FURANODITERPENOIDS FROM THE DORID NUDIBRANCH CASELLA ATROMARGINATA[1]

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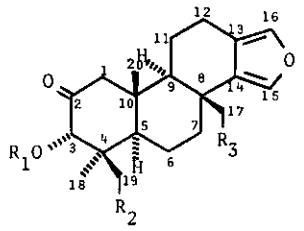
Abstract--The structures of six furanoditerpenoids isolated from a nudibranch, Casella atromarginata, were determined by spectral analysis and chemical interrelation. Two of the compounds, **1** and **5**, are known sponge metabolites of Australian Spongia spp.; two more, **2** and **6**, represent minor structural variants; and two, **3** and **4**, are characterized by a more highly oxidized A-ring, which bears a monoenolized α-diketone function.

The discovery in this Laboratory that nudibranchs,[2] which are gastropod mollusks that lack physical protection, selectively accumulate defense allomones from their highly specific prey, has prompted us to study the chemistry of similar predator-prey pairs.[3] When pertinent field observations that would reveal the dietary origin of these defensive agents are unavailable, we have been studying the secondary metabolism of nudibranchs. Although relatively few predator-prey pairs have been investigated so far, it is already evident that nudibranchs are capable of utilizing a broad spectrum of organic structural types, terpenoid as well as non-terpenoid, for their defense.[3] We now report isolation from a nudibranch and structure determination of a series of furanoditerpenoids, which are related, and in part identical to, compounds known from Spongia spp.

The animals (eight specimens, 25.5 g wet wt), Casella atromarginata, were collected in December, 1980 at Trincomallee, Sri Lanka, and preserved in methanol. The methanolic residue was partitioned between methylene chloride and water, yielding 340 mg of lipids. Chromatography on BioSil A (hexane/EtOAc, 3:2, then EtOAc) furnished five fractions: (a) fats and sterols, (b) sterols and **5**, (c) **1**, **3**, and **6**, (d) **1** and **2**, and (e) compound **4**. HPLC of (b) (Lichrosorb Si 60, hexane/EtOAc, 8:2) yielded 4.6 mg of **5**. HPLC of (c) (Partisil, hexane/EtOAc, 6.5:3.5) furnished mixed **3** and **6** plus 9 mg of **1**. Compounds **3** and **6** were separated (Lichrosorb RP 18, MeOH/H_2O, 8:2) into 3.5 mg of **3** and 3.1 mg of **6**. HPLC of (d) (Partisil, hexane/EtOAc, 6:4) resulted in 40 mg of **1** and 34 mg of **2**. Crude **4** was further purified by HPLC (Lichrosorb Si 60, hexane, EtOAc, 4:6), yielding 2.1 mg.

Spectral characterization[4] of the principal constituent **1** [white amorphous solid, $[\alpha]_D$ +10.1° (c 1.2, $CHCl_3$)] and comparison with literature data proved its identity with spongiatriol triacetate [3α,17,19-triacetoxyspongia-13(16),14-dien-2-one] isolated by Kazlauskas et al.[5] from Great Barrier Reef Spongia spp. Furthermore, one of the minor constituents, compound **5** [colorless



1 R_1 = Ac, R_2 = R_3 = OAc

2 R_1 = H, R_2 = R_3 = OAc

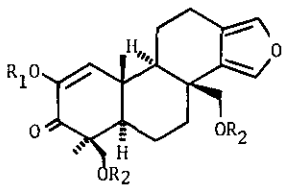
5 R_1 = Ac, R_2 = OAc, R_3 = H

6 R_1 = R_3 = H, R_2 = OAc

glass, $[\alpha]_D$ + 12.9° (c 0.4, $CHCl_3$)], was identical with the known spongiadiol diacetate [3α,19-diacetoxyspongia-13(16),14-dien-2-one] from Australian Spongia spp.[5] Another minor constituent, compound **6** [white amorphous solid, $[\alpha]_D$ +75° (c 0.24, $CHCl_3$)], was recognized as a spongiadiol monoacetate [3α-hydroxy-19-acetoxyspongia-13(16),14-dien-2-one][6] by the upfield shift of the proton at C-3 from δ 5.46 in **5** to δ 4.65 in **6**. This was readily proven by conversion of **6** to **5** with acetic anhydride/pyridine.

