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PLANT ANTIMUTAGENIC AGENTS, 7[1]. STRUCTURE AND ANTIMUTAGENIC PROPERTIES OF CYMOBARBATOL AND 4-ISOCYMOBARBATOL, NEW CYMOPOLS FROM GREEN ALGA (*CYMOPOLIA BARBATA*)

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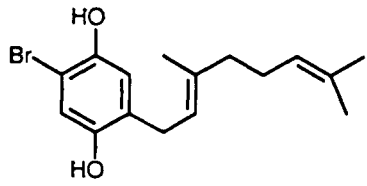
ABSTRACT.—Two new compounds, cymobarbatol and 4-isocymobarbatol, were isolated from the marine alga *Cymopolia barbata*. The complete structures and absolute stereochemistries of these compounds were elucidated by a variety of spectroscopic techniques and X-ray crystallography. Both compounds were found to be nontoxic over a broad concentration range to *Salmonella typhimurium* strains T-98 and T-100. Both compounds exhibited strong inhibition of the mutagenicity of 2-aminoanthracene and ethyl methanesulfonate toward, respectively, the T-98 strain plus a metabolic activator and T-100.

Marine algae have been the subject of several investigations dealing with the structure of various secondary metabolites. Numerous halogenated metabolites have been found in red and brown algae (1,2). Green algae received lesser attention until a group of prenylated bromohydroquinones, called collectively cymopols, were isolated from *Cymopolia barbata* (L.) Lamouroux (Dasycladaceae) by Hogberg *et al.* (3). Of particular interest were two compounds, cymopol [**1**] and cyclocymopol [**2**] (3). Subsequently McConnell *et al.* (4) isolated cyclocymopol from *C. barbata* as a mixture of diastereoisomers. The structure of one of the diastereomers as the monomethyl ether was confirmed by an X-ray crystallographic study (4). In this paper we wish to present the structure of two new cymopols isolated from *C. barbata* guided by antimutagenic assay (5–7). We have named them cymobarbatol [**3**] and 4-isocymobarbatol [**4**]. Com-



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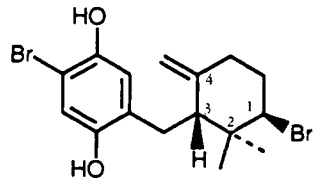

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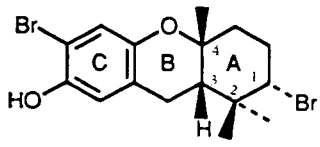

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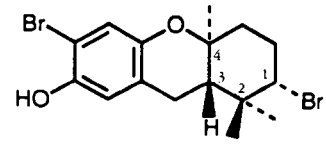

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[1]For Part 6 of this series, see M.E. Wall *et al.*, *J. Nat. Prod.*, **52**, 774 (1989).

pounds **3** and **4** are highly active antimutagens and are nontoxic in the antimutagenic assay (5–7).

EXPERIMENTAL

MUTAGENIC INHIBITION.—Procedures for determining the inhibition of the mutagenic activity of 2-aminoanthracene (2AN) toward *Salmonella typhimurium* (T-98) by crude and purified plant extracts have been described in previous papers (5–7). Similar procedures for determining the inhibition of the mutagenic activity of other mutagens such as acetylaminofluorene (AAF), benzo[*a*]pyrene (B[*a*]P), and ethyl methanesulfonate (EMS) by pure compounds have also been described (5–7). All of these mutagens with the exception of EMS require metabolic activation by the Ames S-9 preparation (8) as described previously (5). In the case of EMS the T-100 strain of *S. typhimurium* was used (9). Toxicity of extracts and pure compounds toward *S. typhimurium* was determined in the absence of mutagen but in the presence of histidine as described previously (5–7).

