6-ACYLLUMAZINES FROM THE MARINE POLYCHAETE, *ODONTOSYLLIS UNDECIMDONTA*

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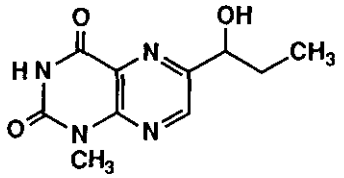
Abstract - Lumazine and its 3-methyl and 1,3-dimethyl derivatives having 6-acyl substituents such as acetyl, propionyl, β-methoxypropionyl, and β-hydroxypropionyl groups were isolated from the marine polychaete, *Odontosyllis undecimdonta.*

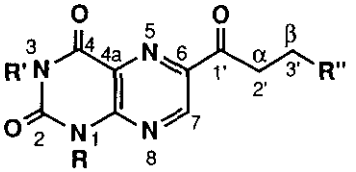
Marine natural lumazine having the C-3 side chain at C-6 position was isolated from *Leucetta microraphis* , a calcareous sponge, by Cardellina and Meinwald in 1981 and named leucettidine.[1] Pfleiderer determined its structure as 6-(1-hydroxypropyl)-1-methyllumazine (**1**).[2] As a part of our ongoing studies on marine natural products, we examined isolation and characterization of the metabolites of a luminous swimming polychaete, *Odontosyllis undecimdonta.*[3,4]

This worm appeared at the surface of the water in some abundance about thirty minutes after sun set and luminesced and spawned for approximately thirty minutes. The spawning can be observed only once a year for about four weeks from the end of September to end of October at Toyama Bay. Collections were made by hand using a fine mesh net at Namerikawa and Uozu sea shores. The specimens (*ca.* 5000 individuals) were lyophilized to give 10 g of dried worms which were ground and subsequently emitted a bright luminescence[5] after being placed in 200 ml of water. The suspended worms were repeatedly ground and squeezed until bioluminescence[6] ceased to be detectable in the dark. This was followed by evaporation, without filtration, to dryness *in vacuo.* The resulting residue obtained was extracted with MeOH. Eight compounds were isolated from the MeOH extract by a combination of silica gel column

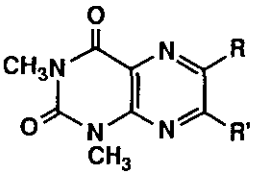
chromatography and successive tlc separations. Structures were determined as 6-acyllumazines (**2-9**) based on the data presented below.



Leucettidine **1**



2 R=R'=CH_3, R"=H
3 R=R"=H, R'=CH_3
4 R=R'=R"=H
6 R=R'=CH_3, R"=OCH_3
7 R=H, R'=CH_3, R"=OCH_3
8 R=R'=CH_3, R"=OH
9 R=H, R'=CH_3, R"=OH



5 R=$COCH_3$, R'=H
10 R=R'=H
11 R=H, R'=$COCH_2CH_3$
12 R=H, R'=SCH_3
13 R=$COCH_2CH_2OCH_3$, R'=SCH_3
14 R=$COCH_3$, R'=SCH_3
15 R=COCH=CH_2, R'=H

Molecular formulae of the metabolites (**2-9**) were determined by HRms as shown in Table 1. The ^{13}C nmr analysis of these metabolites, **2-6**, **8**, and **9**, with six signals in the sp^2 carbon region, given in Table 2, showed that chemical shifts to be consistent with those of the lumazine derivatives and resonances around 199 ppm to possibly be due to carbonyl groups attached to the C-6 position.

