systematic extinctions ($0k0$, absent if $k = 2n + 1$) were uniquely accommodated by space group $P2_1$. A calculated density indicated one molecule of composition $C_{20}H_{32}O_3$ formed the asymmetric unit.

All unique diffraction maxima with $2\theta \leq 114°$ were surveyed on a computer-controlled, four-circle diffractometer by using graphite-monochromated Cu Kα radiation (1.541 78 Å) and a variable-speed, 1° ω scan technique. Periodically monitored check reflections showed no significant crystal decomposition. Of the 1285 reflections surveyed in this manner, 1153 (90%) were considered observed ($|F_o| \geq 3\sigma(F_o)$) after correction for Lorentz, polarization, and background effects.

A phasing model was achieved by using a standard multisolution tangent formula approach, and an E synthesis revealed a plausibly connected 15-atom fragment.[21] The remainder of the nonhydrogen atoms were located on an F synthesis phased by these atoms. Block-diagonal, least-squares refinement followed by a ΔF synthesis revealed the hydrogen atoms which were assigned fixed isotropic temperature factors (4.1 Å^{-2}). Full-matrix, least-squares refinements with anisotropic thermal parameters for the nonhydrogen atoms have currently converged to a standard, unweighted, crystallographic residual of 0.045 for the observed data. Further crystallographic details can be found in the supplementary material described in the paragraph at the end of this paper.

(ii) X-ray Crystallographic Analysis of 7. A clear rectangular parallelepiped of phyllocladane diol **7** with dimensions 0.7 × 0.2 × 0.2 mm was chosen for single-crystal X-ray diffraction analysis. Preliminary X-ray photographs indicated orthorhombic symmetry, and accurate lattice parameters of $a = 17.582$ (4), $b = 14.606$ (4), and $c = 7.025$ (1) Å were determined by a least-squares fit of 15 moderate 2θ values. The systematic extinctions ($h00$, h = $2n + 1$; $0k0$, $k = 2n + 1$; $00l$, $l = 2n + 1$) and presence of chirality were uniquely accommodated by the choice of $P2_12_12_1$ as a space group. A calculated density was also consistent with one molecule of $C_{20}H_{34}O_2$ in the asymmetric unit.

All unique diffraction maxima with $2\theta \leq 60°$ were collected on a computer-controlled, four-circle diffractometer by using graphite-monochromated Mo Kα radiation (0.710 69 Å) and a variable-speed, 1° ω scan technique. Of the 2729 reflections surveyed in this manner, 2325 (85%) were considered observed ($|F_o| \geq 3\sigma(F_o)$) after correction for Lorentz, polarization, and background effects. No crystal decomposition was observed in periodical monitoring of check reflections.

The structure was easily solved by using a multisolution, weighted tangent formula approach for phase determination.[21] An E synthesis calculated from the set of phases with the most favorable figures of merit revealed the entire nonhydrogen framework except the C(18) methyl group. The structure was routinely completed by Fourier methods and all the hydrogen atoms were located in a difference Fourier synthesis calculated from a partially refined ($R \approx 0.10$) set of phases. Full-matrix, least-squares refinements with anisotropic thermal parameters for the nonhydrogen atoms and isotropic thermal parameters for the hydrogen atoms have converged to a standard crystallographic residual of 0.045 for the observed data (0.060 weighted residual). Further crystallographic details can be found in the supplementary material described in the paragraph at the end of this paper.

Acknowledgment. We thank NIH-NCI for support of the work at Northeastern University (Grant No. CA-13001) and that at Cornell University (Grant No. CA-24487). The plant material was collected in Panama for the U.S. Department of Agriculture (collector's no. KEB 3984; USDA no. PR44437), to whom we are indebted for making it available to us. We also thank Professor Paul Vouros and Mr. Hamdy Maksoud, Northeastern University, for some low-resolution mass spectra and Dr. Catherine Costello, MIT, for high-resolution mass spectra obtained with the support of NIH Grant RR 00137 from the Biotechnology Resources Branch, Division of Research Resources. We are grateful to Professor D. G. I. Kingston for an authentic sample of cirsimaritin and to Professor R. C. Cambie for an authentic sample of phyllocladan-16α-ol.

Registry No. 1, 569-80-2; 2, 6601-62-3; 3, 537-73-5; 4, 479-91-4; 4 diacetate, 76215-22-0; 5, 76215-21-9; 6, 76215-23-1; 7, 76248-59-4; 1-monoarachidin, 50906-68-8.

Supplementary Material Available: Tables of fractional coordinates and thermal parameters (Tables I and IV), bond distances (Tables II and V), and bond angles (Tables III and VI) for compounds 5 and 7, respectively (8 pages). Ordering information is given on any current mast head page.

(21) All crystallographic calculations were done on a Prime 400 computer, operated by the Materials Science Center, Cornell University. The principal programs used were as follows: REDUCE and UNIQUE, data reduction programs, M. E. Leonowicz, Cornell University, 1978; BLS, block-diagonal least-squares refinement, K. Hirotsu, Cornell University, 1978; ORFLS (modified), full-matrix least-squares, W. R. Busing, K. O. Martin, and H. S. Levy, Oak Ridge National Laboratory Report No. ORNL-TM305; ORTEP, crystallographic illustration program, C. Johnson, Oak Ridge National Laboratory Report No. ORNL-3794; BOND, structural parameters and errors, K. Hirotsu, Cornell University, 1978; MULTAN-76, direct methods and fast fourier transform, G. Germain, P. Main, and M. Woolfson, University of York.

Diterpenes from the Sponge *Dysidea amblia*

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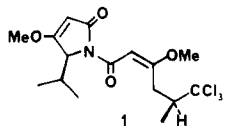
The marine sponge *Dysidea amblia* contained two major metabolites, ambliol-A (8) and ambliol-B (18), and three minor metabolites, ambliofuran (15), ambliolide (13), and dehydroambliol-A (12). The diterpenes are the first to be isolated from a *Dysidea* species. Examination of individual animals indicated that some contained ambliol-A (8) while others contained ambliol-B (18), although the individuals could not be distinguished by means of classical taxonomy.