GENERAL ISOLATION AND CHARACTERIZATION PROCEDURES.—Melting points were determined on a Kofler hotstage microscope and are uncorrected. ^{1}H- and ^{13}C-nmr spectra were obtained with a Bruker WM250 spectrometer using TMS as internal standard. High resolution mass spectra (hrms) were obtained with an AEI MS-902 instrument. Uv spectra were obtained in MeOH with a Varian 2290-UV-VIS spectrometer and ir spectra with a Shimadzu Infrared Spectrophotometer IR-460. X-ray crystallographic data for **3** and **4** were recorded on an Enraf-Nonius CAD-4 diffractometer (CuKα radiation, incident beam graphite monochromator; ω-2θ scans, θ max = 75°). Standard chromatography was carried out on E. Merck 230–40 mesh Si gel, or Baker Flash chromatography Si gel, using in general CH_2Cl_2 as an eluent with a gradient of 0.5–5.0% MeOH, collecting 15-ml fractions with an automatic fraction collector. For tlc determinations precoated Si gel plates were utilized: normal phase, EM precoated Si gel 60, F254, usual solvent 10% MeOH in CH_2Cl_2; reversed-phase, Baker precoated Si gel C_{18}-F plates, usual developer 5–10% H_2O in MeOH. Exposure of plates to iodine vapor was used as a general detection agent; alternatively spraying with phosphomolybdate reagent followed by heating was utilized. Preparative hplc was conducted utilizing a Waters Model Prep-3000 instrument. In most cases a Dynamax reversed-phase C_{18} column (21.5 mm × 25 cm) was utilized, with 10–50% H_2O in MeOH as solvent; for highly nonpolar compounds a similar Dynamax 10μ Silica column was used with CH_2Cl_2 as solvent.

PLANT MATERIAL.—A collection of *C. barbata* was made by W. Gerwick in April 1983, snorkelling off the north coast of Puerto Rico (Jobas). Identification was also made by Dr. Gerwick. A voucher specimen is on deposit in Dr. Gerwick's laboratory.

EXTRACTION AND ISOLATION.—Crude extracts were prepared by homogenization of algae with a mixture of $CHCl_3$-MeOH (2:1), followed by filtration and removal of solvent in vacuo at low temperature. The crude extract was highly active, giving 87% inhibition of 2AN at a dose of 0.6 mg of crude solids per plate. The crude extract was also nontoxic at 0.6 mg per plate. A 28-g sample of crude extract was chromatographed on 800 g of Sephadex LH20 packed in MeOH-CH_2Cl_2 (3:1). Elution was conducted with the same solvent mixture, collecting 50-ml fractions. The fractions were analyzed by normal and reversed-phase tlc. Fractions with similar tlc patterns were combined, solvent removed in vacuo, and the residue weighed and tested for inhibition of 2AN mutagenicity toward *S. typhimurium*. The first six combined samples (12.5 g) were inactive in 2AN inhibition. The next three fractions, 7, 8, and 9, were highly active in 2AN inhibition. The remaining eluates were inactive and had negligible weight. Fractions 7, 8, and 9 contained a number of constituents as indicated by tlc analysis. Fraction 9 (2.2 g) was selected for initial study because tlc analysis indicated that this fraction had fewer constituents than fractions 7 and 8. This fraction was subjected to flash chromatography on Baker Si gel (50 g), using a gradient commencing with 0.5% MeOH in CH_2Cl_2 and ending in 10% MeOH/CH_2Cl_2. Eluates were collected in 15-ml fractions and combined according to tlc pattern. Fractions collected in the first 135 ml were inactive and had low weight. Almost all of the total sample (1.9 g) chromatographed was eluted in the next 150 ml in 0.5% MeOH/CH_2Cl_2: 2AN inhibition = 97% at 0.6 mg per plate. This material was a mixture containing two major constituents. It was subjected to preparative hplc on a Waters Delta Prep-3000 instrument using Dynamax Silical (21.5 mm × 25 cm) column and CH_2Cl_2 as an eluent (uv monitor 254 nm and a flow rate of 10 ml/min).

Two fractions were collected with retention times of 9 and 10 min. The first, weighing 0.24 g, was chromatographically pure and was identified as a new compound, cymobarbatol [**3**]. The second fraction, weighing 0.62 g, was a mixture of **3** and a new compound 4-isocymobarbatol [**4**]. A second preparative hplc of the mixture cleanly separated **3** (168 mg) and **4** (67 mg), retention times 9 and 10 min respectively. Both **3** and **4** were crystallized from aqueous MeOH.

