Journal of Natural Products
Vol. 53, No. 3, pp. 757-760, May-Jun 1990

A NEW BROMINATED DIPHENYL ETHER FROM A PHILIPPINE *DYSIDEA* SPECIES

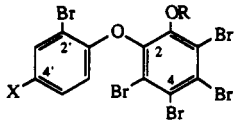
JAVIER SALVÁ[1] and D. JOHN FAULKNER*

Scripps Institution of Oceanography (A-012F), University of California at San Diego, La Jolla, California 92093

ABSTRACT.—A new brominated diphenyl ether, 2-(2′-bromophenoxy)-3,4,5,6-tetrabromophenol [**1**], has been isolated as the major metabolite of a species of *Dysidea* from the Philippines.

Sponges of the genus *Dysidea* are renowned for the variability of their chemical constituents, which include an unusual aziridine (1), monoterpenes (2), sesquiterpenes (3–20), diterpenes (21–23), meroterpenoids (24–32), polyhydroxylated sterols and secosterols (33–38), polychlorinated metabolites derived from amino acids (39–46), and polybrominated diphenyl ethers (47–51). Although terpenoids are found in both temperate and tropical species, the polyhalogenated metabolites appear to be limited to tropical species of *Dysidea* that coincidentally contain large populations of cyanophytes within their tissues. Our interest in the correlation of polyhalogenated metabolites with the presence of cyanophytes has led us to examine several *Dysidea* species from the Philippines. In this paper we report the isolation and characterization of a new polybrominated diphenyl ether, 2-(2′-bromophenoxy)-3,4,5,6-tetrabromophenol [**1**].

Specimens of an unidentified species of *Dysidea* were collected by hand using scuba at a depth of 20 m near San Jose, Batangas, Philippines. The nonpolar material from an MeOH extract of the sponge was chromatographed on Si gel to obtain a uv-active fraction that was separated by hplc on Partisil to obtain 2-(2′-bromophenoxy)-3,4,5,6-tetrabromophenol [**1**] (2% dry wt) as a white crystalline solid, mp 143–145°, and 2-(2′,4′-dibromophenoxy)-3,4,5,6-tetrabromophenol [**2**] (0.3% dry wt). 2-(2′,4′-Dibromophenoxy)-3,4,5,6-tetrabromophenol [**2**] was identified by comparison of the spectral data with those reported by Utkina *et al.* (51). The corresponding methyl ethers **3** and **4**, prepared by treating an Et_2O solution of the intermediate mixture of phenols with ethereal CH_2N_2 solution, were easily separated by Si gel chromatography.



1 X=H, R=H
2 X=Br, R=H
3 X=H, R=Me
4 X=Br, R=Me

The high resolution mass spectrum of 2-(2′-bromophenoxy)-3,4,5,6-tetrabromophenol [**1**] contained the correct cluster of peaks for the molecular formula $C_{12}H_5Br_5O_2$. The uv absorption at 295 nm (ε 4500) was shifted to 310 nm on addition of a drop of base, which indicated the presence of a phenolic group. The ^{1}H-nmr spectrum contained four signals at δ 6.52 (dd, 1H, J=8.2, 1.6 Hz), 6.99 (td, 1H, J=7.9, 1.5 Hz), 7.18 (ddd, 1H, J=8.2, 8.0, 1.6 Hz), and 7.65 (dd, 1H, J=7.9, 1.6 Hz) that must be assigned to four adjacent hydrogens on an aromatic ring. The ^{13}C-nmr spectrum contains 12 signals and therefore supports the proposed structure, 2-(2′-bromophenoxy)-3,4,5,6-tetrabromo-

[1]On leave from Universidad de Cádiz, Departamento de Química Orgánica y Bioquímica, Facultad de Ciencias, Apartado 40–11510 Puerto Real, Cádiz, Spain.

phenol [**1**], rather than the alternative structure with a symmetrically substituted pentabrominated aromatic ring. The spectral data for the corresponding methyl ether **3** is in complete accord with the proposed structure.

The two polybrominated phenols **1** and **2** have approximately equal antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, but the corresponding methyl ethers **3** and **4** are inactive in these assays.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer 783 spectrophotometer. Uv spectra were measured in MeOH solution using a Perkin-Elmer Lambda 3B spectrophotometer. ^{1}H-nmr spectra were recorded at 360 MHz in $CDCl_3$ solution ($\delta_{TMS} = 0$) on a custom-built instrument. ^{13}C-nmr spectra were recorded at 50 MHz in $CDCl_3$ solution ($\delta_{TMS} = 0$) on a Brucker WP-200 spectrometer. Mass spectra were measured on a Hewlett-Packard 5890 spectrometer or were obtained from the UC Riverside regional facility. Melting points (uncorrected) were determined on a Mel-Temp apparatus. All solvents were distilled from glass prior to use.

