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BROMINATED ß-CARBOLINES FROM THE MARINE HYDROID AGLAOPHENIA PLUMA LINNAEUS.

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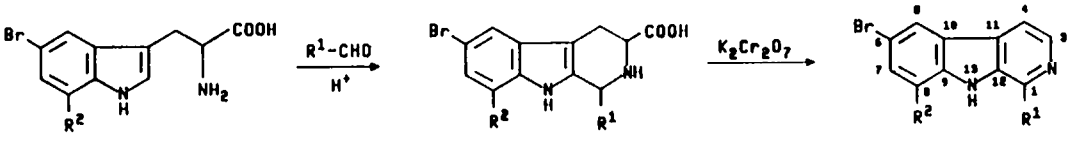
Abstract. - Three new brominated ß-carbolines (1-3) have been isolated from the lipophylic extract of the marine hydroid Aglaophenia pluma Linnaeus and their structures determined on the basis of spectral data including two dimensional proton-carbon shift correlation (direct and long range) NMR spectroscopy. The syntheses of compounds 1-3, starting from the appropriate brominated tryptophan derivatives, are also described.

Recently eudistomins, a new class of metabolites based on a ß-carboline ring system, have been isolated from the colonial Caribbean tunicate Eudistoma olivaceum[1-3]. These compounds, which are considered to be biosynthetically derived from tryptophan, exhibit interesting antiviral and/or antimicrobial activities.

We wish to report here that closely related metabolites are also elaborated by Aglaophenia pluma Linnaeus, a Mediterranean hydroid which during the benthonic phase grows in shallow waters, attached to almost any solid object, assuming a feathery appearance.

From the methanolic extracts of this organism we have isolated three new brominated compounds [6-bromo-1-ethyl-ß-carboline (1), 6-bromo-1-methyl-ß-carboline (2) and 6,8-dibromo-1-ethyl-ß-carboline (3)] whose structure elucidation was based on physico-chemical evidence and confirmed by synthesis.

SCHEME



1 R^1 = Et, R^2 = H
2 R^1 = Me, R^2 = H
3 R^1 = Et, R^2 = Br

A.pluma was collected near Praiano in the Bay of Salerno (Italy) by hand using SCUBA (-5 to -20 m) during the winter 1985. Freshly collected whole specimens were immersed in methanol and allowed to extract for three periods of 2 days at room temperature. The methanol extract was decanted, filtered, evaporated in vacuo to give an aqueous suspension which was extracted with diethyl ether. Compounds 1-3 were isolated by rapid elution chromatography of the ethereal extract using silica gel column and were finally purified by preparative TLC. 6,8-Dibromo-1-ethyl-ß-carboline (3) was the major component comprising 0.4% of the lipid extract, while 1 and 2 each represented less then 0.2% of the above extract.

Data from high resolution mass and ^{13}C-NMR (Table) spectroscopies established a

Table. NMR data for compounds 1 - 3 ($CDCl_3$).

N°	1			2			3		
	δ ^1H[a]	δ ^{13}C[b]	COLOC	δ ^1H[c]	δ ^{13}C[b]	COLOC	δ ^1H[d]	δ ^{13}C[b]	COLOC
1		147.32			142.04	Me-1		147.69	
3	8.38 d	138.09		8.34 d	138.52		8.49 d	139.93	
4	7.75 d	112.90		7.86 d	113.01		7.78 d	113.04	
5	8.20 bd	124.21	7-H	8.22 bd	124.37		8.22 d	123.63	7-H
6		112.48	8-H,5-H		112.01	8-H		112.84	5-H
7	7.60 dd	130.86	5-H	7.72 dd	131.20	5-H,8-H	7.86 d	132.60	5-H
8	7.40 bd	113.10	7-H	7.51 bd	113.12			105.57	7-H
9		139.15	7-H,8-H		138.92			137.63	5-H,7-H
10		123.50	8-H		123.80	8-H,4-H		124.66	
11		127.66	3-H,5-H		127.60	5-H		128.17	
12		134.47	4-H		134.98	Me-1,4-H		133.83	
13	8.52 bs			not observed			8.26 b		
14	3.12 q	27.07		2.82 s	19.89		3.21 q	27.13	
15	1.46 t	12.66	$14\text{-}H_3$				1.54 t	12.28	

