Ambigol A and B: New Biologically Active Polychlorinated Aromatic Compounds from the Terrestrial Blue-Green Alga *Fischerella ambigua*

Beatrix S. Falch,* Gabriele M. König, Anthony D. Wright, and Otto Sticher

Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich, 8057 Zürich, Switzerland

Heinz Rüegger

Laboratory for Inorganic Chemistry, Swiss Federal Institute of Technology (ETH) Zurich, 8092 Zürich, Switzerland

Gérald Bernardinelli

Laboratory of Crystallography, University of Geneva, 24 quai E. Ansermet, 1211 Geneva, Switzerland

Received June 4, 1993•

The isolation and structural elucidation of two novel highly halogenated aromatic compounds, ambigol A (1), 3-(2,4-dichlorophenoxy)-3′,4,5′,6-tetrachloro-2,2′-biphenyldiol, and ambigol B (2), 2,6-bis(2,4-dichlorophenoxy)-3,5-dichlorophenol, from a strain of the terrestrial cyanophyte *Fischerella ambigua* (Näg.) Gomont are described. The structures were established by spectroscopic methods, including the high temperature high resolution 2D-NMR INADEQUATE experiment, and confirmed, for ambigol A, by single-crystal X-ray crystallography. The biological activities of ambigol A and B were determined as antibacterial, antifungal, and molluscicidal. Ambigol A was demonstrated to inhibit the enzymes cyclooxygenase and HIV reverse transcriptase. The indole alkaloid tjipanazole D (3), previously isolated from the blue-green alga *Tolypothrix tjipanasensis*, was also obtained from this cyanobacterium. Tjipanazole D showed no significant biological activities.

Blue-green algae are a rich source of biologically active secondary metabolites.[1] With these resources in mind, a series of laboratory-cultured fresh-water and terrestrial blue-green algae, including strains of *Anabaena planctonica* Brunnth., *Dichothrix orsiniana* Born. et Flah., *Fischerella ambigua* (Näg.) Gom., *Nostoc commune* Vaucher, *Nostoc* sp. *ex Peltigera apthosa* var. *variolosa*, *Nostoc sphaericum* Vaucher, *Oscillatoria rubescens* DC, *Pseudoanabaena catenata* Lauterborn, and *Scytonema lyngbyoides* Gardner, was extracted and investigated for their antibacterial (*Micrococcus luteus*, *Bacillus subtilis*, and *Escherichia coli*), antifungal (*Penicillium oxalicum*), and cytotoxic (KB cell-line) activities as well as brine shrimp toxicity. The results of this screening demonstrated that extracts of *F. ambigua* exhibited the most potent activities, and hence this cyanophyte was chosen for detailed investigation of its secondary metabolite chemistry.

Terrestrial cyanophytes of the genus *Fischerella*, family Stigonemataceae (Stigonematales), have only recently attracted the attention of natural products scientists. To date, examination of *Fischerella* sp. for biologically active compounds resulted in the isolation of two indolinones, containing a spiro-fused cyclopropane and an isonitrile functionality.[2] One of these compounds was described to be an arginine vasopressin inhibitor. Further indole-type alkaloids, substituted with chlorine and isonitrile functionalities, the potent fungicidal ambiguine isonitriles,[3] and fischerindole L,[4] were reported from *F. ambigua* and *F. muscicola*, respectively. These compounds are related to the antibacterial and antimycotic hapalindoles[5] obtained from several strains of *Hapalosiphon* sp., cyanophytes which are part of the same family.

The current report describes the isolation and characterization of the new biologically active ambigol A (1) and ambigol B (2), identified as 3-(2,4-dichlorophenoxy)-3′,4,5′,6-tetrachloro-2,2′-biphenyldiol and 2,6-bis(2,4-dichlorophenoxy)-3,5-dichlorophenol, respectively, and the known tjipanazole D[6] (3), which is structurally related to the potent protein kinase C inhibitor staurosporine,[7] from *F. ambigua*. Ambigol A and B are characterized by their unusually high level of halogenation and represent a structural type not yet encountered in cyanobacteria. Complete spectroscopic data for tjipanazole D are reported for the first time.

The alga was mass cultured as outlined in the Experimental Section. Algal cells were separated from their culture medium, lyophilized, and successively extracted with petroleum ether, dichloromethane, ethyl acetate, methanol, and 80% aqueous methanol. All extracts were subjected to biological screening and, with the exception of the aqueous methanol extract, demonstrated pro-

• Abstract published in *Advance ACS Abstracts*, October 15, 1993.

(1) Moore, R. E. In *Marine Natural Products: Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic Press: New York, 1981; Vol. IV, pp 1–52.

(2) Schwartz, R. E.; Hirsch, C. F.; Springer, J. P.; Pettibone, D. J.; Zink, D. L. *J. Org. Chem.* **1987**, *52*, 3704–3706.

(3) Smitka, T. A.; Bonjouklian, R.; Doolin, L.; Jones, N. D.; Deeter, J. B.; Yoshida, W. Y.; Prinsep, M. R.; Moore, R. E.; Patterson, G. M. L. *J. Org. Chem.* **1992**, *57*, 857–861.

(4) Park, A.; Moore, R. E.; Patterson, G. M. L. *Tetrahedron Lett.* **1992**, *33*, 3257–3260.

