Acetylation of Brianthein X. Brianthein X (5 mg) was dissolved in 0.5 mL of dry pyridine; 0.5 mL of Ac_2O was added, and the mixture was allowed to stand, in a sealed flask, at 50 °C for 1 h. Solvent and excess reagent were removed in vacuo, and the residue was permeated through Bio-Beads (S-X8) with CH_2Cl_2–cyclohexane (3:2) to give, in quantitative yield, a crystalline solid (mp 243–246 °C) whose ^1H NMR spectrum was superimposable on that of brianthein Z (7).

Crystallographic Data and X-ray Structure Analysis of 8. Small single crystals of 8 ($C_{28}H_{37}ClO_{10}$, mol wt 569.0) were grown by slow evaporation of an EtOAc solution. These crystals belong to the orthorhombic space group $P2_12_12_1$ with a = 20.307 (3) Å, b = 10.548 (1) Å, c = 13.444 (1) Å, U = 2880 Å^3, Z = 4, P_{calcd} = 1.312 g cm^{-3}, and P_{measd} (flotation) = 1.30 g cm^{-3}. An octant of data to θ = 65° was collected on a Syntex $P2_1$ automated diffractometer using Ni-filtered Cu Kα (λ = 1.5418 Å) radiation (θ–2θ scan mode). Of the 2189 reflections collected, the 1909 reflections with $I > 2\delta(I)$ were used in the structure solution and refinement. The structure was solved by direct phasing methods (MULTAN 80) and refined by full-matrix least-squares[14] methods. After several cycles of refinement of positional and thermal parameters, anomalous scattering corrections for the chlorine atom were introduced into the structure factor calculations to establish the absolute configuration. For coordinates corresponding to the absolute stereochemistry represented in Figure 1, R was 0.070, whereas for the enantiomer R was 0.073. The highly significant[15] difference indicates that 8 correctly represents the absolute stereochemistry. Further refinement led to convergence at R = 0.050. Positions for hydrogen atoms on C(25) were calculated and given a B of 10.0 Å^2 but were not refined. Hydrogen atoms were not placed on C(13), C(14), C(26), C(34), or C(38).

Acknowledgment. We thank Francis Connor and Dr. Wolfgang Sterrer for assistance in collecting and Dr. Frederick M. Bayer for identifying the specimens of *Briareum polyanthes*. Dr. Saralee Visscher offered helpful advice regarding the insecticidal bioassays. We thank Dr. Donald Stierle for sharing his expertise on the metabolites of *B. asbestinum* and James Campbell for helpful discussions. Field work was supported by grants from the H. B. Wilkinson Foundation, Continental Corp., and Exxon Corp., all awarded through the auspices of the Bermuda Biological Station. Research at Montana State University was supported through the generosity of the Lilly Research Laboratories, the Montana Agricultural Experiment Station, and a State match to NSF-EPSCOR Grant ISP-8011449. Acquisition of the NMR spectrometer was funded by NSF Grant CHE 79-26160; the mass spectrometer was purchased with grant monies from NSF Grant CHE 81-15565 and the M. J. Murdock Charitable Trust. K.D.O. is grateful to the National Science Foundation for funding toward the purchase of the diffractometer.

Registry No. 7, 86105-70-6; 8, 87681-19-4; 9, 87681-20-7.

Supplementary Material Available: Listings of final atomic positional and thermal parameters (anisotropic Cl, O, C; isotropic H) are given in Tables III–V. Bond distances and valency angles are in Table VI, and torsion angles are in Table VII (9 pages). Ordering information is given on any current masthead page.

(14) All crystallographic calculations were carried out on a VAX 11/780 computer. The principal programs used were as follows. FMLS; anisotropic full-matrix least-squares refinement: Ganzel, P. L.; Sparks, R. A.; Trueblood, K. N. (UCLA); modified by McPhail, A. T. (Duke University). MULTAN 80; for description see: Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sect. B* **1970**, *B26*, 274. ORTEP; crystallographic illustration programs: Johnson, C. K. Report ORNL-3794; Oak Ridge National Laboratory: Oak Ridge, TN.

(15) Hamilton, W. C. *Acta Crystallogr.* **1965**, *18*, 502.

Synthesis of (24*R*)- and (24*S*)-5,28-Stigmastadien-3β-ol and Determination of the Stereochemistry of Their 24-Hydroxy Analogues, the Saringosterols

Cesar A. N. Catalan,[1†] W. C. M. C. Kokke,[†] Carmenza Duque,[‡] and Carl Djerassi*[†]

Department of Chemistry, Stanford University, Stanford, California 94305, and Departmento de Quimica, Universidad Nacional de Columbia, Bogota, Colombia

Received June 28, 1983

Both epimers at C-24 of 5,28-stigmastadien-3β-ol (24-vinylcholesterol) were synthesized from 5,24(28)-ergostadien-3β-ol, and their configuration at C-24 was determined by conversion into clionasterol and sitosterol. 24-Vinylcholesterol has not yet been found in nature, but it is a possible intermediate in the biosynthesis of 24-propylcholesterol and 24-propylidenecholesterol. A structurally related compound, 5,28-stigmastadiene-3β,24ξ-diol (saringosterol), first isolated from a brown seaweed, was shown to be a mixture of epimers at C-24. They were separated and their configuration was determined by correlation with fucosterol and isofucosterol 24(28)-epoxides of known stereochemistry.

Analysis of sterol mixtures isolated from marine animals has resulted in the discovery of many sterols with side chain alkylation patterns that have not been found in sterols of terrestrial organisms. Such unusual sterols may result from dietary accumulation by the animal, from de novo synthesis by algal symbionts, or from metabolism of dietary sterols by the animal.[2] An investigation of the mechanisms of the formation of such unusual side chains is clearly of biosynthetic interest.

Work on the biosynthesis of sterols resulting from dietary accumulation had to wait until the primary producers, i.e., species of unicellular marine algae which constitute phytoplankton, had been found. That is the reason why, until now, only one paper on sterol side chain biosynthesis

† Stanford University.

‡ Universidad Nacional de Columbia.

(1) Fellow of the Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Republica Argentina while on leave from the Universidad Nacional de Tucuman, Argentina.

(2) Goad, L. J. *Pure Appl. Chem.* **1981**, *53*, 837. Djerassi, C. *Ibid.* **1981**, *53*, 873.

