Tetrahedron Letters, Vol.31, No.17, pp 2389-2392, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

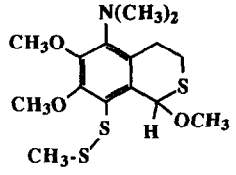
Polycarpamines A-E, Antifungal Disulfides from the Marine Ascidian *Polycarpa auzata.*

Niels Lindquist and William Fenical*
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, CA 92093-0228

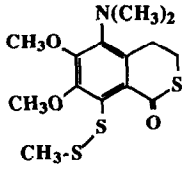
Summary: Five novel benzenoids, polycarpamines A-E (**1-5**), with rare, sulfur-containing functional groups, have been isolated from the solitary marine ascidian *Polycarpa auzata.* The structures of these compounds were determined primarily through interpretation of their NMR characteristics and mass spectral fragmentation patterns. Polycarpamine B (**2**) exhibited significant antifungal activity *in vitro* against *Saccharomyces cerevisiae* and *Candida albicans.*

Prior chemical studies have shown marine ascidians (Urochordata) are a rich source of unique and extremely biologically active secondary metabolites.[1] In our recent chemical and biological studies[2-4], we have proposed and demonstrated that ascidian metabolites deter a variety of potential fish predators. As part of our continuing studies on the chemical adaptations of taxonomically diverse ascidians, our interest was directed to *Polycarpa auzata*, a common solitary ascidian and conspicuous member of the benthic invertebrate fauna on many coral reefs in the Indo-Pacific. Although *P. auzata* has been collected on numerous occasions from locations throughout the Philippines and Indonesia, only our initial collection at Siquijor Island, Philippines, in May 1986, yielded the compounds described in this paper. Solvent partitioning and silica gel flash chromatography were used to fractionate the lipid soluble material from *P. auzata*. Final purification of polycarpamines A-E (**1-5**) was achieved by silica HPLC (92:8 CH_2Cl_2:MeOH) and by preparative TLC.

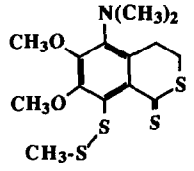
Polycarpamine A (**1**), an oil, analyzed for $C_{15}H_{24}NO_3S_3$ by HRFABMS in conjunction with ^1H and ^{13}C NMR data (Table 1, ref. 5). The high percentage of sulfur in **1** was revealed by the intense M^++2 ion (20% bp) in the HRFAB mass spectrum. A prominent feature of **1** was the six singlet methyl resonances in the ^1H NMR spectrum and the downfield shifts of their corresponding ^{13}C NMR bands. In addition to the methyl groups, DEPT sequence



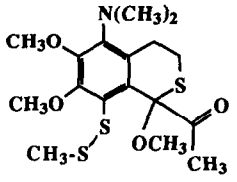
1



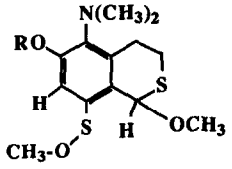
2



3



4



5 R=H

6 R=Ac

experiments also showed one methine carbon (C10) and two methylenes (C7 and C8) accounting for all 23 protons present in this metabolite. The presence of two aromatic methyl ethers, an aliphatic methyl ether and N,N-dimethylamine was apparent from NMR data. A methyl disulfide was indicated by the upfield shift of the ^{13}C band for C11[6] and the difference of 48 mass units between the parent ion (M^++H, HRFABMS) and first fragment ion (M^+-SCH_3, HRDEIMS) of **1**. Considering the methylated functional groups as hydrogen equivalents, five units of unsaturation were indicated for the molecular nucleus. The six quaternary aromatic carbons in the ^{13}C NMR spectrum of **1** were indicative of a fully substituted benzene ring and accounted for four degrees of unsaturation leaving an additional ring in **1**. The UV absorption at 277 nm was also appropriate for a highly substituted benzene ring lacking extended conjugation. A 1,2-disubstituted ethyl group and an acetal functionality were indicated by their ^1H and ^{13}C NMR resonances (for C10, δ 6.88 and δ 91.6).

