Tetrahedron Letters,Vol.27,No.23,pp 2559-2562,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

THE MAJOR POLYPROPIONATE METABOLITES FROM THE SACOGLOSSAN MOLLUSC, ELYSIA CHLOROTICA[1]

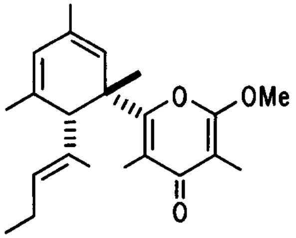
Robert D. Dawe and Jeffrey L.C. Wright*

National Research Council, Atlantic Research Laboratory, 1411 Oxford St., Halifax,
Nova Scotia, Canada, B3H 3Z1.

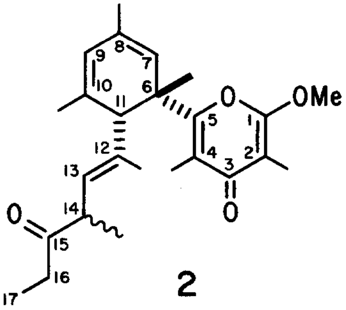
Abstract: The opisthobranch, Elysia chlorotica, contains two major polypropionate-derived metabolites. The least polar component is (1), the enantiomer of a previously reported molluscan metabolite. The second component, elysione (2), possesses the same relative stereochemistry as (1) but contains an additional propionate unit.

Metabolites derived entirely from propionate are rare in nature. A noteable example, the macrolide antibiotic erythromycin from Streptomyces erythreus, is derived solely from seven propionate units [2], but recently many other polypropionates have been found in marine invertebrates, specifically molluscs[3] including the sacoglossan molluscs[4,5] and air-breathing pulmonates[6]. Some of these marine polypropionates have been reported to display antibacterial[3a,6a] and cytotoxic activity[3d]. As part of our search for marine bioactive compounds we have examined the chemical constituents of Elysia chlorotica (Gould), found locally around the Bay of Fundy and in salt marshes[7].

The molluscs (70 g) were extracted with methanol for two days and a portion (65 mg) of the methylene-chloride solubles was chromatographed on silica using hexane-ether mixtures. Following further chromatography by reversed-phase HPLC (ODS-II; 7% H_2O/MeOH) the least polar UV-absorbing fraction (250 nm) was obtained as an oil (1), molecular formula $C_{22}H_{30}O_3$ $[\alpha]_D$ + 400° (c 0.3, $CHCl_3$), positive CD curve, maximum amplitude 270 nm, $[\theta] = + 12{,}000°\ mol^{-1}\ dm^{-1}$. The high ratio of methyl resonances in the ^1H and ^{13}C NMR spectra[8] was indicative of a polypropionate molecule. Upon closer inspection, the ^1H and ^{13}C NMR spectral data were found to be identical with those reported for 9,10-deoxytridachione, a polypropionate previously isolated from the sacoglossan molluscs Tridachiella diomeda[4] and Placobranchus ocellatus[5]. However the specific rotation of the E. chlorotica metabolite had the opposite sign to that reported for 9,10-deoxytridachione[9]. Irradiation of the C-6 methyl group (δ_H 1.43 ppm) of (1) resulted in a nuclear Overhauser enhancement (n.O.e.) of H-7 and H-11 and so established the same relative configuration at the two chiral centres as found in 9,10-deoxytridachione. These data, combined with the fact that the ^1H and ^{13}C NMR shifts are the same for both molecules establishes that the metabolite produced by E. chlorotica is the enantiomer.