Cymobarbatol [**3**].—Mp 166°, $[\alpha]^{23}$D −15.4°; uv max (MeOH) nm (log ϵ) 306 (3.72), 225 sh (4.00),

(MeOH + NaOH) 327 (3.84); ir ν max (KBr) cm^{-1} 3560, 3450, 2960, 2910, 1500, 1490, 1470, 1410, 1160; ^1H nmr ($CDCl_3$) δ 0.80 (3H, s, Me-9), 1.13* (3H, s, Me-7), 1.15* (3H, s, Me-8), 1.55 (1H, d, J = 8.5 Hz, H-3), 1.65 (1H, m, H-5β), 2.0 (1H, m, H-5α), 2.05 (1H, m, H-6α), 2.5 (1H, dq, J = 3.5, 13.0 Hz, H-6β), 2.76 (1H, d, J = 18 Hz, H-10α), 3.04 (1H, dd, J = 8.5 Hz, H-10β), 4.05 (1H, dd, J = 3.5, 13.0 Hz, H-1β), 5.05 (1H, s, 13-OH), 6.74 (1H, s, H-12), 6.88 (1H, s, H-15); ^{13}C-nmr ($CDCl_3$) 16.44 (q, C-9), 24.67 (t, C-10), 26.18* (q, C-7), 29.76* (q, C-8), 29.91 (t, C-6), 39.97 (t, C-5), 44.78 (d, C-3), 66.88 (d, C-1), 74.58 (s, C-4), 107.54 (s, C-14), 114.89 (d, C-12), 119.78 (d, C-15), 122.50 (s, C-11), 145.71 (s, C-16), 148.06 (s, C-13); ms *m/z* (rel. int. %) 401.9831 ($C_{16}H_{22}Br_2O_2$ = 401.9831 Br^{79}) (56), 323 (23), 239 (12), 201 (100), 121 (45). Consecutive values for ^1H and ^{13}C labeled with asterisks may be interchanged.

4-Isocymobarbatol [**4**].—Mp 147°; $[\alpha]^{23}D$ −51.4°; uv max (MeOH) nm (log ϵ) 305 (3.76), 225 sh (3.76), (MeOH + NaOH) 326 (3.79); ir ν max (KBr) cm^{-1} 3510, 2960, 2910, 1490, 1470 1215, 1190; ^1H-nmr ($CDCl_3$) δ 1.01 (3H, s, -Me), 1.16 (3H, s, -Me), 1.20 (3H, s, -Me), 1.67 to 2.32 (5H, m), 2.58 to 2.77 (2H, m), 4.03 (1H, dd, J = 4.2, 13.1 Hz), 5.03 (1H, s, -OH), 6.74 (1H, s, aromatic H), 6.88 (1H, s, aromatic H); ms *m/z* (rel. int. %) 401.9835 ($C_{16}H_{22}Br_2O_2$ = 401.9831 Br^{79}) (44), 323 (42), 201 (100), 121 (50).

CYMOBARBATOL ACETATE.—A sample of cymobarbatol (20 mg, 0.05 mmol) in $CHCl_3$ (1 ml) was treated with Ac_2O (0.5 ml) and a drop of pyridine and stirred for 12 h. The evaporated residue was recrystallized from hexane to yield cymobarbatol acetate (16 mg, 76%): mp 138°; ir ν max ($CHCl_3$) cm^{-1} 1760, 1480, 1370, 1180, 1010, 940, 925; ^1H-nmr ($CDCl_3$) δ 0.79, 1.12, 1.17 (9H, 3s, 3 × Me), 1.56 (1H, d, J = 7.8 Hz, H-3), 1.65 (1H, m, H-5β), 2.06 (2H, m, H-5α, -6α), 2.32 (3H, s, -Ac), 2.45 (dq, J = 3.5, 13.0 Hz, H-6β), 2.78 (1H, d, J = 18.0 Hz, H-10α), 3.0 (1H, dd, J = 8.3 Hz, H-10β), 4.04 (1H, dd, J = 3.5, 13.0 Hz, H-1β), 6.84 (1H, s, H-15), 7.0 (1H, s, H-12); ms *m/z* (rel. int. %) 443.9929 ($C_{18}H_{22}Br_2O_3$ = 443.9937 Br^{79}) (8), 402 (90), 323 (22), 239 (15), 201 (100), 121 (83).

Cymopol [**1**].—Fractions 7 and 8 from the Sephadex chromatography described above were investigated for the presence of other cymopols. Fraction 8 contained primarily **3**, **4**, and a more polar compound. The latter predominated in fraction 7. The entire sample of this fraction (4.0 g) was flash chromatographed on Baker Si gel (50 g) using a gradient of CH_2Cl_2/5% MeOH in CH_2Cl_2. A fraction collected between 165–225 ml weighed 0.50 g. Tlc indicated that the more polar component was concentrated in this fraction. Preparative hplc of this fraction on a reversed-phase Dynamax C_{18} column yielded 0.3 g of purified product which was crystallized from CH_2Cl_2/C_6H_{14} to yield 0.2 g of the known compound cymopol [**1**] (3): mp 60–62° [lit. (3) 59–61°]. ^1H nmr and ms were identical to those reported (3).

