Cystoseirols: Novel Rearranged Diterpenoids of Mixed Biogenesis from *Cystoseiraceae* (Brown Marine Algae)

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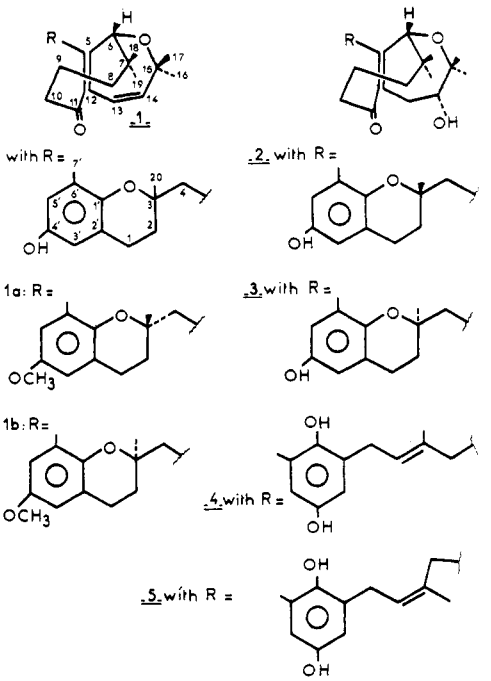
Four new diterpenoid metabolites, cystoseirols B, C, D and E, have been isolated from *Cystoseira mediterranea*. Their structures were determined by spectral and chemical studies and correlated with cystoseirol A, a previously reported irregular diterpene with an oxabicyclo[5.4.1]dodecane ring. Relative and absolute stereochemistries were also described. These compounds were also found in *Cystoseira stricta* and *Cystoseira tamariscifolia*.

Marine algae of the family *Cystoseiraceae* are among the most abundant seaweeds along the Mediterranean coastline. Several chemical investigations have proved that these algae contained regular terpenoids[1,2] with antibacterial and antimitotic activities.[3] We have already described from *Cystoseira mediterranea*[4,5] the novel structure of rearranged diterpenes of mixted biogenesis, mediterraneols A, B, C, and D, with a bicyclo[4.2.1]nonane ring. More recently,[6] we presented the gross structure of a novel irregular metabolite 1, cystoseirol A. In this report, we wish to describe the results of more detailed spectral studies of this novel class of compounds (Chart I), found in various collections of French *Cystoseiraceae*, *Cystoseira mediterranea* (Banyuls sur Mer), *Cystoseira tamariscifolia* (Atlantic coasts), and *Cystoseira stricta* (Nice).

For each *Cystoseiraceae*, a MeOH/$CHCl_3$ extract was partitioned between ether and water. After evaporation to dryness of the ether layer, cystoseirols (0.13% from dry-weight alga) were obtained by standard silica gel chromatography. Each constituent was separated in pure form by HPLC on μ-Porasil (20% EtOAc/isooctane).

Cystoseirol B (2) had the molecular formula $C_{27}H_{38}O_5$ (HRMS, m/z obsd 442.2717; calcd 442.2709) and $[\alpha]_D$ +20° (c 0.3, MeOH). The presence of a hydroquinone moiety was evident, based upon UV absorptions at λ 296 nm (ϵ 3960), ^{1}H NMR bands at 6.50 (1 H, d, J = 3 Hz), 6.38 (1 H, d, J = 3 Hz), and 2.11 ppm (3 H, s) and various ^{13}C NMR aromatic bands (Table I). The first isoprene unit was arranged in a chromane form evidenced from ^{1}H NMR resonances at 2.68 (2 H, m) 1.88–1.80 (2 H, m), and 1.34 ppm (3 H, s) and ^{13}C NMR resonances at 30.9 (t), 22.6 (t), 75.6 (s), and 25.5 ppm (q). Several high-resolution mass spectral fragmentations were in agreement with both of these proposals.[5]

Chart I



Cystoseirol B was recognized to possess an α,β-unsaturated ketone by IR (1680 cm^{-1}) and UV absorptions [λ 230 nm (ϵ 8800), indicating of steric inhibition of resonance] and by ^{13}C NMR singlet resonances (201.5, 144.6, and 138.5 ppm). Further, ^{1}H NMR and ^{13}C NMR bands showed the presence of a secondary hydroxyl group coupled with an allylic methylene group [^{1}H 4.22 (1 H, br t), 2.30 (1 H, dd, J = 13.5 and 6.5 Hz), 2.12 ppm (1 H, dd, J = 13.5 and 5.8 Hz); ^{13}C 80.2 (d), 32.8 ppm (t)]. An oxygen bridge was also suggested by ^{1}H NMR [4.28 ppm (1 H, s)] and ^{13}C NMR [77.4 (s) and 111.4 ppm (d)] analysis.[7]

Both ^{1}H and ^{13}C NMR spectra of 2 (Table I) supported the additional presence of four quaternary methyl and four methylene groups. At least, only one sp^3 quaternary carbon remained in the ^{13}C NMR spectra [43.3 ppm (s)], thus indicating an irregular diterpenoid. Treatment of 2 with Ac_2O/Pyr. followed by HPLC purification (10% EtOAc/isooctane) yielded an oily diacetate (HRMS, m/z obsd 526.2927, $C_{31}H_{42}O_7$ calcd 526.2919) in agreement with

(1) See, for instance: (a) Banaigs, B.; Francisco, C.; Gonzalez, E.; Codomier, L.; Fenical W. *Tetrahedron Lett.* **1982**, *23*, 3271. (b) Amico, V.; Oriente, G.; Piattelli, M.; Ruberto, G.; Tringali, C. *Phytochemistry* **1982**, *2*, 421. (c) Banaigs, B.; Francisco, C.; Gonzalez, E.; Fenical, W. *Tetrahedron* **1983**, *39*, 629.

(2) (a) Sun, H. H.; Ferrara, N. M.; McConnell, O. J.; Fenical, W. *Tetrahedron Lett.* **1980**, *21*, 3123. (b) Amico, V.; Oriente, G.; Piattelli, M.; Ruberto, G.; Fronczek, F. R. *Tetrahedron* **1984**, *40*, 1721. (c) Amico, V.; Oriente, G.; Piattelli, M.; Ruberto, G. *Phytochemistry* **1984**, *23*, 2017.

