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Terpestacin, a Novel Syncytium Formation Inhibitor, Isolated from *Arthrinium* Species

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Keywords : Terpestacin, Complete structure, *Arthrinium* sp., Syncytium formation inhibitor, Biosynthesis.

Abstract : Terpestacin, a novel syncytium formation inhibitor in HIV infection, was isolated from *Arthrinium* sp. metabolites. The structure of terpestacin including the absolute stereochemistry was determined on the basis of spectroscopic analysis and chemical reactions. The biosynthetic pathway of terpestacin was also determined.

In our syncytium formation[1] inhibitor screening, a new sesterterpene antibiotic named as terpestacin (**1**) was isolated from the fermentation metabolites of a fungal strain *Arthrinium* sp.[2]. We report herein determination of its complete structure and biosynthesis from geranylfarnesyl pyrophosphate.

The mycelial cake of *Arthrinium* sp. was extracted with MeOH and the extract was concentrated and re-extracted with EtOAc. **1** was isolated from the extract by consecutive column chromatography on silica gel, reversed phase silica gel and Sephadex LH-20, and crystallized from aqueous methanol to yield monoclinic crystals: m.p. 172 – 173°C, $C_{25}H_{38}O_4$, HRFAB-MS; calcd *m/z* 402.2752; found *m/z* 402.2761, $[\alpha]_D^{22}$ +26° (C 0.5, $CHCl_3$), UV λ_{max} nm (ε) 264(10800) in MeOH, 298(8500) in 0.01N NaOH-MeOH, IR (KBr) cm^{-1} 3350, 1690, 1645, 1450, 1405, 1040, 1020.

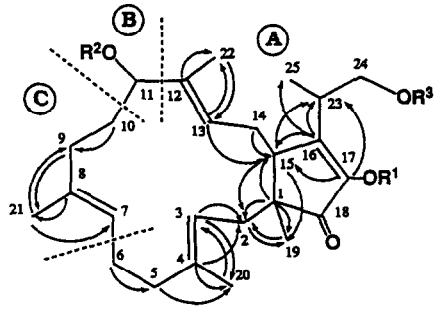
Seven degree of unsaturation in **1** was deduced to be one carbonyl (δ 207.2), four double bonds and two rings from ^{13}C NMR. The four oxygen atoms were determined to be one carbonyl and three hydroxyl groups based on ^1H NMR. Triacetyl-**1** (**2**, EI-MS *m/z* 528 M^+) was obtained upon acetylation confirming the existence of the three hydroxyl groups. The UV spectrum of **1** (264 nm) and its shift by acetylation (**2**, 235 nm) suggested the presence of a 3-alkyl-2-hydroxy-2-cyclopenten-1-one[3] in **1**. Authentic 2-hydroxy-3-methyl-2-cyclopenten-1-one[4] (258 nm) and its acetate (232 nm) showed similar UV and ^{13}C NMR spectra to those of **1** and **2**, respectively.

The ^1H-^1H COSY exhibited a linkage of δ 1.16 (C25-methyl)-2.50(C23-methine)-3.48 & 3.60 (C24-

methylene) protons. The ^{13}C (δ 63.8) and proton chemical shifts of C24-methylene suggested that C24 bore a hydroxyl group. In the long range HETCOR experiments, 23-H showed a contour with δ 150.3 (C16) which also related to δ 2.62 (15-H). Three methyls, C20 (δ 15.3), C21 (δ 15.5) and C22 (δ 10.4), attached to the vinyl carbons were assigned to be on C4, C8 and C12, respectively, by long range HETCOR, and their chemical shifts established that the three double bonds have E-configurations [5]. The extensive ^1H-^1H COSY, long range HETCOR and UV spectral analyses allowed to assign the three partial structures A, B and C (Fig. 1). 2D-INADEQUATE of **2** revealed connectivity between C10 and C11, C11 and C12, and C 6 and C7 establishing the skeleton of **1** as Fig. 1.

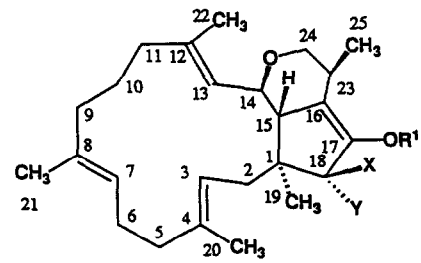
A strong NOE was observed between C19-methyl (δ 1.00) and C14-α-proton (δ 1.92), and the later was coupled with C15-methine (δ 2.71) by J=11.2Hz, indicating a trans relationship of 5- and 15-membered rings.

