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POLYALKYLATED CYCLOPENTINDOLES: CYTOTOXIC FISH ANTIFEEDANTS FROM A SPONGE, AXINELLA SP.

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ABSTRACT: A Western Australian sponge, Axinella sp., yielded three polyalkylated indoles unsubstituted at C3, thus apparently not derived from tryptophan. The three compounds, herbindoles A, B, and C, are cytotoxic against KB cells and are fish antifeedants.

An orange-colored sponge, Axinella sp., was collected in the Gulf of Exmouth, Western Australia, at a depth of 7-10 m. The initial extract (methylene chloride/MeOH 1:1) was successively purified by flash chromatography, HPLC, and crystallization, which eventually led to isolation of one major and two minor constituents in a combined yield of 0.9% based on dry animals.

The major constituent, herbindole A (1)[1], colorless needles (MeOH), mp 120-122°C, had a UV spectrum appropriate for an indole (λmax 210, 226 sh, 272 nm) and composition of $C_{16}H_{19}N$ as determined by HREIMS. Three successive losses of methyl provided the only significant fragments. The IR spectrum confirmed the presence of a secondary amine (3380 cm^{-1}). The structure was solved by interpretation of NMR spectra.

The ^{1}HNMR spectrum (Table I) revealed the presence of four methyl groups, two aromatic (δ 2.45, 2.28) and two on methine carbons (δ 1.34, 1.22). Two doublets of doublets with J values of 1-2 Hz indicated that the indole was unsubstituted in the hetero ring. Absence of benzenoid or olefinic proton signals and an unsaturation value of 7 required that an additional ring was fused to the benzene ring. A COSY experiment proved part structure A, which contains all five carbons other than dimethylindole system. Double quantum filtered COSY, long range COSY, and NOE experiments fully delineated structure 1 for herbindole A (4,5,6,8-tetramethyl-6,7-dihydro-8H- cyclopent [g] indole), see Tables I and II.

Further HPLC separations led to the isolation of herbindole B (2), colorless needles (MeOH), mp 118-120°C. Comparison of the UV, IR, and MS data with those of herbindole A (1) suggested a closely related compound with one more methylene group. This was confirmed by the ^{1}HNMR spectrum of 2 (Table I) and by homonuclear decoupling experiments, which proved that one of the aromatic methyl groups in 1 was replaced in B by an ethyl. Long range COSY (Table III) and NOE experiments proved the regiochemistry shown in 2. Irradiation of the methyl singlet at δ2.34 enhanced the proton signals of H6 and 6-Me and 1'-CH_2-, thus indicating that the irradiated methyl was located at C5. Conversely,

irradiation of the methylene resonance at δ 2.97 enhanced the signals of H3 and C5 aromatic methyl. ^{13}C NMR signals (Table II) were assigned by analogy with those of **1**.

Herbindole C (**3**) was isolated as a colorless oil. Inspection of UV, IR and MS data again suggested a polyalkylated indole of composition $C_{18}H_{23}N$. Additional ^{1}HNMR signals (δ6.90, 1H d, J = 16.2Hz; 6.32, 1H dt, J = 16.2, 6.3Hz; 2.26, 2H qd, J = 7.2, 6.3Hz; and 1.08 (3H t, J = 7.2 Hz) were compatible with a trans-1-butenyl sidechain. Long range COSY and NOE experiments (Table III) proved the structure of **3**. Irradiation of the C5 methyl singlet at δ 2.40 enhanced H1' (δ 6.90), H6(δ 3.33), and 6-Me (δ 1.34), while irradiation of the olefinic proton H1' enhanced the 5-Me singlet (δ 2.40) and the H3 signal (δ 6.93). This signal, downfield by 0.3 ppm from the corresponding resonances in **1** and **2**, is deshielded by the double bond of the butenyl sidechain.

All three herbindoles are cytotoxic against KB cells at MIC of 5 μg/mL for A(**1**); >10 μg/mL for B(**2**) and 10 μg/mL for C(**3**).