(5) (a) Moore, R. E.; Cheuk, C.; Yang, X.-Q. G.; Patterson, G. M. L.; Bonjouklian, R.; Smitka, T. A.; Mynderse, J. S.; Foster, R. S.; Jones, N. D.; Swartzendruber, J. K.; Deeter, J. B. *J. Org. Chem.* **1987**, *52*, 1036–1043. (b) Moore, R. E.; Cheuk, C.; Patterson, G. M. L. *J. Am. Chem. Soc.* **1984**, *106*, 6456–6457.

(6) Bonjouklian, R.; Smitka, T. A.; Doolin, L. E.; Molloy, R. M.; Debono, M.; Shaffer, S. A.; Moore, R. E.; Stewart, J. B.; Patterson, G. M. L. *Tetrahedron* **1991**, *47*, 7739–7750.

(7) (a) Furusaki, A; Hashiba, N.; Matsumoto, T.; Hirano, A.; Iwai, Y.; Omura, S. *J. Chem. Soc., Chem. Commun.* **1978**, 800–801. (b) Gill, M.; Steglich, W. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Tamm, Ch., Eds.; Springer-Verlag: Wien New York, 1987; Vol. 51, pp 216–226.

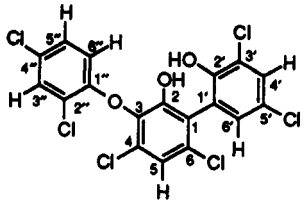
0022-3263/93/1958-6570$04.00/0

Table I. ^{13}C and ^{1}H NMR Spectral Data for Ambigol A

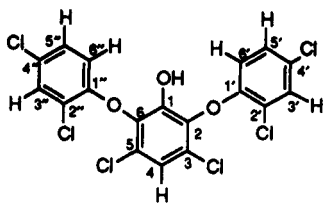
position	δ_H (mult; J, Hz) CD$_3$OD	δ_H (mult; J, Hz) DMSO-d_6[a]	δ_C (mult) CD$_3$OD	δ_C (mult) DMSO-d_6[a]	XHCORR[b]	INADEQUATE[c]
1			125.9 (s)	124.6 (s)		
2			152.3 (s)	151.1 (s)		
3			139.3 (s)	137.9 (s)		
4			128.9 (s)[d]	126.8 (s)[d]		C5
5	7.21 (s)	7.23 (s)	121.8 (d)	119.8 (d)	C1, C2, C3, C4, C6	C5, C6
6			132.8 (s)[d]	130.8 (s)[d]		C5
1′			126.5 (s)	125.1 (s)		C6′
2′			151.6 (s)	150.4 (s)		
3′			123.6 (s)	122.1 (s)		C4′
4′	7.44 (d; 2.6)	7.46 (d; 2.6)	130.1 (d)	128.7 (d)	C2′, C3′, C5′, C6′	C3′, C5′
5′			124.9 (s)	122.3 (s)		C4′, C6′
6′	7.13 (d; 2.6)	7.13 (d; 2.6)	131.1 (d)	129.6 (d)	C1, C2′, C4′, C5′	C1′, C5′
1″			153.3 (s)	151.6 (s)		C6″
2″			124.7 (s)	122.6 (s)		C3″
3″	7.54 (d; 2.5)	7.61 (d; 2.5)	131.1 (d)	129.4 (d)	C1″, C2″, C4″, C5″	C2″, C4″
4″			128.6 (s)	126.3 (s)		C3″, C5″
5″	7.22 (dd; 2.5, 8.9)	7.28 (dd; 2.5, 8.9)	128.8 (d)	127.7 (d)	C1″, C3″	C4″, C6″
6″	6.72 (d; 8.9)	6.81 (d; 8.9)	117.0 (d)	116.3 (d)	C1″, C2″, C4″	C1″, C5″
OH	-	10.1 (br)				

[a] ^{1}H and ^{13}C NMR spectra in DMSO were recorded at 343 K. [b] XHCORR spectra were recorded in CD$_3$OD at ambient temperature (75.5 MHz, J = 5, 10 and 15 Hz). [c] INADEQUATE spectrum was recorded in DMSO at 343 K (125 MHz, J = 58 Hz). [d] Assignments interchangeable.

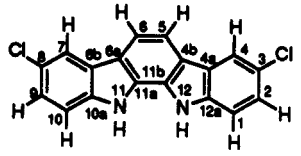
nounced antibacterial and antifungal activity. Moderate toxicity toward brine shrimp was observed for the dichloromethane extract. The petroleum ether and ethyl acetate extracts exhibited immunosuppressive effects. None of these extracts showed a cytotoxic effect against KB cells. Using a bioassay-directed (antibacterial) isolation procedure, the ethyl acetate and dichloromethane extracts were fractionated employing normal-phase vacuum liquid chromatography, followed either by MPLC on silica gel or further purifications employing reversed-phase HPLC, to obtain compounds 1, 2, and 3.



1



2



3

Ambigol A. The molecular formula of ambigol A (1) was deduced as $C_{18}H_8Cl_6O_3$ by HREIMS (m/z 481.8548, Δ 5.7 mmu) in combination with NMR data and the results of elemental analysis. Of the 12 degrees of unsaturation implied by the molecular formula, nine were occupied by carbon–carbon double bonds, indicating the remainder to be involved in ring formations; ambigol A was tricyclic. The EIMS showed an intense molecular ion cluster at m/z (rel intensity) 482 (50), 484 (100), 486 (80), 488 (36), and 490 (9) characteristic of a molecule containing six chlorine atoms. Other significant fragment ions in the EIMS exhibiting chlorine isotope peaks were at m/z 446/448/450/452/454 (M^+ - Cl), 412/414/416/418/420 (M^+ - Cl_2), 320/322/324/326 (M^+ - $C_6H_4OCl_2$), 286/288/290/292 (M^+ - Cl_3 - $C_6H_3OCl_2$), and 161/163/165 (M^+ - $C_6H_3OCl_2$ - $C_6H_2OCl_2$).

