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MAKALUVAMINES H–M AND DAMIRONE C FROM THE POHNPEIAN SPONGE *ZYZZYA FULIGINOSA*

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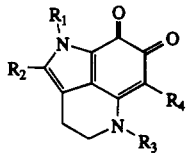
ABSTRACT.—Seven new pyrroloiminoquinone alkaloids, makaluvamines H–M [**19–24**] and damirone C [**25**], together with the known compounds, makaluvamines C [**13**], D [**14**], and G [**17**], were isolated from the sponge *Zyzzya fuliginosa* collected at Nahpali Island, Pohnpei, Micronesia. The structures of the new compounds were elucidated by interpretation of spectral data. The chemotaxonomic relationships involving the makaluvamines and related pyrroloiminoquinone alkaloids are discussed.

Pyrroloiminoquinone alkaloids have been isolated from several sponges and from an ascidian. The simplest of these alkaloids are batzellines A–C [**1–3**] and isobatzellines A–D [**4–7**] from a deep-water Caribbean sponge of the genus *Batzella* (1,2) and damirones A [**8**] and B [**9**] from a *Damiria* sp. from Palau (3). The most complex of these alkaloids are the discorhabdins, exemplified by discorhabdin A [**10**], which have been reported from a New Zealand collection of *Latrunculia* sp. (4–6), *Prianos melanos* from Okinawa (7,8), and *Zyzzya* cf. *marsailis* from Fiji (9). Makaluvamines A–G [**11–17**] are cytotoxic alkaloids from the Fijian *Zyzzya* cf. *marsailis* (9) and an Indonesian *Histodermella* sp. (10) that are intermediate in complexity and may in fact be intermediates in the biosynthetic route to the discorhabdins. In addition, a related pyrroloiminoquinone alkaloid, wakayin [**18**], was isolated from an ascidian of the genus *Clavelina* from Fiji (11). From the viewpoint of chemotaxonomy, this very complex situation has recently been simplified by the taxonomic reassignments noted below. We now report the isolation and identification of seven new alkaloids, makaluvamines H–M [**19–24**], and damirone C [**25**], together with the known metabolites makaluvamines C [**13**], D [**14**], and G [**17**], from a Pohnpeian specimen of *Zyzzya fuliginosa* (order Poecilosclerida) (Carter, 1879).

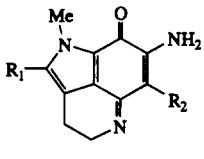
RESULTS AND DISCUSSION

Zyzzya fuliginosa was collected by hand using scuba (−13 meters) from a reef at Nahpali Island, Pohnpei, and was kept frozen until it was lyophilized and extracted. A portion of the MeOH-CH_2Cl_2 (1:1) extract of *Z. fuliginosa* was partitioned between EtOAc and H_2O and the H_2O-soluble material was chromatographed on Sephadex LH-20, using MeOH as eluent, to obtain six major colored fractions. Further purification of these fractions on either Sephadex LH-20 or on a C_{18} reversed-phase Sep Pak column using a MeOH-H_2O (0.1% TFA) gradient, with final purification by hplc on a C_{18} reversed-phase column, yielded makaluvamines C [**13**] (0.51% dry wt), D [**14**] (0.07% dry wt), G [**17**] (0.34% dry wt), H [**19**] (0.47% dry wt), I [**20**] (1.03% dry wt), J [**21**] (0.08% dry wt), K [**22**] (0.02% dry wt), L [**23**] (0.15% dry wt), and M [**24**] (0.03% dry wt), and damirone C [**25**] (0.54% dry wt). Makaluvamines C [**13**], D [**14**], and G [**17**] were identified by comparison of their spectral data with literature values (9,10). Makaluvamines H–M [**19–24**] and damirone C [**25**], which differ from known compounds with respect to the position and number of *N*-methyl groups, were identified by comparison of their spectral data with those of the known members of these series (3,9,10).

