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NEW MARINE CYTOTOXIC BISPYRONES. ABSOLUTE STEREOCHEMISTRY OF ONCHITRIOLS I AND II

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Key Words: *Onchidium sp.*, onchitriols, polypropionates, Mosher-Trost's method, antitumour activity.

Abstract: The complete absolute stereochemistry of two new cytotoxic marine polypropionates isolated from the saponified extract of the pulmonate mollusc *Onchidium sp.*, onchitriol I and II (4, 5), was established using Mosher-Trost's methodology.

In the last few years a number of propionate derivatives with interesting biological properties have been isolated from marine molluscs. They include the polyhydroxylated compounds, peroniatriol I and II (1, 2) and ilikonapyrone (3) (isolated from the saponified extracts of *Peronia peronii*¹ and *Onchidium verruculatum*² respectively), which have a linear structure containing two pyrone rings, three hydroxyl groups and seven asymmetric centers. Although an X-ray crystallographic analysis of the acetonide of 3, established its general structure and relative stereochemistry, its absolute stereochemistry could not be deduced. The structure and relative stereochemistry of 1 and 2 was originally inferred essentially from comparison of their NMR data with those of 3, though a synthesis of the optically active left wing C1-C10 has recently allowed correction at C-43; the absolute stereochemistry of 1 and 2 remains unknown too.

In the course of a study of the cytotoxic components of New Caledonian marine organisms, we isolated from *Onchidium sp.*, a pulmonate mollusc, a mixture of eight acetates and propionates that inhibited the growth of several cell lines *in vitro*. The components were separated by hplc and individually saponified to give two new bis-pyrone polypropionate triols we named onchitriol I (4) and onchitriol II (5). We report here their structure and absolute stereochemistry, as deduced by Mosher-Trost's NMR method.

The skeletons of 4 and 5 and the positions of their functional groups, were deduced by extensive NMR studies. Inverse NMR COSY and ¹³C-¹H correlated (HMQC and HMBC) spectra showed, in addition to the two pyrone rings, the presence of four relevant spin systems (a-d) that could be arranged either as in ilikonapyrone (a-c-b-d) or as in peroniatriol (a-b-c-d). The exact arrangement was deduced from the high resolution mass spectrum (HREIMS), which showed peaks at m/z 365.2333 and m/z 180.1154 due to the loss of the pyrone ring bonded to fragments c and d, and a peak at m/z 307.1928 due to C13-C14 cleavage giving a particularly stable allylic oxonium ion (see Figure 1).

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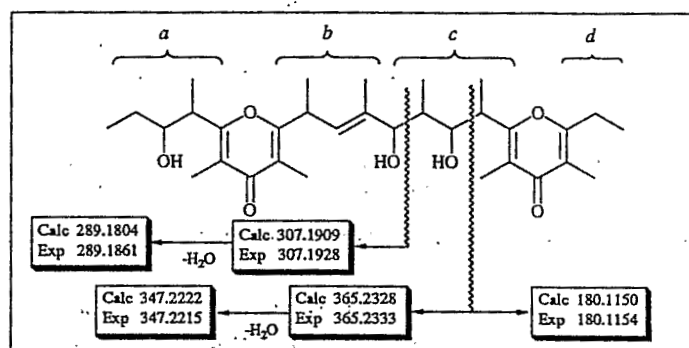
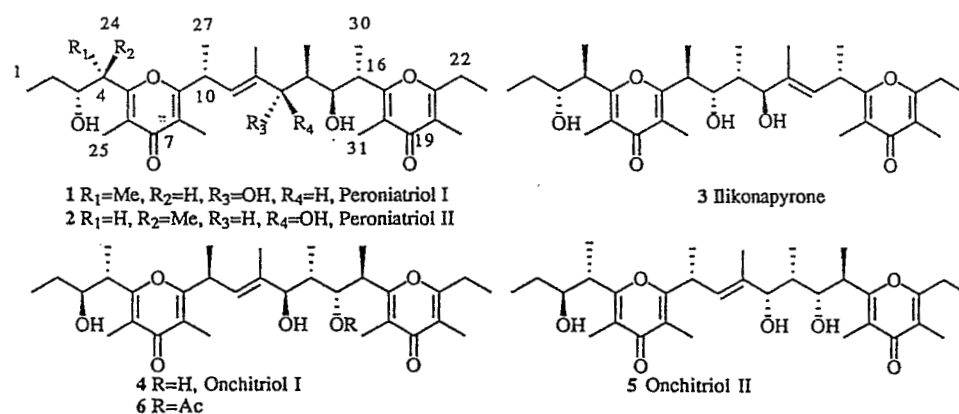


Figure 1. HREIMS of onchitriols I and II.