Cyclocymopol [**2**].—A small quantity of a constituent more polar (by tlc) was isolated as a gum from fraction 7 [hrms *m/z* 401.9839 ($C_{16}H_{22}Br_2O_2$ = 401.9831 Br^{79})]. The mass fragmentation pattern was similar to that of cyclocymopol (3,4). Unfortunately, the suspected **2** was highly unstable in solution and no spectra could be obtained.

X-RAY CRYSTAL DATA.—*Cymobarbatol* [**3**].—$C_{16}H_{20}Br_2O_2$, MW = 404.15, orthorhombic, space group $P2_12_12_1$ (D_2^4)-No. 19, a = 8.403(1), b = 31.110(2), c = 5.937(1) Å (from 25 orientation reflections, 45° <θ< 48°), V = 1552.0(5) $Å^3$, Z = 4, D_c = 1.730 $g \cdot cm^{-3}$, μ (CuKα radiation, λ = 1.5418 Å) = 66.8 cm^{-1}; crystal dimensions 0.06 × 0.16 × 0.26 mm.

4-Isocymobarbatol [**4**].—$C_{16}H_{20}Br_2O_2$, MW = 404.15, monoclinic, space group $P2_1$ (C_2^2)-No. 4, a = 9.153(1), b = 12.928(1), c = 6.906(1) Å, β = 106.12(1)° (from 25 orientation reflections, 43° <θ< 48°), V = 785.1(3) $Å^3$, Z = 2, D_c = 1.710 $g \cdot cm^{-3}$, μ (CuKα radiation) = 66.0 cm^{-1}; crystal dimensions 0.10 × 0.13 × 0.50 mm.

Intensity data ($+h, +k, +l$ for **3**: $+h, -k, \pm l$ for **4**) were recorded on an Enraf-Nonius CAD-4 diffractometer (CuKα radiation, incident-beam graphite monochromator; ω-2θ scans, θ_{max} = 75°). The data were corrected for the usual Lorentz and polarization effects; empirical absorption corrections (T_{max}:T_{min} = 1.00:0.45 for **3**; T_{max}:T_{min} = 1.00:0.82 for **4**) were also applied to both data sets. From totals of 1889 and 1689 nonequivalent reflections for **3** and **4**, respectively, those 1787 and 1573 with $I > 3.0\sigma(I)$ were retained for the analyses.

Both crystal structures were solved by the heavy-atom approach. Initial bromine atom coordinates were derived from Patterson maps. Weighted F_o Fourier syntheses yielded positions for all carbon and oxygen atoms. Non-hydrogen atom positional and anisotropic temperature factor parameters, with hydrogen atoms included at their calculated positions, were adjusted by several rounds of full-matrix least-squares calculations. A secondary extinction correction (g) was included as a variable in the later iterations. The least-squares refinement converged at R = 0.036 (R_w = 0.053) for **3** and R = 0.037 (R_w = 0.058) for **4**: $R = \Sigma||F_o|-|F_c||/\Sigma|F_o|$; $R_w = [\Sigma w(|F_o|-|F_c|)^2/\Sigma w|F_o|^2]^{1/2}$; $\Sigma w\Delta^2$ [$w = 1/\sigma^2(|F_o|)$, $\Delta = (|F_o|-|F_c|)$] was minimized; extinction coefficients 4.7×10^{-6} for **3**, 3.6×10^{-6} for **4**. The absolute configurations of both

compounds were established by incorporating the imaginary contributions to the anomalous dispersion terms into the structure-factor calculations. For cymobarbatol, using parameters corresponding to the absolute stereochemistry represented by structure **3**, R was 0.034 ($R_w = 0.051$) whereas when those for the mirror image were employed R was 0.041 ($R_w = 0.059$). The differences are highly significant (10) and indicate that **3** correctly represents the absolute configuration. Similar calculations for 4-isocymobarbatol yielded R = 0.038 ($R_w = 0.060$) for parameters corresponding to structure **4**, in contrast to R = 0.040 ($R_w = 0.062$) for the mirror image. The determination of the absolute configuration for **3** is significant at the 0.005 level if $R'_w/R_w (= 0.059/0.051) = 1.1569$ equals or exceeds $R_{1,1605,0.005} = 1.00254$. For **4**, corresponding values are $R'_w/R_w (= 0.062/0.060) = 1.0333$, $R_{1,1392,0.005} = 1.00293$. The configurational assignments for **3** and **4** were corroborated by measuring intensities for 80 Friedel pairs of enantiomer-sensitive reflections with $I > 15\sigma(I)$. In all cases, the difference between the calculated values, $|F_c(hkl)|$ and $|F_c(\bar{h}\bar{k}\bar{l})|$, was consistent with that measured. Continuation of the least-squares refinement of atomic parameters for the correct enantiomer for **3** and **4** led to convergence (max. shift $<0.01\sigma$) at R = 0.033 ($R_w = 0.034$, $g = 4.7 \times 10^{-6}$) and R = 0.036 ($R_w = 0.052$, $g = 3.6 \times 10^{-6}$), respectively.[2]

Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package. For the structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from "International Tables for X-Ray Crystallography" (11).

RESULTS AND DISCUSSION

C. barbata, a green marine alga, has received limited attention in regard to the biological activity of extracts from this marine plant, which is found growing near the coasts of Bermuda and Puerto Rico. Et_2O extracts of *C. barbata* have been shown to possess antibiotic and antifungal properties but no specific compounds were reported (12). McConnell *et al.* (4) have reported additional biological activities from such extracts including avoidance by molluscs, inhibition of feeding by sea urchins, and inhibition of plant growth. Inhibition of mutagenicity of 2AN or EMS toward *S. typhimurium* (T-98 or T-100) by extracts and pure compounds isolated from *C. barbata* is reported for the first time in this paper.

After initial extraction of *C. barbata*, chromatography on Sephadex LH-20 rapidly concentrated several active and seemingly nontoxic fractions. Pure compounds were then obtained by Si gel chromatography and finally by preparative hplc.

The first compound isolated was the least polar constituent, which we named cymobarbatol [**3**]. Hrms showed that **3** was a dibrominated compound with the formula $C_{16}H_{20}Br_2O_2$, thus eliminating the monobrominated known cymopol [**1**] ($C_{16}H_{21}BrO_2$) from consideration (3). Although hrms $[M]^+$ values of the known compound cyclocymopol [**2**] (3,4) and **3** were identical ($C_{16}H_{20}Br_2O_2$), it was readily demonstrated that the two compounds were quite dissimilar. Thus on acetylation, **3** forms a monoacetate ($C_{18}H_{22}Br_2O_3$). The ir spectrum of this compound showed absence of a hydroxyl group. Moreover, the ^{1}H-nmr spectrum of **3** showed the presence of three Me groups whereas the spectrum of **2** showed two Me groups and one CH_2 moiety. The uv spectrum of **3** showed the presence of an aromatic phenolic OH as indicated by the bathochromic shift of the uv spectrum in alkaline solution from 306 to 327 nm. The ir spectra also confirmed the presence of an aromatic ring. The presence of two aromatic protons was shown by two singlets at δ 6.74, 6.88, almost identical to those reported for **2** (4). The structure of the tricyclic ether **3** is in agreement with the ms, nmr, ir, and uv spectra shown for this compound. In addition ^{1}H-^{1}H correlation and ^{1}H-^{13}C correlation spectra were obtained, the latter by a HETCOR experiment the results of which are shown in Figure 1. The formation of **3** from **2** is readily rationalized by an intramolecular cyclization of the latter.

[2]Atomic coordinates for compounds **3** and **4** have been deposited with the Cambridge Crystallographic Data Centre and can be obtained from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