Table 1. Mp and HRms Spectral Data for 6-Acyllumazine Derivatives

Compound	mp (°C)	Molecular Formula	Calcd	Found (*m/z*)
2	146-147	$C_{11}H_{12}N_4O_3$	248.0909	248.0905
3	227-228	$C_{10}H_{10}N_4O_3$	234.0752	234.0768
4	278-280 (decomp.)	$C_9H_8N_4O_3$	220.0596	220.0593
5	177-178	$C_{10}H_{10}N_4O_3$	234.0752	234.0756
6	129-130	$C_{12}H_{14}N_4O_4$	278.1014	278.1027
7	193-196 (decomp.)	$C_{11}H_{12}N_4O_4$	264.0858	264.0868
8	168-169 (decomp.)	$C_{11}H_{12}N_4O_4$	264.0858	264.0868
9	-200 (decomp.)	$C_{10}H_{10}N_4O_4$	250.0701	250.0736

From the above data and results of ^1H nmr analysis of the metabolites, structures of the side chain were determined as that of the propionyl group for three of the major metabolites, **2**, **3**, and **4**, the acetyl group for **5**, the β-methoxypropionyl group for **6** and **7** and the β-hydroxypropionyl group for **8** and **9**. Two singlets in and around 3.57 and 3.76 ppm in compounds (**2**, **5**, **6**, and **8**) were assigned as *N*-methyl substituents attached to lactam nitrogen atoms at positions 3 and 1, while the singlet observed at 3.54 ppm

Table 2. ^{13}C Nmr Spectral Data for 6-Acyllumazine Derivatives

Compound	Solvent	N-1 CH_3	N-3 CH_3	C-4a	C-7	C-1'	C-2'	C-3'	OCH_3	Others
2	$CDCl_3$	29.8	29.2	125.7	147.0	200.4	31.3	7.5	-	143.4, 149.7, 150.4, 159.3
3	DMSO-d_6	-	27.6	125.5	146.7	199.6	30.3	7.5	-	142.5, 150.0, 150.1, 160.0
4	DMSO-d_6	-	-	126.3	146.7	199.6	30.1	7.6	-	141.7, 150.9, 152.9, 160.7
5	$CDCl_3$	29.8	29.2	125.8	147.1	197.8	25.8	-	-	143.6, 149.8, 150.4, 159.2
6	$CDCl_3$	29.8	29.2	125.7	147.2	197.9	38.0	67.4	58.8	143.3, 149.8, 150.4, 159.2
8	DMSO-d_6	29.3	28.5	126.4	146.0	198.1	40.6	56.4	-	142.2, 149.7, 150.3, 159.1
9	DMSO-d_6	-	27.6	125.4	146.9	198.1	40.3	56.5	-	141.8, 151.1, 151.5, 160.4

Table 3. ^{1}H Nmr Spectral Data for 6-Acyllumazine Derivatives

Compound	Solvent	N-1 CH_3 (3H, s)	N-3 CH_3 (3H, s)	H-7 (1H, s)	H-2'	H-3'	OCH_3 (3H, s)
2	$CDCl_3$	3.76	3.57	9.28	3.33 (2H) (q, J=7.3 Hz)	1.24 (3H) (t, J=7.3 Hz)	-
3	$CDCl_3$	-	3.54	9.25	3.32 (2H) (q, J=7.3 Hz)	1.25 (3H) (t, J=7.3 Hz)	-
4	DMSO-d_6	-	-	9.08	3.15 (2H) (q, J=7.4 Hz)	1.12 (3H) (t, J=7.4 Hz)	-
5	$CDCl_3$	3.77	3.57	9.29	2.82 (3H) (s)	-	-
6	$CDCl_3$	3.76	3.57	9.29	3.55 (2H) (t, J=5.9 Hz)	3.87 (2H) (t, J=5.9 Hz)	3.36
7	$CDCl_3$	-	3.54	9.24	3.54 (2H) (t, J=6.0 Hz)	3.87 (2H) (t, J=6.0 Hz)	3.36
8	$CDCl_3$	3.77	3.57	9.30	3.54 (2H) (t, J=5.5 Hz)	4.08 (2H) (t, J=5.5 Hz)	-
9	CD_3OD	-	3.54	9.15	3.44 (2H) (t, J=6.0 Hz)	3.99 (2H) (t, J=6.0 Hz)	-

in compounds (**3**, **7**, and **9**) was assigned as N-3 methyl group since it usually resonated in a higher field than that of the N-1 methyl as mentioned by Pfleiderer.[7] This assignment was supported by uv spectral behavior, since uv absorptions of **3**, **7**, and **9** were consistent with those of a known 3-methyllumazine chromophore with a large bathochromic shift of long wavelength absorption band in basic medium.[2b]