The relative stereochemistry of **4** and **5** was obtained by comparison of the NMR data with those of **1-3**. Thus the C10-C16 segment of onchitriol I (**4**) must be identical to the same segment of peroniatriol I (**1**) because of the agreement in carbon and proton chemical shifts and vicinal J values.⁵ Similarly, the C13-C16 fragment of onchitriol II (**5**) has NMR parameters identical to those of C13-C16 in peroniatriol II (**2**); and the C3-C4 fragment of both onchitriols, has the same relative stereochemistry as in peroniatriol II (**2**). Finally, the MM calculations for the acetonides of **4** and **5** afforded vicinal J values for the C13-C16 fragment that agreed with those observed experimentally by NMR.

In view of the presence of three secondary alcohol groups in the onchitriol molecules, we investigated absolute stereochemistry by analysis of the ¹H NMR spectra of the corresponding R- and S-methoxyphenylacetic esters: This approach is based on Mosher-Trost's assumption that the NMR behavior of O-methoxymandelates (RO-MPA) is dominated by the conformation in which the carbonyl proton, the C=O carbonyl bond and the methoxy group are in the same plane.⁴ With this premise, the R/S configuration of the hydroxyl-bearing carbons C3, C13 and C15 can be inferred from the NMR spectra of mono, di and/or tri mandelates of selected natural esters and of the triols **4** and **5** by comparison of the chemical shifts produced by the benzene rings of the R and S mandelates on directly bonded groups. Smaller but significant shifts are also observed for nearby groups, and this information, coupled with the relative orientation, affords the complete absolute stereochemistry of the molecule.

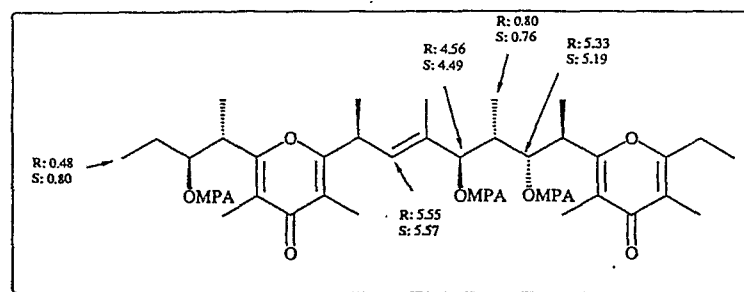


Figure 2. Selected δ values of the S and R trimandelates of onchitriol I (4), and absolute configuration.

Figure 2 shows ^1H NMR chemical shifts of representative signals of the R and S-trimandelates of onchitriol I (4). The C1 methyl group resonates at lower field in the S trimandelate than in the R form, and *viceversa* for H13 and H15. This indicates that the absolute configuration of 4 at these centers is 3S, 13R and 15R. Those of C4, C10, C14 and C16 now follow from the relative orientation of their substituents with respect to the hydroxylated carbons; and proves to be 4S, 10S, 14R, 16R.

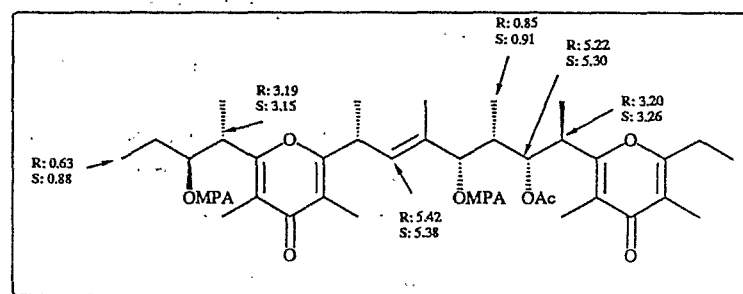


Figure 3. Selected δ values of S and R trimandelates of onchitriol II (5), and absolute configuration.