(3) (a) Caccamese, S.; Azzolina, R.; Furnari, G.; Cormaci, M.; Grasso, S. *Bot. Mar.* **1980**, *23*, 285. (b) Caccamese, S.; Azzolina, R.; Furnari, G.; Cormaci, M.; Grasso, S. *Bot. Mar.* **1981**, *24*, 365.

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(5) Francisco, C.; Banaigs, B.; Teste, J.; Cave, A. *J. Org. Chem.* **1986**, *51*, 1115.

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(7) (a) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic: New York, 1972. (b) Wehrli, F. W.; Wishida, T. *Progress in the Chemistry of Organic Natural Products*; Springer-Verlag: New York, 1979; Vol. 36.

0022-3263/86/1951-2707$01.50/0

Table I. NMR Data for Cystoseirol B (2) and Selected Long-Range Couplings from ^1H–^{13}C Shift-Correlated 2-D NMR in $CDCl_3$

	^1H NMR (360 MHz)				^{13}C NMR (90.5 MHz)		^1H–^{13}C 2-D NMR
	$CDCl_3$		C_6D_6		$CDCl_3$		
C	ppm	mult, J/Hz	ppm	mult, J/Hz	ppm	mult	$^2J_{C-H}$ ($^3J_{C-H}$)
1	2.68	m	2.55	m	22.6	t	
2	1.88–1.80	m	1.95–1.85	m	30.9	t	
3					75.6	s	
4	2.41	AB, 10	2.36	br s	44.6	t	H4–C3, H4–C5
5					144.6	s	
6	4.28	s	4.22	s	111.4	d	H6-C5
7					43.3	s	
8	1.83, 1.31	m	1.70, 1.32	m	36.3	t	
9	1.68–1.43	m	1.80–1.48	m	40.5	t	
10	1.54	m	1.60–1.50	m	20.5	t	
11					201.5	s	
12					138.5	s	
13	2.45	dd, 13.5, 6.5	2.12	dd, 14, 6	32.8	t	
	2.20	dd, 13.5, 5.8	1.96	dd, 14, 6			
14	4.22	br t, 6	3.98	br s	80.2	d	
15					77.4	s	
16	1.29	s	1.25	s	28.1	q	H16–C15
17	1.18	s	1.13	s	22.3	q	H17–C15 (H17–C14)
18	1.09	s	1.30	s	22.8	q	H18–C7 (H18–C8)
19	1.02	s	1.02	s	19.7	q	H19–C7 (H19–C8)
20	1.33	s	1.46	s	25.5	q	H20–C3
1′					147.7	s	
2′					122.3	s	
3′	6.50	d, 3	6.38	br s	112.6	d	
4′					152.0	s	
5′	6.38	d, 3	6.30	br s	115.6	d	
6′					127.4	s	
7′	2.11	s	2.29	s	16.1	q	H7′–C6′

a chromane moiety and a secondary alcohol functionally, thus proving the lack of tertiary hydroxyl group. The 11 degrees of unsaturation (9 degrees for **2**) in the molecular formula were accounted for by three carbonyls, four olefinic bonds, one aromatic cycle, and one chromane cycle, establishing the bicyclic nature of the diterpene skeleton.

Sodium borohydride reduction of **2**, followed by HPLC purification, gave an additional secondary alcohol, characterized by one allylic proton [4.40 ppm (br t)]—shown by spin decoupling to be coupled to a high-field methylene group (1.6 ppm) during ^1H NMR analysis—and by the lack of IR and UV spectral features of the former enone.

After attempts to prepare crystalline derivatives and chemical modifications failed, we turned to 2-D NMR experiments. Total assignment of both ^1H NMR and ^{13}C NMR spectra of **2** (Table I) was accomplished through the use of ^1H–^{13}C shift correlation 2-D spectroscopy, including 2-D long-range ^1H–^{13}C correlations.[8] In this way, partial structural informations have been obtained. The occurrence of H20–C3, H4–C3, H4–C5, and H6–C5 couplings allowed us to connect the row C4–C5–C6 to the chromane moiety. In the same way, H18–C7, H19–C7, H16–C15, and H17–C15 geminal couplings and H19–C8, H18–C8, and H17–C14 vicinal couplings established the presence of two *gem*-dimethyl functions, one bearing a methylene group and the other one the carbon bonded to the secondary alcohol functionality. Careful ^1H NMR decoupling experiments showed three methylene groups to be interrelated (we were not able, however, to determine J values). Consideration of this result, the borohydride reduction and aforementioned spectroscopic evidences led to the formulation of the row C11–C10–C9–C8–C7. The carbons C13–C14–C15 were rapidly connected by a same behavior. These results allowed us to point out the close relationship of **2** with cystoseirol A (**1**) whose structure has been established by similar experiments.[6] In order to prove the presence of this same skeleton, **2** was treated wtih TsCl/Pyr. The result of concomitant dehydration was found to be identical with **1** by spectral analysis, thus providing chemical interrelation between both metabolites.

What remained was to establish the olefin constellation and this was accomplished via the comparison of spectroscopic data of cystoseirols A and B. Long-range ^1H–^{13}C chemical shift correlations of both compounds showed cystoseirol A to possess a supplementary vicinal coupling H6–C12,[6] securely placing the tetrasubstituted olefin at C5 and C12. Direct evidence for the linkage of C12–C13 could not be obtained due to the lack of coupling between H13–C12. This problem was solved by the comparison of UV absorptions in cystoseirol A and its reduction product (λ 240 nm, conjugated diene) with those found in cystoseirol B (λ 230 nm, conjugated enone). This observation provided also considerable support in the position of the ketone at C11, as could be deduced from the lack of corresponding UV chromophore in the reduction product of cystoseirol B. At this point, only C6–C7 and C6–O–C15 junctures remained possible and led to the formulation of two structures, according to the position of C11 and C13 with regard to C5–C12 double bond. As one of the structure contained an unacceptably trans double bond in a cycloheptane ring, we chose the oxabicyclo[5.4.1]dodecane ring for cystoseirol skeleton, taking account of steric hindrance (confirmed by Dreiding models) observed in UV spectra and subsequent NOE results. Another problem was the $^1J_{CH}$ correlation (>144 Hz) observed between the high-field proton H6 and the low-field carbon C6. Chemical shifts (4.30 ppm) displayed by H6 in all ^1H NMR spectra of natural cystoseirols or borohydride reduction products were in agreement with such a skeleton. However, a ^{13}C NMR band at 110 ppm was generally indicative of sp^2 of ketal carbons. As the whole study did not show

(8) Bax, A. *Two-Dimensional NMR in Liquids*; D. Reidel: Dordrecht, Holland, 1982.

