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ENZYME INHIBITORS: NEW AND KNOWN POLYBROMINATED PHENOLS AND DIPHENYL ETHERS FROM FOUR INDO-PACIFIC *DYSIDEA* SPONGES

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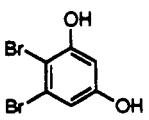
ABSTRACT.—Extracts and pure compounds isolated from four samples of *Dysidea* sp. sponges collected from two geographically distinct regions of the Indo-Pacific (Chuuk Atoll and Fiji) were assayed against five different enzyme assays, four of which are relevant to anticancer drug discovery and one of which (15-lipoxygenase) may detect compounds significant in modulating the development of atherosclerotic plaque. The pure compounds that inhibited various enzymes were polybrominated phenols and polybrominated phenoxyphenols. Fourteen of these phenols were isolated, six of which were new compounds. A variety of the phenols inhibited inosine monophosphate dehydrogenase (IMPDH), guanosine monophosphate synthetase, and 15-lipoxygenase. No activity was observed with protein tyrosine kinase $pp60^{v\text{-}src}$ or matrix metalloprotease.

In recent years, assays incorporating enzymatic reactions or biochemical pathways suspected to be involved with cancer have attracted much interest (1,2). Several years ago we launched a program to test the applicability of this approach to evaluating marine organisms for anticancer leads (3). The broad scope of this effort involved a variety of marine-derived extracts and compounds which were tested for their potential to inhibit enzymes implicated in tumor development. The enzymes used in this program included protein kinase C (PKC) (4–6), protein tyrosine kinase (PTK) (7–9), inosine monophosphate dehydrogenase (IMPDH) (10,11), and guanosine monophosphate synthetase (GMPS) (12). In complementary research, some of the samples were also assayed (13) for inhibition of the enzyme 15-lipoxygenase (15-LO), a 75-kDA iron-containing protein that catalyzes the peroxidation of fatty acids with a *cis-cis*-1,4-pentadiene moiety (14). The peroxidation of fatty acid components in low density lipoproteins (LDL) by 15-LO is thought to play a role in the development of atheroslerotic lesions, so inhibitors of this process could be promising therapeutic agent candidates.

As a result of our joint work on pursuing leads from the above enzyme assays, we have isolated a number of brominated phenolic compounds. In some cases the phenolic compounds proved to be active components in the test system, which prompted fractionation work on these compounds. Six new brominated phenols and phenoxyphenol metabolites were isolated, along with eight known ones from *Dysidea* sponges collected from widely separated locations in the Western Pacific. We report here the structures of the new phenolics and a comparison of the inhibitory activities of various metabolites on different enzymes. Although each of the phenols was not tested against each of the enzymes, the data obtained showed that there is some selectivity in inhibitory activity. The data also showed that in some cases the activity of a crude extract is masked or is

[1]On sabbatical leave from the University of the Virgin Islands, St. Thomas, U.S. Virgin Islands.



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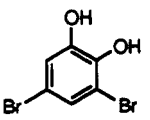

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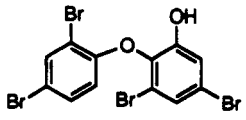

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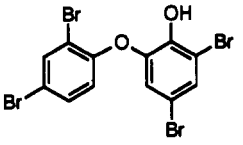

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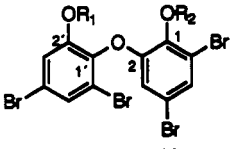

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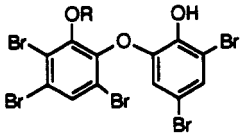

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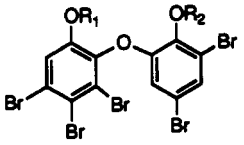

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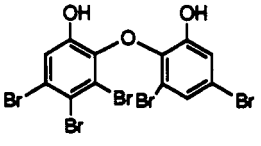
11 R_1=H, R_2=Me



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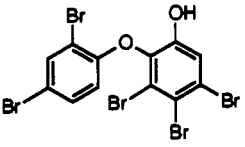

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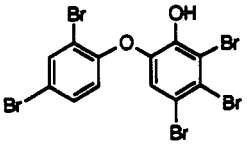

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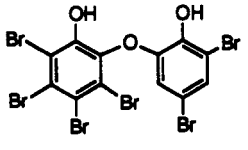

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above the threshold level set for initiating bioassay-directed fractionation. The work also illustrates the wide variability in bromophenol production by the *Dysidea* sponges and/or their associated microfauna.