0022-3263/83/1948-5207$01.50/0

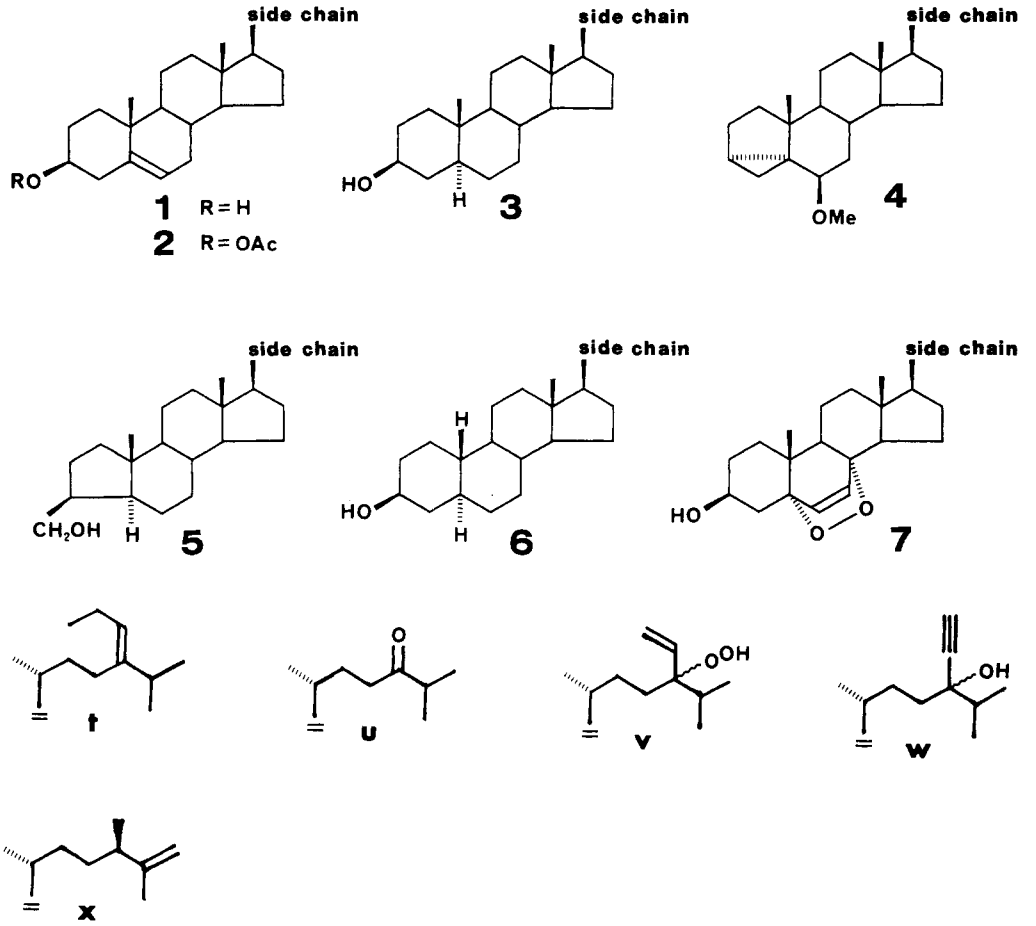


Figure 1. Structures of steroid nuclei and of selected side chains. The structures of side chains **a**–**s** are shown in Schemes I and II.

in a marine alga has been published.[3] In contrast, sterol metabolism in sponges, resulting in sterols with the A-nor skeleton[4] (**5**) and the 19-nor skeleton[5] (**6**) or in the formation of unusual side chains,[6,7] has received considerable attention, notably from the Naples group.[4-6]

In the course of our still ongoing survey of the sterol and phospholipid composition of cultured unicellular marine algae we have discovered several species containing interesting sterols which we are currently using for biosynthetic experiments. Earlier we reported (*E*)-24-propylidenecholesterol (**1t**) to be the main sterol of a still unidentified Chrysophyte alga,[8] which has qualitatively the same sterol composition as *Sarcinochrysis marina*[9] (phylum Chrysophyta, order Ochromonadales, family Sarcinochrysidaceae).[10] This Chrysophyte is photosynthetic, but it has some heterotrophic potential, as methionine labeled with ^{2}H and ^{13}C in the methyl group was taken up from the medium, and sterols labeled in the alkyl substitutents in the side chain were produced.[11]

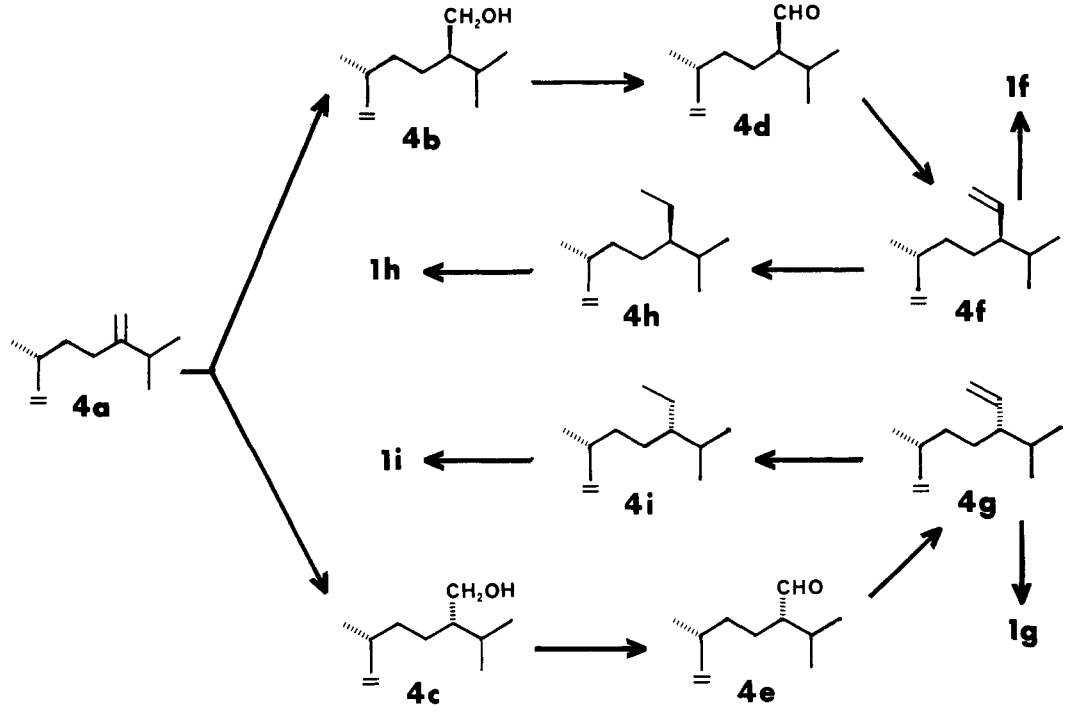
Several plausible mechanisms for the biosynthesis of (*E*)-24-propylidenecholesterol (**1t**) by the Chrysophyte have been considered.[8] To find out which of them is actually operating, and to check the conclusions drawn after our incorporation experiments[11] with labeled methionine, we planned to synthesize selected hypothetical intermediates for two reasons. First, to determine whether they are actually metabolized by the algae, and second, in the case of possible intermediates that have not yet been encountered in nature, to determine their chromatographic behavior (GC, argentic TLC, reverse-phase HPLC). It is possible that some of these intermediates are actually present in the Chrysophyte, but have escaped detection because they were only present in trace amounts, or because they cochromatographed with the main sterols.

In this paper we want to report a synthetic route to both epimers of the most plausible intermediate, viz., 5,28-stigmastadien-3β-ol (24-vinylcholesterol) (**1f,g**). The corresponding radiolabeled compounds will also be synthesized, following the same route, for incubation with a cell-free system prepared from the Chrysophyte in an attempt to prove that 24-vinylcholesterol (**1f,g**) is an intermediate in the biosynthesis of (*E*)-24-propylidenecholesterol (**1t**). Experiments with cell-free system are necessary because we found that the intact cells did not take up radiolabeled 24-methylenecholesterol (**1a**) from the medium.

As outlined below, fucosterol (**1j**) is a useful starting material for the synthesis. When we isolated fucosterol (**1j**) from an extract of the giant kelp *Macrocystis ma-*

(3) Withers, N. W.; Tuttle, R. C.; Goad, L. J.; Goodwin, T. W. *Phytochemistry* **1979**, *18*, 71.
(4) De Rosa, M.; Minale, L.; Sodano, G. *Experientia* **1975** *31*, 408; *Ibid.* **1976**, *32*, 1112. Bibolino, L.; Minale, L.; Sodano, G. *J. Chem. Soc., Chem. Comm.* **1978**, 524. De Stefano, A.; Sodano, G. *Experientia* **1980**, *36*, 630.
(5) Minale, L.; Sodano, G. J. *Chem. Soc., Perkin Trans. 1* **1974**, 1888. De Rosa, M.; Minale, L.; Sodano, G. *Experientia* **1975**, *31*, 758. Minale, L.; Persico, D.; Sodano, G. *Ibid.* **1979**, *35*, 296.
(6) Minale, L.; Riccio, R.; Scalona, O.; Sodano, G.; Fattorusso, E.; Magno, S.; Mayol, L.; Santacroce, C. *Experientia* **1977**, *33*, 1550.
(7) Catalan, C. A. N.; Thompson, J. E.; Kokke, W. C. M. C.; Djerassi, C. *Tetrahedron*, in press.
(8) Rohmer, M.; Kokke, W. C. M. C.; Fenical, W.; Djerassi, C. *Steroids* **1980**, *35*, 219.
(9) Eggersdorfer, M., Stanford University, unpublished results.
(10) Parke, M.; Dixon, P. S. *J. Mar. Biol. As. UK* **1976**, *56*, 527.
(11) Kokke, W. C. M. C.; Shoolery, J. N.; Fenical, W.; Djerassi, C., to be published.

