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Chemical defense of crinoids by polyketide sulphates

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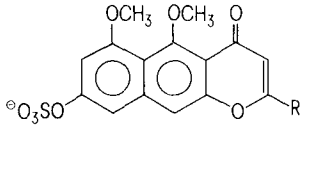
Summary. The range of (presumed) polyketide constituents in comatalid crinoids has been extended to include bianthronyls, meso-naphthodianthrones and sulphate esters of various of the polyketides. Several species of fish are deterred from eating food by the inclusion in it of these sulphates at the concentration found in crinoids. Sodium 2-hydroxy-anthraquinone sulphate and anthraquinone-2-suphonate were also active as antifeedants for the species tested.

Of 20 species of comatalid crinoids examined since 1950, at least 5 contain (presumed) polyketides as monoesters of sulphuric acid. Thus the isolation of **1** and **2** from *Comantheria perplexa* (H. L. Clark) and of **3, 4** and **5** from *Comanthus parvicirrus timorensis* (J. Müller) have been described[3].

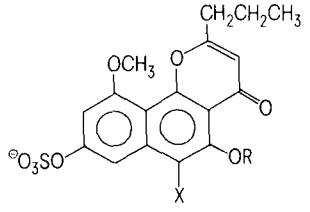
We have now obtained from *Comatula pectinata* (Linnaeus) the previously described[4] polyketide constituents in the form of their sulphate esters, **6, 7** and **8.** Another species, *Lamprometra palmata gyges* (Bell) has provided in addition to anthraquinones and meso-naphthodianthrones, sulphated derivatives of the bianthronyls **9** and **10,** all of which will be described in detail elsewhere. The concentration of the sulphates in these species ranges from c 2% (of wet weight) in *C. pectinata* to c 0.1% in *C. perplexa.* We have also observed various of the sulphates in *Comantheria briareus* (Bell).

Additional still undefined water-soluble components of other crinoids, which apparently hydrolyse to polyketides have been noted in the literature[5] and have also been observed by us in various species, including some which contain the common 3-alkylanthraquinone-type polyketides such as crinemodin[6] (**11**), rhodoptilometrin[7] (**12**) and ptilometric acid[7] (**13**).

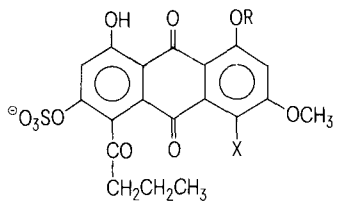
Polyketide sulfates are obtainable from some species of crinoids by immersing the animals directly from the sea



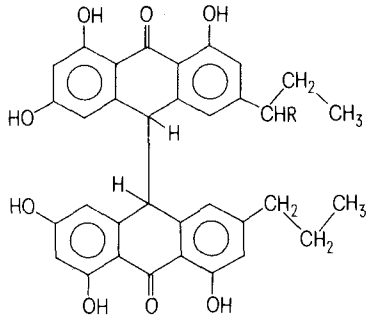
(1) R = CH_3
(2) R = $CH_2 \cdot CH_2 \cdot CH_3$



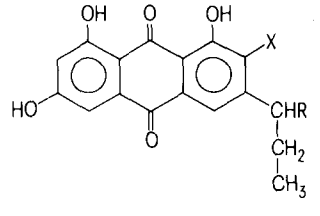
(3) P = X = H
(4) P = H, X = OCH_3 (5) R = CH_3, X = OCH_3



(6) R = X = H
(7) R = CH_3, X = H
(8) R = H, X = OH



(9) R = H
(10) R = OH



(11) R = X = H
(12) R = OH, X = H
(13) R = H, X = COOH

The inhibitory effect of crinoidal polyketide sulfates, etc. on feeding by fish

Test substance	Cation	Concentration of test substance in jelly	Species of fish					
			Gambusia affinis	*Selenotoca multifasciata*	*Monodactylus argenteus*	*Acantho-pagrus australis*	*Platax orbicularis*	*Trachurus mccullochi*
Mixed sulfates ex *C. pectinata*	Na^+	2%	+	+	+	+	+	+
Mixed sulfates ex *C. perplexa*	Na^+	0.2–2%	+	+	+			
Neocomantherin sulfate (synthetic)	Na^+	0.1%		+	+	+		
Anthraquinone-2-sulfonate	Na^+	2%	+	+*	+*			
2-Hydroxyanthraquinone sulfate	Na^+	2%	+	$\delta+$	$\delta+$		$\delta+$	
2-Hydroxynaphthalene sulfate	Na^+	2%	−					
Hexadecyl sulfate	K^+	2%	−					
Cholesteryl sulfate	K^+	2%	−					
Potassium sulfate	K^+	2%	−					
Rhodocomatulin 6-methyl ether	−	2% (as suspension)	$-\phi$					

