The gymnochromes: Novel marine brominated phenanthroperylenequinone pigments from the stalked crinoid Gymnocrinus richeri

ARTICLE *in* THE JOURNAL OF ORGANIC CHEMISTRY · APRIL 2002

Impact Factor: 4.72 · DOI: 10.1021/jo00024a016 · Source: OAI

leum Research Fund, administered by the American Chemical Society, for partial support of this work. We also thank the National Institutes of Health for a Biomedical Research Support grant.

Registry No. **2a**, 136839-81-1; **2b**, 136840-12-5; **2c**, 136839-83-3; **2d**, 136840-13-6; **2e**, 15185-08-7; **2f**, 3212-68-8; **3a**, 40441-34-7; **3b**, 136840-15-8; **3c**, 63577-30-0; **3d**, 42117-26-0; **3f**, 1073-13-8; **4a**, 136839-82-2; **4b**, 136840-16-9; **4c**, 136840-17-0; **4d**, 136840-18-1; **4f**, 25522-79-6; **5a**, 136839-83-3; **5d**, 87064-06-0; **5f**, 5923-10-4; **5g**, 32398-67-7; **6d**, 136839-84-4; **6e**, 136839-98-0; **6f**, 136839-99-1; **7f**, 136839-85-5; **8f**, 136839-86-6; **9a**, 136839-87-7; **9d**, 136840-00-1; **9f**, 136840-23-8; **9g**, 136840-22-7; **10f**, 136839-88-8; **10g**, 100972-00-7; **12d**, 136839-89-9; **14**, 136839-90-2; **15**, 136856-89-8; **17**, 136839-91-3; **18**, 136839-92-4; **19**, 136839-93-5; *cis*-**20**, 136839-94-6; *trans*-**20**, 136840-09-0; **21c**, 136856-90-1; **21d**, 136840-10-3; **23a**, 136839-95-7; **24**, 136839-91-3; **26c**, 136839-96-8; **26d**, 136840-14-7; **27**, 136839-97-9; **32**, 136840-01-2; **33**, 136840-02-3; **35**, 136840-03-4; *cis*-**36**, 136840-04-5; *trans*-**36**, 136840-19-2; *trans*-**37**, 136840-05-6; *cis*-**37**, 136840-20-5; **38**, 136840-06-7; **42**, 95841-57-9; **43**, 136840-07-8; **46**, 136840-08-9; **52a**, 136840-11-4; **52d**, 136840-21-6; sodium dimethyl malonate, 18424-76-5; dimethyl malonate, 108-59-8.

Supplementary Material Available: ^{1}H NMR spectra of all new compounds as well as X-ray parameters for cyclopropanols **14** and **32** (72 pages). Ordering information is given on any current masthead page.

The Gymnochromes: Novel Marine Brominated Phenanthroperylenequinone Pigments from the Stalked Crinoid *Gymnocrinus richeri*†

Francesco De Riccardis,‡ Maria Iorizzi,‡ Luigi Minale,*‡ Raffaele Riccio,‡ Bertrand Richer de Forges,§ and Cecile Debitus§

Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli Federico II, Via D. Montesano, 49, 80131 Napoli, Italy, and ORSTOM, Centre de Nouméa, B.P. A5, Nouméa Cedex, New Caledonia, Italy

Received February 12, 1991 (Revised Manuscript Received August 1, 1991)

Five novel brominated phenanthroperylenequinone pigments, gymnochromes A–D (**1–4**) and isogymnochrome D (**5**), were isolated from the stalked crinoid *Gymnocrinus richeri*. The structures of the compounds were inferred from their spectra (IR, UV–vis, ^{1}H and ^{13}C NMR, FABMS). The presence of both bulky hydroxy groups at positions 10 and 11 and side chains at positions 3 and 4 causes sufficient crowding to force the octacyclic phenanthroperylenequinone system into a nonplanar helical shape. This helicity generates axial chirality in the molecules. The presence of chiral carbon atoms in the side chains gives rise to diastereomers. The absolute configurations of the chiral carbons and the axial chirality of the natural pigments was inferred from CD and NMR data and by correlations made with cercosporin and other naturally occurring perylenequinones. The configurations assigned to the chiral carbons in the side chains of compounds **4** and **5** were confirmed by the results of the application of Horeau's method of kinetic resolution.

Quinoid pigments occur widely in microorganisms and plants.[1] In the animal kingdom they are found in certain insects and in echinoderms,[2] often in echinoids and crinoids. At present, four groups of polyketide-derived pigments are known to occur in crinoids:[3] linear and angular naphtopyrones; 4-acylanthraquinones; 3-alkylanthraquinones; and dimeric bianthrones, bianthraquinones and phenanthroperylenequinones.[4] Apparently, quinoid pigments were biosynthesized many millions of years ago. In support of this belief is the observation that fringelites, described by Blumer[5] as hydroxylated phenanthroperylenequinones, were found in the fossilized remains of a Jurassic crinoid (*Apiocrinus*) discovered near Fringeli in northwestern Switzerland.

We recently had the opportunity to examine a deep-water stalked fossil crinoid, *Gymnocrinus richeri*, discovered by one of us (B.R.F.) at a depth of 520 m during the CHALCAL 2 oceanographic campaign of 1986. The campaign was directed toward the exploration of the bathial zone off the coast of New Caledonia, which is particularly rich in so-called "living fossils". *Gymnocrinus richeri* is one of the best examples of those species to which it is appropriate to apply the description "living fossil".[6] The body of the live crinoid is saffron yellow, whereas its stalk is a darker yellow and the inside of its tentacles is dark yellow-green. However, within a few minutes of collection, outside salt water, the animal turns dark green.

Extraction with hexane and dichloromethane did not remove any pigment from a freeze-dried green sample. Extraction with methanol gave a dark green solution, which on very mild acidification turned violet.

Herein we describe the isolation, structure, and stereochemistry of five violet pigments [gymnochrome A (**1**), gymnochrome B (**2**), gymnochrome C (**3**), gymnochrome D (**4**) and isocymnochrome D (**5**)] which constitute a novel

† Dedicated to Professor G. B. Marini Bettolo on the occasion of his 75th birthday.

‡ University of Naples.

§ ORSTOM.

(1) Thomson, R. H. *Naturally Occurring Quinones*, 2nd ed.; Academic Press: London, 1971; *Naturally Occurring Quinones*, 3rd ed. *Recent Advances*; Chapman and Hall: London, New York, 1987.

(2) Stonik, V. A.; Eliakov, G. B. In *Biorganic Marine Chemistry*; Scheuer, P. J., Ed.; Spring Verlag: Berlin Heidelberg, 1988; Vol. 2, pp 43–86.

(3) Rideout, J. A.; Sutherland, M. D. *Aust. J. Chem.* **1985**, *38*, 793 and references cited therein.

(4) (Hydroxypropyl)propylphenanthroperylenequinone (1% of the total pigment components) was detected in extracts of *Lamprometra palmata*. It may have been formed in very small amounts by air oxidation of the structurally related bianthrone during the extraction. Dipropylphenanthroperylenequinone was found to be a very minor component in extracts of *Himerometra robustipinna*, but in this case bianthroquinones and bianthrones were not present.[3]

(5) Blumer, M. *Microkimie* **1951**, *36/37*, 1048; *Nature (London)* **1960**, *188*, 4756; *Science* **1965**, *149*, 722; *Sci. Amer.* **1976**, *234*, 35.