Scheme I. Synthesis of the 5,28-Stigmastadien-3β-ol Epimers (1f,g) and Determination of Their Configuration at C-24[a]



[a] Structures of the nuclei (1, 4) are shown in Figure 1.

jescula, we also obtained saringosterol (5,28-stigmastadiene-3β,24ξ-diol (**1r,s**)). Although its physical properties and those reported in the literature[12] were identical, 360-MHz NMR spectra clearly showed that we had an epimeric mixture and not a sterically pure compound, as saringosterol was supposed to be. Because the required 24-vinylcholesterol epimers (**1f,g**) and the saringosterol epimers (**1r,s**) are structurally related we also report in this paper the separation of the saringosterol epimers (**1r,s**) as well as the determination of their configuration at C-24.

Results and Discussion

24-Methylenecholesterol (**1a**) was the starting material for the synthesis of (24*R*)- and (24*S*)-5,28-stigmastadien-3β-ol [(2j*R*)- (24*S*)-24-vinylcholesterol] (**1f** and **1g**) (see Scheme I). After protection of the Δ^5 double bond by *i*-methyl ether formation,[13] the double bond in the side chain was hydroborated to give a 1:1 mixture of the two possible 28-hydroxy compounds (**4b,c**) which could be separated by reverse-phase HPLC. Each alcohol was oxidized to the corresponding aldehyde (**4d,e**) with chromium trioxide in pyridine or with pyridinium chlorochromate and the aldehydes were treated with methylenetriphenylphosphorane. Deprotection then gave the desired 24-vinylcholesterols (**1f,g**). Use of a ^{14}C-labeled Witting reagent will allow the synthesis of both epimers of 24-vinylcholesterol, radiolabeled in the 29-position. To determine the configuration at C-24, small samples of the 24-vinylcholesterol *i*-methyl ethers (**4f,g**) were hydrogenated and then deprotected to give sitosterol (**1h**) and clionasterol (**1i**), respectively, which were identified by 360-MHz ^1H NMR spectra.

It is not obvious from the mass spectrum of 24-vinylcholesterol (**1f,g**) that it has a Δ^{25} double bond. Several sterols with a double bond in this position have been isolated from marine organisms, and a comparison of their mass spectra has resulted in the conclusion that a Δ^{25} double bond should give rise to two diagnostic peaks in the mass spectrum at m/z 314 and 328[15] (if there is one degree of unsaturation in the nucleus). Both peaks are caused by loss of part of the side chain by a McLafferty rearrangement. However, the 328 peak is usually low if the Δ^{25} double bond is in a terminal position (as in codiesterol [**1x**]). In the mass spectrum of 24-vinylcholesterol (**1f,g**) the diagnostic peak at m/z 328 is not detectable. There is a peak at m/z 327 (24%) and the intensity of the peak at m/z 328 (6%) indicates that it is just the isotope peak of m/z 327. A peak at m/z 327 is expected in the mass spectra of all Δ^5 sterols of M_r 412 with one degree of unsaturation in the side chain. It is caused by a fragment consisting of C and D ring, the side chain, and part of the A and B ring (C-1, 2, 10, 19).[16] Apparently, cleavage of the C-23–C-24 bond is the most favorable process in the fragmentation of 24-vinylcholesterol, as the base peak in the mass spectrum has m/z 81 (C_6H_9).

360-MHz ^1H NMR data of selected compounds mentioned in this section are included in Table I.

Initially, we planned to use fucosterol (**1j**) as starting material for the synthesis of 24-vinylcholesterol (**1f,g**). We wanted to protect the Δ^5 double bond by *i*-methyl ether formation to give (*E*)-3α,5-cyclo-6β-methoxy-24(28)-stigmastene (**4j**). Ozonolysis to the corresponding 24-ketone (**4u**) followed by a Wittig reaction with methylenetriphenylphosphorane would have given 24-methylene-3α,5-cyclo-6β-methoxycholestane (**4a**) which we also prepared from 24-methylenecholesterol (**1a**) (vide supra). When we isolated fucosterol (**1j**) needed for this synthesis, we also obtained a more polar compound whose mp, mass spectrum, and 60-MHz ^1H NMR spectrum were in excellent agreement with data published by Ikekawa et al.[12] for saringosterol ((24ξ)-5,28-stigmastadiene-3β,24-diol) (**1r** or **1s**). However, the complexity of the olefinic region in the

(12) Ikekawa, N.; Tsuda, K.; Morisaki, N. *Chem. Ind.* **1966**, 1179.
(13) Steele, J. A.; Mosettig, E. *J. Org. Chem.* **1963**, *28*, 571.
(14) Fujimoto, Y.; Morasaki, M.; Ikekawa, N. *J. Chem. Soc., Perkin Trans 1* **1975**, 2302.
(15) Djerassi, C.; Theobald, N.; Kokke, W. C. M. C.; Pak, C. S.; Carlson, R. M. K. *Pure Appl. Chem.* **1979**, *51*, 1815.
(16) Wyllie, S. G.; Amos, B. A.; Tökés, L. *J. Org. Chem.* **1977**, *42*, 725.

Table I. 360-MHz ^{1}H NMR Data ($CDCl_3$) for (24*R*)- and (24*S*)-24-Vinylcholesterol (1f and 1g) and Related Compounds[a]

compd	side chain	C-18-H	C-19-H	C-21-H	C-26,27-H	C-28-H	C-29-H
1f		0.666 (s)	1.000 (s)	0.898 (d) J = 6.5	0.864 (d) J = 7; 0.818 (d) J = 6.7	5.508 (ddd) J = 9.7, 10.2, 17.7	4.983 (dd) J = 10.2, 2.3; 4.900 (dd) J = 17.1, 2.3
1g		0.664 (s)	1.002 (s)	0.913 (d) J = 6.5	0.863 (d) J = 6.7; 0.808 (d) J = 6.7	5.553 (dd) J = 9.1, 10.2, 17.1	4.983 (dd) J = 10.2, 2.2; 4.902 (dd) J = 17.1, 2.2
1h		0.678 (s)	1.007 (s)	0.921 (d) J = 6.6	0.833 (d) J = 6.9; 0.811 (d) J = 6.9		0.844 (t) J = 7.6
1i		0.678 (s)	1.008 (s)	0.924 (d) J = 6.6	0.830 (d) J = 7.0; 0.810 (d) J = 7.0		0.853 (t) J = 7.4
4b		0.713 (s)	1.017 (s)	0.922[b] (d) J = 6.5	0.881[b] (d) J = 6.8; 0.905[b] (d) J = 6.9	3.581 (m)	
4c		0.709 (s)	1.017 (s)	0.927[b] (d) J = 7.1	0.897[b] (d) J = 6.8; 0.887[b] (d) J = 6.8	3.583 (m)	
4d		0.710 (s)	1.017 (s)	0.958[b] (d) J = 6.5	0.958[b] (d) J = 6.5; 0.930[b] (d) J = 6.5	9.576 (d) J = 3.7	
4e		0.705 (s)	1.016 (s)	0.959[b] (d) J = 6.5	0.968[b] (d) J = 6.4; 0.934[b] (d) J = 6.4	9.608 (d) J = 3.1	
4f		0.710 (s)	1.019 (s)	0.899 (d) J = 6.6	0.870 (d) J = 6.7; 0.824 (d) J = 6.8	5.518 (ddd) J = 9.5, 10.3, 17.1	4.988 (dd) J = 10.3, 2.3; 4.908 (dd) J = 17.1, 2.3
4g		0.706 (s)	1.019 (s)	0.912 (d) J = 6.6	0.867 (d) J = 6.7; 0.813 (d) J = 6.8	5.560 (ddd) J = 9.1, 10.3, 17.1	4.985 (dd) J = 10.3, 2.3; 4.905 (dd) J = 17.1, 2.3

[a] Shifts are δ values. Splitting constants are in Hz. [b] Tentative assignments.

