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ANTHRAQUINONE PIGMENTS FROM THE CRINOID *COMANTHUS BENNETTI*

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Abstract—From the crinoid *Comanthus bennetti* we isolated and characterized five pigments. One is the known 3-(1'-hydroxypropyl)-1,6,8-trihydroxy-9,10-anthraquinone (rhodoptilometrin). The remaining four had not previously been isolated from a natural source: 3-propyl-1,6,8-trihydroxy-9,10-anthraquinone; 3-propionyl-1,6,8-trihydroxy-9,10-anthraquinone; 3(2'-hydroxypentyl)-1,6,8-trihydroxy-9,10-anthraquinone; and 2-(1'-hydroxypropyl)-1,4,5,7-tetrahydroxy-9,10-anthraquinone.

Crinoids, in contrast with the familiar sea stars and sea urchins, are perhaps the least known among the five classes of the marine phylum Echinodermata: the crinoids constitute the least numerous class in the phylum and a majority of the living species have been described from a restricted geographical area, the Indo-Pacific.[1] Yet in some ways crinoids are particularly fascinating. They are the most ancient echinoderms with an unequalled fossil record; some eighty of these attached stalked crinoids (sea lilies) still exist today. A majority of the living species are the more modern, free-swimming comatulids (feather stars), some 550 of which have been described. Echinoderm evolution has been the subject of a lively controversy and, not surprisingly, the crinoids play a central role in these considerations. The major viewpoints in echinoderm phylogeny have recently been presented by Grossert.[2]

Interest in crinoid chemistry, whether it be based on considerations of phylogeny or molecular structure, also appears to be well deserved. Because of the spectacularly colorful appearance of many echinoderms, it is only natural that chemical studies of echinoderm pigments began nearly a hundred years ago. Recent reviews by Grossert,[2] Thomson,[3] Chang,[4] and Scheuer[5] have illuminated various facets of this research. The most readily accessible echinoderms, the sea urchins, were long the focal point of these endeavors, which have resulted in the discovery and structural elucidation of a large number of naphthoquinone derivatives. Naphthoquinone pigments have also been reported from the sea cucumbers,[6] the sea stars,[7] and the brittlestars,[7] although the total research effort in these three classes of echinoderms has been minimal. The remaining class of echinoderms, the crinoids, almost entirely through the efforts of Sutherland and his students[8-13] in Australia, have received considerable attention and have yielded a greater structural variety of pigments than have the other classes of echinoderms. In addition to angular and linear[9] naphthopyrones and naphthoquinones,[7,8] a number of anthraquinones[10-15] have been isolated from crinoids and characterized. Anthraquinone pigments, otherwise reported only from plants,[3] microorganisms,[3] insects,[3] and a single marine annelid,[16] have thus emerged as a pigment type characteristic of crinoids. Sutherland and Wells[11] have traced the history of crinoid pigment research.

Our own interest in crinoid pigments is an outgrowth of our earlier attempt at comparative echinoderm pigment research.[7] At that time, it became evident that further crinoid research would be rewarding because of the likelihood of discovery of new structural types and because of the key role of crinoids in evolutionary theory. Since no crinoids have been described from Hawaiian waters, we had to secure animals from the Marshall and Caroline islands in the southwest Pacific.

RESULTS AND DISCUSSION

Our crinoid specimens from the Marshall islands proved to be essentially devoid of pigments. However, two animals from the Carolines, identified[17] as *Comanthus bennetti,* yielded five pigments by acetone extraction, column chromatography on acid-washed silica gel, and preparative TLC on silica gel H. Four of the compounds are derivatives of 1,6,8-trihydroxy-9,10-anthraquinone (**1a**) and one is a 1,4,5,7-tetrahydroxy-9,10-anthraquinone (**2a**).

