Natural Products from Echinoderms

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1 Introduction

Over the past years, the chemistry of natural products from terrestrial organisms such as plants and fungi has received much attention,[1] resulting in the discovery of many exciting and useful molecules. In contrast, marine organisms have received relatively little attention. Some fascinating molecules, such as tetrodotoxin,[2,3] have already been found but, considering that only a small percentage of the approximately 500000 known marine species has been investigated,[4] one becomes aware of the vast area available for exploration by chemists.

This review will discuss the major recent developments in the chemistry of the radially symmetrical invertebrates from the phylum *Echinodermata* (echinoderms), one of the larger phyla of marine invertebrates.[5] The phylogeny of the echinoderms has recently been the subject of some discussion concerning the traditional division into two subphyla[6,7] and a newer proposal to divide the phylum into four subphyla.[8] In the former, the Pelmatozoa encompassed the class Crinoidea (sea lilies) and the Eleutherozoa encompassed the classes Asteroidea (sea stars), Ophiuroidea (brittle stars), Echinoidea (sea urchins) and Holothuroidea (sea cucumbers) (Figure 1). However, the new proposal would have the Echinozoa encompass sea urchins and sea cucumbers, the Homalozoa include the extinct carpoids, the Crinozoa the sea lilies, and then place the sea stars and brittle stars in an Asterozoa subphylum (see Figure 2). This review will be divided into different chemical sections, and these will be discussed in terms of the asteroids (sea stars), crinoids (sea lilies), echinoids (sea urchins), holothurians (sea cucumbers), and ophiuroids (brittle stars). In the conclusion, these discussions will be examined to compare them with suggestions[9,10] that chemical evidence should be used to assist in establishing phylogenetic relationships within the phylum.

[1] *Cf. Fortschr. Chem. org. Naturstoffe*, 1938—1970, **1—28**.
[2] R. B. Woodward and J. Z. Gougoutas, *J. Amer. Chem. Soc.*, 1964, **86**, 5030.
[3] P. J. Scheuer, *Fortschr. Chem. org. Naturstoffe*, 1969, **27**, 322.
[4] A. Der Marderosian, *Lloydia*, 1969, **32**, 438.
[5] L. H. Hyman, 'The Invertebrates', McGraw-Hill, New York, 1955, vol. 4.
[6] R. D. Barnes, 'Invertebrate Zoology', W. B. Saunders, Philadelphia, 1968, 2nd edn., p. 660.
[7] D. Nichols, 'Echinoderms', Hutchinson University Library, London, 1969, 4th edn., p. 159.
[8] H. B. Fell and D. L. Pawson in 'Physiology of Echinodermata', ed. R. A. Boolootian, Interscience, New York, 1966, p. 1.
[9] H. Singh, R. E. Moore, and P. J. Scheuer, *Experientia*, 1967, **23**, 624.
[10] H. I. Bolker, *Nature*, 1967, **213**, 904.

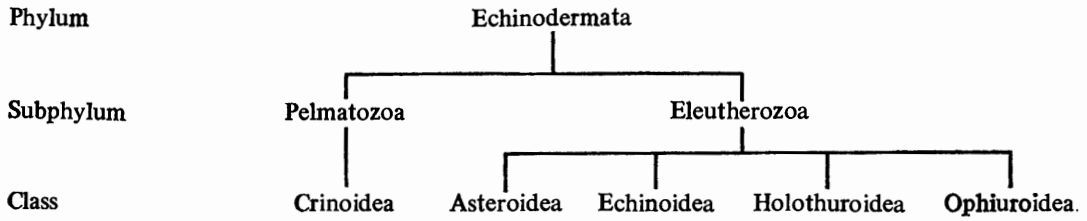


Figure 1 *Traditional Echinoderm phylogeny*

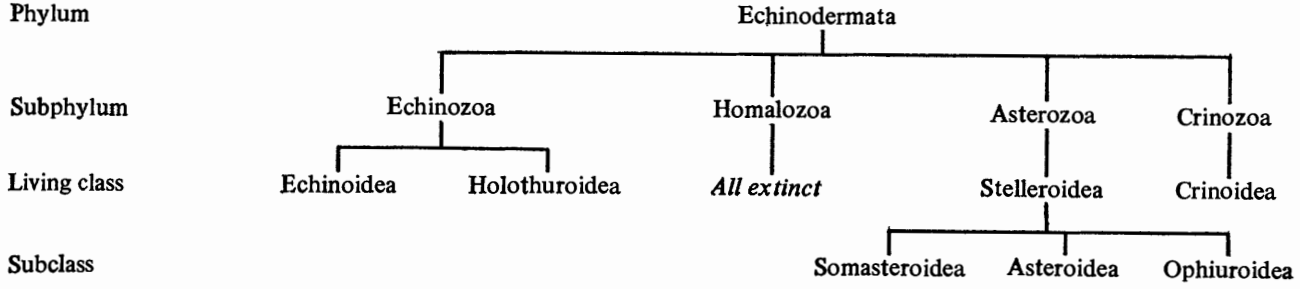


Figure 2 *Fell's proposal for Echinoderm phylogeny*

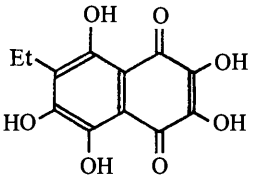
2 Naphthoquinone Pigments

A. Introduction.—Naphthoquinones have been found to occur widely in plants and micro-organisms but, in the animal kingdom, they are chiefly found in the echinoids.[11] The earlier work on naphthoquinones has been reviewed on several occasions,[11–13] and this section will discuss recent advances in the chemistry of these pigments from echinoderms. Much of the work in this area has come from Scheuer's laboratory in Hawaii.[9,14–26]

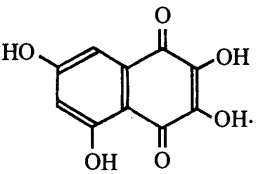
Until the mid-sixties, the chemical literature contained a large array of reports of naphthoquinone pigments from echinoids.[27] Rationalization of these reports began in 1964 with the publication of three notes,[14,15,28] which between them eliminated six supposedly different pigments. In fact, it began to appear possible that echinoids produced one or more of only six pigments, *viz.* echinochrome A (1) and spinochromes A-E (2)—(6).[14,15]

Of importance in the development of this work was the availability of spectroscopic methods, and the development of improved chromatographic techniques in place of the traditional calcium carbonate column chromatography. Of further importance was the use of mass spectrometry for molecular weight determination, because combustion analyses are often unreliable in this series of highly oxygenated molecules, which 'bristle' with solvent traps in the form of both hydrogen-bonded and free hydroxy-groups.

[11] R. H. Thomson in 'Comparative Biochemistry', ed. M. Florkin and H. S. Mason, Academic Press, New York, 1962, vol. 3, p. 686.
[12] R. H. Thomson, 'Naturally Occurring Quinones', Academic Press, London, 1971, 2nd edn., p. 257.
[13] D. L. Fox and T. S. Hopkins in 'Physiology of Echinodermata', ed. R. A. Boolootian, Interscience, New York, 1966, p. 277; H. Gwynne Vevers, *ibid.*, p. 267; T. W. Goodwin in 'Chemical Zoology', ed. M. Florkin and B. T. Scheer, Academic Press, New York, 1969, vol. 3, p. 135.
[14] C. W. J. Chang, R. E. Moore, and P J. Scheuer, *J. Amer. Chem. Soc.*, 1964, **86**, 2959.
[15] C. W. J. Chang, R. E. Moore, and P. J. Scheuer, *Tetrahedron Letters*, 1964, 3557.
[16] I. Singh, R. E. Moore, C. W. J. Chang, and P. J. Scheuer, *J. Amer. Chem. Soc.*, 1965, **87**, 4023.
[17] R. E. Moore and P. J. Scheuer, *J. Org. Chem.*, 1966, **31**, 3272.
[18] R. E. Moore, H. Singh, C. W. J. Chang, and P. J. Scheuer, *J. Org. Chem.*, 1966, **31**, 3638.
[19] R. E. Moore, H. Singh, and P. J. Scheuer, *J. Org. Chem.*, 1966, **31**, 3645.
[20] D. Becher, C. Djerassi, R. E. Moore, H. Singh, and P. J. Scheuer, *J. Org. Chem.*, 1966, **31**, 3650.
[21] L. H. Piette, M. Okamura, G. P. Rabold, R. T. Ogata, R. E. Moore, and P. J. Scheuer, *J. Phys. Chem.*, 1967, **71**, 29.
[22] R. E. Moore, H. Singh, C. W. J. Chang, and P. J. Scheuer, *Tetrahedron*, 1967, **23**, 3271.
[23] I. Singh, R. E. Moore, C. W. J. Chang, R. T. Ogata, and P. J. Scheuer, *Tetrahedron*, 1968, **24**, 2969.
[24] R. E. Moore, H. Singh, and P. J. Scheuer, *Tetrahedron Letters*, 1968, 4581.
[25] I. Singh, R. T. Ogata, R. E. Moore, C. W. J. Chang, and P. J. Scheuer, *Tetrahedron*, 1968, **24**, 6053.
[26] H. Singh, T. L. Folk, and P. J. Scheuer, *Tetrahedron*, 1969, **25**, 5301.
[27] For example, see C. Kuroda and M. Okajima, *Proc. Japan Acad.*, 1960, **36**, 424 and references cited therein.
[28] J. Gough and M. D. Sutherland, *Tetrahedron Letters*, 1964, 269.