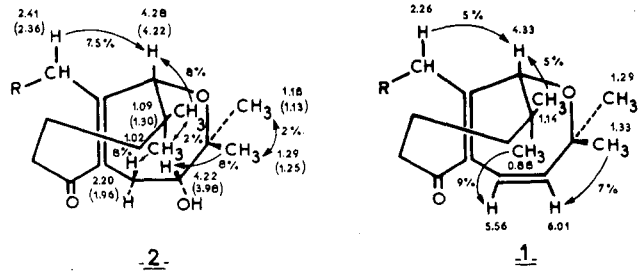


Figure 1. NOE's H (irradiated) → H (enhanced) for compound **2** in $CDCl_3$ (C_6D_6) and **1** in $CDCl_3$.

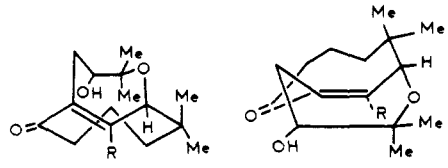


Figure 2. Conformation of the bicyclic system.

evidences for such hypothesis, we concluded that C6 was a sp^3 tertiary carbon with a deshielded value that could be due to the bridgehead position of C6 in a bicyclic ring characterized by a bridgehead, anti-Bredt double bond (in a large enough system to be accommodated).

Nuclear Overhauser enhancements (8%), as measured by using NOE difference spectroscopic techniques, of the C6 proton were observed upon irradiations of the C4 and C18 proton signals (Figure 1). Irradiation of the C19 methyl protons produced marked nuclear Overhauser enhancement (8%) of the β proton at C13. These results were in agreement for the proposed structure **2** in a crown conformation for the cyclooctane ring (Figure 2).

NOE (8%) was also obtained for C14 proton upon irradiation of the C16 methyl protons. As NOE was absent upon irradiation of the C17 methyl protons, the oxacycloheptane ring was assumed to be in a boat-like conformation (Figure 2) corresponding to the minimum steric hindrance for this structure. The hydroxyl group at C14 was deduced to be in an α position upon the basis of NOE results and coupling contants observed between H14β and both C13 protons. Examination of a Dreiding model of cystoseirol B in such a conformation revealed that relative configuratioin at C6 and C14 were identical for these two nuclear centers. A Horeau determination[9] of the absolute configuration of the alcohol indicated a 14*R* configuration for the hydroxyl-bearing carbon. Consequently, C6 was assigned the *R* absolute configuration.

Cystoseirol C (**3**), analyzed for $C_{27}H_{38}O_5$ by HRMS (*m/z* obsd 442.2687), exhibited the same spectral features than **2**, except $[\alpha]_D$ +36° (*c* 0.5, MeOH). The high-field shift of the chromane methyl group in the ^1H NMR spectrum (1.30 ppm) allowed us to postulate that **3** was the epimeric chromane at C3 (*R** by arbitrary choice in our drawing).

Cystoseirol D (**4**) analyzed for $C_{27}H_{38}O_5$ by HRMS (*m/z* obsd 442.2707) in combination with consideration of ^{13}C NMR spectra and showed $[\alpha]_D$ −4° (*c* 1.0, MeOH). ^1H and ^{13}C NMR experiments with **4** allowed the structure to be confidently assigned (see Experimental Section). In particular, the characteristic ^1H bands at 5.37 (1 H, t, J = 7.5 Hz), 3.43 (1 H, dd, J = 18 and 9 Hz), 3.19 (1 H, dd, J = 18 and 6 Hz), 2.68 (2 H, s), and 1.73 ppm (3 H, s) and ^{13}C bands at 30.4 (C1, t), 123.8 (C2, d), 135.9 (C3, s), and 15.5 ppm (C20, q) permitted to characterize the first isoprene unit, with *E* double bond (γ shielding effect). The close structural relationship between **4** and **1**/**2** was verified by

(9) Horeau, A.; Nouaille, A. *Tetrahedron Lett.* **1971**, *12*, 1939.

Table II. ^1H NMR Data for Methylated Derivatives of Cystoseirol A (1a) and Its Stereoisomer at C3 (1b)

	1a		1b	
C	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6
1	2.70	2.73	2.70	2.73
2	1.8	2.11	1.8	2.11
4	2.26	2.41	2.24	2.40
6	4.33	4.31	4.32	4.31
8	1.8, 1.3	1.8–1.5	1.8, 1.3	1.8–1.5
9	1.7, 1.4		1.7, 1.4	
10	1.5	2.5, 1.9	1.5	2.5, 1.9
13	5.56	5.44	5.57	5.44
14	6.01	6.06	6.00	6.04
16	1.33	1.32	1.33	1.34
17	1.29	1.15	1.29	1.16
18	1.14	1.31	1.12	1.29
19	0.88	0.91	0.88	0.91
20	1.36	1.46	1.35	1.46
3′	6.41	6.51	6.41	6.51
5′	6.54	6.74	6.51	6.74
7′	2.07	2.32	2.07	2.33
OCH_3	3.70	3.44	3.70	3.43

dehydration with TsCl and by NOE experiments, thus providing that **4** was of the same carbocyclic structure as **1** and with the same stereochemistry as **2**—Horeau determination confirmed a 14*R* configuration.

