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Elysiapyrones from *Elysia diomedea*. Do Such Metabolites Evidence an Enzymatically Assisted Electrocyclization Cascade for the Biosynthesis of Their Bicyclo[4.2.0]octane Core?

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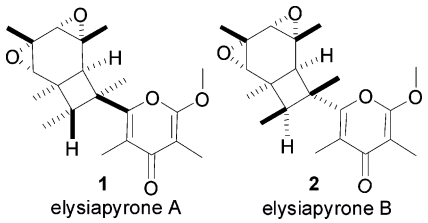
ABSTRACT



Biogenetically interesting polypropionate-derived metabolites 1 and 2, featuring an unprecedented skeleton, have been isolated from the sea slug *Elysia diomedea*. Their enantiomeric character indicates that the current spontaneous electrocyclization cascade biogenetic hypothesis for the bicyclo[4.2.0]octane core must be enzymatically aided. These compounds are isomeric with the 15-nor-9,10-deoxytridachione/15-norphotodeoxytridachione pair of metabolites and encourage speculation about their biosynthetic relationship.

The sacoglossan *Elysia* (= *Tridachiella*) *diomedea* (Bergh) (Mollusca, Opisthobranchia, Sacoglossa) belongs to one of the few metazoan groups that retain chloroplasts harvested from siphonous marine algae to carry out photosynthesis in their own tissues.[1] While the plastids remain photosynthetically active they provide an energy supply, and in some cases, the released organic carbon can totally sustain the sea slugs for several months in the absence of an algal food source,[2] giving rise to the issue of whether lateral gene transfer has occurred between eukaryotic alga and animal

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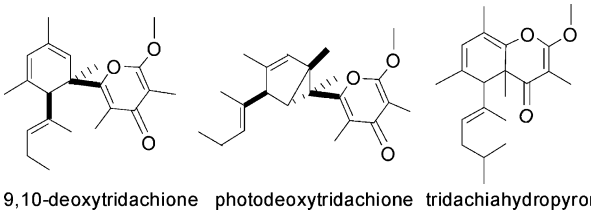
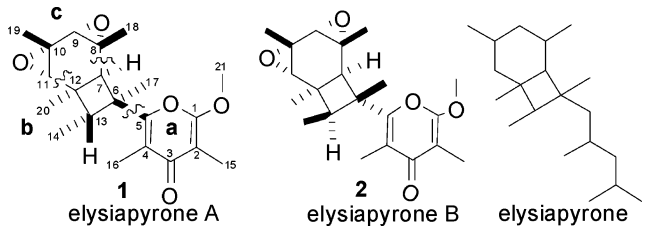
nucleus.[3] Faced with life lacking a protective shell, some sea slugs evolve chemical defense mechanisms against predators based on diet-derived and, mostly, endogenous feeding deterrents[4] produced by joining several propionate units, two of which are involved in the formation of a γ-pyrone ring that has become a common feature of the Elysiidae family.

Naturally occurring metabolites with a backbone of six, seven, or eight propionate units, sometimes elongated by a two-carbon appendage (acetate chain starter unit), have been isolated from the genus *Elysia* and can be grouped within the structural types represented by 9,10-deoxytridachione,[5] photodeoxytridachione,[6,7] or tridachiahydropyrone.[8] Moreover, pairs of cyclohexadiene/bicyclo[3.1.0]hexane isomers containing six, seven, or eight propionate units have been isolated, for instance: 15-nor-9,10-deoxytridachione/15-norphotodeoxytridachione[7,9] (six), 9,10-deoxytridachione/photodeoxytridachione[5−7] (seven), and elysione/crispatene[5,10] (eight propionate units). This isomerization has been proven biosynthetically in vivo as well as in vitro, and it was proposed to proceed as a [$_{\sigma}2_a + {}_{\pi}2_a$] photochemical rearrangement.[6] Nevertheless, it has been suggested that the bicyclic core may also originate from its corresponding acyclic precursor either by thermal [$_{\pi}4_a + {}_{\pi}2_a$][11] or intramolecular photochemical [$_{\pi}4_s + {}_{\pi}2_a$][12] Diels−Alder reactions.



