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THE TRIKENTRINS : NOVEL INDOLES FROM THE SPONGE TRIKENTRION FLABELLIFORME

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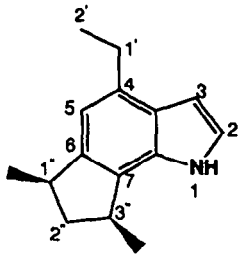
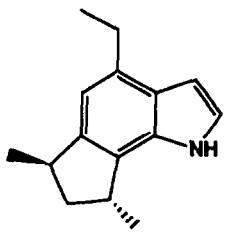
Abstract - Five new indoles, cis-trikentrin A (1), trans-trikentrin A (2), trans-trikentrin B (3), cis-trikentrin B (4) and iso-trans-trikentrin B (5), were isolated from the marine sponge Trikentrion flabelliforme. All possess antimicrobial activity and were identified by detailed spectroscopic analysis.

RESULTS AND DISCUSSION

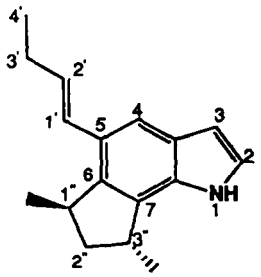
An aqueous acetone extract of the marine sponge Trikentrion flabelliforme[1] collected from the coastal waters off Darwin, Australia, was found to exhibit growth inhibitory activity against the gram positive bacteria Bacillus subtilis. Trituration of the concentrated extract with ethyl acetate/hexane yielded an antimicrobially active lipid soluble fraction, which was further fractionated by rapid elution through silica. High performance liquid chromatography of the resulting active material on silica yielded three pure compounds, 1, 2 and 3, together with an inseparable two component (3:2) mixture of 4 and 5.



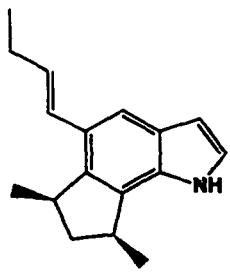
1.
cis - trikentrin A



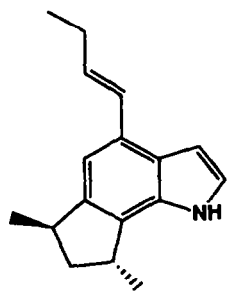
2.
trans - trikentrin A



3.
trans - trikentrin B



4.
cis - trikentrin B



5.
iso-trans - trikentrin B

The mass spectrum of 1 showed an M^+ at m/z 213 with the base peak at m/z 198 (M^+-CH_3). High resolution mass measurements on m/z 213 gave the molecular formula of 1 as $C_{15}H_{19}N$, requiring seven degrees of unsaturation. The presenoe of eight deshielded carbon resonances (C2 to C7a) in the ^{13}C NMR spectrum of 1 (see Table 1) suggested the presence of four double bonds, implying a tricyclic structure.

Table 1

^{13}C NMR ($CDCl_3$) assignments for the trikentrins

Carbon No.	1	2	3	4	5
2	122.9	122.8	123.7	123.7	123.3
3	101.3	101.5	102.8	103.1	101.7
3a	132.4	132.1	132.1	131.9	132.0
4	126.3	126.1	115.1	115.6	112.4
5	114.0	114.1	128.0	128.5	*
6	143.0	142.5	141.4	140.6	141.0
7	127.0	127.2	126.8	127.7	*
7a	135.0	135.0	128.1	129.5	*
1'	26.6	26.5	127.0	127.4	129.1
2'	15.1	15.0	131.0	131.0	132.5
3'	-	-	26.4	26.4	26.4
4'	-	-	14.0	14.1	14.1
1"	37.2[a]	36.0[a]	36.1[a]	37.0[a]	36.1[a]
2"	44.7	43.8	43.5	41.8	43.7
3"	38.9[a]	37.9[a]	37.4[a]	38.4[a]	37.8[a]
1"-CH_3	20.8[b]	20.0[b]	20.0[b]	22.5[b]	20.0[b]
3"-CH_3	21.1[b]	20.8[b]	21.1[b]	24.3[b]	20.1[b]

a) b) Assignments with identical superscripts within a column may be interchanged.

Signals obscured by other carbon resonances in the mixed ^{13}C NMR spectrum of 4 and 5.

Intense and sharp N-H absorption at 3490 cm^{-1} in the IR spectrum of 1 was consistent with a strongly deshielded (δ 8.03) N-H resonance in the ^1H NMR spectrum. This latter signal was observed, by double resonance and homonuclear correlated 2D (COSY) experiments, to be coupled to an isolated spin system consisting of two mutually coupled deshielded protons (δ 6.59 and 7.10), and furthermore, on addition of D_2O was very slow to undergo deuterium exchange. These observations, together with those of UV absorption maxima at 241 (ε 11350) and 271 nm (ε 11200), were consistent with an indole moiety, unsubstituted at positions 1, 2 and 3.