Table 4. Uv Absorption Spectral Data for 6-Acyllumazine Derivatives

Compound	Solvent	λ max (nm)	log ε
2	MeOH	251, 280, 332	4.11, 4.07, 3.98
3	MeOH	249, 272, 328	4.09, 4.03, 4.00
	MeOH-NaOH	256, 313, 372	4.05, 4.24, 3.97
4	MeOH	241, 273, 327	3.87, 4.05, 3.96
	MeOH-NaOH	242, 312, 368	3.90, 4.21, 3.90
5	MeOH	251, 281, 330	4.11, 4.07, 3.96
7	MeOH	250, 273, 326	4.06, 4.01, 4.01
	MeOH-NaOH	256, 315, 371	4.03, 4.23, 3.97
9	MeOH	250, 275, 327	4.00, 3.93, 3.95
	MeOH-NaOH	257, 315, 371	3.97, 4.16, 3.90

Based on the above data, the structures of **2** and **3** were shown to be 1,3-dimethyl-6-propionyllumazine[3] and 3-methyl-6-propionyllumazine,[3] respectively. They were characterized by comparison of physical data with those of authentic samples prepared by a known method.[2,7] The structure of **4** was deduced as 6-propionyllumazine[3] by nmr analysis in conjunction with high resolution mass spectrometry. When **4** was treated with MeI-K_2CO_3 in DMF, 1,3-dimethyl-6-propionyllumazine (**2**) was readily obtained. Based on the above data, the structures of five other metabolites could be easily determined as 6-acetyl-1,3-dimethyllumazine for **5**, 6-(β-methoxypropionyl)-1,3-dimethyllumazine[4] for **6**, 6-(β-methoxypropionyl)-3-methyllumazine[4] for **7**, 6-(β-hydroxypropionyl)-1,3-dimethyllumazine for **8** and 6-(β-hydroxypropionyl)-3-methyllumazine[4] for **9**.

To confirm the above structural assignments for the eight metabolites, three having a different acyl group at the C-6 position, *i.e.* 1,3-dimethy-6-propionyllumazine (**2**), 6-(β-methoxypropionyl)-1,3-dimethyllumazine (**6**) and 6-acetyl-1,3-dimethyllumazine (**5**) were synthesized from 1,3-dimethyllumazines (**10** or **12**) by the procedure of Pfleiderer.[7] The Minisci acylation of 1,3-dimethyllumazine (**10**) with propanal in the presence of *tert*-butyl hydroperoxide and $FeSO_4$ in TFA gave a

separable mixture of 1,3-dimethyl-6-propionyllumazine (**2**) and 1,3-dimethyl-7-propionyllumazine (**11**). The chromatographical and spectral properties of the synthetic 1,3-dimethyl-6-propionyllumazine were identical with those of the natural product (**2**).