The major constituent, which had the lowest R_f on silica gel H, was the known 3-(1'-hydroxypropyl) 1, 6, 8 - trihydroxy - 9, 10 - anthra-

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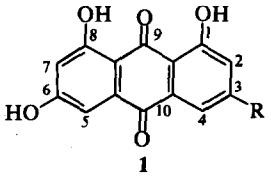
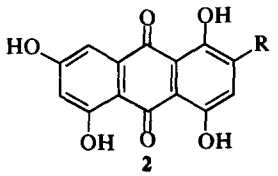


1

a R = H
b R = —CH(OH)—Et
c R = *n*-Pr
d R = —CO—Et
e R = CH_2—CH(OH)—Pr(*n*)
f R = —CH_2—CH(OH)—CH_3



2

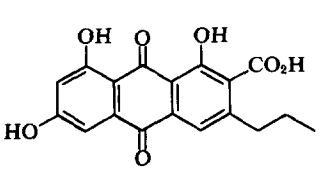
a R = H
b R = Me
c R = CH(OH)—Et

quinone (rhodoptilometrin, **1b**). It had previously been isolated by Powell and Sutherland[13] from *Ptilometra australis* and by Erdman and Thomson[14] from *Heterometra savignii* and from *Lamprometra klunzingeri*. It was identified by spectral and TLC comparison with an authentic sample.

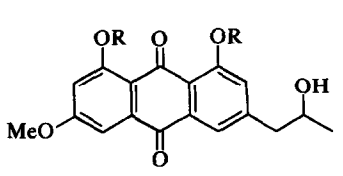
The fastest moving compound proved to be 3-propyl-1,6,8-trihydroxy-9,10-anthraquinone (**1c**). It had not been isolated previously from a natural source, but was known as a degradation product of ptilometric acid (**3**) and of nalgiovensin (**4a**). Nalgiovensin (**4a**) is derived from a microorganism and had been converted to **1c** by phosphorus-hydriodic acid reduction, followed by reoxidation of the resulting anthrone to the quinone.[18] Spectral (electronic, IR) and melting point comparison of **1c** with published data[13,18] of the above degradation products, combined with the NMR and MS properties of **1c** and its 6-methyl ether, demonstrated its structure unambiguously.

The third of the 1,6,8-trihydroxyanthraquinones was the 3-propionyl derivative (**1d**). This pigment is a new compound. Its structure was secured by its electronic spectrum, which clearly showed it to be a 1,6,8-trihydroxyanthraquinone. Assignment of a 1710 cm^{-1} IR band to a side chain CO group was reinforced by the mass spectrum of **1d**, which exhibited a molecular ion peak at *m/e* 312. Loss of ethyl was followed by successive losses of carbon monoxide. The NMR spectrum of **1d** had aromatic signals (1 H doublets at δ 7·92, 7·68, 7·03, and 6·51) characteristic of 3-substituted-1,6,8-trihydroxyanthraquinones, in addition to a 2 H quartet at δ 3·10 and 3 H triplet at δ 1·13 for the side chain protons. Finally Jones oxidation of our major constituent rhodoptilometrin (**1b**) yielded **1d**, identical in all respects with the natural product.

The last of the 1,6,8-trihydroxylated anthraquinones was a minor component and was difficult to free from a persistent aliphatic companion. Its electronic spectrum clearly established its hydroxylation pattern and its IR bands at 1675 and 1615 cm^{-1} were those of an hydroxylated 9,10-anthraquinone. The NMR spectrum, with one proton doublets (or broad singlets) at δ 7·42, 7·05, 7·01, and 6·48, suggested a C-3 substituent. The composition of the substituent was deduced from the mass spectrum. A strong molecular ion peak at *m/e* 342 suggests a $C_5H_{10}O$ side chain. Loss of 72 from the molecular ion led to the base peak at *m/e* 270, a process that may be rationalized by the following scheme. This fragmentation does not locate the position of the side chain OH group. It does exclude the 1′-position since such a structure should have led to a *m/e* peak at 285 by α-cleaveage, in analogy with the observed fragmentation of rhodoptilometrin (**1b**), which has its base peak at *m/e* 285. An hydroxyl group at C-1′ is further excluded by a two proton NMR signal at δ 2·80, which is assigned to two benzylic protons in analogy with the signal at δ 2·83 in nalgiovensin dimethyl ether (**4b**).[19] Further NMR analysis of the side chain signals was impossible because of the interfering signals caused by the aliphatic impurity. We were able