COLLECTION, EXTRACTION AND ISOLATION PROCEDURES.—A small specimen of *Dysidea* sp. (3.4 g dry wt) was collected by hand using scuba near San José, Batangas, Philippines, and was stored in MeOH for 3 months at 4°. Our specimen of *Dysidea* sp. (Scripps Institution of Oceanography Benthic Invertebrate Collection #P1111) is a portion of a sponge of the family Dysideidae, order Dictyoceratida. The specimen is a thin sheet (1–2 mm thick) with a finely conulose surface that was dark purple-gray in life. The endosomal skeleton is a dense reticulation of heavily cored primary and secondary fibers with a tendency to form fascicles.

The MeOH was carefully decanted, and the sponge was washed with fresh MeOH at room temperature over a period of 2 days. The combined MeOH extracts were evaporated to obtain an aqueous suspension that was diluted with distilled H_2O (150 ml) and extracted sequentially with hexane (2 × 200 ml), CH_2Cl_2 (2 × 200 ml), and EtOAc (2 × 200 ml). The extracts were dried over anhydrous Na_2SO_4 and the solvents evaporated to obtain three oily fractions (hexane, 200 mg; CH_2Cl_2, 17 mg; EtOAc, 5 mg). All three fractions showed good antimicrobial activity and similar aromatic signals in the ^{1}H-nmr spectrum; they were therefore combined. The combined organic extracts were chromatographed on Si gel (20 g) under a positive pressure of N_2 using solvents of increasing polarity from hexane to EtOAc. Those fractions that exhibited antimicrobial activity were combined. The tlc of this material showed a single uv-active spot, but the ^{1}H-nmr spectrum indicated the presence of two compounds. A major portion of the material was separated by hplc on Partisil into two fractions that consisted of 2-(2′-bromophenoxy)-3,4,5,6-tetrabromophenol [**1**] (66 mg., 2% dry wt) and 2-(2′,4′-dibromophenoxy)-3,4,5,6-tetrabromophenol [**2**] (10 mg., 0.3% dry wt).

2-(2′-BROMOPHENOXY)-3,4,5,6-TETRABROMOPHENOL [**1**].—Mp 143–145° (EtOAc/hexane); ir ($CHCl_3$) 3500, 1470, 1420, 1375, 1285, 1040 cm^{-1}; uv (MeOH) 295 nm (ε 4500), (MeOH + NaOH) 310 nm; ^{1}H nmr ($CDCl_3$) δ 6.52 (dd, 1H, J = 8.2, 1.6 Hz), 6.99 (td, 1H, J = 7.9, 1.5 Hz), 7.18 (ddd, 1H, J = 8.2, 8.0, 1.6 Hz), 7.65 (dd, 1H, J = 7.9, 1.6 Hz); ^{13}C nmr ($CDCl_3$) δ 152.4 (s, C-1′), 147.3 (s, C-1), 139.5 (s, C-2), 134.0 (d, C-3′), 128.6 (d, C-5′), 125.5 (s, C-4), 124.6 (d, C-4′), 120.9 (s, C-3), 119.4 (s, C-5), 114.5 (d, C-6′), 114.0 (s, C-6), 111.6 (s, C-2′); hrms *m/z* 575.6206 ($C_{12}H_5{}^{79}Br_5O_2$ requires 575.6206); eims *m/z* (intensity, %) 586 (10), 584 (47), 582 (93), 580 (100), 578 (51), 576 (10), 505 (3), 503 (13), 501 (20), 499 (14), 497 (3), 424 (8), 422 (23), 420 (25), 418 (9).

SYNTHESIS OF 2-(2′-BROMOPHENOXY)-3,4,5,6-TETRABROMOANISOLE [**3**].—A freshly prepared solution of CH_2N_2 in Et_2O (excess) was added to a solution of a partially purified mixture of phenols **1** and **2** (20 mg) in Et_2O. Tlc analysis indicated that the reaction was complete after 30 min, at which time the reaction mixture was diluted with Et_2O (30 ml) and washed with 0.1 N HCl (30 ml). The organic layer was washed with H_2O (2 × 10 ml) and dried over anhydrous Na_2SO_4. The reaction product (21 mg) was chromatographed on a Si gel column using 8% EtOAc in hexane as eluent to obtain 2-(2′-bromophenoxy)-3,4,5,6-tetrabromoanisole [**3**] (15 mg) and 2-(2′,4′-dibromophenoxy)-3,4,5,6-tetrabromoanisole [**4**] (2 mg).

