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Violatinctamine, a new heterocyclic compound from the marine tunicate *Cystodytes cf. violatinctus*

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Abstract—A new alkaloid designated violatinctamine and four known metabolites were isolated from the tunicate *Cystodytes cf. violatinctus* collected in Kenya. Violatinctamine has a unique heterocyclic skeleton, which combines a benzothiazole unit and a dihydroisoquinoline unit. The structure of violatinctamine was elucidated by interpretation of MS results as well as 1D and 2D NMR spectra of the alkaloid and of its *O*,*O*′-dimethyl derivative. Analysis of the spectral information also implies that violatinctamine exists as a mixture of two tautomers—the imino-phenol and the amino quinone-methide.

As part of our continuing program to discover bioactive compounds from marine invertebrates,[1,2] we isolated a new alkaloid designated violatinctamine (**1**) having a unique heterocyclic skeleton, which combines a benzothiazole unit and a dihydroisoquinoline unit. Violatinctamine was isolated from the Kenyan tunicate *Cystodytes cf. violatinctus*[3] along with four other known metabolites, that is, styelsamine C (**2**),[4] shermilamine D (**3**),[5] 1,1-dimethyl-5,6-dihydroxyindolinium (**4**)[6] and 3-(2-aminoethyl)phenol (**5**), which has not previously been reported from marine sources and is assumed to be a precursor of **1**, vide infra. In this paper we present an account of the isolation and structure determination of violatinctamine.

Tunicates are rich sources of diverse metabolites derived from amino acids.[7] The amino acid DOPA [2-amino-3-(3′,4′-dihydroxyphenyl)propionic acid] appears to be especially important in the metabolism of these organisms,[8] and serves inter alia as a precursor of isoquinoline alkaloids like the well known lamellarin metabolites.[9] In contrast, benzothiazoles rarely occur as natural products.

Freeze-dried *Cystodytes cf. violatinctus*[3] (17 g) was homogenized and successively extracted with ethyl acetate, ethyl acetate–MeOH (1:1) and MeOH. The ethyl acetate-MeOH (1:1) extract (2.09 g) was subjected to partition by the method of Kupchan et al.[10] to afford five fractions (petroleum ether, CCl_4, chloroform, *n*-BuOH and water). The chloroform fraction (330 mg) was repeatedly chromatographed on a Sephadex LH-20 column, eluting with a mixture of heptane–$CHCl_3$–MeOH (2:1:1) to afford violatinctamine (**1**) (3.2 mg, 0.019% dry weight).[11]

The ES mass spectrum of violatinctamine (**1**) exhibited a molecular ion $[M+H]^+$ at *m/z* 368 and a pseudo molecular ion $[M+Na]^+$ at *m/z* 390. The ^{13}C NMR, ^1H NMR and 2D spectra (Table 1) revealed the presence of the following moieties: (a) two methyl groups, positioned downfield (δ_C 42.7 ppm, δ_H 2.81 ppm, 6H) which were determined to be attached to a nitrogen atom; (b) four methylenes, two of which were positioned downfield (δ_C 40.9, 58.1 ppm, δ_H 3.91 (2H), 3.28 (2H) ppm, respectively) indicating their proximity to nitrogen atoms; (c) 14 sp^2 carbons of which only five were protonated.

Based upon analysis of 2D spectra, three partial structures **a**–**c** could be constructed. Partial structure **a** consists of a β-dimethylaminoethyl moiety, which was established by HMBC correlations from Me-22 to both C-20 and C-22 and a COSY correlation between the adjacent protons H_2-19 and H_2-20. Partial structure **b** consists of a tetrasubstituted aromatic ring, in which protons H-15 and H-17 are *meta* positioned. The carbon

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Table 1. 1D and 2D NMR data for violatinctamine (**1**) in $CDCl_3$ + CD_3OD (5:1)

No.	δ_C, ppm[a,b]	δ_H, ppm (mult)[c,d,e]	COSY[f]	HMBC (H to C)[g]	NOESY	TOCSY
1	N					
2	40.9 (CH_2)	3.91 (t, 7.1)	3	3, 4, 10	3	3, 5
3	25.9 (CH_2)	3.06 (t, 7.1)	2	2, 4, 9	2, 5	2, 5
4	143.0 (C)					
5	116.5 (CH)	6.82 (d, 1.8)		3, 6, 7, 9	3	2, 3
6	167.6 (C)					
7	116.2 (CH)	6.85 (dd, 8.5, 1.8)	8	5, 9	8	8
8	136.3 (CH)	7.95 (d, 8.5)	7	4, 6, 10	7	7
9	115.2 (C)					
10	161.7 (C)					
11	151.7 (C)					
12	N					
13	142.7 (C)					
14	153.4 (C)					
15	113.3 (CH)	6.93 (br s)		13, 14, 17, 19	19, 20	17, 19[h]
16	139.5 (C)					
17	112.4 (CH)	7.38 (br s)		13, 15, 19	19 (weak)	15, 19[h]
18	139.3 (C)					
19	31.1 (CH_2)	3.15 (m)	20	16, 20	15, 17 (weak), 20	15, 17, 20
20	58.1 (CH_2)	3.28 (m)	19		15, 20	19
21	N					
22	42.7 (CH_3)	2.81 (s)		20, 22		

