First Synthesis of the Naturally Occurring Diazocarbonyl Compound Cremeomycin

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Abstract: The first synthesis of cremeomycin (3-diazo-4-methoxy-2-oxocyclohexa-4,6-dienecarboxylic acid), a naturally occurring diazocarbonyl compound, is described. Starting from methyl 2-hydroxy-4-methoxybenzoate, nitration, hydrolysis, and reduction gave 3-amino-2-hydroxy-4-methoxybenzoic acid as the key aminophenol precursor, diazotization of which gave the diazoquinone natural product.

Key words: natural products, total synthesis, diazo compounds, electrophilic aromatic substitution

Although diazocarbonyl compounds are probably best known for their involvement as versatile intermediates in modern synthetic organic chemistry,[1] a number of such compounds also occur naturally. Many of the early examples, originally isolated in the 1950s, have antitumor properties and consist of modified α-amino acids. These include azaserine (**1**),[2–4] 6-diazo-5-oxonorleucine (DON, **2**),[5,6] alazopeptin (**3**),[7,8] duazomycins A and B (**4** and **5**),[9–11] *N*-alanylazaserine (LL-D05139β, **6**),[12] and thrazarine (FR900840, **7**)[13–16] (Figure 1). More recently, other diazocarbonyl compounds have been isolated from natural sources including SQ30,957 (**8**),[17] and the more complex natural product lagunamycin.[18,19] We now report the first synthesis of another diazocarbonyl natural product, cremeomycin (**9**).

Cremeomycin (**9**) was originally described in 1967 as a photodegradable antibiotic, isolated from *Streptomyces cremeus* by the Upjohn Company, although no details of the structure were reported.[20] The unusual structure **9** of the natural product was finally solved by Rinehart and coworkers using X-ray crystallography some 28 years later.[21] The compound is an example of a diazoquinone, also known as quinone diazides,[22] and as such should be available by diazotization of the appropriately substituted aminophenol. The starting point for the synthesis was the nitration of methyl 2-hydroxy-4-methoxybenzoate (**10**),[23] that gave a mixture of the 5-nitro compound **11** (42%) together with the desired 3-nitro isomer **12** (23–32%). Reduction of **12** gave the corresponding amino ester **13**, and this was followed by diazotization to give cremeomycin methyl ester **14** in modest yield. The diazoketone **14** was an orange solid that quickly darkened on exposure to light, but could be characterized spectroscopically, with the presence of the diazo group being confirmed by IR (2145 cm^{-1}) and ^{13}C NMR (diazo carbon at δ = 84.5) spectroscopy. Perhaps not surprisingly, attempted hydrolysis of methyl ester **14** to cremeomycin (**9**) itself was not successful, and therefore the hydrolysis step was carried out earlier in the sequence. Thus, the nitro ester **12** was hydrolyzed to the nitro acid **15**, reduction of which gave the corresponding amino acid **16** (Scheme 1). Finally, diazotization of aminophenol **16** gave, after workup and chromatography on phosphate buffered silica gel, cremeomycin (**9**) as a bright yellow solid with spectroscopic properties consistent with those reported for the natural product. In particular, the diazo group showed a strong IR absorbance at 2166 cm^{-1} and the diazo carbon at δ = 84.2 in the ^{13}C NMR spectrum, although the spectrometer relaxation delay had to be increased to 10 seconds to ob-