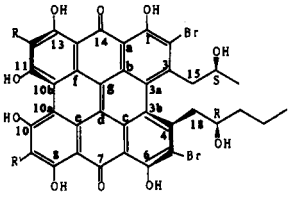
(6) Bureau, J. P.; Ameziane-Cominardi, N.; Roux, M. C. R. *Acad. Sci. Paris III*, **1987**, *305* (49), 595.

0022-3263/91/1956-6781$02.50/0

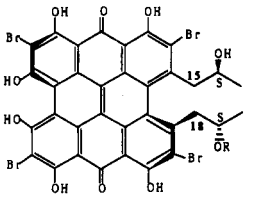
group of brominated phenanthroperylenequinones. Their discovery in a "living fossil" species further supports the belief that quinoid pigments originated in crinoids in ancient times. It has been postulated that the pigments, especially the sulfate forms, are used by the animals as a defense against predatory fishes.[7]

Results and Discussion

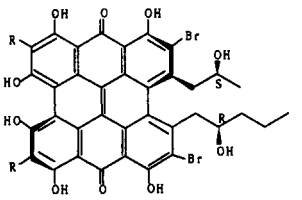
A sample of the freeze-dried crinoid was extracted successively with *n*-hexane, CH_2Cl_2, and MeOH. The methanol extracts, dark green in color, were concentrated. The residue was suspended in dilute aqueous HCl, and the suspension was extracted successively with Et_2O and *n*-BuOH. TLC of the extracts showed four violet spots, of which the major (R_f = 1) was due to gymnochromes A (1)



1, R = R' = Br; gymnochrome A
2, R = H, R' = Br or R = Br, R' = H; gymnochrome B

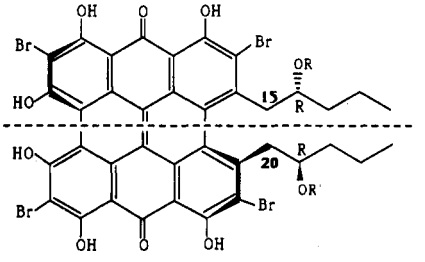


3, R = SO_3H; gymnochrome C

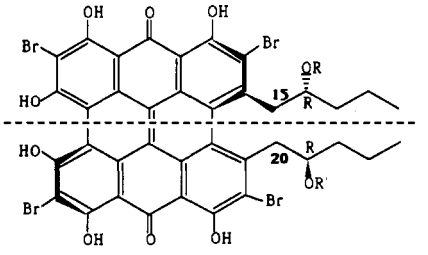


2a, R = H, R' = Br, or R = Br, R' = H

and B (2) and the minor (R_f = 0.85, 0.68, and 0.50) were due to the sulfated gymnochromes C (3) and D (4) and isogymnochrome D (5) respectively. The Et_2O extract



4, R = R' = SO_3H; gymnochrome D
4a, R = SO_3H, R' = H
4b, R = R' = H



5, R = R' = SO_3H; isogymnochrome D
5a, R = SO_3H, R' = H
5b, R = R' = H

(7) Rideout, J. A.; Smith, N. B.; Sutherland, M. D. *Experientia* **1979**, *35*, 1273.

Table I. Chemical Shifts of the Protons and Carbon Atoms of the Side Chains of Gymnochromes A (1), B (2), and C (3)[a]

position	gymnochrome A (1) and B (2)[b] δ_H	gymnochrome A (1) and B (2)[b] δ_C	gymnochrome C (3) δ_H
C_3 side chain			C_3 side chain
15	4.10 dd (11.5, 2.5), 3.70 dd (11.5, 10)	48.7	4.12 dd (11.5, 2.5), 3.70 m
16	3.75 m	68.5	3.70 m
17	−0.05 d (5)	21.5	−0.07 d (5)
C_5 side chain			C_3 side chain
18	3.95 dd[c] (13, 4), 3.75 m	48.1	4.45 dd (12.0, 4), 3.78 dd (12.0, 10.0)
19	3.75 m	73.0	4.62 m
20	1.62 m, 1.51 m	41.3	0.01 d (6.5)
21	1.42 m, 1.34 m	19.9	
22	0.94 t (7)	14.3	

[a] The ^1H NMR are of methanol-d_4 solutions recorded at 250 MHz; the spectrum of 2 was recorded at 500 MHz. Multiplicity: d = doublet; t = triplet; m = multiplet. Coupling constants reported in Hz. The ^{13}C NMR spectra are of methanol-d_4 solutions recorded at 62.9 MHz. Assignments were aided by DEPT measurements. [b] The spectra of gymnochrome A (1) and gymnochrome B (2) show virtually identical chemical shifts for the side-chain signals; these data were extracted from the spectrum of 2. [c] Distorted signal.

(which contained mainly 1 and 2) and the *n*-BuOH extract (which contained mainly the sulfated 3–5) phases were evaporated to dryness. The residues were combined and were dissolved in MeOH. Column chromatography of the solution on Sephadex LH-20 gave the sulfated gymnochromes C (3) and D (4) and isogymnochrome D (5) in the first fractions to be eluted and the sulfur-free pigments 1 and 2 in the later fractions. Small amounts of the monosulfated gymnochrome D (4a) and isogymnochrome D (5a), artifacts produced by partial hydrolysis of 4 and 5 during concentration of the still slightly acidic *n*-BuOH extracts, were also isolated. Each fraction was then purified by reversed-phase HPLC to yield gymnochrome A (1; 0.0019% by dry weight of the original freeze-dried sample of crinoid), B (2; 0.0031%), C (3; 0.0002%), D (4; 0.0017%), isogymnochrome D (5; 0.0013%), and the artifacts 4a and 5a.

Structures. The IR spectrum of the most abundant pigment, gymnochrome B (2), shows a carbonyl stretching band at low frequency (ν_{max} = 1634 cm^{-1}), characteristic of hydrogen-bonded quinone. The UV–vis spectrum (MeOH) shows maxima at 216 (ε 48 000), 233 (ε 51 500), 295 (ε 32 000), 329 (ε 27 200), 485 (ε 13 800), 553 (ε 19 800), and 596 (ε 39 300) nm, very close to those that appear in the spectra of hypericin and related phenanthroperylenequinones.[1] When dissolved in base (aqueous NH_3) the pigment yields a bright green solution. The UV–vis spectrum of the solution shows maxima at 236, 281, 358, 500, and 669 nm, characteristic of a chelated extended quinone.

The FAB (negative ion mode) mass spectrum of 2 shows a complex multiplet between *m/z* 853 and 859 (four peaks, at *m/z* 853, 855, 857, and 859 $[M - H]^-$, relative intensity 1:2.9:2.8:0.9), suggestive of the presence of three bromine atoms. The 500-MHz ^1H NMR spectrum and spin decoupling experiments identified two separate spin systems, which could be ascribed to 2′-hydroxypropyl and 2′-hydroxypentyl side chains (Table I). The remaining signal in the spectrum is an upfield aromatic singlet at ca. δ 6.50,[8] which indicates the presence of an aromatic proton located between two phenolic hydroxyl groups.[1] In addition to

(8) The chemical shift of this signal varies from δ 6.74 to 6.35 ppm in the spectra of different samples. It is also concentration dependent.