360-MHz ^{1}H NMR spectrum clearly showed it to be an epimeric (1:1) mixture as the 1 H multiplet at about δ 5.7 ($CDCl_3$) consisted of 8 peaks, whereas only 4 peaks would have been expected in the case of a sterically pure compound. Also, the methyl region of the 360-MHz ^{1}H NMR spectrum showed that the saringosterol sample was a mixture (**1r** and **1s**), which we were able to separate by reverse-phase HPLC. The configuration at C-24 of the saringosterol epimers (**1r,s**) was determined by chemical correlation with the 24(28)-epoxides (**2l–o**) of fucosterol and isofucosterol acetate (**2j,k**). The stereochemistry of these epoxides is known through the work of Ikekawa et al.[17] and Russo et al.[18]

Lithium aluminum hydride reduction of (24*S*,28*S*)-24,28-epoxy-5-stigmasten-3β-ol acetate (**2l**) and of (24*S*,28*R*)-24,28-epoxy-5-stigmasten-3β-ol acetate (**2n**) afforded the higher melting epimer of 5-stigmastene-3β,24-diol (**1p**) which was also obtained as one of the catalytic reduction products of the saringosterol epimer with the shorter retention time in reverse-phase HPLC. Thus this saringosterol epimer has the 24*S* configuration (**1r**). The lower melting epimer of 5-stigmastene-3β,24-diol (**1g**) was obtained by LAH reduction of (24*R*,28*R*)-24,28-epoxy-5-stigmasten-3β-ol (**2m**) and also by catalytic reduction of the saringosterol epimer (**1s**) with the longer retention time in reverse-phase HPLC. This result confirms that the latter saringosterol epimer has the 24*R* configuration (**1s**). 360-MHz ^{1}H NMR data of the saringosterol epimers (**1r,s**) and of related compounds are included in Table II.

The mass spectrum of 5-stigmasten-3β,24-diol (**1p,g**) includes a peak of low intensity for the molecular ion at m/z 430 (1%); a molecular ion was not observed in the mass spectrum of its saturated analogue, 5α-stigmastane-3β,24-diol (**3p,q**). In both spectra the base peak occurs at m/z 101 ($C_6H_{13}O$), caused by cleavage of the C-23–C-24 bond.

It has not yet been demonstrated that saringosterol (**1r,s**) is a natural product. Like several other sterols which are oxygenated in the side chain saringosterol (**1r,s**) must be considered an artifact unless one can present evidence to the contrary.[19,20] The hydroperoxide (**1v**) corresponding to saringosterol has been isolated from tunicates[21] and is not an artifact because it was found in fresh material. The same tunicates also contained sterol epidioxides (**7**).[22] It

(17) Fujimoto, Y.; Murakami, K.; Ikekawa, N. *J. Org. Chem.* **1980**, *45*, 566.
(18) Nicotra, F.; Ronchetti, F.; Russo, G.; Toma, L. *Gazz. Chim. Ital.* **1980**, *110*, 151.
(19) Knights, B. A. *Phytochemistry* **1970**, *9*, 903.
(20) Francisco, C.; Combaut, G.; Teste, J.; Tarchini, C.; Djerassi, C. *Steroids* **1979**, *34*, 163.
(21) Guyot, M.; Davoust, D.; Belaud, C. *Tetrahedron Lett.* **1982**, *23*, 1905.
(22) Guyot, M.; Durgeat, M. *Tetrahedron Lett.* **1981**, *22*, 1391. Gunatilaka, A. A. L.; Gopichand, Y.; Schmitz, F. J.; Djerassi, C. *J. Org. Chem.* **1981**, *46*, 3860 and references therein.

Table II. 360-MHz ^{1}H NMR Data ($CDCl_3$) for the Saringosterols (1r,s) and Related Compounds[a]

compd	side chain	C-18-H	C-19-H	C-21-H	C-27,27-H	C-28-H	C-29-H	other
1p		0.681 (s)	1.006 (s)	0.938 (d) J = 6.5	0.886 (d) J = 6.9; 0.890 (d) J = 6.9		0.850 (t) J = 7.5	
1q		0.681 (s)	1.006 (s)	0.942 (d) J = 6.4	0.885 (d) J = 6.8; 0.891 (d) J = 6.8		0.863 (t) J = 7.4	
1r		0.670 (s)	1.003 (s)	0.916 (d) J = 6.6	0.870 (d) J = 6.9; 0.897 (d) J = 6.9	5.795 (dd) J = 17.4, 10.9	5.181 (dd) J = 17.4, 1.5; 5.130 (dd) J = 10.9, 1.5	
1s		0.665 (s)	1.000 (s)	0.919 (d) J = 6.5	0.867 (d) J = 6.9; 0.887 (d) J = 6.9	5.808 (dd) J = 17.4, 10.9	5.188 (dd) J = 17.4, 1.4; 5.136 (dd) J = 10.9, 1.4	
2l		0.676 (s)	1.016 (s)	0.938[b] (d) J = 6.6	0.907[b] (d) J = 7.0; 0.913[b] (d) J = 6.9	2.903 (q) J = 5.6	1.269 (d) J = 5.6	2.032 (s) (acetate)
2m		0.678 (s)	1.016 (s)	0.933[b] (d) J = 6.5	0.884[b] J = 6.9; 0.919[b] (d) J = 6.8	2.900 (q) J = 5.5	1.266 (d) J = 5.5	2.032 (s) (acetate)
2n		0.665 (s)	1.012 (s)	0.907 (d) J = 6.5	0.956 (d) J = 7.1; 1.047 (d) J = 6.9	2.942 (q) J = 5.6	1.290 (d) J = 5.6	2.032 (s) (acetate)
2o		0.666 (s)	1.012 (s)	0.907 (d) J = 6.6	0.957 (d) J = 7.1; 1.051 (d) J = 6.9	2.942 (q) J = 5.6	1.292 (d) J = 5.6	2.031 (s) (acetate)
3p		0.648 (s)	0.799 (s)	0.920 (d) J = 6.5	0.883 (d) J = 6.9; 0.887 (d) J = 6.9		0.847 (t) J = 7.5	
3q		0.648 (s)	0.799 (s)	0.923 (d) J = 6.5	0.881 (d) J = 6.9; 0.887 (d) J = 6.8		0.859 (t) J = 7.5	

[a] Shifts are δ values. Splitting constants are given in Hz. [b] Tentative assignments.

seems feasible that saringosterol might occur as a natural product in animals that also contain epidioxides (**7**) such as some sponges, tunicates, and a sea hare.[22] In those animals saringosterol might be formed via the hydroperoxide **1v**.

Saringosterol (**1r,s**) has been synthesized by Ikekawa et al.[12,14] by catalytic reduction of stigmast-5-en-28-yne-3β,24-diol (**1w**) and by treatment of the corresponding monoacetate (**2w**) with LAH–$AlCl_3$. A sample prepared by the second method was kindly supplied by Professor Ikekawa. Surprisingly, the 360-MHz ^{1}H NMR spectrum showed that it was not a 1:1 mixture (**1r,s**) as expected but an 85:15 mixture. This followed from the relative intensities of the overlapping 4-line patterns at about δ 5.7. The main component was the epimer with this 4-line pattern at lowest field (**1s**).

Experimental Section

General. We used Waters HPLC equipment (M6000, M6000A and M45 pumps, R401 and R403 refractometers, U6K injector) and also a Valco Model CV-6-UHPa-N60 and a Rheodyne Model 7120 injector, two Waters semipreparative μPorasil columns in series, a Whatman Partisil M9 10/50 ODS-2 column, and two Altex Ultrasphere ODS columns (5 μm, 10 mm I.D. × 25 cm) in series. For GC we used a Hewlett-Packard Model 402 gas chromatograph with FID (3% SP2250 column, 2 mm I.D. × 1.80 m, 260 °C). The point of injection and not the beginning of the solvent peak was used to calculate relative (Rt_R) and absolute retention times (t_R) in HPLC. Low-resolution mass spectra were recorded on a Finnigan MAT-44 spectrometer [probe or GC/MS (capillary SE54 column, 9 m, 260 °C)]. High-resolution mass spectra were recorded using an MS50 instrument at the Midwest Center for Mass Spectrometry, University of Nebraska—Lincoln. 360-MHz ^{1}H NMR spectra were recorded on a Brucker HXS-360 NMR spectrometer and 300-MHz ^{1}H NMR spectra on a Nicolet NMC-300 widebore NMR spectrometer.