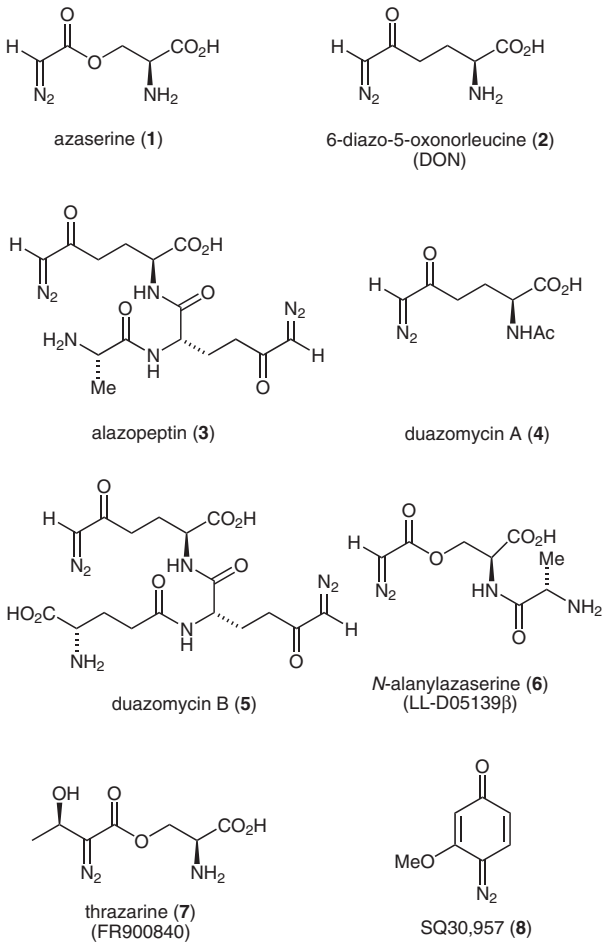

Figure 1 Some naturally occurring diazocarbonyl compounds

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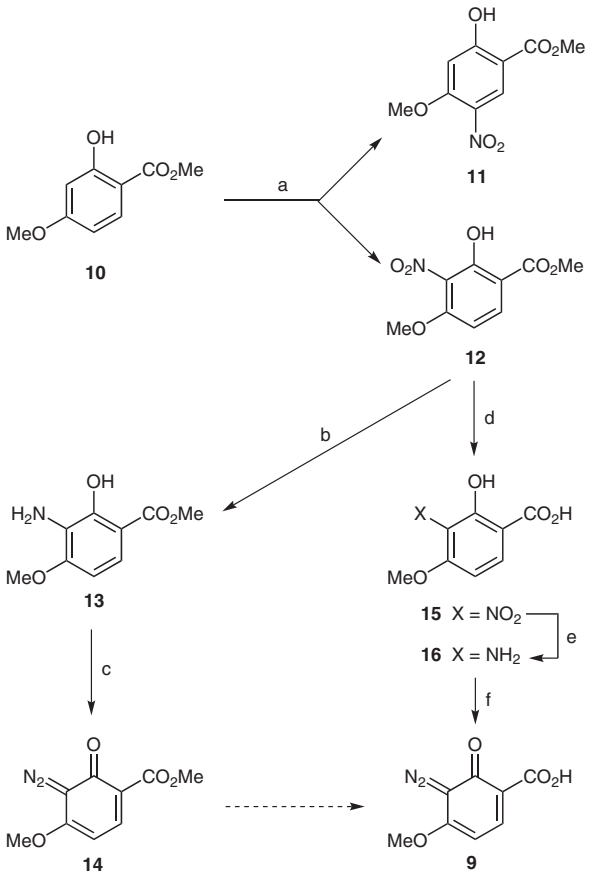

Scheme 1 *Reagents and conditions*: (a) HNO_3, Ac_2O, AcOH (42% of **11**, 23–32% of **12**); (b) H_2, Pd/C, EtOH (51%); (c) $NaNO_2$, aq HCl, 0 °C, then Na_2CO_3 (58%); (d) LiOH, aq THF (94%); (e) H_2, Pd/C, EtOH (81%); (f) $NaNO_2$, aq HCl, 0 °C, then Na_2CO_3 (38%).

serve the diazo carbon. As reported for the natural product, the EI mass spectrum showed a molecular ion that lost CO_2 and then N_2.

Commercially available reagents were used throughout without purification unless otherwise stated. Light petroleum refers to the fraction with bp 40–60 °C. Reactions were routinely carried out under N_2 or argon. Analytical TLC analyses were carried out on aluminum-backed plates coated with silica gel, and visualized under UV light at 254 and/or 360 nm. Chromatography was carried out on silica gel unless otherwise stated. Fully characterized compounds are chromatographically homogeneous. IR spectra were recorded in the range of 4000–600 cm^{-1}. NMR spectra were recorded at 400 and 500 MHz (^1H frequencies, corresponding ^{13}C frequencies at 100 and 125 MHz). Chemical shifts are quoted in ppm and are referenced to residual H in the deuterated solvent as the internal standard. *J* values are recorded in Hz. High- and low-resolution mass spectra were recorded on a time-of-flight mass spectrometer.