The more minor metabolite, cystoseirol E (**5**), had the molecular formula $C_{27}H_{38}O_5$ (HRMS, *m/z* obsd. 442.2718) and showed $[\alpha]_D$ +10°/(*c* 0.4, MeOH). The high degree of similarity between the chemical shift of nuclear proton in **5** and **4** indicated that they were almost identical. The C2–C3 olefin stereochemistry was assigned as *Z* upon the basis of the complete lack of allylic coupling found between the protons H2 and H20 when either were irradiated and the chemical shift observed for H20 (1.57 ppm) in ^1H NMR experiments.

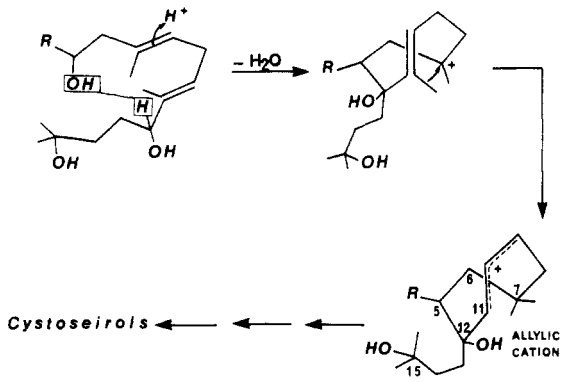
The structure of cystoseirol A (**1**), described in an earlier paper,[6] was already used in the discussion of cystoseirol B, and we would like now to present briefly the stereochemical assignments.

During purification of natural compound **1**, an epimeric mixture was found in a part of the HPLC peak. Attempts to isolate the two epimers, **1a** and **1b**, was made on methylated (ICH_3/K_2CO_3) or acetylated derivatives. **1a** was obtained in a pure form, but only enrichment was achieved for **1b** by HPLC purifications. However, comparison of ^1H NMR results of the enriched sample of **1b** with those of **1a** allowed us to identify C3 as the only epimeric center (see Table II) in agreement with doubleting recently described in an other meroditerpenoid.[10] As for cystoseirols B and C, the ether formation at C3 did not appear to be stereospecific. NOE measurements (Figure 1) supported the fact that **1a** presented similar carbocyclic conformation as **2**. Thus, absolute configuration at C6 was assigned as *R* by comparison with the result of dehydration process already described from **2**. We were not able, however, to establish the stereochemistry at C3 in **1a** (and therefore in **1b**).

The presence of mediterraneol, cystoseirol, and bifurcarenone in *Cystoseira mediterranea*, *Cystoseira stricta*, and *Cystoseira tamariscifolia* led us to conclude the occurence of a particular enzymatic complex in these algae. Biosynthesis of such compounds like mediterraneol or cystoseirol is still unclear, and we try now to isolate minor metabolites involved in the same metabolic pathways in order to explain the various stages conducive to the dis-

(10) Amico, V.; Cunsolo, F.; Oriente, G.; Piattelli, M.; Ruberto, G. *J. Nat. Prod.* **1984**, *47*, 947.

Scheme I. Proposal for Partial Biosynthesis of Cystoseirols



placement of one methyl group from probable *trans*-cyclooctene precursors (like in Scheme I).

Experimental Section

General. IR spectra were recorded on a Perkin-Elmer Model 621 spectrophotometer and optical rotations were measured on a Roussel Jouan (T71) polarimeter using a 0.5-cm microcell. UV spectra were recorded on a Perkin-Elmer 551 spectrophotometer, and high-resolution mass spectra were obtained through the Bioorganic and Biomedical Mass Spectrometry Resource Center, Space Sciences Laboratory, University of California, Berkeley. ^1H NMR and ^{13}C NMR spectra (respectively at 360 and 90.5 MHz) were recorded on a Bruker instrument. All chemical shifts are reported in ppm relative to Me_4Si (δ 0) and coupling constants are in hertz.

All solvents used were either spectral grade or were distilled from glass prior to use. Purifications of all metabolites and reaction products were achieved by HPLC on preparative silica gel column using various proportions of EtOAc and isooctane.

Cystoseira mediterranea (150 g dry weight) was collected in June and July (1981, 1982) at Banyuls-sur-Mer, France. The freez-dried material was ground and extracted with $CHCl_3$/MeOH (1:1). After filtration and evaporation, the extract was partitioned between water and ether. The ether-soluble material was dried over $MgSO_4$ and filtered, and the filtrate was evaporated to yield 2.24 g of a crude organic extract. From various collections, compounds were eluted from a silica gel column with 30% hexane in ether and further purified as oily substances by HPLC (20% EtOAc in isooctane). This typical separation scheme was used for *Cystoseira stricta* and *Cystoseira tamariscifolia*. Acetylated compounds were obtained as usual (Ac_2/Pyr) and purified by HPLC (10% EtOAc in isooctane).

Cystoseirol B (2) (1.8% from ether extract): IR (film) 3400, 2960, 2920, 2860, 1680, 1605, 1460, 1215 cm^{-1}; UV (MeOH) λ 230 (ϵ 8800), 296 nm (ϵ = 3960); ^1H NMR and ^{13}C NMR see Table I. ^1H–^{13}C shift correlations: the applied pulse sequence was (π/2, ^1H) – $t_{1/2}$ – (π, ^{13}C) – $t^{1/2}$ – τ_1 – (π/2, ^1H; π/2, ^{13}C) – τ_2 – (B.B., ^1H; FID, t_2) with τ_1 = 3.3 ms and τ_2 = 1.67 ms. Spectral width in F_1 was W_1 = ±500 Hz and in F_2, W_2 = ±6024 Hz. ^1H–^{13}C long-range shift correlations: same as ^1H–^{13}C shift correlations except τ_1 = τ_2 = 41.7 ms (maximum polarization for J = 12 Hz). HRMS (relative intensity) m/z 442.2717 (11.5, $C_{27}H_{38}O_5$) 424.2623 (7, $C_{27}H_{36}O_4$), 406.2487 (0.6, $C_{27}H_{34}O_3$), 384.2336 (1.6, $C_{24}H_{32}O_4$), 274.1577 (41.3, $C_{17}H_{22}O_3$), 256.1454 (11.2, $C_{17}H_{20}O_2$), 233.1537 (6.2, $C_{15}H_{21}O_2$), 216.1136 (5.5, $C_{14}H_{16}O_2$), 190.1348 (11.5, $C_{13}H_{18}O$), 178.0833 (14.2, $C_{11}H_{14}O_2$), 175.1122 (12.0, $C_{11}H_{11}O_2$), 168.1147 (58.2, $C_{10}H_{16}O_2$), 155.1079 (8.4, $C_9H_{15}O_2$), 150.0899 (100, $C_{10}H_{14}O_2$), 137.0867 (20.7, $C_9H_{13}O$), 137.1015 (12.2, $C_8H_9O_2$), 135.0822 (9.2, $C_9H_{11}O$), 123.0800 (5.4, $C_8H_{11}O$), 113.0598 (12.7, $C_6H_9O_2$), 95.0487 (13.8, C_6H_7O).