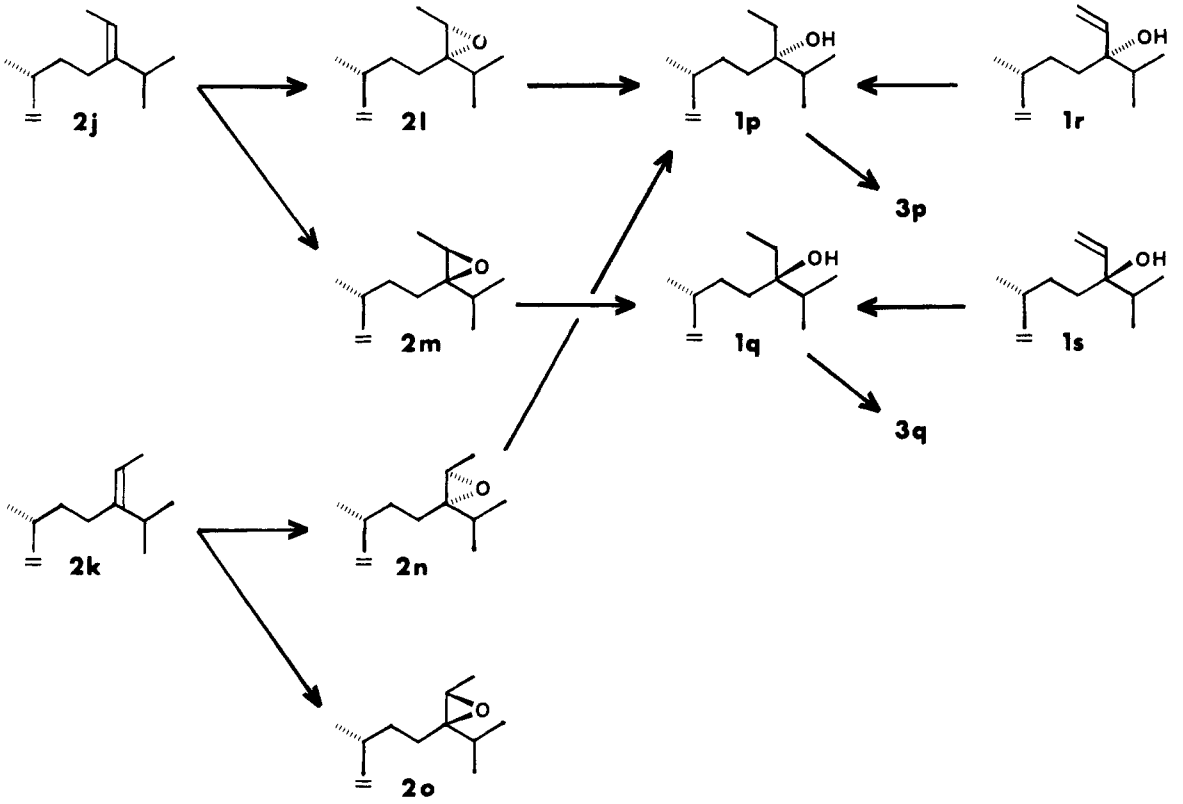
24-Methylenecholesterol (1a) was isolated by argentic TLC in the manner of Idler[23] from sterol fractions of soft corals from the Indo-Pacific (Palau) in which it occurs as the main sterol.

24-Methylene-3α,5-cyclo-6β-methoxycholestane (24-Methylenecholesterol *i*-Methyl Ether) (4a). 24-Methylenecholesterol (**1a**) (143 mg) in anhydrous pyridine (2 mL) was treated with *p*-toluenesulfonyl chloride (220 mg) at 0 °C. After 24 h at room temperature the mixture was poured into 5% $NaHCO_3$ (15 mL), stirred for 15 min, and then extracted with ether. After the usual workup 191 mg of the tosylate was obtained. It was dissolved in absolute MeOH (8 mL) and fused KOAc (210 mg) was added, and the mixture was refluxed for 24 h. After evaporation of the solvent the residue was partitioned between ether and water. Column chromatography (silica gel, eluent hexane) afforded 159 mg of **4a**: MS 70 eV (probe), m/z (relative intensity) 412 (M^+, 1.5), 397 (2), 380 (2), 357 (5), 328 (3), 313 (4), 296 (4), 285 (6), 253 (11), 227 (5), 213 (6), 201 (4), 55 (100).

(24*R*)- and (24*S*)-24-(Hydroxymethyl)-3α,5-cyclo-6β-methoxycholestane [(24*R*)- and (24*S*)-24-(Hydroxymethyl)cholesterol *i*-Methyl Ether] (4b and 4c). A solution of **4a** (90 mg, 0.22 mM) in THF (70 mL) under nitrogen was cooled

(23) Idler, D. R.; Safe, L. M. *Steroids* **1972**, *19*, 315.

Scheme II. Determination of the Configuration at C-24 of the 5,28-Stigmastadiene-3β,24-diol Epimers (1r,s)[a]



[a] Structures of the nuclei (1-3) are shown in Figure 1.

in an ice bath and 10 mL of a 1 M solution of BH_3–THF complex (Aldrich) was added with magnetic stirring. After 1 h at 0 °C and 24 h at room-temperature the mixture was cooled again, and 16 mL of water was added dropwise, followed by 6 mL of 3 N sodium hydroxide, and finally by the slow addition of 16 mL of 30% hydrogen peroxide. The mixture was stirred at room temperature for 2 h then extracted with chloroform. The chloroform extract was washed with water, dried, and evaporated. The reaction product was isolated from the residue of the chloroform extract by silica gel column chromatography (eluent hexane: ether 8:2). The yield was 78 mg (83%); the purity was checked by TLC and GC (RRT 1.50, standard cholesterol). The 360-MHz ^1H NMR spectrum showed the product to be a 1:1 mixture of both epimers (**4b** and **4c**). The epimers were separated by reverse-phase HPLC (Altex columns, MeOH–water 97:3). Reinjection of the material corresponding to the first and second peak gave the pure epimers: **4b** (24*R*), t_R 60 min (flow 3.0 mL/min), 28 mg. **4c** (24*S*), t_R 62 min, 33 mg. GC/MS 70 eV, *m/z* (relative intensity) 430 (M^+, 78), 415 (44), 398 (100), 375 (64), 315 (11), 277 (8), 255 (14), 229 (10), 213 (11).

(24*R*)- and (24*S*)-3α,5-Cyclo-6β-methoxyergostan-28-al (4d and 4e). Method A.[24] To a solution of anhydrous pyridine (0.15 mL) in CH_2Cl_2 (2 mL) was added chromium trioxide (80 mg) with magnetic stirring. After 20 min, alcohol **4b** (or **4c**) (20 mg) in CH_2Cl_2 (1 mL) was added dropwise. The reaction was complete after 10 min (TLC). The solution was decanted and the residue was washed with ether. The combined decantate and washings were filtered through a short silica gel column. The solvent was evaporated and the residue was purified by HPLC (ODS-2 column, MeOH, t_R about 26 min at a flow of 3.0 mL/min). 360-MHz ^1H NMR spectra (Table I) of the products showed that no epimerization had taken place during the reaction.

Method B.[25] To 17 mg of pyridinium chlorochromate suspended in 0.5 mL of CH_2Cl_2 was added with stirring 22 mg of alcohol **4b** (or **4c**) in 0.5 mL of CH_2Cl_2. After 2 h, 2 mL of ether was added and the supernatant was decanted. The residue was washed twice with ether. The combined decantate and washings were worked up as above. The yield of **4d** (or **4e**) was 17–19 mg (77–86%). The product was sterically pure (360 MHz ^1H NMR, see Table I). MS 70 eV (probe), *m/z* (relative intensity) 428 (M^+, 1.5), 413 (1.5), 396 (3), 381 (0.5), 373 (3), 255 (5), 229 (3), 213 (8), 201 (4), 199 (3), 55 (100).