(1)



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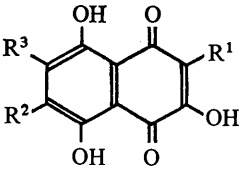
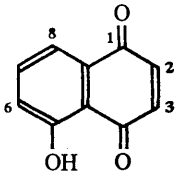
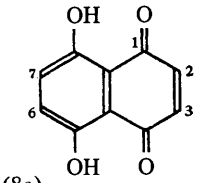


	R^1	R^2	R^3
(2)	Ac	OH	H
(4)	OH	Ac	OH
(5)	OH	OH	H
(6)	OH	OH	OH

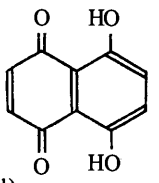
After the earlier rationalization of structures, it was somewhat of a shock when, unlike sea urchins that had heretofore been examined, two *Echinothrix* species were discovered to contain at least 30 different pigments.[19] These may be accounted for by considering the possible arrangements of ethyl, acetyl, and hydroxy-groups on either a juglone (7) or a naphthazarin (8a—d) skeleton; most arrangements now appear to have been found.



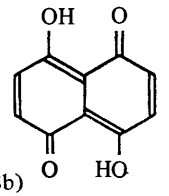
(7)



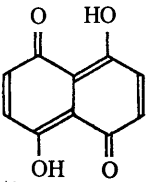
(8a)



(8d)



(8b)

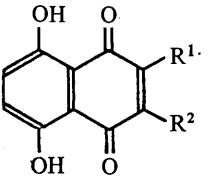


(8c)

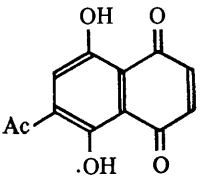
B. Physical Properties of Naphthoquinones.—The naphthazarin skeleton is actually a complex one, despite its apparently simple symmetry. This complexity manifests itself especially in substituted naphthazarins, *e.g.* is 2-hydroxynaphthazarin correct or is it 7-hydroxynaphthazarin, or a tautomeric mixture of several structures? This question was answered by Moore and Scheuer,[17] using detailed ^1H n.m.r. studies on a large number of substituted juglones and naphthazarins, both synthetic and natural. Comparison of the ^1H n.m.r. spectra of 1,4-naphthoquinone, juglone (7), and naphthazarin (8a—d) showed that the

single resonance exhibited by naphthazarin at δ 7·13 is intermediate between the resonances exhibited by the C-6 hydrogen (δ 7·25) and the quinone hydrogens (δ 6·97) of juglone. It is also between the resonances of the aromatic (δ 7·31) and the quinone (δ 6·75) protons in 5,8-dimethoxy-1,4-naphthoquinone. This nicely illustrates the rapid tautomerism of the naphthazarin system.

When naphthazarin is substituted at one of its hydrogen atoms, with an ethyl, acetoxy-, hydroxy-, or methoxy-group, then the principal tautomer in chloroform solution is (9).* This is based on the fact that the C-3 proton in (9) resonates at the same chemical shift as in 1,4-naphthoquinone, whereas the C-6 and C-7 protons have been shifted downfield to a chemical shift similar to those protons in juglone. In addition, when R = Et, the C-3 proton appears as a sharp triplet (J = 1·5 Hz), indicating allylic coupling across a fixed double bond. The signal is only a slightly broadened singlet when the double bond is delocalized, as in 2-ethylnaphthalene. When naphthazarin is substituted by an acetyl group then (10) is the best representation of the molecule.



(9) R^1 = OAc, OH, OMe, or Et;
R^2 = H



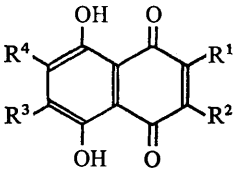
(10)

(11) R^1, R^2 = OAc, OH, OMe, or Et

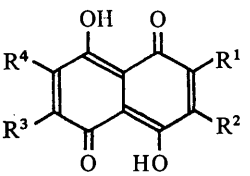
Using the same ^{1}H n.m.r. approach, Moore and Scheuer showed that for disubstitution, if the groups are acetoxy, ethyl, hydroxy, or methoxy, and are on the same ring, then (11) is the best representation of molecular structure, as might well be expected. However, if one group is an acetyl then several structures are possible, and the predominant one will depend on the 'quinoidal attraction' or 'aromatic attraction' of the other group. It appears that the groups studied have 'quinoidal attractions' in the order HO > MeO ≫ AcO > Et ≫ H ≫ Ac, which means that a 2-hydroxy-3-acetylnaphthazarin should be represented in solution by (12), 2-methoxy-3-acetylnaphthazarin by (13), 2-ethyl-3-acetylnaphthazarin by (14), 2,6-dimethoxynaphthazarin by (15), and 2,6-diacetylnaphthazarin by (16), *etc.*

Obviously, other situations will exist in the case of tri- and tetra-substitution, and Moore and Scheuer discuss these in detail.[17] They were thus able to propose correlations of the chemical shifts of acetyl, ethyl, methoxy, and acetoxy sub-

*For a novel chemical demonstration of this point, see S. Alvarado, F. Fariña, and J. L. Martin, *Tetrahedron Letters*, 1970, 3377.



	R^1	R^2	R^3	R^4
(12)	OH	Ac	H	H
(14)	H	H	Ac	Et



	R^1	R^2	R^3	R^4
(13)	OMe	Ac	H	H
(15)	OMe	H	OMe	H
(16)	H	Ac	H	Ac

stituents with their positions on the ring, and then show how these may be used to determine the substitution patterns of new pigments.

The availability of a wide range of samples also permitted Scheuer and his group to study the u.v. spectra of naphthoquinones.[25] Independently, Thomson[29] has published a list of pigments and their electronic spectra, although without comment. As with their n.m.r. studies, Scheuer's group discussed the spectra of 1,4-naphthoquinone, juglones, and naphthazarins in detail, and assigned bands in the 240—600 nm range to either benzenoid or quinonoid electronic excitations. These correlations should be useful in corroborating structural assignments of new pigments.

Because of the minute quantities required, mass spectrometry has the potential to open a new dimension in the study of naphthoquinone pigments. It should be possible to determine pigment structures in many cases using the material obtained from relatively few specimens if it is processed by analytical t.l.c., and then analysed by u.v. spectroscopy* as well as mass spectrometry. In order to correlate mass spectra with pigment structures, Djerassi and Scheuer[20] studied the electron-impact fragmentations of a considerable number of natural and synthetic naphthoquinones. They concluded that the acetyl function exerts a powerful effect on fragmentation direction, and even overshadows hydroxy- or methoxy-groups. Furthermore, hydrogen bonding between adjacent acetyl and hydroxy-groups plays a noticeable role in favouring ring breakdown rather than fission of the acetyl group. However, if the acetyl group is on the benzenoid portion of the molecule, loss of methyl, followed by loss of CO is the favoured fragmentation pathway.

In a continuing quest for sensitive physical methods to handle both the subtle differences in structure and the minute quantities of the natural naphthoquinone pigments, Piette and Scheuer investigated the potential of e.s.r. spectroscopy[21] in which 1,4-naphthoquinones were polarographically reduced to radical species. A gain of 10^2 in sensitivity over n.m.r. techniques was achieved by the use of e.s.r., and a rough correlation could be made between spin coupling constant

*This has been done by Thomson's group.[29]

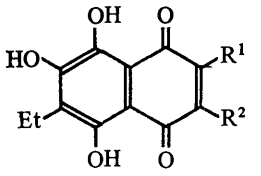
[29] H. A. Anderson, J. W. Mathieson, and R. H. Thomson, *Comp. Biochem. Physiol.*, 1969 **28**, 333.

and the quinoidal character of the ring protons. The study of a large number of molecules revealed marked lineshape sensitivity to changes in substitution or steric modifications of the molecule, but no further work on this approach has appeared.