An unusually diverse array of secondary metabolites has been isolated from *Dysidea* species. Various samples of *Dysidea herbacea* contained brominated diphenyl esters,[1] chlorinated metabolites such as dysidin (1),[2] dysidenin (2),[3] isodysidenin[4] and the dioxopiperazine derivative 3,[5] and some unusual sesquiterpenes.[6] An Australian *Dysidea*

(1) Sharma, G. M.; Vig, B. *Tetrahedron Lett.* **1972**, 1715.
(2) Hofheinz, W.; Oberhänsli, W. E. *Helv. Chim. Acta* **1977**, *60*, 660.
(3) Kazlauskas, R.; Lidgard, R. O.; Wells, R. J.; Vetter, W. *Tetrahedron Lett.* **1977**, 3183.
(4) Charles, C.; Braekman, J. C.; Daloze, D.; Tursch, B.; Karlsson, R. *Tetrahedron Lett.* **1978**, 1519.

0022-3263/81/1946-1098$01.25/0

species was the source of two sulfur-containing sesquiterpenes 4 and 5 that were closely related to the sesquiterpenes found in *D. herbacea*.[7] Sesquiterpene furans have also been isolated from *D. fragilis*[8] and *D. pallescens*.[9] Compounds of mixed biosynthesis such as avarol (6) from *D. avara*[10] and disidein (7) from *D. pallescens*[11] represent further examples of the diversity of *Dysidea* metabolites. In this paper we report the first isolation of diterpenes from a *Dysidea* species.



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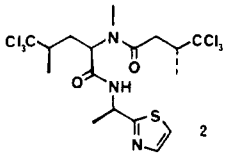

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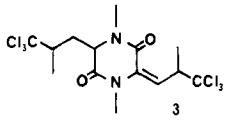

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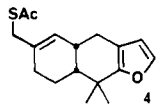

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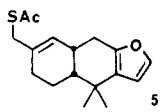
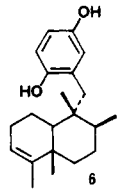


5



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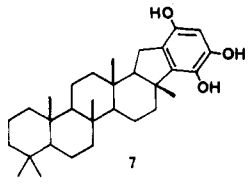
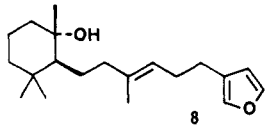


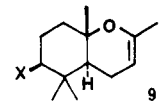
7

Dysidea amblia (de Laubenfels)[12] was collected by hand, using SCUBA (−30 m) at Scripps Canyon, La Jolla, CA. The hexane-soluble material from a methanolic extract of the sponge was chromatographed on silica gel to obtain two major diterpene alcohols, ambliol-A (8) and ambliol-B (18), and three minor metabolites. Subsequent analyses of individual animals have revealed that there are two chemical varieties of *D. amblia*, one containing ambliol-A the other ambliol-B, that cannot be distinguished by classical taxonomic methods.[13]

The major diterpene alcohol, ambliol-A (8) (1.1% dry



8



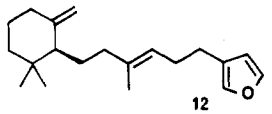
9 X = H

10 X = Br

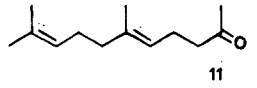
weight) had the molecular formula $C_{20}H_{32}O_2$. The infrared spectrum contained a hydroxyl band at 3500 cm^{-1} and bands at 1385 and 1365 cm^{-1} due to a *gem*-dimethyl group. An absorption at 213 nm (ϵ 7450) in the ultraviolet spectrum indicated the presence of a furan group. The ^{13}C NMR spectrum contained signals at δ 142.8 (d), 139.2 (d), 125.2 (s), and 111.3 (d) due to a β-substituted furan, at 137.0 (s) and 124.1 (d) assigned to a trisubstituted olefin, and at 73.7 (s) due to a tertiary alcohol carbon. Ambliol-A (8) must therefore contain one additional carbocyclic ring.

The ^1H NMR spectrum contained three furan proton signals at δ 7.25 (br s, 1 H), 7.14 (br s, 1 H), and 6.18 (br s, 1 H) and a vinyl proton signal at δ 5.18 (br t, 1 H, J = 7 Hz) coupled to a two-proton signal at δ 2.21 (q, 2 H, J = 7 Hz) which was in turn coupled to a signal at δ 2.43 (t, 2 H, J = 7 Hz) assigned to the methylene adjacent to the furan ring. The ^1H NMR spectrum also contained four methyl signals at δ 1.60 (s, 3 H), 1.10 (s, 3 H), 0.93 (s, 3 H), and 0.80 (s, 3 H). On the assumption that we were dealing with a regular diterpene skeleton, we assigned the structure 8 to ambliol-A.

Ozonolysis of ambliol-A (8) in ethyl acetate solution at −78 °C, followed by reduction of the ozonide with dimethyl sulfide, gave the enol ether 9 as the major product. The mass spectrum [m/z 194 (M^+)] and ^{13}C NMR spectrum both indicated that the molecular formula of the enol ether 9 was $C_{13}H_{22}O$. The ^{13}C NMR spectrum contained signals at δ 148.6 (s) and 94.7 (d) due to the enol carbon atoms and at δ 76.2 (s) due to the tertiary ether carbon. The ^1H NMR spectrum, with an olefinic proton signal at δ 4.30 (br d, 1 H, J = 4 Hz) and four methyl signals at δ 1.60 (s, 3 H), 1.12 (s, 3 H), 0.92 (s, 3 H), and 0.81 (s, 3 H), was reminiscent of the ^1H NMR spectrum of the brominated ether 10 that we had previously synthesized by bromonium ion induced cyclization of geranylacetone (11).[14] Acid-



12



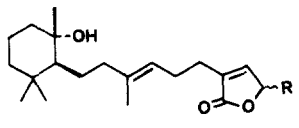
11

catalyzed cyclization[15] of geranylacetone (11) gave an enol ether 9 that was identical in all respects except optical rotation with the ozonolysis product. The formation of enol ether 9 indicated that the hydroxyl group and the side chain of ambliol-A (8) were both equatorial with respect to the cyclohexane ring. The methyl signals in the ^{13}C NMR spectrum of ambliol-A (8) were assigned by comparison with data for model compounds.[16] The chemical shift of the olefinic methyl signal at δ 16.2 (q) indicated that the olefinic bond had the *E* geometry.