(24*R*)- and (24*S*)-3α,5-Cyclo-6β-methoxystigmast-28-ene [(24*R*)- and (24*S*)-24-Vinylcholesterol *i*-Methyl Ether] (4f and 4g). In a small vial provided with a serum stopper and containing methyltriphenylphosphonium iodide (14 mg, 35 μM) and a stirring bar under nitrogen, 0.5 mL of anhydrous ether was introduced followed by 15 μL (36 μM) of a 2.4 M solution of *n*-butyllithium in hexane. After stirring for 2.5 h at room temperature, 43 mg (100 μM) of the aldehyde **4d** or **4e** in 0.5 mL of ether was introduced. The mixture was stirred at room temperature for 65 h. It was then diluted with hexane–ether 97:3 (1 mL) and stirred for 15 min. The supernatant was introduced into a small silica gel column and the reaction product was eluted with hexane-ether 97:3. Further purification using Altex columns (MeOH, flow 3.0 mL/min) gave the desired olefin (**4f**, t_R 100 min, **4g**, t_R 102.1 min.). Yield (based on the phosphonium salt, i.e., the limiting reagent), 43–57% (6.4–8.5 mg). A yield of 75–80% is obtained by this procedure if a 100% excess of phosphonium salt is employed. We have shown by coinjection that both epimers of 24-vinylcholesteryl *i*-methyl ether (**4f** and **4g**) can be separated by reverse-phase HPLC (Altex columns, MeOH). Thus we could use reverse-phase HPLC to prove that no epimerization had taken place during the Wittig reaction. MS 70 eV (probe), *m/z* (relative intensity) 426 (M^+, 1), 411 (0.6), 400 (0.2), 394 (1), 379 (0.1), 371 (1), 368 (0.7), 345 (0.2), 313 (0.1), 255 (1), 213 (2), 55 (100).

Hydrogenation of the 24-Vinylcholesterol *i*-Methyl Ethers (4f and 4g). The *i*-methyl ether **4f** (2 mg) and PtO_2 (Aldrich) (2.3 mg) in EtOAc (2 mL) were stirred under hydrogen for 2 h. Filtration, evaporation of the solvent, and purification by reverse-phase HPLC (ODS-2, MeOH) yielded 1.6 mg of the *i*-methyl ether of 24-ethylcholesterol (**4h**). Deprotection[26] gave (24*R*)-24-ethyl-5-cholesten-3β-ol (**1h**) (sitosterol) as was determined by 360-MHz ^1H NMR (Table I). This result permitted the assign-

(24) Ratcliffe, R.; Rodehorst, R. *J. Org. Chem.* **1970**, *35*, 4000.
(25) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647.
(26) Theobald, N.; Wells, R. J.; Djerassi, C. *J. Am. Chem. Soc.* **1978**, *100*, 7677.

ment of the configuration at C-24 for the family **4b**, **4d**, **4f**, and **1f**. Similarly, hydrogenation of **4g** followed by deprotection[26] gave (24*S*)-24-ethyl-5-cholesten-3β-ol (clionasterol) (**1i**) (stereochemistry at C-24 determined by 360-MHz ^{1}H NMR) thus establishing the configuration at C-24 for the family **4c**, **4e**, **4g**, and **1g**.

(24*R*)- and (24*S*)-5,28-Stigmastadien-3β-ol [(24*R*)- and (24*S*)-24-vinylcholesterol] (1f and 1g) were prepared by deprotection[26] of the corresponding *i*-methyl ethers (**4f** and **4g**), followed by purification of the free sterol by reverse-phase HPLC (Altex columns, MeOH). (24*R*)-Vinylcholesterol (**1f**): mp 132–133 °C (from MeOH); Rt_R (HPLC) 0.91; Rt_R (GC) 1.53 (standard cholesterol). (24*S*)-24-Vinylcholesterol (**1g**): mp 141–142 °C (from MeOH); Rt_R (HPLC) 0.92; Rt_R (GC) 1.53. A reference sample of 24-vinylcholesterol, kindly supplied by Professor N. Ikekawa,[14] had mp 123–127 °C, and was shown to be a 1:1 mixture of both epimers by 360-MHz ^{1}H NMR. High-resolution MS 70 eV of **1f** (probe), *m/z* (assignment, relative intensity) 412.3703 ($C_{29}H_{48}O$, M^+, 70), 397.3461 ($C_{28}H_{45}O$, 25), 394.3588 ($C_{29}H_{46}$, 35), 379.3355 ($C_{28}H_{43}$, 25), 369.3172 ($C_{26}H_{41}O$, 2), 352.3114 ($C_{26}H_{40}$, 7), 340.3121 ($C_{25}H_{40}$, 3), 339.3054 ($C_{25}H_{39}$, 2), 327.3041 ($C_{24}H_{39}$, 24), 314.2607 ($C_{22}H_{34}O$, 9), 301.2878 ($C_{22}H_{37}$, 19), 299.2380 ($C_{21}H_{31}O$, 10), 283.2427 ($C_{21}H_{31}$, 2), 281.2273 ($C_{21}H_{29}$, 3), 273.2214 ($C_{19}H_{29}O$, 19), 271.2064 ($C_{19}H_{27}O$, 22), 255.2115 ($C_{19}H_{27}$, 27), 246.1984 ($C_{17}H_{26}O$, 7), 241.1955 ($C_{18}H_{25}$, 9), 231.1748 ($C_{16}H_{23}O$, 25), 229.1947 ($C_{17}H_{25}$, 10), 228.1874 ($C_{17}H_{24}$, 12), 215.1791 ($C_{16}H_{23}$, 13), 213.1643 ($C_{16}H_{21}$, 45), 81.0715 (C_6H_9, 100).

Isolation of (24*S*)- and (24*R*)-5,28-Stigmastadiene-3β,24-diol [(24*S*)- and (24*R*)-24-Saringosterol] (1r and 1s). Method A. Workup of the extract of giant kelp by silica gel column chromatography afforded the free sterols (mainly fucosterol [**1j**]) and a more polar compound (R_f 0.1, silica gel TLC, hexane–ether 1:1) of mp 160 °C (from hexane) which was identified as a 1:1 mixture of the saringosterol[12] epimers (**1r** and **1s**) on the basis of its mass spectrum and its 360-MHz ^{1}H NMR spectrum. The epimers could be separated by reverse-phase HPLC (Altex columns, MeOH–water 9:1), and they were further purified by recrystallization from hexane.

(24*S*)-Saringosterol (1r): t_R (HPLC, flow 3.0 mL/min) 98.0 min; mp 168.5–169.0 °C; $[\alpha]_D$ −55° (*c* 6 × 10^{-3}, $CHCl_3$); 300 MHz ^{1}H NMR (C_6D_6) δ (multiplicity, splitting constant(s) in Hz, assignment) 5.706 (dd, 10.9 and 17.3, C-28–H), 5.354 (d, 5.0, C-6–H), 5.259 (dd, 1.8 and 17.3, C-29–H_a), 5.095 (dd, 1.8 and 10.8, C-28–H_b), 0.976 (d, 6.5, C-21–H), 0.859 and 0.943 (d's, 6.8, C-26–H and C-27–H), 0.944 (s, C-19–H), 0.648 (s, C-18–H).

(24*R*)-Saringosterol (1s): t_R (HPLC, flow 3.0 mL/min) 101.5 min; mp 167.0–167.5 °C; $[\alpha]_D$ −27° (*c* 5 × 10^{-3}, $CHCl_3$); 300 MHz ^{1}H NMR (C_6D_6) δ (multiplicity, splitting constant(s) in Hz, assignment) 5.716 (dd, 10.9 and 17.3, C-28–H), 5.356 (m, C-6–H), 5.250 (dd, 1.7 and 17.3, C-29–H_a), 5.096 (dd, 1.7 and 10.8, C-29–H_b), 0.977 (d, 6.5, C-21–H), 0.945 and 0.860 (d's, 6.8, C-26,27–H), 0.947 (s, C-19–H), 0.655 (s, C-18–H).