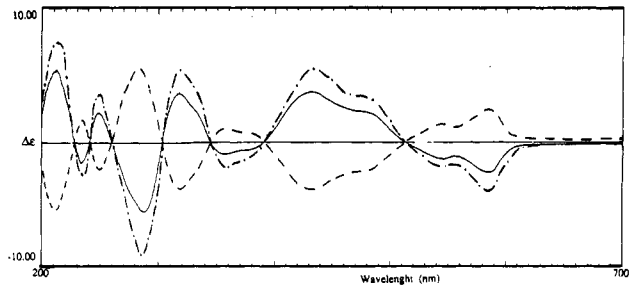


Figure 1. CD curves of gymnochrome B (-·-), gymnochrome D (—), and isogymnochrome D (--).

signals that could be assigned to 2′-hydroxypropyl and 2′-hydroxypentyl side chains, the ^{13}C NMR spectrum shows a low-field CH signal at 106.9 ppm and 27 other low-field signals due to quaternary carbon atoms. The chemical shifts of the signals are consistent with a hexahydroxy phenanthroperylenequinone system (six signals appearing between 160.4 and 172.0 ppm and assignable to aromatic *C*-OH carbons and two carbonyl carbon signals, at 184.7 and 186.4 ppm). The assignments reported in Table III are based on a comparison of the ^{13}C NMR spectrum of **2** with those of model compounds, anthraquinone emodin and 5,5′-linked bianthraquinone skyrin,[9] perylenequinones,[10] and phenanthroperylenequinones[11] and the application of known chemical shift rules.[12] Thus, both the ^{13}C NMR spectrum and the FABMS, which indicates the presence in the molecule of three bromine atoms, suggested for gymnochrome B (**2**) a molecular formula of $C_{36}H_{25}Br_3O_{10}$. The spectra and biogenetic considerations further suggest that **2** is 1,6,8,10,11,13-hexahydroxy-2,5,9-tribromo-3(or 4)-(2′-hydroxypropyl)-4(or 3)-(2′-hydroxypentyl)phenanthroperylene-7,14-quinone. That the side chains are located on the same side of the molecule (i.e., at positions 3 and 4) is suggested by analogy with gymnochrome D (**4**) and isogymnochrome D (**5**), in which the locations of the side chains have been determined (see below). It should be noted that the chemical shifts of carbons 3, 4, 3a, 3b and 10, 11, 10a, 10b, which would be expected to be sensitive to the location of the side-chains, are virtually identical in the spectra of all the compounds (Table III). Moreover, all naturally occurring phenanthroperylenequinones bear the side chains at positions 3 and 4 and hydroxyl groups at positions 10 and 11. The relative positions of the side chains, i.e., whether the C_3 chain is at C-3 and the C_5 chain is at C-4 or vice versa, remain to be established. The overlapping of the substituents at C-3 and C-4 and at C-10 and C-11 causes twisting of the ring system, as the CD (circular dichroism) curve of **2** reveals (Figure 1). Therein the strong peaks are evidently due to the inherently dissymmetric chromophore of the phenanthroperylenequinone system. As a consequence the molecule possesses axial chirality. When it was heated in pyridine at 160 °C, gymnochrome B (**2**) was partially converted into a diastereomer, "isogymnochrome B" (**2a**), which has a CD curve, the $\Delta\epsilon$ of which is opposite in sign to that of **2**. This fact implies that the two compounds are both configurational helices, one left-handed and the other right-handed. However, the

Table II. Chemical Shifts of the Protons and Carbon Atoms of the Side Chain of Gymnochrome D(4) and Isogymnochrome D (5)[a]

	gymnochrome D (4)		isogymnochrome D (5)	
position	δ_H	δ_C	δ_H	δ_C
15, 20	3.94 dd (12.5, 3.5), 3.88 dd (12.5, 7.5)	44.3	4.38 dd (12.5, 4.5), 3.91 dd (12.5, 10.0)	43.3
16, 21	4.36 m	80.1	4.50 m	77.9
17, 22	1.66 m, 1.50 m	38.7	0.56 m, 0.04 m	35.7
18, 23	1.44 m	18.9	0.83 m, 0.25 m	17.4
19, 24	0.89 t (7)	14.4	−0.05 t (7)	13.6

[a] ^1H and ^{13}C NMR spectra are of methanol-d_4 solutions, recorded at 250 and 62.9 MHz, respectively.

configurations of the side chain chiral centers cannot be modified by the thermal isomerization. The inversion of helicity[13] is accompanied by a change in the conformations of the side chains relative to the chromophore system as the change in the chemical shifts of the side chain protons show. The large upfield shift (δ −0.05 ppm, Table I) of the doublet due to the methyl group of the C_3 side chain of gymnochrome B (**2**) indicates that the group lies sufficiently above the phenanthroperylenequinone ring system to be exposed to a strong shielding effect, whereas the methyl group of the C_5 side chain, the protons of which resonate "downfield" (Table I), is outside the zone affected by the ring current. In the spectrum of the diastereomer, "isogymnochrome B", the methyl doublet is observed downfield, at δ 1.14, which implies that the C_3 side chain is outside the zone affected by the ring current, whereas the triplet due to the methyl group of the C_5 side chain resonates at high field (δ 0.07), which indicates that the C_5 side chain lies above the aromatic system (i.e., within the shielding zone). The absolute configuration [i.e., *P* (or *S*)[14] axial chirality, *S* configuration at C-16 and *R* configuration at C-19] of gymnochrome B (**2**) was inferred from the experimental evidence and the arguments given below.

Gymnochrome A (**1**) is related to **2** in that an additional bromine atom is present at position 12. The UV–vis spectrum closely resembles that of gymnochrome B. The IR spectrum reveals an absorption band at 1635 cm^{-1}, characteristic of a chelated quinone. The FABMS (negative ion mode) shows a complex multiplet between *m/z* 931 and 939 (peaks at *m/z* 931, 933, 935, 937, and 939 [M − H]$^-$, relative intensity 1:3.8:6:4:1.2) suggestive of the presence of four bromine atoms, and is consistent with a molecular formula of $C_{36}H_{24}Br_4O_{10}$. In the ^1H NMR spectrum of **1**, signals due to 2′-hydroxypropyl and 2′-hydroxypentyl side chains are still observed. However, the aromatic singlet at ca. δ 6.50, that in the spectrum of **2** was assigned to H-12, is missing. Likewise, in the ^{13}C NMR spectrum of **1**, the low-field *CH* signal that is present at δ 106.9 in the spectrum of **2** is missing and a quaternary carbon atom signal at 103.7 ppm is present. The positions of the remaining signals in the spectrum are virtually identical with those in the spectrum of **2**. $\Delta\epsilon$ of the CD spectrum of **1** is of the same sign as that of **2**, which implies that the phenanthroperylenequinone chromophore of both molecules possesses the same axial chirality.

The UV–vis spectrum of gymnochrome D (**4**) is superimposable on those of gymnochromes A (**1**) and B (**2**). The IR spectrum still shows a carbonyl band at 1631 cm^{-1} (chelated quinone) but also shows an intense band at 1240

(9) Gill, M.; Gimenez, A., McKenzie, R. W. *J. Nat. Prod.* **1988**, *51*, 1251.
(10) (a) Kurobane, I.; Vining, L. C.; McInnes, A. G.; Smith, D. G.; Walter, J. A. *Can. J. Chem.* **1981**, *59*, 422. (b) Iida, T.; Kobayashi, E.; Yoshida, M.; Sano, H. *J. Antibiotics* **1989**, *42*, 1475.
(11) Brockmann, H.; Lackner, H. *Tetrahedron Lett.* **1979**, 1575.
(12) Stothers, G. B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York, San Francisco, London, 1972.
(13) Equilibration of the isomers is slow, even at elevated temperatures. Also, at the temperature required for thermal inversion of the helix of gymnochrome B (160 °C), partial degradation occurs. For these reasons it was not possible equilibrate the diastereomers.
(14) Cahn, R. S.; Ingold, C. K.; Prelog, V. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 385.