2-(2′-BROMOPHENOXY)-3,4,5,6-TETRABROMOANISOLE [**3**].—White solid, mp 132–134°; ir ($CHCl_3$) 1475, 1445, 1390, 1350, 1045, 1010 cm^{-1}; uv (MeOH) 221 nm (ε 5850), (MeOH + NaOH) 221 nm; ^{1}H nmr ($CDCl_3$) δ 3.86 (s, 3H), 6.43 (dd, 1H, J = 8.2, 1.6 Hz), 6.95 (td, 1H, J = 7.9, 1.6 Hz), 7.16 (ddd, 1H, J = 8.2, 8.0, 1.6 Hz), 7.63 (dd, 1H, J = 7.9, 1.6 Hz); ^{13}C nmr ($CDCl_3$) δ 152.8 (s, C-1′), 151.0 (s, C-1), 145.6 (s, C-2), 133.9 (d, C-3′), 128.5 (d, C-5′), 125.8 (s, C-4), 124.2 (s, C-6), 124.1 (d, C-4′), 122.0 (s, C-3), 121.8 (s, C-5),

114.2 (d, C-6'), 111.0 (s, C-2'), 61.7 (q); eims *m/z* (intensity, %) 600 (9), 598 (45), 596 (99), 594 (100), 592 (48), 590 (10), 504 (5), 502 (19), 500 (31), 498 (21), 496 (6).

ACKNOWLEDGMENTS

The sponge was collected by Mia Unson and Anna Pison and was identified by Mary Kay Harper. The research was supported by grants from the California Sea Grant College Program (NA-85-D-SG140, R/MP40) and by a fellowship (to J.S.) from Ministerio de Educación y Ciencia (PF89/31219143).