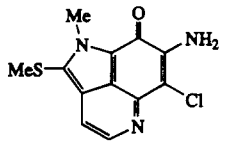
Makaluvamine H [**19**] was obtained as the TFA salt, which is a red-brown solid. The molecular formula of the protonated base was found by hrms to be $C_{12}H_{14}N_3O$. The ^{1}H-



	R_1	R_2	R_3	R_4
1	Me	Cl	H	SMe
2	H	Cl	H	SMe
3	Me	Cl	H	H
8	Me	H	Me	H
9	H	H	Me	H
25	H	H	H	H



	R_1	R_2
4	SMe	Cl
5	SMe	H
6	H	Cl



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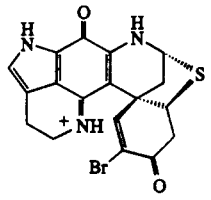

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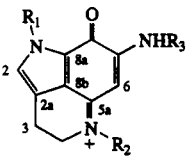
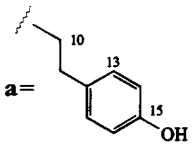
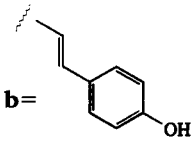
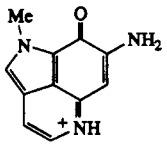


	R_1	R_2	R_3
11	Me	H	H
13	H	Me	H
14	H	H	**a**
15	Me	H	**b**
17	Me	Me	**b**
19	Me	Me	H
20	H	H	H
21	H	Me	**a**
22	Me	H	**a**
23	H	Me	**b**
24	H	H	**b**







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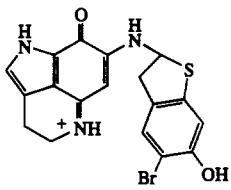

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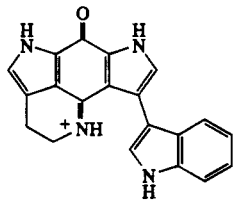

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nmr spectrum (Table 1) contained signals at δ 7.26 (1H, s, H-2), 5.65 (1H, s, H-6), 3.81 (2H, t, J=7.5 Hz, H-4), and 2.86 (2H, t, J=7.5 Hz, H-3), which were assigned by comparison with the spectral data of makaluvamines A [**11**] and C [**13**] (9), both of which have one fewer methyl group than makaluvamine H [**19**]. The two methyl signals at δ 3.83 (3H, s, Me-1) and 3.27 (3H, s, Me-5) correspond in chemical shift to the *N*-methyl signals in makaluvamines A [**11**] and C [**13**], respectively. A complete analysis of HMQC and HMBC nmr experiments allowed the assignment of the ^{13}C-nmr data (Table 1) and confirmed the positions of the two *N*-methyl groups.

Makaluvamine I [**20**], obtained as the TFA salt, is a green solid that has the molecular formula $C_{10}H_{10}N_3O$. The ^{1}H-nmr spectrum is similar to that of makaluvamine H [**19**] except that it lacks signals corresponding to *N*-methyl groups. The ^{1}H- and ^{13}C-nmr data of makaluvamine I [**20**] (Table 1) were compared with those of makaluvamines A [**11**] and C [**13**] and the resulting assignments were confirmed using DEPT and HMBC experiments.

The TFA salts of makaluvamine J [**21**] and makaluvamine K [**22**] were obtained as red-brown solids. The protonated bases of both compounds have the same molecular formula, $C_{19}H_{20}N_3O_2$. The ^{1}H- and ^{13}C-nmr spectra of both compounds (Table 2) contain signals that were assigned to a tyramine residue, and the structures must therefore be

TABLE 1. Comparison of the ^{1}H- and ^{13}C-Nmr Data of Makaluvamines H [**19**] and I [**20**] with those of Makaluvamines A [**11**] and C [**13**].

Position	Makaluvamine H [**19**]			Makaluvamine I [**20**]			Makaluvamine A [**11**]			Makaluvamine C [**13**]		
	δ_H	mult., J	δ_C	δ_H	mult., J	δ_C	δ_H	mult., J	δ_C	δ_H	mult., J	δ_C
2	7.26	s	131.1	7.29	s	127.0	7.30	s	131.0	7.28	s	126.6
2a			118.7			118.9			117.8			123.4
3	2.86	t, 7.5	18.8	2.84	t, 7.5	18.5	2.83	t, 7.5	18.0	2.92	t, 7.5	18.9
4	3.81	t, 7.5	52.4	3.76	t, 7.5	42.7	3.75	t, 7.5	42.0	3.90	t, 7.5	52.6
5a			158.1			156.4			156.0			155.7
6	5.65	s	85.6	5.63	s	87.0	5.61	s	86.4	5.73	s	85.5
7			158.8			157.7			156.7			156.5
8			167.7			168.4			168.2			167.4
8a			123.2			124.2			123.0			117.9
8b			122.5			123.0			122.3			123.2
N(1)H				13.02						13.10		
N(1)Me	3.83	s	35.9				3.88	s	35.8			
N(5)H				10.47			10.44					
N(5)Me	3.27	s	39.6							3.31	s	39.0
$N(9)H_2$	8.61			8.54			8.37			8.65		
	9.40			8.92			9.09			9.53		

TABLE 2. ^{1}H- and ^{13}C-Nmr Data of Makaluvamines J [**21**], K [**22**], L [**23**], and M [**24**].