Our interest in benthic organisms from both sides of the isthmus of Panama[13−15] prompted us to study the Pacific sacoglossan *E. diomedea*. In this paper, we report the isolation of two complex polyketide derivatives elysiapyrone A **1** and elysiapyrone B **2** containing a bicyclo[4.2.0]octane core that features an unprecedented carbon skeleton, elysiapyrone. These compounds were isolated together with the known metabolites tridachione, 9,10-deoxytridachione,[5] iso-9,10-deoxytridachione,[7] and 15-norphotodeoxytridachione.[9]



Elysiapyrone A, **1**, was isolated as a colorless oil, $[\alpha]_D^{25} = +37°$ (*c* 0.08, $CHCl_3$). The molecular formula suggested by the HREIMS, $[M]^+$ 360.1883 (calcd for $C_{21}H_{28}O_5$, 360.1936), indicates eight degrees of unsaturation. The ^{13}C NMR data showed signals for 21 carbons, and DEPT spectral data indicated the presence of 8 methyl groups, 4 methine carbons (2 bearing oxygen), 4 quaternary olefinic carbons, 1 carbonyl, and 4 sp^3 quaternary carbons. The ^1H NMR spectrum showed the following 8 methyl group signals: 1 methoxy group (δ 3.98), 2 olefinic methyls (δ 1.89 and δ 1.93), 4 methyls on quaternary carbon (δ 1.03, δ 1.20, δ 1.38, δ 1.55), and a secondary methyl group (δ 1.19 d, $J =$ 7.5 Hz). Additional signals at δ 2.43 (q, $J = 7.5$ Hz) for a methine quartet (COSY coupled with a secondary methyl group) as well as three methines, two of them bearing oxygen (δ 2.39, s; δ 3.18, s), complete all the protons of **1**.

All C−H correlations for **1** were detected in the HSQC spectrum. The comparison of the chemical shifts of carbons C-1−C-5 and the H_3-15, H_3-16, and H_3-21 methyls with those of the corresponding polypropionate congeners[7] indicates the presence of an α-methoxy-γ-pyrone fragment **a**. This fragment was confirmed by the following HMBC correlations: H_3-15, H_3-16/C=O; H_3-15, H_3-21/C-1 and H_3-16/C-4, C-5.

Considering that one carbon of the molecular formula corresponds to a methoxy group, the remaining 20 carbons account for a maximum of 6 propionate units, 2 of them forming a γ-pyrone ring. The IR spectrum suggested that no oxygen of the molecular formula was a hydroxyl group and revealed absorption for a dienone at 1548 $cm.^{-1}$ Since the γ-pyrone ring contains all the unsaturations and 5 of the residual 13 carbons are methyl groups, the molecule must be tricyclic and the characteristic cyclohexane side-chain must form part of a ring.

In the absence of hydroxyls, the remaining two oxygens must be epoxidic, which is concordant with the ^{13}C NMR chemical shifts for each methine bearing oxygen (δ 61.1 and 62.1). The vicinal bis-epoxide fragment **c** was confirmed by a HMBC experiment, particularly by the long-range correlations H_3-18, H_3-19/C-9; H-9/C-11 and H_3-19/C-11.

The key to establishing the presence of a cyclobutane ring fragment **b** was the HMBC correlations of the H_3-17 with C-6, C-7, and C-13 crossed with H_3-14/C-6 and H_3-20/C-7, C-13. In addition to biogenetic considerations, the HMBC correlations of C-5 with H-7, H-13, H_3-17 established the

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regiochemistry of the γ-pyrone ring on fragment **b**. Finally, fragments **b** and **c** were linked through the selected HMBC correlations: H_3-18/C-7; H_3-20/C-11. This completes the planar structure of elysiapyrone A as depicted in **1**.

Elysiapyrone B, **2**, colorless oil, $[\alpha]_D^{25} = +225°$ (*c* 0.08, $CHCl_3$) HREIMS $[M]^+$ 360.1925 (calcd for $C_{21}H_{28}O_5$, 360.1936) possesses the same molecular formula, and similar IR spectrum and ^1H NMR data, as **1**, suggesting that the difference between both compounds must be stereochemical in nature.