The UV and IR spectra of ambigol A contained absorptions characteristic of an extended aromatic ring system containing hydroxyl functionalities [λ_{max} nm (ϵ) 324 (7700), 291 (5700), 231 (37 300) and 3400, 3080, 1580, 1470 cm^{-1}]. The ^{13}C NMR spectrum indicated the presence of 18 carbon atoms, six of which were identified as being aromatic methine groups. Of the remaining 12 quaternary carbons, six were substituted with chlorine, four with oxygen-containing functionalities, and two formed a carbon–carbon bond. The ^{1}H NMR spectrum (CD$_3$OD) exhibited signals for six aromatic protons, representing three isolated spin systems: a pair of doublets (δ 7.13, 7.44; J = 2.6 Hz) for two *meta* coupled protons, three signals at δ 6.72 (J = 8.9 Hz), 7.22 (J = 2.5 and 8.9 Hz), and 7.54 (J = 2.5 Hz) for *ortho*, *ortho-meta*, and *meta* coupled protons, and one singlet (δ 7.21) for an uncoupled proton. These results clearly indicated the presence of three aromatic rings and the position of the hydrogen atoms on them.

Connections between rings and sites of substitution with oxygen-containing and chlorine functionalities as well as the complete assignment of all ^{1}H and ^{13}C NMR resonances were established from the results of extensive two-dimensional NMR studies in CD$_3$OD and DMSO (Table I). The results of several carbon-detected ^{1}H–^{13}C long range correlation experiments, optimized for 5, 10, and 15 Hz, confirmed the presence of three aromatic rings but did not facilitate the assignment of these as the two-, three-, and four-bond ^{1}H–^{13}C couplings could not be clearly distinguished in these experiments; hence no further conclusions concerning the unequivocal positioning of substituents could be made at this point of the structure solution.

The feasibility of recording an INADEQUATE spectrum was assessed in an attempt to resolve the structure of ambigol A. A preliminary experiment indicated that an interpretable result could not be obtained in a reasonable time (48–72 h); additionally some carbon signals were overlapping. Further experiments revealed that heating of the NMR (DMSO) sample to 343 K permitted all carbon resonances to be observed as individual signals. Pulse repetition rates of the order of 100 ms also provided acceptable signal-to-noise ratios in the ^{13}C NMR spectrum

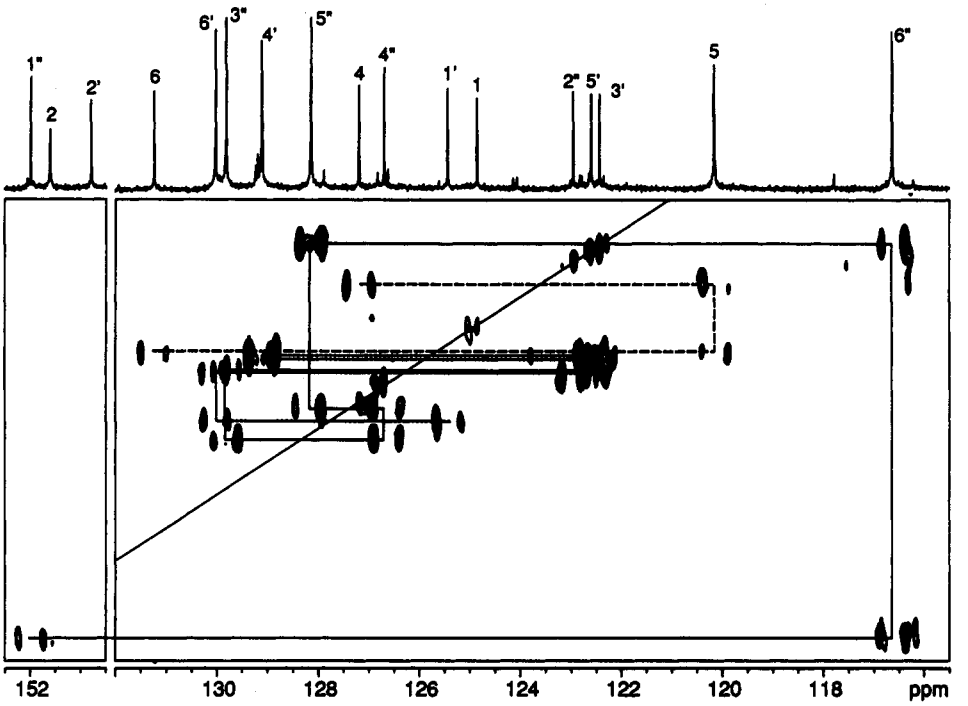
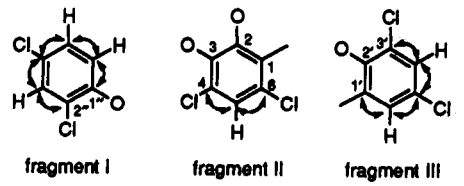


Figure 1. INADEQUATE experiment of ambigol A. The full, dashed, and dotted lines correspond to fragment I, II, and III, respectively.