Similarly, the natural monoacetate 6 was converted to the corresponding R and S dimandelates. Figure 3 shows selected chemical shifts indicating that onchitriol II (5) has the configuration 3S, 4S, 13S, 14R, 15R and 16R. The configuration as C10 follows from noting that compounds 2 and 5 have either the same or the opposite configuration at C3, C4, C13, C14, C15 and C16, and that the only way for 2 and 5 to be diastereoisomers (as they are: compound 2 $[\alpha]_D = +224.8$ and $[\alpha]_D = -33.0$ for 5) is for the absolute configuration of the remaining asymmetric centre to be 10R.

Compounds 4 and 5 showed antitumour activity (IC_{50} 10 and 20 $\mu\text{g}/\text{ml}$ respectively) when tested *in vitro* against cell lines P388, A-549 and HT-29. Both compounds were also found to possess moderate antiviral activity against cell lines HSV-1 and VSV at 20 $\mu\text{g}/\text{ml}$.

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5. Onchitriol I 4: $[\alpha_D] = -20.0^{\circ}$ (Cl_2CH_2 , $c=0.01$); UV (MeOH) λ_{max} : 260 nm; IR ν_{max} (cm^{-1}): 1665, 1620. HREIMS: $\text{C}_{32}\text{H}_{48}\text{O}_7$: Calc 544.3395; Exp.: 544.3400. EIMS m/z (%): 544 (7), 515 (2), 486 (6), 459 (4), 365 (10), 319 (7), 307 (23), 248 (34), 219 (13), 191 (16), 180 (100); 179 (54), 151 (20), 123 (16), 97 (17), 83 (34). NMR ^1H (250.13 MHz, Cl_3CD): 0.95 (t, 3H, $J=7.3$ Hz, H1); 1.43, 1.40 (m, 2H, H2); 3.55 (ddd, 1H, $J=7.2$, 8.3 and 3.5 Hz, H3); 2.97 (dq, 1H, $J=7.2$ and 7.0 Hz, H4); 3.90 (dq, 1H, $J=9.2$ and 6.9 Hz, H10); 5.59 (dq, 1H, $J=9.2$ and 1.1 Hz, H11), 4.05 (d, 1H, $J=7.4$ Hz, H13); 1.92 (ddq, 1H, $J=7.4$, 7.0 and 1.9 Hz, H14); 4.12 (dd, 1H, $J=9.6$ and 1.9 Hz, H15); 3.13 (dq, 9.6, 6.9 Hz, H16); 2.56 (m, 2H, H22); 1.15 (t, 3H, $J=7.5$ Hz, H23); 1.26 (d, 3H, $J=7.0$ Hz, H24); 1.98 (s, 3H, H25); 1.97 (s, 3H, H26); 1.26 (d, 3H, $J=7.0$ Hz, H27); 1.70 (d, 3H, $J=1.1$ Hz, H28); 0.92 (d, 3H, $J=7.0$ Hz, H29); 1.14 (d, 3H, $J=6.9$ Hz, H30); 1.95 (s, 3H, H31); 1.92 (s, 3H, H32). NMR ^{13}C (62.83 MHz, Cl_3CD): 9.6 (q, C1), 27.9 (t, C2), 75.4 (d, C3), 41.4 (d, C4), 164.7 (s, C5), 119.8 (s, C6), 179.8 (s, C7), 118.8 (s, C8), 164.4 (s, C9), 39.1 (d, C10), 127.3 (d, C11), 137.6 (s, C12), 79.8 (d, C13), 34.4 (d, C14), 72.4 (d, C15), 36.4 (d, C16), 164.3 (s, C17), 118.0 (s, C18), 179.8 (s, C19), 117.4 (s, C20), 164.3 (s, C21), 24.7 (t, C22), 11.4 (q, C23), 13.9 (q, C24), 10.1 (q, C25), 9.3 (q, C26), 18.7 (q, C27), 14.4 (q, C28), 9.4 (q, C29), 12.0 (q, C29), 12.0 (q, C30), 9.1 (q, C31), 9.2 (q, C32).