a. J (Hz) 3-4: 5.5; 5-7: 2.0; 7.8: 8.7; 14-15: 7.0.
b. Assignments are based on carbon-proton shift correlated 2D-NMR spectroscopy.
c. J (Hz) 3-4: 5.6; 5-7: 1.7; 7-8: 9.0.
d. J (Hz) 3-4: 5.6; 5-7: 1.5; 14-15: 7.0.

molecular formula $C_{13}H_{11}N_2Br$ for 1 and indicated that the molecule was highly unsaturated. The ^1H-NMR spectrum contained signals relative to five aromatic protons [δ 8.38 (1H, d, J= 5.5 Hz; 3-H), 7.75 (1H, d, J= 5.5 Hz, 4-H), 8.20 (1H, bd, J= 2.0 Hz, 5-H), 7.60 (1H, dd, J= 8.7 and 2.0 Hz, 7-H) and 7.40 (1H, bd, J= 8.7 Hz, 8-H)] which were shown to belong to two separate spin systems by double resonance experiments. The remaining signals were interpretated as an ethyl group attached to an sp^2 carbon [δ 3.12 (2H, q, J= 7.0 Hz, $14\text{-}H_2$), 1.46 (3H, t, J= 7.0 Hz, $15\text{-}H_3$)] and a $>$NH function [δ 8.52 (1H, bs, D_2O exchangeable)].

The above data and ^{13}C-NMR spectrum, which contained eleven resonances in the sp^2 region due to five methines and six non protonated carbons as deduced from DEPT (Distortionless Enhancement by Polarization Transfer)[4], suggested that 1 was a brominated 1-ethyl-ß-carboline. This was also consistent with UV spectrum which displayed absorption at λ_{max} (CH_3OH) 237 (ϵ = 33854), 281 (7436), 254sh (19454), 290 (10927), 345 (4382), and 358 nm (4818) typical of ß-carbolines[5]. The bromine atom must be positioned on the ring A at C-6 or C-7 as indicated by the coupling pattern of the protons resonating at δ 8.20, 7.60 and 7.40 (see Table). The second possibility was ruled out on the basis of comparison of the above mentioned chemical shift values with those of model compounds[3]. Further support for structure 1 was provided by ^{13}C-^1H shift correlated 2D-NMR spectroscopy[6] *via* ^1J, which established direct correlations, and that *via* ^2J and ^3J [COLOC (Correlation by Long Range Coupling)] [7] (Table). The latter experiment showed a set of intense heteronuclear peaks, through which confident and unequivocal assignments for non protonated carbons were also accomplished.

Compound 2 (6-bromo-1-methyl-ß-carboline) was formulated as $C_{12}H_9N_2Br$ (from HRMS and ^{13}C-NMR spectrum) and showed UV absorptions [λ_{max} (CH_3OH) 236 (ϵ = 33870), 253sh (19460), 281 (7460), 290 (11100), 345 (4380), and 358 nm (4820)] that clearly defined its similarity with 1. The two metabolites differed only in the high field region of their respective ^1H- and ^{13}C-NMR spectra (see Table), indicating that the ethyl group at C-1 had been replaced by a methyl. This was supported by a COLOC experiment which showed, *inter alia*, the correlation of the two fully

substituted carbons C-12 (δ 134.98) and C-1 (δ 142.04) with the methyl protons resonating at δ 2.82, thus confirming that 2, and consequently 1, are 1-alkylated ß-carbolines.