Method B. The free sterol fractions of giant kelp (vide supra) were combined and evaporated. The residue was saponified; the neutral unsaponifiables were obtained as an oil. GC/MS showed the sterol components of the oil to be a 9:1 mixture of fucosterol (**1j**) and 24-methylenecholesterol (**1a**); saringosterol (**1r,s**) was not present. The oil, in a white glass vial, was left on a bench in contact with air and light for about two months. Workup by silica gel chromatography using hexane–ether then afforded pure regular sterols (**1a,j**) together with the autooxidation product saringosterol (**1r,s**).[19,20] The weight of the autooxidation product was twice that of the unchanged sterols.

Method C. The sponge *Xestospongia muta* was collected at −25 m off Punta Agujas, Colombian Caribbean coast. It was identified by Dr. Sven Zea, El Instituto de Investigaciones Marinas de Punta Betin (Colombia). The sun-dried sponge (1000 g) was extracted with $CHCl_3$–MeOH (2:1) according to the Bligh and Dyer[27] procedure. The yield was 22.76 g of lipids. Part of this mixture (16.3 g) was carefully chromatographed over silica gel (eluent benzene–EtOAc 10:2) to give two fractions: XM1 (143 mg, R_f 0.24 in the above solvent mixture) and XM2 (460 mg, R_f 0.28). Cholesterol has R_f 0.28 under these conditions. Saringosterol (**1r,s**) was isolated by reverse-phase HPLC (one Altex column, MeOH) from fraction XM1; it accounted for 31.3% of this fraction. This saringosterol sample was a 1:1 mixture of both epimers (**1r,s**). It is probably an artifact[19,20] formed from fucosterol (**1j**) or isofucosterol (**1k**) when the sponge was sun-dried.

Hydrogenation of (24*S*)-Saringosterol (1r). A 6.0 mg sample of **1r** in EtOAc (4 mL) was hydrogenated for 1 h with PtO_2 at room temperature. Filtration, evaporation of the solvent and separation of the reaction products (a 2:1 mixture) by reverse-phase HPLC (Altex columns, MeOH–7.5% water) gave (24*S*)-5-stigmastene-3β,24-diol (**1p**), t_R 47 min (flow 3.0 mL/min.), mp 178–179 °C (from hexane), and (24*S*)-5α-stigmastane-3β,24-diol (**3p**), t_R 53 min, mp 194–195 °C (from hexane). **1p** was also obtained by LAH reduction of (24*S*,28*S*)-24,28-epoxy-stigmasten-3β-ol acetate (**2l**) and of (24*S*,28*R*)-24,28-epoxy-5-stigmasten-3β-ylacetate (**2n**) (vide infra). The stereochemistry of these epoxides is known.

Hydrogenation of (24*R*)-Saringosterol (1s). The 24*R* isomer **1s** was hydrogenated under the same conditions as **1r**. Separation of the products (as above) gave (24*R*)-5-stigmastene-3β,24-diol (**1q**), t_R 48.5 min, mp 163–164 °C (from hexane) and (24*R*)-5α-stigmastane-3β,24-diol (**3q**), t_R 54.0 min, mp 182–183 °C (from hexane). **1q** was also obtained by LAH reduction of (24*R*,28*R*)-24,28-epoxy-5-stigmasten-3β-ol acetate (**2m**) (vide infra). The stereochemistry of that epoxide is known. High-resolution MS of **3q**, 70 eV (probe), *m/z* (assignment, relative intensity) 432 (M^+, not observed), 414.3844 ($C_{29}H_{50}O$, 2), 399.3629 ($C_{28}H_{47}O$, 1), 389.3411 ($C_{26}H_{45}O_2$, 4), 385.3460 ($C_{27}H_{45}O$, 4), 371.3314 ($C_{26}H_{43}O$, 13), 353.3197 ($C_{26}H_{41}$, 6), 316.2756 ($C_{22}H_{36}O$, 19), 301.2534 ($C_{21}H_{33}O$, 7), 299.2733 ($C_{22}H_{35}$, 2), 285.2581 ($C_{21}H_{33}$, 3), 283.2427 ($C_{21}H_{31}$, 1), 273.2221 ($C_{19}H_{29}O$, 16), 257.2270 ($C_{19}H_{29}$, 12), 233.1901 ($C_{16}H_{25}O$, 5), 229.1955 ($C_{17}H_{25}$, 3), 217.1949 ($C_{16}H_{25}$, 4), 215.1798 ($C_{16}H_{23}$, 8), 101.0967 ($C_6H_{13}O$, 100).

(24*S*,28*S*)- and (24*R*,28*R*)-24,28-Epoxy-5-stigmasten-3β-ol acetate (2l and 2m) were prepared from fucosterol acetate (**2j**) using a procedure for the epoxidation of fucosterol benzoate.[17] From 42 mg of fucosteryl acetate (**2j**) 34.2 mg of a 1:1 mixture of the epoxide **2l** and **2m** was obtained and 11.3 mg of starting material was recovered. Products and starting material were separated using a small silica gel column and hexane–toluene (3:2) and benzene as eluents. The epoxides were separated by HPLC (two Waters semipreparative μPorasil columns in series, hexane–2.5% EtOAc); **2m** was eluted faster then **2l**. The stereochemistry of these epoxides is known through the work of Ikekawa et al.[17,28]

(24*S*)-5-Stigmastene-3β,24-diol (1p). Method A. (24*S*,28*S*)-24,28-Epoxy-5-stigmasten-3β-ol acetate (**2l**) (the 24,28-epoxide of fucosterol acetate with the lower R_f value on silica gel) (17 mg) was reduced with LAH (200 mg) in ether (25 mL) (reflux, 12 h). The product was worked up by silica gel column chromatography (eluent hexane–ether 1:1), purified by reverse-phase HPLC (Altex columns, MeOH–7.5% water), and recrystallized from hexane. In this manner the higher melting epimer of 5-stigmastene-3β,24-diol was obtained: mp 178.5–180.0 °C, $[\alpha]_D$ −30.7 (*c* 5 × 10^{-3}, $CHCl_3$).

Method B. A sample of (24*S*,28*R*)-24,28-epoxy-5-stigmasten-3β-yl acetate (**2n**) (the 24,28-epoxide of isofucosterol acetate with the higher R_f value on silica gel) was kindly supplied by Professor G. Russo, University of Milan, Italy. The stereochemistry of this epoxide has been determined by Russo et al.[18] LAH reduction and workup as above afforded **1p**: mp 177.5–179.0 °C (from hexane); high-resolution MS, 70 eV (probe), *m/z* (assignment, relative intensity) 430.3799 ($C_{29}H_{50}O_2$, M^+, 1), 412.3686 ($C_{29}H_{48}O$, 7), 401.3409 ($C_{27}H_{45}O_2$, 1), 397.3462 ($C_{28}H_{45}O$, 4), 387.3254 ($C_{26}H_{43}O_2$, 11), 379.3354 ($C_{28}H_{43}$, 3), 369.3139 ($C_{26}H_{41}O$, 19), 351.3034 ($C_{26}H_{39}$, 6), 314.2590 ($C_{22}H_{34}O$, 39), 312.2787 ($C_{22}H_{35}$, 7), 299.2378 ($C_{21}H_{31}O$, 9), 296.2493 ($C_{22}H_{32}O$, 3), 283.2408 ($C_{21}H_{31}$, 3), 281.2261 ($C_{21}H_{29}$, 7), 273 (8), 271.2059 ($C_{19}H_{27}O$, 21), 255.2097 ($C_{19}H_{27}$, 15), 253.1945 ($C_{19}H_{25}$, 4), 231 (8), 229 (11), 217 (4), 215.1764 ($C_{16}H_{23}$, 5), 213.1639 ($C_{16}H_{21}$, 11), 101.0959 ($C_6H_{13}O$, 100).