Positive results were obtained with extracts for four sponges in the genus *Dysidea* during initial screening with enzymes including PTK, IMPDH, and 15-LO. These active crude extracts were from samples collected by either the University of Oklahoma or the University of California, Santa Cruz groups and a parallel study was begun on them in both laboratories. Separate fractionation of the extracts yielded fourteen compounds which could be classified as either polybrominated phenols or polybrominated phenoxyphenols. We now report on their structures, which include the new metabolites **1**, **6**, **8**, **9**, **11**, and **12**, accompanied by the known compounds **2**–**5**, **7**, and **13**–**15**. Several of these compounds were inhibitors of IMPDH or 15-LO, but none were active against PTK.

RESULTS AND DISCUSSION

The activity profile of the crude extracts obtained from the five *Dysidea* sponges studied is outlined in Table 1. Two of these sponges yielded crude extracts which significantly inhibited 15-LO. These were collections 5-T-92 and 35-T-92 obtained by the Oklahoma group from different locations in Chuuk State, Federated States of Micronesia. The extracts of the other two sponges included one that was active against

TABLE 1. Enzyme Inhibition Profile for Crude Extracts and Pure Compounds.[a]

Collection No./ Compound No.	15-LO (IC_{50}, μM)	IMPDH (EC_{50}, μM)	PTK (EC_{50})	MMP (EC_{50})	GMPS (Radioactive Assay) % Inhibition @ 50 μM)
88098	—	—	2.4 μg/ml	—	—
91009	—	1.3 μg/ml	3.0 μg/ml	16 μg/ml	—
5-T-92	100% inhibition @ 40 μg/ml	NA	—	—	—
35-T-92	100% inhibition @ 40 μg/ml	NA	—	—	—
1/2	100% inhibition @ 40 μg/ml	NA	—	—	—
3	15	5.71	NA	NA	56
4	—	—	NA	—	—
5	—	—	NA	—	—
6	—	—	NA	—	—
7	2.5	7.8	NA	NA	70
9	—	—	NA	NA	89
12	100% inhibition @ 40 μg/ml	—	—	—	—
13	7.4	4.0	NA	NA	99
14	7.4	4.4	NA	NA	71
15	1.3	2.79	NA	NA	96

[a]For explanation of bioassay acronyms, see text.

PTK and one that was active against PTK, IMPDH, and MMP. These sponges were collected by the University of California, Santa Cruz group and included collections 88098 and 91009 from Fiji. It is relevant to note that the genus *Dysidea* has been the subject of intense chemical study and its members have been reported previously as a source of polybrominated phenoxyphenols (15–20), terpenoids (21,22), and polychlorinated peptides (23). For the most part, the bioactivity data shown in Table 1 were used to direct the fractionation of the various crude extracts. The fourteen compounds that we isolated overlapped in their occurrence from the different sponges as follows: coll. No. 35-T-92 yielded **3**, **4** **13**, and **14**; coll. No. 91009 yielded **6–8**, **11**, and **15**; coll. No. 88098 yielded **4–6** and **15**; and coll. No. 5-T-92 yielded **1**, **2**, **7**, **9**, **12**, and **15**.

Inspection of the aromatic region of the ^{1}H-nmr spectra of the chromatographic fractions suspected to contain pure compounds or simple mixtures provided extremely useful initial data. The multiplet patterns plus the count of aromatic protons in the regions δ 7.2–7.8 and δ 6.2–6.4 provided quick information about the presence of and relative orientation of the bromine and oxygen substituents. The known compounds **3–5**, **7**, and **13–15** were identified by comparison of their spectral data with published values (16, 19, 20, 24). ^{13}C-Nmr data for **13** are reported here for the first time.