Methyl 2-Hydroxy-4-methoxy-3-nitrobenzoate (12)

Methyl 2-hydroxy-4-methoxybenzoate (**10**; 8.00 g, 43.92 mmol) was dissolved in a mixture of glacial AcOH (57.6 mL) and Ac_2O (29.6 mL). After cooling the mixture to 0 °C, a mixture of fuming HNO_3 (70%; 4.3 mL, 48.32 mmol) in glacial AcOH (26.4 mL) was added with stirring over 5 min. When addition was complete, the light yellow solution was allowed to reach r.t. and was stirred for 25 min, after which a light brown suspension had formed. H_2O (110 mL) was added and the mixture was allowed to stand without stirring for a further 30 min, after which the precipitate was filtered, rinsed with small amounts of H_2O and dried under vacuum to yield a light brown solid, that was purified by chromatography on silica gel (15:1 light petroleum–EtOAc, gradient to 100% EtOAc) to yield methyl 2-hydroxy-4-methoxy-5-nitrobenzoate (**11**) as a light yellow solid (4.17 g, 42%) and the title compound **12** (2.28 g, 23%) as a light yellow solid.

11

Mp 156–158 °C (Lit.[24] mp 156–157 °C).

^1H NMR (400 MHz, $CDCl_3$): δ = 11.43 (1 H, s, OH), 8.58 (1 H, s, 6-H), 6.59 (1 H, s, 3-H), 4.00 (3 H, s, OCH_3), 3.99 (3 H, s, OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.5 (C), 166.9 (C), 159.6 (C), 132.5 (C), 129.9 (CH), 105.1 (C), 101.4 (CH), 57.2 (CH_3), 53.1 (CH_3).

MS (ESI): *m*/*z* (%) = 228 ($M + H^+$, 100%), 227 (30), 223 (39), 217 (26), 210 (26), 196 (26), 192 (26), 190 (27), 174 (25), 150 (27), 149 (52).

HRMS-ESI: *m*/*z* calcd for $C_9H_9NO_6$ + H $[M + H]^+$: 228.0509; found: 228.0506.

12

Mp 183–186 °C (Lit.[25] mp 185–187 °C).

IR ($CHCl_3$): 2958, 1682, 1627, 1583, 1541, 1509, 1441, 1377, 1333, 1284, 1240, 1183, 1160, 1144, 1098, 996, 890 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 11.41 (1 H, s, OH), 7.92 (1 H, d, *J* = 9.1 Hz, 6-H), 6.57 (1 H, d, *J* = 9.1 Hz, 5-H), 3.98 (3 H, s, OCH_3), 3.97 (3 H, s, OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.1 (C), 156.0 (C), 154.2 (C), 132.3 (CH), 130.4 (C), 106.6 (C), 102.5 (CH), 56.4 (CH_3), 52.4 (CH_3).

MS (ESI): *m*/*z* (%) = 250 ($M + Na^+$, 11%), 197 (9), 196 (100), 149 (5).

HRMS-ESI: *m*/*z* calcd for $C_9H_9NO_6$ + Na $[M + Na]^+$: 250.0328; found: 250.0320.

When the reaction was repeated on a smaller scale (3.0 g of starting material), the yield of **12** was 32%.

Methyl 3-Amino-2-hydroxy-4-methoxybenzoate (13)

Methyl 2-hydroxy-4-methoxy-3-nitrobenzoate (**12**; 0.20 g, 0.88 mmol) was hydrogenated under an atmosphere of H_2 over 10% Pd/C (0.10 g) in EtOH (20 mL) for 17 h. The resulting suspension was filtered through Celite and rinsed with EtOH (3 × 20 mL) and the solvent was removed to give a brown solid, which was purified by column chromatography on silica gel (10:1 light petroleum–EtOAc) to give the title compound (88 mg, 51%) as a light brown solid; mp 72–75 °C (Lit.[25] mp 72–74 °C).

