Tetrahedron Vol. 45, No. 3, pp. 617 to 622, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00

KUMEPALOXANE, A REARRANGED TRISNOR SESQUITERPENE FROM THE BUBBLE SHELL HAMINOEA CYMBALUM[1]

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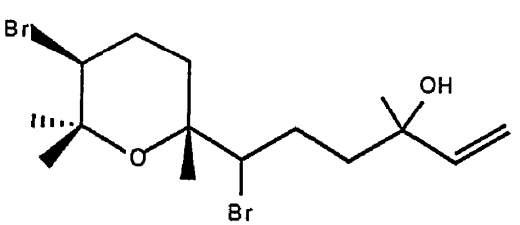
(*Received in USA* 4 *October* 1988)

ABSTRACT: Kumepaloxane (3), a feeding deterrent to generalist carnivorous fishes and to the pufferfish Canthigaster solandri was isolated from the Guamanian mollusc Haminoea cymbalum; its structure was elucidated by spectral analysis.

Among numerous halogenated sesquiterpenes reported from the red algal genus Laurencia[2] are only two tetrahydropyranyl derivatives, obtusenol (1)[3] and 3β-bromo-8-epicaparrapi oxide (2)[4], both from L. obtusa. The latter compound (2) is the brominated epimer of an essential oil constituent from a Colombian tree.[5] We wish to report the structure of a truncated (C_{12}) rearranged sesquiterpene encompassing an oxane ring. It was isolated from a bubble shell and was shown to be a fish antifeedant.

The brightly colored bubble shell Haminoea cymbalum (Family Atyidae, Order Cephalaspidea, Subclass Opisthobranchia) was collected from the reef flat at Pago Bay, Guam. Approximately 100 animals were collected from the green alga Caulerpa racemosa; however, the animals did not appear to graze on Caulerpa but may eat microbial films or epiphytes on the algal surfaces. The whole animals were extracted three times with acetone, which was evaporated to yield 0.3 g organic extract from 13.4 g of dry mass of Haminoea including shells. Kumepaloxane[6] (3) was the major metabolite in the extracts and comprised 40% of the organic extract or 0.9% of the dry mass of the animals. This nonpolar metabolite was isolated by silica gel flash chromatography followed by HPLC.



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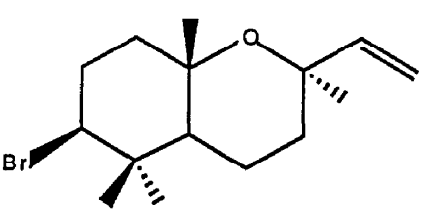

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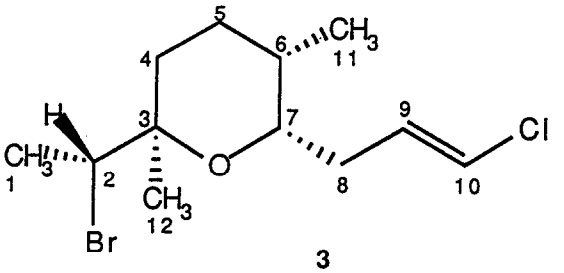

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Kumepaloxane was obtained as an optically active colorless oil, $[\alpha]_D$+22.6°. The molecular formula $C_{12}H_{20}BrClO$, was determined from HRCIMS, which gave a molecular ion cluster with the isotope pattern $MH^+/MH+2^+/MH+4^+$ in the ratio 3:4:1, which is typical for compounds containing Br and Cl. The IR spectrum (υ_{max}740 and 650 cm^{-1}) confirmed the presence of both halogens, while the ^{13}C NMR and DEPT data (Table 1) indicated the presence of 12 carbons and 20 protons. The structure of kumepaloxane (**3**) was established through interpretation of 1-D and 2-D NMR data. The relative stereochemistry of 3 was deduced from n.O.e. measurements.