Isomers **1** and **2** were obtained as an inseparable mixture in a ratio of 1:7, respectively, as estimated by the integration of the ^{1}H-nmr spectrum. The ir spectrum (3480, 1585, 1466 cm^{-1}) of the mixture revealed the presence of hydroxyl groups and phenyl rings. A cluster of peaks at *m/z* 270, 268, and 266 in the eims established the molecular composition $C_6H_4O_2Br_2$ for the isomers **1** and **2**. Two sets of signals for meta-coupled protons were observed in the ^{1}H-nmr spectrum: δ 6.64 (d, 2), 6.84 (d, 2) for the minor component and δ 6.99 (d, 2), 7.12 (d, 2) for the major component. Of the four possible isomeric phenol structures for the formula $C_6H_4O_2Br_2$ that have meta-oriented protons, two are symmetrical and can therefore be ruled out. The remaining two are

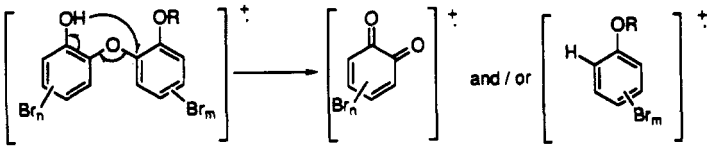
structure **1**, assigned to the minor component on the basis of its relatively higher field ^{1}H-nmr absorptions, and structure **2** assigned to the major compound. Phenol **2** has been synthesized (25), but this is the first report of it as a natural product. Phenol **1** has not been reported previously.

Three other new compounds were OMe analogues of previously reported metabolites. The first of this trio was **6**, whose formula of $C_{13}H_8O_3Br_4$ and ^{1}H-nmr multiplets showed that it was isomeric to **5**. An nOe difference spectrum for **6** revealed that the OMe (δ 3.78) and H-3′ (δ 7.09) were ortho and thereby supported the placement of the OMe groups in both **6** and **5**. The second member of this series was **8**, $C_{13}H_7O_3Br_5$, which differed from **7** by having one of the OH groups methylated. The methoxy group could be assigned to the C-2′ position by comparison of the proton nmr data of **8** with those of **7** and the dimethylated derivative of **7** which were described by Carté and Faulkner (20). The proton chemical shifts of H-3 and H-5 change very little (+0–0.04 ppm) when **7** is dimethylated, whereas the H-5′ resonance changes from δ 7.57 to 7.73.

Because the chemical shift of the singlet resonance of **8** occurs at δ 7.73, the methyl ether group must be at C-2′. As shown in Scheme 1, the mass spectral fragment ion clusters at *m/z* 264, 266, and 268 indicate that the three oxygens are all ortho to one another. The fragmentation leading to these clusters of ions has been noted previously to depend on the ortho-hydroxy ether arrangement (24). The final member of this series, **11**, with a formula of $C_{13}H_7O_3Br_5$, was clearly isomeric with **8** (nmr data). The aromatic proton nmr signals of **11** were nearly identical to those of **9** and **10** and this leads to the conclusion that the two have the same substitution pattern. The methoxy group was tentatively assigned to the C-1 position based on an nOe difference spectrum that showed no correlations to the OMe group, and the intense mass spectral fragment ion clusters at *m/z* 264 and 342 as discussed above. Although the failure to observe an nOe is not definitive evidence, the facile observation of an nOe in other related cases, e.g., **6**, gives weight to this negative information in these simple structures.

The molecular formula determined by hreims for the polybrominated phenol **9**, $C_{12}H_5O_3Br_5$, was the same as that of compound **7**, indicating that the two phenols are isomers. The ^{1}H-nmr spectrum of **9** in CD_3OD contained doublets at δ 6.46 (J=2 Hz) and 7.23 (J=2 Hz) due to meta-situated aromatic protons like those in **7**, and a singlet at δ 7.26 assigned to a single proton on the second aromatic ring. Methylation of phenol **9** with methyl iodide and K_2CO_3 in dry Me_2CO yielded a dimethyl ether which exhibited mp, ^{1}H-nmr, and ms spectra matching those reported for **10** (19), and hence structure **9** was confirmed.