Cystoseirol B acetate: IR (film) 2960, 2920, 2860, 1750, 1730, 1460, 1350, 1240, 1180 cm^{-1}; UV (MeOH) λ 228 (ϵ 9100), 287 nm (ϵ 1690); ^1H NMR (360 MHz, $CDCl_3$) 6.54 (1 H, d, J = 2.9 Hz), 6.49 (1 H, d, J = 2.9 Hz), 5.04 (1 H, dd, J = 7.2 and 4.3 Hz), 4.35 (1 H, s), 2.72 (2 H, m), 2.45 (1 H, dd, J = 7.2 and 14.4 Hz), 2.27 (2 H, s), 2.25 (3 H, s), 2.20 (1 H, dd, J = 4.3 and 14.4 Hz), 2.14 (3 H, s), 2.06 (3 H, s), 1.87–1.65 (6 H, m), 1.56 (2 H, br s), 1.35 (3 H, s), 1.15 (3 H, s), 1.09 (3 H, s), 1.00 (3 H, s); HRMS, m/z (relative intensity 526.2927 (6.6, $C_{31}H_{42}O_7$), 484.2752 (0.4, $C_{29}H_{40}O_6$), 466.2656 (5.5, $C_{29}H_{38}O_5$), 442.2736 (0.5, $C_{27}H_{38}O_5$), 424.2654 (1.3, $C_{27}H_{36}O_4$), 316.1658 (9.4, $C_{19}H_{24}O_4$), 274.1567 (9.4, $C_{17}H_{22}O_3$), 256.1461 (3.2, $C_{17}H_{20}O_2$), 233.1503 (5.3, $C_{15}H_{21}O_2$), 216.1140 (4.6, $C_{14}H_{16}O_2$), 210.1255 (37.5, $C_{12}H_{18}O_3$), 205.1592 (3.6, $C_{14}H_{21}O$), 190.1360 (9.7, $C_{13}H_{18}O$), 177.0812 (45.4, $C_{11}H_{13}O_2$), 175.0760 (8.8, $C_{11}H_{11}O_2$), 168.1156 (4.5, $C_{10}H_{16}O_2$), 150.1031 (100, $C_{10}H_{13}O$), 137.0867 (31.2, $C_9H_{10}O$), 137.0605 (11.3, $C_8H_9O_2$), 135.0809 (15.0, $C_9H_{11}O$), 123.0806 (6.0, $C_8H_{11}O$), 121.0649 (8.3, C_8H_9O), 109.0654 (8.7, C_7H_9O), 95.0489 (15.8, C_6H_7O), 93.0663 (10.7, C_7H_9), 83.0494 (11.8, C_5H_7O).

Cystoseirol C (3) (1.1% from ether extract): IR (film) 3400, 2960, 2920, 2860, 1680, 1605, 1460, 1215, cm^{-1}; UV (MeOH) λ 229 (ϵ 8800) 296 nm (ϵ 4140); ^1H NMR (360 MHz, $CDCl_3$) 6.48 (1 H, d, J = 3 Hz), 6.39 (1 H, d, J = 3 Hz), 4.32 (1 H, s), 4.27 (1 H, br s), 2.73 (2 H, m), 2.29 (1 H, dd, J = 13, 7 Hz), 2.22 (2 H, s), 2.17 (1 H, dd, J = 13, 7 Hz), 1.84 (2 H, m), 1.70–1.45 (6 H, m), 1.30 (6 H, s), 1.17 (3 H, s), 1.10 (3 H, s), 1.03 (3 H, s); ^1H NMR (360 MHz, C_6D_6) 6.38 (1 H, br s), 6.30 (1 H, br s), 4.30 (1 H, s), 4.00 (1 H, br t), 2.60 (2 H, m), 2.35 (2 H, s), 2.28 (3 H, s), 2.13 (1 H, dd, J = 14.3 and 6.1 Hz), 1.98 (1 H, dd, J = 14.3 and 7 Hz) 1.80–1.40 (8 H, m), 1.39 (3 H, s), 1.25 (3 H, s), 1.22 (3 H, s), 1.10 (3 H, s), 0.99 (3 H, s); HRMS, m/z (relative intensity) 442.2687 (5.7, $C_{27}H_{38}O_5$), 424.2604 (3.8, $C_{27}H_{36}O_4$), 274.1575 (17.8, $C_{17}H_{22}O_3$), 256.1468 (5.1, $C_{17}H_{20}O_2$), 190.1359 (5.8, $C_{13}H_{18}O$), 177.0910 (67.7, $C_{11}H_{13}O_2$), 175.0758 (11.5, $C_{11}H_{11}O_2$), 168.1152 (26.3, $C_{10}H_{16}O_2$), 150.1045 (100, $C_{10}H_{14}O$), 137.0870 (14.1, $C_9H_{13}O$), 137.0605 (9.8, $C_8H_9O_2$), 135.0822 (8.9, $C_9H_{11}O$), 121.0653 (6.8, C_8H_9O), 111.1167 (10.4, C_8H_{15}), 95.0862 (26.6, C_7H_{11}), 95.0502 (12.5, C_6H_7O), 83.0497 (18.0, C_5H_7O), 82.0418 (14.6, C_5H_6O).