+, Substantial preference for control sample; −, no statistically significant preference for either sample; $\delta+$, marginal preference for control sample; *, less effective than polyketide sulfates; ϕ, possibly (from 1 observation) a slow acting toxin.

into acetone, evaporating the acetone to yield an aqueous residue which is extracted with hexane and ether to remove fats and free polyketides. The aqueous residue is then saturated with sodium chloride which precipitates the sulfates almost completely, the remainder being extractable with butanol or butanone. The crude preparations can be stored at −20° indefinately. The sulphate 7 forms orange microcrystals, $C_{20}H_{17}SO_{10}Na$. 1.5 H_2O from water or methanol/ethyl acetate, decomposes without melting and shows solubilities, elemental composition, and UV, PMR and IR-spectra consistent with the proposed structure which has been confirmed by degradative studies also.

A feature of Indo-West-Pacific and Caribbean coral reefs is the sight of richly-coloured crinoids fully exposed on coral surfaces[8] in the presence of a variety of predatory fish species, although there are some other crinoid species which do inhabit cavities in the coral. Various surveys[9] of the stomach contents of reef fish have revealed that crinoidal fragments are typically absent. Echinoderm authorities[10] are agreed that crinoids in general are not subject to attack by fish[11]

The possibility that the sulphated polyketides might provide crinoids with a chemical defense mechanism by rendering them unpalatable to likely predators was first studied with the readily available fresh-water fish, *Gambusia affinis*. The effect of test substances on palatability was examined by offering to a group of fish a choice between 2 samples of prawn homogenate coloured with blood to minimise colour differences and set with gelatine around lead weights but differing in that 1 also contained the substance under test. Both samples were introduced simultaneously into the fish tank some 10–20 cm apart. The numbers of attacks on each sample were counted over an interval of some min after which the samples were interchanged and new counts started. Statistical analysis confirmed the obvious distaste of the fish for various of the test samples. Further, a much greater number of bitten-off fragments were dropped by the fish under some test samples as compared with control samples.

It was found that the polyketide sulphates of *Comatula pectinata* and *Comantheria perplexa* tested at the concentrations found in the crinoids, were very effective in reducing the palatability of otherwise attractive food to *Gambusia*. Also strongly inhibitory were the sodium salts of the sulphate ester of 2-hydroxyanthraquinone and of anthraquinone-2-sulfonic acid. The sulphates of β-naphthol, hexadecanol, cholesterol and potassium were ineffective.

5 marine fish species (table) were tested with results that confirmed the inhibitory activity of the natural sulphates from *C. pectinata* and *C. perplexa* and of anthraquinone-2-sulphonic acid. A sample of synthetic neocomantherin sulphate proved active at 0.1% concentration, demonstrating that the activity attributed to the natural sulphate is due to the polyketide sulphate and not to some impurity in the natural material.

The results presented, although only exploratory in scope, support the view that constituent sulfate esters of polyketides provide a chemical defense mechanism against fish for some species of crinoids.

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6 The trivial name crinemodin is suggested for **11** isolated from *Comanthus bennetti* by G.L. Bartolini, T.R. Erdman and P.J. Scheuer (Tetrahedron *29*, 3699 (1973)) and by us from *Ptilometra australis* (Wilton) and from many other crinoid species so that with rhodoptilometrin, it is one of the commonest crinoidal polyketides.

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9 e.g., J.E. Randall, Stud. trop. Oceanogr., Miami *5*, 665 (1967); R.W. Hiatt and D.W. Strasburg, Ecol. Monogr. *30*, 65 (1960); R.H. Lowe, J. Linn. Soc. London Zool.; *44*, 669 (1962).

10 For the views of H.L. Clark, A.H. Clark and T. Mortensen, see H.B. Fell in: Physiology of Echinodermata, p.53. Ed. R.A. Boolootian, Interscience, New York 1966.

11 Exceptions have been observed by Ms J. Marshall of the Australian Museum who has encountered a fish eating *Colobometra perspinosa* and Mr A. Birtles of the James Cook University of North Queensland who has witnessed attacks on *Amphimetra tessellata tessellata* (personal communications to J.A.R.).