6. In contrast to the perfect agreement of NMR data for the C10-C16 fragments of 1 and 4, there is small difference between the C10 ^{13}C chemical shifts of 2 and 5. Onchitriol II 5 $[\alpha_D] = -33.0^{\circ}$ (Cl_2CH_2 , $c=0.01$); UV (MeOH) λ_{max} : 260 nm; IR ν_{max} (cm^{-1}): 1660, 1610. HREIMS: [544 (5) $\text{C}_{32}\text{H}_{48}\text{O}_7$: Calc 544.3395, Exp: 544.3399]; [486 (5) $\text{C}_{29}\text{H}_{42}\text{O}_6$: Calc 486.2981, Exp: 486.2981]; [365 (10) $\text{C}_{21}\text{H}_{33}\text{O}_5$: Calc 365.2328, Exp: 365.2333]; [347 (3) $\text{C}_{21}\text{H}_{31}\text{O}_4$: Calc 347.2222, Exp: 347.2215]; [307 (15) $\text{C}_{18}\text{H}_{27}\text{O}_4$: Calc 307.1909, Exp: 307.1928]; [289 (5) $\text{C}_{18}\text{H}_{25}\text{O}_3$: Calc 289.1804, Exp: 289.1861], [248 (10) $\text{C}_{15}\text{H}_{20}\text{O}_3$: Calc 248.1412, Exp: 248.1413]; [180 (100) $\text{C}_{11}\text{H}_{16}\text{O}_2$: Calc 180.1150, Exp: 180.1154]. NMR ^1H (250.13 MHz, Cl_3CD): 0.87 (t, 3H, $J=7.3$ Hz, H1); 1.60, 1.40 (m, 2H, H2); 3.55 (ddd, 1H, $J=7.9$, 8.3 and 3.0 Hz, H3); 2.79 (dq, 1H, $J=8.3$ and 6.9 Hz, H4); 3.95 (dq, 1H, $J=9.6$ and 6.8 Hz, H10); 5.84 (dq, 1H, $J=9.6$ and 1.2 Hz, H11), 4.03 (d, 1H, $J=3.3$ Hz, H13); 1.90 (ddq, 1H, $J=7.0$, 3.3 and 2.0 Hz, H14); 3.68 (dd, 1H, $J=9.7$ and 2.0 Hz, H15); 3.08 (dq, 1H, $J=9.7$ and 7.1 Hz, H16); 2.55, 2.24 (m, 2H, H22); 0.98 (t, 3H, $J=7.5$ Hz, H23); 1.14 (d, 3H, $J=76.9$ Hz, H24); 1.85 (s, 3H, H25); 1.87 (s, 3H, H26); 1.31 (d, 3H, $J=7.1$ Hz, H27); 1.63 (d, 3H, $J=1.2$ Hz, H28); 1.15 (d, 3H, $J=7.1$ Hz, H29); 1.02 (d, 3H, $J=7.1$ Hz, H30); 1.93 (s, 3H, H31); 2.04 (s, 3H, H33). NMR ^{13}C (62.83 MHz, Cl_3CD): 9.7 (q, C1), 27.6 (t, C2), 75.2 (d, C3), 41.8 (d, C4), 165.6 (s, C5), 120.2 (s, C6), 179.8 (s, C7), 118.5 (s, C8), 164.9 (s, C9), 39.1 (d, C10), 125.5 (d, C11), 137.3 (s, C12), 79.9 (d, C13), 34.3 (d, C14), 73.1 (d, C15), 34.0 (d, C16), 164.5 (s, C17), 117.4 (s, C18), 180.0 (s, C19), 116.0 (s, C20), 164.1 (s, C21), 24.5 (t, C22), 10.5 (q, C23), 13.7 (q, C24), 9.5 (q, C25), 9.3 (q, C26), 19.5 (q, C27), 13.6 (q, C28), 9.2 (q, C29), 12.0 (q, C29), 15.3 (s, C30), 9.7 (q, C31), 9.3 (q, C32).

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