New 4-Methoxybenzoyl Derivatives from the Ascidian *Polycarpa aurata*[1]

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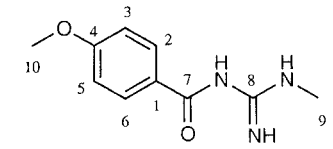
From the hydrophilic extract of the ascidian *Polycarpa aurata* three new compounds, *N*-(4-methoxybenzoyl)-*N*′-methylguanidine (**1**), butyl 2-(4-methoxyphenyl)-2-oxoacetate (**2**), and 2-(4-methoxyphenyl)-*N*-methyl-2-oxoacetamide (**3**), together with the known compounds methyl 2-(4-methoxyphenyl)-2-oxoacetate (**4**) and 4-methoxybenzoic acid were isolated. The structures of all isolates were determined from their spectroscopic data (NMR, MS, IR, UV).

Chemical and biological investigations have shown ascidians to be a useful source of biologically active secondary metabolites,[2−5] the majority of which contain nitrogen. Ascidians of the genus *Polycarpa* are known to produce antifungal disulfides[6] and cytotoxic disulfide alkaloids,[7−9] compounds that are reported to partially degrade on silica gel.[9]

In the present study a sample of the solitary ascidian *Polycarpa aurata* (Quoy and Gaimard, 1834), collected from Kelso Reef (Great Barrier Reef, Australia) in March 1993, was investigated. Separations and purifications were carried out employing a combination of liquid−liquid distribution and column chromatography, including HPLC, and were guided by TLC and ^{1}H NMR to yield three new aromatic compounds (**1**−**3**) and two previously reported ones (**4** and 4-methoxybenzoic acid).

The ^{13}C NMR and mass spectral data of **1** indicated it to have the molecular formula $C_{10}H_{13}N_3O_2$ and, thus, to have six degrees of unsaturation. Further analysis of its ^{13}C NMR spectrum showed these elements of unsaturation to be present in the form of a carbonyl (168.5, s ppm) and an imine (156.3, s ppm) group and a 1,4-disubstituted benzene ring (164.6, s; 2 × 131.3, d; 122.4, s; 2 × 114.4, d ppm). The ^{13}C NMR spectrum also revealed the presence of a methoxyl (55.6, q ppm) and an N-CH_3 (28.3, q ppm) group. Analysis of the ^{1}H NMR spectrum showed the N-CH_3 to be present as a doublet resonance (3.13 [d, 3H, J = 4.6 Hz] ppm), which indicated the nitrogen to be secondary. This spectrum also contained resonances consistent with the aforementioned methoxyl group, the 1,4-disubstituted benzene ring, as well as two other deuterium exchangeable resonances (9.74, 12.09 ppm) associated with nitrogen.

After all protons had been assigned to their directly bonded carbon atoms, aided by an HMQC spectrum, it was possible to deduce two major molecular fragments by interpretation of the HMBC spectrum. Thus, starting with the methoxyl group, H_3-10 showed heteronuclear multiple bond couplings with C-4, which has HMBCs with H-2 and H-6, which in turn have HMBCs with each other and the carbonyl C-7. H-3 and H-5 have HMBCs with each other's carbon and C-1, affording a *p*-methoxybenzoyl fragment. This fragment is also clearly responsible for the base peak (*m*/*z* 135) in the EIMS of **1**. The second major molecular fragment was deduced starting from the methyl H_3-9,



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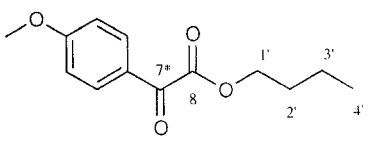

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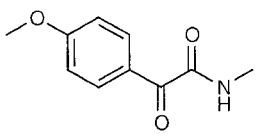

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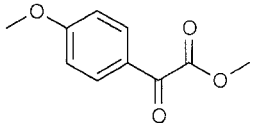

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*For the purpose of naming compounds **2-4** above position 7 is referred to as 2.

which shows a HMBC with C-8, clearly leaving the remaining NH group to reside between C-7 and C-8, giving rise to the *N*-methyl guanidine moiety. Comparison of the ^{13}C NMR data of C-8 (156.3 ppm) with that of other compounds containing a guanidine group (e.g., 158.6 ppm[10] and 157.2 ppm[11]) further supported this deduction. Compound **1** is *N*-(4-methoxybenzoyl)-*N*′-methylguanidine.