Compound **2**, the second most abundant constituent, was related to **1** as **6** is to **5**, i.e. the 3α-OH occurs unacetylated. This was seen by the ^1H NMR shift of the proton at C-3, which resonates at δ 5.46 in **1** and at δ 4.70 in **2**. It was proven by acetic anhydride/pyridine transformation of **2** to **1**. Compound **2** [colorless glass, $[\alpha]_D$ +13.5° (c 0.74, $CHCl_3$)] therefore is a spongiatriol monoacetate [3α-hydroxy-17,19-diacetoxyspongia-13(16),14-dien-2-one].[7]

The remaining two metabolites of C. atromarginata **3** and **4** differed from the other four by the presence of an enolized α-diketone system, as evidenced by a uv chromophore at 272 nm (5400) [**3**], or 271 nm (3560) [**4**], shifted in base to 310 nm (3500) or 308 nm (1950),[8] in addition to the furan bands at 221 nm (4300) [**3**], or 219 (3500) [**4**]. The ^1H NMR spectrum of compound **3** [white amorphous solid, $[\alpha]_D$ +17.4° (c 0.23, $CHCl_3$)] further lacked the two sharp doublets arising from the C-1 methylene group as well as the characteristic singlet of the proton at C-3. Instead, there were new signals at δ 6.53 (1 H s) and a broad, D_2O-exchangeable singlet at δ 5.90. These and the remaining spectral data[9] are fully compatible with expression **3**, which therefore is 17,19-diacetoxyspongia-13(16),14-dien-2,3-dione. This was proven by oxidizing **2** with bismuth trioxide in acetic acid[10] to **3**, identical in all respects with the natural product.



3 R_1 = H, R_2 = Ac

4 R_1 = R_2 = H

7 R_1 = R_2 = Ac

The ^{1}H NMR spectrum of **4** resembled that of **3**, but lacked methyl singlets arising from acetyl and exhibited upfield shifts of the oxygenated C-17 and C-19 methylenes. Compound **4** [white, amorphous solid, $[\alpha]_D$ +50° (c 0.2, $CHCl_3$)], therefore, is 17,19-dihydroxyspongia-13(16),14-dien-2,3-dione.[11] This was proven by acetylating **3** and **4** (Ac_2O/pyridine) to 2,17,19-triacetoxyspongia-1,13(6),14-trien-3-one (**7**).[17]

Although Kazlauskas et al.[5] were not concerned with sponge ecology and we lack information on the preferred diet of C. atromarginata, it is likely that the nudibranch obtains its metabolites from a Spongia sp.

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3. G. R. Schulte and P. J. Scheuer, Tetrahedron, in preparation.
4. ^{1}H NMR spectra were recorded on a Varian XL 100 spectrometer. UV spectra were measured on a Beckman ACTA III and IR spectra on a Perkin Elmer 710 spectrometer. Optical rotations were determined on a Bendix-Ericcson Model ETL-NPL polarimeter. High resolution mass spectra were determined at the Berkeley, CA Mass Spectrometry Resource on an AEI instrument; low resolution spectra on a Finnigan 3000 spectrometer.
5. R. Kazlauskas, P. T. Murphy, R. J. Wells, K. Noack, W. E. Oberhänsli, and P. Schönholzer Aust. J. Chem. 1979, **32**, 867.
6. Cpd **6**: UV (MeOH) 224 nm (3150). IR ($CHCl_3$) 3500, 2960, 2870, 1740, 1715, 1380, 1240, 1035 cm^{-1}. MS: m/z 374.2086 (100%); calcd for $C_{22}H_{33}O_5$ 374.2093; 359(6), 332(15), 314(12), 301(69), 299(39), 285(27), 281(26), 241(22), 201(30), 161(36), 147(78), 135(54), 91(68). ^{1}H NMR ($CDCl_3$): δ 7.1, (2H m), 4.65 (1H s), 4.21 (1H d, J = 12 Hz), 4.02 (1H d, J = 12 Hz), 2.64 (1H d, J = 19 Hz), 2.14 (1H d, J = 19 Hz), 2.09 (3H s), 1.28 (3H s), 1.22 (3H s), 0.83 (3H s).