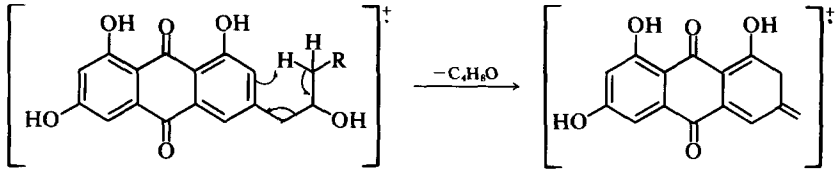


3



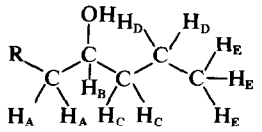
4

a R = H
b R = Me



m/e 342 *m/e* 270

to remove virtually all of the aliphatic contaminant when we prepared a monomethyl ether of the pigment by brief diazomethane treatment. The ether could be purified by TLC and its NMR spectrum established the structure of the side chain (part structure **5**). The benzylic protons (H_A) appear as a complex multiplet at δ 2·82. The multiplicity probably arises from nonequivalence of the two protons and is further complicated by long range coupling with the aromatic protons and with H_C. The proton



5

geminal to the OH group, H_B, is just visible as a broad hump under the OMe singlet at δ 3·94. Protons, H_C and H_D appear as a broad band centered at δ 1·50. The methyl protons, H_E, appear at δ 0·95 as a broadened triplet. The broadening arises through virtual coupling[20] with protons H_C and lends support to the proposed structure of the pigment, 3 - (2' - hydroxypentyl) - 1, 6, 8 - trihydroxy - 9, 10 - anthraquinone (**1e**). If, on the other hand, the side chain hydroxy group were at C-3', virtual coupling would be absent and the methyl protons would appear as a distinct triplet, as e.g. in rhodoptilometrin (**1b**). Lack of material did not allow us to measure the optical rotation of this pigment.

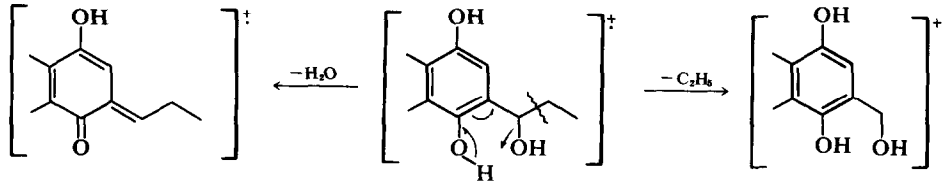
The fifth pigment was a minor constituent (about 1 mg) and was difficult to separate from accompanying **1d** and **1e**, which preceded and followed it on TLC. its color on TLC, however, is strikingly different from those of the other pigments: it appears as rose-pink while the others are various shades of orange. Its electronic spectrum is very similar to that of the microbial pigment catenarin (**2b**)[21] which is a 1,4,5,7-tetrahydroxy-9,10-anthraquinone. The mass spectrum of the pigment elucidated the nature of the side chain. A molecular ion at *m/e* 330 shows that the side chain composition is C_3H_7O. Fragmentation, as shown in the following scheme, reveals the structure. Loss of C_2H_5·, from the molecular ion gives rise to the base peak at *m/e* 301, similar to an identical loss in **1b** (rhodoptilometrin) and **1d**, thereby suggesting a 1-hydroxypropyl moiety. Loss of water from the molecular ion (330→312) is well documented in molecules where a phenolic hydroxyl group is β to an aliphatic OH group.[22] Rhodoptilometric (**1b**), which lacks the appropriate phenolic OH group, does not exhibit a similar loss of water. The attachment at C-3 of the hydroxypropyl substituent cannot be unequivocally determined. By analogy with its four companion pigments it is reasonable that this pigment is 2 - (1' - hydroxypropyl) - 1, 4, 5, 7 - tetrahydroxy - 9, 10 - anthraquinone.

Four of the pigments of *Comanthus bennetti* (**1b, 1c, 1d, 2c**) have three carbon side chains and differ only in degree of oxidation. All may well be derived from a common polyketide precursor[23] as has been pointed out by Powell and Sutherland.[13] Pigment **1e**, however, appears to be the first reported natural anthraquinone with a five carbon side chain. It is closely related to isorhodoptilometrin (**1f**),[13] from which it differs by an additional ethyl group, and it may well be derived from a C_{20} polyketide chain with loss of one carbon atom.