Dehydration of ambliol-A (8) with phosphorus oxychloride in pyridine gave dehydroambliol-A (12), identical in all respects with a minor metabolite (0.04% dry weight). Signals in the ^{13}C NMR spectrum at δ 149.4 (s) and 109.4 (t) required the presence of the exocyclic methylene group.

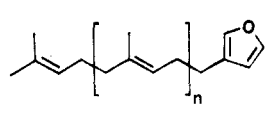
A second minor metabolite, ambliolide (13) (0.04% dry weight), had the molecular formula $C_{21}H_{34}O_4$. The infrared spectrum contained bands at 3600 and 1775 cm^{-1} due to a hydroxyl and an α,β-unsaturated γ-lactone respectively. The ^1H NMR spectrum contained methyl signals at δ 0.78 (s, 3 H), 0.93 (s, 3 H), 1.09 (s, 3 H), and 1.60 (s, 3 H) and an olefinic proton signal at δ 5.07 (br t, 1 H, J = 7 Hz) that

(5) Kazlauskas, R.; Murphy, P. T.; Wells, R. J. *Tetrahedron Lett.* **1978**, 4945.
(6) Kazlauskas, R.; Murphy, P. T.; Wells, R. J. *Tetrahedron Lett.* **1978**, 4949. Charles, C.; Braekman, J. C., Daloze, D., Tursch, B.; Declercq, J. P.; Germain, G.; Van Meerssche, M. *Bull. Soc. Chim. Belg.* **1978**, *87*, 481.
(7) Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; Daly, J. J.; Schönholzer, P. *Tetrahedron Lett.* **1978**, 4951.
(8) Armstrong, R. W., unpublished data. Schulte, G.; Scheuer, P. J.; McConnel, O. J., unpublished data, communicated by G. Schulte.
(9) Cimino, G.; De Stefano, S.; Guerriero, A.; Minale, L. *Tetrahedron Lett.* **1975**, 1417, 1421, 1425. Matsumoto, T.; Usui, S. *Chem. Lett.* **1978**, 105.
(10) De Rosa, S.; Minale, L.; Riccio, R.; Sodano, G. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1408 and ref 4 and 5 cited therein.
(11) Cimino, G.; De Luca, P.; De Stefano, S.; Minale, L. *Tetrahedron* **1975**, *31*, 271.
(12) De Laubenfels, M. W. *Proc. U.S. Nat. Mus.* **1932**, *81*, 123.
(13) De Laubenfels, M. W. "Allen Hancock Foundation Occasional Papers"; 1948, Vol. 3, p 137.
(14) Wolinsky, L. E.; Faulkner, D. J. *J. Org. Chem.* **1976**, *41*, 597.
(15) Smit, W. A.; Semenovsky, A. V.; Brunovlenskaja, I. I.; Portnova, C. L.; Kucherov, V. F. *Dokl. Acad. Nauk* **1977**, *160*, 849.
(16) Signals at δ 33.0 (q) and 21.6 (q) were assigned to *gem*-methyl groups and at δ 23.5 (q) to a methyl on a carbon bearing oxygen. Cf. assignments for 3β-bromo-8-epicaparrapi oxide: Sims, J. J.; Rose, A. F.; Izac, R. R. "Marine Natural Products"; Scheuer, P. J., Ed.; Academic Press: New York, 1978; Vol II, p 348.



13 R = OMe

14 R = H



15 n = 2

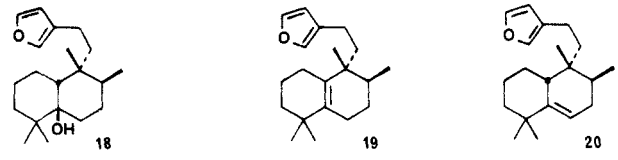
16 n = 1

17 n = 3

were assigned to a molecule having the same basic structure as ambliol-A (8) but containing a lactone ring in place of the furan ring. The signals at δ 3.51 (s, 3 H), 5.61 (d, 1 H, J = 1 Hz), and 6.66 (d, 1 H, J = 1 Hz) were assigned to a γ-methoxy α,β-unsaturated γ-lactone. Reduction of ambliolide (13) with sodium borohydride in methanol at 25 °C for 10 min gave the lactone 14. The ^{1}H NMR spectrum of the lactone lacked the methoxy signal and contained signals at δ 4.68 (d, 2 H, J = 2 Hz) and 6.98 (t, 1 H, J = 2 Hz), the latter being assigned to the β proton of an α,β-unsaturated γ-lactone.[17] Since γ-methoxy α,β-unsaturated γ-lactones may be formed by air oxidation of furans in methanolic solution, ambliolide (13) could be an artifact of the isolation procedure.

