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THE STEREOCHEMISTRY OF EUDISTOMINS C,K,E,F AND L

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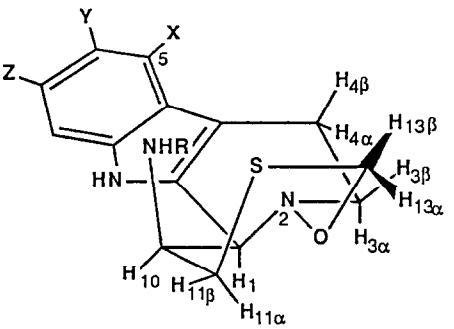
Summary: Eudistomin K has been isolated from the New Zealand ascidian *Ritterella sigillinoides* (Brewin 1956). Nmr studies have shown that the stereochemistry of the N-O bond is 2α, not 2β as previously suggested.

Much interest has been generated by the recent report on the structures of the eudistomins, a series of β-carboline derivatives isolated from the Caribbean ascidian *Eudistoma olivaceum*.[1] Some of these, in particular eudistomin K (**1a**) which contains the novel oxathiazepine ring, have shown potent *in vitro* antiviral properties, and are now the subject of considerable interest as synthetic targets (see e.g.[2]). During the course of investigations of the New Zealand marine biota for new classes of antiviral and antitumour compounds,[3] a compound which showed marked activity against *Herpes simplex* Type I and *Polio* vaccine Type I viruses *in vitro* was isolated from the compound ascidian *Ritterella sigillinoides* (Brewin 1956). Spectroscopic investigations of this compound revealed marked similarities with the data reported for eudistomin K (**1a**).[1] The *Ritterella* -derived compound has now been shown to be the salt (**1b**) of eudistomin K, but in the course of these studies it became necessary to revise the stereochemistry originally suggested for eudistomin K.

		X	Y	Z	R
Eudistomin K	1a	H	H	Br	H
	1b	H	H	Br	H_2^+
	1c	H	H	Br	Ac
Eudistomin C	2a	H	OH	Br	H
	2b	H	OAc	Br	Ac
Eudistomin E		Br	OH	H	H
Eudistomin F		H	OH	Br	$C_2H_3O_2$
Eudistomin L		H	Br	H	H



Extraction of 2.6 kg of *R. sigillinoides* with methanol/toluene followed by partitioning between ethyl acetate/water yielded an organic-soluble crude extract (5.3 g). This was subjected to bioassay-directed reverse phase flash chromatography[4] and preparative reverse phase high pressure liquid chromatography on C-18 packings with H_2O/CH_3OH/0.05%TFA mixtures to yield 33 mg of the trifluoroacetate salt (**1b**) of eudistomin K. The ^1H nmr spectrum of this salt was identical with that of the TFA salt prepared from *Eudistoma* -derived eudistomin K.[5] Treatment of both salts with IRA400 (^-OH form) each gave **1a** with identical ^1H nmr characteristics. Furthermore, acetylation of the salt **1b** from *R. sigillinoides* in pyridine/acetic anhydride afforded an acetyl derivative (**1c**) whose spectroscopic properties were identical with those reported[1] for the diacetyl derivative of eudistomin C (**2b**), except for those features associated with the differently substituted aromatic ring.

An examination of the ^1H, ^{13}C, homonuclear and heteronuclear correlated nmr spectra[5] of the *Ritterella* -derived salt (**1b**) showed that it was necessary to revise the reported assignments[1] for the protons at positions 3 and 4, and the carbons at positions 1 and 10. The full assignments[6] were assisted by an analysis of the proton-proton couplings, made by inspection of the ^1H nmr spectra and by simulations of the spin systems to extract the δ_H and J_{HH} values. These analyses confirmed the connectivities required by the structure (**1**), but did not resolve the issue of stereochemistry at the N at position 2, which had only been assigned tentatively by Rinehart *et al.*[1] as 2β-O. A full

investigation of the proton-proton nOe's was undertaken for each of **1a-c**. For the salt (**1b**) only the following nOe connectivities, in addition to the expected vicinal and geminal connections, were observed using the difference nOe technique with low-power frequency cycling on multiplets in order to achieve high selectivity.[7]

$$H_1\{H_{3\alpha}\};\ H_{3\alpha}\{H_1\};\ H_1\{H_{11\alpha}\};\ H_{11\alpha}\{H_1\};\ H_{3\beta}\{H_{13\beta}\};\ H_{13\beta}\{H_{3\beta}\};\ H_{11\alpha}\{H_{13\alpha}\};\ H_{13\alpha}\{H_{11\alpha}\};\ H_5\{H_{4\alpha}\}$$

The presence of the nOe connectivity between H_1 and $H_{3\alpha}$ necessitates that these protons be present in a diaxial relationship. Similarly, H_1 and $H_{11\alpha}$ must also be in close spatial proximity. These requirements, together with the observation of an nOe effect between $H_{11\alpha}$ and *one* of H_{13}, and between $H_{3\beta}$ and the *other* H_{13}, can only be accomodated by the existence of the conformation shown in the diagram, which has the 2α-O configuration. This conformation also contains vicinal dihedral H-H angles[8] consistent with the J_{HH} values found. Since similar nOe connectivities were observed for **1a** and **1c**, in particular for the critical H_1-$H_{3\alpha}$, H_1-$H_{11\alpha}$, $H_{11\alpha}$-$H_{13\alpha}$ and $H_{3\beta}$-$H_{13\beta}$ pairs, it follows that eudistomin K and its acetyl derivative also have the conformation and configuration shown in the diagram. It is thus probable that eudistomins E, C, F and L also have this stereochemistry. Because of the low barrier to inversion at trivalent nitrogen[9], the eudistomins with the 2β-O configuration almost certainly exist in equilibrium with those with the 2α-O configuration shown here. However, the equilibrium clearly favours the 2α-O configuration, which exists in a preferred conformation for the 7-membered ring.