IR ($CHCl_3$): 3457, 3371, 3193, 3011, 2956, 2912, 2844, 1670, 1624, 1603, 1577, 1508, 1461, 1440, 1396, 1312, 1291, 1253, 1193, 1147, 1071, 1020 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 10.83 (1 H, s, OH), 7.30 (1 H, d, *J* = 9.0 Hz, 6-H), 6.45 (1 H, d, *J* = 9.0 Hz, 5-H), 3.97 (6 H, s, 2 × OCH_3), 3.84 (2 H, br s, NH_2).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9 (C), 151.5 (C), 149.5 (C), 124.1 (C), 119.2 (CH), 106.1 (C), 102.3 (CH), 55.8 (CH_3), 52.0 (CH_3).

MS (ESI): *m*/*z* (%) = 198 ($M + H^+$, 8%), 194 (15), 167 (9), 166 (100).

HRMS-ESI: *m*/*z* calcd for $C_9H_{11}NO_4$ + H $[M + H]^+$: 198.0766; found: 198.0749.

Methyl 3-Diazo-4-methoxy-2-oxocyclohexa-4,6-dienecarboxylate (Cremeomycin Methyl Ester, 14)

To a stirred solution of methyl 3-amino-2-hydroxy-4-methoxybenzoate (**13**; 77 mg, 0.39 mmol) in aq 2 M HCl (0.86 mL), cooled to 0 °C was slowly added a solution of $NaNO_2$ (27 mg, 0.39 mmol) in H_2O (1.4 mL). The mixture was stirred at 0 °C with the exclusion of light for 1.5 h, and then solid Na_2CO_3 was added to neutralize the reaction. The resulting slurry was then reacidified to pH 2 with aq 2 M HCl and extracted with CH_2Cl_2 (3 × 10 mL). Drying (Na_2SO_4) and removal of the solvent under reduced pressure yielded a bright orange solid. Purification by chromatography on silica gel (100% CH_2Cl_2, then 100:1 CH_2Cl_2–MeOH) yielded the title compound (47 mg, 58%) as a bright orange solid that quickly turned brown upon exposure to light; mp 150–153 °C.

IR ($CHCl_3$): 3010, 2953, 2145, 1720, 1694, 1623, 1566, 1526, 1445, 1424, 1336, 1295, 1278, 1193, 1171, 1104 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 8.16 (1 H, d, *J* = 8.6 Hz, 6-H), 5.74 (1 H, d, *J* = 8.6 Hz, 5-H), 3.99 (3 H, s, OCH_3), 3.88 (3 H, s, OCH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.5 (C), 166.0 (C), 161.2 (CH), 116.9 (C), 92.7 (CH), 84.5 (C), 57.0 (CH_3), 52.0 (CH_3).

MS (EI): *m*/*z* (%) = 208 (M^+, 100%), 182 (7), 177 (14), 165 (92), 149 (34), 137 (97), 109 (23).

HRMS-EI: *m*/*z* calcd for $C_9H_8N_2O_4$ + H $[M]^+$: 208.0484; found: 208.0476.

UV (MeCN): λ_{max} (log ε) = 270 (3.10), 423 nm (2.76).

2-Hydroxy-4-methoxy-3-nitrobenzoic Acid (15)

To a solution of **12** (0.50 g, 2.20 mmol) dissolved in a mixture of THF (100 mL) and H_2O (25 mL) was added LiOH (2.90 g, 121.05 mmol). The resulting mixture was heated under reflux with stirring for 18 h. After cooling to r.t., the mixture was acidified to pH 2 with aq 2 M HCl (2 M), and then extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were combined, dried (Na_2SO_4), and concentrated in vacuo to yield the title compound (0.44 g, 94%) as a light yellow solid; mp 210–211 °C.

IR ($CHCl_3$): 3690, 3606, 3507, 3012, 2928, 2855, 1688, 1602, 1542, 1508, 1458, 1374, 1302, 1240, 1181, 1159, 1126, 1098, 904 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 7.95 (1 H, d, *J* = 9.1 Hz, 6-H), 6.86 (1 H, d, *J* = 9.1 Hz, 5-H), 3.94 (3 H, s, OCH_3).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.8 (C), 155.3 (C), 153.7 (C), 133.2 (CH), 130.2 (C), 107.6 (C), 103.6 (CH), 57.1 (CH_3).

MS (ESI): *m*/*z* (%) = 258 (M + Na_2, 100%), 236 (M + Na, 30), 234 (37), 227 (35), 223 (64), 196 (60), 150 (47), 149 (36), 131 (31).