Table III. ^{13}C NMR Chemical Shifts of the Aromatic Carbons of Gymnochrome A (1), B (2), D (4), and Isogymnochrome D (5)[a]

aromatic carbons	1	2	4	5
1,6	160.7, 160.6	160.4, 160.4	160.5	161.0
2,5	116.4, 116.6	116.1, 116.2	116.6	117.0
3,4	146.4, 144.2	145.9, 143.9	144.8	144.4
3a,3b	126.3, 126.7	126.5, 127.0	126.9	126.5
6a,14a	111.0, 110.6	111.0, 110.9	111.2	110.7
7,14	185.7, 185.3	186.4, 184.7	186.5	185.9
7a,13a	104.0, 103.7	104.6, 103.2	104.6	104.0
8,13	165.7, 165.6	165.8, 171.0	165.9	166.2
9,12	103.7, 103.4	103.4, 106.9	103.5	104.0
10,11	169.4, 169.3	169.4, 172.0	170.1	170.8
10a,10b	118.8, 118.8	118.7, 119.7	119.4	119.6
14b,14c	122.6, 122.8	122.7, 122.9	123.4	122.7
14d,14g	127.3, 127.3	128.6, 127.0	128.1	127.6
14f,14e	123.8, 123.5	123.7, 123.5	124.5	123.1

[a] The spectra are of methanol-d_4 solutions recorded at 62.9 MHz.

cm^{-1} (sulfate). The FABMS (negative ion mode) shows a quintet of molecular ion peaks at m/z 1119, 1121, 1123, 1125, 1127 ($[M - H]^-$) indicative of the presence of four bromine atoms in the compound. A molecular formula of $C_{38}H_{26}Br_4O_{10}(SO_3H)_2$ is inferred from the FABMS, the ^{13}C NMR spectrum (Tables II and III), and chemical data, which indicate the presence of two sulfate groups. Solvolysis of **4** in dioxane/pyridine mixture afforded a sulfur-free derivative (**4b**). Its FABMS shows pseudomolecular ion peaks at m/z 959, 961, 963, 965, and 967, each of which is 160 mass units less than the corresponding peak in the FABMS of **4**. Gymnochrome D (**4**) is a chiral molecule but is symmetric by virtue of the presence of a C_2 axis of symmetry in its structure, as the symmetry of its NMR spectra, which show only half of the expected signals, shows. The ^1H NMR spectrum (Table II) shows only one proton spin system, which can be assigned to a 2′-oxygenated pentyl side chain, whereas the ^{13}C NMR spectrum (Tables II and III) shows only 19 carbon resonances, due to one methyl, three methylene, one methine, and fourteen quaternary carbon atoms. The chemical shift of the >*C*H(O) carbon signal (80.1 ppm) in the spectrum of **4**, when compared with the chemical shift of the signal due to >*C*(19)HOH (73.0 ppm) in the spectra of **1** and **2**, strongly suggests that the sulfate groups are located in the side chains. Moreover, the upfield shift of the oxymethine proton signal, from δ 4.36 (m) in the spectrum of **4** to 3.63 ppm in the spectrum of sulfur-free **4b**, further indicates that the side-chain hydroxyl groups are sulfated. The helicity (axial chirality) of gymnochrome D (**4**) must be the same as that of gymnochromes A and B, because $\Delta\epsilon$ of its CD spectrum (Figure 1) is of the same sign as those of the gymnochromes A and B. Moreover, the presence of a C_2 axis of symmetry requires that the chiral carbon atoms of the side chains must be of the same absolute configuration. The 2′-sulfoxypentyl chains of **4** preferentially orient themselves outside the zone affected by the ring current because the signals due to the terminal methyl group protons and the C-17 and C-18 methylene protons are observed "downfield" (Table II).

Isogymnochrome D (**5**) is isomeric with gymnochrome D (**4**). They show identical FAB mass spectra and virtually identical UV–vis and IR spectra. In contrast, their ^1H NMR spectra are substantially different. In the spectrum of **5**, the signals due to the terminal methyl group protons and the C-17 and C-18 methylene protons of the chain, are located far upfield, at δ –0.05 (t), 0.04 (m)–0.56 (m), and 0.25 (m)–0.83 (m), respectively. The ^{13}C NMR spectra of the two isomers (Tables II and III) are also significantly different. The presence of only half of the expected signals in both the ^1H and ^{13}C NMR spectra (Tables II and III) indicates that isogymnochrome D (**5**) also possesses a symmetric structure, which, like gymnochrome D (**4**), possesses a C_2 axis of symmetry. However, $\Delta\epsilon$ of the CD spectrum (Figure 1) of isogymnochrome D (**5**) is opposite in sign to that of gymnochrome D (**4**). These findings suggest that isogymnochrome D (**5**) is a diastereomer of gymnochrome D (**4**), the helicity of which is opposite to that of **4**.

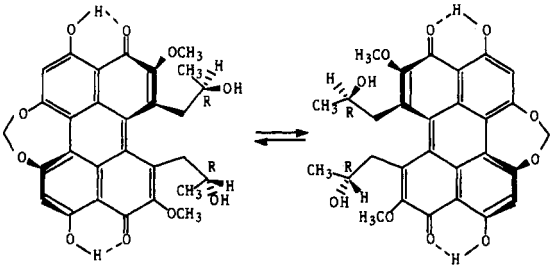
Solvolysis of **5** in dioxane/pyridine afforded sulfur-free **5b** (FABMS, m/z 959, 961, 963, 965, and 967; $\Delta\epsilon$ of the CD curve of the same sign as that of **5**). Heating **5b** in pyridine at 160 °C partially converted it to sulfur-free gymnochrome D (**4b**). Analogously, heating **4b** in pyridine at 160 °C gave some **5b**. Because the configuration of the side-chain chiral centers cannot be modified by thermal isomerization, these findings definitively established that isogymnochrome D (**5**) is a diastereomer of gymnochrome D (**4**). The difference between the two is only in the helicity of the phenanthroperylenequinone ring. Because heating interconverts the two diastereomers, the two chiral carbons of the side chains of gymnochrome D (**4**) and isogymnochrome D (**5**) must be of the same configuration. It should be noted, once again, that inversion of helicity is accompanied by a change in the orientations of the side-chains, as shown by ^1H NMR (Table II). In gymnochrome D (**4**) the chains preferentially assume conformations outside the zone affected by the ring current, whereas in isogymnochrome D (**5**) the large upfield shifts of the signals due to the terminal methyl group protons and of the C-18 and C-17 methylene protons (Table II) implies that the side chains are located above the aromatic system (i.e., inside shielding zone).

As mentioned above, partial hydrolysis of **5** occurred during its isolation and yielded the monosulfated derivative **5a** (FABMS, m/z 1039, 1041, 1043, 1045, 1047). The 500-MHz ^1H NMR spectrum of **5a** reveals separate signals due to a 2′-hydroxypentyl and a 2′-sulfoxypentyl side chain. Spin decoupling experiments identified two ABX spin systems for the protons of the $ArCH_2CH(O)$– groupings, with signals at δ 4.48, 3.91 (each dd, J = 12.5, 5 and 12.5, 4 Hz, CH_2) and 4.58 (m) (CH), ($ArCH_2CH(OSO_3H)$–) and 4.04, 3.84 (each dd, J = 12.5, 5 and 12.5, 8 Hz, CH_2) and 3.60 (m) (CH), ($ArCH_2CH(OH)$–) ppm. In a NOE experiment, irradiation of the signal at δ_H 4.48 ppm resulted in the enhancement of the double doublet at δ 4.04, whereas irradiation at δ 4.04 enhanced the double doublet at δ 4.48. The results of this experiment indicated that the side chains are in close proximity and thus gave support to the belief that the side chains are located at positions 3 and 4.

