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TRYPTOPHAN DERIVATIVES FROM A MEDITERRANEAN ANTHOZOAN, *ASTROIDES CALYCULARIS*

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ABSTRACT.—In addition to aplysinopsin (**1**), the anthozoan *Astroides calycularis* produces 6-bromoaplysinopsin (**6**) and smaller amounts of propionyl derivatives of **1** (**7**) and **6** (**8**).

Aplysinopsin (**1**), a yellow tryptophan derivative that showed cytotoxic activity against some cancer cell lines, has been isolated from three different marine sponges [*Thorecta* sp. (1), *Verongia spengelii* (2), and *Dercitus* sp. (3)]. The last organism also produces 2′-de-*N*-methylaplysinopsin (**2**) and 6-bromo-2′-de-*N*-methylaplysinopsin (**3**). More recently (4), the marine sponge *Smerospongia aurea* was shown to contain the closely related bromo compound **4**. In addition, the *N*-methylaplysinopsin (**5**) has also been isolated from a marine sponge (5). This compound is an interesting antidepressant, acting as a competitive inhibitor of monoamine oxidase.

We report that metabolites of this type, which until now seemed to be produced by Porifera only, are also present in the Mediterranean anthozoan *Astroides calycularis* Pallas which, in addition to **1**, elaborates the newly discovered 6-bromoaplysinopsin (**6**). In the extracts of this organism, we have also found smaller amounts of their *N*-propionyl derivatives **7** and **8**, from which possibly **1** and **6** could have been generated during the extraction procedure.

EXPERIMENTAL

INSTRUMENTAL.—^{1}H-nmr and ^{13}C-nmr spectra were recorded on a Bruker WM-250 instrument. Eims were obtained on an AEI MS-902 mass spectrometer. Uv spectra were recorded on a Beckman DB-GT instrument. Mps were measured on a Kofler hot stage apparatus and are uncorrected.

Ten medium-sized colonies of *A. calycularis* were collected in the Bay of Naples, Italy, in April 1984 (the voucher specimen is located in Dipartimento di Chimica delle Sostanze Naturali), and immediately freeze-dried. The biological material, freed by hand from macroscopic epibionts, was extracted four times with MeOH at room temperature. The aqueous methanolic solution was decanted, filtered, and concentrated to afford a dark brown oil (1.12 g), which was chromatographed on a SiO_2 column, under pressure, using as eluent, solvent mixtures of increasing polarities from C_6H_6 to MeOH through CH_2Cl_2. The fractions eluted with CH_2Cl_2-MeOH (97:3), (95:5), (9:1), and (85:15) afforded crude **8, 7, 6,** and **1,** respectively. Each of these fractions was chromatographed on Sephadex LH-20 columns using MeOH as eluent to give pure **8** (4 mg), **7** (5 mg), **6** (18 mg), and **1** (25 mg).

Compound **6**, mp 283-285° (from MeOH), λ max (MeOH) 385 nm, (ϵ=24000), M^+ *m/z* 332.0268, $C_{14}H_{13}N_4O\,^{79}Br$ requires 332.0273, ^{1}H-nmr and ^{13}C-nmr data are reported in Table 1.

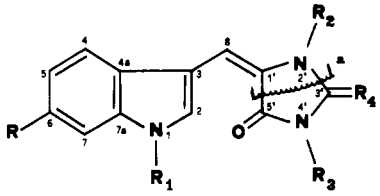
Compound **7**, mp >300° (from MeOH), M^+ *m/z* 310.1438, $C_{17}H_{18}N_4O_2$, requires 310.1431, ^{1}H-nmr data are reported in the Results and Discussion section.

Compound **8**, mp >300° (from MeOH) M^+ *m/z* 388.0543, $C_{17}H_{17}N_4O_2\,^{79}Br$, requires 388.0536, ^{1}H-nmr data are reported in the Results and Discussion section.

ACETYLATION OF **1**.—A mixture of **1** (8 mg) and excess of Ac_2O (0.3 ml) in 0.3 ml of dry pyridine was kept at room temperature overnight. Successive purification of the reaction mixture by plc on SiO_2, using C_6H_6 as the eluent, gave pure **9** (6 mg).

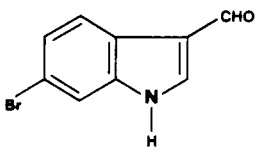
PROPIONYLATION OF **1**.—The mixture of **1** (10 mg) and excess of propionic anhydride (0.4 ml) in 0.4 ml of dry pyridine was kept at room temperature overnight. The excess reagents were evaporated under vacuum, and the residue, dissolved in $CHCl_3$, was chromatographed on plc (SiO_2; CH_2Cl_2-MeOH 9:1). The eluate was evaporated to give pure **10** (5 mg, mp >300°, M^+ *m/z* 366, ^{1}H-nmr spectrum is described in the Results and Discussion) and **11** (3 mg, mp >300°, M^+ *m/z* 310, ^{1}H-nmr spectrum is described in the Results and Discussion section).

