, 317, 370, *475*, *489*, 500, 523, 537 mμ (log ε 4·31, 4·41, 4·22, 3·90, 4·12, 3·26, 4·04, 4·12, 4·21, 4·06, 4·10); ν_{max} 1673, 1635, 1593 cm^{-1} (Nujol).

(*k*) *Rubrocomatulin Pentamethyl Ether*

A solution of the quinone (43 mg) in ethyl methyl ketone (25 ml) was refluxed for 25 hr with dimethyl sulphate (1 ml) and potassium carbonate (2 g). After evaporation of the solvent, the residue was treated with water and the mixture kept for 2 days. The benzene-soluble fraction was chromatographed on magnesium oxide whence a major yellow zone was eluted with chloroform. Evaporation of the solvent yielded the *quinone* as an orange oil (35 mg) which crystallized from methanol as orange-yellow needles, m.p. 152–153·5° and 214–215° (Found: C, 64·2; H, 5·7; OCH_3, 35·4. $C_{23}H_{24}O_8$ requires C, 64·5; H, 5·6; $5 \times OCH_3$, 36·2%). Light absorption: λ_{max} 226, *270*, 287, *380*, 419 mμ (log ε 4·58, 4·28, 4·37, 3·70, 3·88); ν_{max} 1682, 1660, 1644, 1584, 1550 cm^{-1} (Nujol).

(*l*) *Rubrocomatulin Monomethyl Ether Tetraacetate*

A solution of rubrocomatulin monomethyl ether (40 mg) in acetic anhydride (2·5 ml) containing triethylamine (3 drops) on standing at room temperature for 2 days changed from purple to pale yellow in colour and yielded a tan precipitate (42 mg) when the reaction mixture was poured into ice-water. Recrystallization from methanol gave the *acetate* as lemon yellow needles, m.p. 203–205° (dec.) (Found: C, 59·9; H, 4·5; OCH_3, 5·7. $C_{27}H_{24}O_{12}$ requires C, 60·0; H, 4·5; $1 \times OCH_3$, 5·7%). Light absorption: λ_{max} 254, 270, 360 mμ; ν_{max} 1756, *1702*, 1692, 1657, 1587, *1570*, *1548* cm^{-1} (Nujol).

Pigments of C. cratera

The extraction of the animals and the isolation of the anthraquinonoid pigments was carried out as described in (*a*) and (*b*) above. The three principal pigments of m.p. 229·5–230·5° (and 208–209°), 248–250° (dec.), and 290–292° (dec.) were identified by chromatographic mobilities, by mixed melting point tests with rhodocomatulin 6,8-dimethyl ether, rhodocomatulin 6-methyl ether, and rubrocomatulin monomethyl ether, and by conversion into either rhodocomatulin tetramethyl ether or rubrocomatulin pentamethyl ether on methylation. One minor component of *C. cratera* not present in *C. pectinata* was irreversibly adsorbed on magnesium carbonate and gave a brown spot (red-orange when dry) of R_F 0·07 in the butanol/ammonia system.

Acknowledgments

The authors are indebted to Miss A. M. Clark, of the British Museum of Natural History, for the identification of crinoid specimens, to various friends for assistance in collection of crinoids, to Mr E. C. M. Grigg for infrared spectra, to Professor J. C. Brand for providing a p.m.r. spectrum of rhodocomatulin tetramethyl ether, to the Australian Atomic Energy Commission for financial support for one of them (J.W.W.), and to Mr G. Lahey, for drawing their attention to *Comatula pectinata* as a source of pigments.