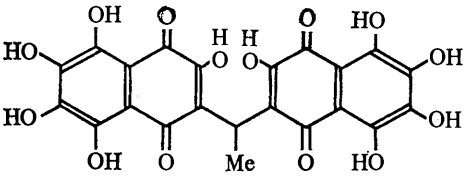
C. Isolation Techniques and Distribution of Naphthoquinones.—As mentioned previously, modern chromatographic techniques have been responsible for much of the rapid development in this area since 1964. The universally used extraction technique[14,19,29–32] is to digest the echinoid shell and/or spines with concentrated hydrochloric acid to dissolve the calcite material. After filtration, which may take several hours,[19] the crude pigments are eventually obtained in an ether solution. Partial separation of the crude pigments on silica gel deactivated by dilute hydrochloric acid was pioneered by the Hawaiian workers,[14,18] although Thomson has used cellulose powder as well as acid-washed silica gel.[32] More-precise separations have been carried out by preparative thick-layer chromatography using deactivated silica gel[19] or cellulose,[32] or by preparative paper chromatography.[31]

Despite much progress in the techniques of analysis of the spinochromes from echinoderms, new problems have become apparent. It has been the practice of the Hawaiian workers to methylate the more reactive hydroxy-groups in partially purified spinochrome mixtures, which enabled much more efficient separations to be achieved. The same group had also developed demethylation procedures and could thus study what they hoped to be the original pigments.[22] This was done on the grounds that no methoxylated spinochromes were known to be present in sea urchins, and in any case methylated alcohols or phenols occur rarely in animals,[9,33] although it was stated by Scheuer that 'these apparently clear-cut reactions, methylation and demethylation, are not without ambiguity nor without intrinsic interest'.[22] This is especially true in the light of the fact that a methoxylated spinochrome had been isolated from a sea cucumber,[34] and Scheuer himself had isolated a 3,7-dimethoxyspinochrome from a sea star.[9,22] In addition, Thomson has now isolated a pair of monomethoxylated spinochromes, (17) and (18), from the Caribbean sea urchin *Diadema antillarum* (Philippi).[29,32] The discovery of two dimeric naphthoquinone pigments [*cf.* (19)] which are unstable on acid-treated silica gel, but are stable in cold concentrated HCl,[32] indicates that care needs to be taken in the handling of these pigments. It is noteworthy that no 2,3-dimethoxynaphthoquinones have yet been reported from the spines of echinoids. If such compounds are indeed present, they may well be monodemethylated during the relatively slow initial HCl digestion of the calcareous material, in view of the rapidity with which this reaction is reported

[30] F. Fariña and W. Heimlich, *Anales de Quím.*, 1969, **65B**, 713.
[31] J. H. Gough and M. D. Sutherland, *Austral. J. Chem.*, 1967, **20**, 1693.
[32] J. W. Mathieson and R. H. Thomson, *J. Chem. Soc.* (*C*), 1971, 153.
[33] W. Bergmann and M. F. Stempien, jun., *J. Org. Chem.*, 1957, **22**, 1575.
[34] M. Yamaguchi, T. Mukai, and T. Tsumaki, *Mem. Fac. Sci. Kyushu Univ., Ser. C.*, 1961 **4**, 193 (*cf. Chem. Abs.*, 1963, **58**, 4486); T. Mukai, *Bull. Chem. Soc. Japan*, 1960, **33**, 453, 1234.



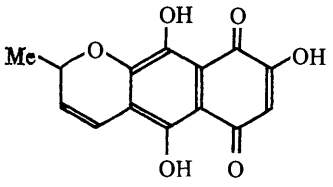
(17) R^1 = OH; R^2 = OMe
(18) R^1 = OMe; R^2 = OH



(19)

to occur in refluxing ethanol–hydrochloric acid[22] (see Section 2D). There does not appear to be a simple solution to this problem.

One other new type of spinochrome skeleton (20) has now been described; it was isolated from the mixture of more than 30 pigments Scheuer found in *Echinothrix diadema* Linn.,[24] which has also been found to include (21), the first benzoquinone in a marine invertebrate.[19] The structure of (20) was deduced by standard spectroscopic techniques.



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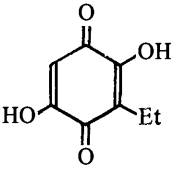


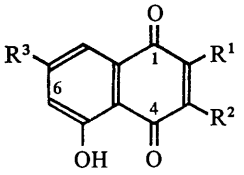
(21)

The distribution of naphthoquinone pigments has recently been comprehensively reviewed by Thomson.[12,29] Suffice it to add that these pigments appear to be widely distributed in sea urchins of the Atlantic and Pacific Oceans, both north and south[30] of the equator. Little work has been done on their existence in other echinoderms.[9,34]

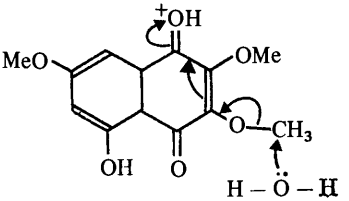
D. Reactions of Naphthoquinone Pigments.—The reactions of echinoid naphthoquinone pigments may be classed either as relatively simple or as very complex. In the first category are the methylations,[22] demethylations,[22] and deacetylations;[31] in the second category is what one would normally assume to be an apparently ordinary sodium borohydride reduction reaction, but which on spinochrome A (2) yields an array of at least 11 products.[18]

Reactions of the first category are primarily controlled by the number of hydrogen-bonding hydroxy–carbonyl interactions. The quinonoid hydroxy-groups are generally more reactive than the phenolic hydroxy-groups. For example, 2,3,7-trihydroxyjuglone [spinochrome B, (3)] reacts rapidly with diazomethane to give the 2,3-dimethoxy-compound (22), which further reacts

slowly with an excess of diazomethane to give (23). Reaction of (23) with hot ethanolic HCl for two minutes gives 90% of (24), presumably *via* (25). Prolonged further hydrolysis of (24) gives (26) in poor yield,[22] owing to the greater difficulty in protonating the bonded carbonyl at C-4.

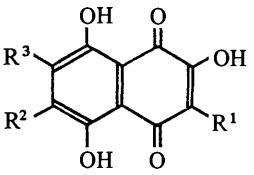


	R^1	R^2	R^3
(22)	OMe	OMe	OH
(23)	OMe	OMe	OMe
(24)	OMe	OH	OMe
(26)	OH	OH	OMe



(25)

The sodium borohydride reduction of spinochrome A (2) is of interest.[18] The purpose of the experiment was an attempt to characterize the pigment spinochrome P which had been reported to occur in the spines of the Mediterranean sea urchin, *Paracentrotus lividus* Lam., and to have structure (27). It was hoped to reduce (2) to (28), which would in fact have the properties ascribed to spinochrome P. Using a slight excess of sodium borohydride, it was found that the



	R^1	R^2	R^3
(27)	OH	MeCHOH	H
(28)	MeCHOH	H	OH

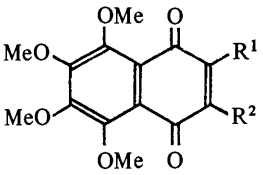
quinone system was readily reduced, and could then be quantitatively air-oxidized back to spinochrome A. With a large excess of borohydride, the product isolated was not (28), but a complex mixture which was eventually analysed and shown to contain at least 11 components, among which were four naphthazarins and four juglones, each with the acetyl group reduced to ethyl, as well as one naphthazarin and two juglones with intact acetyl groups. These molecules had also lost no, one, or two hydroxy-groups. No compounds containing the hydroxyethyl group were found, and it appears that the ready hydrogenolysis of the hydroxyethyl side-chain and the ring hydroxy-groups are competing

reactions and explicable on the basis of hydrogen-bonding assistance from vicinal hydroxy-groups and ready tautomerism of the naphthoquinone ketones. Part of this mechanistic proposal was originally made by Thomson from studies on the reductive removal of hydroxy-groups in naphthoquinones using Sn^{II} salts.[35] However, the details of these interesting reductive cleavages remain to be resolved. One useful discovery from this reaction, however, was the fact that quite a number of products were naturally occurring pigments in two Hawaiian *Echinothrix* species, *viz.*, *E. diadema* Linn. and *E. calamaris* Pallis.

E. Syntheses of Naphthoquinone Pigments.—The synthesis of these pigments is a relatively simple problem in that it is entirely structural and devoid of stereochemical intricacies. The synthesis may be considered to consist of two parts, *viz.* the construction of the skeleton and the introduction of the appropriate functional groups.

Skeletal construction has been achieved in several ways. The Zahn–Ochwat reaction,[36] which involves the condensation of an appropriately substituted 1,4-dihydroxy- or 1,4-dialkoxy-benzene with an appropriately substituted maleic anhydride in a 1 : 2 NaCl–$AlCl_3$ melt, has been used by both Wallenfels[37] and Scheuer[23] for this purpose. Another skeletal synthesis has been achieved by Thomson[38] by condensation of 3,4,5,6-tetramethoxyphthalaldehyde with glyoxal to yield the methylated naphthazarin (29), which could be demethylated to give spinochrome E (6).