The reaction of 1,3-dimethyl-7-methylthiolumazine (**12**) with 3-methoxypropanal under Minisci conditions gave 6-(β-methoxypropionyl)-1,3-dimethyl-7-methylthiolumazine (**13**) in 74% yield. Desulfurization of **13** with Raney Ni (W-2) in acetone-EtOH under reflux afforded 6-(β-methoxypropionyl)-1,3-dimethyllumazine (**6**) in 70% yield. The acetylation of **12** with acetaldehyde in place of propanal under the same Minisci conditions followed by desulfurization with Raney Ni gave 6-acetyl-1,3-dimethyllumazine (**5**) (65%, 2 steps). The physical properties of the synthetic 6-acyl-1,3-dimethyllumazines were identical with those of natural products, 6-(β-methoxypropionyl)-1,3-dimethyllumazine (**6**) and 6-acetyl-1,3-dimethyllumazine (**5**), respectively. Heating **6** in 90% TFA at 70 °C for 3 h resulted in hydrolysis of the methoxypropionyl substituent to give a 3:1 equilibrium mixture of 6-(β-hydroxypropionyl)-1,3-dimethyllumazine (**8**) and its dehydrated form (**15**). On heating **8** in 1N HCl-MeOH under reflux, the hydroxypropionyl group in **8** was easily converted to the β-methoxypropionyl group to form **6**. Isolation of these lumazine derivatives possessing propionyl or acetyl substituents from the natural source is the first example. Whether these lumazine derivatives are related to *Odontosyllis* bioluminescence or its biorhythm is not yet known. Studies on the roles of these lumazines in *Odontosyllis* and on characterization of other metabolites are presently being conducted.

EXPERIMENTAL

Melting points were taken in capillary tubes and uncorrected. Spectra were recorded on the following instruments; ir spectra, Jasco IRA-1 spectrometer, uv spectra, Jasco UVIDEC-610C; ms spectra, Hitachi M-80B spectrometer; nmr spectra, Jeol JNM GX-400 (400 MHz) spectrometer. Chemical shifts of nmr spectra are given in ppm from tetramethylsilane as the internal standard. Preparative thin layer chromatography was conducted on Merck Kiselgel 60F_{254} or 60F_{254}S and column chromatography was preformed on Merck Kiselgel 60 (70-230 mesh).

Isolation of 6-Acyllumazines (2-9)

Freeze dried worms (10 g, *ca.* 5000 individuals) were ground and stimulated to bright luminescence by placing them in 200 ml of water. Suspended worms were repeatedly ground and squeezed until brilliant luminescence could no longer be detected in the dark, all contents were then evaporated, *without filtration*, to dryness *in vacuo* and the residue was extracted with MeOH. The contents (*ca.* 4.1 g) were taken up in MeOH-CH_2Cl_2 (1:1) to remove the insoluble portion and solvents were evaporated to dryness *in vacuo*. The reddish brown residue (2.1 g) obtained was chromatographed on a silica gel column using a MeOH-CH_2Cl_2 (1:5) solvent system into the two fractions, A (367 mg) and B (185 mg). Further tlc separation of fraction A using MeOH-CH_2Cl_2 (1:20) gave crude compounds (**2**, **3**, **4**, **5**, and **6**) which were purified by the following tlc; **2** (AcOEt:benzene=2:3, 30 mg), **3** (AcOEt:benzene=1:1, 29 mg), **4** (AcOEt:benzene=2:1, 8 mg), **5** [i) AcOEt:benzene=2:3 ii) MeOH:CH_2Cl_2=1:20, 0.4 mg], and **6** [i) AcOEt:hexane=3:1 ii) MeOH:CH_2Cl_2=1:10, 0.5 mg].

Further tlc separation of fraction B using MeOH-CH_2Cl_2 (1:5) gave crude compounds (**7**, **8**, and **9**) which were purified as follows; **7** [i) AcOEt:benzene:MeOH=10:6:1 ii) MeOH:CH_2Cl_2=1:10, 0.5 mg], **8** [i) AcOEt:benzene=2:3 ii) MeOH:CH_2Cl_2=1:10, 1.0 mg], and **9** [i) 80% CH_3CN ii) AcOEt:benzene:MeOH=10:6:1 iii) MeOH:CH_2Cl_2=1:10, 0.9 mg].