Gymnochrome C (**3**) is a very minor component of the mixture of phenanthroperylenequinones. Its UV–vis spectrum is virtually identical with those of the gymnochromes previously described. Its FABMS shows a quintet of pseudomolecular ion peaks at m/z 983, 985, 987, 989, 991. The ^1H NMR spectrum (Table I) shows two separate proton spin systems, due to two C_3 side chains. The chemical shifts of the methine protons (δ 3.70 (m) and 4.62 (m), respectively) indicate, in agreement with the FABMS, that one of the side-chain hydroxyl groups is sulfated. In addition to a carbonyl stretching band at 1632 cm^{-1}, the IR spectrum shows an intense band at 1258 cm^{-1} (sulfate). Solvolysis of **3** in dioxane/pyridine afforded a sulfur-free derivative, **3a**. Its FABMS (negative ion mode) shows peaks at m/z 903, 905, 907, 909, and 911. Each is 80 mass units less than the corresponding peak in the FABMS of **3**. Its ^1H NMR spectrum shows half of the expected signals

(typical of a symmetric molecule). $\Delta\epsilon$ of the CD curve of gymnochrome C (3) is of the same sign as those of gymnochromes A, B, and D, which implies that 3 possesses the same axial chirality as 1, 2, and 4. The upfield position, δ −0.07 and 0.01 ppm, of the two doublets due to the two methyl group protons in the ^{1}H NMR spectrum implies that both of the side chains are oriented above the phenanthroperylenequinone system.

Stereochemistry. Recently, a group of natural pigments, produced by a wide variety of molds, was intensively studied because of their intriguing stereochemistry. The molecules are axially dissymmetric because the perylenequinone ring system has been forced into a nonplanar helical shape.[15] Cercosporin (6) is representative.



6 7

When heated in various solvents, cercosporin (6) isomerizes into a diastereomer, isocercosporin (7), which possesses the opposite helicity; i.e., $\Delta\epsilon$ of its CD spectrum is opposite in sign to that of 6.[16a] Inversion of the helix is accompanied by a change in the orientation of the side chains because, in the ^{1}H NMR spectrum of 6, the doublet due to the methyl group protons appears at δ 0.60 whereas in the spectrum of 7 it is shifted downfield to δ 0.96. X-ray crystallographic analysis of a natural ester of cercosporin established the helicity of the ring (*R* axial chirality (or *M*)) and confirmed the *R* configuration of the chiral carbons of the side chains.[16b]

These results enabled Arnone et al. to establish the axial chirality possessed by other perylenequinones, based on their CD curves and that of cercosporin.[17] After inspection of the configurations and the NMR spectra of each pair of isomers, it was concluded that the orientation of the side chains is regulated by both the helicity of the ring system and the configurations of the chiral centers in the side chains.[15] That is, when the helicity of the ring system and the configuration of the side-chain chiral carbon are *M* and *R* (or *P* and *S*), respectively, the side chain is oriented above the ring system (i.e., inside shielded zone), whereas when the helicity of the ring system and the configuration of the side-chain chiral carbon are *M* and *S* (or *P* and *R*), respectively, the side chain is directed away from the aromatic ring (i.e., outside shielded zone).

Because the CD curves of the gymnochromes are very similar in shape to those of cercosporin and isocercosporin,[16a] it was assumed that the sign of $\Delta\epsilon$ of the CD curve is indicative of the helicity of the ring system. Thus, because $\Delta\epsilon$ of the CD curves of gymnochromes A (1), B (2), C (3), and D (4) are opposite in sign to that of cercosporin, those four compounds are believed to be *P* isomers (i.e., they possess *S* axial chirality[14]), in contrast to cercosporin,[16] which is an *M* isomer. On the other hand, because $\Delta\epsilon$ of the CD curve of isogymnochrome D is of the same sign as that of cercosporin, that compound is believed to be an *M* isomer, like cercosporin[16] is. Furthermore, based on correlation between the conformations of the side chains, the ^{1}H NMR and CD data, and by analogy with cercosporin,[16] phleichromes,[17a] and other perylenequinones,[17b,c] the configurations of the chiral carbons of the side chains are believed to be those depicted in structures 1–5.

An attempt was also made to obtain confirmation of the configurations assigned to the chiral carbons of the side chains. In both gymnochrome D (4) and isogymnochrome D (5), the two chiral carbons of the C_5 side chains must be of the same absolute configuration. Thus, it was decided to determine the configurations of the two carbon atoms by applying the empirical approach of Horeau[18] to appropriate derivatives in which all the phenolic hydroxyl groups were protected. Diazomethane methylation of the sulfur-free gymnochrome D (**4b**) gave a mixture of methyl ethers, from which the major component was isolated and was shown to be a hexamethyl derivative (FABMS, *m/z* 1043, 1045, 1047, 1049, 1051 $[M - H]^-$). Its ^{1}H NMR spectrum shows only three methoxyl singlets, at δ 3.53, 4.23, and 4.24 ppm, half of the expected signals as is typical of a symmetric molecule. Treatment with alkali did not shift the positions of the UV absorptions, which indicated that all the phenolic hydroxyl groups had been methylated. The hexamethyl ether was then allowed to react with D,L-phenylbutyric anhydride, according to the gas chromatographic modification of Horeau's method.[19] The relative proportions of the (+)-(*R*)-α-phenylethylamides of (−)-(*R*)- and (+)-(*S*)-α-phenylbutyric acid were determined and showed a peak increment for the *S* acid (+4%). If it is assumed that the aryl-CH_2 group (in which aryl is the entire quinone ring system) is larger than a propyl group, the absolute configuration of both carbons is *R*, which is in agreement with the assignment made on the basis of CD and ^{1}H NMR data and by analogy with the perylenequinone pigments. The same procedure was then applied to the sulfur-free isogymnochrome D (5). As expected, the absolute configuration of the chiral side-chain carbon was found to be *R*.

The green color of the animals suggests that the pigments are present as phenoxide salts, probably in the form of metal complexes. Indeed, atomic absorption spectroscopy of a partially purified sample of the green form of gymnochromes A and B revealed the presence of significant amounts of zinc. The yellow color of the live animals suggests that the phenanthroperylenequinone system of the gymnochromes may have been formed, at least in part, by oxidation and exposure to sunlight of structurally related bianthrones or other dimeric precursors. Cameron et al.[20] have shown that the autoxidation of emodin 10,10′-bioanthrone in an acetone solution containing a catalytic amount of hydroxide causes progressive darkening of the solution and is accompanied by the production of hypericin. Chelation with Zn could also catalyze such oxidation. How the stereochemistry of the bianthrones or structurally related dimeric precursors are related to the helicity of the phenanthroperylenequinones is also an interesting question. An elegant, recently reported model

(15) Weiss, U.; Merlini, L.; Nasini, G. In *Fortschr. Chem. Org. Naturstoffe*; Springer Verlag: Berlin, 1987; Vol. 52, pp 1–71.
(16) (a) Lousberg, R. J. J. Ch.; Weiss, U.; Salemink, C. A.; Arnone, A.; Merlini, L.; Nasini, G. *J. Chem. Soc., Chem. Commun.* **1971**, 1463. (b) Nasini, G.; Merlini, L.; Andreetti, G. D.; Bocelli, G.; Sgarabotto, P. *Tetrahedron* **1982**, *38*, 2787.
(17) (a) Arnone, A.; Camarda, L.; Nasini, G.; Merlini, L. *J. Chem. Soc., Perkin Trans. 1* **1985**, 1387. (b) Arnone, A.; Assante, G.; Di Modugno, V.; Merlini, L.; Nasini, G. *Phytochemistry* **1988**, *27*, 1675. (c) Arnone, A.; Assante, G.; Merlini, L.; Nasini, G. *Gazz. Chim. Ital.* **1989**, *199*, 557.
(18) Horeau, A. *Tetrahedron Lett.* **1961**, 506.
(19) Brooks, C. J. W.; Gilbert, J. D. *J. Chem. Soc., Chem. Commun.* **1973**, 194.
(20) Cameron, D. W.; Edmonds, J. S.; Raverty, W. D. *Aust. J. Chem.* **1976**, *29*, 1535.

study by Feringa and Wynberg[21] showed that the oxidative coupling of the chiral tetrahydronaphtol moieties proceeded with complete stereospecificity (in the helical sense) and stereoselectivity.