Introduction of hydroxy-groups into the juglone (7) or naphthazarin (8) skeletons has been accomplished in numerous ways,[23,26,35] including the Thiele reaction (*cf.* ref. 36, p. 484). Acetyl groups have primarily been introduced by Fries rearrangements (*cf.* ref. 36, p. 28) on the reductive acetylation products



(29) $R^1 = R^2 = OH$
(30) $R^1 = OH$; $R^2 = H$

(leucoacetates) of the quinones.[23,26,39] Hydrolysis and air oxidation regenerate the appropriate quinone system. Finally, introduction of an ethyl group is

[35] J. F. Garden and R. H. Thomson, *J. Chem. Soc.*, 1957, 2483.
[36] For a discussion on this reaction, see L. F. Fieser and M. Fieser, 'Reagents for Organic Synthesis', Wiley, New York, 1967, p. 1027.
[37] K. Wallenfels and A. Gauhe, *Chem. Ber.*, 1943, **76**, 325.
[38] H. A. Anderson and R. H. Thomson, *J. Chem. Soc.* (*C*), 1966, 426.
[39] J. H. Gough and M. D. Sutherland, *Austral. J. Chem.*, 1970, **23**, 1839.

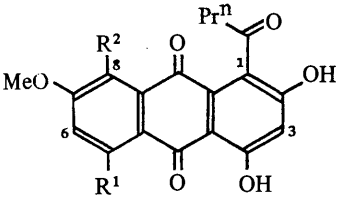
required for the synthesis of echinochrome A (1) and several of the pigments isolated from two Hawaiian *Echinothrix* species.[19] Although the sodium borohydride reduction of spinochrome A (2) gave products with ethyl groups,[18] this is not an ideal reaction for synthetic work owing to low yields and the difficulty of obtaining starting material. However, greater success was achieved on simpler molecules, such as 6-acetyl-2,3-dihydroxyjuglone which gave the corresponding 6-ethyl compound in 41% yield.[26] The introduction of the ethyl group was achieved in a mechanistically interesting synthesis of echinochrome A (1) by Thomson.[40] This involves the oxidation of the enolate anion of 5,6,7,8-tetramethoxy-1-tetralone with molecular oxygen to give (30). The ethyl group was then ingeniously introduced by heating (30) with dipropionyl peroxide in acetic acid, in a reaction which proceeds *via* intermediate ethyl radicals.[41]

3 Other Pigments

A. Anthraquinones.—Six anthraquinone pigments from Australian crinoids have been characterized by Sutherland.[42–44] Superficially, the crinoids or sea lilies appear to be 'plants' but are indeed living echinoderms, often brightly coloured. Until this work, no serious chemical studies on pigments of living crinoids had appeared.

The pigments were extracted simply by immersing the freshly caught animal in acetone. Purification by solvent partition, followed by chromatography on magnesium carbonate and multiple recrystallization gave the pure pigments.[43]

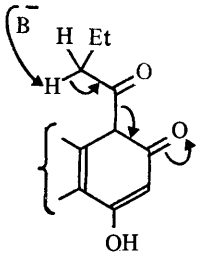
Both free-swimming crinoids, *Comatula pectinata* L., and *C. cratera* A. H. Clark, gave rhodocomatulin 6-methyl (31) and 6,8-dimethyl ethers (32) and rubrocomatulin 7-methyl ether (33) as major pigments. An interesting reaction of the rhodocomatulin molecule is the ready loss of the butyryl side-chain under acidic or basic conditions, provided that at least one hydroxy-group is present



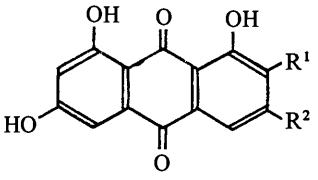
(31) R^1 = OH; R^2 = H
(32) R^1 = OMe; R^2 = H
(33) R^1 = OH; R^2 = OH

[40] H. A. Anderson, J. Smith, and R. H. Thomson, *J. Chem. Soc.*, 1965, 2141.
[41] For a discussion on the mechanism of this reaction, see E. S. Gould, 'Mechanism and Structure in Organic Chemistry', Henry Holt, New York, 1959, p. 717.
[42] M. D. Sutherland and J. W. Wells, *Austral. J. Chem.*, 1967, **20**, 515.
[43] V. H. Powell, M. D. Sutherland, and J. W. Wells, *Austral. J. Chem.*, 1967 **20**, 535.
[44] V. H. Powell and M. D. Sutherland, *Austral. J. Chem.*, 1967, **20**, 541.

on the ring bearing the butyryl group. This is evidently due to steric hindrance between the C-10 carbonyl and the butyryl group, which is forced out of the plane of the ring, and to the possibility of ketonization of the hydroxy-group, followed by cleavage of the resulting β-diketone system, as in (34). Three other anthraquinones, rhodoptilometrin (35), isorhodoptilometrin (36) and ptilometric acid (37) were isolated from *Ptilometra australis* Wilton, whereas (37) was the major pigment in both yellow and black specimens of *Tropiometra afra* Hartlaub. All these anthraquinones, except (35), obey the Birch polyketide rule and all appear to be endogenous in nature.[44]



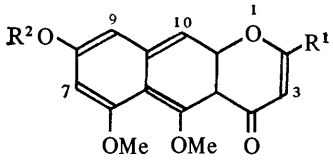
(34)



(35) $R^1 = H$; $R^2 = (S)$–CHOH Et
(36) $R^1 = H$; $R^2 = CH_2CHOH$ Me
(37) $R^1 = COOH$; $R^2 = Pr^n$

B. Naphthopyrones.—Sutherland has also reported the isolation of naphthopyrones from two previously unexamined species.[45,46]

The mustard-yellow crinoid, *Comantheria perplexa* H. L. Clark, gave a mixture of two water-soluble *O*-sulphates which were shown to be the linear naphthopyrones (38) and (39). Mild acidic hydrolysis gave the substituted 4*H*-naphtho-[2,3-*b*]pyran-4-ones, comantherin (40), and neocomantherin (41), together with a small amount of 5-demethylcomantherin. Refluxing (40) for 1 min in

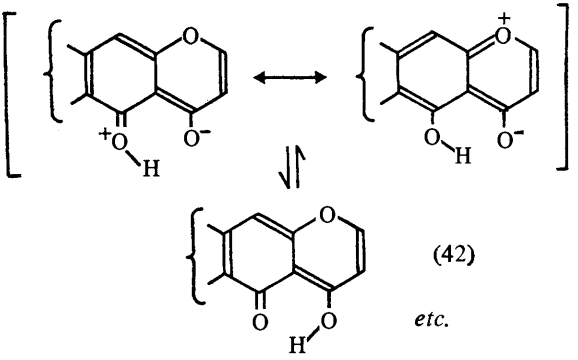


(38) $R^1 = Me$; $R^2 = SO_3^-$
(39) $R^1 = Pr^n$; $R^2 = SO_3^-$
(40) $R^1 = Me$; $R^2 = H$
(41) $R^1 = Pr^n$; $R^2 = H$

HBr–HOAc caused demethylation at C-5, presumably assisted by resonance stabilization of the protonated C-4 ketone, similarly to (25). Also, the C-5

[45] R. A. Kent, I. R. Smith, and M. D. Sutherland, *Austral. J. Chem.*, 1970, **23**, 2325.
[46] I. R. Smith and M. D. Sutherland, *Austral. J. Chem.*, 1971, **24**, 1487.

hydroxy-group of these naphthopyrones is readily methylated by diazomethane, in direct contrast to apparently similar hydroxy-groups in juglone (7) or naphthazarin (8) which do not react with diazomethane.[22] This may be rationalized in terms of resonance participation by the heterocyclic oxygen which increases the acidity of the C-5 hydroxy-group, as depicted in (42).



(42)

etc.

More recently, Sutherland[46] has isolated three angular naphthopyrone *O*-sulphates from the crinoid *Comanthus parvicirrus timorensis* J. Müller.

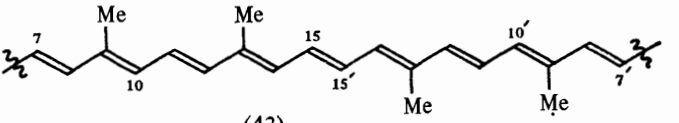
C. Porphyrins and Melanins.—Porphyrins and melanins have been detected in various species of echinoderms,[13] but little detailed chemical work has been reported.