The ^{1}H-nmr spectrum of **12**, $C_{12}H_5O_3Br_5$ by hreims, in CD_3OD showed a singlet at δ 7.24, and doublets (J=2 Hz) at δ 7.10 and 7.39. The chemical shift of the singlet at δ 7.24 differed significantly from that of compound **7** (δ 7.36, s), but was nearly identical to that observed for compounds **9** (δ 7.26, s) and **10** (δ 7.25, s). Hence, the tribrominated ring of **12** was assigned the same substitution pattern as found in



Br$_2$: *m/z* 264
Br$_3$: *m/z* 342

Br$_2$OH: *m/z* 250
Br$_2$OMe: *m/z* 264
Br$_3$OMe: *m/z* 342

SCHEME 1. Mass spectral fragments observed from polybrominated phenyl ethers.

compound **9**. The hydroxyl group in the dibrominated ring was assigned to the position ortho to the ether linkage on the basis of ms data as discussed for **8** above. Significant peaks at *m/z* 250, 252, and 254 ($C_6H_4OBr_2$) were noted and these were attributed to rearrangement of the molecular ion as shown in Scheme 1 (24). Because the chemical shifts (CD_3OD) of the meta-coupled protons of this ring are similar to those of the A ring of **3** (δ 7.03, 7.22) and quite different from those of the analogous protons in ring A of **7**, **9**, and **15** (δ 6.51, 7.26), the protons in ring A of **12** were assigned to the positions ortho and para to the hydroxyl group. Further support for this assignment was derived from the observation that all of the proton chemical shifts of **12** in DMSO-d_6 were shifted upfield by the same amount, ca. 0.45–0.44 ppm, when 1 drop of 40% $NaOD/D_2O$ was added, as expected for protons ortho- and para- to phenolic hydroxyl groups (20,26).

The results of a spectrum of enzyme inhibition assays are summarized in Table 1. Bioactivity-directed fractionation of sponges 5-T-92 and 35-T-92 led efficiently to the isolation of a variety of phenols that inhibited 15-LO in the low micromolar range. The possibility that the inhibitory effects observed were due to an antioxidant effect of the phenols was ruled out for phenols **3**, **7**, and **13–15** by control experiments in which a known peroxide was substituted for the enzyme in the assay (13). Since the crude extracts of 5-T-92 and 35-T-92 were not active at the threshold level set for crudes in the IMPDH assay, screening of chromatographic fractions for this activity was not pursued. However, because phenols might be suspected to interact with various proteins, several of the brominated phenols isolated via bioassay guidance for 15-LO activity were checked for selectivity by evaluating them for inhibition of IMPDH, tyrosine kinase (TK), matrix metalloprotease (MMP), and guanosine monophosphate synthetase (GMPS). Inhibition of IMPDH and GMPS activity was observed for a number of the phenols, but no activity was noted with TK or MMP. The phenols in extracts of sponges 88098 and 91009 are not the source of the PTK inhibition exhibited by these extracts. Phenol **15** was also found to be inactive in a protein kinase C assay.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra at the University of Oklahoma were obtained on a Varian XL-300 spectrometer; ms were measured with either Hewlett-Packard 5985B or VG ZAB mass spectrometers; ir spectra were recorded on a Bio-Rad 3240-spc Ft instrument. Prep. hplc was performed using a Spherex 5 C_{18} column (300×10 mm) with uv detection. Flash chromatography was carried out on Si gel 60-H (230–400 mesh).

ANIMAL MATERIAL.—Sample 5-T-92 was collected from beneath overhangs on the reef crest at a depth of 2–3 meters inside Kuop Atoll, Chuuk State, Federated States of Micronesia, in June 1992. The sponge forms an extremely thin spreading sheet less than 1 mm thick and is fairly easily peeled from the underlying coral. The surface is very smooth with extremely delicate conules. The sponge is fleshy to the touch, rubbery, and grayish-green in life. The primary and secondary fibers are heavily cored in the lower half of the sponge, but less frequently cored in the upper surface. Some areas of fiber are completely devoid of coring material. The sponge is packed with chain-forming cyanobacteria. The sample is an undescribed species of *Dysidea* (order Dictyoceratida; family Dysideidae). A voucher specimen has been deposited at the Natural History Museum, London (BMNH: 1992.6.2.1).

