A New Tricarbocyclic Diterpene Structure from the Soft Coral *Xenia florida*

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Floridicin 1, a diterpene containing a [4.3.1] bicyclic ring system, has been isolated from the soft coral *Xenia florida*, and represents a new class of tricarbocyclic diterpenes.

Coelenterates belonging to the order Alcyonacea (soft corals), Gorgonacea (gorgonians) or Pennatulacea (sea pens), are a rich source of unusual diterpenes, representing a diverse group of structural types.[1,2] This diversity reflects a different folding pattern of a common diterpenoid precursor, and diterpenes isolated from the soft coral *Xenia* spp. usually contain mono- or di-carbocyclic structures.[3] During our investigations of the soft coral *Xenia florida*,† collected in the area of Bonotsu, Kagoshima Prefecture, a major component of the lipid-soluble extract was identified as the tricarbocyclic diterpene floridicin **1**.

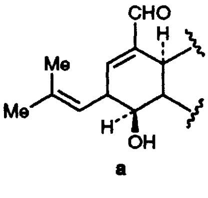
Frozen coral (12 kg) was extracted with methanol, and this initial extract was partitioned against methylene chloride. Primary purification of this extract was achieved by Si-gel adsorption chromatography and elution with mixtures of hexane, methylene chloride and methanol. The fractions eluted with methanol–methylene chloride mixtures gave oily residues that were purified by further Si-gel chromatography and finally C_{18} reversed-phase HPLC and elution with water–methanol mixtures. Following these steps, **1** was obtained as colourless needles (300 mg). The mass spectral data suggested a molecular formula of $C_{20}H_{30}O_4$ (6 double bond equivalents)‡ that was supported by the ^{13}C NMR data which showed twenty identifiable resonances. The assignment of a single carbonyl (δ 195.45), and four olefinic resonances (δ 124.8, 137.69, 146.46, 153.82), required three ring systems to account for the remaining degrees of unsaturation.

Further inspection of the ^1H and ^{13}C NMR data revealed two olefinic methyl groups (δ 1.74, 18.72; 1.78, 26.15), weakly coupled to an olefinic proton (δ 4.96, 124.8), characteristic of a dimethyl allyl group. The remaining double bond was identified as part of a conjugated aldehyde system (UV λ_{max}/nm 235; IR ν/cm^{-1} 2700 and 1680; ^1H NMR δ 9.30; ^{13}C NMR δ 195.45) and the low-field olefinic resonance at δ 6.38 was assigned to the β proton (H-11) of this conjugated system. The 2D COSY, TOCSY and HMQC data of **1** established the partial structure **a**. An identical moiety is found in a portion of the diterpene **3** isolated from another soft coral species, *Efflatounaria* sp.[4] Structure **3** was established on the basis of NMR and X-ray crystallographic data which at the time represented a new class of coral diterpene molecules. The 1D NOE and 2D NOESY data confirmed that the stereochemistry of structure **a** was similar to that of **3**; irradiation of H-9 resulted in a strong NOE (7.5%) with H-13, as well as with H-17 (7%). However, despite this structural similarity between portions of **1** and **3**, there was little commonality of NMR data for the remainder of the molecule. An important observation was that in **1**, two ring systems remained unaccounted for, and this was significant since coral diterpenoids possessing more than two carbocyclic rings are extremely rare.[2]

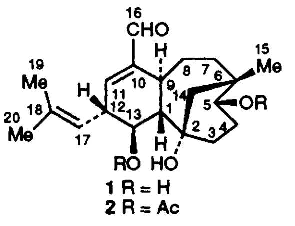
Acetylation of **1** yielded a crystalline diacetate **2** which provided better resolved NMR spectra and so this was used for most of the remaining structural elucidation studies.§ The unsaturated aldehyde grouping was unperturbed, as judged from the UV (λ_{max}/nm 235), IR (ν/cm^{-1} 2700 and 1680) and NMR (^1H δ 9.34) data, which also confirmed the acetate groups (IR: ν/cm^{-1} 1745; ^1H NMR δ 2.05, 3H, and δ 2.06, 3H) and a remaining tertiary alcohol group (ν/cm^{-1} 3600). The ^{13}C NMR DEPT spectral data supported a molecular formula of $C_{24}H_{34}O_6$ (8 double bond equivalents), and confirmed the presence of two acetate groups (δ 21.22, 168.95 and 21.27, 170.63), an aldehyde group (δ 192.70) and two double bonds (δ 121.69, 137.07 and 145.09, 150.78).