D. Carotenoids.—The carotenoids constitute, in addition to the naphthoquinones, a major source of pigmentation in the echinoderms. The carotenoids as a group have recently been excellently reviewed,[47] and specialist reports on echinoderm pigments[13] have been published. All classes of echinoderm contain carotenoids,[13] which may be either 'carotenes' or 'xanthophylls'.[47] In the asteroids, alcoholic xanthophylls are often bound to integumentary proteins, differences between which probably account for the wide variations in colours often observed among different specimens from the same species of asteroid.

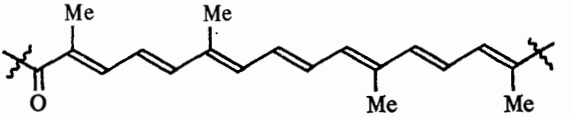
Recent work on echinoderm carotenoids has originated from four laboratories. The holothurian, *Psolus fabrichii*, Duben and Koren, is observably red even at depths of 70—90 feet, by which depth most other species have little colouring.[48] Preparative t.l.c. gave ten bands, workable quantities being obtained from all fractions except the second least polar (fraction 2). Fraction 1 was a carotene, *very similar* to β-carotene (43bb); fractions 3 and 7 appeared to be keto-carotenoids; fractions 4 and 6 appeared to be a diester and a monoester

[47] J. B. Davis in 'Rodd's Chemistry of Carbon Compounds', ed. S. Coffey, Elsevier, Amsterdam, 1968, 2nd edn., vol. 2B, chap. 7.
[48] E. Bullock and C. J. Dawson, *Comp. Biochem. Physiol.*, 1970, **34**, 799.

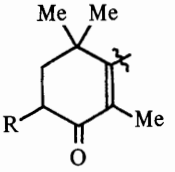
respectively of astaxanthin (43aa); fraction 5 was echinenone (43bc); fraction 8, canthaxanthin (43cc); fraction 9, phoenicoxanthin (43ac), and fraction 10 was astaxanthin (43aa). These body-wall carotenoids do not occur as a protein complex, and they represent a series of oxidation products of a carotene.



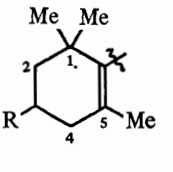
(43)



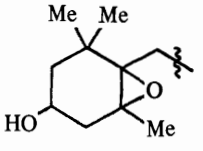
(44)



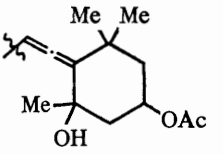
(a) R = OH
(c) R = H



(b) R = H
(g) R = OH



(d)



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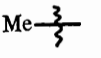


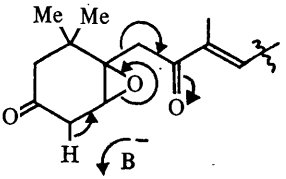
(f)

Matsuno has shown that the gonads of the 'trepang', *Stichopus japonicus* Selenka, contain a mixture of carotenoids, including β-carotene (43bb), echinenone (43bc), canthaxanthin (43cc), zeaxanthin (43gg), and astaxanthin (43aa).[49] Both the deep-orange ovaries and the pale-orange testes contain the same mixture, but in different proportions, and Matsuno mentions that a similar mixture occurs in the gonads of *H. leucospilota*. No reference is made as to whether these

[49] T. Matsuno, T. Ishida, T. Ito, and A. Sakushima, *Experientia*, 1969, **25**, 1253; T. Matsuno and T. Ito, *ibid.*, 1971 **27**, 509.

carotenoids are protein-bound, but this series is obviously very similar to that found by Bullock.[48]

While reinvestigating some early work by Lederer, Weedon and Lederer have found fucoxanthin (44de), its 3′-deacetyl derivative, fucoxanthinol, and a new allenic polyene, paracentrone (44fe), in the Mediterranean sea urchin *Paracentrotus lividus*.[50] Paracentrone was synthesised by Oppenauer oxidation of the 3-OH group in fucoxanthin which permitted a retro-aldol reaction to occur as in (45).[51]



(45)

The structure of 'asterinsäure', the characteristic carotenoid from carotenoprotein of *Asterias rubens*, has been shown by Liaaen-Jensen[52] to be the acetylenic xanthophyll, 7,8-didehydroastaxanthin [*cf.* (43aa)].

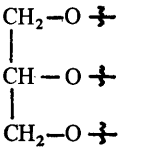
4 Non-phosphatide Lipids

For the purposes of this review, the term 'lipids' is used to cover long-chain compounds that are not polyisoprenoid in nature. Primarily this includes long-chain hydrocarbons and their terminal oxidation products, along with ester and ether derivatives of these.

The lipids of echinoderms have recently been reviewed by Giese[53] and by Fagerlund.[54] A recent review on the lipid biochemistry of marine organisms has been published,[55] and for an introduction to the general literature of lipid chemistry, the reader is referred to a recent monograph edited by Gunstone.[56] It is convenient to consider lipids as being saponifiable or non-saponifiable; the former group includes the fats [triglycerides (46ccc)], waxes [fatty alcohol esters (47c)] and neutral plasmalogens (46bcc) or (46bdd), whereas the latter category includes long-chain hydrocarbons and the α-glyceryl ethers (46add).

The triglycerides are generally very complex mixtures and are not amenable to easy separation. However, they are easily hydrolysed and converted into their constituent fatty-acid methyl esters. These esters may be readily analysed by

[50] G. Galasko, J. Hora, T. P. Toube, B. C. L. Weedon, D. André, M. Barbier, E. Lederer and V. R. Villanueva, *J. Chem. Soc.* (*C*), 1969, 1264.
[51] J. Hora, T. P. Toube, and B. C. L. Weedon, *J. Chem. Soc.* (*C*), 1970, 241.
[52] G. W. Francis, R. R. Upadhyay, and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1970, **24**, 3050.
[53] A. C. Giese, *Physiol. Rev.*, 1966, **46**, 244.
[54] U. H. M. Fagerlund in 'Chemical Zoology', ed. M. Florkin and B. T. Scheer, Academic Press, New York, 1969, vol. 3, p. 123.
[55] D. C. Malins and J. C. Wekell, *Progr. Chem. Fats and Lipids*, 1970, **10**, 339.
[56] 'Topics in Lipid Chemistry', ed. F. D. Gunstone, Logos Press, London, 1970, vol. 1.



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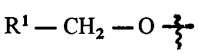
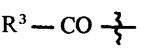
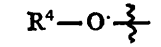


(a)



(c)



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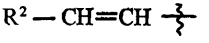
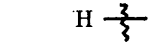


(b)



(d)

v.p.c.[57] and most work on triglycerides has been done in this manner. Despite much work on triglycerides from diverse natural sources, little has been done on echinoderm fats. Rodegker and Nevenzel[58] studied lipids from the ochre star, *Pisaster ochraceus*, along with its mussel and barnacle prey, and found differences among the various lipid samples, suggesting that the sea star did modify the lipids it had ingested. Allen[59] has made a comparative study of the fatty-acid composition of an asteroid, an echinoid, and a holothurian, and has included the incubation of tissues with [1-^{14}C]acetate. This showed that the incorporation decreased with the unsaturation of the acid, *i.e.* saturates > monoenes > dienes ≫ polyenes, the analyses being made with the help of t.l.c. on $AgNO_3$-impregnated plates. On the other hand, Kōchi[60] has used urea fractionation techniques[61] together with v.p.c. to detect 39 or 40 fatty acids in the lipids from several species of sea urchin gonads. He found only slight variations in fatty-acid composition among species and sources of sea urchins.

It appears that no wax esters have been isolated from echinoderms.[62] Neutral plasmalogens (46bcc), however, have been found in an inner tissue of the sea star *Asterias forbesi*.[63] Most of the knowledge of α-glyceryl ethers in asteroids (46add) has been accumulated by Karnovsky, with his most recent paper[64] discussing results from incubating radioactively labelled acetate, stearic acid (n-$C_{17}H_{35}CO_2H$), stearaldehyde and stearyl alcohol with pieces of digestive gland from the sea star, *Asterias forbesi*. The results were that acetate, stearic acid, and stearyl alcohol incorporated better into the alkyl glycerol ethers, whereas stearaldehyde incorporated better into the alkenyl glycerol ethers. This

[57] G. R. Jamieson, in ref. 56, p. 107.
[58] W. Rodegker and J. C. Nevenzel, *Comp. Biochem. Physiol.*, 1964, **11**, 53.
[59] W. V. Allen, *J. Mar. Biol. Ass. U.K.*, 1968, **48**, 521.
[60] M. Kōchi, *Suisan Daigakko Kenkyu Hokoku*, 1968, **17**, 9 (*cf. Chem. Abs.*, 1969, **71**, 2262b).
[61] For an example of this method, see J. L. Iverson and R. W. Weik, *J. Assoc. Offic. Analyt. Chemists*, 1967, **50**, 1111.
[62] J. C. Nevenzel, *Lipids*, 1970, **5**, 308.
[63] J. Eichberg, J. R. Gilbertson, and M. L. Karnovsky, *J. Biol. Chem.*, 1961, **236**, PC 15.
[64] J. Ellingboe and M. L. Karnovsky, *J. Biol. Chem.*, 1967, **242**, 5693.

evidence mitigates against a direct precursor–product relationship between alkyl and alkenyl glycerol ethers in this asteroid.

