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GROSSULARINE, A NOVEL INDOLE DERIVATIVE FROM THE MARINE TUNICATE, *DENDRODOA GROSSULARIA*

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Summary. *A new marine metabolite, derived from indole and dimethylguanidine, was isolated from the tunicate Dendrodoa grossularia. The proposed structure was based upon spectroscopic evidence.*

The tunicate *Dendrodoa grossularia* grows in dark red clusters along the coast of Brittany. This marine animal differs from others tunicates already investigated, in that it produces indole derivatives(1); among them, Dendrodoine 1(2) offered the first known example of a S(II)-N bond in natural products, owing to its 1,2,4-thiadiazole nucleus.

In this paper we describe isolation and structure elucidation of a new indole derivative: Grossularine 2 from the same tunicate.

Freeze-dried animals were first extracted by hexane, then by dichloromethane and ethyl-acetate. Combined dichloromethane and ethyl-acetate extracts were submitted to the following chromatographic separations: a) silicagel column (chloroform-acetone: 80-20, v/v); b) LH 20 column (chloroform-methanol: 60-40); c) alumina column (THF-acetone: 20-80 or chloroform-acetone: 70-30). Pure 2 was isolated as a yellow powder: 40mg from 830g of freeze-dried (i.e. about 5kg wet weight) animals (mp 350°C; no dec.).

The electron-impact mass spectrum displayed a molecular ion M^+: 394 (confirmed by C.I.M.S.), and addtional peaks at *m/e* 350, 277, 250, 234, 180, 144, 116. High resolution mass measurements gave the empirical formula $C_{23}H_{18}N_6O$ (3) and some key fragmentation ions: *m/e* 350: $C_{21}H_{12}N_5O$, M^+-$N(CH_3)_2$; 277: $C_{15}H_{11}N_5O$; 250: $C_{14}H_{12}N_5$; 180: $C_{11}H_6N_3$ and 144: C_9H_6NO (3). The fragmentation ions at 144 and 116 led us to suspect a 3-carbonyl indole nucleus, which was then confirmed by NMR (^1H and ^{13}C).

In addition to resonances for a N-$(CH_3)_2$ group a δ 3.35 ppm, the ^1H NMR spectrum (table I) (250 MHz, DMSO-d_6,δ ppm) contained signals for three **N-H** at δ 12.16 (1H,d,J=3 Hz), 11.6 (1H,s) and 10.94 (1H,s) exchangeable by D_2O and nine aromatic protons: five of them were readily assigned to the 3-carbonyl-indole protons: a 1H doublet 9.44 (J=3 Hz) (H-2) which collapsed in a singlet by addition of D_2O; multiplets at 8.56 (1H) (H-4); 7.56 (1H) (H-7); 7.29 (2H) (H-6) and (H-5). The remaining four signals: 8.24 (1H,d broad, J=8 Hz); 7.51 (1H,d broad,J=8 Hz); 7.42 (1H,dt, J=8 & 1 Hz) were assigned to the aromatic protons 4', 7', 5', 6' of a 2'-3'-disubstituted indole nucleus(4).

Table 1 - ^1H NMR data of compounds 2 (DMSO)

H-1	12.16 d	H-2	9.445 d	H-7	7.565 m	H-6+5	7.29 m
H-2'	11.59 s	H-4	8.56 m	H-7'	7.51 d br	H-5'	7.23 d t
H-19	10.94 s	H-4'	8.24 d br	H-6'	7.42 d t	N-$(CH_3)_2$	3.35 s

Treatment of 1 with Ac_2O-pyridine (100°C,4h) gave a diacetate 3, m.p. 230°C, M.S. : M^+ 478; ^{1}NMR (80 MHz, δ ppm): δ 10.0 (non acetylated N-H); 8.50 (4H,m) (H-4, 4', 7,7') and 7.50 (4H,m) (H-5,6, 5',6'); 3.12 (3H,s) and 2.72 (3H,s) (two $N\text{-}COCH_3$)

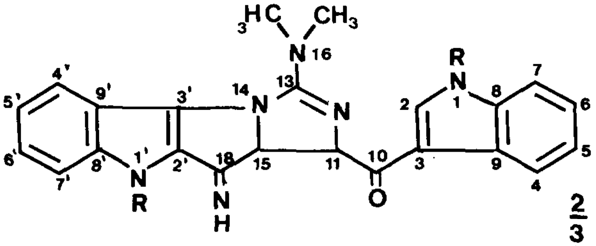
^{13}C NMR data (table 2) compared with those of model compound 1 and indole confirmed the presence of indole moities a and b, of a $N(CH_3)_2$ group and suggested that the carbonyl was ketonic. The signals at δ 159.4 and 159.2 were assigned to (-C=N-); owing to the presence of a $N\text{-}(CH_3)_2$, one of them could be attributed to the carbon of a *N*-dimethyl guanidine while the other, due to the presence of weak acidic (since non-acetylable) N-H, could be attributed to a C=NH group. The signals at 146.8, 145.7 and 139.3 ppm could be attributed to aromatic carbons bonded to nitrogen (=C-N)-$^{(5)}$. To assemble these elements we have considered the chemical shift of one of the N-acetyl (δ 2.72 ppm) and therefore placed the C=NH in the vicinity of the NH of the 2-3-disubstituted indole nucleus. The only possibility for assembling these partial structures was therefore structure 2. This was substained by the $\nu_{C=O}$1600 cm^{-1} in the infrared spectrum, and by a bathochromic affect observed when the UV spectrum was recorded with the addition of $AlCl_3$, both indicating chelation of the carbonyl $^{(6-7)}$.

Table 2 - ^{13}C NMR data of compound 2 (DMSO) (20 MHz, Bruker, δ ppm)

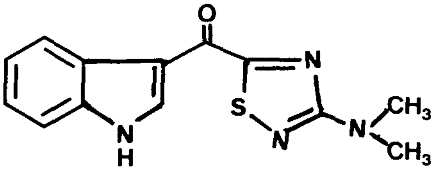
C-10	186.8 s	C-8	135.7 s	C-4'	121.2 d		
C-13 *a*	159.4 s	C-8'	132.9 s	C-4	121.5 d		
C-18 *a*	159.2 s	C-9'	127.1 s	C-3'	119.5 s		
C-2' *b*	146.8 s	C-9	127.0 s	C-5'	118.6 d	$N\text{-}(CH_3)_2$	39.9 q
C-15 *b*	145.7 s	C-5	125-4 d	C-3	114-3 s		
C-11	139.3 s	C-6'	122.4 d	C-7	111.8 d		
C-2	137.2 d	C-6	122.3 d	C-7'	110.3 d		

a, b : Assignments may be reversed

Degradation in EIMS are in good agreement with the proposed structure and bring evidence for the presence of the two ring systems : m/e 180 and 144 and of the dimethylguanidine : m/e 350.



2 R = H
3 R = OAc



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