Fig. 1 ^1H-^{13}C Long Range Connectivities of **1** and Partial Structures A, B and C



Compound	R^1	R^2	R^3
1	H	H	H
2	Ac	Ac	Ac
4	H	H	Trityl
5a	H	(*R*)-O-Methylmandeloyl	Trityl
5b	H	(*S*)-O-Methylmandeloyl	Trityl

Fig. 2



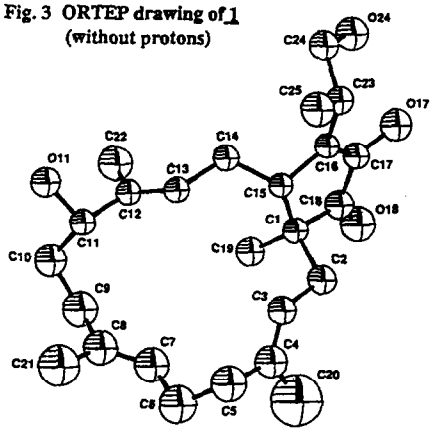
Compound	R^1	X	Y
3	H	=O (X & Y)	
6	Me	=O (X & Y)	
7a	Me	OH	H
7b	Me	H	OH
8a	Me	Benzoyloxy	H

Relative stereochemistry of C23 was determined by the analysis of **3** ($C_{25}H_{36}O_3$, Fig. 2) formed on treatment of **1** with *p*-TsOH in CH_2Cl_2. ^1H-^1H COSY exhibited that a methine proton at δ 3.68 (14-H) coupled with vinyl proton at δ 5.45 (13-H, J=7.3 Hz) and methine proton at δ 2.72 (15-H, J=10.3 Hz) indicating a trans relationship of 14-H and 15-H. C14 carbon assigned as δ 78.4 by HETCOR was determined to be an oxygene-bearing methine carbon which correlated to 24-Hα (δ 3.85) in long range HETCOR. NOE between 15-methine and 25-methyl protons in **3** confirmed the assigned relative stereochemistry of the four asymmetric carbons (C1, C14, C15 and C23) of **3**.

The allylic benzoate chirality method[6] was applied for determination of the absolute configurations of C1, C15 and C23. When **3** was treated with diazomethane (**6**) and then reduced with LAH, two epimeric alcohols **7a** (major) and **7b** (minor) were obtained. A strong NOE between C18-methine proton and C19-methyl protons (NOE difference spectroscopy) in **7a** indicated that C18-hydroxyl group and C19-methyl were

in a trans orientation. No NOE was observed between those protons in **7b**. The CD spectrum of the C18–O–benzoate of **7a(8a)** exhibited a negative Cotton effect (λ_{max} 225nm, $\Delta\varepsilon$ = –22.3), indicating that the C18–O–benzoate and the Δ^{16} double bond were in anticlockwise direction.

Fig. 3 ORTEP drawing of **1** (without protons)

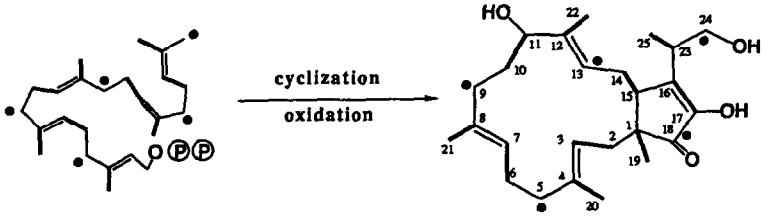


For determination of the absolute stereochemistry at C11 by Trost's method[7], the primary hydroxyl group at C24 of **1** was protected by a trityl group (**4**) and then converted to C11(*R*)– and –(*S*)–O–methylmandelates (**5a** and **5b**, respectively). Upon calculation of $\Delta\delta$ (δS– δR, in ppm), the positive values of C9–methylene (α, +0.23 & ß, +0.23), C10–methylene (+0.20) and C21–methyl (+0.08), and the negative values of C13–methine (–0.09), C14–methylene (α, –0.11 & ß, –0.19) and C22–methyl (–0.21) established 11*S* configuration. Therefore the absolute stereochemistry of **1** was established to be 1*S*, 11*S*, 15*R* and 23*S*.

A single–crystal X–ray diffraction analysis[8] of **1** determined the structure as Fig. 3, unambiguously establishing the structure and absolute stereochemistry obtained by the spectral analysis.

[1–^{13}C]–, [2–^{13}C]–, and [1,2–$^{13}C_2$]–acetates were fed to the culture of *Arthrinium* sp., and ^{13}C–enriched antibiotics were isolated. Their ^{13}C NMR spectra showed that five isoprenoid units condensed in "head–to–tail" fashion and the biogenetic pathway of **1** from geranylfarnesyl pyrophosphate was deduced as in scheme 1. The absolute configurations at the bridge head carbons of **1** (C1 and C15) were opposite to those of two biogenetically related sesterterpenes, retigeranic acid [9] and variculanol[10].

Scheme 1. Biosynthesis of **1**



Terpestacin (**1**)

Terpestacin (**1**) showed potent syncytium formation inhibitory activity and the potency (IC_{50}: 0.46μg/ml) was much higher than that of dextran sulfate.

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