The mass spectral data of **2** indicated it to have the molecular formula $C_{13}H_{16}O_4$ and to contain a *p*-methoxybenzoyl fragment (base peak *m*/*z* 135). Its IR spectral data showed the presence of two carbonyl groups (ν_{max} 1730, 1675 cm^{-1}) and the para-substituted benzene ring (ν_{max} 1600, 820 cm^{-1}). The ^{1}H NMR data further supported the presence of the 1,4-disubstituted benzene ring as the only cyclic element within the molecule (7.99 [d, 2H, J = 9.0 Hz] and 6.98 [d, 2H, J = 9.0 Hz] ppm) and also showed the presence of the methoxyl (3.90 [s, 3H] ppm), a methyl

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Table 1. ^{1}H NMR Data of Compounds **1**−**3** ($CDCl_3$, 400 MHz, δ relative to residual $CHCl_3$ [δ 7.26] in $CDCl_3$, J in Hz)[a]

proton	1	2	3
2	8.31 (d, 1H, J = 8.6)	7.99 (d, 1H, J = 9.0)	8.43 (d, 1H, J = 9.2)
3	7.01 (d, 1H, J = 8.6)	6.98 (d, 1H, J = 9.0)	6.95 (d, 1H, J = 9.2)
5	7.01 (d, 1H, J = 8.6)	6.98 (d, 1H, J = 9.0)	6.95 (d, 1H, J = 9.2)
6	8.31 (d, 1H, J = 8.6)	7.99 (d, 1H, J = 9.0)	8.43 (d, 1H, J = 9.2)
9	3.13 (d, 3H, J = 4.6)	3.90 (s, 3H)	2.96 (d, 3H, J = 5.1)
10	3.87 (s, 3H)		3.89 (s, 3H)
1′		4.38 (t, 2H, J = 6.8)	
2′		1.76 (tt, 2H, J = 7.5, 6.8)	
3′		1.45 (qt, 2H, J = 7.5, 7.5)	
4′		0.97 (t, 3H, J = 7.5)	
NHs	9.74 (brs, 1H) 10.16 (brs, 1H, N*H*-Me) 12.09 (brs, 1H)		7.12 (brs, 1H)

[a] All assignments for **1** and **3** are based on extensive 1D and 2D NMR measurements (HMBC, HMQC, COSY).

(0.97 [t, 3H, J = 7.5 Hz] ppm), and three methylene (4.38 [t, 2H, J = 6.8 Hz], 1.76 [tt, 2H, J = 6.8, 7.5 Hz], 1.45 [qt, 2H, J = 7.5, 7.5 Hz] ppm) groups. The triplet multiplicity of the methyl group implied a vicinal methylene group. This methylene group has its resonances centered at 1.45 (qt, 2H, J = 7.5, 7.5 Hz) ppm, indicating a methyl and a methylene as direct neighbors. The attached CH_2 group was identified as that with the 1.76 (tt, 2H, J = 6.8, 7.5 Hz) ppm resonance, which in turn coupled with the CH_2 group whose resonance is at 4.38 (t, 2H, J = 6.8 Hz [−O−CH_2−]) ppm. This left the second carbonyl group to be positioned between the *p*-methoxybenzoyl moiety and the butoxyl group and thus completed the structure of **2** [butyl 2-(4-methoxyphenyl)-2-oxoacetate]. Comparison of the spectral data of **2** with the corresponding data for α-oxobenzeneacetic acid butyl ester and 4-methoxy-α-oxobenzeneacetic acid ethyl ester (compounds **1f** and **1k** in the work of Hu and Neckers,[12] respectively) further supported the structure proposed for **2**. ^{13}C and 2D NMR spectra were not recorded for **2**, as it had almost completely decomposed prior to the measurements being undertaken.