Sample 35-T-92 was collected from a near vertical wall at a depth of 25–30 meters at Satawan Atoll, Chuuk State, Federated States of Micronesia, in June 1992. The sponge was ramose-encrusting, irregularly lobate, and frequently digitate. The surface was finely conulose with scattered prominent oscules. The texture was firm, slightly fleshy to the touch, and the sponge is whitish purple in life. The primary and secondary fibers are heavily cored and form a tight reticulation, and spongin is visible on the edges of the fibers. The sample is an undescribed species of *Dysidea* (order Dictyoceratida; family Dysideidae). A voucher specimen has been deposited at the Natural History Museum, London (BMNH: 1992.6.7.1).

Sample 88098 was collected from Vitu Levu, Fiji and was identified by Ms. C.C. Diaz [University of California, Santa Cruz, Institute of Marine Sciences (USCS IMS)] as *Dysidea? herbacea* (order Dictyoceratida; family Dysideidae). This whitish-greenish-grey sponge has a thin lobate shape with plate-like projections. It has a soft and slimy consistency. The surface is finely conulose with small oscules on top of the projections.

Its choanosome is fibroticulated and highly charged with foreign material. The fibers are 40–100 μm in size. The greenish interior was assumed to be due to the presence of blue-green algal symbionts. A voucher sample is available from P.C.

Sample 91009 was a recollection of sample 88098 and it was identified by Ms. C.C. Diaz (UCSC IMS) as *Dysidea? herbacea* (order Dictyoceratida; family Dysideidae). The interior of this sponge is loaded with blue-green algal symbionts. A voucher sample is available from P.C.

EXTRACTION AND ISOLATION (5-T-92).—The chopped sponge (90 g wet wt; 5.5 g dry wt after extraction) was extracted with MeOH (3 times) followed by MeOH-CH_2Cl_2 (1:1) (3 times). The extracts were evaporated to dryness under reduced pressure and submitted for enzyme assays. Both the MeOH and MeOH/CH_2Cl_2 extracts showed 15-lipoxygenase activity (100% inhibition at 40 μg/ml), and were therefore combined. The combined extract (1.97 g) was subjected to Kupchan partitioning (27) to give, after evaporation of solvents under reduced pressure, hexane (328 mg), CH_2Cl_2 (383 mg), *n*-BuOH (1.02 g), and aqueous MeOH (197 mg) fractions. Analysis of the first two fractions by tlc and ^{1}H-nmr spectroscopy indicated similar interesting components and they were therefore pooled and chromatographed over Si gel eluting with a step gradient of EtOAc in hexane. The fractions which contained phenolic compounds were combined and rechromatographed over Si gel with a step gradient of Me_2CO in hexane. Six fractions were collected. Reversed-phase hplc of fractions 2 and 3, eluted by 20% H_2O in MeOH, yielded two pure compounds **15** (30 mg), **12** (2 mg), and two mixtures, one consisting of **7** and **9**, the other (~3 mg) containing **1** and **2**. Compounds **7** (14 mg) and **9** (6 mg) were separated by reversed-phase hplc using H_2O-MeOH-AcOH (25:75:0.2) as eluent.

Chopped specimens of sponge 35-T-92 (635 g wet wt; 104 g dry wt after extraction) were extracted and the extracts partitioned in the same manner as described above for sponge 5-T-92. The hexane and CH_2Cl_2 fractions obtained by Kupchan partitioning showed similar inhibitory activity against 15-lipoxygenase (100% inhibition at 40 μg/ml) and similar ^{1}H-nmr spectra. Thus, they were combined and subjected to vacuum-flash chromatography over Si gel using a step gradient of EtOAc in hexane. Six fractions were collected. A major compound, **13** (ca. 2 g), was obtained from the third fraction by recrystallization in CH_2Cl_2. The mother liquor of fraction 3 was pooled with fraction 2 and the resulting mixture was subjected to C_{18} reversed-phase hplc using 16% H_2O in MeOH as eluent to afford compounds **3** (12 mg), **4** (0.5 mg), **5** (0.5 mg), and **14** (4 mg).

The samples collected by University of California, Santa Cruz workers were processed in a similar manner to yield **4**–**6** and **15** (sample 88098) and **6**–**8**, **11**, and **15** (sample 91009).