Synthesis and Reactions of 6-Acyllumazines

1,3-Dimethyl-6-propionyllumazine (2) and 1,3-Dimethyl-7-propionyllumazine (11)

Compounds (**2** and **11**) were prepared essentially by the same way as reported by Pfleiderer.[7] To a solution of **10** (100 mg, 0.521 mmol) in TFA (2 ml) containing propanal (0.4 ml, 5.55 mmol) were added gradually and alternately 78% *tert*-butyl hydroperoxide (0.4 ml, 3.07 mmol)) and finely pulverized $FeSO_4.7H_2O$ (900 mg, 3.24 mmol). Temperature was kept at 10 °C during the addition, and the mixture thus obtained was allowed to warm to room temperature. After being stirred for 4 h at room temperature, the mixture was evaporated to dryness *in vacuo* and the residue was taken up in CH_2Cl_2. The extracts were washed with H_2O, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was fractionated by silica gel preparative tlc (AcOEt:benzene=1:2) to give three fractions, A, B, and C. The less polar fraction A (18.2 mg, mp 146-147 °C) was characterized as 1,3-dimethyl-6-propionyllumazine (**2**) based on spectral data reported by Pfleiderer.[7] This compound was identical with natural product (**2**) in all respects. The more polar fraction B (22.4 mg, mp 137-138 °C) was identified as 1,3-dimethyl-7-propionyllumazine (**11**)

by comparison of spectral data in the literature.[7] The starting material (58.8 mg) was recovered from the most polar fraction C.

Methylation of 4

To a stirred solution of **4** (1 mg) in DMF (0.1 ml) containing finely powdered K_2CO_3 (2 mg) was added MeI (0.05 ml) at room temperature. After a few minutes, the reaction mixture was evaporated to dryness *in vacuo*. The residue was purified by silica gel tlc (MeOH:CH_2Cl_2=3:97) to give a colorless crystalline solid (0.7 mg). This compound was identical with 1,3-dimethyl-6-propionyllumazine (**2**) in all respects.

6-(β-Methoxypropionyl)-1,3-dimethyl-7-methylthiolumazine (13)

To a solution of **12** (300 mg, 1.26 mmol) in aqueous acetic acid (18.9 ml of AcOH and 6.3 ml of H_2O) was added 3-methoxypropanal (1.26 ml, 14.3 mmol) at room temperature with stirring. To this a solution of $FeSO_4.7H_2O$ (2.21 g, 7.95 mmol) in H_2O (10 ml) and 78% *tert*-butyl hydroperoxide (1 ml, 7.70 mmol) were added dropwise gradually and alternately, and stirring was continued for 20 min. The mixture was evaporated to dryness *in vacuo*. The residue was treated with a small amount of water and extracted with CH_2Cl_2 and the CH_2Cl_2 layer was dried over Na_2SO_4. Removal of the solvent left an oily product which crystalized on adding MeOH. The crystals were collected by filtration and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (AcOEt:benzene=1:1) to give the pure product which was combined with the first crop of crystals (301 mg, 74%); light yellow needles; mp 147-148 °C; uv (MeOH) λ_{max} 257 (log ε 4.31), 311 (4.09), 366 (4.21) nm; ^{1}H nmr (400 MHz, $CDCl_3$) δ 2.58 (3H, s), 3.36 (3H, s), 3.53 (2H, t, J=5.9 Hz), 3.54 (3H, s), 3.75 (3H, s), 3.84 (2H, t, J=5.9 Hz); *Anal.* Calcd for $C_{13}H_{16}N_4O_4S$: C, 48.14; H, 4.97; N, 17.27. Found: C, 48.17; H, 4.94; N, 17.18.

6-(β-Methoxypropionyl)-1,3-dimethyllumazine (6)

Raney Ni [W-2, 1.6 g (wet)] was deactivated by refluxing in acetone (25 ml) for 1 h. To this was added a solution of **13** (50 mg) in EtOH (0.25 ml) and the mixture was refluxed for 2 h with stirring. After cooling, the mixture was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column (AcOEt:benzene=1:1) to give a single product which was recrystallized from MeOH to light yellow needles (30 mg, 70%); mp 129-130 °C; *Anal.* Calcd for $C_{12}H_{14}N_4O_4$: C, 51.80; H, 5.07; N, 20.14. Found: C, 51.55; H, 5.05; N, 19.90.