The ^{13}C NMR and mass spectral data of the third new natural product, **3**, indicated it to have the molecular formula $C_{10}H_{11}NO_3$ and to also contain a *p*-methoxybenzoyl fragment (base peak *m*/*z* 135). Its IR spectral data also revealed the presence of two carbonyl groups (ν_{max} 1685, 1645 cm^{-1}) and the para-substituted benzene ring (ν_{max} 1600, 846 cm^{-1}), deductions that were also supported by the ^{1}H and ^{13}C NMR data. The NMR data also contained resonances consistent with the presence of an NH-CH_3 moiety (^{13}C NMR: 26.0, q; ^{1}H NMR: 2.96 [d, 3H, J = 5.1 Hz], 7.12 [br, 1H, D exchangeable] ppm). This then showed **3** to contain the *p*-methoxybenzoyl moiety, another carbonyl group, and the NH-CH_3. These three molecular fragments can only be combined in one way: the structure proposed for **3**. A structure-based literature search led to two recently published articles where synthetic studies of α-oxoketones were reported.[13,14] In both these articles 2-(4-methoxyphenyl)-*N*-methyl-2-oxoacetamide (**3**) was described as one of a variety of products, but no supporting spectral data were published in either article.

Together with the new natural products **1**−**3** the known metabolites methyl 2-(4-methoxyphenyl)-2-oxoacetate (**4**) and 4-methoxybenzoic acid were also isolated. As well as being constituents of ascidians,[7] compounds **4** and 4-methoxybenzoic acid are known constituents of the essential oil of several terrestrial plant species.[15]

A GC−MS analysis of the lipophilic extract of the current sample revealed it to contain elementary sulfur S_8 and a number of sterols as the main components. Sulfur was also detected as a minor impurity in many of the hydrophilic fractions from which compounds **1**−**5** were isolated. Earlier reports on *Polycarpa* spp. also described the presence of sulfur-containing secondary metabolites[6,7] and S_8 within the animals.[7] As discussed previously, isolates from ascidians tend to contain nitrogen and range from peptide and amino acid derived metabolites to polycyclic alkaloids and amino lipids.[2,4] Guanidine derivatives with antimicrobial and cytotoxic properties have been described from ascidians of the genera *Polyandrocarpa*[11,16] and *Didemnum*,[10] but to date not from *Polycarpa*. In this investigation two nitrogen-containing and three non-nitrogenous methoxybenzoyl derivatives were isolated. The tunichromes, a series of unstable hydroxy-Dopa-containing peptides,[17,18] contain a similar moiety. Thus, in a biosynthetic sense, it is likely that all of these compounds derive, at least partially, from shikimic acid.

The isolated compounds were tested in several bioassays for their biological activities,[19] but due to their apparent instability no meaningful results were obtained from these assays.

Experimental Section

General Experimental Procedures. The general experimental procedures were carried out as previously described.[20]

Animal Material. Samples of the ascidian *Polycarpa aurata* were collected in the 3−9 m depth range from Kelso Reef, Great Barrier Reef, Australia, in March 1993. Collected material was stored at −20 °C until used. A voucher specimen, number CT293P, is stored at the Institute for Pharmaceutical Biology, University of Bonn, Nussallee 6, 53115 Bonn, Germany.