The 2D-COSY and TOCSY data of **2** revealed a major spin system that contained H-7, H-8, H-9, H-1, the unsaturated aldehyde group, the dimethyl allyl group, as well as H-12 and H-13, the latter characteristically deshielded following acetylation of the hydroxy group at C-13. This corresponds to the partial structure **a** already identified in **1**, and a characteristic homoallylic coupling between H-9 and H-12 (*J* 3.7 Hz), also reported in **3**, was now clearly observed in the diacetate. The 2D-NOESY data of the diacetate also confirmed that the stereochemistry of this partial structure was unchanged. In a second spin system, the low-field proton at δ 4.65 was assigned to H-5, geminal to the second acetate group and part of a system that included the methylenes H-4 (δ 1.64) and H-3 (δ 1.71 and 1.98). There remained a singlet methyl resonance at δ 0.95 and an isolated methylene group H-14, which appeared as an AB quartet at δ 1.67 and 1.88 (*J* 14.5 Hz) and was reminiscent of a methylene bridge in a bicyclic ring system.

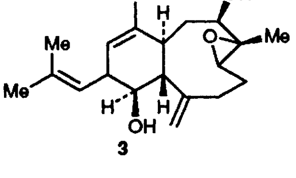
In both **1** and **2** (excluding the two acetate groups), the partial structure **a** accounts for all but two of the double bond equivalents, which can only be accommodated by two additional ring systems. These ring systems were established



a



1 R = H
2 R = Ac



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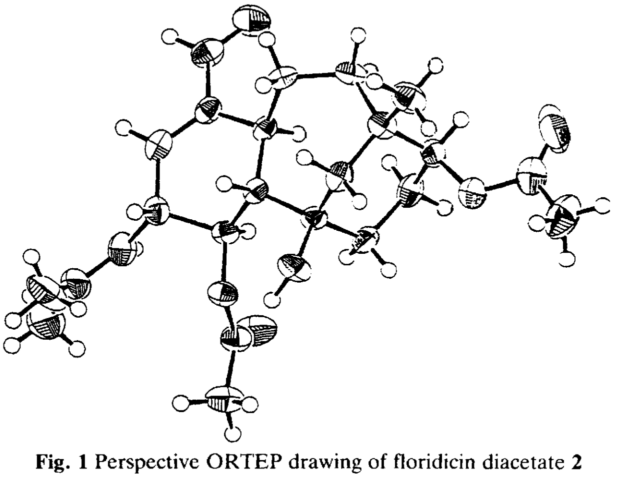

Fig. 1 Perspective ORTEP drawing of floridicin diacetate **2**

by interconnection of the major spin systems through a series of ^{1}H-detected long-range ^{1}H–^{13}C correlation experiments (HMBC). A key result was the correlation of the singlet methyl resonance H-15 (δ 0.95), with C-14 (δ 43.94), C-7 (δ 38.10), C-6 (δ 35.76) and C-5 (δ 75.50) which established that *all* the spin systems terminated at the quaternary carbon C-6. Another crucial correlation was between the tertiary hydroxy carbon C-2 (δ 73.57) and the methine H-1 (δ 2.10) as well as H-14 (δ 1.67, 1.88), which thus linked these two spin systems through C-2. The [4.3.1] bicyclic structure was completed by linking the spin system containing H-3, H-4 and H-5, through the same low-field quaternary C-2.

The relative orientation of the groups and the *trans*-fusion of the cyclohexene ring system of **2** was established by 2D-NOE correlations between H-1 and H-12 (but not between H-1 and H-13) and between H-9 and H-13. Further proof of the orientation of the isobutenyl group came from an NOE correlation between H-13 and H-17. The stereochemistry of the bicyclic ring system was established by the observation of a crucial NOE correlation between the H-14 methylene bridge proton at δ 1.88 and H-1, and between the singlet methyl resonance H-15 and both protons (δ 1.67 and δ 1.88) of the methylene bridge. No correlation was observed between H-15 and H-5.

Final confirmation of the structure for this new tricarbocyclic diterpene came from X-ray analysis of a crystal of the diacetate¶ and the 3-D structure obtained from the analysis is depicted in Fig. 1. This structure represents a new class of tricarbocyclic diterpenes among the plethora of diterpenoid compounds already reported from corals. Interestingly, careful examination of other related *Xenia* spp. from around the Kagoshima area, using the same chemical procedures, failed to uncover any other tricarbocyclic compounds. Further investigations are underway to determine if other examples of the [4.3.1] bicyclic system are produced by *X. florida*.

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Footnotes

† Specimen sample was identified as *Xenia florida* by Mr F. Iwase, Kushimoto Marine Park Centre, and a voucher sample is deposited at this institute.

