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STEREOCHEMISTRY OF ICHTHYOTOXIC DIACYLGLYCEROLS FROM OPISTHOBRANCH MOLLUSCS

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Abstract. The absolute configuration of verrucosin-A and -B and of umbraculumin-A and -C has been established by chemical correlation with 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerol.

The defence chemicals isolated from opisthobranchs, shell-less marine molluscs, include feeding deterrents, ichthyotoxins and alarm pheromones. Unusual diacylglycerols are the most powerful among the ichthyotoxins so far isolated from Mediterranean opisthobranchs[2]. To this class of compounds belong the verrucosins, isolated from the nudibranch *Doris verrucosa*[3] and the umbraculumins isolated from the opisthobranch *Umbraculum mediterraneum*[4]. We wish to report now the determination of the absolute configuration of these compounds which was not investigated during the previous structural work.

The strategy for the determination of the absolute configuration consisted in the transformation of the naturally occurring diacylglycerols in to the diastereomeric urethanes **1** and **2** which were compared (HPLC[5]; ^{1}H-NMR[6]) with those prepared by reaction (75°; 18 hr.) of commercially available[7] 1,2- and 2,3-O-isopropylidene-*sn*-glycerol, respectively, with (*R*)-(-)-1-(1-naphthyl)ethyl isocyanate The method was tested with 1,2-dipalmitoyl-*sn*-glycerol[8] in the conditions used for the umbraculumins (see below) which afforded mainly (92%) the urethane **1**.

Verrucosins. The relative stereochemistry of verrucosin-B (**4**) was determined by X-rays; verrucosin-A (**3**) arises, at least in part, by migration of the acetyl group of **4**[3]. To establish the acyl substitution at the two enantiotopic -CH_2O- positions in **3**, the different reactivity of the two ester functions was exploited. Reaction of **3** (38 mg) with 2,2-dimethoxypropane (2 ml; 18 hr., r.t.) in the presence of a crystal of *p*-TsOH, resulted in the selective cleavage of the acetate affording two acetonides (21 and 2.7 mg), both retaining the diterpenoidic acyl residue, which were separated by silica gel chromatography. Reduction of the major acetonide ($LiAlH_4$, THF; 3.5 hr., reflux), destruction of $LiAlH_4$ excess with little MeOH and subsequent reaction *in situ* with the isocyanate afforded the urethane **2**, while the minor one (2.7 mg) in similar conditions afforded **1**. Thus, taking into account the resistance of the diterpenoidic acyl residue to the reaction conditions, verrucosin-A should be naturally a mixture of two diastereomers, the major one being represented by **3**. A similar sequence of reactions on verrucosin-B (**4**) afforded the urethane **2** contaminated only by trace amounts of **1**. Thus the relative stereochemistry shown in **4** is also the absolute stereochemistry.

Umbraculumins. A 4 mg sample of umbraculumin-A (**5**) was transformed into the corresponding urethane (15 µl isocyanate, 0.5 ml toluene; 76°C; 48 hr.) which was isolated by preparative TLC. Methanolysis of the latter (MeOH, Na_2CO_3) and reaction with 2,2-dimethoxypropane (0.5 ml; p-TsOH; 48 hr.; r.t.) afforded pure **1**. Similarly, umbraculumin-C (**6**; 2 mg) afforded the same urethane **1**, thus establishing that **5** and **6** are both 1,2-*sn*-diacylglycerols.

The approach described here could find wide application for the stereochemical analysis of suitable mono- and diacylglycerols. A minor drawback is the difficult chromatographic separation of the diacylglyceryl urethane from unreacted isocyanate and by-products when a small sample (1-2 mg) of diacylglycerol is processed.

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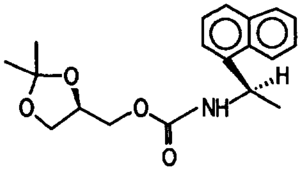

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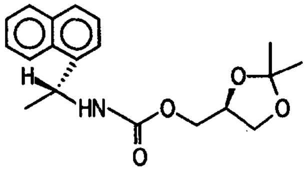

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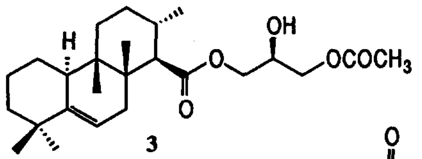

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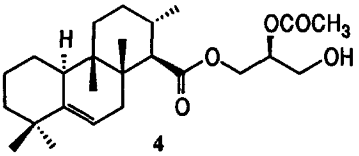

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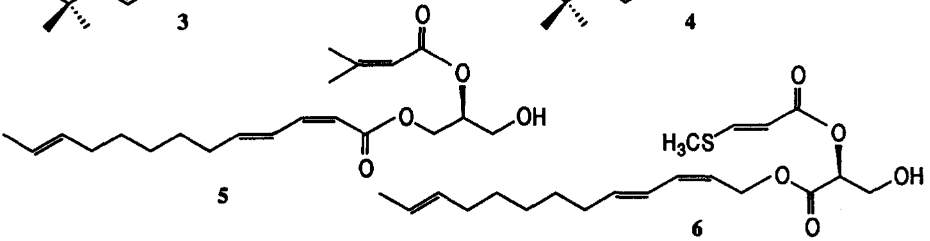

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3. G. Cimino, M. Gavagnin, G. Sodano, R. Puliti, C.A. Mattia and L. Mazzarella, *Tetrahedron 44*, 2301, 1988.
4. G. Cimino, A. Crispino, A. Spinella and G. Sodano, *Tet. Letters 29*, 3613, 1988.
5. Two μ-Porasil columns connected in series; n-hexane/2-propanol, 99:1.
6. The major distinguishing features of the ^{1}H-NMR spectra of **1** and **2** (500 MHz; CD_3OD) resided in the chemical shift values of the acetonide methyls (δ 1.38 and 1.33 in **1**; δ 1.41 and 1.35 in **2**).
7. Sigma. The commercial samples were found contaminated each one by the other enantiomer, after transformation in the corresponding urethanes **1** and **2**. **1** contained 15% of **2**, while **2** contained 32% of **1**. Pure **1** and **2** were obtained by preparative HPLC.
8. Sigma.

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