Cystoseirol C acetate: IR (film) 2960, 2920, 2860, 1750, 1730, 1460, 1350, 1250, 1180 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) 6.67 (1 H, d, J = 3 Hz), 6.61 (1 H, d, J = 3 Hz), 5.16 (1 H, dd, J = 6.8 and 5.7 Hz), 4.35 (1 H, s), 2.75 (2 H, m), 2.43 (1 H, dd, J = 14.5 and 6.8 Hz), 2.25 (3 H, s), 2.20 (1 H, dd, J = 14.5 and 5.7 Hz), 2.18 (2 H, s), 2.13 (3 H, br s), 2.07 (3 H, s), 1.90–1.40 (8 H, m), 1.35 (3 H, s), 1.30 (3 H, s), 1.15 (3 H, s), 1.09 (3 H, s), 1.00 (3 H, s); HRMS, m/z (relative intensity) 526.2915 (5.4, $C_{31}H_{42}O_7$), 466.2709 (3.7, $C_{29}H_{38}O_5$), 424.2635 (2.0, $C_{27}H_{36}O_4$), 316.1669 (6.9, $C_{19}H_{24}O_4$), 274.1566 (8.2, $C_{17}H_{22}O_3$), 256.1454(3.0, $C_{17}H_{20}O_2$), 233.1536 (3.9, $C_{15}H_{21}O_2$), 219.1012 (60, $C_{13}H_{15}O_3$), 217.0870 (10.5, $C_{13}H_{13}O_3$), 210.1256 (26.5, $C_{12}H_{18}O_3$), 205.1588 (3.2, $C_{14}H_{21}O$), 190.1364 (7.4, $C_{13}H_{18}O$), 177.0808 (34.0, $C_{11}H_{13}O_2$), 175.0756 (11.2, $C_{11}H_{11}O_2$), 168.1149 (3.0, $C_{10}H_{16}O_2$), 150.1050 (100, $C_{10}H_{14}O$), 137.0864 (25.8, $C_9H_{13}O$), 137.0596 (10, $C_8H_9O_2$), 135.0807 (13.9, $C_9H_{11}O$), 123.0816 (5.4, $C_8H_{11}O$), 108.0651 (8.1, C_7H_9O), 95.0495 (11.3, C_6H_7O), 83.0497 (9.0, C_5H_7O).

Cystoseirol D (4) (2.0% from ether extract): IR (film) 3400, 2960, 2925, 2860, 1675, 1595, 1460, 1215, 1180 cm^{-1}; UV (MeOH) λ 229 (ϵ 8910), 293 (ϵ 2140); ^1H NMR (360 MHz, $CDCl_3$) 6.50 (2 H, s), 5.37 (1 H, br t), 4.32 (1 H, s), 4.26 (1 H, br t), 3.43 (1 H, dd, J = 18 and 9 Hz), 3.19 (1 H, dd, J = 18 and 6 Hz), 2.68 (2 H, s), 2.44 (1 H, dd, J = 13 and 7 Hz), 2.19 (3 H, s) 2.10 (1 H, dd, J = 13 and 7 Hz), 1.85–1.40 (6 H, m), 1.73 (3 H, s), 1.23 (3 H, s), 1.14 (3 H, s), 1.10 (3 H, s), 1.02 (3 H, s); ^1H NMR (360 MHz, C_6D_6) 6.70 (1 H, br s), 6.58 (1 H, br s), 5.40 (1 H, br s), 4.25 (1 H, t), 4.18 (1 H, s), 3.36 (1 H, dd, J = 16 and 7.5 Hz), 3.21 (1 H, dd, J = 16 and 6.5 Hz), 2.50 (2 H, s), 2.28 (1 H, dd, J = 13 and 7 Hz), 2.21 (3 H, s), 2.00 (1 H, dd, J = 13 and 7 Hz), 1.80–1.40 (6 H, m), 1.59 (3 H, s), 1.31 (3 H, s), 1.14 (3 H, s), 1.11 (3 H, s), 1.04 (3 H, s); ^{13}C NMR (90.5 MHz, $CDCl_3$) 202.0 (s), 148.6 (s), 146.2 (s), 145.1 (s), 135.9 (s), 132.5 (s), 127.4 (s), 125.8 (s), 123.8 (d), 115.5 (d), 114.0 (d), 109.5 (d), 95.1 (s), 83.8 (d), 46.3 (s), 45.3 (t), 42.0 (t), 40.4 (t), 36.1 (t), 30.4 (t), 28.1 (q), 22.9 (q), 22.2 (q), 20.4 (t), 19.6 (q), 16.1 (q), 15.5 (q). HRMS, m/z (relative intensity) 442.2707 (3.3, $C_{27}H_{38}O_5$), 424.2615 (19.5, $C_{27}H_{36}O_4$), 406.2505 (9.0, $C_{27}H_{34}O_3$), 274.1573 (11.0, $C_{17}H_{22}O_3$), 256.1469 (4.1, $C_{17}H_{20}O_2$), 233.1539 (20.1, $C_{15}H_{21}O_2$), 215.1427 (6.7, $C_{15}H_{19}O$), 205.1596 (6.1, $C_{14}H_{21}O$), 190.1360 (10.6, $C_{13}H_{18}O$), 177.0810 (34.0, $C_{11}H_{13}O_2$), 175.0755 (33.4, $C_{11}H_{11}O_2$), 168.1117 (53.2, $C_{10}H_{16}O_2$), 150.0929 (100, $C_{10}H_{14}O$), 137.0859 (32.8, $C_9H_{13}O$), 137.0593 (23.5, $C_8H_9O_2$), 135.0805 (26.4, $C_9H_{11}O$), 123.0818 (13.1, $C_8H_{11}O$), 113.0605 (17.5, $C_6H_9O_2$), 109.0657 (11.1, C_7H_9O), 107.0861 (12.8, C_8H_{11}), 95.0494 (27.1, C_6H_7O), 83.0499 (18.9, C_5H_7O), 82.0421 (27.5, C_5H_6O).