[a] Bruker Avance-400 instrument, chemical shifts refer to $CDCl_3$ (δ_C = 77.0).
[b] Multiplicities were determined by DEPT and HMQC experiments.
[c] Bruker ARX-500 instrument, chemical shifts refer to TMS (δ_H = 0).
[d] The CH correlations were assigned by a HMQC experiment.
[e] Multiplicity and coupling constants are indicated in parentheses.
[f] A and B denote downfield and upfield resonances, respectively, of a geminal pair.
[g] HMBC data are a summary of two experiments: with a delay of 55 ms optimized for 8 Hz coupling, and with a delay of 90 ms optimized for 5.5 Hz coupling.
[h] TOCSY correlations were observed although no splitting of the signal was apparent.

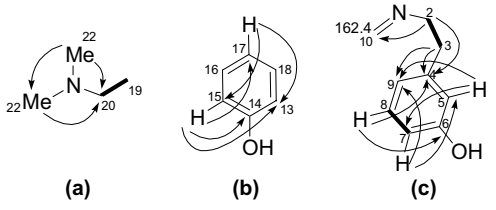

Figure 1. COSY and HMBC correlations in partial structures **a**–**c** of **1**.

at δ_C 153.4 ppm was determined to be oxygenated, and was assigned to position 14 based on the upfield shift exhibited by H-15 (δ_H 6.93 ppm) as well as HMBC correlations depicted in Figure 1. Partial structure **c** consists of a trisubstituted benzene ring, in which H-7 (δ_H 6.85 ppm) is a double doublet presenting coupling constants of 8.5 and 1.8 Hz and H-8 (δ_H 7.95 ppm) is a doublet presenting a coupling constant of 8.5 Hz indicating they are *ortho* positioned. H-5 (δ_H 6.82, d 1.8 Hz) is positioned *meta* to H-7. As in the case of H-15, H-5 and H-7 are both upfield shifted (δ_H 6.82, 6.85 ppm, respectively) therefore, and on the basis of HMBC correlations (Fig. 1) it was determined that both are situated α to the oxygenated carbon at δ_C 167.6 ppm, vide infra, which was assigned as C-6. The resulting unit can be further extended by other HMBC correlations from H-3 to both C-4 and C-9 and from H-2 to both C-4 and C-10 to afford partial structure **c**.

Partial structures **a**–**c** account for all the protons observed in the ^1H NMR spectrum and 16 out of the 17 carbon resonances observed in the ^{13}C NMR spectrum. Unaccounted for was another quaternary sp^2 carbon at δ_C 151.7 ppm, to which no correlations were observed in HMBC experiments with delays of 55–140 ms (optimized for J_{CH} of 4–9 Hz). The total mass of the above fragments, including the carbon at δ_C 151.7 ppm, was only 321, 46 mass units short of the molecular mass, which was found to be 367. Since there were no additional carbon atoms, the presence of a nitrogen atom and a sulfur atom could be suggested based on the 46 mass units difference. Indeed, HRMS confirmed the molecular formula to be $C_{20}H_{21}O_2N_3S$.[11] Additional correlations that were observed in the HMBC spectrum, that is, a correlation from H-8 to C-10, correlations from both H-15 and H-17 to C-19 and from H-19 to C-16, as well as a correlation observed in the TOCSY spectrum between H-17 and H-19 led to the construction of two alternative structures **I** and **II** (Fig. 2) that both agree with the 2D experiments data, the mass and the HRMS requirements. Structure **I** differs from **II** in the orientation of the thiazole ring relative to the phenol.

Initially, structure **I**, in which N-12 is connected to C-13, was preferred over structure **II**, in which the sulfur atom is connected to C-13, based on the chemical shift of C-13. The hydroxyl group at C-14 induces an upfield shift

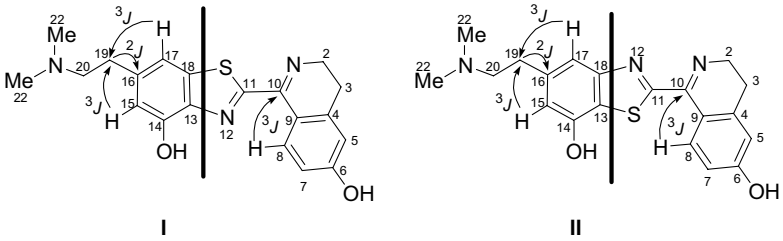

Figure 2. Two alternative structures for compound **1**.

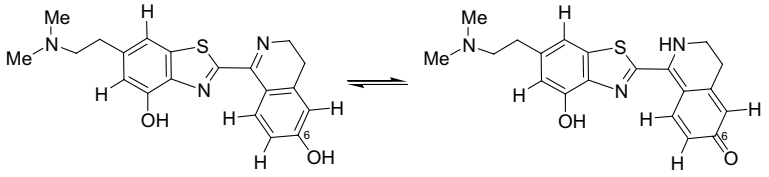

Figure 3. Equilibrium presentation of compound **1**.