Experimental Section

The purity of the title compounds was judged to be >90% by ^1H NMR analysis. The ^1H NMR spectra of all the title compounds and the ^{13}C NMR spectra of compounds **2** and **4** are available as supplementary material.

Extraction and Isolation of Pigments. Samples of the crinoid *Gymnochrinus richeri*, collected in 1987 off the coast of New Caledonia at a depth of 520 m, were freeze-dried and dispatched to the laboratory. Reference samples were deposited at the Museum National d'Histoire Naturelle in Paris, 57, rue Cuvier. A sample (2.6 kg) was extracted successively with *n*-hexane and CH_2Cl_2 (Soxhlet extraction) and then with MeOH (2 × 5 L) at room temperature. The dark green methanolic extracts were concentrated in vacuo. The residue (40 g) was divided into 4-g portions. Each was suspended in aqueous HCl (50 mL of H_2O + 50 mL of 0.3 N aqueous HCl). The suspension was then extracted successively with Et_2O and *n*-BuOH. The extracts were washed with water and were concentrated in vacuo. The residues were combined, and the whole was purified by column chromatography on 100 g of Sephadex LH-20 (methanol). Fractions (8 mL) were collected and were analyzed by TLC on SiO_2 (*n*-BuOH/AcOH/H_2O (12:3:5)). Fractions 37–43 contained gymnochrome D (**4**), fractions 46–53 contained isogymnochrome D (**5**), fractions 54–69 contained gymnochrome C (**3**), fractions 70–75 contained a mixture of the monosulfated **4a** and **5a**, fractions 76–100 contained gymnochrome B (**2**) and a small amount of gymnochrome A (**1**), and fractions 101–115 contained gymnochrome A (**1**) and a small amount of gymnochrome B (**2**).

Each fraction was further purified by HPLC (column, 7.8 mm i.d. × 30 cm C_{18} μ-Bondapak; flow rate, 5 mL/min). MeOH/H_2O (65:35), eluted the sulfur-free pigments (**1**) and (**2**); 50:50 MeOH/H_2O eluted the monosulfated pigments **3**, **4a** and **5a**; and 40:60 MeOH/H_2O eluted the disulfated pigments **4** and **5**. The amount of each pigment is that isolated from 4 g of crude MeOH extract.

The ^1H and ^{13}C NMR data are shown in Tables I–III. FABMS were recorded in the negative ion mode.

Gymnochrome A (1): 4.9 mg; UV (MeOH) λ_{max} 216 (sh), 235, 256, 298, 332, 500, 553 and 597 nm (ϵ = 41 000, 44 000, 42 000, 31 300, 24 000, 12 700, 17 000, and 33 800); CD (MeOH, c = 5.72 × 10^{-4} g/mL): 212 ($\Delta\epsilon$ = +8.44), 233 ($\Delta\epsilon$ = −4.42), 250 ($\Delta\epsilon$ = +5.90), 290 ($\Delta\epsilon$ = −13.9), 318 ($\Delta\epsilon$ = +8.24), 358 ($\Delta\epsilon$ = −1.98), 380 (sh) ($\Delta\epsilon$ = −0.96), 435 ($\Delta\epsilon$ = +7.71), 477 ($\Delta\epsilon$ = +4.56), 542 ($\Delta\epsilon$ = −2.70), and 587 ($\Delta\epsilon$ = −5.16) nm; IR (KBr) 1635, 1240, 1128 cm^{-1}; FABMS (assignment, relative intensity) m/z 931, 933, 935, 937, 939 [(M − H)$^-$, 1:3.8:6:4:1.2].

Gymnochrome B (2): 8.1 mg; UV (MeOH) λ_{max} 216, 233, 295 (sh), 329, 485, 553, and 596 nm (ϵ = 48 000, 51 500, 32 000, 27 200, 13 800, 19 800, and 39 300); CD (MeOH, c = 2.75 × 10^{-4} g/mL) reported in Figure 1; IR (KBr) 1634, 1240, 1125 cm^{-1}; FABMS (assignment, relative intensity) m/z 853, 855, 857, 859 [(M − H)$^-$, 1:2.9:2.8:0.9].

Gymnochrome C (3): 0.5 mg; UV (MeOH) λ_{max} 215, 234, 297, 338, 500, 553, and 597 nm (ϵ = 36 800, 34 000, 21 000, 15 700, 8500, 8000, and 19 000); CD (MeOH, c = 5.36 × 10^{-4} g/mL): 210 ($\Delta\epsilon$ = +2.87), 233 ($\Delta\epsilon$ = −0.54), 250 ($\Delta\epsilon$ = +1.89), 290 ($\Delta\epsilon$ = −3.44), 318 ($\Delta\epsilon$ = +1.96), 358 ($\Delta\epsilon$ = −0.61), 380 ($\Delta\epsilon$ = −0.28), 435 ($\Delta\epsilon$ = +1.96), 477 ($\Delta\epsilon$ = +1.15), 542 ($\Delta\epsilon$ = −0.82), and 587 ($\Delta\epsilon$ = −1.47) nm; IR (KBr) 1632, 1258, 1128 cm^{-1}; FABMS (assignment, relative intensity) m/z 983, 985, 987, 989, 991 [(M − H)$^-$, 1:3.6:5.6:3.8:1].

Gymnochrome D (4): 4.5 mg; UV (MeOH) λ_{max} 216, 233, 298, 326, 500, 553, and 598 nm (ϵ = 39 600, 39 600, 24 400, 22 800, 11 500, 12 700, and 24 700); CD (MeOH, c = 4.1 × 10^{-4} g/mL) reported in Figure 1; IR (KBr) 1631, 1240, 1129 cm^{-1}; FABMS (assignment, relative intensity) m/z 1119, 1121, 1123, 1125, 1127 [(M − H)$^-$, 1:3.9:5.8:4.2:1.2].

Isogymnochrome D (5): 3.0 mg; UV (MeOH) λ_{max} 218, 234, 297, 338, 500, 553, and 597 nm (ϵ = 44 600, 44 000, 28 700, 24 200, 12 400, 15 200, and 29 000); CD (MeOH, c = 3.3 × 10^{-4} g/mL) reported in Figure 1; IR (KBr) 1630, 1238, 1128 cm^{-1}; FABMS (assignment, relative intensity) m/z 1119, 1121, 1123, 1125, 1127 [(M − H)$^-$, 1:4.0:5.6:4.1:1.1].