PROPIONYLATION OF **7**.—Compound **7** (5 mg), treated by the method described above, gave **10** (4 mg).



	R	R_1	R_2	R_3	R_4
1	H	H	CH_3	CH_3	NH
2	H	H	H	CH_3	NH
3	Br	H	H	CH_3	NH
4	Br	H	H	H	O
5	H	H	CH_3	CH_3	NCH_3
6	Br	H	CH_3	CH_3	NH
7	H	CH_3CH_2CO	CH_3	CH_3	NH
8	Br	CH_3CH_2CO	CH_3	CH_3	NH
9	H	CH_3CO	CH_3	CH_3	CH_3CON
10	H	CH_3CH_2CO	CH_3	CH_3	CH_3CH_2CON
11	H	H	CH_3	CH_3	CH_3CH_2CON

SYNTHESIS OF **6**.—6-Bromoindole-3-carboxaldehyde (6) (**12**, 100 mg) and 1,3-dimethyl-2-iminoimidazolidin-4-one (7) (**13**, 75 mg) were mixed and heated at 150° for 5 min. The crude material was purified by plc [SiO_2; CH_2Cl_2-MeOH (9:1) as eluent]. The yellow band Rf 0.5 was scraped and eluted with MeOH to give **6** (122 mg), which was recrystallized from aqueous MeOH. It was identical to natural **6** by comparison of their physical properties (mixed mp, ^{1}H nmr, ^{13}C nmr, ms, uv).



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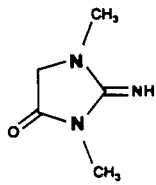

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RESULTS AND DISCUSSION

The identity of aplysinopsin (**1**), the major indole pigment, was proved by a comparison of its physical properties (mp, ^{1}H nmr and ^{13}C nmr, uv and ms) and those of its diacetate (**9**) with the data reported in the literature (1,2).

The mass spectrum of the optically inactive compound **6** (mp 283-285°) exhibited molecular ions of equal intensity at *m/z* 334 and 332; hence, the compound must contain one bromine atom; hrms at *m/z* 332 showed a molecular formula $C_{14}H_{13}N_4OBr$. The absorption maximum in the uv spectrum is similar to that of aplysinopsin (1,2). Also the ^{1}H-nmr spectrum (Table 1) is strongly reminiscent of that of **1** and showed signals at δ 3.05 (s, 3H) and 3.24 (s, 3H) attributed to N-methyl groups, at δ 6.38 (s, 1H), due to an olefinic proton and at δ 7.21 (dd, 1H, J=8, 1.5 Hz), 7.60 (d, 1H, J=1.5 Hz) and 7.85 (d, 1H, J=8 Hz), due to aromatic protons, suggesting that the compound under investigation could be a 6-bromoaplysinopsin. This was confirmed by comparison of its ^{13}C-nmr spectrum, which contains twelve distinct lines with two degenerate signals at δ 114.0 and δ 126.9 for two olefinic carbons, respectively, with that of **1**, whose signals, reported in Table 1, have been assigned by us on the basis of selective decoupling experiments and comparison to models. The definitive proof for the structure **6** was obtained from total synthesis; condensation of 6-bromoindole-3-carboxaldehyde (**12**) with 1,3-dimethyl-2-iminoimidazolidin-4-one (**13**) gave com-

TABLE 1. Nmr Data for **1** and **6**[a]

C chemical shifts		Assignment	H chemical shifts	
1	**6**		**1**	**6**
127.2	127.7	2	8.72 (bs)	8.67 (bs)
108.7	108.7	3		
119.3	119.6	4	7.45 (bd)	7.85 (d, J=8 Hz)
121.6	121.8	5	715 (m)	7.21 (dd, J=8, 1.5 Hz)
117.9	114.0	6	7.15 (m)	
116.6	114.0	7	7.89 (bd)	7.60 (d, J=1.5 Hz)
127.6	126.9	4a		
135.5	136.2	7a		
102.6	101.5	8	6.46 (bs)	6.38 (s)
126.4	126.9	1′		
162.1	162.1	3′		
150.6	150.4	5′		
24.7[b]	24.4[b]	2′	3.07[b]	3.05[b]
26.8[b]	26.6[b]	4′	3.26[b]	3.24[b]

[a]The ^{1}H-nmr spectrum of **6** was recorded on a Bruker 250 WM instrument in DMSO-d_6 solution and the assignments were confirmed by decoupling experiments. The ^{13}C-nmr spectra of **1** and **6** were taken at 62.9 MHz in DMSO-d_6 solution. Multiplicities were determined from single-frequency off-resonance decoupling and assignments were made based upon selective decoupling experiments and/or a comparison to models. The δ values are in ppm downfield from TMS.

[b]Signals within a column may be interchanged.

pound **6** in 70% yield. The synthetic material was identical in all respect to the natural product.