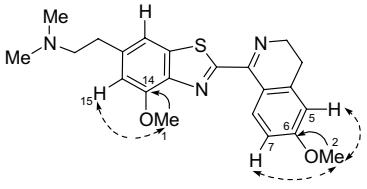

Figure 4. HMBC (⟶) and NOESY (⇢) correlations in **1b**.

on the *ortho* carbons, that is, C-13 and C-15. C-13 resonates at δ_C 142.7 ppm, therefore, unless the nitrogen atoms were connected at C-13, the latter carbon would resonate at a much higher field. This conclusion was further corroborated by comparison to literature ^{13}C NMR values of 4-hydroxy-6-(2-amino-2-carboxyethyl)benzothiazole (δ_C 152.80, 151.35, 112.73, 133.76, 113.02, 135.28, 142.13 ppm),[12] which are in close agreement with those of the benzothiazole part of compound **1** (δ_C 151.7, 153.4, 113.3, 139.5, 112.4, 139.3 and 142.7 ppm, respectively).

Noteworthy is the very high chemical shift of C-6 at δ_C 167.6 ppm. It is suggested that compound **1** exists in two tautomers of which in one, C-6 is substituted by a hydroxyl and in the other, C-6 is part of a carbonyl moiety (Fig. 3).

Methylation of compound **1** with CH_2N_2 yielded the *O*,*O*′-dimethyl product **1b**[13] (Fig. 4), confirming the dihydroxylated structure. After methylation, two methoxyl groups were clearly observed (δ_H 4.05 and 3.80 ppm, δ_C 56 and 55 ppm, respectively). Correlations in the HMBC spectrum were observed from OMe-1 to C-14 and from OMe-2 to C-6, which after methylation resonated at δ_C 162 ppm (−5.6 ppm, while the expected shift from a OH to OMe replacement is +4.5). Correlations in the NOESY spectrum were observed between OMe-1 and H-15 and between OMe-2 and both H-5

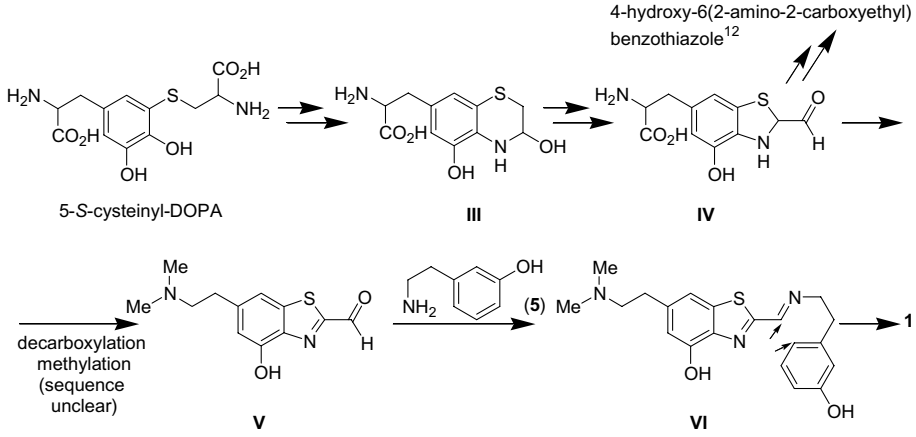

Figure 5. Suggested biogenesis for violatinctamine (**1**).

and H-7. This information provided further corroboration to the structure suggested.

A suggested biogenetic pathway for compound **1** is depicted in Figure 5. As part of a biosynthetic study of the pheomelanins it was demonstrated that 5-*S*-cysteinyl-DOPA undergoes oxidative cyclization to the benzothiazine **III**, which in turn reacts in the presence of copper or iron ions at physiological pH to form 4-hydroxy-6-(2-amino-2-carboxyethyl)benzothiazole. In this study it was proposed that the latter transformation proceeds via several intermediates, amongst which is the aldehyde **IV** (Fig. 5).[12] It is now proposed that **IV** undergoes decarboxylation and methylation (the reaction sequence is unclear) to yield the aldehyde **V**, which in turn reacts with 3-(2-aminoethyl)phenol (a metabolite isolated from this organism in this work) to afford the Schiff base **VI**. The latter undergoes a radical cyclization reaction (*para* to the phenolic OH and next to the imine nitrogen) to afford compound **1**.

Benzothiazoles rarely occur as marine natural products. The first benzothiazoles from the marine biosphere were isolated from fermentation culture extracts of *Micrococcus* sp., a marine bacterium obtained from the tissues of the sponge *Tedania ignis*.[14] The latter compounds included 2-mercaptobenzothiazole, 2-methylbenzothiazole, 2-hydroxybenzothiazole and 6-hydroxy-3-methyl-2-benzothiazolone. Another benzothiazole derivative designated S1319 was isolated from *Dysidea* sp. and exhibited bronchodilating activity.[15]

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