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Villagorgin A and B. New Type of Indole Alkaloids with Acetylcholine Antagonist Activity from the Gorgonian *Villagorgia rubra*

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Abstract: Two new indoloquinolizidine alkaloids incorporating an imidazol ring, villagorgin A and villagorgin B, along with caffeine, tryptamine, *Nb*-methyltryptamine, and 1, 2, 3, 4 tetrahydro-ß-carboline, were isolated from the gorgonian *Villagorgia rubra*, and determined by spectral data. Villagorgin A has a calmodulin-related antagonist activity.

Gorgonians have been shown to contain a fair variety of terpene metabolites[1] mainly diterpenes (cembranoids), sesquiterpenes and steroids. *Paramuricea chamaeleon* represents the only case of a gorgonian rich in indole alkaloids[2] . In connection with our investigations of biologically active marine metabolites, we focused our attention on the gorgonian *Villagorgia rubra*, selected for study because the methanol extract was very active in the guinea-pig ileum contraction test. In this paper we report that *V. rubra* is a rich source of nitrogenous metabolites that were isolated from the butanolic fraction and identified as caffeine (**1**), the simple indoles, tryptamine (**2**), *Nb*-methyltryptamine (**3**), 1, 2, 3, 4 tetrahydro-ß-carboline (**4**), and two new complex indole alkaloids named as villagorgin A (**5**) and villagorgin B (**6**). The structure and absolute configuration of villagorgins were deduced by extensive use of 2D-NMR, FABMS and HREIMS, and CD data.

The gorgonian *Villagorgia rubra*, a genus which has never been studied before, was collected in New Caledonia. The lyophilized material gave a methanol extract which was then partitioned into n-hexane, CH_2Cl_2 and n-BuOH. The n-butanol was passed through amberlite XAD-2 and the methanolic eluates (1 g) fractionated on a Sephadex LH-20 column eluted with methanol to produce eight fractions. Fractions 7-8 contained compounds **5** and **6** that were isolated by reversed phase HPLC (MeOH-OH_2 60:40; r.t = 14 and 24 min., respectively). Fractions 4-6 contained known compounds **1-4** isolated by HPLC as before.

Villagorgin A (**5**, 12 mg), was obtained as a yellow powder $[\alpha]_D^{20}$ = +7.8 °. The molecular formula $C_{16}H_{16}N_4$, determined by HREIMS ([M+] = 264.1378, Δ 0.3 mmu of calcd) inferred 11 degrees of unsaturation. The presence of six quaternary, six methine and four methylene carbon signals was deduced from the ^{13}C and DEPT NMR spectra. The HMQC experiment allowed us to assign and correlate all the protons to the corresponding carbons.

The UV spectra of **5** (λ (nm) 224 (ε 25600) and 280 (ε 52409))[3] and ^1H NMR signals in the 7.5-6.9 ppm region revealed the presence of an indole moiety in the molecule.

The proton and carbon chemical shifts for the C-5 and C-6 methylenes and the C-3 methine indicated that the indole system constitutes a part of a tetrahydro ß-carboline moiety[4a] . The m/z 169 (100%) ion in the EIMS of **5** is characteristic of this type of structure.[4b]

The ^1H-^1H COSY NMR spectrum showed a coupling between the highly deshielded H-3 methine proton at δ 3.83 ppm and the H-14 methylene protons at δ 3.36 and 2.72 ppm . Relay-COSY displayed five bond correlations between H-14 and both H-20 methylene protons at δ 3.99 and 3.62 ppm . Furthermore, HMBC experiments showed that the olefinic quaternary carbons C-15 and C-19 are correlated with H-14 and H-20. All these data indicate that **5** has the indoloquinolizidine system characteristic of well known plant alkaloids like yohimbine and corynantheine.[5]

The remaining two unsaturations and the CHN_2 atoms were assumed to comprise an imidazol[6] system on the basis of the carbon signal at δ 135.2 (C-17, d, J_{CH} = 207.5 Hz) bonded to the proton at δ 7.57 ppm. HMBC correlations and particularly those between C-19 and H-17 corroborated the D/E ring connections and the structure of villagorgin A as **5**.[7]

The trans quinolizidine stereochemistry was determined by the strong ROESY correlation between H-3 and the axial proton H-20. Furthermore, the circular dichroism curve of **5** presents a negative Cotton effect at 277 nm, (Δε -0.19) and this allowed us to determine the 3ß-H absolute sterochemistry[8] for villagorgin A (**5**).