Remaining functionalities identified in 1 included an isolated ethyl group (δ 2.94 and 1.36), two secondary methyls (δ 1.37 and 1.47) together with attendant methine protons (δ 3.42 and 3.23), a methylene bearing non-equivalent protons (δ 1.31 and 2.60) and an aromatic proton (δ 6.84)(see Table 2). Long range couplings (<1Hz) were observed between the latter and both the methine proton at δ 3.23, and the methylene protons at δ 2.94. Combining this evidence with the observation of NOEs between the methylene protons at δ 2.94 and both the aromatic proton singlet (δ 6.84, 2.6%) and the C3 proton multiplet (δ 6.59, 2.7%) permitted formulation of the structure for 1 as shown. The cis relationship between the two secondary methyls could be deduced from non-equivalence of the methylene protons on C2". This effect had previously been observed with appropriately methylated indanes[2].

The minor component 2 (M^+, m/z 213) was isomeric with 1 and possessed many common spectroscopic features (see Tables 1 and 2). Homonuclear correlated 2D and NOE analyses on 2 confirmed the presence of an indole moiety with a substitution pattern identical to that of 1. Unlike 1, 2 did not exhibit the non-equivalent methylene protons at C2" indicative of cis secondary methyls. Instead, the relevant methylene protons in the ^{1}H NMR spectrum of 2 resonated as a complex multiplet at δ 2.02, identifying 2 as the 'trans' analogue of 1[3]. Although both compounds were optically active, absolute stereochemistries were not determined for either 1 or 2. Thus, 1 and 2 have been named cis-trikentrin A and trans-trikentrin A, respectively.

Table 2

^{1}H NMR ($CDCl_3$) assignments for cis and trans trikentrin A

Proton No.	1	2
1	8.03, bs, $W_{½}$ = 13 Hz	8.02, bs, $W_{½}$ = 14 Hz
2	7.10, dd, J = 2.7,3.2 Hz	7.16, dd, J = 2.6,3.2 Hz
3	6.59, dd, J = 2.0,3.2 Hz	6.12, dd, J = 2.0,3.2 Hz
4	-	-
5	6.84, bs, $W_{½}$ = 2 Hz	6.85, s, $W_{½}$ = 2 Hz
1'	2.94, q, J = 7.4 Hz	2.95, q, J = 7.6 Hz
2'	1.36, t, J = 7.4 Hz	1.37, t, J = 7.6 Hz
1"[a]	3.23, dq, J = 7.4,7.4 Hz	3.43, dq, J = 7.0,7.0 Hz
2"	2.60, ddd, J = 12.4,7.4,7.4 Hz	2.02, bm
	1.31, ddd, J = 12.4,3.6,3.6 Hz	
3"[a]	3.42, dq, J = 7.4,7.4 Hz	3.54, dq, J = 4.4,7.0 Hz
1"-CH_3[b]	1.47, d, J = 7.4 Hz	1.22, d, J = 7.0 Hz
3"-CH_3[b]	1.37, d, J = 7.4 Hz	1.34, d, J = 7.0 Hz

a) b) Assignments with identical superscripts may be interchanged.

For reasons similar to those discussed for cis and trans trikentrin A, **3** could be formulated as an indole unsubstituted at positions 1,2 and 3. High resolution accurate mass measurements established its molecular formula as $C_{17}H_{21}N$, an addition of C_2H_2 to that observed for **1** and **2**. Resonances in the ^1H NMR (δ 6.61 and 6.18) and ^{13}C NMR (ppm 127.0 ; 131.0) spectra of **3** (see Tables 1 and 3) indicated the presence of a 1-but-1-enyl substituent, instead of the ethyl substituent observed in both cis and trans trikentrin A. A deshielded aromatic proton (δ 7.61) in the ^1H NMR spectrum of **3**, lacking in long range couplings, was located at C4 rather than at C5 as in **1** and **2**. Confirmation of this and the substitution of a 1-but-1-enyl moiety at C5 came from the measurement of NOE's between the aromatic proton singlet at δ 7.61 and those resonances attributed to the C3 and C2' protons (9% and 16%, respectively). A lack of non-equivalence between the two methylene protons adjacent to the secondary methyls was taken[3] as evidence for a trans configuration about these centres (c.f. **2**). Thus **3** was assigned the structure of trans-trikentrin B, as shown.