It appears that little is known of the details of lipids present in echinoderms, and further work is required to determine if there are significant variations among classes or if new exotic lipids, *e.g.* prostaglandins, could be discovered.

5 Sterols

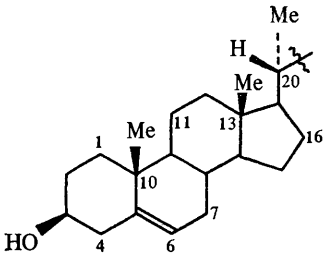
A. Distribution.—The recognition that the animal kingdom contained a variety of steroids, which varied from one species to another, was first detailed in a classic paper by Dorée.[65] Since then, progress on echinoderm-sterol research has been reported by Toyama[66] and Bergmann;[67] recently, Brooks comprehensively reviewed the chemistry of sterols.[68]

Since Bergmann's review was published, relatively little work has appeared in this area. Fagerlund and Idler have reported the isolation of 5α-ergosta-7,24(28)-dien-3β-ol[69] from the asteroid *Pisaster ochraceus*,[70] and Bolker has claimed[71] the isolation of (24*S*)-24-methyl-5,22-cholestadien-3β-ol (C-24 epimer of brassicasterol) from an unidentified Pacific crinoid, probably of the *Comatula genus*. The homogeneity of this sterol must be questioned in the light of later work (see below), as Bolker used no chromatography in his isolation procedure.

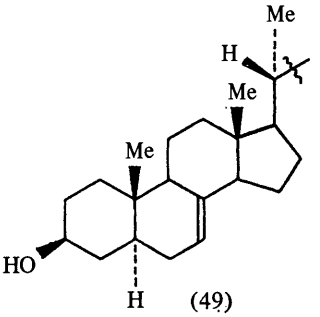
Scheuer has reported a detailed study of the sterols from one species of each of the five echinoderm classes.[72] In general, the sterols were isolated by column chromatography of the unsaponifiable acetone extracts from the echinoderm, followed by preparative v.p.c. of the recrystallized sterol trimethylsilyl ethers. The pure sterols were analysed by mass spectrometry and, where quantities permitted, melting points and rotations were determined also. The asteroid and the holothurian gave mixtures of various cholest-7-enols (49a), (49b), (49c+d), (49e), and (49f), whereas the other echinoderms contained only various mixtures of cholest-5-enols (48a), (48c+d), (48e), (48g), (48h), and (48i). Identifications were made primarily on the basis of careful v.p.c. comparisons with standards.

The holothurians *Stichopus japonicus* and *Holothuria tubulosa* have been extracted according to standard techniques.[73] The non-saponifiable portions of the extracts yielded mixtures of both Δ^5 and Δ^7 sterols, in a ratio of *ca.* 1 : 9, based on a separation using t.l.c. with $AgNO_3$-impregnated layers of alumina. Mass spectral analyses of the propionates from these sterol fractions showed

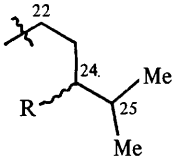
[65] C. Dorée, *Biochem. J.*, 1909, **4**, 72.
[66] Y. Toyama, *Fette, Seifen, Anstrichm.*, 1958, **60**, 909.
[67] W. Bergmann in 'Comparative Biochemistry', eds. M. Florkin and H. S. Mason, Academic Press, New York, 1962, vol. 3, p. 103.
[68] C. J. W. Brooks in 'Rodd's Chemistry of Carbon Compounds', ed. S. Coffey, Elsevier, Amsterdam, 1970, 2nd edn., vol. 2D, p. 1.
[69] For nomenclature of sterols, see ref. 68, pp. 422—454.
[70] U. H. M. Fagerlund and D. R. Idler, *J. Amer. Chem. Soc.*, 1959, **81**, 401.
[71] H. I. Bolker, *Nature*, 1967, **213**, 905.
[72] K. C. Gupta and P. J. Scheuer, *Tetrahedron*, 1968, **24**, 5831.
[73] T. Nomura, Y. Tsuchiya, D. André, and M. Barbier, *Nippon Suisan Gakkaishi* (*Bull. Japan Soc. Sci. Fisheries*), 1969 **35**, 293.



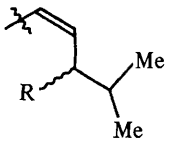
(48)



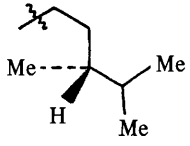
(49)



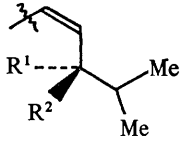
(a) R = H
(b) R = Me
(c) R = Et
(d) R = MeCH=



(e) R = H
(f) R = Me

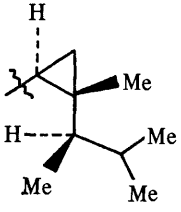


(g)



(h) R^1 = H
R^2 = Me

(i) R^1 = Et
R^2 = H



(j)

that they were mixtures containing mono- and di-unsaturated C_{27}, C_{28}, and C_{29} sterols. Also isolated from both holothurians were lanosterol,* cycloartenol, and D-xylosides of the Δ^5 series of sterols. It is interesting to compare these results with those of Scheuer,[72] and with those obtained by the author (in collaboration with Dr. I. M. Campbell) on the mudstar, *Ctenodiscus crispatus* Retzius.[75] This last study used a combined v.p.c.–m.s. approach[76] and detected the presence

*Lanosterol and 5-cholesten-3β-ol have also been found in a whole series of Mediterranean sea cucumbers.[74]

[74] G. Habermehl and G. Volkwein, *Naturwiss.*, 1968, **55**, 83.

[75] Unpublished data.

[76] For details, see B. A. Knights, *J. Gas Chromatog.*, 1967, **5**, 273.

of approximately 15 different sterols, primarily Δ^7 isomers. The virtue of this approach is that the m.s. may be scanned several times during the passage of the v.p.c. peak, whereas isolation of compounds by preparative v.p.c. may yield isomeric mixtures which are very difficult to separate given the small quantities available. It appears therefore that echinoderm sterols are very complex mixtures, and that complete analyses will require at least the combination of argentation t.l.c. and detailed v.p.c.–m.s. studies.

A new sterol was discovered by Scheuer in the study already referred to,[72] and as it occurred in the 'crown of thorns' sea star, *Acanthaster planci* Linn., it was named acanthasterol; it was suggested to be the Δ^7 analogue of a coral sterol, gorgosterol, but the structures of neither sterol were known at that time. Gorgosterol has since been shown to be (48j),[77] and Djerassi has independently determined the structure of 'acansterol', which name he has now changed to acanthasterol (49j),[78] in recognition of Scheuer's prior work. Acanthasterol, with 30 carbons and a side-chain cyclopropyl ring, is obviously a very unusual and biogenetically interesting molecule. Although the sterol composition of the corals on which this steroid feeds does not appear to have been determined, it is intriguing to speculate that acanthasterol is a metabolic product of ingested gorgosterol (see below).

B. Biosynthesis.—A number of complementary studies on sterol biosynthesis in echinoderms have been reported.[79–82] It was shown that three species of asteroid are able to convert cholest-5-en-3β-ol into 5α-cholest-7-en-3β-ol,[79,82] and in one case, that this proceeded *via* 5α-cholestan-3β-ol.[82] (The separation of these three compounds by t.l.c. was readily achieved by converting the olefins into their epoxides.[82])

Furthermore, the feeding of [1,2-$^{14}C_2$]acetate to the echinoid *Paracentrotus lividus* yielded no radioactivity in either the sterols or in the squalene.[80] A similar situation occurred with two holothuroids, although in this case squalene was labelled.[81] It thus appears probable that sterols in echinoderms are of dietary origin (hence generally Δ^5) but that the asteroids and holothurians possess metabolic systems capable of converting Δ^5 into Δ^7 sterols.

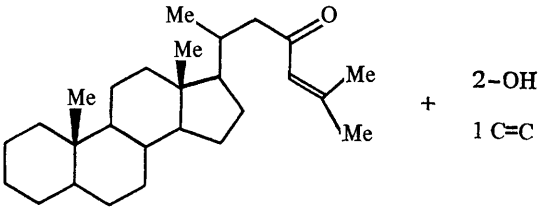
6 Saponins

The presence of toxic saponins in both asteroids and holothurians has been known for quite a few years, and has been discussed by Hashimoto[83] and by Scheuer.[3] Since these reports, a number of advances have been reported, and discussion will centre on these.