Scheme I. Fragments of Ambigol A Deduced from 2D NMR Measurements (arrows indicate ^{13}C–^{13}C coupling observed in the INADEQUATE experiment)



to permit the measurement of an INADEQUATE spectrum (at 500 MHz), the results of which are depicted in Figure 1 and Scheme I. Correlation of C-1″ to C-6″, C-6″ to C-5″, C-5″ to C-4″, C-4″ to C-3″, and C-3″ to C-2″ established the nature of all carbon atoms found in fragment **I** (Scheme I). For fragment **II**, connectivities between C-4 and C-5 and C-5 and C-6 could be established unequivocally from the results of the INADEQUATE experiment. From this information and the results of the ^{1}H–^{13}C carbon-detected long range correlation experiments (J = 5, 10, and 15 Hz) (Table I) it was possible to deduce the chemical shifts of all carbon atoms involved in fragment **II**. For fragment **III**, connectivities between C-1′ and C-6′, C-6′ and C-5′, C-5′ and C-4′, and C-4′ and C-3′ could be concluded from the INADEQUATE experiment, as indicated in Scheme I. The remaining connectivities between C-2′ and C-3′ and C-2′ and C-1′ were clearly evident from the results of the carbon-detected ^{1}H–^{13}C experiment (J = 10 Hz). With these data and the ^{1}H–^{13}C correlations observed between the protons on C-6′ and C-5 to the C-1 carbon, it was possible to establish unequivocally the connectivity between fragments **II** and **III** as being a C-1, C-1′ carbon–carbon bond.

The question of which way fragment **I** was connected and the position of the two hydroxyl groups still remained. In accordance with its chemical shift (139.3 ppm in CD_3OD), the C-3 carbon was suggested to be in direct neighborhood to an hydroxyl group and bonded to oxygen. Thus a connection between fragments **I** and **II** over an ether bridge between C-3 and C-1″ was proposed; consequently C-2 had to be hydroxylated. To prove this contention a single-crystal X-ray crystallographic analysis was undertaken.

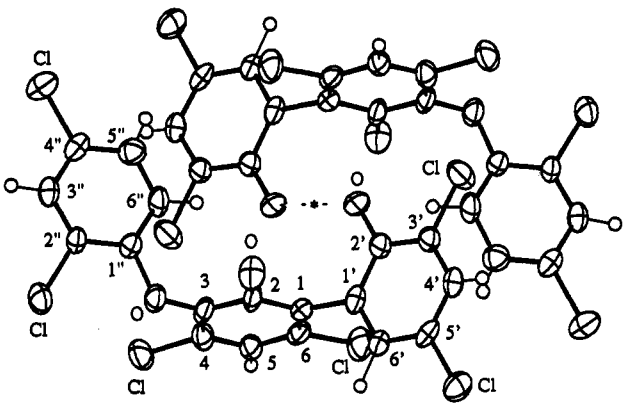


Figure 2. ORTEP stereodrawing of ambigol A showing a pair of molecules associated by a hydrogen bond around a center of inversion. Ellipsoids are represented with 50% probability.

X-ray Crystallography. Crystals of ambigol A suitable for single-crystal X-ray analysis were obtained from a dichloromethane solution. The X-ray crystallographic study revealed that the molecule is centrosymmetric. The molecules were associated in pairs by a hydrogen-bond interaction involving the oxygen on C-2′ around a center of inversion. A residual electronic density of about 1.6 e Å^{-3} was observed on the atomic site of H-6′ and it was proposed that the phenyl C adopted two distinct positions by rotation of 180° around the C-1–C-1′ bond. An occupancy factor of 81% (position as shown in Figure 2) and 19%, respectively, was found. A corresponding drawing of a pair of this molecule as it occurred in the crystal is shown in Figure 2.

The results of this measurement thus confirmed all previous findings and established the structure of ambigol A (1) as that of 3-(2,4-dichlorophenoxy)-3′,4,5′,6-tetrachloro-2,2′-biphenyldiol.

Table II. ^{13}C and ^{1}H NMR Spectral Data for Ambigol B in CD_3OD

position	δ_H (mult; J, Hz)	δ_C (mult)	XHCORR[a]
1		151.6 (s)	
2/6		141.2 (s)	
3/5		126.3 (s)	
4	7.0 (s)	117.8 (d)	C2, C3, C5, C6
1′/1″		153.4 (s)	
2′/2″		124.4 (s)	
3′/3″	7.51 (d; 2.4)	130.9 (d)	C1′, C2′, C4′, C5′, C1″, C2″, C4″, C5″
4′/4″		128.1 (s)	
5′/5″	7.21 (dd; 2.4, 8.9)	128.8 (d)	C1′, C3′, C1″, C3″
6′/6″	6.68 (d; 8.9)	116.9 (d)	C2′, C4′, C2″, C4″

[a] XHCORR spectra were recorded at ambient temperature (75.5 MHz, J = 10 Hz).

No optical rotation could be observed, indicating that there is no hindered rotation around the C-1–C-1′ bond in solution.

