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Luffolide, a novel anti-inflammatory terpene from the sponge *Luffariella* sp.

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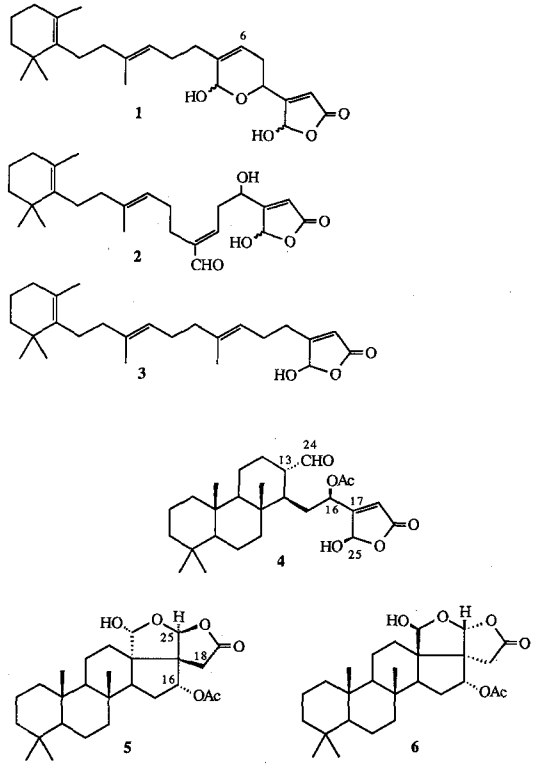
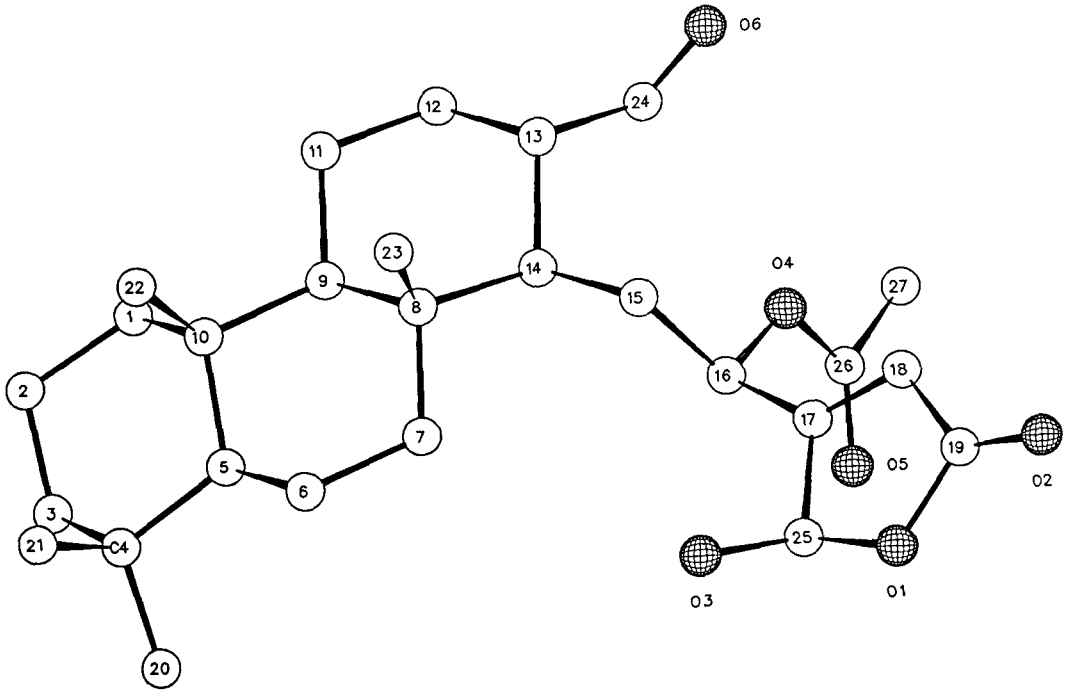
Summary. Luffolide (**4**) is a minor metabolite of the sponge *Luffariella* sp. from Palau. The structure of luffolide was determined by single crystal X-ray analysis. Luffolide is relatively unstable and undergoes a complex cyclization reaction to give the hexacyclic products **5** and **6**. Luffolide (**4**) has some of the anti-inflammatory properties of manoalide (**1**): this may help to define the chemical reaction between manoalide (**1**) and phospholipase A_2.
Key words. Luffolide; *Luffariella* sp.; X-ray structure determination; anti-inflammatory; phospholipase A_2.