The third minor metabolite was ambliofuran (15) (0.05% dry weight) which had the molecular formula $C_{20}H_{30}O$. Comparison of the ^{1}H NMR spectrum of ambliofuran (15) with those of dendrolasin (16)[18] and furospinulosin-1 (17)[19] suggested that ambliofuran (15) was the diterpene analogue. The ^{1}H NMR spectrum contained signals at δ 7.24 (br s, 1 H), 7.12 (br s, 1 H), and 6.17 (br s, 1 H) due to the furan protons, a vinyl proton signal at δ 5.11 (br t, 1 H, J = 7 Hz) coupled to a methylene signal at δ 2.17 (q, 1 H, J = 7 Hz) which was in turn coupled to the signal at δ 2.42 (t, 2 H, J = 7 Hz) due to the methylene protons adjacent to the furan ring, overlapping vinyl proton signals at δ 5.03 (br t, 2 H, J = 7 Hz), and four methyl signals at δ 1.65 (s, 3 H) and 1.58 (s, 9 H) that implied the E geometry for the two trisubstituted olefinic bonds.[20]

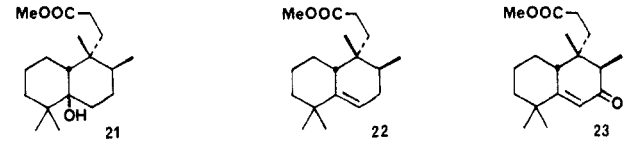
The remaining major metabolite, ambliol-B (18) (1.0% dry weight), had the molecular formula $C_{20}H_{32}O_2$. The infrared spectrum contained a hydroxyl band at 3640 cm^{-1}. The ultraviolet absorption at 214 nm (ε 5260) could be assigned to a furan ring. The ^{1}H NMR spectrum contained signals at δ 7.34 (br s, 1 H), 7.20 (br s, 1 H), 6.26 (br s, 1 H), and 2.28 (m, 2 H), indicative of a β-substituted furan ring attached to a $-CH_2-CH_2-$ side chain, and four methyl signals at δ 1.00 (s, 3 H), 0.86 (s, 6 H), and 0.86 (d, 3 H, J = 7 Hz). The chemical shifts of the methyl signals implied that the hydroxyl group was not attached to a carbon bearing a methyl group. The ^{13}C NMR spectrum contained signals at δ 142.9 (d), 138.8 (d), 125.9 (s), and 111.3 (d) for the furan carbons and at δ 76.0 (s) due to a tertiary alcohol carbon. Since the remaining signals were all in the range δ 16.2–41.3, we proposed that ambliol-B (18) contained a cis-fused bicyclic ring system[21] with the alcohol group at either C-5 or C-10.



18 19 20

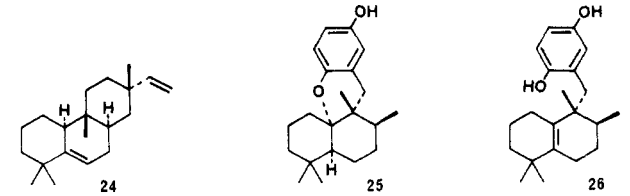
Dehydration of ambliol-B (18) with p-toluenesulfonic acid in refluxing benzene gave the tetrasubstituted olefin 19, again indicating that the hydroxyl group was at C-5 or C-10. Dehydration of ambliol-B (18) with phosphorus oxychloride in pyridine at 80 °C for 12 h, a reaction that required a trans-diaxial transition state, gave only a trisubstituted olefin 20, confirming that the hydrogen at the bridgehead was cis to the hydroxyl group in a cis-fused bicyclic ring system.

To determine whether the hydroxyl group was at C-5 or C-10, we attempted an allylic oxidation of the trisubstituted olefin 20 but obtained an inseparable mixture of products resulting from oxidation reactions about the furan ring. The furan ring was therefore removed by ozonolysis of ambliol-B (18) in ethyl acetate solution at −78 °C, followed by oxidation of the ozonide with Jones' reagent and methylation of the product with diazomethane in ether to obtain the methyl ester 21 in 84% overall yield. Dehydration of the methyl ester 21 with thionyl chloride in pyridine at −5 °C resulted in a 95% yield of the olefinic ester 22. Allylic oxidation of the olefinic ester 22 with chromium trioxide–dimethylpyrazole in dichloromethane at 0 °C gave a modest yield of the α,β-unsaturated ketone 23. The ^{1}H NMR spectrum of the ketone 23 contained a complex three-proton signal at δ 2.23 which comprised the signals of the methylene protons adjacent to the ester group and the single proton at C-8 adjacent to the C-7 ketone. Spin-decoupling experiments showed that the C-8 proton at δ 2.23 was coupled to the methyl signal at δ 0.95 (d, 3 H, J = 7 Hz). If ambliol-B (18) had contained a C-10 alcohol, this sequence of reactions would have given a 2-oxo-1(10) olefin with four protons adjacent to carbonyl groups.



21 22 23

The stereochemistry of ambliol-B (18) was established from spectral data and by a chemical interconversion. We had established from the thionyl chloride dehydration reaction that ambliol-B had an axial hydroxyl group at C-5 and an equatorial hydrogen at C-10 (both with respect to ring B). The stereochemistry at C-8 was determined by a careful decoupling experiment. Subtraction of the ^{1}H NMR spectrum of ambliol-B from the spectrum obtained on irradiation of the methyl signal at δ 0.86 (d, 3 H, J = 7 Hz) resulted in the appearance of a doublet of doublets with coupling constants of 11.5 and 2.0 Hz at δ 1.52. This signal was assigned to an axial proton at C-8. The ^{1}H NMR spectrum of the trisubstituted olefin 20 contained a methyl signal at δ 0.64 (s, 3 H). A similar chemical shift (δ 0.62) had been observed for the axial C-9 methyl group in rimuene (24), due to the shielding effect of the Δ^5 olefin.



24 25 26

(17) See ref 7 and 9 in: Cimino, G.; De Stefano, S.; Minale, L. *Experientia* **1974**, *30*, 18.

(18) Vanderah, D. J.; Schmitz, F. J. *Lloydia* **1975**, *38*, 271 and references cited therein. We isolated a sample from the mollusc *Cadlina luteomarginata*.