Table 3

^1H NMR ($CDCl_3$) assignments for trans, cis and iso-trans trikentrin B

Proton No.	3	4‡	5‡
1	7.91, bs, $W_{\frac{1}{2}}$ = 10 Hz	7.94, bs, $W_{\frac{1}{2}}$ = 15 Hz	8.03, bs, $W_{\frac{1}{2}}$ = 15 Hz
2	7.03, dd, J = 2.6,2.6 Hz	7.14, dd, J = 2.5,3.0 Hz	7.20, dd, J = 2.6, 3.0 Hz
3	6.49, dd, J = 2.0,2.6 Hz	6.54, dd, J = 2.0,3.0 Hz	6.75, dd, J = 2.0, 3.0 Hz
4	7.62, bs, $W_{\frac{1}{2}}$ = 2 Hz	7.62, bs, $W_{\frac{1}{2}}$ = 2 Hz	-
5	-	-	7.09, bs, $W_{\frac{1}{2}}$ = 2 Hz
1'	6.61, bd, J = 15.8 Hz	6.60, bd, J = 15.8 Hz	6.79, bd, J = 15.6 Hz
2'	6.18, dt, J = 15.8,6.4 Hz	6.17, dt, J = 15.8,6.4 Hz	6.40, dt, J = 15.6,6.6 Hz
3'	2.26, dq, J = 6.4,7.6 Hz	2.30, bm	2.30, bm
4'	1.10, t, J = 7.6 Hz	1.12, t, J = 7.2 Hz	1.15, t, J = 7.0 Hz
1"[a]	3.46, dq, J = 5.2,6.8 Hz	3.5, m*	3.5, m*
2"	2.0, bm	2.72, ddd, J = 13.0,9.2,9.2 Hz 1.55, ddd, J = 13.0,2.4,2.4 Hz	2.0, bm
3"[a]	3.61, dq, J=7.2,6.8 Hz	3.5, m*	3.5, m*
1"-CH_3[b]	1.40, d, J = 6.8 Hz	1.36, d, J = 7.4 Hz	1.29, d, J = 6.6 Hz
2"-CH_3[b]	1.17, d, J = 6.8 Hz	1.46, d, J = 7.2 Hz	1.33, d, J = 7.2 Hz

a) b) Assignments with identical superscripts may be interchanged.

* Overlapping multiplets.

‡ Assignments made from ^1H NMR spectra taken on a 3:2 mixture of **4** and **5**.

Attempts to resolve the mixture of minor components 4 and 5 proved unsuccessful. The presence of 4 and 5 in a 3:2 ratio in the mixture, together with extensive homonuclear decoupling and NOE experiments, permitted complete ^{1}H NMR spectral assignments to be made for each component (see Table 3). Similarly, complete and partial ^{13}C NMR assignments could be made for 4 and 5 respectively. Consequently, after examination of NMR data and mixed UV, IR and mass spectral data, 4 and 5 were identified as isomers of 3. Furthermore, ^{1}H NMR comparisons between 3 and the major component 4, revealed the only significant difference to be the non-equivalence of the C2" methylene protons in 4 relative to 3 (see Table 3). This, together with similar NOE measurements in 4 as observed for 3, established 4 as the cis analogue of 3.

Unlike the aromatic proton singlet in the ^{1}H NMR spectra of 3 and 4 (δ 7.62) the corresponding signal in 5 resonated at higher field (δ 7.09) and exhibited no NOE to the C3 proton, similar to that observed for C5 in 1 and 2 (δ 6.84 and 6.85 respectively). Instead, NOEs were observed between the C2' proton and the C3 proton (4%), and also between the C2' proton and the aromatic proton (1%). Thus 5 possessed the same substitution pattern as both cis and trans trikentrin A but with the ethyl group replaced by a 1-but-1-enyl substituent. Lack of non-equivalence in the methylene protons adjacent to the secondary methyls pointed[3] to a trans configuration about these centres. Consequently 5 was assigned the structure, iso-trans-trikentrin B, as shown. As in the case of 1 and 2, absolute stereochemistries were not determined for either 3, 4 or 5. As such, the structures represented (1 to 5) should not be interpreted as favouring a C3" epimeric relationship between cis and trans isomers over epimerisation about the alternate C1" centre.

Secondary metabolites incorporating indole moieties are not uncommon among marine natural products isolated from sponges[4]. The unusual aspect of the trikentrins, apart from their aromatic ring substitutions, is their lack of substitution at position 3. All other indole containing secondary metabolites from marine sponges identified to date bear substituents at C3, reminiscent of their probable biosynthetic precursor, tryptophan. This may indicate that the biosynthesis of the trikentrins does not involve tryptophan as a precursor.