Mixture (1:7) of 2,3-dibromo-5-hydroxyphenol [**1**] *and 3,5-dibromo-2-hydroxyphenol* [**2**] (25).—Ir ν max (neat) 3480, 1585, 1466 cm^{-1}; ^{1}H nmr (Me_2CO)-d_6/CD_3OD) δ 6.64 (1H, d, J=2 Hz), 6.84 (1H, d, J=2 Hz) (assigned to compound **1**), 6.99 (1H, d, J=2 Hz), 7.12 (1H, d, J=2 Hz) assigned to compound **2**; eims (70 eV) *m/z* [M]$^+$ 270 (504), 268 (100), 266 (53), 188 (8), 186 (8); 160 (18), 158 (19), 79 (39).

4,6-Dibromo-2-(2′-methoxy-4′,6′-dibromophenoxy)phenol [**6**].—Viscous oil; ^{1}H nmr ($CDCl_3$) assignments based on ^{1}H-^{1}H COSY nmr data, δ 7.40 (1H, d, J=2 Hz, H-5′), 7.33 (1H, d, J=2 Hz, H-5), 7.09 (1H, d, J=2 Hz, H-3′), 6.54 (1H, d, J=2 Hz, H-3), 3.78 (s, OMe); ^{13}C nmr ($CDCl_3$) δ 153.4 (s, C-2′), 145.2 (s, C-2), 142.8 (s, C-1), 139.9 (s, C-1′), 129.2 (d, C-5), 127.5 (d, C-5′), 119.8 (s, C-4′), 118.6 (s, C-6′), 116.5 (d, C-3′), 115.7 (d, C-3), 111.5 (s, C-6), 110.0 (s, C-4), 56.6 (q, OCH_3); lreims *m/z* [M]$^+$ 528, 530, 532, 534, 536 (1.0:3.0:4.8:3.0:1.0).

4,6-Dibromo-2-(3′,4′,6′-tribromo-2′-methoxyphenoxy)phenol [**8**].—Viscous oil; ^{1}H nmr ($CDCl_3$) δ 7.73 (1H, s, H-5′), 7.34 (1H, d, J=2 Hz, H-5), 6.48 (1H, d, J=2 Hz, H-3), 3.8 (s, OMe); lreims *m/z* [M]$^+$ 606, 608, 610, 612, 614, 616 (1.0:4.0:8.0:8.0:4.0:1.0).

4,6-Dibromo-2-(4′,5′,6′-tribromo-2′-hydroxyphenoxy)phenol [**9**].—Fine needles from MeOH; mp 132–133°; ir ν max (neat) 3480, 1585, 1470 cm^{-1}; ^{1}H nmr (CD_3OD) δ 6.46 (1H, d, J=2 Hz, H-3), 7.23 (1H, d, J=2 Hz, H-5), 7.26 (1H, s, H-3′); eims (70 eV) identical with that of compound **7**; hreims *m/z* 593.6180 (48) (calcd for $C_{12}H_5O_3{}^{79}Br_4{}^{81}Br$ 593.6135), 595.6081 (97) (calcd for $C_{12}H_5O_3{}^{79}Br_3{}^{81}Br_2$ 595.6115), 597.6072 (100) (calcd for $C_{12}H_5O_3{}^{79}Br_2{}^{81}Br_3$ 597.6094), 599.6107 (50) (calcd for $C_{12}H_5O_3{}^{79}Br^{81}Br_4$ 599.6074).

Methylation of **9**.—A mixture of 2.5 mg of **9**, 0.5 ml of methyl iodide and 20 mg of K_2CO_3 in 3 ml of dry Me_2CO was heated under reflux for 24 h. Excess K_2CO_3 solid was filtered off, the solvent was evaporated, and the residue was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 fraction was separated, the solvent evaporated, and the residue subjected to reversed-phase hplc (2% H_2O in MeOH as eluent) to yield ca. 2 mg of material which exhibited mp and ^{1}H-nmr and ms spectra which match those reported for **10** (19).

4,6-Dibromo-2-(4′,5′,6′-tribromo-2′-hydroxyphenoxy)phenol-1-methyl ether [**11**].—Viscous oil; ^{1}H nmr ($CDCl_3$) δ 7.37 (1H, d, J=2 Hz, H-5), 7.31 (1H, s, H-3′), 6.54 (1H, d, J=2 Hz, H-3), 3.8 (3H, s, OMe); lreims *m/z* [M−H]$^-$ 605, 607, 609, 611, 613, 615 (1.0:4.0:8.0:8.0:4.0:1.0).