1



2

The other major UV-absorbing component, elysione (2), was also obtained as an oil, molecular formula $C_{25}H_{34}O_4$ $[\alpha]_D$ + 213° (c 0.31, $CHCl_3$), positive CD curve, maximum amplitude 270 nm, $[\theta]$ = + 9600° mol^{-1} dm^{-1}. The appearance of eight methyl resonances in the ^1H NMR spectrum[10] indicated the polypropionate origin of the molecule, and indeed the spectral data were similar to those of (1). The molecular formula of the new compound revealed an extra C_3 unit compared with (1), and the additional oxygen is present as a ketone (δ_C 212.13 ppm; IR, 1720 cm^{-1}). In addition to this extra carbonyl resonance the ^{13}C NMR spectrum displayed eleven low field signals. By comparison with other spectra[4,11] five of these signals (δ_C 181.51 (s), 161.78 (s), 160.76 (s), 119.70 (s) and 98.87 (s)) could be assigned to the α-methoxy-β,β'-dimethyl-γ-pyrone ring system. The upfield region of the spectrum contained the resonances of the two vinyl methyls at (δ_C 6.87 and 12.45 ppm) of the pyrone ring[4,11]. More importantly it also contained the characteristic resonances of the methine at C-11 (δ_C 59.64 (d) ppm) and the quaternary carbon at C-6 (δ_C 47.21 (s) ppm). The presence of these two signals together with the olefinic resonances at δ_C135.61 (s), 127.81 (s), 124.14 (d) and 122.85 (d) ppm indicated the substituted cyclohexadiene moiety was preserved in elysione (2). From the ^1H and ^{13}C NMR data it was concluded that the additional C_3 unit had been added as an ethyl ketone grouping to the side chain of (1) to produce a 1,3 dimethyl-1-hexen-4-one side chain. The new proton resonances at δ_H 0.88 (t, 3 H, J = 7.2 Hz) and δ_H 2.09 (q, 2 H, J = 7.2 Hz) together with the ^{13}C NMR resonances at δ_C 212.13 (s), 34.35 (t) and 7.63 (q) ppm were consistent with an ethyl ketone moiety. This was further supported by the mass spectrum which showed a strong M^+-57 ion at m/z 341. The methyl at δ_H 1.0 ppm appeared as a doublet through coupling with a methine at δ_H3.19 ppm, which in turn was coupled to an olefinic proton at δ_H 5.06 (J = 9.75 Hz). Irradiation of the C-12 methyl group (δ_H 1.46 ppm) resulted in n.O.e. of H-14 and so confirmed the trans configuration of the double bond. Irradiation of the C-6 methyl group (δ_H1.43 ppm) resulted in n.O.e. of H-7 (δ_H 5.61 ppm) and H-11 (δ_H 2.78 ppm), establishing the cis relationship of H-7 and the C-6 methyl group. It is known that non-planar conjugated cis dienes display optical activity[12]. The maxima exhibited for the CD spectra of these chromophores generally occur in the 260-280 nm region with amplitudes ranging from ± 10,000 to ± 90,000° mol^{-1} dm^{-1}. The CD curves of both (1) and (2) are positive and almost superposeable, displaying similar maxima at 270 nm. Thus from this evidence as well as the ^{1}H NMR data, it is concluded that the spatial distribution of atoms around the diene chromophore is the same in both E. chlorotica metabolites[13].

Although the discovery of naturally occurring enantiomers is not unknown, it is unusual. The phenomenon is perhaps more frequently encountered among mevalonate-derived metabolites though cases have been found among the polyketides[14]. The occurrence of the enantiomer (1) of 9,10-deoxytridachione (and presumably elysione (2)) is perhaps more easily rationalized by consideration of the biogenesis of polypropionates which essentially mimics the steps of polyketide biogenesis[2]. Thus the two chiral centres in (1) and (2) are formed concomitantly during the aldol condensation of the polypropionate chain which results in formation of the cyclohexyl ring.

In standard disc-diffusion assays (0.5 mg/disc) both metabolites inhibited the growth of the gram (+) bacterium, Micrococcus luteus, but only (1) was active against another gram (+) bacterium, Bacillus subtilis.

Acknowledgements: We thank Dr. John Faulkner, Scripps Institution, La Jolla, for his advice and encouragement, Dr. Sherman Bleakney, Acadia University, Wolfville, who collected and identified the molluscs and Cheryl Craft for excellent technical assistance. We thank Dr. Fritz Schmitz, University of Oklahoma, Norman, for informing us of his research on Tridachia crispata collected from the Caribbean. We also thank Dr. Jab Verpoorte, Dalhousie University, Halifax, for recording the CD spectra. One of us (RDD) is grateful to the Natural Sciences and Engineering Research Council of Canada for financial assistance.

References and Notes

1. NRCC 25175
2. Cane, D. E., H. Hasler, P. B. Taylor and T.-C. Liang. Tetrahedron 39, 3449 (1983).
3. (a) Biskupiak, J. E. and C. M. Ireland. Tetrahedron Let., 3055 (1983); (b) Ireland, C. M, J. E. Biskupiak, G. J. Hite, M. Rapposch, P. J. Scheuer, and J. R. Ruble. J. O. Chem., 49, 559, (1984); (c) Cimino, G., G. Sodano, A. Spinello and E. Trivellone. Tetrahedron Let., 3389, (1985); (d) Biskupiak, J. E. and C. M. Ireland. Tetrahedron Let., 4307 (1985).
4. Ireland, C. and D. Faulkner. Tetrahedron 37, Suppl. 1, 233 (1981).
5. Ireland, C. and P. J. Scheuer. Science 205, 922 (1979).

