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Preliminary note

Conformational effects in the biological activity of fluorinated analogs of β-thujaplicin

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Abstract

Fluorination of β-thujaplicin with diethylaminosulfur trifluoride (DAST) gave 2-fluoro-4-isopropyltropone and 2-fluoro-6-isopropyltropone (1:1 mixture; 60%). The relationships between the antibacterial activities and conformations of β-thujaplicin and its fluorinated analogs are discussed.

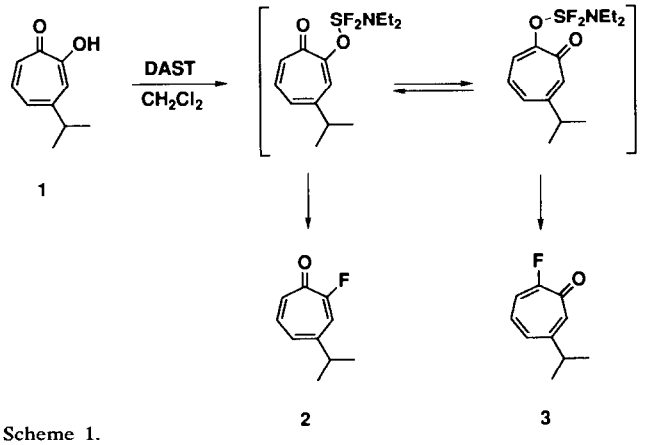
Keywords: Conformational effects; Biological activity; β-Thujaplicin; Fluorination; NMR spectroscopy; Semi-empirical MO calculations

Research on the incorporation of fluorine into organic molecules which can lead to profound and unexpected results on biological activity [1–4] has been extensive in recent years. The fluorine atom has been regarded as an isoelectric replacement for the hydroxy group [1]. However, few comparisons of molecules and their fluorine derivatives have included the relationship between structural conformations and biological activities. Accordingly, we are studying such relationships.

We describe herein the synthesis of fluorinated analogs of β-thujaplicin (**1**), 2-fluoro-4-isopropyltropone (**2**) and 2-fluoro-6-isopropyltropone (**3**), and the relationship between the antibacterial activities and structural conformations. β-Thujaplicin (**1**) (Hinokitiol) is a natural product isolated from the extract of certain plants of the Cupressaceae family [5]. Because of its antibial and antifungal activity [6], compound **1** has found use as a bactericide, fungicide, antiseptic, etc. [7]. Recently, the antimethicillin resistant *Stphylococcus aureus* (MRSA) activity of compound **1** has been discovered [6,7].

Fluorination of β-thujaplicin (**1**) (Hinokitiol) with diethylaminosulfur trifluoride (DAST) in methylene chloride gave a 1:1 mixture (60%) of the fluorinated analogs 2-fluoro-4-isopropyltropone (**2**) and 2-fluoro-6-isopropyltropone (**3**). They were thought to arise as shown in Scheme 1.

Compounds **2** and **3** could not be separated completely. For this reason, we distinguished compounds **2** and **3** via their corresponding ^1H and ^{19}F NMR spectra coupling constants, and hence the mixture was examined in vitro. A bacterium, *E. coli* K-12 or *S. aureus* ATCC-6538P, was cultivated until $OD^{660} = 0.1$ was indicated by the biophotorecorder, and was then diluted 10^3 times with sterilized water. One loop of the medium containing **1** and/or the mixture of **2** and **3** was inoculated onto Muller Hinton Broth (Difco), and the whole incubated



1 2 3

Scheme 1.

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for 15 h at 37 °C. The antibacterial activity was estimated by the inhibition of bacterial growth. The results obtained, as shown in Table 1, compare the fluorinated mixture with the established antimetabolite, β-thujaplicin (**1**). A comparison of the minimum inhibitory concentration (MIC) values (μg ml^{-1}) demonstrates the potential of the fluorinated analogs as antibacterials, although they were less effective than β-thujaplicin (**1**) (Hinokitiol) in inhibiting cell growth of *E. coli* K-12 or *S. aureus* ATCC-6538P.

Although the mechanism of the biological activity of β-thujaplicin (**1**) has not been clear until now, the inhibitory effects of **1** on several enzymes such as catechol-*O*-methyltransferase [8] and dopamine β-hydroxylase [9] have been reported. Biochemical isostericity between the tropolone ring and catechol or dopamine is an important factor in such effects. To make clear the relationships between structural conformations and antibacterial activity, we examined the geometry optimizations of compounds **1**, **2** and **3***. PM-3 calculations through multiconformer analysis of compounds **1**, **2** and **3**, as shown in Fig. 1, gave optimized conformations.

Based on the results of the PM-3 calculations, the ratio of the conformations **1a** and **1b** for β-thujaplicin (**1**) is **1a**/**1b** = 78:22. Obviously, the hydroxy group of **1a** and the carbonyl group can form a hydrogen bond which allows the oxygen atom of the hydroxy group to conjugate with the ring system of **1**, thus increasing the degree of delocalization. However, the optimized conformations of **2** and **3** are twist conformations, and hence the fluorine atoms cannot be regarded as isopolar and isosteric replacements for the hydroxy group.