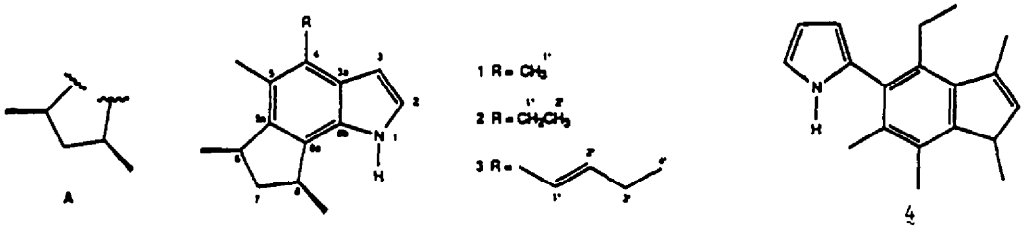

Table I. ^{1}H NMR Data for Herbindoles A, B and C (benzene-d_6)

proton no.	A	B	C
1	6.8 (bs,1H)	6.79 (bs,1H)	6.82 (bs,1H)
2	6.65 (dd,2.5,0.5,1H)	6.66 (dd,3.0,2.4,1H)	6.69 (dd,3.0,2.4,1H)
3	6.61 (dd,2.5,1.0,1H)	6.61 (dd, 3.0,2.1,1H)	6.93 (dd,3.0,2.1,1H)
6	3.34 (dqd,9.0,7.0,2.0,1H)	3.34 (dqd,9.3,6.9,1.8,1H)	3.33 (dqd,9.0,7.2,1.8,1H)
7α	2.60 (ddd,12.8,9.0,9.0,1H)	2.59 (ddd,12.9,9.3,9.3,1H)	2.59 (ddd,13.2,9.0,9.0,1H)
7β	1.46 (ddd,12.8,2.0,2.0,1H)	1.46 (ddd,12.9,1.8,1.8,1H)	1.45 (ddd,13.2,1.8,1.8,1H)
8	3.18 (dqd,9.0,7.0,2.0,1H)	3.16 (dqd,9.3,7.2,1.8,1H)	3.18 (dqd,9.0,7.8,1.8,1H)
5-Me	2.45 (s,3H)	2.34 (s,3H)	2.40 (s,3H)
6-Me	1.34 (d,7.0,3H)	1.35 (d,6.9,3H)	1.34 (d,7.2,3H)
8-Me	1.22 (d,7.0,3H)	1.21 (d,7.2,3H)	1.22 (d,7.8,3H)
1'	2.28 (s,3H)	2.97 (q,7.5,2H)	6.90 (d,16.2,1H)
2'	-	1.27 (t,7.5,3H)	6.32 (dt,16.2,6.3,1H)
3'	-	-	2.26 (qd,7.2,6.3,2H)
4'	-	-	1.08 (t,7.2,3H)

Table II.
^{13}C NMR Data for Herbindoles A, B and C (Benzene-d_6)

carbon no.	A	B	C
2	123.0	122.39	123.11
3	101.7	101.06	103.11
3a	126.5	127.03	127.36
4	128.7	128.65	128.56
5	122.9	121.44	122.67
5a	126.4	126.34	127.03
6	39.5	39.21	39.45
7	42.2	41.80	42.07
8	37.5	36.95	37.41
8a	141.8	141.64	141.79
8b	130.9	132.59	134.92
5-Me	15.6	14.40	14.38
6-Me	24.0	23.33	23.99
8-Me	23.0	22.55	22.89
1'	15.4	14.59	129.00
2'	-	23.72	135.78
3'	-	-	16.15
4'	-	-	27.18

Table III.
Long Range COSY Data for Herbindoles B and C (Benzene-d_6)

proton no.	B L.R. COSY	C L.R. COSY
1		
2	H3	H3
3	H2	H2
6	H7α,H7β,5-Me,6-Me	H7α,5-Me,6-Me
7α	H6,H7β,6-Me	H6,6-Me
7β	H6,H8,H7α	
8	H7β,5-Me,8-Me,$H_2$1'	5-Me,8-Me
5-Me	H6,H8,$H_2$1'	H6,H8,H1',H2'
6-Me	H7α,H6	H7α,H6
8-Me	H8	H8,H7α
1'	$H_3$2',Me5,H8	H2',$H_2$3'
2'	$H_2$1'	H1',$H_2$3',$H_3$4'
3'		H1',H2',$H_3$4'
4'		H2',$H_2$3'

In field experiments conducted at Fingers Reef, Apra Harbor, Guam, the combined herbindoles proved to possess significant feeding deterrence against generalist fishes including wrasses, triggerfishes, and sergeant majors.