Position	Makaluvamine J [**21**]			Makaluvamine K [**22**]			Makaluvamine L [**23**]			Makaluvamine M [**24**]		
	δ_H	mult., *J*	δ_C	δ_H	mult., *J*	δ_C	δ_H	mult., *J*	δ_C	δ_H	mult., *J*	δ_C
2	7.31	s	126.8	7.33	s	131.4	7.28	s	126.2	7.33	s	126.5
2a			118.1			118.0			117.6			118.4
3	2.91	t, 7.5	18.9	2.84	t, 7.5	18.0	2.93	t, 7	18.9	2.89	t, 7	18.2
4	3.89	t, 7.5	52.7	3.78	t, 7.5	42.2	3.92	t, 7	52.8	3.87	t, 7	42.8
5a			155.4			156.5			154.7			157.8
6	5.59	s	83.4	5.47	s	84.2	6.28	s	85.6	6.12	s	86.6
7			153.3			152.9			146.7			147.1
8			167.0			167.7			167.3			167.7
8a			123.2			123.0			123.5			122.4
8b			123.4			122.3			123.1			124.0
10	3.59	m	45.0	3.48	m	45.1	7.58	m	121.7	6.99	d, 13.5	121.4
11	2.81	t, 7	32.7	2.77	t, 7	32.4	7.05	d, 13.5	124.2	7.40	d, 13.5	123.5
12			128.3			128.2			126.2			126.2
13,17	7.04	d, 8	129.8	7.03	d, 8	129.6	7.41	d, 8	128.4	7.38	d, 8	128.2
14,16	6.68	d, 8	115.2	6.68	d, 8	115.3	6.76	d, 8	115.8	6.77	d, 8	115.7
15			156.0			156.0			158.0			156.3
N(1)H	13.09	br s					13.10	br s		13.12	br s	
N(1)Me				3.89	s, 3H	35.9						
N(5)H				10.47	br s					10.63	br s	
N(5)Me	3.37	s, 3H	39.3				3.47	s, 3H	39.6			
N(9)H	9.13	t, 6		8.97	t, 6		10.78	d, 11		10.63	br s	
OH	9.1	br s		9.3	br s		9.8	br s		9.8	br s	

related to that of makaluvamine D [**14**], which does not contain an *N*-methyl group. The ^{1}H-nmr signal at δ 3.37 (3H, s) in the spectrum of makaluvamine J [**21**] was assigned to a methyl group at N-5 and the signal at 3.89 (3H, s) in the spectrum of makaluvamine K [**22**] was assigned to a methyl group at N-1. The assignments were confirmed by analysis of HMBC and HMQC results.

The TFA salt of makaluvamine L [**23**] was obtained as a green solid. The molecular formula, $C_{19}H_{18}N_3O_2$, reveals that it is isomeric with makaluvamine E [**15**], which is also a green solid with a similar uv-visible spectrum. The ^{1}H-nmr spectrum of **23** contained signals at δ 7.58 (1H, m), 7.41 (2H, d, J=8 Hz), 7.05 (1H, d, J=13.5 Hz), 6.76 (2H, d, J=8 Hz) that were assigned to a *N*-(*p*-hydroxy-*trans*-styryl) group, similar to that found in makaluvamine E [**15**]. The major differences between the ^{1}H-nmr data of **23** and **15** were in the chemical shifts of the *N*-methyl signals at δ 3.47 in **23** and 3.97 in **15** indicating that the *N*-methyl group in makaluvamine L [**23**] was at N-5 rather than N-1. Analysis of HMQC and HMBC data allowed assignment of the ^{13}C-nmr signals (Table 2) and confirmed the location of the *N*-methyl group at N-5.

A second green compound, makaluvamine M [**24**], was obtained as a very minor metabolite. The molecular formula of the protonated base, $C_{18}H_{16}N_3O_2$, coupled with the lack of *N*-methyl signals in the ^{1}H- and ^{13}C-nmr spectra (Table 2), indicated that makaluvamine M [**24**] is the *N*-demethyl derivative of makaluvamine L [**23**].