HRMS-ESI: *m*/*z* calcd for $C_8H_7NO_6$ + Na $[M + Na]^+$: 236.0171; found: 236.0148.

Anal. Calcd for $C_8H_7NO_6$: C, 45.1; H, 3.3; N, 6.6. Found: C, 45.0; H, 3.3; N, 6.3.

3-Amino-2-hydroxy-4-methoxybenzoic Acid (16)

2-Hydroxy-4-methoxy-3-nitrobenzoic acid (**15**; 0.20 g, 0.94 mmol) was hydrogenated under an atmosphere of H_2 over 10% Pd/C (0.10 g) in EtOH (20 mL) for 20 h. The resulting suspension was filtered through Celite and rinsed with EtOH (3 × 20 mL), and the solvent was removed to give the title compound as a brown solid (0.14 g, 81%), which was recrystallized from toluene to give a colorless solid; mp 192–194 °C.

IR ($CHCl_3$): 3691, 3607, 3523, 3012, 2930, 2338, 1681, 1602, 1543, 1508, 1443, 1289, 1240, 1181, 1139, 1067, 927, 852, 821 cm^{-1}.

^1H NMR (400 MHz, DMSO-d_6): δ = 7.19 (1 H, d, *J* = 8.8 Hz, 6-H), 6.57 (1 H, d, *J* = 8.8 Hz, 5-H), 3.85 (3 H, s, OCH_3).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.9 (C), 151.3 (C), 149.8 (C), 124.2 (C), 119.3 (CH), 106.7 (C), 103.0 (CH), 56.2 (CH_3).

MS (ESI): *m*/*z* (%) = 182 (M – H, 100%), 181 (42), 175 (7), 167 (41), 123 (44), 122 (20).

HRMS-ESI: *m*/*z* calcd for $C_8H_9NO_4$ – H $[M – H]^+$: 182.0453; found: 182.0457.

Anal. Calcd for $C_8H_9NO_4$: C, 52.5; H, 5.0; N, 7.7. Found: C, 52.2; H, 4.8; N, 7.3.

3-Diazo-4-methoxy-2-oxocyclohexa-4,6-dienecarboxylic Acid (Cremeomycin, 9)

To a stirred solution of 3-amino-2-hydroxy-4-methoxybenzoic acid (**16**; 20 mg, 0.11 mmol) in aq 2 M HCl (1.0 mL), cooled to 0 °C was slowly added a solution of $NaNO_2$ (7.5 mg, 0.11 mmol) in H_2O (0.2 mL). The mixture was stirred at 0 °C with the exclusion of light for 40 min, and then solid Na_2CO_3 was added to neutralize the reaction. The resulting slurry was then reacidified to pH 2 with aq 2 M HCl and extracted with CH_2Cl_2 (3 × 5 mL). Drying (Na_2SO_4) and removal of the solvent under reduced pressure yielded a brown solid. Purification by chromatography on phosphate buffered silica gel [prepared using KH_2PO_4 (2.45 g) and Na_2HPO_4 (0.024 g) in H_2O for silica gel (45 g), eluent: 100% CH_2Cl_2, then 99:1 CH_2Cl_2–MeOH)] yielded the title compound (8 mg, 38%) as a bright yellow solid; mp 139–141 °C (Lit.[20] mp 142–143 °C).

IR ($CHCl_3$): 3012, 2166, 1736, 1604, 1538, 1459, 1443, 1339, 1270, 1239, 1168, 1101, 966 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 13.75 (1 H, s, OH), 8.37 (1 H, d, *J* = 8.6 Hz, 6-H), 5.98 (1 H, d, *J* = 8.6 Hz, 5-H), 4.06 (3 H, s, OCH_3).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 177.2 (C), 165.9 (C), 162.0 (C), 146.5 (CH), 114.6 (C), 95.0 (CH), 84.2 (C), 57.6 (CH_3).

MS (ESI): *m*/*z* (%) = 195 (M + H^+, 8%), 177 (53), 153 (6), 150 (9), 149 (100), 139 (13), 134 (31).

HRMS-ESI: *m*/*z* calcd for $C_8H_6N_2O_4$ + H $[M + H]^+$: 195.0406; found: 195.0397.

UV (MeOH): λ_{max} (log ε) = 206 (3.73), 260 (3.14), 289 (3.04), 413 nm (3.04).

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