Ambigol B (2) was closely related to ambigol A. The molecular formula of ambigol B was deduced as $C_{18}H_8Cl_6O_3$ by HREIMS (m/z 481.8628, Δ 2.3 mmu) and NMR spectroscopy. As for ambigol A, these data indicated ambigol B to be tricyclic. Its EIMS revealed a molecular ion cluster at m/z (rel intensity) 482 (55), 484 (100), 486 (78), 488 (37), and 490 (8) characteristic for a molecule containing six chlorine atoms. Other fragment ion clusters were similar to those found for ambigol A; m/z 446/448/450/452/454 ($M^+ - Cl$), 412/414/416/418/420 ($M^+ - Cl_2$), 376/378/380 ($M^+ - Cl_2$), 320/322/324/326 ($M^+ - C_6H_4OCl_2$), 302/304/306 ($M^+ - Cl - C_6H_3Cl_2$), 286/288/290/292 ($M^+ - Cl - C_6H_3OCl_2$), and 162/164/166 ($M^+ - C_6H_3OCl_2 - C_6HOCl_2$). The UV and IR spectra contained absorptions characteristic for aromatic and hydroxyl moieties [λ_{max} nm (ε) 340 (2740), 300 (4200), 288 (6640), 282 (6700) and 3450, 1580, 1470 cm^{-1}].

The ^{13}C NMR spectrum (CD_3OD) contained only ten resonances. Integration of the ^{1}H NMR spectrum (CD_3OD) revealed that the signals at δ 7.51 (d, J = 2.4), 7.21 (dd, J = 2.4, 8.9), and 6.68 (d, J = 8.9) each represented two hydrogen atoms whereas the signal at δ 7.0 (s) derived from a single hydrogen atom, giving seven aromatic methine signals. Interpretation of the mass spectral data and the results of ^{1}H–^{13}C long and short range correlation experiments showed that all ^{13}C NMR signals except those at 117.8 (d) and 151.6 (s) ppm were doubled, indicating a total of 18 carbons. Thus ambigol B was a symmetrical molecule, possessing a C_{2v} symmetry.

Close comparison of the ^{13}C and ^{1}H NMR data of ambigol B (Table II) with those of ambigol A (Table I) revealed chemical shifts virtually identical to those observed for fragment **I** of ambigol A (Scheme I). Additionally similar resonances as observed for C-2, C-3, C-4, C-5, and H-5 of fragment **II** of ambigol A were found. In the ^{1}H NMR spectrum of ambigol B, signals at δ 7.44 (d) and 7.13 (d) were lacking, indicating fragment **III** of **1** not to be part of ambigol B. From these data it could be concluded that ambigol B consisted of two moieties identical to fragment **I** of **1** (Scheme I). These were connected to a third aromatic ring, closely related to fragment **II** of **1**, over two ether bridges. Consideration of chemical shifts and symmetry requirements lead to the conclusion that the two ether bridges were located between C-6 (141.2 ppm) and C-1″ (153.4 ppm) and C-2 (141.2 ppm) and C-1′ (153.4 ppm). Ambigol B is thus 2,6-bis(2,4-dichlorophenoxy)-3,5-dichlorophenol.

Tjipanazole D (3) is a known metabolite, previously obtained from the blue-green alga *Tolypothrix tjipanasensis*[6] and related to the potent protein kinase C inhibitor, staurosporine.[7] The literature[6] provided ^{1}H NMR, UV, and IR data for tjipanazole D, which proved identical to those observed for compound **3**. ^{13}C NMR data were in agreement with those described for closely related derivatives of tjipanazole D.[6] HMQC, XHCORR, and NOESY measurements performed with **3** permitted the unambiguous assignment of all carbon and proton resonances for this compound, which is described for the first time (see Experimental Section).

Ambigol A and B are the first examples of this structural class of secondary metabolites in cyanobacteria. Structurally related compounds, however not chlorinated, were encountered in macroalgae (Phycophyta). Phlorotannins, polyhydroxylated phenols, are characteristic metabolites of brown algae with moderate antimicrobial activity.[8] Phlorotannin-type compounds from the brown alga *Ecklonia kurome* are considered promising antithrombotic agents.[9] The brominated phenols vidalol A and B occur in the red alga *Vidalia obtusaloba* and were shown to possess antiinflammatory potential.[10] The aromatic moiety of hydrolyzable tannins, as found in higher plants,[11] also resembles the structures described here for blue-green algae. For many polyphenols, antiviral and antimutagenic activities have been reported.[12]

Biological Testing. Ambigol A exhibited a strong inhibition of cyclooxygenase (in the range of indometacin) and of the HIV-1 reverse transcriptase as well as a potent antibacterial activity against *Bacillus subtilis* and a strong molluscicidal activity toward *Biomphalaria glabrata*. Ambigol B showed moderate activities in the antimicrobial, molluscicidal, and cytotoxic assays. For tjipanazole D, a moderate antibacterial but no antifungal, cytotoxic, or molluscicidal activity could be detected. Details of the results of the biological testing will be described elsewhere.

Experimental Section

Spectral Analysis. NMR spectra were measured on Bruker AMX instruments operating at basic frequencies of 500 and 300 MHz, respectively. ^{1}H and ^{13}C NMR chemical shifts are referenced to solvent peaks: δ_H 7.26 (residual $CHCl_3$) and δ_C 77.0 for $CDCl_3$, δ_H 3.35 (residual CHD_2OD) and δ_C 49.0 for methanol-d_4, and δ_H 2.50 (residual DMSO-d_5) and δ_C 39.7 for DMSO-d_6. Heteronuclear ^{1}H–^{13}C correlations were determined by HMQC, XHCORR, and HMBC experiments. The INADEQUATE experiment of ambigol A was recorded in DMSO (AMX 500, 125 MHz for ^{13}C) at 343 K with delays optimized for J = 58 Hz. In this experiment 1024 scans for each of the 64 t_1 increments were made. Mass spectra, including the HREIMS, were determined on a VG Tribrid mass spectrometer operating in the EI or FAB mode. UV spectra were measured in MeOH at 25 °C. IR spectra were recorded on a Perkin-Elmer 781 IR spectrophotometer (KBr

(8) Glombitza, K.-W.; Grosse-Damhues, J. *Planta Med.* **1985**, *48*, 42–46.