NOESY experiments, Figure 1, aided in establishing the

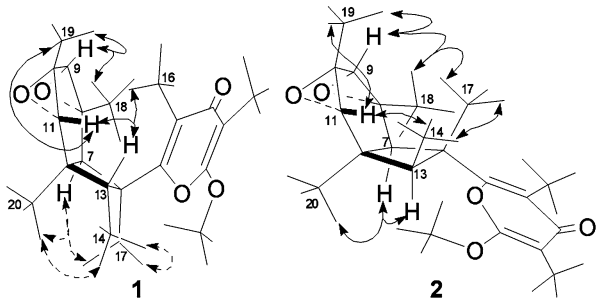


Figure 1. Selected NOEs of **1** and **2**.

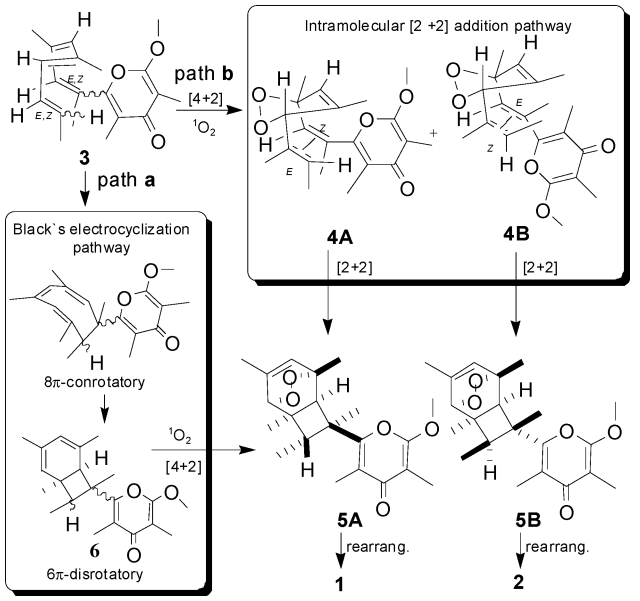
stereochemistry of the substituents on the bicyclo[4.2.0]-octane core of **1**. NOESY experiments of **1** revealed a syn-periplanar relationship between three vicinal angular methyl groups (Me-14, Me-17, Me-20); thus, the six-membered rings are face-to-face on the opposite side of the cyclobutane ring. Additional NOEs, particularly H_3-18, H_3-19/H-9 and H_3-19, H-13/H-11, fixed the stereochemistry of the epoxide rings.

To obtain an energy-minimized conformation of **1** to justify the observed NOE between H_3-16 and H-13, molecular mechanics energy minimization was performed. The minimized structure **1**, Figure 1, led to a H_3-16−H-13 interatomic distance (2.071 Å) appropriate for the strong NOE observed.

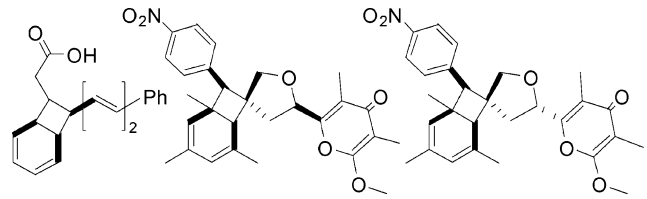
In elysiapyrone B, **2**, Figure 1, the NOEs H-7/H_3-20, H-13, and H_3-17/H_3-14 indicate an antiperiplanar relationship between the γ-pyrone ring and both the cyclohexane ring and methyl groups H_3-14 and H_3-17. The NOEs indicate that the stereochemistries of the epoxide rings were identical to those in compound **1**. The minimized conformation **2** is also in accord with the NOEs depicted in Figure 1. Thus, the overall stereochemistry of elysiapyrone A is 6**S*,7**R*, 8**R*,9**R*,10**S*, 11**S*,12**R*,13**R*, and the stereochemistry of elysiapyrone B is 6**R*,7**R*,8**R*,9**R*,10**S*,11**S*,12**R*,13**S*.