The major metabolite [6,8-dibromo-1-ethyl-ß-carboline, (3)] has the composition $C_{13}H_{10}N_2Br_2$, deduced from HRMS and ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum of 3 is reminiscent of that of 1, the only significant difference being confined to the protons of the ring A, which now resonate as a pair of mutually coupled signals [δ 8.22 (1H, d, J= 1.5 Hz, 5-H) and 7.86 (1H, d, J= 1.5 Hz, 7-H)]. The similarity between 1 and 3 was also evident from UV [λ max (CH_3OH) 233 (ε = 45267) 252 (27311), 284 (10689), 291 (17200),and 341 (4778),and 355 nm (4822)] and ^{13}C-NMR spectra. Particularly, in the latter spectrum the major difference was in the aromatic region where a methine resonance had been replaced by a non protonated carbon signal.

The above data clearly indicated that both the bromine atoms were present in the ring A, but their location remained to be established. In fact the meta coupling between the protons resonating at δ 8.22 and 7.86 is compatible with the positioning of the bromine atoms at C-6 and C-8 or at C-5 and C-7. ^{1}H- and ^{13}C-NMR spectra are more favourable to the former hypothesis, since the chemical shift values are in good agreement with those calculated starting from the data of compound 1, taking into account the effects produced by the introduction of a further bromine atom at C-8. Confirmatory evidence arose from a COLOC experiment (Table) which showed, inter alia, a correlation of C-9 with the protons resonating at δ 8.22 and 7.86, which can be explained only assuming that the unsubstituted carbon atoms in the ring A are C-5 and C-7 instead of C-6 and C-8.

Final proof for the correctness of structure 3 was provided by its synthesis starting from 5,7-dibromotryptophan (prepared according to Roverty et al.[8]) by reaction with propionaldehyde followed by acidic cyclization. The resulting tetrahydro-ß-carbolinecarboxylic acid, without any further purification, was treated with potassium dichromate which caused the aromatization of the ring C and the concomitant decarboxylation thus obtaining 6,8-dibromo-1-ethyl-ß-carboline (see scheme) whose spectral and chromatographic properties were identical to those of natural 3.

Following analogous procedures compounds 1 and 2 were synthesized starting from 5-bromo-tryptophan and propionaldehyde or acetaldehyde, respectively, thus unambiguously proving the proposed structures.

In keeping with the increasing pharmacological interest on brominated ß-carbolines[9] work is in progress to evaluate biological activities of metabolites 1 - 3. This can be more easily accomplished since substantial amounts of compounds are available through chemical synthesis.

EXPERIMENTAL

Electron impact (70 eV) mass spectra were taken on a Kratos MS 50 instrument. ^{1}H- and ^{13}C-NMR spectra were performed on a Bruker WM-250 spectrometer in $CDCl_3$ solns at 250.13 and 62.9 MHz respectively. Two DEPT experiments were performed using polarization transfer pulses of 90° and 135°, respectively, obtaining in the first case only signals for CH groups and in the other case positive signals for CH and CH_3 and negative ones for CH_2 groups.

The shift correlation with polarization transfer via ^1J and via ^2J and ^3J (COLOC) experiments were performed using Bruker microprograms on a 256x1024 data matrix adjusting the fixed delays to give maximum polarization for J_{C-H} = 135 Hz and 6.25 Hz, respectively.

UV spectra were measured on a Beckman DU-40 spectrophotometer. M.p.'s were measured on a Kofler apparatus and are uncorrected.

Isolation of 1, 2, and 3. - Colonies of the hydroid A.pluma identified by Dr. Pansini (University of Genova, Italy) were collected in the Bay of Salerno, Italy (Nov.-Dec. 1985) and freed by hand from macroscopic epibionts. A voucher specimen is deposited in the

Dipartimento di Chimica delle Sostanze Naturali, University of Naples.