Sponges of the genus *Luffariella* have provided a series of sesterterpenes that are potent anti-inflammatory agents. Both manoalide (**1**) and seco-manoalide (**2**), isolated from the sponge *Luffariella variabilis*[3], are irreversible inhibitors of the enzyme phospholipase A_2 (PLA_2)[4] while luffariellolide (**3**) is a partially reversible PLA_2 inhibitor that was isolated as a major metabolite of a Palauan species of *Luffariella*[5]. The same specimen of *Luffariella* sp. also contained a minor polycyclic sesterterpene, luffolide (**4**), that also inhibits hydrolysis of phosphatidylcholine by bee venom PLA_2.

The hexane-soluble material from a methanol extract of *Luffariella* sp. was chromatographed on silica gel, using solvents of increasing polarity from hexane to ethyl acetate, to obtain two antimicrobial fractions containing luffariellolide (**3**, 15.4% dry weight) and luffolide (**4**, 0.0037% dry weight). Quite unexpectedly, luffolide (**4**) crystallized from $CDCl_3$ in an NMR tube to produce colorless rods, mp. 123 °C, and a crystal was reserved for an X-ray study. Crystals formed in space group $P2_1$ with $a = 8.317(2)$, $b = 20.238(7)$, $c = 10.259(2)$ Å, and $\beta = 111.30(1)°$ with an asymmetric unit of $C_{28}H_{40}O_6 \cdot CHCl_3$. All diffraction maxima with $2\theta < 114°$ were collected on a computer-controlled four-circle diffractometer using 1° ω-scans and CuK$\bar{\alpha}$ radiation (1.54178 Å). Of the 2257 symmetry unique reflections, 1931 (94%) were judged observed ($|F_o| \geqq 3\sigma(F_o)$) after correcting for background, Lorentz, and polarization effects[6]. The structure was solved routinely using direct methods and was refined, using anisotropic nonhydrogens and fixed isotropic hydrogens, to a conventional crystallographic residual of 0.083 for the observed reflections. Additional crystallographic details have been deposited at the Cambridge Crystallographic Data Centre. The figure is a computer-generated perspective drawing of the final X-ray model of luffolide (**4**). Hydrogens have been omitted from the drawing, and the absolute configuration was not established by the X-ray experiment. The





A computer-generated perspective drawing of the final X-ray model of luffolide (**4**). Hydrogens are omitted for clarity and no absolute configuration is implied.

C14-C25-C16-C17 fragment is in the completely extended or antiperiplanar conformation with a central torsional angle of 170°. The butenolide ring is planar with all endocyclic torsional angles less than 3°. The three six-membered rings are all in the chair conformation with the bridgehead substituents having axial orientations. The aldehyde group at C13 is equatorial.

The spectral data of luffolide (**4**) were completely in accord with the X-ray structure. The infrared spectrum exhibited bands at 3600–3300 (hydroxyl), 1765 (butenolide), 1740 (acetate), 1720 (aldehyde) and 1600 cm^{-1} (olefinic). The ^1H NMR spectrum contained signals at δ 9.44 (d, 1 H, J = 5 Hz, H-24), 6.09 (br s, 1 H, H-18), 5.96 (s, 1 H, H-25), 5.29 (dd, 1 H, J = 10.8, 2.8 Hz, H-16), 3.49 (s, 1 H, OH), 2.15 (s, 3 H, OAc), 0.87 (s, 3 H), 0.85 (s, 3 H), 0.83 (s, 3 H), 0.81 (s, 3 H).