The core bicyclo[4.2.0]octane skeleton has been previously identified in endriandic acids, a series of natural compounds isolated by Black et al. from an Australian *Laureacea*.[16] In 1980, Black proposed a biogenetic hypothesis[17] for the formation of the bicyclo[4.2.0]octane moiety with the aim of justifying the unusual finding that endriandic acids occur in nature in racemic rather than enantiomeric form. Black's suggestive hypothesis postulated successive nonenzymatic electrocyclization reactions (path **a**, Scheme 1) from a

Scheme 1. Possible Biogenetic Pathways for **1** and **2**



polyunsaturated achiral substrate. This hypothesis was later experimentally supported by a biomimetic synthesis[18,19] of these compounds and also inspired a biomimetic synthetic strategy toward the bicyclo[4.2.0]octane core of SNF4435 C and SNF4435 D,[20] two natural products recently isolated from a strain of *Streptomyces spectabilis*.[21]



The genesis of elysiapyrones A and B may follow Black's biogenetic route leading to **6** by an 8π conrotatory followed by a 6π disrotatory electrocyclization reaction from the achiral polyene precursor **3**, path **a**, Scheme 1. This pathway would provide the bicyclo[4.2.0]octane network of **6** and fits with the finding of the diastereomeric mixture of **1** and **2**. However, unlike the endriandic acid family, which occurs

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in racemic form, elysiapyrone metabolites are optically active, thus indicating that the electrocyclization cascade must be enzymatically assisted, as it should be for the optically active SNF4435 C and D.

We think that path **b**, Scheme 1, involving an intramolecular [2 + 2]-addition of the endoperoxides **4A** and **4B**, providing the carbobicyclic network of **5A** and **5B**, respectively, should not be excluded. The known facile rearrangement of endoperoxide into vicinal *syn*-epoxides[22] will afford **1** and **2**. Moreover, some naturally occurring endoperoxide polypropionate pyrones have been isolated from sacoglossans.[23]

Chloroplast redox signals have widespread repercussions for gene expression, helping to acclimate the organism to stressful conditions. Changes in environmental factors such as light intensity, temperature, salinity, and nutrient deficiency may limit CO_2 fixation, producing an imbalance between the light energy absorbed and the energy utilized through metabolism. This can result in an overreduction of electron-transport carriers, sensitizing photosystems (PSs) to photoinhibition, which leads to damaging reactive oxygen species (ROS).[24] Singlet oxygen O_2 ($^1\Delta_g$) is one such reactive oxygen species.

It is difficult to present evidence for the occurrence of singlet oxygen in a living whole body because of its short lifetime in a water system, i.e., 1−2.5 μs. Trapping methods with chemicals such as β-carotene that specifically react with singlet oxygen succeeded in isolating and identifying β-carotene 5,8-endoperoxide and β-carotene 5,6-epoxide ex vivo and in vivo systems.[25] On the basis of these precedents, the alkenyl open-chain of **3** assembled using polyketide synthase complex (PKS) may well also be a chemical quencher of biologically excited singlet oxygen, leading to chiral endoperoxides **4A** and **4B** in an overall 1,4-addition, path **b**, Scheme 1. Intramolecular [2 + 2]-cycloadditions, although infrequent in marine organisms, occur in algae,[26] sponges,[27] marine ciliates,[28] and corals.[29] Thus, acting on an unsaturated polyketide substrate **3** may be an alternative pathway for elysiapyrones.

Since toxic levels of ROS can damage the PS protein-dependent chloroplast activity,[30] the generation of elysiapyrones by either path **a** or path **b** would alleviate the symbiotic plastids from light-induced damage, conferring advantages in adaptive responses of *E. diomedea*.

Elysiapyrones, with their bicyclo[4.2.0]octane core, are structural isomers of both 15-nor-9,10-deoxytridachione and 15-norphotodeoxytridachione (six propionate units) and encourage speculation about their biosynthetic relationship, raising the question of why bicyclo[4.2.0]octane compounds formed by seven or eight propionate units have not been isolated from sacoglossans. It may be that elongated polyene chains with seven and eight propionate units cannot meet the appropriate geometry requirements to undergo a 8π conrotatory electrocyclization reaction or to undergo an intramolecular [2 + 2]-addition. Or it may be simply that they are produced in such a small amount as to prevent isolation.

The intriguing biosynthesis of the potent immunosupressants SNF4435 and elysiapyrones, both polypropionate-derived pyrones, poses the question of whether **1** and **2** are microbial in origin and add attractiveness as interesting biological, biomedical, and synthetic targets.

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Supporting Information Available: Spectral data and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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