‡ Data for **1**: Colourless needles (mp 182–183 °C); $[\alpha]_D$ −94.5 (MeOH, *c*. 0.635); UV (MeOH) λ/nm 235 (ε 8880); IR (Nujol) ν/cm^{-1} 3300, 2700, 1680, 1630, 980, 940, 870; EIMS *m/z* 334; HREIMS *m/z* 316.2037 [(M − H_2O)$^+$, calc. $C_{20}H_{28}O_3$, Δ 0 mmu]; ^{1}H NMR (400 MHz; CD_3OD) δ (rel. to $SiMe_4$) 0.99 (s, H-15), 1.4, 1.58 (m, H-7), 1.42, 2.14 (m, H-8), 1.75, 2.07 (m, H-4), 1.72 (m, H-14), 1.74 (d, *J* 1.47 Hz, H-19), 1.78 (m, H-10), 1.78 (d, *J* 1.47, H-20), 1.84 (br dd, *J* 5.1 and 15.4 Hz, H-3), 2.02 (br t, *J* 13.9 Hz, H-3), 2.11 (dd, *J* 4.7 and 12.4 Hz, H-1), 2.54 (m, H-9), 3.33 (br s, H-5), 3.36 (dd, *J* 1.5 and 9.2 Hz, H-12), 3.58 (dd, *J* 9.2 and 10.4 Hz, H-13), 4.96 (dq, *J* 1.5 and 9.2 Hz, H-17), 6.38 (t, *J* 1.8 Hz, H-11), 9.3 (s, H-16); ^{13}C NMR (100 MHz; CD_3OD) δ ($SiMe_4$) 18.72 (q, C-19), 26.15 (q, C-20), 28.86 (t, C-3), 29.32 (t, C-4), 29.48 (t, C-8), 31.27 (q, C-15), 37.99 (s, C-6), 39.85 (t, C-7), 40.44 (d, C-9), 45.05 (t, C-14), 48.08 (d, C-12), 57.67 (d, C-1), 74.84 (d, C-5), 75.3 (d, C-13), 76.39 (s, C-2), 124.8 (d, C-17), 137.69 (s, C-18), 146.46 (s, C-10), 153.82 (d, C-11), 195.45 (s, C-16).

§ Data for **2**: colourless needles (mp 214–216 °C); $[\alpha]_D$ −80 ($CHCl_3$, *c* 0.05); UV (MeOH) λ_{max}/nm 231 ε (20 600); IR (Nujol) ν/cm^{-1} 3600, 2700, 1745, 1680, 1640, 1455, 1220; EIMS *m/z* 418; HREIMS *m/z* 418.2350 (M$^+$, calc. $C_{24}H_{34}O_6$, Δ −0.5 mmu); ^{1}H NMR (500 MHz; $CDCl_3$) δ 0.95 (s, H-15), 1.45, 2.18 (m, H-8), 1.48, 1.69 (m, H-7), 1.64 (m, H-4), 1.67, 1.88 (br d, *J* 14.5 Hz, H-14), 1.70 (br s, H-19), 1.71, 1.98 (m, H-3), 1.73 (br s, H-20), 2.05 (s, Ac *Me*), 2.06 (s, Ac *Me*), 2.10 (ddd, *J* 1.3, 11.1 and 11.1 Hz, H-1), 2.63 (m, H-9), 3.56 (dddd, *J* 1.9, 3.7, 9.8 and 10.1 Hz, H-12), 4.65 (t, *J* 2.6 Hz, H-5), 4.85 (dq, *J* 1.4 and 10.1 Hz, H-17), 5.06 (dd, *J* 9.5 and 10.8 Hz, H-13), 6.34 (t, *J* 2.0 Hz, H-11), 9.34 (br s, H-16); ^{13}C NMR (125 MHz; $CDCl_3$) δ 18.19 (q, C-19), 21.22 (q, C-22), 21.27 (q, C-24), 25.68 (t, C-3), 25.80 (q, C-20), 27.57 (t, C-8), 28.70 (t, C-4), 30.21 (q, C-15), 35.76 (s, C-6), 38.1 (t, C-7), 40.54 (d, C-9), 43.94 (t, C-14), 45.29 (d, C-12), 56.34 (d, C-1), 73.57 (s, C-2), 75.38 (d, C-13), 75.50 (d, C-5), 121.69 (d, C-17), 137.07 (s, C-18), 145.09 (s, C-10), 150.78 (d, C-11), 168.95 (s, Ac *C*O), 170.63 (s, Ac *C*O), 192.70 (s, C-16).

¶ *Crystal data* for **2**: $C_{24}H_{34}O_6$, colourless prisms, 0.1 × 0.5 × 0.1 mm, orthorhombic space group $P2_12_12_1$, $a = 11.293(1)$, $b = 25.753(4)$, $c = 7.876(1)$ Å, $U = 2290.4(5)$ Å^3, $Z = 4$, μ(Cu-Kα) = 6.64 cm^{-1}. Data were collected on an AFC5R Rigaku diffractometer, with graphite monochromated Cu-Kα radiation and a 12 kW rotating anode generator; 2004 reflections, of which 1626 had $I > 3\sigma$ (**1**), were collected in the range $6 < \theta < 120.2°$. The structure was solved by MITHRIL and extended with DIRDIF.[5] It was refined by full-matrix least-squares using the TEXSAN system. It converged with $R = 0.06$, $R_w = 0.076$ for the 1626 reflections. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

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