Table 1. ^1H and ^{13}C NMR assignments for polycarpamines A-E (1-5) and derivative 6.[a]

	polycarpamine A (1)			polycarpamine B (2)		polycarpamine C (3)	
#	^{13}C	^1H	long range correlations from ^1H at C #	^{13}C	^1H	^{13}C	^1H
1	132.4[c]		7	132.4[e]		127.6[f]	
2	133.0		10	132.4[e]		133.7[f]	
3	147.5[d]		12	144.4		146.1	
4	153.9[d]		13	153.0		152.7	
5	129.9		7, 14	132.0[e]		130.9[f]	
6	133.6[c]		7, 10	135.9[e]		134.0[f]	
7	34.0	3.12, 2H, m		33.1	3.07, 2H, m	33.5	3.10, 2H, m
8	59.2	2.49, 2H, m		58.4	2.48, 2H, m	58.3	2.49, 2H, m
10	91.6	6.88, 1H, s	16	NR[b]		NR[b]	
11	19.6	2.37, 3H, s		19.2	2.42, 3H, s	19.4	2.42, 3H, s
12	60.7	3.88, 3H, s		60.7	3.95, 3H, s	60.6	3.94, 3H, s
13	61.4	3.89, 3H, s		60.8	3.95, 3H, s	60.9	3.95, 3H, s
14	45.1 (2C)	2.35, 6H, s		45.1 (2C)	2.35, 6H, s	45.1 (2C)	2.36, 6H, s
15	51.7	3.22, 3H, s	10				

	polycarpamine D (4)		polycarpamine E (5)		polycarpamine E acetate (6)
#	^{13}C	^1H	^{13}C	^1H	^1H
1	130.6[g]		124.1[h]		
2	130.4[g]		141.2		
3	145.6		111.9	6.67, 1H, s	6.81, 1H, s
4	152.6		147.5		2.27, 3H, s (OAc)
5	129.2[g]		129.1		
6	132.0[g]		125.7[h]		
7	33.5	3.06, 2H, m	34.2	2.79, 2H, m	2.86, 2H, m
8	58.1	2.49, 2H, m	59.8	2.69, 2H, m	2.70, 2H, m
10	110.8		92.3	6.86, 1H, s	6.98, 1H, s
11	19.5	2.36, 3H, s	57.1	3.84, 3H, s	3.80, 3H, s
12	60.3	3.90, 3H, s	45.0 (2C)	2.43, 6H, s	2.41, 6H, s
13	60.8	3.91, 3H, s	51.4	3.18, 3H, s	3.18, 3H, s
14	45.0 (2C)	2.38, 6H, s			
15	51.9	3.44, 3H, s			
16	196.5				
17	25.8	2.37, 3H, s			

[a] ^1H and ^{13}C NMR spectra were recorded at 360 and 50 MHz, respectively. NMR spectra for **1**-**4** were recorded in $CDCl_3$ and for **5**-**6** in methanol-d_4. Chemical shifts are reported in δ units (downfield of Me_4Si). Assignments for polycarpamine A were aided by DEPT sequence experiments and J_{C-H} and $^{2,3}J_{C-H}$ correlation experiments. Assignments for polycarpamines B-E (**2**-**5**) were based on DEPT sequence experiments and comparison with the assignments for **1**. [b] No resonances were observed for these carbons due to limited sample quantities. [c-h] Assignments within a column may be exchanged.

The N, N-dimethylamine was unambiguously placed at C5 by the long range ^{1}H -^{13}C NMR correlations from the N-Me protons, and from the C7 protons, to C5. The thio acetal center at C10 was confirmed by correlations from the C15 methyl protons to C10 and from the methine proton at C10 to C15. The C10 acetal proton correlated to the carbon resonances assigned to C2 and C6, while the C7 protons correlated to C1 and C6. This pattern of correlations placed the methyl disulfide at C2. The two remaining methoxy groups could thus be placed at C3 and C4 completing the structural assignment of polycarpamine A. The lack of optical activity for **1** revealed that C10 was racemic, thus raising the question of solvent incorporation during preservation, extraction and/or isolation procedures.

Polycarpamines B and C (**2-3**) shared many of the spectral features of **1**. Conspicuously absent were ^{1}H and ^{13}C NMR resonances for the thio acetal. The presence of a methyl disulfide in **2** and **3** was established by interpretation of their mass spectral fragmentation patterns and by ^{13}C NMR data. HRFABMS established a molecular formula of $C_{14}H_{19}NO_3S_3$ for **2** (6 units of unsaturation). The UV spectrum of **2** exhibited a λ max of 305 nm indicating further conjugation of the aromatic ring. A strong absorption at 1665 cm^{-1} in the FTIR spectrum was appropriate for assignment of a thioester[7] at C10 completing the structural assignment of polycarpamine B.