Freshly collected material (wet weight 900 g) was freeze-dried and extracted at room temperature three times with MeOH for periods of two days. The hydro-methanolic solution was decanted, filtered, concentrated in vacuo and extracted with diethyl ether (three times). The organic phase was taken to dryness affording a brown oil (6.3 g). The crude product was fractionated by flash chromatography on a silica gel (Merck, 230-400 mesh) column (40 x 4 cm) under a moderate pressure of N_2, using as eluent 40°-70° light petroleum-diethyl ether (7:3, 1.5 l) followed by 40°-70° light petroleum-diethyl ether (3:7, 1.5 l) and then diethyl ether. Fractions of 50 ml were collected; fractions 41-61, taken to dryness, afforded 400 mg of a residue which was further chromatographed on preparative TLC. (Silica gel, Merck, 0.2 mm, 10 plates eluent diethyl ether-benzene, 6:4). Bands Rf 0.2, 0.3, and 0.5 (evidenced by UV light), scraped and eluted with diethyl ether, yielded crude 2, 1, and 3 respectively, which were finally purified by preparative TLC (silica gel, Merck 0.2 mm, 1 plate; eluent diethyl ether-benzene 7:3) affording pure 1 (10 mg) [λ_{max} (CH_3OH) 237 (ϵ = 33854), 254sh (19454), 281 (7436), 290 (10927), 345 (4382), and 358 nm (4818); HRMS: found m/z 274.0116 (base peak) calc. for $C_{13}H_{11}N_2{}^{79}Br$ 274.0106] ; 2 (11 mg) [λ_{max} (CH_3OH) 236 (ϵ = 33870) 253sh (19460), 281 (7460), 290 (11100), 345 (4380), and 358 nm (4820); HRMS: found m/z 259.9942 (base peak) calc. for $C_{12}H_9N_2{}^{79}Br$ 259.9950] ; and 3 (25 mg) [λ_{max} (CH_3OH) 233 (ϵ = 45267), 252 (27311), 284 (10689), 291 (17200), 341 (4778), and 355 nm (4822); HRMS: found m/z 353.9210 (base peak) calc. for $C_{13}H_{10}N_2{}^{79}Br{}^{81}Br$ 353.9190].

6-Bromo-1-ethyl-ß-carboline (1).

5-Bromogramine was prepared from commercial 5-bromoindole, dimethylamine and formaldehyde and converted into 5-bromotryptophan by published methods[9,10]. This aminoacid (340 mg) was dissolved in a mixture of 40 ml of 0.1 N H_2SO_4 and 15 ml of aqueous propionaldehyde (10%, w/v). After stirring for 18 hrs at 40°C, 4 ml of glacial acetic acid and 20 ml of an aqueous solution of potassium dichromate (10%, w/v) were added and the solution kept under reflux. After 5 min 20 ml of an aqueous saturated solution of sodium sulphite and 20 ml of an aqueous saturated solution of sodium carbonate were poured in the warm solution. The resulting blue solution was cooled and extracted with diethyl ether (3x100 ml). The crude organic phase was dried over anhydrous Na_2SO_4 and taken to dryness to give 120 mg of crude 1, which was crystallized from CH_2Cl_2 (m.p. 155-157°C) (found: C, 56.71; H, 4.28; N, 10.16. $C_{13}H_{11}N_2Br$ requires C, 56.75; H, 4.03; N, 10.18 %). Its physico-chemical and chromatographic properties matched those of natural 1.

6-Bromo-1-methyl-ß-carboline (2).

200 mg of 5-bromotryptophan and 6.8 ml of aqueous acetaldehyde (10%, w/v) were converted into 75 mg of 2, m.p. 155-157°C (from CH_2Cl_2) following the procedure used for the preparation of 1 (found: C, 55.13; H, 3.43; N, 10.76. $C_{12}H_9N_2Br$ requires C, 55.20; H, 3.47; N, 10.73%). Synthetic 2 was identical in all respect to the metabolite 2 isolated from A.pluma.

6,8-Dibromo-1-ethyl-ß-carboline (3).

5,7-Dibromotryptophan (3.1 g) prepared from isatin according to the method described by Roverty et al.[8], was treated with aqueous propionaldehyde (100 ml, 10% w/v) yielding 75 mg of 3, m.p. 150-152°C (from CH_2Cl_2) through the same experimental procedure as for 1 and 2. The purification of synthetic 3 required column chromatography on silica gel (eluent diethyl ether-benzene 7:3) (found: C, 44.12; H, 2.82; N, 7.89. $C_{13}H_{10}N_2Br_2$ requires C, 44.10; H, 2.84; N, 7.91%). Spectral and chromatographic properties were identical to naturally occurring 3.

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