Damirone C [**25**] was isolated as a red-brown solid of molecular formula $C_{10}H_8N_2O_2$. In contrast with data for the makaluvamines, the ^{13}C-nmr spectrum of damirone C [**25**] contained two signals between δ 170–180, and was similar to the spectra of the damirones (3). The ^{1}H- and ^{13}C-nmr data, assigned using HMQC and HMBC experiments, were fully compatible with the proposed structure, which lacks the *N*-methyl group of damirones A [**8**] and B [**9**].

The rather complicated chemotaxonomic picture described in the introductory paragraph has recently been simplified by taxonomic revisions proposed by van Soest *et al.* (12). The Fijian sponge *Zyzzya* cf. *marsailis* (9), which is an incorrect spelling of *Z. massalis,* and the Palauan *Damiria* sp. (3) have been reassigned to *Z. fuliginosa*. The Indonesian *Histodermella* sp. (10) is also now considered to be a species of *Zyzzya* (M. Kelly-Borges, Harbor Branch Oceanographic Institution, personal communication). Thus, all the makaluvamines and damirones described to date have been obtained from sponges of the genus *Zyzzya*.

Some of the makaluvamines (A, C, E, and F) were reported to be cytotoxic against the human colon tumor line HCT-116 and to inhibit topoisomerase II activity (9). Makaluvamines D, G, H, and J–M were inactive against topoisomerase in an assay that employs three genetically engineered yeast strains (13); one strain was least sensitive to DNA damaging agents, one was hypersensitive with a DNA repair gene deleted, and one had its topoisomerase I and DNA repair genes deleted. We recognize that this assay is not comparable with that used previously (9). However, the makaluvamines were cytotoxic against HCT-116 in vitro. In a semi-quantitative assay, makaluvamines I [**20**] and L [**23**] were an order of magnitude more active than makaluvamines C [**13**], G [**17**], H [**19**], and K [**22**], and makaluvamines D [**14**] and M [**24**] were the least active. The new makaluvamines showed only mild activity against *Bacillus subtilis* and no activity against other standard test organisms in our panel.

EXPERIMENTAL

ANIMAL MATERIAL.—*Zyzzya fuliginosa* (collection No. POH 93-027, SIO Invertebrate Collection No. P1155) was collected by hand using scuba (−13 m) from Nahpali Island, Pohnpei, Federated States of Micronesia, and was frozen within 1 h.

EXTRACTION AND ISOLATION.—The sponge was stored frozen until it was lyophilized. The sponge (34

g dry wt) was extracted exhaustively with CH_2Cl_2-MeOH (1:1) to obtain a black extract (7.5 g). A portion (3.8 g) of the extract was partitioned between H_2O and EtOAc. The darkly pigmented aqueous layer was lyophilized and the residue chromatographed on Sephadex LH-20, using MeOH as eluent, to obtain several colored fractions that were further purified by chromatography on a reversed-phase C_{18} Sep Pak (Waters Bondapak) column using a gradient of 20–70% MeOH in H_2O containing 0.1% TFA as eluent to obtain a mixture of makaluvamines C [**13**], H [**19**], and I [**20**], and damirone C [**25**], as the TFA salts. The mixture was purified on Sephadex LH-20 using 100% MeOH as eluent to obtain makaluvamines C [**13**] (88 mg, 0.51% dry wt), H [**19**] (81 mg, 0.47% dry wt), and I [**20**] (177 mg, 1.03% dry wt), and damirone C [**25**] (93 mg, 0.54% dry wt). An impure fraction containing makaluvamines G and I was further purified by hplc on a prep. Dynamax-C_{18} column using 40% MeOH in 0.1% aqueous TFA solution as eluent to obtain makaluvamine G [**17**] (59 mg, 0.34% dry wt) and makaluvamine L [**23**] (26.4 mg, 0.15% dry wt). A second impure fraction was separated on hplc using 30% MeOH in 0.1% aqueous TFA solution as eluent to obtain makaluvamines D [**14**] (12 mg, 0.07% dry wt), J [**21**] (14 mg, 0.08% dry wt), and K [**22**] (3.4 mg, 0.02% dry wt). A third impure fraction was purified on hplc using 22% CH_3CN in 0.1% aqueous TFA solution as eluent to obtain makaluvamine M [**24**] (5 mg, 0.03% dry wt).

Makaluvamine H [**19**].—Red-brown solid; ir (film) ν max 3500–3000 (br), 2975, 1675, 1610, 1530, 1200 cm^{-1}; uv (MeOH) λ max (ϵ) 240 (19 400), 345 (13500), 522 (1100) nm, (MeOH+$NaHCO_3$) 209 (15 700), 241 (21 200), 334 (14 200) nm; ^{1}H-nmr data (DMSO-d_6), see Table 1; ^{13}C-nmr data (DMSO-d_6), see Table 1; hrfabms *m/z* 216.1101 ($C_{12}H_{14}N_3O$ $[M+H]^+$ requires 216.1137).