These results indicate that the differences in antibacterial activities between β-thujaplicin (**1**) and its fluorinated analogs, **2** and **3**, are probably explained by the flat conformation of the tropolone ring in **1**, which matches well at the binding site, and/or the donating ability of the hydroxy group and the degree of π-electron delocalization [10,11].

Table 1
Minimum inhibitory concentration (μg ml^{-1})

Compound	Bacterium	
	E. coli	*S. aureus*
1	25	0.63
2+**3**	200	25

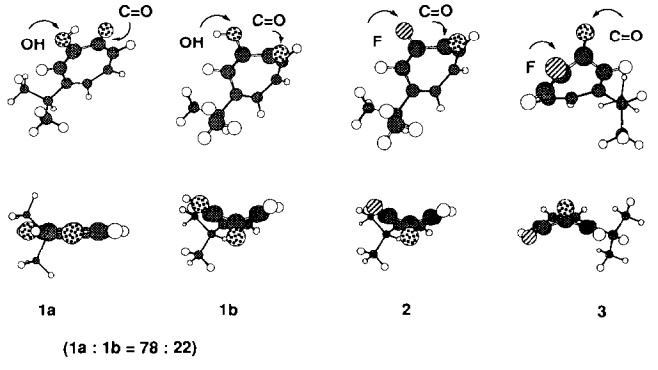

Fig. 1. The lowest energy conformers of compounds **1**, **2** and **3**.

*Calculations were performed by MOPAC v 6.10 (PM-3) program implemented using the CAChe Worksystem (Sony/Tektronix Corporation) for conformers obtained from the rigid search method followed by optimization by the eigenvector following the minimization (EF) method with the extra keyword 'PERCISE', the final gradient norm being less than 0.01 kcal $Å^{-1}$.

1. Experimental details

1.1. General procedures

All commercially available reagents were used without further purification. The ^1H (500 MHz; internal Me_4Si) and ^{19}F (470 MHz; internal C_6F_6) NMR spectra were recorded on sample solutions in $CDCl_3$ using a VXR 500 instrument. Yields quoted are those of the products actually isolated.

1.2. Fluorination of β-thujaplicin (1) (Hinokitiol)

To a flask containing DAST (0.81 g, 5 mmol) in methylene chloride (3 ml) under an atmosphere of nitrogen, a solution of **1** (0.82 g, 5 mmol) in methylene chloride (3 ml) was added slowly at −78 °C. After 30 min stirring at that temperature, the whole solution was allowed to warm to room temperature and stirred for 4 h. The mixture was quenched with water (5 ml). Oily materials were extracted with methylene chloride (5 ml×3) and the extract was dried ($MgSO_4$). On removal of the solvent, the residual oil was purified by flash chromatography on silica gel to afford a mixture of **2** and **3** (1:1, 0.51 g) in 60% yield. For the mixture: high-resolution MS: Calc. for $C_{10}H_{11}OF$, 166.0794. Found: 166.0787. For measurement of the NMR spectra, the mixture (6:4 or 4:6) as obtained by chromatography on silica gel was employed.

Compound **2**: ^1H NMR δ: 1.25–1.26 (6H, d, $J_{H,H}$ = 6.8 Hz); 2.82–2.84 (1H, sep, $J_{H,H}$ = 6.8 Hz); 6.97 (1H, ddd, $J_{H,H}$ = 1.0, 1.6, 8.8 Hz); 7.21 (1H, dd, $J_{H,H}$ = 1.6 Hz, $J_{H,F}$ = 22.2 Hz); 7.22 (1H, ddd, $J_{H,H}$ = 1.0, 12.5 Hz, $J_{H,F}$ = 8.8 Hz); 7.28 (1H, dd, $J_{H,H}$ = 8.8, 12.3 Hz) ppm. ^{19}F NMR δ: 67.2 (dd, $J_{F,H}$ = 8.4, 22.1 Hz) ppm from internal C_6F_6.

Compound **3**: ^1H NMR δ: 1.25–1.26 (6H, d, $J_{H,H}$ = 6.8 Hz); 2.82–2.84 (1H, sep, $J_{H,H}$ = 6.8 Hz); 6.95 (1H, dddd, $J_{H,H}$ = 0.6, 9.3, 11.4 Hz, $J_{H,F}$ = 4.2 Hz); 7.00 (1H, ddd, $J_{H,H}$ = 1.3, 1.5, 11.4 Hz); 7.14 (1H, ddd, $J_{H,H}$ = 1.3, 9.0 Hz, $J_{H,F}$ = 18.8 Hz); 7.27 (1H, ddd, $J_{H,H}$ = 0.6, 1.5 Hz, $J_{H,F}$ = 9.2 Hz) ppm. ^{19}F NMR δ: 65.5 (ddd, $J_{F,H}$ = 3.8, 9.2, 19.1 Hz) ppm.

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