Monosulfated gymnochrome D (4a): 1.0 mg; UV (MeOH) λ_{max} 216, 234, 298, 328, 500, 553, and 597 nm (ϵ = 40 000, 40 000, 25 400, 23 800, 11 900, 13 600, and 25 700); CD (MeOH, c = 6.16 × 10^{-4} g/mL); 212 ($\Delta\epsilon$ = +4.61), 233 ($\Delta\epsilon$ = −1.43), 250 ($\Delta\epsilon$ = +1.84), 290 ($\Delta\epsilon$ = −4.7), 318 ($\Delta\epsilon$ = +3.28), 358 ($\Delta\epsilon$ = −0.71), 374 (sh) ($\Delta\epsilon$ = −0.51), 435 ($\Delta\epsilon$ = +3.28), 477 ($\Delta\epsilon$ = +1.95), 542 ($\Delta\epsilon$ = −1.13), and 587 ($\Delta\epsilon$ = −2.05); FABMS (assignment, relative intensity) m/z 1061, 1063, 1065, 1067, 1069 [(M − H of the Na salt)$^-$, 30], 1039, 1041, 1043, 1045, 1047 [(M − H)$^-$, 1.0:3.8:5.7:3.9:1.5, 30], 959, 961, 963, 965, 967 [(M − H − SO_3)$^-$, 60], 941, 943, 945, 947, 949 [(M − H − H_2SO_4)$^-$, 100]; ^1H NMR (CD_3OD) δ 0.87 and 0.89 (each t, J = 6.2 and 5.5 Hz, CH_3), 1.32–1.66 (m's, CH_2), 3.60 (2 H, broad signal), 3.90 (3 H, complex signal), 4.34 (m, >C*H*(OSO_3H)) ppm.

Monosulfated isogymnochrome D (5a): 0.7 mg; UV (MeOH) λ_{max} 218, 235, 298, 328, 500, 554, and 598 nm (ϵ = 43 500, 43 000, 28 100, 23 200, 11 900, 14 600, and 28 600); CD (MeOH, c = 6.4 × 10^{-4} g/mL) 212 ($\Delta\epsilon$ = −4.26), 233 ($\Delta\epsilon$ = +1.00), 250 ($\Delta\epsilon$ = −3.65), 290 ($\Delta\epsilon$ = +5.64), 318 ($\Delta\epsilon$ = −3.3), 358 ($\Delta\epsilon$ = +0.7), 375 (sh) ($\Delta\epsilon$ = +0.2), 435 ($\Delta\epsilon$ = −3.37), 477 ($\Delta\epsilon$ = −2.18), 542 ($\Delta\epsilon$ = +0.99), and 587 ($\Delta\epsilon$ = +1.88); FABMS (assignment, relative intensity) m/z 1061, 1063, 1065, 1067, 1069 [(M − H of the Na salt)$^-$, 25], 1039, 1041, 1043, 1045, 1047 [(M − H)$^-$, 1.0:4.2:6.1:4.6:1.5, 20], 959, 961, 963, 965, 967 [(M − H − SO_3)$^-$, 25], 941, 943, 945, 947, 949 [(M − H − H_2SO_4)$^-$ 100]; 500-MHz ^1H NMR (CD_3OD) δ −0.12 and 0.17 (each t, J = 7 and 6 Hz, CH_3), 0.3–1.66 (m's, CH_2), 3.60 (m, 21-H), 3.84 (dd, J = 12.5, 8 Hz, 20-H), 3.91 (dd, J = 12.5, 4 Hz, 15-H), 4.04 (dd, J = 12.5, 5 Hz, 20-H′), 4.48 (dd, J = 12.5, 5 Hz, 15-H′), 4.58 (m, 16-H) ppm.

A portion of the methanolic extract (4 g) was applied directly to a column of Sephadex LH-20 (100 g) and was eluted with MeOH. Fractions (8 mL) were collected. The first fractions contained green pigments. The green-colored fractions 24–37, which contained the ionic forms of gymnochrome A (**1**) and B (**2**), were analyzed by atomic absorption spectroscopy, which showed the presence of 0.054 mg of Zn. The amount of pigments (estimated by UV analysis of the mixture after acidification) was 0.4 mg.

Solvolysis of Gymnochromes C (3), D (4), and Isogymnochrome D (5). A solution of **4** (10 mg), pyridine (500 μL), dioxane (500 μL), and H_2O (10 μL) was heated in a stoppered reaction vial at 140 °C for 3 h. After the mixture cooled, H_2O (5 mL) was added and the solution was extracted with Et_2O (2 × 4 mL). The combined extracts were evaporated to dryness and the residue was subjected to HPLC (30 cm × 3.9 mm i.d. C_{18} μ-Bondapak column). Elution with 72:28 MeOH/H_2O gave sulfur-free **4b**: UV (MeOH) λ_{max} 234 (sh), 257 (sh), 298, 328, 500, 554, and 598 nm (ϵ = 56 000, 48 000, 36 000, 30 600, 11 500, 14 400, and 27 500); CD (MeOH, c = 6.80 × 10^{-4} g/mL) 212 ($\Delta\epsilon$ = +4.03), 233 ($\Delta\epsilon$ = −1.54), 250 ($\Delta\epsilon$ = +2.06), 290 ($\Delta\epsilon$ = −4.89), 318 ($\Delta\epsilon$ = +3.17), 358 ($\Delta\epsilon$ = −0.69), 380 ($\Delta\epsilon$ = −0.51), 435 ($\Delta\epsilon$ = +3.17), 477 ($\Delta\epsilon$ = +1.80), 542 ($\Delta\epsilon$ = −1.20), and 587 ($\Delta\epsilon$ = −1.97) nm; FABMS (assignment, relative intensity) m/z 959, 961, 963, 965, 967 [(M − H)$^-$, 1.0:3.8:5.8:3.7:1.1]; ^1H NMR (CD_3OD) δ 0.89 (3 H, t, J = 7 Hz), 1.34–1.62 (4 H, m's, CH_2), 3.63 (2 H, complex m), 3.89 (1 H, broad d, J = 12 Hz).

Isogymnochrome D (**5**, 5 mg) was desulfated in an identical manner to give **5b**: UV (MeOH) λ_{max} 234 (sh), 257 (sh), 299, 332, 498, 553, and 597 nm (ϵ = 45 200, 41 000, 28 300, 22 500, 21 100, 15 300, and 29 000); CD (MeOH, c = 5.92 × 10^{-4} g/mL) 212 ($\Delta\epsilon$ = −5.54), 233 ($\Delta\epsilon$ = +0.89), 250 ($\Delta\epsilon$ = −3.67), 290 ($\Delta\epsilon$ = +6.16), 318 ($\Delta\epsilon$ = −4.06), 358 ($\Delta\epsilon$ = +0.69), 380 (sh) ($\Delta\epsilon$ = +0.19), 435 ($\Delta\epsilon$ = −4.04), 477 ($\Delta\epsilon$ = −2.36), 542 ($\Delta\epsilon$ = +1.18), and 587 ($\Delta\epsilon$ = +2.18). FABMS (assignment, relative intensity) m/z 959, 961, 963, 965, 967 [(M − H)$^-$ 1.0:3.8:5.8:4.0:1.1]; ^1H NMR (CD_3OD) δ 0.12 (3 H, t, J = 7 Hz), 0.24–0.94 (4 H, m's, CH_2), 3.60 [m, >CH(OH)], 3.86 (dd, J = 12.5, 8 Hz) −4.02 (dd, J = 12.5, 5 Hz, ArCH_2CHOH–).

Gymnochrome C (**3**, 2 mg) was desulfated in an identical manner to give **3a**: FABMS m/z 903, 905, 907, 909, 911 [(M − H)$^-$, 1.0:3.6:5.8:3.8:0.8], ^1H NMR (CD_3OD) δ −0.08 (3 H, d, J = 5 Hz, CH_3), 3.70 (2 H, m), 4.10 (1 H, dd, J = 12.5, 3 Hz).