(9) (a) Fukuyama, Y.; Miura, I.; Kinzyo, Z.; Mori, H.; Kido, M.; Nakayama, Y.; Takahashi, M., Ochi, M. *Chem. Lett.* **1985**, 739–742. (b) Fukuyama, Y., Kodama, M., Miura, I.; Kinzyo, Z.; Kido, M.; Mori, H.; Nakayama, Y.; Takahashi, M. *Chem. Pharm. Bull.* **1989**, *37*, 349–353. (c) Fukuyama, Y.; Kodama,. M.; Miura, I.; Kinzyo, Z.; Mori, H.; Nakayama, Y.; Takahashi, M. *Chem. Pharm. Bull.* **1989**, *37*, 2438–2440.

(10) Wiemer, D. F.; Idler, D. D.; Fenical, W. *Experienta* **1991**, *47*, 851–853.

(11) Yoshida, T.; Nakata, F.; Hosotani, K.; Nitta, A.; Okuda, T. *Phytochemistry* **1992**, *31*, 2829–2833.

(12) Huang, M.-T., Ferraro, In *Phenolic Compounds in Food and their Effects on Health II*; Huang, M.-T., Ho, C.-T., Lee, C. Y., Eds.; ACS Symposium Series 507, 1992; pp 9–34.

disks). Optical rotations were determined in MeOH at 20 °C at the sodium D line in a 10-cm microcell.

Culture Conditions. *F. ambigua* (Näg.) Gomont, designated strain number 108b, was obtained from the Swiss Federal Institute for Water Resources and Water Pollution Control (EAWAG) Culture Collection of Algae, 8600 Dübendorf, Switzerland. The blue-green alga has been isolated from a sample obtained in 1965 from a shallow hollow in Mellingen, Switzerland.

The cyanophyte was mass cultured in 10-L glass bottles containing a modified inorganic medium, designated Z, in which the major salt concentrations were (mg/L) for $NaNO_3$, 467.0; $Ca(NO_3)_2 \cdot 4H_2O$, 59.0; K_2HPO_4, 31.0, $MgSO_4 \cdot 7H_2O$, 25.0, Na_2CO_3, 21.0; FeEDTA solution, 1.0 mL/L; trace element solution, 0.08 mL/L. The FeEDTA solution was composed of (mL/L) $FeCl_3 \cdot 6H_2O$ 0.1 M solution in 0.1 N HCl, 10.0; Na_2EDTA 0.1 M solution, 10.0. The trace element solution consisted of (mg/100 mL) H_3BO_3, 310.0; $MnSO_4 \cdot 4H_2O$, 223.0; $Na_2WO_4 \cdot 2H_2O$, 3.3; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 8.8; KBr, 11.9; KJ, 8.3; $ZnSO_4 \cdot 7H_2O$, 28.7; $Cd(NO_3)_2 \cdot 4H_2O$, 15.4; $Co(NO_3)_2 \cdot 6H_2O$, 14.6; $Cu(SO_4) \cdot 5H_2O$, 12.5; $NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$, 19.8; $Cr(NO_3)_3 \cdot 7H_2O$, 3.7, $VOSO_4 \cdot 2H_2O$, 2.0, $Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$, 47.4. Cultures were illuminated continuously at a light intensity of 1500 lux and shaken at 60 rpm and 25 °C. The headspace of the culture vessel was continuously flushed with air enriched with 2% CO_2. The axenic alga was harvested by decanting of the supernatant after 30 to 40 days to give yields of lyophilized cells ranging from 0.4 to 0.5 g/L.

Isolation. Freeze-dried algal material (300 g) was extracted successively with petroleum ether (15 L), dichloromethane (20 L), ethyl acetate (15 L), MeOH (25 L), and a mixture of 4:1 methanol/H_2O (15 L). Extracts were concentrated and dried under reduced pressure to give 1.0, 4.5, 1.0, 90.0, and 15.0 g, respectively. The dichloromethane and ethyl acetate extracts each were dissolved in the appropriate solvents and each applied to a 60 × 100 mm VLC-column filled with silica gel (Merck, 15 μm). After successive elution with 100 mL of each of (a) petroleum ether, (b) 1:99 ethyl acetate/petroleum ether, (c) 3:97 ethyl acetate/petroleum ether, (d) 5:95 ethyl acetate/petroleum ether, (e) 1:9 ethyl acetate/petroleum ether, (f) 2:8 ethyl acetate/petroleum ether, (g) 3:7 ethyl acetate/petroleum ether, (h) 1:1 ethyl acetate/petroleum ether, (i) 3:1 ethyl acetate/petroleum ether, (j) ethyl acetate, (k) 1:9 methanol/ethyl acetate, (l) 1:4 methanol/ethyl acetate, (m) 3:7 methanol/ethyl acetate, (n) 1:1 methanol/ethyl acetate, (o) 3:1 methanol/ethyl acetate, and methanol, 11 fractions were obtained. Antibacterial activity was detected for fractions 2 (286 mg), 3 (158 mg), 4 (314 mg), 8 (1159 mg), 9 (940 mg), and 10 (1025 mg).