Makaluvamine I [**20**].—Green solid; ir ν max (film) 1665, 1660 cm^{-1}; uv (MeOH) λ max (ϵ) 240 (23 900), 340 (13 500), 534 (1000) nm, (MeOH+$NaHCO_3$) 214 (16 000), 327 (15 500), 458 (1400) nm; ^{1}H-nmr data (DMSO-d_6), see Table 1; ^{13}C-nmr data (DMSO-d_6), see Table 1; hrfabms *m/z* 187.0737 ($C_{10}H_9N_3O$ $[M]^+$ requires 187.0746).

Makaluvamine J [**21**].—Red-brown solid; ir (film) ν max 3250 (br), 1680, 1620, 1550, 1200 cm^{-1}; uv (MeOH) λ max (ϵ) 220 (11 600), 241 (18 600), 354 (13 900), 534 (1400) nm; (MeOH+$NaHCO_3$) 217 (9600), 257 (1200), 370 (5600) nm; ^{1}H-nmr data (DMSO-d_6), see Table 2; ^{13}C-nmr data (DMSO-d_6), see Table 2; hrfabms *m/z* 322.1564 ($C_{19}H_{20}N_3O$ $[M+H]^+$ requires 322.1555).

Makaluvamine K [**22**].—Red-brown solid; ir (film) ν max 3250 (br), 1685, 1675, 1560 cm^{-1}; uv (MeOH) λ max (ϵ) 222 (18 000), 246 (29 800), 347 (19 200), 536 (2600) nm; (MeOH+$NaHCO_3$) 224 (10 900), 242 (10 200), 335 (8800) nm; ^{1}H-nmr data (DMSO-d_6), see Table 2; ^{13}C-nmr data (DMSO-d_6), see Table 2; hrfabms *m/z* 322.1568 ($C_{19}H_{20}N_3O$ $[M+H]^+$ requires 322.1555).

Makaluvamine L [**23**].—Green solid; ir (film) ν max 3280, 2970, 1675, 1615, 1545, 1200 cm^{-1}; uv (MeOH) λ max (ϵ) 276 (1050), 344 (11 700), 451 (9200), 638 (10 400) nm, (MeOH+$NaHCO_3$) 271 (7800), 365 (2900), 608 (5200) nm; ^{1}H-nmr data (DMSO-d_6), see Table 2; ^{13}C-nmr data (DMSO-d_6), see Table 2; hrfabms *m/z* 320.1387 ($C_{19}H_{18}N_3O$ $[M+H]^+$ requires 320.1399).

Makaluvamine M [**24**].—Green solid; ir (film) ν max 3290 (br), 1675, 1605, 1545, 1205 cm^{-1}; uv (MeOH) λ max (ϵ) 274 (14 900), 330 (11 000), 445 (8400), 623 (9200) nm, (MeOH+$NaHCO_3$) 217 (11 300), 302 (12 600), 596 (5800) nm; ^{1}H-nmr data (DMSO-d_6), see Table 2; ^{13}C-nmr data (DMSO-d_6), see Table 2; lrms *m/z* 306 ($C_{18}H_{16}N_3O$ $[M+H]^+$ requires 306).

Damirone C [**25**].—Red-brown solid; ir (film) ν max 1725, 1670, 1650, 1535, 1280, 1120 cm^{-1}; uv (MeOH) λ max (ϵ) 240 (23 200), 330 (10 200), 528 (900) nm, (MeOH+$NaHCO_3$) 240 (23 900), 330 (10 800), 518 (1100) nm; ^{1}H nmr (DMSO-d_6) δ 2.72 (2H, t, J=7.5 Hz, H-3), 3.49 (2H, t, J=7.5 Hz, H-4), 5.02 (1H, s, H-6), 7.08 (1H, s, H-2), 8.24 (1H, s, NH-5), 12.39 (1H, s, NH-1); ^{13}C nmr (DMSO-d_6) δ 19.3 (C-3), 41.5 (C-4), 92.7 (C-6), 117.0 (C-2a), 124.2 (C-2, C-8b), 125.3 (C-8a), 154.5 (C-5a), 171.3 (C-7), 178.4 (C-8); hreims *m/z* 188.0587 ($C_{10}H_8N_2O_2$ $[M]^+$ requires 188.0586).

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