EXPERIMENTAL

General experimental details

^{1}H and ^{13}C NMR spectra, together with homo and heteronuclear correlated 2D spectra and NOE measurements, were recorded on a Varian XL-200-E spectrometer. Electron impact mass spectra were recorded on a VG Micromass 7070F instrument at 70 eV, with chemical ionization mass spectra being recorded on the same instrument using ammonia as the reagent gas. High resolution accurate mass measurements were determined under electron impact conditions on an AEI MS 902 mass spectrometer. Optical rotations were recorded on a Perkin Elmer 121 polarimeter. A Varian DMS 90 UV-Visible spectrophotometer was used to obtain ultraviolet absorption spectra while infrared absorption spectra were recorded on a Perkin Elmer 683 Infrared spectrophotometer. ^{13}C and ^{1}H NMR spectra are tabulated in Tables 1, 2 and 3.

Collection, extraction and isolation

A specimen of Trikentrion flabelliforme (15 g dry wt) collected by hand (SCUBA) was packed in dry ice and transported to the laboratory. The diced sponge was steeped in acetone at-20°C for approximately one week and the acetone extract decanted. The acetone extract was then reduced to a thick syrup and re-extracted with 50% ethylacetate:hexane to yield an antimicrobially active fraction (600 mgs). Antimicrobial activity was determined in a standard disc assay, against cultures of Bacillus subtillis, by measuring zones of inhibition.

Rapid silica filtration (stepwise elution; hexane to ethylacetate) of the resulting active material yielded a non polar material (433 mgs) of which half was subsequently subjected to HPLC fractionation on a 'Whatman' Partisil 10 M9/50 column (50 cm x 1.28 cm, 10 μ particle size, 5% ethylacetate/hexane as eluant). Fractions collected, in order of increasing polarity, corresponded to cis-trikentrin A (1, 74 mgs), trans-trikentrin A (2, 13 mgs), trans-trikentrin B (3, 118 mgs) and a 3:2 mixture of cis-trikentrin B and iso-trans-trikentrin B (4 and 5, 9 mgs).

Thus percentage dry weight yields for the trikentrins in the examined specimen of Trikentrion flabelliforme were 1 (0.099%), 2 (0.017%), 3 (0.160%), 4 (0.007%) and 5 (0.005%).

cis-Trikentrin A (1)

An unstable colourless oil which darkens on storage: $[\alpha]_D$ + 48° (c 2.47, $CHCl_3$); λ_{max} (MeOH) (nm), 241 (ε 11350), 271 (11200); ν_{max} ($CHCl_3$) 3490 cm^{-1}; EIMS m/z (%), 213 (M^+, 35), 198 (100); HRMS 213.1517 (M^+ requires 213.1517, $C_{15}H_{19}N$).

trans-Trikentrin A (2)

A stable colourless oil which crystallizes on standing: $[\alpha]_D$ + 23.3 (c 1.0, $CHCl_3$); λ_{max} (MeOH) (nm), 222 (ε 68400), 271 (12600); ν_{max} ($CHCl_3$), 3480 cm^{-1}; EIMS m/z (%), 213 (M^+, 50), 198 (100); HRMS 213.1517 (M^+ requires 213.1517, $C_{15}H_{19}N$).

trans-Trikentrin B (3)

An unstable colourless oil which darkens on storage: $[\alpha]_D$ -13° (c 1.97, $CHCl_3$); λ_{max} (MeOH) (nm), 249 (ε 24200), 275 (8800); ν_{max} (CCl_4), 3495 cm^{-1}; EIMS m/z (%), 239 (M^+, 83), 224 (100); HRMS 239.1674 (M^+ requires 239.1674, $C_{17}H_{21}N$).

Mixture of cis and iso-trans trikentrin B (4 and 5)

An unstable oil which darkens on storage: λ_{max} (MeOH) (nm), 240 (ε 44900), 298 (12300); ν_{max} (MeOH) (nm), 240 (ε 44900), 298 (12300); ν_{max} ($CHCl_3$), 3480 cm^{-1}; EIMS m/z (%), 239 (M^+, 86), 224 (100); HRMS 239.1673 (M^+ requires 239.1674, $C_{17}H_{21}N$).

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REFERENCES

1 A type sample of the specimen of Trikentrion flabelliforme examined in detail during this investigation is lodged at the Northern Territory Museum of Arts and Sciences, Darwin, Australia, under the registry No. 2711. Other specimens of T. flabelliforme known to contain trikentrins are lodged under the registry numbers 2525, 2169, 2383, 2528 and 2247.

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