Table 1. NMR Data for Kumepaloxane (**3**)

Position	^{13}C			^{1}H		
	δ	mult.	^{1}J(Hz)	δ	mult.	J(Hz)
1	19.29	q	128.8	1.62	d	6.8
2	49.61	d	148.1	4.66	q	6.8
3	76.04	s	-	-	-	-
4α	27.64	t	127.1	1.50	m	-
β				2.04	m	-
5α	26.35	t	123.4	1.44	dddd	13.0,3.5,3.5,3.5
β				1.81	dddd	13.4,12.8,4.3,4.3
6	29.57	d	128.1	1.62	m	-
7	71.91	d	138.4	3.47	ddd	8.2,5.3,2.7
8a	35.03	t	128.3	1.99	ddd	14.2,8.1,5.3
b				2.14	dddd	14.2,8.2,7.0,1.1
9	130.42	d	161.1	5.85	ddd	13.3,8.1,7.0
10	118.65	d	193.7	5.97	dd	13.3,1.1
11-Me	11.25	q	124.4	0.99	d	7.0
12-Me	23.25	q	126.3	1.25	s	-

The double quantum filtered COSY (DQFC) spectrum (see Table 2) established that C-4, -5, -6, and 11Me are contiguous, as are C-7,-8,-9,-10. In addition, H-2 was coupled only to the methyl doublet Me-1. The connectivity between H-6 and H-7 was not seen in the DQFC spectrum as these hydrogens are nearly orthogonal and hence have a small mutual coupling; however, the connection of C-6 to C-7 was established from relayed coherence transfer COSY (RCT COSY) results (Table 2); in particular, 11-Me showed relayed proton connectivity to H-7. There were signals corresponding to two olefinic carbons (C-9,10) and 3 heteroatom-substituted carbons (C-2,-3,-7) in the ^{13}C NMR spectrum. From their chemical shifts (76.04, 71.91 ppm), C-3 and C-7 are attached to the oxygen atom and are therefore ether-linked, which required that 12-Me and C-2 must be attached to the quaternary carbon, C-3. The proton coupling between H-9 and H-10 (13.3 Hz) is typical of a trans vinyl halide,[8] while the carbon chemical shift of C-10 (118.65 ppm) indicated a vinyl chloride;[9] hence bromine is situated at the remaining hetero-substituted carbon C-2. Additional evidence for this assignment came from the EIMS, which recorded a peak at 187 amu corresponding to the loss of 1-bromoethyl radical from the molecular ion.

Table 2. DQFC and RCT COSY Data for Kumepaloxane (3)

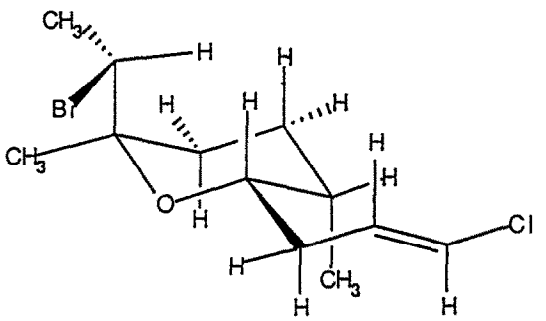
Position	DQFC Correlations	RCT COSY Correlations
H4α	H4β,H5α,H5β	-
H4β	H4α,H5α,H5β,H6	H6,11-Me
H5α	H4α,H4β,H5β,H6	11-Me
H5β	H4α,H4β,H5α,H6	11-Me
H6	H4β,H5α,H5β	H4β
H7	H_2-8	H9,11-Me
H2	H_3-1	-
H_3-1	H2	-
H_a-8	H7,H_a-8,H9	H10
H_b-8	H7,H_a-8,H9,H10	-
H9	H_2-8	H7
H10	H8,H9	H_2-8
11-Me	H6	H4β,H5α,H5β,H7

Table 3. Nuclear Overhauser Enhancements for Kumepaloxane (3)

Irradiated	Enchanced (%)
H7	H5β(1.2),H2(8.8),H8β(0.8),H8α(0.9),H9(2.0),H10(0.7)
H2	H5β(3.2),H7(8.5),H_3-1(4.3)
H8a	H6(8.0),H7(2.2),H9(2.3),H10(1.1)
H9	H7(1.8),H8β(1.4),H8α(1.6)
H_3-1	H4β(3.6),H2(15.0),12Me(1.1)
12-Me	H4α(1.4),H4β(1.8),(1.2)(H_3-1)
11-Me	H4α+H6(9.6)*,H8β(3.4),H9(1.9),H10(0.7)

*These signals are nearly coincident; hence the percentages are combined.