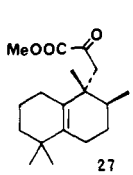
(19) Cimino, G.; De Stefano, S.; Minale, L. *Tetrahedron* **1972**, *28*, 1315. We isolated a sample from an unidentified Caribbean sponge.

(20) Faulkner, D. J. *Synthesis* **1971**, 175.

(21) The trans-fused drimane ring system requires signals in the 50–60-ppm region. We had encountered similar chemical shifts in the spectrum of aureol (25).[22]

These data suggested that ambliol-B (18) had an axial methyl group at C-9 and an equatorial methyl group at C-8, the same relative stereochemistry as was found for the hydroquinone 26 obtained from aureol (25).[22]

Oxidation of the hydroquinone 26 with potassium per-



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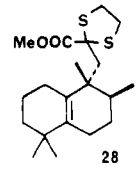

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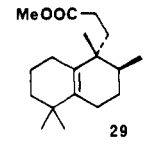

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manganate in aqueous acetone, followed by esterification of the acids produced with diazomethane in ether, gave the α-keto ester 27 in 20% yield. The α-keto ester 27 was converted into an ethylene thioketal 28 with ethane dithiol and boron trifluoride. The ethylene thioketal 28 was desulfurized with deactivated Raney nickel (W-2) in methanol at 60 °C to obtain the olefinic ester 29 in good yield. Dehydration of the methyl ester 21 with *p*-toluenesulfonic acid in refluxing benzene also gave the olefinic ester 29, identical in all respects with the material prepared from aureol (25). We had therefore established the absolute configuration of ambliol-B (18) as 5*R*,8*S*,9*R*,10*R*.

Experimental Section[23]

Extraction and Chromatography. *Dysidea amblia* (de Laubenfels) was collected by hand, using SCUBA (-30 m) at Scripps Canyon, La Jolla, in March 1979. The sponge (159 g dry weight) was steeped in methanol at 0 °C for 2 weeks, then homogenized, and Soxhlet extracted with fresh methanol (3 L) for 2 days. The combined extracts were evaporated under vacuum to obtain an oily suspension that was partitioned between water (500 mL) and hexane (4 × 1 L), then ethyl acetate (3 × 1 L), and finally *n*-butanol (3 × 1 L). Each extract was evaporated under vacuum and the residues were examined by TLC and ^{1}H NMR.

The hexane-soluble material (7.27 g, 4.6% dry weight) was chromatographed on a column (100 × 5 cm i.d.) of silica gel, using eluants of increasing polarity from hexane to ether. Fractions eluted with hexane gave dehydroambliol-A (12, 67 mg, 0.04% dry weight). Fractions eluted with 1% ether in hexane contained ambliofuran (15, 80 mg, 0.05% dry weight). Ambliol-B (18, 1.6 g, 1.0% dry weight) was eluted with 5% ether in hexane and the major diterpene alcohol, ambliol-A (8, 1.74 g, 1.1% dry weight), was eluted with 10% ether in hexane. The fraction eluted with 50% ether in hexane was rechromatographed by LC on μPorasil, using ether as eluant, to obtain ambliolide (13, 64 mg, 0.04% dry weight). Analytically pure samples of each compound were prepared, using LC on μPorasil.

Ambliol-A (8): oil; $[\alpha]^{20}_D$ -3.9° (*c* 2.5, $CHCl_3$); IR (KBr) 3500, 1385, 1365 cm^{-1}; UV (MeOH) 213 nm (ε 7450); ^{1}H NMR (CCl_4) δ 0.80 (s, 3 H), 0.93 (s, 3 H), 1.10 (s, 3 H), 1.60 (s, 3 H), 2.21 (q, 2 H, *J* = 7 Hz), 2.43 (t, 2 H, *J* = 7 Hz), 5.13 (br t, 1 H, *J* = 7 Hz), 6.18 (br s, 1 H), 7.14 (br s, 1 H), 7.25 (br s, 1 H); ^{13}C NMR (C_6D_6) δ 142.8 (d), 139.2 (d), 137.0 (s), 125.2 (s), 124.1 (d), 111.3 (d), 73.7 (s), 56.7 (d), 43.9 (t), 43.2 (t), 41.8 (t), 35.6 (s), 33.0 (q), 28.8 (t), 25.3 (2 t), 23.5 (q), 21.6 (q), 20.8 (t), 16.2 (q); high-resolution mass spectrum, obsd *m*/*z* 286.2317 ($C_{20}H_{30}O$ ($M^+ - H_2O$) requires 286.2297).

Dehydroambliol-A (12): oil; $[\alpha]^{20}_D$ -1.3° (*c* 1.8, $CHCl_3$); IR 1650, 1385, 1365 cm^{-1}; UV (hexane) 223 (ε 6030); ^{1}H NMR (CCl_4) δ 0.83 (s, 3 H), 0.90 (s, 3 H), 1.55 (s, 3 H), 2.18 (q, 2 H, *J* = 7 Hz), 2.42 (t, 2 H, *J* = 7 Hz), 4.49 (br s, 1 H), 4.71 (br s, 1 H), 5.08 (br t, 1 H), 6.17 (br s, 1 H), 7.12 (br s, 1 H), 7.24 (br s, 1 H); ^{13}C NMR (C_6D_6) δ 149.4 (s), 142.8 (d), 139.2 (d), 136.2 (s), 125.2 (s), 124.2 (d), 111.3 (d), 109.4 (t), 53.8 (d), 38.6 (t), 36.6 (t), 35.0 (t), 32.8 (s), 28.9 (t), 28.6 (q), 26.4 (q), 25.4 (t), 25.1 (t), 24.1 (t), 16.2 (q); high-resolution mass spectrum, obsd *m*/*z* 286.2272 ($C_{20}H_{30}O$ requires 286.2297).