The illustrated *E*-configuration for the C-8 double bond is tentatively assigned from consideration of previous work by Hollenbeak and Schmitz (2), which proved this configuration for aplysinopsin diacetate on the basis of nOe experiments. Compound **7** had an elemental composition $C_{17}H_{18}N_4O_2$ as established by hrms on the molecular ion at *m/z* 310. The 250 MHz ^{1}H-nmr spectrum ($CDCl_3$) of **7** revealed the presence of a propionyl residue that gave rise to signals at δ 3.09 (q, 2H, J=7 Hz, CH_3-CH_2-CO-$N_1<$) and 1.38 (t, 3H, J=7 Hz, CH_3-CH_2-CO-$N_1<$). The remaining signals [δ 9.14 (s, 1H, 2-H), 8.54 (bd, 1H, J=7 Hz, 7-H), 7.65 (bd, 1H, J=7 Hz, 4-H), 7.2-7.5 (m, 2H, 5-H and 6-H), 6.20 (s, 1H, 8-H), 3.33 and 3.24 (s, 3H each, 2′-CH_3 and 4′-CH_3)] were remarkably similar to those of **1**. This suggested that both compounds possess the same skeleton, the only difference being the presence in **7** of a CH_3-CH_2-CO residue linked to a nitrogen atom. This hypothesis was supported by the fact that **7** easily undergoes a hydrolytic cleavage by atmospheric moisture at room temperature to afford **1**. The location of the propionyl residue on the indole nitrogen atom was deduced from the mass spectrum of **7**: the peaks at *m/z* 226 (a) and 225 (a-H) exclude the possibility that the propionyl residue could be linked to the imino nitrogen atom.

The structure of **7** was confirmed by treatment with propionic anhydride in pyridine which gave compound **10** in 78% yield [mp>300°; ^{1}H nmr ($CDCl_3$) δ 9.26 (s, 1H, 2-H), 8.51 (m, 1H, 7-H), 7.67 (m, 1H, 4-H), 7.1-7.5 (m, 2H, 5-H and 6-H), 6.55 (s, 1H, 8-H), 3.35 and 3.22 (s, 3H each, 2′-CH_3 and 4′-CH_3), 3.14 (q, 2H, J=7 Hz, CH_3-CH_2-CO-$N_1<$), 2.54 (q, 2H, J=7 Hz, CH_3-CH_2-CO-N=C<), 1.43 (t, 3H, J=7 Hz, CH_3-CH_2-CO-$N_1<$) and 1.27 (t, 3H, J=7 Hz, CH_3-CH_2-CO-N=C<); ms M^+ *m/z* 366]. This compound was identical in all respects to the compound obtained from aplysinopsin by the same treatment. In addition to **10**, the reaction of **1** with propionic anhydride/pyridine afforded the monopropionyl derivative **11** [mp > 300°; ^{1}H nmr ($CDCl_3$) δ 9.07 (d, 1H, J=2 Hz, 2-H), 8.88 (s, 1H, N-H), 7.78

(m, 1H, 7-H), 7.47 (m, 1H, 4-H), 7.1-7.35 (m, 2H, 5-H and 6-H), 6.75 (bs, 1H, 8-H), 3.25 and 3.11 (s, 3H each, 2′-CH_3 and 4′-CH_3), 2.50 (q, 2H, J=7 Hz, CH_3-CH_2-CO-N=C<) and 1.23 (t, 3H, J=7 Hz, CH_3-CH_2-CO-N=C<); ms: M^+ *m/z* 310] in which the propionyl residue was proved to be linked to the imino nitrogen on the basis of its ^{1}H-nmr spectrum: HC-2 signal (δ 9.07) resonates as a doublet (J=2 Hz) and was simplified to a singlet by irradiation at δ 8.88 (indole N-H).

The methanolic extract of *A. calycularis* also contains smaller amounts of **8** ($C_{17}H_{17}N_4O_2$ Br from hrms, mp >300°), which easily hydrolyzes to **6** by atmospheric moisture. Its structure, analogous to **7**, was based on ^{1}H-nmr correlation ($CDCl_3$) δ 9.08 (s, 1H, 2-H), 8.77 (bs, 1H, 7-H), 7.49 (m, 2H, 4-H and 5-H), 6.10 (s, 1H, 8-H), 3.32 and 3.23 (s, 3H each, 2′-CH_3 and 4′-CH_3) with the parent compound (**6**, Table 1). The location of the propionyl residue was indicated from the intense fragmentation ions in mass spectrum *m/z* 304.306 (a) and 303.305 (a-H) and from the values of the propionyl protons in the ^{1}H-nmr spectrum [δ 3.08 (q, 2H, J=7 Hz, CH_3-CH_2-CO-N_1<) and 1.37 (t, 3H, J=7 Hz, CH_3-CH_2-CO-N_1<)], which are significantly downfield shifted when this functionality is linked to an indole nitrogen as in **7**.

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