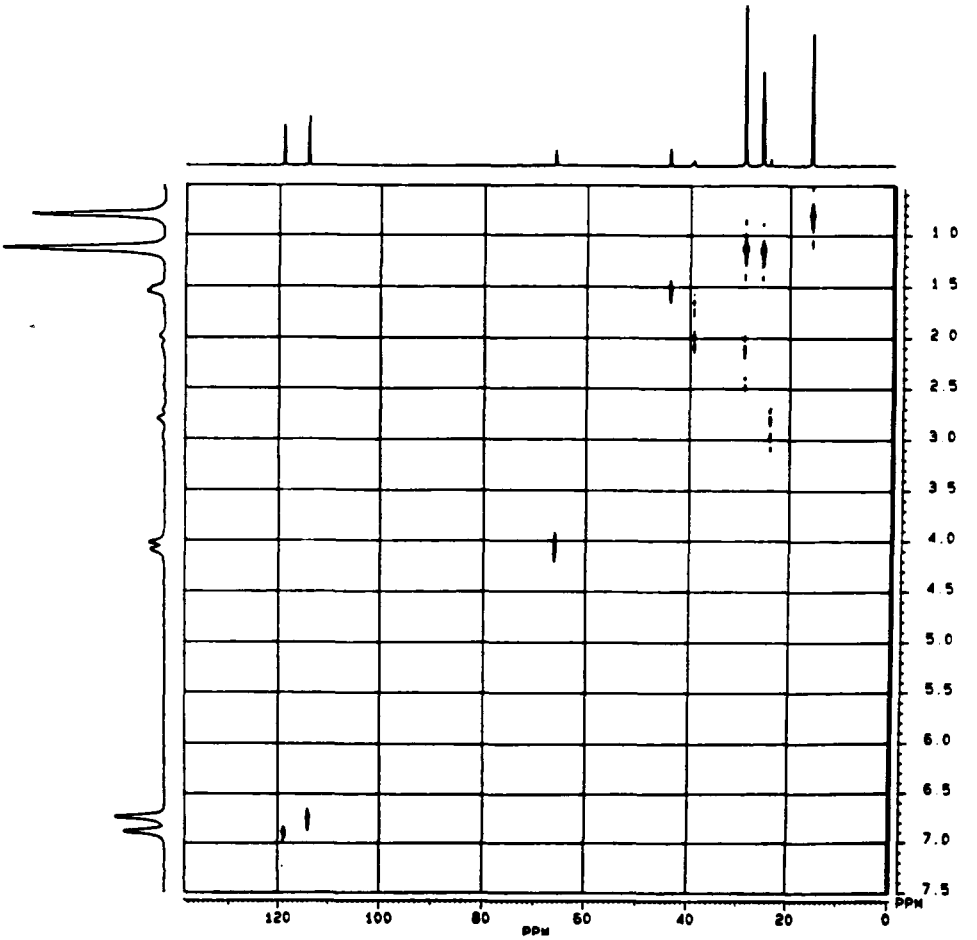

FIGURE 1. ^{1}H-^{13}C correlation of cymobarbatol [**3**].

Remaining to be elucidated were the configuration of **3** at ring AB fusion points at C-3 and C-4 and the configuration of the bromine moiety at C-1. In addition the conformation of rings AB of cymobarbatol required clarification. Because **3** and an isomer **4** formed crystals suitable for X-ray crystallography, it was decided to settle all the above issues unequivocally by means of this technique.

The complete structures and absolute configurations of **3** and **4** were established by single-crystal X-ray analyses. Both crystal structures were solved by the heavy-atom approach. Full-matrix least-squares refinement of atomic parameters converged at $R = 0.033$ ($R_w = 0.047$) over 1787 reflections for **3** and $R = 0.036$ ($R_w = 0.052$) over 1573 reflections for **4**. Fractional coordinates for the non-hydrogen atoms of **3** and **4** are provided in Tables 1 and 2, respectively. Views of the solid-state conformations of **3** and **4** are presented in Figures 2 and 3, respectively. The absolute configurations, represented by structures **3** and **4**, were determined by use of the anomalous scattering of X-rays (see Experimental section).

Bond lengths in **3** and **4** lie close to expected values (13). The cyclohexane ring in each compound has a chair conformation with that in AB cis fused **3** being somewhat flattened around C-3 and C-4 relative to that in **4**, which is ring AB trans fused. Endocyclic torsion angles (ω_{ij}, $\sigma \pm 0.4$–$0.6°$ for **3**, ± 0.6–$0.8°$ for **4**) about the bonds between atoms i and j in **3**, with corresponding values for **4** in parentheses, follow: $\omega_{1,2}$

TABLE 1. Fractional Coordinates and Equivalent Isotropic Thermal Parameters for the Non-hydrogen Atoms of Cymobarbatol [3] (with estimated standard deviations in parentheses).