[77] C. Djerassi, *Intra-Sci. Chem. Reports*, 1970, **4**, 165.
[78] Y. M. Sheikh, C. Djerassi, and B. M. Tursch, *Chem. Comm.*, 1971, 217, 600.
[79] U. H. M. Fagerlund and D. R. Idler, *Canad. J. Biochem. Physiol.*, 1960, **38**, 997.
[80] A. Salaque, M. Barbier, and E. Lederer, *Comp. Biochem. Physiol.*, 1966, **19**, 45.
[81] T. Nomura, Y. Tsuchiya, D. André, and M. Barbier, *Nippon Suisan Gakkaishi*, 1969, **35**, 299.
[82] A. G. Smith and L. J. Goad, *F.E.B.S. Letters*, 1971, **12**, 233.
[83] T. Yasumoto, M. Tanaka, and Y. Hashimoto, *Nippon Suisan Gakkaishi*, 1966, **32**, 673

A. Asteroid Saponins.—Hashimoto has obtained a mixture of six saponins from the Japanese sea star *Asterias amurensis*, and has partially characterized two of these.[3,84] In the meantime, Mackie has published work, primarily on the saponins from the British sea star, *Marthasterias glacialis*.[85] A major part of this work has been the development of a sensitive biological assay, which can detect micrograms of the toxic materials. This assay is simply a live snail, *Buccinum undatum*, which reacts by withdrawing its foot muscle into its shell when the toxin is introduced into its proximity. Isolation of the saponin was accomplished simply by collecting the copious exudate as the frozen sea stars thawed out. This was purified by ethanol and butanol extraction procedures, eventually being isolated as a water-soluble powder.

This powder was further purified on DEAE-cellulose, leading to the isolation of a reasonably homogeneous sample of glycoside. Hydrolysis of the glycoside gave the aglycone, together with glucose, quinovose, fucose, and sulphate in 1 : 2 : 1 : 1 molar proportions. The aglycone could be diacetylated and, from spectroscopic evidence, possesses a cholestane skeleton with a novel $\alpha\beta$-unsaturated ketone function in the side-chain (50). The position of the hydroxy-



(50)

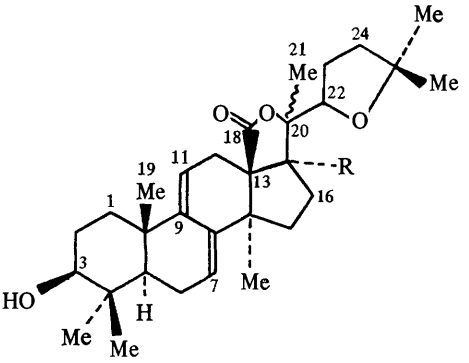
groups, double bond, sugars, and sulphate groups remains to be determined and, as the glycoside is obtainable in reasonable quantities, this should be readily possible. The evidence thus far accumulated supports a similarity to the known asterosaponins, whereas they are obviously different from the holothurins (see below).

A biologically important finding from Mackie's work is that the *M. glacialis* saponin acts on the chemosensory cells in the snail's foot muscle in the same manner as do some synthetic non-ionic surfactants. Maintaining the snail in a low concentration of these substances causes loss of response to the toxin, and this is relevant to the use of detergents in cleaning up ocean oil spills, which could result in molluscs losing their defences against predatory asteroids.[85b]

[84] T. Yasumoto and Y. Hashimoto, *Agric. and Biol. Chem.* (*Japan*), 1965, **29**, 804; 1967, **31**, 368.
[85] (*a*) A. M. Mackie, R. Lasker, and P. T. Grant, *Comp. Biochem. Physiol.*, 1968, **26**, 415; (*b*) A. M. Mackie, *J. Exp. Mar. Biol. Ecol.*, 1970, **5**, 63; (*c*) A. M. Mackie and A. B. Turner, *Biochem. J.*, 1970, **117**, 543.

B. Holothurian Saponins.—Among the toxic marine animals are the holothurians, and these secrete, presumably as a defence mechanism, water-soluble glycosides, which possess greater haemolytic activity than saponins of plant origin.[86] Since their discovery, the holothurian saponins (holothurins) have been the subject of considerable investigation, both chemical and pharmacological.[3] The Cuviers glands of the Caribbean sea cucumber, *Actinopyga agassizi*, have been a major source of holothurin,[3] and Chanley has recently published details of further work on this saponin mixture.[87]

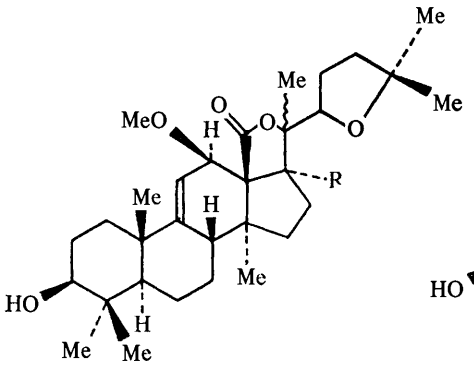
Holothurin itself is a mixture of glycoside sulphates, and hydrolysis of one of these, holothurin A, in concentrated HCl gave an aglycone mixture that yielded *inter alia* the two oxidoholothurinogenins (51) and (52). These holo-



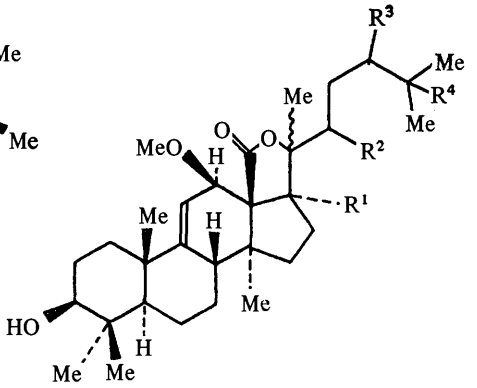
(51) R = OH: 22,25-oxidoholothurinogenin
(52) R = H: 17-deoxy-22,25-oxido-holothurinogenin

thurinogenins contain a 7:8,9:11 heteroannular diene system which is absent in the parent holothurin A, and an obvious question is whether this is the only change that occurs in the aglycone moiety as the hydrolysis occurs. By careful acidic methanolysis of holothurin A,[87a] Chanley has isolated a series of 12β-methoxy-7,8-dihydroholothurinogenin acetates (neoholothurinogenins) (53)—(57). When dihydroholothurin A was reacted under the same conditions, (53), (54), (57), and (58) were obtained, proving the existence of only three different side-chains in the molecule, (56) having arisen by the addition of methanol to the 24,25 double bond (holothurin A contains only one methoxy-group which is attached to a glucose residue). Structural proof for the neoholothurinogenins was obtained by conversion into their corresponding known holothurinogenins, and also by detailed ^{1}H n.m.r. studies on them. The configuration of the 12-methoxy-group in the neoholothurinogenins is clearly shown to be β (equatorial) by the

[86] B. W. Halstead, 'Poisonous and Venomous Marine Animals of the World', U.S. Govt. Printing Office, Washington, D.C., 1965, Vol. I, p. 575.
[87] (*a*) J. D. Chanley and C. Rossi, *Tetrahedron*, 1969, **25**, 1897; (*b*) *ibid.*, p. 1911.



(53) R = OH
(54) R = H



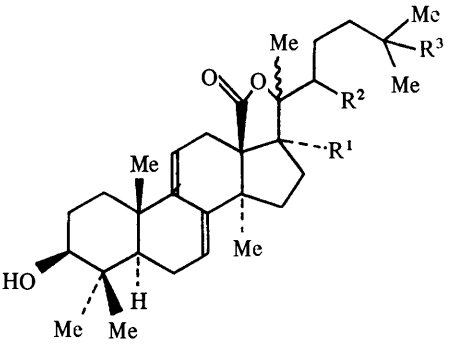
	R^1	R^2	R^3	R^4
(55)	OH	H	double	bond
(56)	OH	H	H	OMe
(57)	OH	OAc	H	H
(58)	OH	H	H	H

^{1}H n.m.r. spectrum. However, enzymic hydrolysis of a desulphated sample of holothurin A[87b] gave a low yield of a mixture which contained 12α (axial) oxygen functions. This led Chanley to propose[87b] that the aglycone portions in holothurin A are as in the neoholothurinogenins, apart from possessing a 12α-hydroxy-function in place of the 12β-methoxy-groups in the latter compounds.