Ambliolide (13): oil; $[\alpha]^{20}_D$ -4.0° (*c* 2.2, $CHCl_3$); IR (CCl_4) 3600, 1775 cm^{-1}; ^{1}H NMR (CCl_4) δ 0.78 (s, 3 H), 0.93 (s, 3 H), 1.09 (s, 3 H), 1.60 (s, 3 H), 2.06 (m, 2 H), 2.30 (m, 4 H), 3.51 (s, 3 H), 5.07 (t, 1 H, *J* = 7 Hz), 5.61 (d, 1 H, *J* = 1 Hz), 6.66 (d, 1 H, *J* = 1 Hz); ^{13}C NMR (C_6D_6) δ 170.9 (s), 142.1 (d), 138.1 (s), 128.0 (s), 122.8 (d), 102.3 (d), 73.6 (s), 56.8 (d), 56.0 (q), 43.9 (t), 43.2 (t), 41.8 (t), 35.6 (s), 33.0 (q), 26.0 (t), 25.6 (t), 25.3 (t), 23.5 (q), 21.6 (q), 20.9 (t), 16.3 (q); high-resolution mass spectrum, obsd *m*/*z* 332.2366 ($C_{21}H_{32}O_3$ ($M^+ - H_2O$) requires 332.2351).

Amblofuran (15): oil; ^{1}H NMR (CCl_4) δ 1.59 (s, 9 H), 1.65 (s, 3 H), 2.17 (q, 2 H, *J* = 7 Hz), 2.42 (t, 2 H, *J* = 7 Hz), 5.03 (m, 2 H), 5.11 (t, 1 H, *J* = 7 Hz), 6.17 (br s, 1 H), 7.12 (br s, 1 H), 7.24 (br s, 1 H); high-resolution mass spectrum, obsd *m*/*z* 286.2286 ($C_{20}H_{30}O$ requires 286.2297).

Ambliol-B (18): oil; $[\alpha]^{20}_D$ -3.4° (*c* 1.5, $CHCl_3$); IR (KBr) 3640, 1380, 1365 cm^{-1}; UV (MeOH) 214 nm (ε 5260); ^{1}H NMR ($CDCl_3$) δ 0.86 (d, 3 H, *J* = 7 Hz), 0.86 (s, 6 H), 1.00 (s, 3 H), 2.28 (m, 2 H), 6.26 (br s, 1 H), 7.20 (br s, 1 H), 7.34 (br s, 1 H); ^{13}C NMR (C_6D_6) δ 142.9 (d), 138.8 (d), 125.9 (s), 111.3 (d), 76.0 (s), 41.3 (d), 39.1 (s), 39.0 (s), 38.3 (t), 37.1 (t), 36.8 (d), 32.4 (t), 26.8 (t), 24.7 (q), 24.1 (q), 22.5 (t), 21.9 (t), 18.5 (t), 17.6 (q), 16.2 (q); high-resolution mass spectrum, obsd *m*/*z* 304.2424 ($C_{20}H_{32}O_2$ requires 304.2402).

Ozonolysis of Ambliol-A (8). A stream of ozone in oxygen was bubbled into a solution of alcohol 8 (90 mg, 0.29 mmol) in ethyl acetate (25 mL) at -78 °C until a light blue colored solution was obtained. Excess ozone and oxygen were removed in a stream of nitrogen. Dimethyl sulfide (0.5 mL) was added and the solution was allowed to warm to room temperature. After being stirred for 30 min, the solution was washed with water (3 × 10 mL) and dried over sodium sulfate and the solvent evaporated to leave a yellow oil. The major product was purified by preparative TLC on silica gel to obtain the enol ether 9 (18 mg, 32% theoretical) as a clear oil: ^{1}H NMR (CCl_4) δ 0.81 (s, 3 H), 0.92 (s, 3 H), 1.12 (s, 3 H), 1.60 (s, 3 H), 4.30 (br d, 1 H, *J* = 4 Hz); ^{13}C NMR (C_6D_6) δ 148.6 (s), 94.7 (d), 76.2 (s), 48.7 (d), 42.1 (t), 40.5 (t), 33.3 (s), 32.4 (q), 20.9 (q), 20.7 (q), 20.1 (t), 19.7 (t), 19.4 (q); mass spectrum, *m*/*z* 194 (M^+). This material was identical in all respects with a sample of the enol ether 9 prepared by the method of Smit et al.[15]

Dehydration of Ambliol-A (8) Using Phosphorus Oxychloride in Pyridine. Ambliol-A (8; 79 mg, 0.026 mmol) was dissolved in a mixture of phosphorus oxychloride (0.5 mL) and pyridine (2.0 mL) and the resulting solution was stirred at room temperature for 4 h. The reaction mixture was poured onto ice and the organic material extracted with ether (3 × 25 mL). The combined extracts were dried over sodium sulfate and the solvents evaporated under vacuum to obtain dehydroambliol-A (12; 68 mg, 91% theoretical), identical in all respects with the natural material.

Reduction of Ambliolide (13) with Sodium Borohydride. Sodium borohydride (25 mg, 0.74 mmol) was added to a stirred solution of ambliolide (13; 3 mg, 0.009 mmol) in methanol (5 mL). After 10 min, the reaction mixture was quenched with 5% hydrochloric acid (5 mL), diluted with water (10 mL), and extracted with dichloromethane (3 × 5 mL). The combined organic extracts were dried over sodium sulfate and the solvent was evaporated to give a crude oil that was purified by LC on Partisil, using ether as eluant, to obtain the lactone 14 (2 mg, 70% theoretical): IR (film 3550, 1755 cm^{-1}; ^{1}H NMR (CCl_4) δ 0.81 (s, 3 H), 0.93 (s, 3 H), 1.09 (s, 3 H), 1.61 (s, 3 H), 2.07 (m, 2 H), 2.25 (m, 2 H), 2.32 (m, 2 H), 4.68 (d, 2 H, *J* = 2 Hz), 5.09 (t, 1 H, *J* = 6.5 Hz), 6.98 (t, 1 H, *J* = 2 Hz); mass spectrum, *m*/*z* 320 (M^+), 302.