Villagorgin B (**6**, 2mg), has molecular formula $C_{16}H_{13}N_4$. The positive ion FABMS showed the molecular ion M^+ at m/z 261. Its uv spectrum showed absorbances due to an indole system and an additional chromophore.[9]

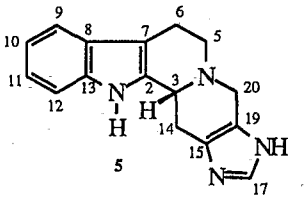
The ^{13}C and ^1H -NMR data of compound **6** indicate the presence of the indole system. The 5, 6 dihydro β-carboline system with a positively charged nitrogen atom was deduced from the chemical shifts of the C-6 and C-5 ethylene group[10] . In accordance with that, the positive ion FABMS of villagorgin B gives a prominent ion m/z 171 (100 %).

In addition, the ^{13}C and ^1H-NMR spectra of **6** displayed signals for three isolated aromatic methines (C-14, C-17 and C-20). Five bond correlations were found among those protons by RELAY COSY experiments. HMBC spectra displayed the correlations showed in Table 1. Particularly relevant are those of C-17 to H-14, C-20 to H-17, and C-3 to H-20. All these data suggest an imidazo [4, 5-c] pyridine system for the D/E rings. The ions at m/z 259 (M^+-2, fully aromatic structure), m/z 235 (M^+ -CN), and 207 (M^+ -2 HCN) in the (+) FABMS confirms the presence of the imidazol group and structure **6** for villagorgin B. The small amount available and its fast decomposition impeded the realization of further experiments.

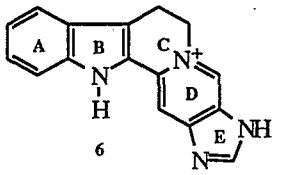
The known alkaloids caffeine (**1**, 4 mg), tryptamine (**2**, 5 mg) , *Nb*-methyltryptamine (**3**, 4 mg), and 1, 2, 3, 4 tetrahydro-ß-carboline (**4**, 2 mg) were also isolated from *V. rubra* and identified by comparison with published data and authentic samples.

Villagorgin A (**5**) was shown to produce strong inhibition on the acetylcholine induced contraction of guinea-pig ileum and a dose-dependent inhibitory effect against human platelet aggregation induced by thrombin and calcium ionophore A23187. Since this biological process is a calcium-calmodulin mediated event, the antiaggregatory activity of **5** could be due to the inhibition of that enzyme. It should be mentioned that villagorgin A and B are structurally related to the β-carboline marine alkaloid eudistomidin-A, isolated from a tunicate, and a strong calmodulin antagonist .[11]

Indole alkaloids have been isolated in a fair number from marine sources and some of them present outstanding pharmacological activity, but this is the first report of complex indoles in gorgonians.



5



6

Pos	δ H, m, J (Hz) (500 MHz)[a]	Relay-COSY	δ C, m (60 MHz)	HMBC (J_{C-H}= 7 Hz)
NH	10.86 s[a]			
2			135.1 s	H-6[b]
3	3.83 dt, (3.7, 11.0)	H-14, H-14'	58.4 d	H-14[b], H-14', H-5, 5', H-20
5	3.33 m	H-5', H-6, H-6'	54.0 t	H-6, 6', H-20'
5'	2.87 ddd (4.0, 12.0, 22.5)	H-5, H-6, H-6'		
6	3.00 m	H-5, H-5', H-6'	22.3 t	H-5, 5'[b]
6'	2.79 dt (2.0, 16.0)	H-5, H-5', H-6		
14	3.36 m	H-3, H-14', H-20'	29.2 t	
14'	2.72 m	H-3, H-14, H-20		
15			129.7 s	H-14, H-20, 20'
17	7.57 s		135.2 d	
19			127.3 s	H-17[b], H-20, 20'
20	3.99 d, (13.0)	H-14', H-20'	53.4 t	H-3
20'	3.62 dt, (13.0)	H-14, H-14', H-20		