Habermehl[88] has studied the six aglycones derived from *Holothuria polii* (Delle Chiaje). These were shown to have structures (51), (52), (59)—(62); (59) is the known holothurinogenin, griseogenin.[89] The molecular structures of (60)—(62) were deduced from ^{1}H n.m.r. and mass spectral data [but see praslinogenin (61) and ternaygenin (62) below].

The Indian Ocean sea cucumber, *Bohadschia koellikeri*, has proved to be a useful source of holothurins, from the hydrolysis of which seychellogenin (63), koellikerigenin (64), ternaygenin (62), and praslinogenin (61) were isolated.[90] Koellikerigenin proved to be usefully reactive as, after monoacetylation, its 25-OH group could be smoothly dehydrated. Hydrogenation of the resulting olefin gave seychellogenin monoacetate. Reaction of koellikerigenin (64) with methanolic HCl under the conditions employed for hydrolysis of the glycoside gave ternaygenin (62), leading to the suggestion that both praslinogenin (61) and ternaygenin may be artefacts produced during the hydrolysis of the glycoside mixture.[90b] As Habermehl has employed similar hydrolysis conditions,[74] it is

[88] G. Habermehl and G. Volkwein, *Annalen*, 1970, **731**, 53.
[89] B. Tursch, I. S. de Souza Guimarães, B. Gilbert, R. T. Aplin, A. M. Duffield, and C. Djerassi, *Tetrahedron*, 1967, **23**, 761.
[90] (*a*) P. Roller, C. Djerassi, R. Cloetens, and B. Tursch, *J. Amer. Chem. Soc.*, 1969, **91**, 4918; (*b*) B. Tursch, R. Cloetens, and C. Djerassi, *Tetrahedron Letters*, 1970, 467.



	R^1	R^2	R^3
(59)	OH	OH	H
(60)	OH	H	H
(61)	OH	H	OMe
(62)	H	H	OMe
(63)	H	H	H
(64)	H	H	OH

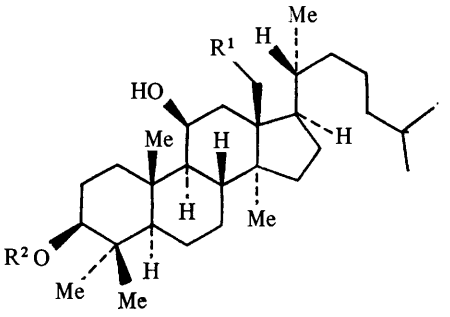
probable that his compounds (61) and (62) are also artefacts. It may be worth suggesting at this point the desirability of naming compounds (61)—(64) as substituted holothurinogenins (as done by Habermehl),* rather than introducing new trivial names, especially for compounds that are found in several sea cucumbers or which may only be artefacts.

Roller, Tursch, and Djerassi have carried out an essential part of holothurinogenin research in that they have chemically converted 17-deoxyholothurinogenin [seychellogenin, (63)] into lanostane-3β,11β,18-triol (65).[91] This triol was also prepared from the known 11β-hydroxylanostan-3β-yl acetate (66), *via* the pentacyclic ether (67). As a result, the stereochemistry of all centres, except C-20, in 17-deoxyholothurinogenin (63) has been unequivocally determined.

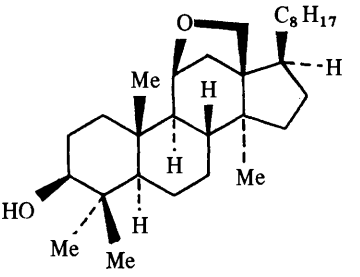
The C-17 hydroxy-group has been shown[87a] to be *cis* to C-32 in various holothurinogenins by the technique of comparing the ^{1}H n.m.r. spectra in chloroform with the spectra in pyridine or in benzene. It was found that a downfield shift in the resonance of C-32 in the pyridine or benzene solutions occurred only when the C-17 hydroxy-group was present. If the C-17 hydroxy-group and C-32 were *trans* they would be too far apart for any preferentially bound pyridine solvent molecules to influence the chemical shift of C-32, and hence they must be in a *cis* configuration. Chanley has applied the same concepts[87] to determine the configuration at C-20, and he states that C-21 must be 'oriented β (behind the plane of the lactone ring)'. Presumably this means[89] *S* in (51) and hence *R* in (52). On the other hand, Habermehl[88] concluded that because C-21 has the

* Habermehl has now proposed a new system of nomenclature for holothurinogenins: G. Habermehl and G. Volkwein, *Toxicon*, 1971, **9**, 319.

[91] P. Roller, B. Tursch, and C. Djerassi, *J. Org. Chem.*, 1970, **35**, 2585



(65) R^1 = OH; R^2 = H
(66) R^1 = H; R^2 = Ac



(67)

same chemical shift in (51) and in (61), therefore C-21 and the C-17 hydroxy-group are *trans*, *i.e.* the opposite of Chanley's conclusion. Chanley's approach is probably more convincing, but further work appears needed.

To conclude this section, mention must be made of Matsuno's work on *Holothuria leucospilota*,[92] which has included studies on the seasonal variation of holothurin content within different body organs, and of work on holothurins from *Stichopus japonicus*. Shimada[93] has isolated a material 'holotoxin' (no structure proposed) which he claims to be the first-known animal antifungal glycoside. On the other hand, Elyakov[94] has published structures for two aglycones, stichopogenin A_2 and stichopogenin A_4. These were claimed to have a 5:6,8:9 unconjugated homoannular diene system, and were prepared by HCl hydrolysis of two glycosides isolated from the holothurian.

7 Conclusion

In this review, most emphasis has been placed on the chemistry of molecules found in echinoderms. Mention has been made also of the few biosynthetic studies that have been carried out. Obviously, there is still much scope for similar work to be done, and whether this will yield only known compounds or some new family of molecules is of course pure speculation. However, marine natural products have obvious potential for the development of new drugs,[95] and of the compounds discussed, only the pharmacology of the saponins has been

[92] T. Matsuno and J. Iba, *Yakugaku Zasshi* (*J. Pharm. Soc. Japan*), 1966, **86**, 637; T. Matsuno and T. Ishida, *Experientia*, 1969, **25**, 1261.
[93] S. Shimada, *Science*, 1969, **163**, 1462.
[94] G. B. Elyakov, T. A. Kuznetsova, A. K. Dzizenko, and Yu. N. Elkin, *Tetrahedron Letters*, 1969, 1151; G. B. Elyakov, T. A. Kuznetsova, and V. E. Vas'kovskii, *Chem. Natural Compounds*, 1968, **4**, 253.
[95] A. H. Der Marderosian in 'Food–Drugs from the Sea, Proceedings, 1969', ed. H. W. Youngken, jun., Marine Technology Society, Washington, D.C., 1970; M. H. Baslow, *Ann. Rev. Pharmacol.*, 1971, **11**, 447.

described.[85b,93,96] Many echinoderms are known to exhibit toxicity of one form or another[97] and few of these toxins have been characterized.

Two other possibilities for further work are apparent. Echinoderms have unique symmetry properties and their skeletons contain calcite with unusual strength properties.[98] As calcium carbonate is apparently used with unusually high efficiency, further studies on how these skeletons are formed could be fruitful. Secondly, there has been considerable discussion on the phylogeny of echinoderms, and whether comparative biochemistry can contribute to this.[9,10,72] This possibility was dismissed by Fell[8] as leading to 'absurd results', but there is a considerable body of evidence which cannot be neglected. Although the sterols found in echinoderms appear to be exogenous, only the asteroids and the holothurians appear to possess the enzyme systems necessary to modify Δ^5 sterols to Δ^7 sterols. Only these classes have the ability to elaborate saponins, and their naphthoquinone pigments also appear to be similar. Echinoids and ophiuroids elaborate similar sterol and naphthoquinone mixtures. On the other hand, asteroids appear to contain protein-bound carotenoids and α-glyceryl ethers in quantity, whereas the holothurians have free carotenoids, and no reports of α-glyceryl ethers have appeared. Not much work has been done on crinoids, but they appear to be the only class to contain anthraquinones.

Probably, the most realistic assessment at this time is that inter-class relationships seem probable, but much more detailed work on more species, together with checking their feeding habits and metabolic processes will be required before definite conclusions can be drawn. In the process of doing this, there will undoubtedly be the reward of discovering new, interesting, and useful molecules.

I am grateful to Miss P. M. Lutley and other staff of Dalhousie University Library for help with the bibliography.

[96] S. L. Friess, R. C. Durant, and J. D. Chanley, *Toxicon*, 1968, **6**, 81; S. L. Friess, *Aldrichimica Acta*, 1970, **3**, 16 (*Chem. Abs.*, 1970, **73**, 43 387f).
[97] Ref. 86, p. 537, *et seq.*
[98] J. Weber, R. Greer, B. Voight, E. White, and R. Roy, *J. Ultrastruct. Res.*, 1969, **26**, 355.