Polycarpamine C (**3**) analyzed for $C_{14}H_{19}NO_2S_4$ (six units of unsaturation) which related it to **2** by the replacement of an oxygen atom with sulfur. The UV spectrum of **3** had a λ max at 375 nm indicative of the expected absorption of a substituted benzyldithio lactone.[8] However, perhaps because of long relaxation times and small sample sizes, no carbonyl absorptions were observed in the ^{13}C NMR spectrum of this compound. Based on these data and a comparison of the spectral data for **1-3**, C10 in **3** was formulated as the dithiolactone. IR absorptions for dithioesters are weak and commonly occur between 1200 and 1250 cm^{-1}. An absorption at 1280 cm^{-1} was noted in the FTIR spectrum of **3**. Dithiolactones and dithioesters have apparently not been reported in secondary metabolites from marine or terrestrial sources.

Polycarpamine D (**4**), which analyzed for $C_{17}H_{25}NO_4S_3$ (6 units of unsaturation), was similar to **1-3**, however, it had additional NMR resonances not previously seen in the other metabolites. The UV spectrum of **4** was virtually identical to that of **1** indicating no extended conjugation of the aromatic ring. However, a strong absorption at 1733 cm^{-1} in the FTIR spectrum of **4** and a quaternary carbon at δ 196.5 in its ^{13}C NMR spectrum indicated the presence of a ketone. Proton and ^{13}C NMR resonances for an additional methyl group at δ 2.37 and 25.8, respectively, established the compound as a methyl ketone. Since the C10 carbon appeared at δ 110.6 with no attached protons, and with the reappearance of the aliphatic methyl ether, the methyl ketone was assigned to the thio ketal at C10 in **4**. As in **1**, compound **4** displayed no optical activity (Na D line) indicating it could have been formed by methanol addition to an unknown reactive precursor.

Polycarpamine E (**5**), which analyzed for $C_{13}H_{19}NO_3S_2$, had some structural similarities to **1**, but lacked both methyl ethers and the methyl disulfide. The bathochromic shift observed in the UV spectrum of **5**, upon the addition of base, was indicative of a free phenol. In addition to the C10 acetal proton in the ^{1}H NMR spectrum of **5**, the appearance of an aromatic proton denoted the replacement of an aromatic methyl ether with hydrogen. A thio acetal was assigned at C10 in **5** by comparison with the ^{1}H and ^{13}C NMR resonances of **1**. The aromatic proton was assigned to C3 by the relative downfield shift noted for the C2 carbon resonance. In NOEDS experiments, irradiation of the C3 proton produced a 12% enhancement of the C11 methyl protons, while this irradiation produced no effect on the N,N-dimethyl amine methyl protons. This NOE enhancement provided corroborating evidence for the substituent assignments for **5**, which were based primarily on ^{13}C NMR chemical shift considerations. Acetylation of **5** yielded the monoacetate **6** (^{1}H NMR, δ 2.27, 3H, s). The acetate carbonyl absorption observed at 1770 cm^{-1} was assigned to a phenolic acetate. The parent ion (M^{+}+H) at *m/z* 344.1005 in the HRFABMS of **6** confidently analyzed for

$C_{15}H_{22}NO_4S_2$, indicating no tendency for cleavage of the S-O bond. The spectral and chemical data for polycarpamine E established a plausible structure as **5**, a molecule possessing an unusual esterified sulfinic acid at C2.

Only the initial collection of *P. auzata* yielded polycarpamines suggesting that the ascidian may not be the true source of these unique compounds. All ascidians are filter feeders, straining planktonic organisms and suspended organic matter from seawater. The polycarpamines may thus have been derived from some planktonic organisms or microorganism ingested by *P. auzata*. Polycarpamines A, D and E are quite plausably produced by methanol incorporation into an unknown precursor. Studies are now in progress to find the true origin of these compounds and to probe their reactivities. Polycarpamine B (**2**) was found to be a significant inhibitor *in vitro* of the fungi *Saccharomyces cerevisiae* and *Candida albicans*. No significant antifungal activities were found for polycarpamines A and C-E.

Acknowledgements

This research is a result of financial support from the National Science Foundation, Chemistry Division, under grant CHE86-20217. We wish to thank Dr. Ernani Meñez of the Smithsonian Institution, and Dr. Angel Alcala, Silliman University Marine Laboratory, for the opportunity to perform research in the Philippines. The faculty and staff of the Silliman University Marine Laboratory are also acknowledged for their generous assistance. We also thank Dr. Françoise Monniot for identifying *Polycarpa auzata* and P. Jensen for biotesting these compounds.

References and Notes

1. Faulkner, D. J., *Nat. Prod. Rep.*, 1984, **1**, 551-98; *ibid.*, 1986, **3**, 1-33; *ibid.*, 1987. **4**, 539-76; *ibid.*, **5**, 613-63.

2. Lindquist, N., W. Fenical, G. D. Van Duyne and J. Clardy. 1988. *J. Org. Chem., 53*, 4570.

3. Lindquist, N., W. Fenical, D. F. Sesin, C. M. Ireland, G. D. Van Duyne, C. J. Forsyth and J. Clardy. 1988. *J. Am. Chem. Soc., 110*, 1308.