Fraction 4 was subjected to reversed phase HPLC (Spherisorb S5 ODS II, 5 μm, 250 × 16 mm = column A) with UV detection at 254 nm and 85:15 MeOH/H_2O as eluant (8 mL/min) to give 100 mg (0.03% of dried algal material) of crude ambigol A (t_R 14 min) as a pale yellow oily substance. Repurification of ambigol A after heating to 343 K for the INADEQUATE experiment was carried out by HPLC on normal phase (Lichrosorb Si 60, 5 μm, 250 x 16 mm) with RI detection and 20:1 hexane/EtOAc as eluant (7mL/min) obtaining 45 mg of ambigol A (1) (t_R 19 min) as a white powder.

Fractions 2 and 3 were combined and then subjected to reversed phase HPLC (column A) with 80:20 MeOH/H_2O as eluant (8 mL/min) to give 33 mg (0.011%) of ambigol B (2) (t_R 32 min) as a pale yellow amorphous powder.

Fraction 6 (854 mg) was subjected to a Büchi MPLC column (1.5 × 47 cm) dry-packed with TLC silica gel HF 254 (Merck, 15 μm) followed by successive elution with hexane (120 mL), 1:1 hexane/dichloromethane (650 mL), and dichloromethane (860 mL). One of the fractions (740–1240 mL) contained 18 mg (0.006%) of tjipanazole D (3).

Ambigol A (1) (3-(2,4-dichlorophenoxy)-3′,4,5′,6-tetrachloro-2,2′-biphenyldiol): mp 181.5–183.5 °C; $[\alpha]_D$ 0° (*c* 6.0, MeOH): FABMS (3-NOBA) *m/z* 504 ($MNa^+ - 1$), 482 ($MH^+ - 1$); HREIMS *m/z* 481.8548 (M^+) ($C_{18}H_8Cl_6O_3$, mmu error Δ 5.7); IR (KBr) ν_{max} 3400, 3080, 2950, 2920, 1720, 1580, 1470, 1430, 1390 cm^{-1}; UV (MeOH) λ_{max} (ε) 231 (37 300), 291 (5700), 324 (7700). Anal. Calcd for $C_{18}H_8Cl_6O_3$: C, 44.86; H, 1.67; N, 0. Found: C, 45.37; H, 1.73; N, 0.1.^{1}H NMR (300 MHz, DMSO-d_6 at 343 K) and ^{1}H NMR (300 MHz, MeOH-d_4): see Table I. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.47 (d, *J* = 2.5 Hz, H-4′), 7.44 (d, *J* = 2.5 Hz, H-3″), 7.23 (s, H-5), 7.14 (d, *J* = 2.5 Hz, H-6′), 7.13 (dd, *J* = 2.5 and 8.8 Hz, H-5″), 6.62 (d, *J* = 8.9 Hz, H-6″). ^{13}C NMR (75 MHz, DMSO-d_6 at 343 K) and ^{13}C NMR (75 MHz, MeOH-d_4): see Table I. ^{13}C NMR (75 MHz, $CDCl_3$): δ150.9 (s), 148.4 (s), 148.0 (s), 137.3 (s), 131.8 (s), 130.6 (d), 130.4 (d), C 129.3 (d), 128.8 (s), 128.1 (s), 127.9 (d), 125.5 (s), 123.8 (s), 122.5 (s), 122.4 (d), 122.4 (s), 121.3 (s), 115.8 (d).

Ambigol B (2) (2,6-bis(2,4-dichlorophenoxy)-3,5-dichlorophenol): FABMS (3-NOBA) *m/z* 527 ($MNa_2^+ - 1$), 482 ($MH^+ - 1$); HREIMS *m/z* 481.8628 ($C_{18}H_8Cl_6O_3$, mmu error Δ 2.3); IR (KBr) ν_{max} 3450, 2950, 2920, 1710, 1580, 1470, 1445, 1385 cm^{-1}; UV (MeOH) λ_{max} (ε) 282 (6700), 288 (6640), 300 (4200), 340 (2740). ^{1}H NMR (300 MHz, MeOH-d_4) and ^{13}C NMR (75 MHz, MeOH-d_4): see Table II.

Tjipanazole D (3) (3,8-dichloroindolo[2,3-a]carbazole): EIMS *m/z* 324 ($C_{18}H_{10}N_2Cl_2$); IR (KBr) ν_{max} 3400, 2920, 1570, 1460, 1445, 1430, 1380, 1318, 1270, 1220 cm^{-1}; UV (MeOH) λ_{max} (ε) 259 (63 100), 268 (54 100, shoulder), 291 (25 500), 331 (31 000), 345 (7300, shoulder), 364 (3900). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.31 (s, N–H), 8.26 (d, *J* = 2.1 Hz, H-4, H-7), 7.96 (s, H-5, H-6), 7.72 (d, *J* = 8.6 Hz, H-1, H-10), 7.40 (dd, *J* = 2.1, 8.6 Hz, H-2, H-9). ^{13}C NMR (75 MHz, DMSO-d_6) δ (multiplicity, carbon assignment, ^{1}H–^{13}C long range correlations) 137.6 (s, C-10a, C-12a, H-2,4,7,9,NH), 126.5 (s, C-11a, C-11b, H-5,6,NH), 125.1 (s, C-6b, C-4a, H-1,5,6,10,NH), 124.6 (d, C-9, C-2, H-7,4), 123.6 (s, C-8, C-3, H-1,2,4,7,9,10), 119.9 (s, C-6a, C-4b, H-4,5,6,7,NH), 119.5 (d, C-4, C-7), 113.3 (d, C-2,C-10), 112.4 (d, C-5, C-6).