The relative stereochemistry at C-3,-6,-7,-8, was assigned from n.O.e. data (Table 3). The six-membered oxane ring adopts the expected chair configuration (3a) with 11-Me and H-4α axially oriented (n.O.e. from C-11Me to H-4α and confirmed by a W connectivity between the equatorial protons H-6 and H-4β in the DQFC). N.O.e's from H-7 to H-5β and H-2, from H-2 to H-7 and H-5β, and from H_3-1 to H-4β (and not to H-4α) indicated that both the C-3,2 bond and H-7 are axial, necessitating that the allyl group and 12-Me are equatorial. The axial orientation of C-2 causes restricted rotation around the C-3-2 bond because of bromine and methyl substitution at C-2. H-2 points towards H-7 and H-5β, as it shows n.O.e.'s to both protons and not to 12-Me, while H_3-1 gave n.O.e.'s to H-4β and 12-Me; consequently, if C-3 has R configuration, 2 must have likewise.



3a

Kumepaloxane appears to function in chemical defense. It is exuded in the mucus of the animals when they are disturbed and is an effective feeding deterrent toward carnivorous fishes in field and aquarium assays on Guam. Field assays were conducted at Finger's Reef on Guam and a variety of fishes including wrasses, triggerfish, sergeant majors, and

emperors fed during the assays. The metabolite significantly reduced grazing by 32% relative to control pieces coated with ether only (control $\bar{X}$=68.6%+SE5.8% eaten; treated 46.9% + 10.2% eaten; N=15, p=0.05, Wilcoxon signed-ranks test for paired comparisons).[10] In aquarium assays with the pufferfish, Canthigaster solandri, kumepaloxane significantly reduced grazing on an artifical diet of brine shrimp and krill in agar. The compound was coated onto the surface of the food at 1.8% dry mass, which approximates concentrations found in the soft tissues of H. cymbalum (control $\bar{X}$=0.842 g eaten, treated $\bar{X}$=0.716 g eaten; N=7, p=0.015, paired t-test).

We do not know if kumepaloxane is of dietary origin or is synthesized by H. cymbalum. The compound is not found in Caulerpa racemosa, where the mollusks graze.

EXPERIMENTAL PART

Isolation - H. cymbalum (approximately 100 animals) were collected in July 1987, in Pago Bay, Guam, from the surfaces of the green alga Canlerpa racemosa. Whole animals were extracted three times with acetone at room temperature. Removal of the solvent furnished a residue (0.3 g, 2.25% of dry wt. of the animals), which was flash-chromatographed on silica gel (hexane), followed by HPLC (Silica, Alltech). Kumepaloxane (3) was isolated as a colorless oil (0.12 g, 0.91% of animal).

Structure - $[\alpha]_D$ + 22.6° (C 0.32, $CHCl_3$); IR ($CHCl_3$): υ_{max} 2950, 2850, 1640, 1450, 1380, 1070, 1060, 1030, 940, 750, 740, 670 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3/C_6D_6$, 7:3) see Table 1; ^{13}C NMR (75 MHz), $CDCl_3/C_6D_6$, 7:3): see Table 1; CIMS: m/z 295.0405; $C_{12}H_{21}BrClO$ (M+H) requires 295.0464; EIMS: m/z 187.0808; $C_{10}H_{16}ClO$ ($M^+ - C_2H_4Br$) requires 187.0889.

Bioassays - For the field assay, kumepaloxane was dissolved in ether and the resulting solution was coated on thin strips of squid at a concentration of 1% of **3** relative to dry mass of squid. As previously described[11], four pieces of treated squid were attached by paper clips to polypropylene lines. Four pieces of control squid were also attached to lines; and treated and control lines were attached to the reef as a paired sample. Sixteen replicate pairs were placed on the reef for approximately 20 minutes. Results were scored as the number of pieces of squid completely eaten.

For the laboratory assay, two or three puffer fish, Canthigaster solandri, were placed in individual chambers of a large outdoor aquarium at the University of Guam Marine Laboratory and were fed a diet consisting of brine shrimp and krill in agar. The fish were allowed to graze for approximately 30 min. on strips of diet either coated with kumepaloxane or with ether (controls).

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Acknowledgments. This research was supported by the National Science Foundation and the University of Hawaii Sea Grant College Program under Institutional Grant NA81AA-D-0070 from NOAA, Office of Sea Grant, U.S. Department of Commerce. Research on Guam was supported by the National Institutes of Health (GM38624). We thank Scott Sherman for assistance with the isolation of kumepaloxane and Shelly Rogers with the bioassays. This is contribution #261 of the University of Guam Marine Laboratory.