During an attempt to run a ^{13}C NMR spectrum overnight in $CDCl_3$ solution, luffolide (**4**) decomposed into two new products **5** and **6** that were isomeric with luffolide (**4**). The isomeric lactones **5** and **6** were separated by LC on Partisil. The infrared spectra of lactones **5** and **6** were similar, each having a band at 1795 cm^{-1}, due to a saturated γ-lactone ring, and 1745 cm^{-1}, due to the acetate carbonyl, but did not contain a saturated aldehyde band. The ^1H and ^{13}C NMR spectra of **5** and **6** also confirmed the absence of an aldehyde group. The ^1H and ^{13}C NMR spectra of lactone **5** contained signals due to a methine carbon bearing an acetate group [δ 5.41 (d, 1 H, J = 3 Hz), 2.15 (s, 3 H); 73.0 (d), 21.4 (q)], two acetal or hemiacetal groups [δ 6.22 (s, 1 H), 5.61 (d, 1 H, J = 1 Hz); 113.0 (d), 103,2 (d)] and an isolated methylene group adjacent to a carbonyl [δ 2.93 (d, 1 H, J = 18 Hz), 2.78 (d, 1 H, J = 18 Hz)]. A similar array of signals were observed in the ^1H and ^{13}C NMR spectra of lactone **6**. We therefore proposed that the lactones were two geometrical isomers formed by cyclization between C-13 and C-17 in a Michael fashion to form two pentacyclic spiro-lactones, followed by hemiacetal formation to give the two hexacyclic lactones **5** and **6**. This sequence of reactions requires the formation of four new chiral centers; but, due to the constraints imposed by ring formation, only four isomers are possible. Although isomerization at the hemiacetal carbon is possible, the fact that the two pure isomers would not equilibrate tended to eliminate this possibility. We therefore propose the structures shown for lactones **5** and **6**. The two structures could be differentiated by NOEDS measurements: irradiation of the H-16 signal at δ 5.17 in the spectrum of lactone **6** caused a 24% enhancement of the H-25 signal at 5.72 and an 8% enhancement of the H-18β signal at 2.70 while in the spectrum of lactone **5**, irradiation of the H-16 signal at δ 5.41 caused a 6% enhancement of the H-18α signal and no enhancement of the H-25 signal. The relative configuration at C24 remains uncertain although the complete absence of nuclear Overhauser enhancements involving the H-24 signals suggests that H-24 is *trans* to H-25.

An initial evaluation that was limited by the paucity of material indicated that luffolide (**4**) has some of the pharmacological properties of manoalide (**1**). Luffolide is an

effective antagonist of PMA-induced mouse ear inflammation (Dose PMA = 2 µg/ear): topical application of luffolide at 50 µg/ear significantly inhibited inflammation (55% inhibition). The hydrolysis of phosphatidylcholine by bee venom PLA_2 is completely inhibited by luffolide at a concentration of 3.5 µM (100%). These data may shed some light on the chemical structure of the covalent adduct between manoalide (**1**) and PLA_2. It has been proposed[7] that a lysine residue of PLA_2 reacts at C-6 of manoalide in a Michael addition to the α,β-unsaturated aldehyde that results from opening the pyran ring. The fact that luffolide (**4**) has a saturated aldehyde in place of the 'unsaturated aldehyde' of manoalide suggests that the lysine residue may react at the aldehyde group to form, at least initially, a Schiff base[8].

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Protective chemicals in caterpillar survival

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Summary. Using two species of caterpillars; larvae of the swallowtail butterfly, which produce large amounts of iso-butyric and 2-methylbutyric acids, and larvae of the puss moth, which produce no such detectable volatile compounds, it was shown that the insect which utilizes a chemical defense is better protected from its natural enemy, a praying mantis.
Key words. Iso-butyric and 2-methylbutyric acids; *Papilio memnon heronus; Cerura erminea menciana; Hirodula patellifera;* osmeteria; mimicry.

In Taiwan, during the summer, we can find two types of lepidopterous larvae with eversible tissues used in defense behavior. One, the caterpillar of the swallowtail butterfly *Papilio memnon heronus* Frunstorfer, has an eversible cervical gland or osmeterium in an anterior position on the head, whereas the larva of the puss moth, *Cerura erminea menciana* Moore, has an eversible tube in a posterior position on the abdominal end (fig., a and b). Although in response to irritation the hidden structures of both these species will evert, and the same bright red color becomes visible, only the former species seemed capable of producing a strong odor. Therefore, we hypothesized that the larva of the puss moth may mimic the caterpillar of the swallowtail butterfly, to avoid their common predator, the praying mantis *Hirodula patellifera* Serville. Close mimicry, where a harmless species mimics the warning coloration of one that harms or sickens predators, is very common in caterpillars[1, 2], so these 2 insects are good subjects for experiment. The results we report here, however, show that in the case of the puss moth, caterpillar mimicry is not a valuable protection against one of its natural predators, the praying mantis. Caterpillars of *Papilio memnon heronus* were collected from the Yangmingsan area in the vicinity of Taipei. Secretions discharged from the osmeterial glands of 25 individuals were dissolved in methylene chloride. One µl of the extract, corresponding to one tenth of an individual discharge, was injected into a 3% OV 101 capillary