EXPERIMENTAL

Crinoid collection. The animals were collected at the west entrance of Malakal Harbor, Palau, Western Caroline islands and freeze-dried. They were identified[17] as *Comanthus bennetti* (J. Müller), family Comasteridae.

Extraction. A freeze-dried animal (67 g) was extracted (Soxhlet) overnight with acetone. The acetone was changed and extraction continued for another 4 d. The combined acetone extracts were concentrated to give 2·06 g of dark brown oil which solidified on standing. Further extraction of the crinoid with ethanol for 4 d afforded another 4·02 g of black solid, but which contained no quinone pigments and was not investigated further. The acetone extracts were chromatographed on a



column of acid-washed silica gel (Baker, 60–200 mesh) in CH_2Cl_2 with increasing amounts of methanol. Fractions containing the pigments were combined to give 0·824 g (1·3% of the dried animal weight) of material. Similar extraction of a second crinoid (105 g) afforded 0·943 g of the pigment mixture. Repeated preparative thin layer chromatography on silica gel H in 4% MeOH/CH_2Cl_2 yielded five anthraquinone pigments (R_f = 0·58, 0·53, 0·31, 0·29, 0·21).

Rhodoptilometrin (**1b**). This pigment, R_f = 0·20, 80–85% of total pigment, formed dark red crystals, mp 216–218° (from MeOH) 496 (TLC, NMR, MS) with an authentic sample of rhodoptilometrin, supplied by Professor R. H. Thomson. Found: M, 314·0790.* $C_{17}H_{14}O_6$ requires: 314·0790; UV: λ_{max} (MeOH) 253, 266, 271, 437 nm; IR: ν_{max} (KBr) 3400, 1665, 1630 cm^{-1}; NMR: δ (DMSO-d_6) 7·56 (1H, broad s), 7·17 (1H, broad s), 6·96 (1H, d, J = 2·0 Hz), 6·41 (1H, d, J = 2·0 Hz), 4·56 (1H, perturbed t), 1·65 (2H, perturbed quintet), 0·91 (3H, t, J = 8·0 Hz). MS: *m/e* (70 eV) 315 (8%), 314 (42), 286 (17), 285 (100), 258 (8), 257 (30), 243 (5), 241 (3), 215 (12), 187 (5).

3-*Propyl*-1,6,8-*trihydroxy*-9,10-*anthraquinone* (**1c**). The fastest moving pigment, $R_f \approx 0{\cdot}58$, 10–15% of total pigment, formed orange plates, mp 219·5–221·5° (MeOH/$CHCl_3$). Found: M, 298·0841.* $C_{17}H_{14}O_5$ requires 298·0841; UV: λ_{max} (MeOH) 254 (14,600), 264 sh, 291 (13,800), 302 infl., 400 (7,000) nm; IR: ν_{max} (KBr) 3400, 1675, 1625 cm^{-1}; NMR: δ (DMSO-d_6) 11·90 (1H, s), 11·82 (1H, s), 11·26 (1H, broad s), 7·28 (1H, d, J = 1·5 Hz), 6·96 (2H, broad d, J = 1·5–2·5 Hz), 6·43 (1H, d, J = 2·5 Hz), 2·52 (2H, t, J ≅ 8 Hz), 1·55 (2H, perturbed quintet, J ≅ 7 Hz), 0·91 (3H, t, J ≅ 7·5 Hz); MS: *m/e* (79 eV) 299 (16) 298 (100), 282 (13), 271 (9), 270 (54); 269 (11), 242 (7), 241 (26), 213 (7).

1, 8-*Dihydroxy*-6-*methoxy*-3-*propyl*-9, 10-*anthraquinone* was prepared by brief treatment of **1c** in MeOH with ethereal diazomethane. TLC of the product yielded a mixture of dimethoxyanthraquinones and pure monomethylated **1c** mp 159–160·5 (from MeOH); UV: λ_{max} (MeOH) 256 (17,300), 266 (17,900), 288 (16,300), 436 (10,600) nm; IR: ν_{max} (KBr) 3400–3500, 1681, 1632, 1611 cm^{-1}; NMR: δ ($CDCl_3$) 7·64 (1H, d, J = 1·5 Hz), 7·37 (1H, d, J = 2·5 Hz), 7·09 (1H, d, J = 1·5 Hz), 6·69 (1H, d, J = 2·5 Hz), 3·90 (3H, s), 2·64 (2H, t, J = 7·5 Hz), 1·63 (2H, perturbed quintet, J ≅ 7·5 Hz), 0·94 (3H, t, J = 7 Hz); MS: *m/e* (70 ev) 313 (21%), 312 (100), 310 (7), 298 (4), 297 (10), 285 (11), 284 (60), 283 (10), 256 (9), 255 (33), 217 (8), 212 (9).