Dehydration of Ambliol-B (18) with *p*-Toluenesulfonic Acid. A solution of ambliol-B (18; 16 mg, 0.052 mmol) in benzene (10 mL) containing *p*-toluenesulfonic acid (1 crystal) was boiled under reflux for 2 h. After cooling, the reaction mixture was washed with sodium bicarbonate solution (5 mL) and then water (2 × 5 mL) and dried over sodium sulfate and the solvent evaporated to obtain the olefin 19 (8 mg, 47% theoretical): ^{1}H NMR (CCl_4) δ 0.83 (s, 3 H), 0.87 (d, 3 H, *J* = 7 Hz), 0.98 (s, 3 H), 0.99 (s, 3 H), 6.14 (br s, 1 H), 7.09 (br s, 1 H), 7.23 (br s, 3 H); ^{13}C NMR (C_6D_6) δ 142.9 (d), 138.8 (d), 137.5 (s), 132.9 (s), 126.1 (s), 111.3 (d), 41.0, 40.3, 36.9, 34.7, 33.9, 29.4, 27.9, 27.6, 26.2, 25.6, 21.3, 20.4, 19.9, 16.3; mass spectrum, *m*/*z* 286 (M^+), 191.

Dehydration of Ambliol-B (18) with Phosphorus Oxychloride in Pyridine. Ambliol-B (18; 326 mg, 1.1 mmol) was

(22) Djura, P.; Stierle, D. B.; Sullivan, B.; Faulkner, D. J.; Arnold, E.; Clardy, J. *J. Org. Chem.* **1980**, *45*, 1435.
(23) For general procedures see ref 22.

dissolved in a mixture of phosphorus oxychloride (1 mL) and pyridine (7 mL) and the solution was stirred for 12 h at 80 °C. After cooling, the solution was poured onto ice and the organic material was extracted with ether (3 × 50 mL). The combined extracts were dried and the solvents (including pyridine) evaporated under vacuum to obtain the olefin **20** (258 mg, 84% theoretical): ^{1}H NMR (CCl_4) δ 0.64 (s, 3 H), 0.86 (d, 3 H, J = 7 Hz), 0.99 (s, 3 H), 1.06 (s, 3 H), 2.20 (br d, 1 H, J = 12 Hz), 2.28 (m, 2 H), 5.41 (br d, 1 H, J = 5 Hz), 6.16 (br s, 1 H), 7.12 (br s, 1 H), 7.24 (br s, 1 H); ^{13}C NMR (C_6D_6) δ 146.2 (s), 142.9 (d), 138.8 (d), 125.9 (s), 116.7 (d), 111.2 (d), 41.3 (t), 40.3 (d), 37.4 (s), 37.2 (t), 36.3 (s), 33.7 (d), 32.0 (t), 30.1 (q), 29.2 (q), 27.8 (t), 22.6 (t), 18.5 (t), 16.3 (q), 15.3 (q); mass spectrum, m/z 286 (M^+).

Ozonolysis of Ambliol-B (18). A stream of ozone in oxygen was bubbled through a solution of ambliol-B (**18**; 138 mg, 0.45 mmol) in acetone (75 mL) at −78 °C until a light blue colored solution was formed. Excess ozone and oxygen were removed in a stream of nitrogen while the solution was allowed to warm to 0 °C. Jones' reagent (2 mL of 1.34 M solution) was added and the solution was stirred at 0 °C for 1 h. The reaction mixture was extracted with ether (3 × 25 mL) and the combined extracts were washed with water (3 × 25 mL) and sodium bicarbonate solution (3 × 50 mL). The bicarbonate extracts were acidified with 3 N hydrochloric acid and the organic material was extracted with ether (3 × 50 mL). The ether extract was washed with water and dried over sodium sulfate and the solvent evaporated to obtain the acid (108 mg, 84% theoretial) as white prisms, mp 123–125 °C. The acid was dissolved in dry ether and treated with excess ethereal diazomethane solution to give the methyl ester **21** (113 mg, quantitative): IR (CCl_4) 3600, 1740 cm^{-1}; ^{1}H NMR (CCl_4) δ 0.82 (d, 3 H, J = 7 Hz), 0.84 (s, 6 H), 0.97 (s, 3 H), 2.05 (m, 2 H), 3.61 (s, 3 H).

Dehydration of Methyl Ester 21 with Thionyl Chloride in Pyridine. Thionyl chloride (0.5 mL) was added to a cooled solution of the methyl ester **21** (67 mg, 0.23 mmol) in dry pyridine (15 mL) and the resulting solution was stirred for 1 h at −5 °C (ice/salt bath). The reaction mixture was poured onto ice and extracted with ether (3 × 25 mL). The combined organic extracts were dried over sodium sulfate and the solvents evaporated under vacuum to obtain the olefinic ester **22** (60 mg, 95% theoretical): IR (CCl_4) 1740, 1380, 1365 cm^{-1}; ^{1}H NMR (CCl_4) δ 0.64 (s, 3 H), 0.83 (d, 3 H, J = 7 Hz), 0.99 (s, 3 H), 1.05 (s, 3 H), 2.01 (br d, 1 H, J = 12 Hz), 2.14 (t, 2 H, J = 8 Hz), 3.61 (s, 3 H), 5.34 (br s, 1 H).