LITERATURE CITED

1. T.F. Molinski and C.M. Ireland, *J. Org. Chem.*, **53**, 2103 (1988).
2. I. Mancini, G. Guella, A. Guerriero, A. Boldrin, and F. Pietra, *Helv. Chim. Acta*, **70**, 2011 (1987).
3. G. Cimino, S. De Stefano, A. Guerriero, and L. Minale, *Tetrahedron Lett.*, 1417 (1975).
4. G. Cimino, S. De Stefano, A. Guerriero, and L. Minale, *Tetrahedron Lett.*, 1421 (1975).
5. G. Cimino, S. De Stefano, A. Guerriero, and L. Minale, *Tetrahedron Lett.*, 1425 (1975).
6. R. Kazlauskas, P.T. Murphy, and R.J. Wells, *Tetrahedron Lett.*, 4949 (1978).
7. R. Kazlauskas, P.T. Murphy, R.J. Wells, J.J. Daly, and P. Schönholzer, *Tetrahedron Lett.*, 4951 (1978).
8. C. Charles, J.C. Braekman, D. Daloze, B. Tursch, J.P. Declercq, G. Germain, and M. Van Meerssche, *Bull. Soc. Chim. Belg.*, **87**, 481 (1978).
9. G. Schulte, P.J. Scheuer, and O.J. McConnell, *J. Org. Chem.*, **45**, 552 (1980).
10. G. Schulte, P.J. Scheuer, and O.J. McConnell, *Helv. Chim. Acta*, **63**, 2159 (1980).
11. R.W. Dunlop, R. Kazlauskas, G. March, P.T. Murphy, and R.J. Wells, *Aust. J. Chem.*, **35**, 95 (1982).
12. G. Guella, A. Guerriero, P. Traldi, and F. Pietra, *Tetrahedron Lett.*, **24**, 3897 (1983).
13. S.H. Grode and J.H. Cardellina II, *J. Nat. Prod.*, **47**, 76 (1984).
14. G. Guella, A. Guerriero, and F. Pietra, *Helv. Chim. Acta*, **68**, 39 (1985).
15. G. Guella, I. Mancini, A. Guerriero, and F. Pietra, *Helv. Chim. Acta*, **68**, 1276 (1985).
16. T.J. Schram and J.H. Cardellina II, *J. Org. Chem.*, **50**, 4155 (1985).
17. N.S. Sarma, M. Rambabu, A.S.R. Anjaneyulu, C.B.S. Rao, and I. Saito, *Ind. J. Chem.*, **25B**, 1001 (1986).
18. R.J. Capon and J.K. MacLeod, *J. Nat. Prod.*, **50**, 1136 (1987).
19. J.H. Cardellina II and D.E. Barnekow, *J. Org. Chem.*, **53**, 882 (1988).
20. B. Carté, S. Mong, B. Poehland, H. Sarau, J.W. Westley, and D.J. Faulkner, *Tetrahedron Lett.*, **30**, 2725 (1989).
21. R.P. Walker and D.J. Faulkner, *J. Org. Chem.*, **46**, 1098 (1981).
22. R.P. Walker, R.M. Rosser, D.J. Faulkner, L.S. Bass, C.-H. He, and J. Clardy, *J. Org. Chem.*, **49**, 5160 (1984).
23. S. Carmely, M. Cojocaru, Y. Loya, and Y. Kashman, *J. Org. Chem.*, **53**, 4801 (1988).
24. L. Minale, R. Riccio, and G. Sodano, *Tetrahedron Lett.*, 3401 (1974).
25. G. Cimino, P. De Luca, S. De Stefano, and L. Minale, *Tetrahedron*, **31**, 271 (1975).
26. G. Cimino, S. De Stefano, and L. Minale, *Experientia*, **31**, 117 (1975).
27. S. De Rosa, L. Minale, R. Riccio, and G. Sodano, *J. Chem. Soc., Perkin Trans. 1*, 1408 (1976).
28. G. Cimino, S. De Rosa, S. De Stefano, L. Cariello, and L. Zanetti, *Experientia*, **38**, 896 (1982).
29. F.J. Schmitz, V. Lakschmi, D.R. Powell, and D. van der Helm, *J. Org. Chem.*, **49**, 241 (1984).
30. N. Fusetani, M. Sugano, S. Matsunaga, K. Hashimoto, H. Shikama, A. Ohta, and H. Nagano, *Experientia*, **43**, 1233 (1987).
31. G. Cimino, S. De Rosa, S. De Stefano, R. Puliti, G. Strazzullo, C.A. Mattia, and L. Mazzarella, *Tetrahedron*, **43**, 4777 (1987).
32. A. Crispino, A. De Guilio, S. De Rosa, and G. Strazzullo, *J. Nat. Prod.*, **52**, 646 (1989).
33. S.P. Gunasekera and F.J. Schmitz, *J. Org. Chem.*, **48**, 885 (1983).
34. R.J. Capon and D.J. Faulkner, *J. Org. Chem.*, **50**, 4771 (1985).
35. Y. Fujimoto, T. Yamada, and N. Ikekawa, *Chem. Pharm. Bull.*, **33**, 3129 (1985).
36. R.R. West and J.H. Cardellina II, *J. Org. Chem.*, **53**, 2782 (1988).
37. J.C. Braekman, D. Daloze, B. Moussiaux, G. Vandervyver, and R. Riccio, *Bull. Soc. Chim. Belg.*, **97**, 293 (1988).
38. R.R. West and J.H. Cardellina II, *J. Org. Chem.*, **54**, 3234 (1989).
39. W. Hofheinz and W.E. Oberhänsli, *Helv. Chim. Acta*, **60**, 660 (1977).
40. R. Kazlauskas, R.O. Lidgard, R.J. Wells, and W. Vetter, *Tetrahedron Lett.*, 3183 (1977).
41. C. Charles, J.C. Braekman, D. Daloze, B. Tursch, and R. Karlsson, *Tetrahedron Lett.*, 1519 (1978).
42. R. Kazlauskas, P.T. Murphy, and R.J. Wells, *Tetrahedron Lett.*, 4945 (1978).

43. C. Charles, J.C. Braekman, D. Daloze, and B. Tursch, *Tetrahedron*, **36**, 2133 (1980).
44. K.L. Erickson and R.J. Wells, *Aust. J. Chem.*, **35**, 31 (1982).
45. J.E. Biskupiak and C.M. Ireland, *Tetrahedron Lett.*, **25**, 2935 (1984).
46. T. Gebreyesus, T. Yosief, S. Carmely, and Y. Kashman, *Tetrahedron Lett.*, **31**, 3863 (1988).
47. G.M. Sharma and B. Vig, *Tetrahedron Lett.*, 1715 (1972).
48. R.S. Norton and R.J. Wells, *Tetrahedron Lett.*, **21**, 3801 (1980).
49. B. Carté and D.J. Faulkner, *Tetrahedron*, **37**, 2335 (1981).
50. R.S. Norton, K.D. Croft, and R.J. Wells, *Tetrahedron*, **37**, 2341 (1981).
51. N.K. Utkina, M.V. Kazantseva, and V.A. Denisenko, *Nat. Prod. Chem.*, **23**, 508 (1987).

Received 9 February 1990