6-Acetyl-1,3-dimethyllumazine (5)

1,3-Dimethyl-7-methylthiolumazine (**12**) (350 mg, 1.47 mmol) was acetylated with acetaldehyde (1.47 ml, 26.3 mmol) in the presence of 78% *tert*-butyl hydroperoxide (1.11 ml, 8.54 mmol) and $FeSO_4.7H_2O$ (2.58 g, 9.28 mmol) in the same way as for **12**. The resulting 6-acetyl-1,3-dimethyl-7-methylthiolumazine (**14**) [344 mg, 84% yield, ^{1}H nmr (400 MHz, $CDCl_3$) δ 2.58 (3H, s), 2.79 (3H, s), 3.54 (3H, s), 3.75 (3H, s)] was desulfurized with W-2 Raney Ni (2.5 g) in acetone-EtOH (10:1, 50 ml) in the usual manner. The crude product (**5**) was purified by silica gel column chromatography (AcOEt:benzene=1:2). Recrystallization from MeOH afforded colorless needles (222 mg, 65% over all yield, mp 177-178 °C).

The uv and ^{1}H nmr spectra of **5** were in accord with those of 6-acetyl-1,3-dimethyllumazine reported by Pfleiderer.[7]

6-(β-Hydroxypropionyl)-1,3-dimethyllumazine (8)

A solution of 6-(β-methoxypropionyl)-1,3-dimethyllumazine (**7**) (100 mg) in 90% TFA (8 ml) was heated at 70 °C for 3 h to afford an equilibrium mixture of **8** and its dehydrated form **15**. The mixture was evaporated to dryness *in vacuo* and the residue was fractionated by silica gel tlc (MeOH:CH_2Cl_2=1:20) to give two fractions.

Fraction 1 (more polar portion): pale yellowish-brown crystals (60 mg), identical to natural product and characterized as 6-(β-hydroxypropionyl)-1,3-dimethyllumazine (**8**) as described above.

Fraction 2 (less polar portion): a colorless crystalline solid (20 mg), characterized as **15**, a dehydrated form of **8**, as indicated by the following physical data: mp 170-171 °C (decomp.); uv (MeOH) λ_{max} 223 (log ε 4.01), 250 (4.11), 297 (4.15), 326 (4.10) nm; uv (MeOH-NaOH) λ_{max} 251 (log ε 4.18), 282 (4.15), 330 (4.03) nm; ^{1}H nmr (400 MHz, $CDCl_3$) δ 3.57 (3H, s), 3.78 (3H, s), 6.04 (1H, dd, J=1.8, 10.6 Hz), 6.72 (1H, dd, J=1.8, 17.6 Hz), 7.92 (1H, dd, J=10.6, 17.6 Hz), 9.38 (1H, s).

6-(β-Methoxypropionyl)-1,3-dimethyllumazine (6) from 8

A solution of 6-(β-hydroxypropionyl)-1,3-dimethyllumazine (**8**) (10 mg) in 1N HCl-MeOH (1 ml) was heated under reflux with stirring. After 1.5 h, the reaction mixture was neutralized with solid $NaHCO_3$ and evaporated to dryness *in vacuo*. The residue was purified by silica gel tlc (MeOH:CH_2Cl_2=1:20) to give **6** (7.7 mg) as a colorless crystalline solid.

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5. A luminescent substance (colorless nonfluorescent solid) was isolated from the 70% MeOH extract of the dried worm. Its luciferin-luciferase reaction as well as the chemiluminescence reaction with DMSO in the presence of *tert*-BuOK are positive. The emission spectra of the bio- and the chemiluminescence were observed at 507 nm, respectively.
6. The light-emitter was isolated as a water soluble orange-yellow crystalline solid. Fluorescence spectrum; λ_{max} 507 nm (excited by uv at 350 nm).
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