Allylic Oxidation of Olefinic Ester 22. 3,5-Dimethylpyrazole (346 mg, 3.6 mmol) was added to a suspension of chromium trioxide (360 mg, 3.6 mmol) in dry dichloromethane (25 mL) at −20 °C and the reagent mixture was stirred for 15 min. A solution of the olefinic ester **22** (40 mg, 0.14 mmol) in dichloromethane (15 mL) was added dropwise and the reaction mixture was stirred for 3 h at −15 to −20 °C. Sodium hydroxide solution (5 N, 10 mL) was added and the reaction mixture was stirred for 1 h at 0 °C. The organic material was extracted with ether (3 × 50 mL) and the combined ether extracts were washed with 5% hydrochloric acid (3 × 50 mL), brine (2 × 25 mL), and water (2 × 25 mL). The extract was dried over sodium sulfate and the solvent evaporated under vacuum to yield to a crude product (44 mg) that was chromatographed by high-performance LC on μPorasil, using 25% ether in hexane as eluant, to obtain the α,β-unsaturated ketone **23** (16 mg, 38% theoretical): IR (CCl_4) 1740, 1680 cm^{-1}; UV (CCl_4) δ 0.72 (s, 3 H), 0.95 (d, 3 H, J = 7 Hz), 1.13 (s, 3 H), 1.18 (s, 3 H), 2.23 (m, 3 H, 2 signals), 2.43 (m, 1 H), 3.63 (s, 3 H), 5.83 (d, 1 H, J = 3 Hz).

Dehydration of Methyl Ester 21 with *p*-Toluenesulfonic Acid. A solution of the methyl ester **21** (20 mg, 0.07 mmol) and *p*-toluenesulfonic acid (1 mg) in benzene (15 mL was refluxed under nitrogen for 24 h. The cooled solution was washed with water (3 × 5 mL) and dried over sodium sulfate and the solvent evaporated to obtain an oil. The oil was chromatographed by LC on μPorasil, using 10% ether in hexane as eluant, to obtain the olefinic ester (**29**; 16 mg, 85% theoretical): $[\alpha]_D$ −52.9° (*c* 0.7, $CHCl_3$); IR (film) 1740 cm^{-1}; ^{1}H NMR (CCl_4) δ 0.84 (s, 3 H), 0.86 (d, 3 H, J = 7 Hz), 0.97 (s, 3 H), 0.98 (s, 3 H), 3.59 (s, 3 H); mass spectrum, m/z 278 (M^+).

Oxidation of Hydroquinone 26. A solution of potassium permanganate (700 mg, 4.43 mmol) in water (25 mL) was added to a stirred solution of the hydroquinone (**26**; 330 mg, 1.05 mmol) in 3:1 acetone/water (150 mL) at 0 °C over a period of 5 min. The solution was stirred at 0 °C for 40 min when the reaction was quenched by the addition of a saturated solution of sodium bisulfite in 5% hydrochloric acid until the purple color had been discharged. The solution was diluted to 350 mL with water and extracted with ether (4 × 150 mL). The combined extracts were washed with saturated sodium bicarbonate solution (4 × 100 mL), the bicarbonate extracts acidified with concentrated hydrochloric acid, and the organic acids extracted with ether (4 × 100 mL). The combined ether extracts were dried over sodium sulfate and the solvent was evaporated to obtain an oil that was treated with excess ethereal diazomethane solution. The resulting mixture of methyl esters was chromatographed by LC on Partisil, using 10% ether in hexane as eluant, to obtain the α-keto ester **27** (71 mg, 23% theoretical): IR (CCl_4) 1740 cm^{-1} (br); ^{1}H NMR (CCl_4) δ 0.88 (d, 3 H, J = 7 Hz), 0.92 (s, 3 H), 0.97 (s, 3 H), 0.99 (s, 3 H), 2.81 (d, 1 H, J = 16 Hz), 2.91 (d, 1 H, J = 16 Hz), 3.78 (s, 3 H); mass spectrum, m/z 292 (M^+), 191 (base peak).

Ethylene Thioketal 28. Boron trifluoride etherate (100 μL) was added to a solution of the keto ester **27** (9 mg, 0.03 mmol) in ethanedithiol (150 μL) and the mixture was stirred for 2 min at room temperature. The reaction mixture was quenched with water (25 mL) and extracted with ether (4 × 10 mL). The combined organic extracts were washed with 5% potassium hydroxide solution (10 mL), saturated brine (10 mL), and water (10 mL) and then dried over sodium sulfate and the solvent was evaporated to obtain the ethylene thioketal **28** (4.5 mg, 40% theoretical): IR (film) 1740 cm^{-1}; ^{1}H NMR (CCl_4) δ 0.79 (d, 3 H, J = 7 Hz), 0.83 (s, 3 H), 0.96 (s, 3 H), 0.98 (s, 3 H), 2.46 (d, 1 H, J = 15 Hz), 2.66 (d, 1 H, J = 15 Hz), 3.19 (m, 2 H), 3.26 (m, 2 H), 3.68 (s, 3 H); mass spectrum, m/z 368 (M^+), 191 (base peak).

Desulfurization of the Ethylene Thioketal 28. Raney nickel (W-2)[24] was deactivated in refluxing acetone for 20 min. The deactivated Raney nickel (50 mg) was added to a solution of the ethylene thioketal **28** (4.5 mg, 0.012 mmol) in methanol (10 mL) and the suspension was stirred at 60 °C for 8 h. The Raney nickel was removed by filtration through Celite and the solvent evaporated. The residue was purified by LC on Partisil, using 2% ether in hexane as eluant, to obtain the ester **29** (3 mg, 88% theoretical), $[\alpha]^{20}_D$ −48.4° (*c* 0.4, $CHCl_3$), identical in all respects with the material obtained previously.

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Registry No. **8**, 76215-30-0; **9**, 76248-60-7; **12**, 76249-87-1; **13**, 76215-28-6; **14**, 76215-31-1; **15**, 76215-29-7; **18**, 76215-27-5; **19**, 76215-32-2; **20**, 76215-33-3; **21**, 76215-34-4; **22**, 76215-35-5; **23**, 76215-36-6; **26**, 72853-85-1; **27**, 76215-37-7; **28**, 76215-38-8; **29**, 76215-39-9.

(24) Pettit, G. R.; Van Tamelen, E. E. *Org. React.* **1962**, *12*, 2481.