X-ray crystallographic analysis of ambigol A: $C_{18}H_8Cl_6O_3$, M_w = 485; μ = 0.963 mm^{-1}, *F*(000) = 484, d_x = 1.76 g cm^{-3}, triclinic *P*1, *Z* = 2, *a* = 7.391(2), *b* = 10.913(2), and *c* = 12.224(3) Å, α = 94.584(9), β = 98.865(9), and γ = 108.65(1)°, *V* = 914.2(4) Å^3, from 26 reflections (21° < 2θ < 38°), colorless prism 0.10 × 0.20 × 0.25 mm mounted on a quartz fiber. Cell dimensions and intensities were measured at room temperature on a Philips PW1100 diffractometer with graphite-monochromated Mo[Kα] radiation (λ = 0.71069 Å), ω–2θ scans, scanwidth 1.2° + 0.2 tan θ, scan speed 0.06 deg/s. Two reference reflections measured every 45 min showed a decrease of about 3.3%, all intensities were corrected for this drift; $-8 < h < 8$; $-11 < k < 11$; $0 < l < 13$; 2553 unique reflections measured of which 1916 (75%) were observable ($|F_o| > 4\sigma(F_o)$). Data were corrected for Lorentz and polarization effects and for absorption[13] (*A** min, max = 1.093, 1.209). The structure was solved by direct methods using MULTAN 87;[14] all other calculations used the XTAL[15] system and ORTEP[16] programs. Atomic scattering factors and anomalous dispersion terms were taken from ref 17. Full-matrix least-squares refinement based on *F* using a weight of $1/\sigma^2(F_o)$ gave final values *R* = 0.049, R_w = 0.029, and *S* = 2.47 for 265 variables and 1912 contributing reflections. Hydrogen atoms were observed (excepted these of the hydroxyl groups) and refined with fixed isotropic value of atomic displacement parameters. The final difference electron density map showed a maximum of +0.50 and a minimum of −0.53 e Å^{-3}.

Biological Testing. Test organisms used for the antibacterial assays were *M. luteus* ATCC 9341, *B. subtilis* ATCC 6633, and *E. coli* ATCC 25922.[18] For the antifungal test *P. oxalicum* CBS 219.30[18] was employed. Cytotoxicity[19] was determined with a KB cell-line (human nasopharyngal carcinoma) and brine

(13) Blanc, E.; Schwarzenbach, D.; Flack, H. D. *J. Appl. Cryst.* **1991**, *24*, 1035–1041.
(14) Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J.-P.; Woolfson, M. M. In *A System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data*; University of York, England, and Louvain-la-Neuve, Belgium, 1987.
(15) Hall, S. R.; Stewart, J. M. In *Eds XTAL3.0 User's Manual*, Universities of Western Australia and Maryland, 1992.
(16) Johnson, C. K. In *ORTEP II*, report ORNL-5138; Oak Ridge National Laboratory: Oak Ridge, TN, 1976.
(17) *International Tables for X-Ray Crystallography*, Vol. IV, Birmingham; Kynoch Press, 1974.
(18) Baumgartner, B.; Erdelmeier, C. A. J.; Wright, A. D.; Rali, T.; Sticher, O. *Phytochemistry* **1990**, *29*, 3327–3330.
(19) Swanson, S. M.; Pezzuto, J. M. In *Drug Bioscreening: Drug evaluation techniques in pharmacology*; Thompson, E. B., Ed.; VCH Publishers: New York, 1990; pp 273–297.

shrimp[20] (*Artemia salina*). Inhibitory activity of cyclooxygenase and that of HIV reverse transcriptase were measured as described.[21,22] The molluscicidal assay (test organism *Biomphalaria glabrata*) was carried out as reported.[23]

Acknowledgment. We thank Mrs. Dr. M. Bossli and Mr. H. Bachmann (EAWAG, Dübendorf/Switzerland) for providing and cultivating the algal material, Dr. W. Amrein, Mr. R. Häfliger, and Mr. O. Greter (ETH Zurich) for all mass spectral measurements, Dr. E. Zass (ETH Zurich) for performing literature searches, Mr. D. Manser (ETH Zurich) for elemental analyses, and Prof. J. Pezzuto and Dr. C. Angerhofer (University of Illinois, Chicago) and Mrs. A. Pröbstle (University of Munich) for the biological testing. We are also extremely grateful to Prof R. E. Moore (University of Hawaii), Dr. S. Carmeli (University of Tel-Aviv), Dr. R. Joshi (ETH Zurich), and Dr. J. Orjala (ETH Zurich) for providing useful suggestions concerning various aspects of this research.

Supplementary Material Available: ^{1}H NMR, ^{13}C NMR, HMQC, XHCORR, and two-dimensional COSY spectra of compounds 1 and 2 (15 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information. Atomic coordinates and geometrical data for compound 1 have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

(20) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31–34.
(21) Wagner, H.; Wierer, M.; Bauer, R. *Planta Med.* **1986**, *52*, 184–187.
(22) Tan, G. T.; Pezzuto, J. M.; Kinghorn, A. D.; Hughes, S. H. *J. Nat. Prod.* **1991**, *54*, 143–154.
(23) Hostettmann, K.; Kizu, H.; Tomimori, T. *Planta Med.* **1982**, *44*, 34–35.