Isolation. Animal tissue was freeze-dried (dry wt 74.0 g) and then exhaustively extracted with CH_2Cl_2 (4 L), followed by MeOH (4 L). Solvents were removed in vacuo and the extracts filtered, using the respective solvents, through a pad (10 mm thick) of Si gel. The MeOH extract was partitioned between H_2O and CH_2Cl_2, and the two lipophilic fractions combined to yield 0.8 g (1.1%) of CH_2Cl_2-soluble and 29.6 g (40.0%) of MeOH−H_2O-soluble material. After drying, the MeOH−H_2O-soluble material was extracted first with EtOAc and then BuOH. Repeated chromatography of the EtOAc-soluble material over Sephadex LH-20 (elution with MeOH−CH_2Cl_2, 1:4), followed by HPLC (Si gel, 100% EtOAc), led to the isolation of compounds **1**−**4** and 4-methoxybenzoic acid.

***N*-(4-Methoxybenzoyl)-*N*′-methylguanidine (1):** oil (3.1 mg; 0.004%); UV λ_{max} (MeOH) 276 nm (ϵ 4754); IR ν_{max} 3480−3100, 2920, 1695, 1605, 1255, 845, cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; EIMS *m*/*z* 207 (82) [M]$^+$, 206 (92), 135 (100) [M − $C_2H_6N_3$]$^+$, 107 (14), 100 (37), 92 (20), 83 (12), 77 (20); FABMS (NBA, Xe) *m*/*z* 208 [M + H]$^+$; CIMS (NH_3) *m*/*z* 208 [M + H]$^+$; HREIMS *m*/*z* 207.1004 (calcd for $C_{10}H_{13}N_3O_2$, 207.1005).

Butyl 2-(4-methoxyphenyl)-2-oxoacetate (2): oil (1.7 mg; 0.002%); UV λ_{max} (EtOH) 292 nm (ϵ 7284), 225 nm (ϵ 5020); IR ν_{max} 2920, 1730, 1675, 1600, 1205, 1160, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) see Table 1; EIMS *m*/*z* 236 (6) [M]$^+$, 135

Table 2. ^{13}C NMR Data of Compounds **1** and **3** (100 MHz, δ in ppm relative to $CDCl_3 = 77.0$)'

carbon	**1**[a]	**3**[b]
1	122.4 (s)[c]	126.5 (s)
2	131.3 (d)	134.0 (d)
3	114.4 (d)	113.9 (d)
4	164.6 (s)	164.8 (s)[d]
5	114.5 (d)	113.9 (d)
6	131.3 (d)	134.0 (d)
7	168.5 (s)	185.6 (s)
8	156.3 (s)	163.0 (s)[d]
9	28.3 (q)	26.0 (q)
10	55.6 (q)	55.6 (q)

[a] Assignments are based on extensive 1D and 2D NMR measurements (HMBC, HMQC, COSY). [b] Assignments are based on 1D NMR measurements (^1H, ^{13}C, DEPT). [c] Implied multiplicities determined by DEPT (s = C, d = CH, t = CH_2, q = CH_3). [d] Assignments may be interchanged.

(100) [M − $C_5H_9O_2$]$^+$, 107 (15), 92 (18), 77 (25); HREIMS *m*/*z* 236.1044 (calcd for $C_{13}H_{16}O_4$, 236.1044).

2-(4-Methoxyphenyl)-*N*-methyl-2-oxoacetamide (3): an oil (0.6 mg; 0.0008%); UV λ_{max} (EtOH) 293 nm (ϵ 1675), 225 nm (ϵ 8738); IR ν_{max} 3380, 2920, 1685, 1645, 1600, 1260, 845 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; EIMS *m*/*z* 193 (6) [M]$^+$, 135 (100) [M − C_2H_4N]$^+$, 107 (8), 92 (11), 77 (14); HREIMS *m*/*z* 193.0735 (calcd for $C_{10}H_{11}NO_3$, 193.0736).

Methyl 2-(4-methoxyphenyl)-2-oxoacetate (4): an oil (3.8 mg; 0.005%). The physical and spectral properties of **4** compare well with published values.[7]

4-Methoxybenzoic acid: oil (22.1 mg; 0.030%). The physical and spectral properties are in agreement with published values.[21]

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