4. Paul, V. J., N. Lindquist and W. Fenical. 1989. *Mar. Ecol. Prog. Ser.* in press.

5. Additional spectral data: For **1**; yellow oil; $[\alpha]_D$ 0.00° ($CHCl_3$, c 5.2); UV (MeOH) 277 (ε 5500), 238 nm (29100); FTIR (neat, NaCl) 2929, 2855, 2818, 2779, 2766, 1451, 1398, 1367, 1310, 1065, 1027, 969, 900 cm^{-1}; HRFABMS, obsd. (M^++H) *m/z* 362.0918, $C_{15}H_{24}NO_3S_3$ requires 362.0920, Δ 0.6; HRDEIMS obsd. (M^+-SCH_3) *m/z* 314.0865, $C_{14}H_{20}NO_3S_2$ requires 314.0886, Δ 6.7; ^1H and ^{13}C NMR (Table 1). For **2**: yellow oil; UV (MeOH) 305 (sh), 270 (ε 5200), 240 (sh), 228 nm (22600); FTIR (neat, NaCl) 1665, 1455, 1400, 1364, 55, 1070, 1022, 967 cm^{-1}; HRFABMS, obsd. (M^++H) *m/z* 346.0618, $C_{14}H_{20}NO_3S_3$ requires 346.0607, Δ -3.2; HRDEIMS, obsd. (M^+-SCH_3) *m/z* 298.0539, $C_{13}H_{16}NO_3S_2$ requires 298.0573, Δ 11.4; ^1H and ^{13}C NMR (Table 1). For **3**: yellow oil; UV (MeOH) 375 (ε 13200), 305 (sh), 278 (4400), 256 (sh), 236 nm (17400); FTIR (neat, NaCl) 2900, 2840, 1460, 1400, 1360, 1280, 1075, 1024, 968 cm^{-1}; HRFABMS obsd. (M^++H) *m/z* 362.0378, $C_{14}H_{20}NO_2S_4$ requires 362.0378, Δ 0.00; HRDEIMS obsd. (M^+-SCH_3) *m/z* 314.0309, $C_{13}H_{16}NO_2S_3$ requires 314.0345, Δ 11.5; ^1H and ^{13}C NMR (Table 1). For **4**: yellow oil; $[\alpha]_D$ 0.00° ($CHCl_3$, c 3.5); UV (MeOH) 277 (ε 6100), 238 nm (32900); FTIR (neat, $CHCl_3$) 2928, 2854, 2819, 1733, 1452, 1398, 1368, 1356, 1294, 1282, 1263, 54, 15, 1096, 1073, 1026, 969, 892 cm-^{1}; HRFABMS, obsd. (M^++H) *m/z* 404.1041, $C_{17}H_{26}NO_4S_3$ requires 404.1026, Δ -3.7; HRDEIMS obsd. (M^+-SCH_3) *m/z* 356.0968, $C_{16}H_{22}NO_4S_2$ requires 356.0991, Δ 6.5; ^1H and ^{13}C NMR (Table 1). For **5**: yellow oil; UV (MeOH) 255 (sh), 230 (ε 10800), 207 nm (11000); UV (MeOH + NaOH) 313 (ε 40), 260 (sh), 233 (8700), 204 nm (54200); FTIR (neat, NaCl) 3550 -2900, 2958, 2955, 2942, 1598, 1566, 1488, 1464, 1398, 1282, 38, 27, 1075, 1057 cm^{-1}; HRDEIMS, obsd. (M^+) *m/z* 301.0803, $C_{13}H_{19}NO_3S_2$ requires 301.0808, Δ 1.7; ^1H and ^{13}C NMR (Table 1). For **6**: IR (neat, NaCl) 2940, 2860, 1770, 1760, 1365, 1340, 1310, 1055 cm^{-1}; HRFABMS obsd. (M^++H) *m/z* 344.1005, $C_{15}H_{22}NO_4S_2$ requires 344.0992, Δ -3.8; ^1H NMR (Table 1).

6. Shin, J. and W. Fenical. 1987. *Phytochemistry, 26*, 3347.

7. Dolphin, D. and A. E. Wick. 1977. *Tabulation of Infrared Spectral Data.* John Wiley and Sons, New York. 549 p.

8. Kato, S., H. Shibahashi, T. Katada, T. Takagi, I. Noda, M. Mizuta and M. Goto, *Liebigs Ann. Chem.*, 1982., (7), 1229.

(Received in USA 16 February 1990)