(24*R*)-5-Stigmastene-3β,24-diol (1q). (24*R*,28*R*)-24,28-Epxoy-5-stigmasten-3β-yl acetate (**2m**) (the 24,28-epoxide of fucosterol acetate with the higher R_f value on silica gel) was reduced with LAH and worked up as above to give the lower melting

(27) Bligh, E. G.; Dyer, W. J. *Can. J. Biochem. Physiol.* **1959**, *37*, 911.

(28) Chen, S.-M.; Nakanishi, K.; Atawa, N.; Morisaki, M.; Ikekawa, N.; Shimizu, Y. *J. Am. Chem. Soc.* **1975**, *97*, 5297.

epimer of 5-stigmastene-3β-24-diol (**1q**): mp 161.0–162.0 °C (from hexane); $[\alpha]_D$ −34.2° (c 5 × 10^{-3}, $CHCl_3$).

Acknowledgment. We are indebted to Dr. William Fenical, Scripps Institution of Oceanography, La Jolla, for sterol samples of soft corals from Palau, to Professor N. Ikekawa, Tokyo Institute of Technology, for samples of 24-vinylcholesterol and saringosterol, to Professor G. Russo, University of Milan, for samples of the acetates of the isofucosterol 24,28-epoxides, to Dr. Sven Zea, El Instituto de Investigacions Marinas de Punta Betin, Columbia, for the identification of *X. muta*, and to Dr. Howard Jones, Merck, Sharp & Dohme, Rathway, New Jersey, for the extract of giant kelp. CANC thanks the Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Republica Argentia for a postdoctoral fellowship. Financial support to C. Duque was provided by Fondo Colombiano de Investigaciones Cientificas y Proyectos Especiales 'Francisco Jose de Caldas' COLCIENCIAS (Grant 10000-1-126-80) and by El Programa Multinacional de Quimica of the OAS. The research at Stanford was supported by Grants GM-06840 and GM-28352 from the National Institutes of Health. 360-MHz ^1H NMR spectra were recorded in the Stanford Magnetic Resonance Laboratory which is supported by grants from the National Science Foundation (GP-23633) and the National Institutes of Health (RR-00711). The acquisition of a Nicolet 300-MHz NMR spectrometery by the Stanford Chemistry Department was made possible by a grant from the National Science Foundation (CHE 81-09064). High-resolution mass spectra were recorded at the Midwest Center for Mass Spectrometry, University of Nebraska—Lincoln, which is supported by grants from the National Science Foundation.

Registry No. **1a**, 474-63-5; **1a**·tosylate, 87801-42-1; **1f**, 58507-55-4; **1g**, 87859-96-9; **1h**, 83-46-5; **1i**, 83-47-6; **1p**, 71208-86-1; **1q**, 87859-97-0; **1r**, 87859-98-1; **1s**, 87859-99-2; **2j**, 51297-12-2; **2l**, 57173-62-3; **2m**, 57173-63-4; **2n**, 57173-69-0; **2o**, 57173-70-3; **3p**, 87801-43-2; **3q**, 87860-00-2; **4a**, 68844-31-5; **4b**, 68844-34-8; **4c**, 68889-65-6; **4d**, 87801-44-3; **4e**, 87860-01-3; **4f**, 87801-45-4; **4g**, 87860-02-4; **4h**, 53139-46-1; methyltriphenylphosphonium iodide, 2065-66-9.

Phospholipid Synthesis Based on New Sequential Phosphate and Carboxylate Ester Bond Formation Steps

C. Jeffrey Lacey and Leslie M. Loew*

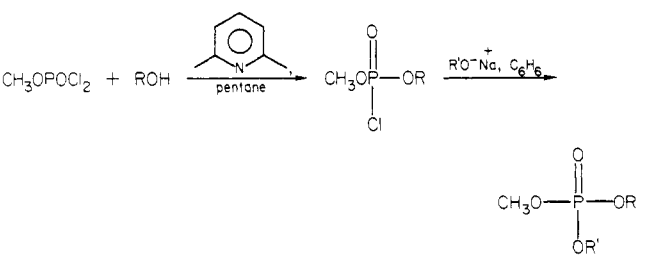
Department of Chemistry, State University of New York at Binghamton, Binghamton, New York 13901

Received May 19, 1983

A total synthesis of racemic phosphatidylcholine is based on two novel but straightforward reaction sequences. The phosphate diester portion is constructed by successive displacement of the chlorines on methyl dichlorophosphate by allyl alcohol and dimethylethanolamine. The resulting triester isomerizes smoothly to allyl choline phosphate. The double bond is then converted to the bromohydrin to allow the sequential introduction of the two acyl ester linkages. Esterification of the hydroxyl with palmitoyl chloride produces 2-bromo-2-deoxylysophosphatidylcholine as the only isomer. The bromide is displaced in the final step upon treatment with the carboxylate form of an anion-exchange resin. The distinctive ^{31}P–^{13}C coupling patterns in the ^{13}C resonances of the glycerol backbone allow the regiochemistry of the various steps to be conveniently monitored. Also, employment of palmitic-*1*-^{13}C acid in the final step indicated a 70% rearrangement accompanied formation of the mixed acid phosphatidylcholine.

The synthesis of phospholipids has been actively and successfully pursued for over 70 years.[1] With our focus restricted to the preparation of phosphatidylcholine (PC), the available methods can be approximately divided into three approaches: (1) the formation of phosphate ester linkages to a diglyceride and choline as the last steps;[2] (2) the introduction of both fatty acid ester linkages simultaneously into glycerophosphorylcholine to provide symmetrical PC;[3] (3) the preparation of mixed carboxy ester PC by acylation of lysophosphatidylcholine (the latter is obtained by enzymatic hydrolysis of a symmetrical PC).[3c,4]

Scheme I



(1) A. Grun and I. Kade, *Chem. Ber.*, **45**, 3367 (1912).

(2) (a) E. Baer and M. Kates, *J. Am. Chem. Soc.*, **72**, 942 (1950); (b) E. Baer and J. Maurukus, *Ibid.*, **74**, 158 (1952); (c) T. Malkin and T. H. Bevan in "Progress in the Chemistry of Fats and Lipids," Vol. IV, R. T. Holman, W. O. Lundberg, and T. Malkin, Eds., Pergamon Press, London, 1957, pp 97–138; (d) E. Baer and A. Kindler, *Biochemistry*, 1, 518 (1962); (e) V. R. Hirt and R. Berchtold, *Pharm. Acta Helv.*, **33**, 349 (1958); (f) T. Mukaiyama, N. Morito, and Y. Watanabe, *Chem. Lett.*, 521 (1979); (g) P. E. Verkade, *Bull. Soc. Chim. Fr.*, 1993 (1963); (h) V. I. Shveits, L. T. Dorofeeva, M. A. Grum-Grzhimailo, I. S. Shmidt, L. V. Volkova, and N. A. Preobrazhenskii, *Zh. Obshch. Khim.*, **34**, 3983 (1964); (i) N. A. Bogoslovskii, G. I. Samokhvalov, and N. A. Preobrazhenskii, *Ibid.*, **32**, 2210 (1962); (j) Yu. G. Molotkovskii, T. Yu. Lazurkina, and L. D. Bergel'son, *Izv. Akad. Nauk SSSR Ser. Khim.*, 8, 1784 (1969); (k) G. H. de Haas and L. L. M. van Deenen, *Tetrahedron Lett.*, 1 (1960); (l) G. H. de Haas and L. L. M. van Deenen, *Recl. Trav. Chem. Pays-Bas*, **80**, 951 (1961).

(3) (a) E. Baer, D. Buchnea, and A. G. Newcombe, *J. Am. Chem. Soc.*, **78**, 232 (1956); (b) N. H. Tattrie and C. S. McArthur, *Can. J. Biochem. Physiol.*, **35**, 1165 (1957); (c) E. Baer and D. Buchnea, *Ibid.*, **37**, 953 (1959); (d) E. Cubero Robles and D. van den Berg, Biochim. *Biophys. Acta*, **187**, 520 (1969); (e) E. L. Pugh and M. Kates, *J. Lipid Res.*, **16**, 392 (1975).

0022-3263/83/1948-5214$01.50/0