6. (a) Hochlowski, J. E. and D. J. Faulkner. *Tetrahedron Let.*, 1917, (1983); (b) Capon, R. J. and D. J. Faulkner. *J. O. Chem.*, **49**, 2506, (1984); (c) Hochlowski, J. E. and D. J. Faulkner. *J. O. Chem.*, **49**, 3838, (1984); (d) Hochlowski, J. E., J. C. Coll, D. J. Faulkner, J. E. Biskupiak, C. M. Ireland, Q.-T. Zheng, C.-H. He, and J. Clardy. *J. Am. Chem. Soc.*, **106**, 6748 (1984).
7. Bleakney, J. S. and K. B. Meyer. *Proc. N. S. Inst. Sci.* **29**, 353 (1979).
8. Oil; $[\alpha]_D$ + 400° (c 0.3, $CHCl_3$); CD (MeOH) Molar ellipticity $[\theta]_{325}$ 0$[\theta]_{270}$ + 12000 $[\theta]_{240}$ + 3800° mol^{-1} dm^{-1}; IR (CCl_4) 1660, 1580, 920 cm^{-1}; UV (MeOH) 255 nm (ε 15,000): ^{1}H NMR ($CDCl_3$) δ_H 5.67 (bs, 1 H), 5.58 (bs, 1 H), 5.05 (t, 1 H, J = 7Hz), 3.99 (s, 1 H), 2.71 (s, 1 H), 2.06 (s, 3 H), 1.83 (s, 3 H), 1.78 (s, 3 H), 1.76 (m, 2 H), 1.72 (s, 3 H), 1.43 (s, 3 H), 1.32 (s, 3 H), 0.70 (t, 3 H, J = 7 Hz), ^{13}C NMR ($CDCl_3$) δ_C181.84 (s), 161.64 (s), 161.04 (s), 134.86 (s), 132.13 (s), 130.94 (d), 127.8 (s), 124.27 (d), 122.4 (d), 120.02 (s), 98.78 (s), 59.51 (d), 55.34 (q), 47.58 (s), 26.85 (q), 22.27 (q), 21.49 (q), 21.08 (t), 13.78 (q), 13.67 (q), 12.19 (q), 6.80 (q); MS, m/z 342 (M$^+$), 327, 313, 199, 155.
9. The value reported by Ireland and Faulkner (reference 4) for deoxytridachione is $[\alpha]_D$ - 194° (c 0.27, $CHCl_3$). The specific rotation of the *E. chlorotica* metabolite was checked several times with different preparations and even with material isolated from molluscs collected from different locations.
10. Oil; $[\alpha]_D$ + 213° (c 0.31, $CHCl_3$); CD (MeOH) Molar ellipticity $[\theta]_{325}$0 $[\theta]_{305}$ - 1000 $[\theta]_{295}$0 $[\theta]_{270}$ + 9600 $[\theta]_{240}$ + 3800° mol^{-1} dm^{-1}; IR (CCl_4) 1720, 1667, 1605, 1315, 1170 cm^{-1}; UV (MeOH) 255 nm (ε 16,000); ^{1}H NMR ($CDCl_3$) δ_H5.70 (bs, 1 H), 5.61 (1 H, bs), 5.06 (bd, 1 H, J = 9.7 Hz), 3.97 (s, 3 H), 3.19 (m, 1 H), 2.78 (s, 1 H), 2.09 (s, 3 H), 2.09 (q, 2 H, J = 7.2 Hz), 1.81 (s, 3 H), 1.80 (s, 3 H), 1.71 (s, 3 H), 1.46 (d, 3 H, J = 1.2 Hz), 1.43 (s, 3H), 1.00 (d, 3 H, J = 6.9 Hz), 0.88 (t, 3 H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$), δ_C 212.13 (s), 181.51 (s), 161.78 (s), 160.76 (s), 135.61 (s), 134.01 (s), 128.8 (d), 127.81 (s), 124.14 (d), 122.85 (d), 119.70 (s), 98.87 (s), 59.64 (d), 55.45 (q), 47.21 (s), 45.57 (d), 34.35 (t), 27.12 (q), 22.09 (q), 21.50 (q), 16.25 (q), 12.45 (q), 7.63 (q), 6.87 (q); MS, m/z 398.25041 (M+, $C_{25}H_{34}O_4$ requires 398.24679), 383, 341, 327, 313.
11. Kakinuma, K, C. A. Hanson and K. L. Rinehart, Jr. *Tetrahedron* **32**, 217 (1976).
12. Weiss, V., H. Ziffer and E. Charney. *Tetrahedron* **21**, 3105 (1965).
13. During preparation of this manuscript we learned from Ksebati and Schmitz that a re-investigation of *Tridachia crispata* had resulted in the isolation of the propionyl homologue of 9,10-deoxytridachione. Although no optical data were recorded, it was assumed that the stereochemistry of this compound is the same as that reported for 9,10-deoxytridachione, and is hence the enantiomer of (2). A full report has been published: Ksebati, M. S. and F. J. Schmitz. *J. O. Chem.* **50**, 5637 (1985).
14. Omura, S., H. Tanaka, Y. Okada and H. Marumo. *J. C. S. Chem. Commun.* 320 (1976).

(Received in USA 10 January 1986)