Methylation of the Desulfated Pigments 4b and 5b. To a solution of **4b** (5 mg) in 4:1 acetone/MeOH (2.5 mL) was added

(21) Feringa, B.; Wynberg, H. *J. Org. Chem.* **1981**, *46*, 2547.

ethereal CH_2N_2 (5 mL). The mixture was left at room temperature for 2 h. The solvent was evaporated and the residue was subjected to HPLC (30 cm × 3.9 mm i.d. C_{18} μ-Bondapak column). Elution with 80:20 MeOH/H_2O gave two main compounds. The first eluted (the major product) was collected and was analyzed by FABMS: M–H peaks at m/z 1043, 1045, 1047, 1049, 1051 (quintet); ^1H NMR (CD_3OD) δ 0.89 (t, J = 7 Hz), 3.53, 4.23 and 4.24 (each s, $-OCH_3$); UV (MeOH) λ_{max} 221, 277, 316, and 451 nm (ε 13 000, 7900, 6000, and 5300).

Compound **5b** (5 mg) was methylated with CH_2N_2 in a similar manner. HPLC as described above gave four compounds. The major (first eluted) compound was collected and was analzyed by FABMS: $[M - H]^-$ ions at m/z 1043, 1045, 1047, 1049, 1051 (quintet); ^1H NMR (CD_3OD) δ_H 0.23 (t, J = 7 Hz), 3.62, 4.23 and 4.24 (each s, $-OCH_3$); UV (MeOH), λ_{max} 221, 277, 316, and 451 nm (ε 12 000, 13 000, 7900, and 6000).

Isomerization of Gymnochrome B (2). A pyridine (0.5 mL) solution of gymnochrome B (8 mg) was heated at 160 °C for 20 h. The reaction was monitored by TLC (*n*-BuOH/$CHCl_3$ (1:1)). Evaporation of the pyridine gave a mixture of gymnochrome B (**2**) and its isomer, isogymnochrome B (**2a**). Purification by HPLC (30 cm × 3.9 mm i.d. C_{18} μ-Bondapak column; MeOH/H_2O (65:35)) gave pure gymnochrome B (**2**) and a mixture of isogymnochrome B **2a** and a small amount of **2**. The latter was analyzed without further purification. In addition to signals due to **2**, the ^1H NMR spectrum of the mixture showed signals at δ 1.14 (d, J = 5 Hz) and 0.07 (t, J = 7 Hz) due to the methyl protons of the C_3 and C_5 chain, respectively, characteristic of **2a**; other signals appeared at δ 4.10 and 3.70 [$ArCH_2CH(OH)-$] and δ 0.10 and 1.6 (methylene protons at C-20 and C-21 of the C_5 chains); CD (MeOH, c = 4.21 × 10^{-4} g/mL): 212 (Δε = −4.20), 233 (Δε = +2.21), 250 (Δε = −2.90), 290 (Δε = +7.03), 318 (Δε = −4.10), 358 (Δε = +1.01), 380 (sh) (Δε = +0.45), 435 (Δε = −3.8), 477 (Δε = −2.25), 542 (Δε = +1.35), and 587 (Δε = +2.62). The lower intensity of the CD spectrum is consistent with the presence of **2** (Δε of the CD curve of **2** is opposite in sign to that of **2a**).

Isomerization of 4b to Give 5b. A pyridine solution of sulfur-free gymnochrome D (**4b**, 8 mg) was heated at 160 °C. The reaction was monitored by TLC (*n*-BuOH/$CHCl_3$ (1:1)). After 20 h, the pyridine was evaporated and the residue was subjected to HPLC (30 cm × 3.9 mm i.d. C_{18} μ-Bondapak). Elution with 72:28 MeOH/H_2O gave **4b** and **5b**.

Isogymnochrome D (**5b**) was isomerized to **4b** in a similar manner.

Reaction with (±)-2-Phenylbutyric Anhydride. (±)-2-Phenylbutyric anhydride (0.5 μL) was added to a pyridine solution (30 μL) of the hexamethyl derivative (0.45 mg) formed by the methylation of **4b**. The solution was warmed at 55 °C for 6 h in a sealed vial. A parallel reaction was performed with cyclohexanol. (+)-(*R*)-α-Phenylethylamine (0.58 μL) was added to both solutions. After 30 min, the solutions were diluted with EtOAc (40 μL) and samples were analyzed by GLC–MS (0.20 mm × 25 m fused silica capillary column coated with a 0.33 μm thick film of HP-5 (cross-linked phenyl methyl silicone, 5%) temperature programmed from 120 to 220 °C at 5.00 °C/min).

The relative proportions of the amides of (−)-(*R*)- and (+)-(*S*)-α-phenylbutyric acid (retention times 28 and 29 min, respectively) were indicated by the areas of their respective GLC peaks, which were corrected by subtracting the corresponding peak areas of the product from reaction with cyclohexanol. The increment of the (*S*)-(+)-acid was 4%. When the reaction was applied to the hexamethyl derivative of **5b**, the increment of the (*S*)-(+)-acid was 8%.

Acknowledgment. This contribution is from the Project SMIB ("Substances Marines d'Interêt Biologique"), ORSTOM-CNRS, Noumeâ, New Caledonia. The chemical studies were supported by C.N.R. (P.F. "Chimica Fine II" and Contributo 90.00332.03), Rome, Italy. FAB MS spectra were recorded by Servizio di Spettrometria di Massa del C.N.R. e dell' Università di Napoli. The assistance of the staff is gratefully acknowledged. We are also grateful to Professor G. Nota (Università di Napoli) for performing the atomic absorption spectroscopic analyses. We are indebted to Professor P. Salvadori (Università di Pisa) and Professor L. Merlini (Università di Milano) for discussions.

Supplementary Material Available: ^1H NMR spectra of the obtained compounds (15 pages). Ordering information is given on any current masthead page.

Synthesis of the Helicopodands: Novel Shapes for Chiral Clefts†

Kurt Deshayes, Richard D. Broene, Ito Chao, Carolyn B. Knobler, and François Diederich*

Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90024-1569

Received June 4, 1991

Helicopodands are a new class of chiral nonmacrocyclic ("podand") receptors with a helicene backbone ("helico"). At the termini of the helix, they form a preorganized cleft of pronounced asymmetric character which is aligned with convergent hydrogen bonding functionality. The synthetic routes to the two helicopodands **2** and **3** each include two photocyclodehydrogenation reactions. The X-ray crystal structure of **14**, a direct helical precursor to **2** and **3**, confirms the main structural features of the helicopodands. MMP2 calculations give a geometric description of **14** which is in reasonable agreement with the X-ray results.

Introduction

In the mid 1980's, molecular clefts aligned with convergent functional groups for small substrate recognition through hydrogen bonding were introduced as a versatile new class of receptors by Rebek et al.[1] In subsequent rapid developments, several other research groups prepared cleft-type receptors shaped by a wide variety of structural elements and investigated the selective hydrogen bonding recognition of a diversity of substrates.[2-7] These

† We dedicate this paper to Professor Vladimir Prelog on the occasion of his 85th birthday.

(1) (a) Rebek, J., Jr. *Angew. Chem.* **1990**, *102*, 261–272; *Angew Chem., Int. Ed. Engl.* **1990**, *29*, 245–255. (b) Rebek, J., Jr. *Acc. Chem. Res.* **1990**, *23*, 399–404. (c) Novick, J. S.; Ballester, P.; Ebmeyer, F.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 8902–8906.

(2) Adrian, J. C., Jr.; Wilcox, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 678–680.

0022-3263/91/1956-6787$02.50/0