3-*Propionyl*-1, 6, 8-*trihydroxy*-9, 10-*anthraquinone* (**1d**). This pigment, about 21% of total pigment, R_F = 0·53, formed orange needles, mp 265–6° from EtOH. Found: M, 312·0646.* $C_{17}H_{12}O_6$ requires: 312·0634; UV: λ_{max} (MeOH) 230, 256, 265 sh, 338, 442, 515 sh nm; IR: ν_{max} (KBr) 3400, 1710, 1630 cm^{-1}; NMR: δ (DMSO-d_6) 7·92 (1H, d, J = 1·5 Hz), 7·68 (1H, d, J = 1·5 Hz), 7·03 (1H, d, J = 2·5 Hz), 6·51 (1H, d, J = 2·5 Hz), 3·10 (2H, q, J = 7 Hz), 1·13 (3H, t, J = 7 Hz); MS: *m/e* (70 ev) 312 (42%), 284 (22), 283 (100), 255 (46), 199 (13), 171 (13).

3-(2′)*Hydroxy*-*n*-*pentyl*)-1, 6, 8-*trihydroxy*-9, 10-*anthraquinone* (**1e**). This compound, about 0·1% of total pigment, R_F = 0·29, could not be obtained free from a small amount of highly aliphatic material as observed by NMR; UV: λ_{max} (MeOH) 224, 254, 268, 290, 439 nm; IR: ν_{max} (KBr) 3400, 1705 (impurity), 1675 sh, 1615 cm^{-1}; δ DMSO-d_6) 11·97 (1H, s) 11·87 (1H, s), 11·29 (1H, broad s), 7·42 (1H, broad s), 7·05 (1H, broad s), 7·01 (1H, d, J = 2·5 Hz), 6·48 (1H, d, J = 2·5 Hz), 2·75 (2H, m), 1·6–0·8 (broad envelope) (impurity); MS: *m/e* (70 eV) 342 (73%), 270 (100), 241 (23), 213 (11). High resolution MS:† Found: M, 342·1089. $C_{19}H_{18}O_6$ requires 342·1103.

1,8-*Dihydroxy*-3-(2′-*hydroxy*-*n*-*pentyl*)-6-*methoxy*-9,10-*anthraquinone* was prepared by brief treatment of **1e** with ethereal CH_2N_2. The product was purified by TLC to give 1·1 mg of organge solid; UV: λ_{max} (MeOH) 226 (24,000), 255 (15,000), 263 (14,800), 286 (12,100), 433 (8400) nm; IR: ν_{max} ($CHCl_3$) 1680, 1628, 1615 sh cm^{-1}; NMR: δ ($CDCl_3$) 12·28 (1H, s), 12·12 (1H, s), 7·68 (1H, d, J ≅ 1·5 Hz), 7·37 (1H, d, J = 2·7 Hz), 7·15 (1H, d, J ≅ 1·5 Hz), 6·69 (1H, d, J = 2·7 Hz), 3·94 (3H, s), ≈ 3·95 (broad m), 2·82 (2H, complex m), 1·62 (broad s); 1·50 (broad hump), 0·95 (3H, broad t).

2-(1′)*hydroxypropyl*)-1,4,5,7-*tetrahydroxy*-9,10-*anthraquinone* (**2c**). Approximately 1 mg of this pigment, corresponding to about 0·1% of total pigment, was isolated; UV: λ_{max} (MeOH) 231, 257, 271, 300 sh, 465 sh, 481 sh, 493, 508 sh, 525 nm; IR: ν_{max} (p-dioxane) 3500, 1620 $c,^{-1}$; MS: *m/e* (70 ev) 331 (8%), 330 (39), 312 (20), 302 (24), 301 (100); 286 (33), 273 (11).

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