Cystoseirol D acetate: IR (film) 2960, 2925, 2860, 1755, 1740, 1460, 1360, 1220, 1180 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) 6.82 (1 H, br s), 6.78 (1 H, br s), 5.30 (1 H, dd, J = ?), 5.13 (1 H, dd, J ?), 4.26 (1 H, s), 3.18 (2 H, br t), 2.65 (2 H, s), 2.45 (1 H, dd, J = 12.6 and 6.8 Hz), 2.32 (3 H, s), 2.26 (3 H, s), 2.20 (1 H, dd, J = 12.6 and ? Hz), 2.14 (3 H, s), 2.06 (3 H, s), 1.63 (3 H, s), 1.31 (3 H, s), 1.14 (3 H, s), 1.10 (3 H, s), 0.99 (3 H, s); HRMS, m/z (relative intensity) 568.3053 (1.7, $C_{33}H_{44}O_8$), 526.2881 (2.2, $C_{31}H_{42}O_7$), 508.2801 (4.2, $C_{31}H_{40}O_6$), 466.2713 (2.0, $C_{29}H_{38}O_5$), 448.2587 (0.3, $C_{29}H_{36}O_4$), 424.2583 (0.7, $C_{27}H_{36}O_4$), 358.1750 (2.5, $C_{21}H_{26}O_5$), 316.1661 (5.0, $C_{19}H_{24}O_4$), 298.1553 (2.4, $C_{19}H_{22}O_3$), 274.1545 (5.2, $C_{17}H_{22}O_3$), 261.1125 (2.2, $C_{15}H_{17}O_4$), 256.1451 (2.8, $C_{17}H_{20}O_2$), 233.1514 (23.5, $C_{15}H_{21}O_2$), 219.1026 (28.5, $C_{13}H_{15}O_3$), 215.1434 (3.9, $C_{15}H_{19}O$), 210.1254 (38.9, $C_{12}H_{18}O_3$), 205.1590 (6.6, $C_{14}H_{21}O$), 190.1347 (12.9, $C_{13}H_{18}O$), 177.0805 (21.1, $C_{11}H_{13}O_2$), 175.0753 (10.8, $C_{11}H_{11}O_2$), 168.1160 (2.3, $C_{10}H_{16}O_2$), 150.1002 (100, $C_{10}H_{14}O$), 137.0866 (46.0, $C_9H_{13}O$), 137.0600 (14.3, $C_8H_9O_2$), 135.0806 (21.5, $C_9H_{11}O$), 123.0803 (14.3, $C_8H_{11}O$), 109.0651 (8.8, C_7H_9O), 95.0498 (25.5, C_6H_7O), 83.0499 (10.7, C_5H_7O).

Cystoseirol E (5) (0.6% from ether extract): IR (film) 3400, 2960, 2920, 2860, 1680, 1595, 1460, 1210, 1185 cm^{-1}; UV (MeOH) λ 229 (ϵ 8700), 296 nm (ϵ 2260); ^1H NMR (360 MHz, $CDCl_3$) 6.62 (1 H, br s), 6.56 (1 H, br s), 5.23 (1 H, br t), 4.34 (1 H, t, J = 8 Hz), 4.26 (1 H, s), 3.15 (2 H, d, J = 7 Hz), 2.68 (2 H, s), 2.24 (1 H, dd, J = 15 and 6 Hz), 2.14 (1 H, dd, J = 15 and 7 Hz), 2.07 (3 H, s), 1.80–1.40 (6 H, m), 1.57 (3 H, s), 1.23 (3 H, s), 1.14 (3 H, s), 1.10 (3 H, s), 1.02 (3 H, s); ^1H NMR (360 MHz, C_6D_6) 6.56 (1 H, br s), 6.01 (1 H, br s), 5.15 (1 H, br t), 4.40 (1 H, t, J = 7.5 Hz), 4.27 (1 H, s), 3.02 (2 H, d, J = 7.5 Hz), 2.69 (2 H, s), 2.27 (1 H, dd, J = 11 and 6.8 Hz), 2.18 (1 H, dd, J = 11 and 7.5 Hz), 1.90–1.40 (6 H, m), 1.58 (3 H, s), 1.51 (3 H, s), 1.34 (3 H, s), 1.31 (3 H, s), 1.24 (3 H, s), 1.05 (3 H, s); HRMS, m/z (relative intensity) 442.2718 (1.5, $C_{27}H_{38}O_5$), 424.2615 (7.8, $C_{27}H_{36}O_4$), 406.2510 (4.5, $C_{27}H_{34}O_3$), 274.1573 (6.4, $C_{17}H_{22}O_3$), 256.1471 (2.9, $C_{17}H_{20}O_2$), 233.1555 (10.3, $C_{15}H_{21}O_2$), 216.1133 (12.6, $C_{14}H_{16}O_2$), 190.1429 (9.9, $C_{13}H_{18}O$), 177.0907 (38.0, $C_{11}H_{13}O_2$), 175.0832 (100, $C_{11}H_{11}O_2$), 168.1138 (77.0, $C_{10}H_{16}O_2$), 150.1032 (90.1, $C_{10}H_{14}O$), 137.0966 (33.2, $C_9H_{13}O$), 137.0604 (17.3, $C_8H_9O_2$), 135.0809 (17.2, $C_9H_{11}O$), 123.0811 (10.1, $C_8H_{11}O$), 95.0495 (21.6, C_6H_7O), 83.0496 (14.4, C_5H_7O), 82.0420 (22.6, C_5H_6O).

Cystoseirol E acetate: IR (film) 2960, 2920, 2860, 1755, 1740, 1595, 1460, 1210 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) 6.82 (1 H, br s), 6.78 (1 H, br s), 5.30 (1 H, br t), 5.10 (1 H, dd, J = 12.5 and 7 Hz), 4.25 (1 H, s), 3.20 (2 H, br t, J = ?), 2.70 (2 H, s), 2.43 (1 H, dd, J = 12.5 and 7 Hz), 2.31 (3 H, s), 2.25 (3 H, s),2.19 (1 H, ?), 2.18 (3 H, s), 2.06 (3 H, s), 1.55 (3 H, s), 1.23 (3 H, s), 1.14 (3 H, s), 1.09 (3 H, s) 0.99 (3 H, s).

Cystoseirol A (1 or 1a) (1.9% from ether extract): $[\alpha]_D$ +14.9° (c 1.6, CCl_4); IR (film) 3350, 2980, 2965, 2935, 1680, 1610, 1470, 1220, 980 cm^{-1}; UV ($CHCl_3$) λ 241 (ϵ 4600), 296 nm (ϵ 4400); ^1H and ^{13}C NMR yet described (see ref 6).