Atom	*x*	*y*	*z*	B_{eq} (Å^2)
C-1	0.6356(5)	0.18908(13)	0.3187(7)	3.10(7)
C-2	0.5976(5)	0.14376(12)	0.4066(7)	2.81(6)
C-3	0.4405(5)	0.14913(12)	0.5486(7)	2.83(6)
C-4	0.3036(5)	0.17353(12)	0.4330(8)	3.20(7)
C-5	0.3586(6)	0.21528(12)	0.3248(9)	3.75(8)
C-6	0.5043(6)	0.20933(13)	0.1778(9)	3.92(9)
C-7	0.5821(6)	0.11037(13)	0.2166(8)	3.42(7)
C-8	0.7321(6)	0.12895(16)	0.5663(9)	3.96(9)
C-9	0.1719(6)	0.18330(15)	0.6003(9)	4.24(9)
C-10	0.3758(5)	0.10693(14)	0.6425(8)	3.50(7)
C-11	0.2512(5)	0.08672(12)	0.4920(8)	2.93(7)
C-12	0.1938(5)	0.04559(14)	0.5421(8)	3.47(8)
C-13	0.0831(5)	0.02541(12)	0.4005(9)	3.48(7)
C-14	0.0323(5)	0.04614(13)	0.2097(7)	3.06(7)
C-15	0.0854(5)	0.08756(12)	0.1564(8)	3.05(7)
C-16	0.1935(4)	0.10751(11)	0.3024(7)	2.77(6)
O-17	0.2392(4)	0.14845(8)	0.2482(5)	2.99(5)
O-18	0.0279(5)	−0.01521(9)	0.4510(7)	4.59(7)
Br-19	0.83718(6)	0.19095(2)	0.1428(1)	4.55(1)
Br-20	−0.11397(6)	0.01890(2)	0.0136(1)	4.66(1)

−58.0 (−55.7), $\omega_{2,3}$ 49.6 (51.1), $\omega_{3,4}$ −47.7 (−52.6), $\omega_{4,5}$ 49.5 (53.6), $\omega_{5,6}$ −56.0 (−57.5), $\omega_{6,1}$ 63.5 (61.3) for ring A; $\omega_{3,10}$ 36.7 (−49.0), $\omega_{10,11}$ −6.1 (16.7), $\omega_{11,16}$ −3.9 (1.9), $\omega_{16,17}$ −20.7 (14.2), $\omega_{17,4}$ 53.5 (−46.6), $\omega_{4,3}$ −60.9 (64.5) for ring B. The bromine substituent at C-1 has an α configuration, and it is equatorially oriented in both isomers. It should be noted that in cyclocymopol [2], the bromo sub-

TABLE 2. Fractional Coordinates and Equivalent Isotropic Thermal Parameters for the Non-hydrogen Atoms of Isocymobarbatol [4] (with estimated standard deviations in parentheses).

Atom	*x*	*y*	*z*	B_{eq} (Å^2)
C-1	−0.1752(6)	0.1980(6)	0.2698(8)	4.0(1)
C-2	−0.0722(6)	0.1064(6)	0.3663(8)	3.8(1)
C-3	0.0519(6)	0.1000(5)	0.2458(7)	3.4(1)
C-4	0.1347(6)	0.2008(5)	0.2271(8)	3.5(1)
C-5	0.0221(7)	0.2869(6)	0.1444(9)	4.3(1)
C-6	−0.0929(7)	0.3000(6)	0.2684(10)	4.6(1)
C-7	−0.0036(7)	0.1200(7)	0.5949(9)	4.8(1)
C-8	−0.1629(7)	0.0075(7)	0.3303(10)	4.8(1)
C-9	0.2570(6)	0.2343(6)	0.4164(9)	4.3(1)
C-10	0.1678(6)	0.0131(5)	0.3165(8)	3.9(1)
C-11	0.2785(6)	0.0114(5)	0.1905(7)	3.4(1)
C-12	0.3660(7)	−0.0747(5)	0.1856(8)	3.8(1)
C-13	0.4652(7)	−0.0792(5)	0.0621(8)	3.8(1)
C-14	0.4685(6)	0.0038(6)	−0.0608(7)	3.5(1)
C-15	0.3840(6)	0.0930(5)	−0.0558(7)	3.3(1)
C-16	0.2920(6)	0.0963(5)	0.0730(8)	3.4(1)
O-17	0.2106(4)	0.1866(4)	0.0680(6)	4.0(1)
O-18	0.5496(5)	−0.1666(4)	0.0707(7)	5.2(1)
Br-19	−0.33641(7)	0.2222(1)	0.40479(11)	5.68(2)
Br-20	0.59041(7)	0.0000(−)[a]	−0.24103(8)	4.82(1)

[a]The *y*-coordinate of Br-20 was held constant throughout to define the origin in this direction.