Pos	δ H, m, J (Hz) (500 MHz)	Relay-COSY	δ C, m (60 MHz)	HMBC (J_{C-H}= 7 Hz)
NH	10.98 s[a]			
2			128.4 s	H-6, H-14
3			142.4 s	H-14, H-20
5	4.86 t (7.0)		57.3 t	H-6
6	3.35 t (7.0)		21.2 t	
14	8.05 s	H-17, H-20	107.1 d	
15			164.4 s	H-14, H-20
17	8.93 s	H-14, H-20	135.2 d	H-14
19			155.6 s	H-20, H-17
20	8.38 s	H-14,H-17	163.5 d	H-17

Table 1. Selected NMR data of villagorgin A (**5**) and villagorgin B (**6**) in CD_3OD; a) In DMSO-d_6 b) HMBC with a J_{C-H} = 3.5 Hz.

Indole alkaloids containing an imidazol ring are known from either marine or terrestrial origin, but compounds **5** and **6** are the first examples with the imidazol ring attached to an indoloquinolizidine and constitute a new skeleton among this type of alkaloids. A biogenetic route to **5** and **6** can be formulated from tryptophane and histidine as starting aminoacids, as in some Penicillium alkaloids such as oxaline and roquefortine[12], but requires an additional carbon unit (C-20). The isolation of caffeine (**1**) from *Villagorgia rubra*, is noteworthy because it has been considered to be a terrestrial metabolite. This is, as far as we know, the first report on its presence in a marine organism.

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7. **5**: Red amorphous solid. IR $\nu_{max.}$: 3880, 2923, 1650, 1541 cm^{-1}. ^{1}H-NMR (CD_3OD) δ (m, J in Hz): 7.41 (d, 7.5, H-9), 7.30 (d, 7.5, H-12), 7.06 (ddd, 1.0, 7.5, 16.0, H-11), 6.98 (ddd, 0.5, 7.5, 14.5, H-10) and Table 1. ^{13}C-NMR (CD_3OD): 138.5 (C-13), 128.1 (C-8), 122.3 (C-11), 118.8 (C-9), 111.9 (C-10), 112.0 (C-12), 108.5 (C-7) and Table 1. HREIMS: $C_{16}H_{16}N_4$ 264.1375: calc. 264.1378. EIMS m/z (%): 264 (55), 234 (10), 220 (6), 207 (4), 169 (100), 154 (6), 142 (9), 129 (5), 115 (11), 94 (8). (-) FABMS, m/z, (%): 263 ($[M-H]^-$, 100).
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9. **6**: Red amorphous solid. U.V. (MeOH) $\lambda_{max.}$: 228, 244, 280, 338 nm. UV (MeOH, HCl 1N) $\lambda_{max.}$: 222, 242, 332 nm. IR $\nu_{max.}$: 3480, 1648, 1632 cm^{-1}. ^{1}H-NMR (CD_3OD) δ (m, J in Hz): 7.61 (d, 8, H-9), 7.46 (d, 8.5, H-12), 7.26 (ddd, 1.5, 7.5, 15, H-10), 7.12 ddd (1, 8, 15, H-10) and Table 1. ^{13}C-NMR (CD_3OD): 139.9 (C-13), 126.9 (C-8), 125.4 (C-11), 121.4 (C-10), 120.3 (C-9), 113.8 (C-7), 112.5 (C-12) and Table 1. (+) FABMS (Glycerol matrix), m/z (%): 261 (M^+, $C_{16}H_{16}N_4$, 50), 205 (60), 171 (100), 135 (80); (+) FABMS (Magic +NaCl matrix), m/z, (%): 261 (M^+, $C_{16}H_{16}N_4$, 20), 235 (M^+-CN, 22), 215 (37), 207 (M^+-2HCN, 15), 177 (53), 115 (100).
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