A series of similar polyalkylated cyclopentindoles, the trikentrins had been isolated by Capon et al.[2] from a north Australian sponge, Trikentrion flabelliforme.

The unusual feature common to these compounds as was pointed out earlier,[2] is the lack of substitution at C3, which points to a biogenetic pathway not involving tryptophan. A clue to a possible α-phenylindole origin of these compounds was recently provided by a metabolite (4) which Kornprobst and coworkers[4] isolated from an axinellid sponge, Trikentrion loeve.

Experimental Part

The sponge (756 g dry weight) was extracted with methylene chloride/MeOH 1:1 yielding a solid residue (29.1 g. 3.85%). Flash chromatography of ~1 g extract on silica gel with a hexane/ethyl acetate gradient yielded the herbindole mixture concentrated in the nonpolar fractions. HPLC on silica (hexane/ethyl acetate 9:1) resulted in a dark reddish brown residue (428 mg), which was further purified by vacuum liquid chromatography (VLC)

on reverse phase silica (RP-18, 75 x 65 mm, elution with methanol/water, 4:1) yielding the herbindoles as a purple fraction (229.5 mg). Further purification on reverse phase HPLC (RP-18, 5μm, Lichrosorb, methanol/water, 4:1) yielded pure herbindole A and herbindole C and a crude mixture of herbindole A and herbindole B. HPLC of this crude fraction (RP-18, 5 μm, Lichrosorb, methanol/water, 3:1) yielded pure herbindole A and herbindole B.

Herbindole A - Colorless needles from methanol mp 120-122°C. UV (MeOH): λ_{max} 210 (log ∈ 4.74). 226 sh (4.68, 272 nm (3.93); IR 3380, 2990, 2960, 1480, 1470, 1130, 740 cm^{-1}; HREIMS m/z 213. ($C_{15}H_{19}N$ requires 213). EIMS m/z 213 (45%), 198 (100), 183 (26), 168 (12).

Herbindole B - Colorless needles from methanol mp 118-120 °C. UV (MeOH): λ_{max} 222 (log ∈ 4.67). 272 nm (4.09); IR 3410, 2970, 2920, 2880, 1450, 1400, 1130, 730 cm^{-1}; HREIMS m/z 227.1679 ($C_{16}H_{21}N$ requires 227.1684), 212.1437 ($C_{15}H_{18}N$ requires 212.1435). EIMS m/z. 227 (53%), 212 (100), 198 (9), 183 (12), 168 (9).

Herbindole C - Colorless oil. UV (MeOH): λ_{max} 212 (log ∈ 4.70), 226 (4.63), 294 nm (4.00); IR 3420, 2980, 2940, 2880, 1460, 1270, 1100, 1020, 800, 730 cm^{-1}; HREIMS m/z. 253.1844 ($C_{18}H_{23}N$ requires 253.1857), 238.1611 ($C_{17}H_{20}N$ requires 238.1626) EIMS m/z. 253(80%), 238 (100).

Antifeedant Assays - The herbindole mixture was coated on thin strips of squid at a concentration of 1.2% of extract per squid dry wt. Control pieces of squid were coated with diethyl ether. Four pieces of treated squid were attached with paper clips to a polypropylene line, while four pieces of control squid were similarly attached to another line. The lines were paired, treated and untreated and fastened to the reef for 15-30 min. A total of 13 pairs were used. The results for N=11 pairs, p=0.005, were (X±SE) 48.1 ± 10% for treated and 80.8 ± 6.4% for control squid that were completely eaten. Further experimental details were described previously.[3]

References and Notes

1. The name is derived from the first author's family name.
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