3,5-Dibromo-2-(4',5',6'-tribromo-2'-hydroxyphenoxy)phenol [**12**].—Amorphous powder; ir ν max (neat) 3400, 1580, 1475 cm^{-1}; ^1H nmr (DMSO-d_6) δ 6.93 (1H, d, J=2.3 Hz, H-6), 7.08 (1H, s, H-3'), 7.51 (1H, d, J=2.3 Hz, H-4); ^1H nmr (DMSO-d_6, 1 drop 40% NaOD in D_2O) δ 6.47 (1H, d, J=2.3 Hz, H-6), 6.64 (1H, s, H-3), 7.04 (1H, d, J=2.3 Hz, H-4); ^1H nmr (CD_3OD) δ 7.10 (1H, d, J=2 Hz, H-6), 7.24 (1H, s, H-3), 7.39 (1H, d, J=2 Hz, H-4); ^1H nmr (CD_3OD+1 drop of 40% NaOD in D_2O) δ 6.66 (1H, d, J=2.4 Hz, H-6), 6.80 (1H, s, H-3), 7.16 (1H, d, J=2.4 Hz, H-4); hreims (70 eV) *m/z* 593.6095 (48) (calcd for $C_{12}H_5O_3{}^{79}Br_4{}^{81}Br$ 593.6135), 595.6055 (94) (calcd for $C_{12}H_5O_3{}^{79}Br_3{}^{81}Br_2$ 595.6115), 597.6142 (100) (calcd for $C_{12}H_5O_3{}^{79}Br_2{}^{81}Br_3$ 597.6094), 599.6094 (56) (calcd for $C_{12}H_5O_3{}^{79}Br{}^{81}Br_4$ 599.6073); eims (70 eV) *m/z* 602 (1), 600 (6), 598 (13), 596 (13), 594 (7), 592 (2) $[M]^+$, 440 (6), 438 (18), 436 (20), 434 (6), 334 (30), 332 (98), 330 (100), 328 (40), 270 (18), 268 (35), 266 (19), 254 (18), 252 (33), 250 (23).

3,4,5-Tribromo-2-(2',4'-dibromophenoxy)phenol [**13**].—Crystals from CH_2Cl_2; mp 195–196° [lit. (16,20) mp 185–186°]; ^1H nmr ($CDCl_3$) δ 6.38 (1H, d, J=8 Hz, H-6'), 7.27 (1H, dd, J=8 and 2 Hz, H-5'), 7.42 (1H, s, H-6), 7.77 (1H, d, J=2 Hz, H-3'); ^1H nmr (CD_3COCD_3) δ 6.63 (1H, d, J=8 Hz, H-6'), 7.39 (1H, dd, J=8 and 2 Hz, H-5'), 7.51 (1H, s, H-6), 7.82 (1H, d, J=2 Hz, H-3'), 9.73 (br, OH); ^1H nmr (DMSO-d_6), δ 4.25 (br, OH), 6.42 (1H, d, J=8 Hz, H-6'), 7.28 (1H, dd, J=8 and 2 Hz, H-5'), 7.38 (1H, s, H-6), 7.78 (1H, d, J=2 Hz, H-3'); ^{13}C nmr (DMSO-d_6) δ 111.9, 114.4, 116.0, 116.1, 120.9, 121.6, 121.7, 131.6, 135.3, 139.7, 150.9, 152.4; eims (70 eV) *m/z* 586, 584, 582, 580, 578, 576 $[M]^+$, 506, 504, 502, 500, 498 $[M^+-Br]$, 426, 422, 420, 418 $[M^+-2\ Br]$.

ENZYME ASSAYS.—Tyrosine kinase and inosine mono-phosphate dehydrogenase assays were conducted as described previously (7,10). The assay for 15-lipoxygenase inhibition and antioxidant activity followed essentially the protocol described by Sigal *et al.* (14). Matrix metalloprotease inhibition was assayed by the procedure of Park *et al.* (28) and guanosine monophosphate synthetase inhibition by the protocol described by Hirst *et al.* (12).

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