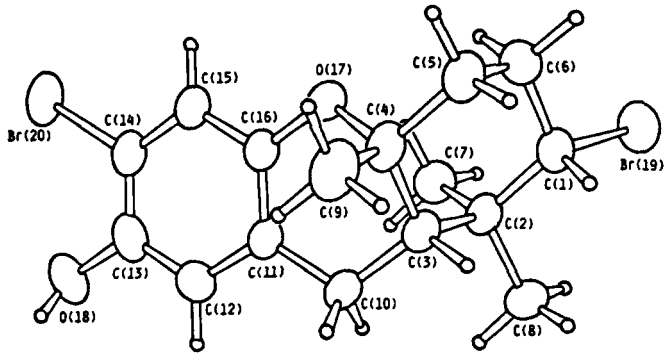

FIGURE 2. Structure and solid-state conformation of cymobarbatol [**3**]; small circles represent hydrogen atoms.

stituent is in the β configuration (4). The C-4 methyl substituent in **3** is equatorial, whereas in the C-4 epimer **4**, this group is axially disposed. Endocyclic torsion angles characterizing the ring B conformation in **4** are related by an approximate C_2 symmetry axis passing through the midpoints of the C-3–C-4 and C-11–C-16 bonds and, with a value (1.9°) close to 0° around the latter, this ring has a half-chair form. In contrast, analysis of ring B torsion angles in **3** in terms of deviations from symmetry-related values for half-chair and 1,2-diplanar (envelope, half-boat) forms indicates that it lies intermediate between these two conformations. The different conformations adopted by ring B in each of these compounds are a consequence of the different nature of their A/B ring fusions and reflect the torsional changes necessary to minimize non-bonded 1,3-diaxial interactions between the axial methyl group at C-2 and the C-4 substituent (O-aryl in **3**; methyl in **4**). In crystals of **3**, molecules are linked by an O-18–H-18 . . . Br-20 hydrogen bond. [Hydrogen-bonded distances follow: O-18 . . . Br-20 = 3.500(4) Å, H-18 . . . Br-20 = 3.09 Å, Br-20 at -1/2-*x*, -*y*, 1/2 + *z* in crystals of **3**; O-18 . . . Br-20 = 3.140(5) Å, H-18 . . . Br-20 = 2.55 Å in crystals of **4**. Exocyclic bond angles subtended at C-13 reflect the different hydrogen atom orientations with respect to the aromatic ring C plane: C-12–C-13–O-18 = 120.2(4)° ≃ C-14–C-13–O-18 = 120.4(4)° for the out-of-plane H-18 in **3**; C-12–C-13–O-18 = 117.5(6)° ≪ C-14–C-13–O-18 = 124.5(6)° for the co-planar H-18 in **4**.] In **4**, the hydroxy hydrogen atom is oriented such that it is involved in an intramolecular O-18–H-18 . . . Br-20 hydrogen bond and the shortest intermolecular separations in the crystals correspond to normal van der Waals distances.

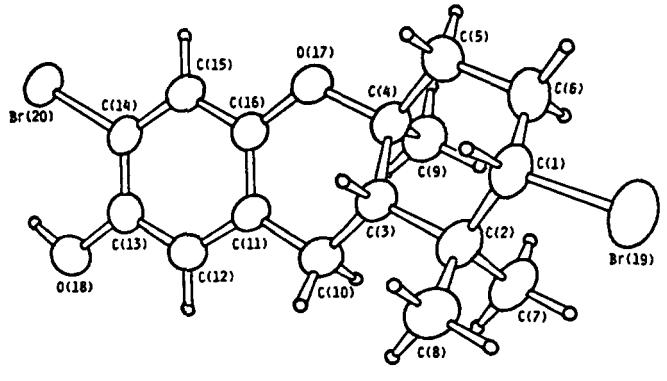

FIGURE 3. Structure and solid-state conformation of 4-isocymobarbatol [**4**]; small circles represent hydrogen atoms.

BIOLOGICAL ACTIVITY.—Compounds **3** and **4** were nontoxic at doses from 300 to 75 μg per plate in both *S. typhimurium* T-98 and T-100 strains. Compound **1**, cymopol, was very toxic in both T-98 and T-100 at doses from 600 to 75 μg per plate and hence was not further investigated. Both compounds **3** and **4** were highly active in inhibition of 2AN mutagenicity toward *S. typhimurium* using the T-98 stain at doses of 300, 150 and 75 μg per plate. Even greater activity in the inhibition of EMS (T-100), was shown with inhibition values of 80–95% at concentrations ranging from 32.5 μg to 300 μg per plate. Unfortunately, quantities of a compound believed to be **2** were too low to permit testing. Further studies will be required to elucidate the surprising differences in the toxicities of **1** from **3** and **4**.

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