7. Cpd **2**: UV (MeOH) 223 nm (2200). IR ($CHCl_3$) 3520, 3050, 2975, 2875, 1740, 1718, 1375, 1250, 1038 cm^{-1}. MS: m/z 432.2146 (17%). Calcd for $C_{24}H_{32}O_7$ 432.2148, 372(45), 359(81), 299(100), 281(54), 253(23), 199(28), 165(38), 147(88), 135(56), 133(55), 121(35), 105(44), 91(51). ^1H NMR ($CDCl_3$): δ 7.10 (1H s), 7.08 (1H s), 4.70 (1H s), 4.33 (1H d, J = 10 Hz), 4.15 (1H d, J = 10 Hz), 4.13 (1H d, J = 11 Hz), 4.03 (1H d, J = 11 Hz), 2.66 (1H d, J = 19 Hz), 2.13 (1H d, J = 19 Hz), 2.10 (3H s), 2.00 (3H s), 1.32 (3H s), 0.82 (3H s).

8. A. I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon Press, 1964, p. 60.

9. Cpd **3**: UV (MeOH) 221 (4300), 272 (5400) nm; (MeOH/OH^-) 220 (5040), 310 (3500) nm. IR ($CHCl_3$) 3480, 3050, 2975, 2875, 1740, 1675, 1650 sh, 1410, 1385, 1370, 1235, 1038 cm^{-1}. MS: m/z 430.1985 (17%); calcd for $C_{24}H_{30}O_7$ 430.1992, 388(14), 370(13), 357(26), 319(14), 297(50), 279(25), 269(15), 223(23), 163(23), 151(100), 143(73), 135(31), 91(28). ^1H NMR ($CDCl_3$): δ 7.09 (2H s), 6.53 (1H s), 5.90 (1H br s), 4.36 (1H d, J = 11 Hz), 4.35 (1H d, J = 11 Hz), 4.19 (1H d, J = 11 Hz), 4.08 (1H d, J = 11 Hz), 2.05 (3H s), 1.99 (3H s), 1.30 (3H s), 1.28 (3H s).

10. W. Rigby, J. Chem. Soc. 1951, 793.

11. Cpd **4**: UV (MeOH) 219 (3500), 271 (3560) nm; (MeOH/OH^-) 219 (5500), 308 (1950) nm. IR ($CHCl_3$) 3480, 2955, 2875, 1745, 1715, 1670, 1645, 1410, 1260, 1230, 1038 cm^{-1}. MS: m/z 346.1778 (4%); calcd for $C_{20}H_{26}O_5$ 346.1780, 316(38), 315(30), 285(70), 269(11), 267(12), 257(9), 239(6), 185(11), 181(14), 161(78), 151(100), 147(83), 135(50), 125(45), 91(45). ^1H NMR ($CDCl_3$): δ 7.17 (2H s), 6.56 (1H s), 5.87 (1H br s), 3.90 (1H d, J = 11 Hz), 3.78 (1H d, J = 11 Hz), 3.60 (1H, d, J = 11 Hz), 3.52 (1H d, J = 11 Hz), 1.35 (3H s), 1.18 (3H s).

12. Cpd **7**: UV (MeOH) 240 (6000) nm. IR ($CHCl_3$) 1748, 1700 cm^{-1}. MS: m/z 472, 430, 412. ^1H NMR ($CDCl_3$): δ 7.12 (2H s), 6.85 (1H s), 4.25 (4H m), 2.20 (3H s), 2.05 (3H s), 2.00 (3H s), 1.25 (6H s).

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