Cystoseirol A acetate: IR (film) 2980, 2965, 2865, 1760, 1680, 1475, 1370, 1210 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) 6.68 (1 H, br s), 6.60 (1 H, br s), 6.05 (1 H, d, J = 6.5 Hz), 5.60 (1 H, d, J = 6.5 Hz), 4.35 (1 H, s), 2.70 (2 H, m), 2.27 (2 H, s), 2.25 (3 H, s), 2.13 (3 H, s), 1.95–1.45 (8 H, m), 1.37 (3 H, s), 1.33 (3 H, s), 1.29 (3 H, s), 1.14 (3 H, s), 0.88 (3 H, s).

Epimeric Mixture of Cystoseirol A. Enrichment for **1b** was obtained by usual methylation (ICH_3/K_2CO_3) of the mixture followed by HPLC separation (65% CH_2Cl_2 in isooctane). Methoxycystoseirol A: IR (film) 2960, 2920, 2860, 1675, 1605, 1480, 1220, 975 cm^{-1}; ^1H NMR for **1a** + **1b** see Table II.

$NaBH_4$ Reduction of Cystoseirol A. The compound was reduced with excess $NaBH_4$ in MeOH (0 °C, 30 min) and purified by HPLC (20% EtOAc in isooctane): IR (film) 3500, 2980, 2960, 2860, 1620, 1605, 1480, 1370 cm^{-1}; UV ($CHCl_3$) λ 240 (ϵ 4700), 296 nm (ϵ 4500). ^1H NMR (360 MHz, $CDCl_3$) 6.54 (1 H, d, J = 3 Hz), 6.41 (1 H, d, J = 3 Hz), 6.10 (1 H, d, J = 6 Hz), 5.52 (1 H, d, J = 6 Hz), 4.41 (1 H, br t), 4.33 (1 H, s), 2.70 (2 H, m), 2.20 (2 H, s), 2.09 (3 H, s), 1.90–1.30 (8 H, m), 1.36 (3 H, s), 1.33 (3 H, s), 1.29 (3 H, s), 1.14 (3 H, s), 0.90 (3 H, s).

$NaBH_4$ Reduction of Cystoseirol B and D. Reductions were performed as described above with similar results except for the following: UV (MeOH) λ 296 nm (ϵ 4100); ^1H NMR ($CDCl_3$) [for reduced cystoseirol B] 6.50 (1 H, d, J = 3 Hz), 6.38 (1 H, d, J = 3 Hz), 4.45 (1 H, br t), 4.28 (1 H, °), 4.21 (1 H, br t, J = 6 Hz), 2.69 (2 H, m), 2.40 (2 H, AB pattern, J = 10 Hz), 2.35 (1 H, dd, J = 14 and 7 Hz), 2.25 (1 H, dd, J = 14 and 7 Hz), 2.13 (3 H, s), 1.90–1.32 (8 H, m), 1.32 (3 H, s), 1.29 (3 H, s), 1.17 (3 H, s), 1.10 (3 H, s), 1.05 (3 H, s); selected ^1H NMR bands (in $CDCl_3$) [for reduced cystoseirol D] 5.36 (1 H, br t), 4.43 (1 H, br t), 3.40 (1 H, dd, J = 18 and 8.5 Hz), 3.20 (1H, dd, J = 18 and 6.5 Hz), 2.70 (2 H, s), 1.75 (3 H, s).

Dehydration of Cystoseirol B. A solution of cystoseirol B (20 mg) and tosyl chloride (1 mL) in pyridine (0.5 mL) was stirred at room temperature overnight. The mixture was poured into water and then extracted with ether and the crude product purified by HPLC (20% EtOAc in isooctane). The major compound exhibited $[\alpha]_D$ and IR and ^1H NMR ($CDCl_3$) bands similar to **1**.

Dehydration of Cystoseirol D. The reaction was performed as described above and the dehydrated product showed $[\alpha]_D$ −9° (c 0.7, MeOH): IR (film) 3400, 2960, 2860, 1680, 1620, 1595, 1460, 1370, 1220 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) 6.79 (1 H, d, J = 3 Hz), 6.77 (1 H, d, J = 3 Hz), 6.00 (1 H, d, J = 7 Hz), 5.59 (1 H, d, J = 7Hz), 5.31 (1 H, bt, J = 6 Hz), 4.29 (1 H, s), 3.16 (2 H, br t), 2.66 (2 H, AB pattern, J = 14.5 Hz), 2.1 (3 H, s), 1.63 (3 H, s), 1.30 (3 H, s), 1.27 (3 H, s), 1.13 (3 H, s), 0.88 (3 H, s).

Horeau Determination of Cystoseirol B. The alcohol **2** (10 mg) in dry pyridine (150 μL) was treated with (±)-2-phenylbutyric anhydride (32 mg) and left at room temperature overnight. Water (500 μL) was added and the mixture warmed for 30 min until a homogeneous solution was obtained. Water (2 mL) and benzene (3 mL) were added, and the mixture was titrated with NaOH solution (0.1 M) until basic (phenolphtalein). Benzene (10 mL) was added, and the layers were separated. The benzene layer was washed with water, and the combined aqueous phases were acidified to pH 1.5 (10 M HCl) and extracted with benzene (2 × 10 mL). Benzene extracts were washed with water (10 mL), dried over $MgSO_4$, and concentrated to 1 mL and the rotation measured to be +0.012°. Acid excess was dextrogyre. Thus the molecule has the 14R configuration.

Horeau Determination of Cystoseirol A and D. In same conditions as described above, cystoseirol D lead to 14R configuration (+0.02°) and cystoseirol A (reference) was found to be inactive.

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Registry No. **1**, 102396-17-8; **1** (acetate), 102396-20-3; **2**, 102396-15-6; **2** (acetate), 102396-18-9; **3**, 102491-66-7; **3** (acetate), 102490-78-8; **4**, 102396-16-7; **4** (acetate), 102396-19-0; **5**, 102490-77-7; **5** (acetate), 102490-79-9.