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5 All nmr spectra were obtained on a Varian XL300 spectrometer operating at 300MHz for ^1H and 75.4 MHz for ^{13}C, except for the ^1H nmr spectra for *Eudistoma* -derived **1a** and **1b** which were recorded at 360MHz on a Nicolet EM360 instrument.

6 **1a** δ_H (CD_3CN ppm relative to CHD_2CN at 2.00): 7.57, d, J=1.8, H_8; 7.39, d, J=8.5, H_5; 7.21, dd, J=1.8,8.5, H_6; 4.96, d, J=9.2, $H_{13\alpha}$; 4.82, d, J=9.2, $H_{13\beta}$; 4.10, bs, H_1; 3.62, ddd, J=9.8,5.0,2.2, $H_{3\beta}$; 3.58, bd, H_{10}; 3.31, d, J=14.4, $H_{11\alpha}$; 3.11, ddd, J=11.5,9.8,4.0, $H_{3\alpha}$; 2.86, m, J=15.8,11.5,5.0,2.4, $H_{4\beta}$; 2.83, m, J=15.8,4.0,2.2,0.9, $H_{4\alpha}$; 2.82, dd, J=14.4,5.6, $H_{11\beta}$. δ_C (CD_3CN ppm relative to CD_3CN at 117.7): 138.3, C_{8a}; 131.6, C_{9a}; 129.1, C_{4b}; 122.3, C_6; 119.7, C_5; 114.5, C_7; 114.2, C_8; 110.0, C_{4a}; 71.4, C_{13}; 70.3, C_1; 54.1, C_3; 50.6, C_{10}; 35.0, C_{11}; 20.6, C_4. **1b** δ_H (CD_3OD ppm relative to CHD_2OD at 3.30): 7.53, d, J=1.9, H_8; 7.34, d, J=8.4, H_5; 7.14, dd, J=1.9,8.4, H_6; 4.97, d, J=9.0, $H_{13\alpha}$; 4.89, d, J=9.0, $H_{13\beta}$; 4.32, bs, H_1;4.18, bd, H_{10}; 3.62, ddd, J=9.8,4.6,2.2, $H_{3\beta}$; 3.48, d, J=15.8, $H_{11\alpha}$; 3.12, ddd, J=11.8,9.8,4.0, $H_{3\alpha}$; 3.01, m, J=15.8,6.2, $H_{11\beta}$; 2.95, m, J=15.3,11.8,4.6,2.2, $H_{4\beta}$; 2.80, m, J=15.3,4.0,2.2,0.9, $H_{4\alpha}$. δ_C (CD_3OD ppm relative to CD_3OD at 49.3): 140.0, C_{8a}; 129.5, C_{9a}; 126.5, C_{4b}; 123.6, C_6; 120.7, C_5; 116.5, C_7; 115.5, C_8; 112.4, C_{4a}; 71.9, C_{13}; 67.2, C_1; 54.6, C_3; 51.8, C_{10}; 30.5, C_{11}; 21.2, C_4. **1c** δ_H ($CDCl_3$ ppm relative to TMS at 0.00): 8.81, H_9; 7.45, d, J=1.9, H_8; 7.27, d, J=8.5, H_5; 7.16, dd, J=1.9,8.5, H_6; 6.63, d, J=9.7, NH; 5.02, m, J=9.7,5.5, H_{10}; 4.96, d, J=8.9, $H_{13\alpha}$; 4.83, d, J=8.9, $H_{13\beta}$; 4.13, bs, H_1; 3.61, ddd, J=10.2,5.0,1.7, $H_{3\beta}$; 3.33, d, J=14.5, $H_{11\alpha}$; 3.14, ddd, J=11.5,10.2,4.8, $H_{3\alpha}$; 2.92, m, J=15.8,11.5,5.0,2.4, $H_{4\beta}$; 2.80, m, J=15.8,4.8,1.7,0.9, $H_{4\alpha}$; 2.78, dd, J=14.5,5.5, $H_{11\beta}$; 1.75, s, CH_3. δ_C ($CDCl_3$ ppm relative to TMS at 0.00): 170.4, **C**=O; 137.9, C_{8a}; 131.2, C_{9a}; 125.1, C_{4b}; 122.7, C_6; 119.1, C_5; 115.4, C_7; 114.5, C_8; 109.3, C_{4a}; 71.0, C_{13}; 69.0, C_1; 54.8, C_3; 46.7, C_{10}; 32.1, C_{11}; 23.3, CH_3; 20.6, C_4.

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