Norselic Acids A−E, Highly Oxidized Anti-infective Steroids that Deter Mesograzer Predation, from the Antarctic Sponge *Crella* sp.

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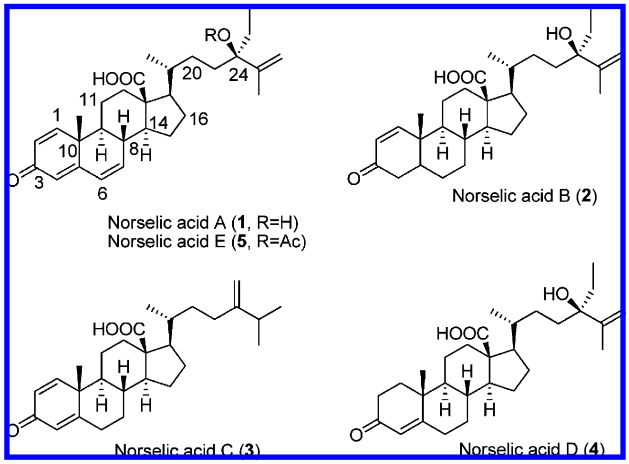
Five new steroids, norselic acids A−E (**1**−**5**), were isolated from the sponge *Crella* sp. collected in Antarctica. The planar structures of the norselic acids were established by extensive NMR spectroscopy and mass spectrometry studies, and the configuration of norselic acid A (**1**) was elucidated by X-ray crystallography. Norselic acid A displays antibiotic activity against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA), vancomycin-resistant *Enterococcus faecium* (VRE), and *Candida albicans* and reduces consumption of food pellets by sympatric mesograzers. Compounds **1**−**5** are also active against the Leishmania parasite at low micromolar levels.

Natural products from the marine environment represent a rich source of bioactive compounds for the use in pharmaceutical drug discovery. Among marine natural products reported to date, fewer than 3% have been isolated from organisms collected from a polar region.[1] Herein, we report five new steroids, norselic acids A−E (**1**−**5**), that were isolated from the *Crella* sp. collected in the cold waters of Antarctica. Their structures were elucidated primarily by NMR spectroscopy and mass spectrometry, while one, norselic acid A (**1**), was analyzed by X-ray crystallography. The A-ring oxidation patterns of the norselic acids are reminiscent of monomers that comprise the crellastatins, a series of asymmetric dimeric steroids that were isolated from an undescribed *Crella* species collected from the tropic waters of the Vanuatu islands.[2−4]



Result and Discussion

The thinly encrusting, bright red sponge *Crella* sp. was first collected from Norsel Point on what was once Anvers Island, Antarctica, near the U.S. Antarctic Program's Palmer Station. With the retreat of the Marr Glacier, which covers Anvers Island, Norsel Point was revealed in 2004 as a prominence of a new island, now named Amsler Island, of the Anvers archipelago. *Crella* sp. has since been found frequently at other sites near Palmer Station, occupying vertical rock faces below 30 m depth. Freeze-dried *Crella* sp. was exhaustively extracted with 1:1 CH_2Cl_2/MeOH to produce a lipophilic extract, which was partitioned between EtOAc and H_2O. The EtOAc partition was subjected to repeated silica gel flash column chromatography followed by both normal- and reversed-phase HPLC to afford the norselic acids.

Norselic acid A (**1**) was found to have a molecular formula of $C_{29}H_{40}O_4$ by HRESIMS ($[M + H]^+$ 453.3000). The multiplicities of carbon atoms were established from the DEPT spectrum (Table 2), which revealed seven quaternary carbons, 10 methines, eight methylenes, including one vinyl methylene, and four methyl groups, leaving two unassigned protons as heteroatom-bound. The terpene character of the compound was foretold by the high-field methyl groups in the ^{1}H NMR spectrum, but the steroidal character was masked by the lack of the typical C-3 hydroxymethine. Characterization began with a ketone carbonyl observed at δ_C 186.6 in the ^{13}C NMR spectrum, which showed HMBC correlations (Table 1) to the olefinic region at δ_H 7.03 (H-1), which in turn correlated to two olefinic carbons, δ_C 163.4 (C-5) and 127.5 (C-2). H-1 showed correlation to the angular methyl C-19, at δ_C 20.8, as well as C-10 (δ_C 41.5). H-1 is further correlated, by COSY, to H-2 (δ_H 6.25), the latter of which showed HMBC correlation to C-4 (δ_C 123.9). H-4, a singlet at δ_H 6.01, shows HMBC correlation only to C-2. Taken with H_3-19 correlations in the HMBC spectrum to C-5 and C-1 (δ_C 153.4), as well as C-9 (δ_C 48.4) and C-10, the A ring (Figure 1) could be assigned as that of a very highly oxidized steroid.

The remaining steroid rings could be accounted for by HMBC (Table 1) and COSY, with key correlations of H-6 (δ_C 6.21) to C-5, C-7 (δ_C 138.3) and C-4, and of H-7 (δ_H 6.07) to C-5 and C-6. Surprisingly, two ring C protons, H-12a (δ_H 1.31) and H-14 (δ_H 1.58), correlated in the HMBC to a carboxylic acid equivalent carbon at δ_C 178.6. The absence of a second angular methyl ^{1}H NMR absorption secured the position of that carbon at C-18; the carboxylic acid nature of the carbon was supported by the mass spectrum, which established the oxygen count.

The steroidal side chain of norselic acid A (**1**) followed from analysis of the remaining proton and carbon signals. Briefly, the as-yet unaccounted oxygen-bearing carbon at δ_C 78.8 (C-24) displayed HMBC correlations (Table 1, Figure 1) from olefinic protons on a terminal methylene, H-26a/H-26b (δ_H 4.86/δ_H 4.91), as well as those on H_3-27 (δ_H 1.62), H-28a (δ_H 1.49), and H_3-29

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Table 1. ^{1}H NMR Spectroscopic Data for Norselic Acids A−E (**1**−**5**)[a]

	A (**1**)		B (**2**)		C (**3**)		D (**4**)		E (**5**)	
pos	δ_H (*J*, Hz)	HMBC	δ_H (*J*, Hz)	HMBC	δ_H (*J*, Hz)	HMBC	δ_H (*J*, Hz)	HMBC	δ_H (*J*, Hz)	HMBC
1	7.03, d (10.2)	2,3,5	7.11, d (10.2)	2,3,5,9,10	7.03, d (11.0)	3,4,5,6,10,19	1.98, m	3,5	7.04, d (10.3)	1,3
							1.68, m	3,6,10		
2	6.25, dd (10.1, 1.8)	4	5.86, d (10.3)	4,10	6.25, d (10.3)	5	2.35, m	3	6.27, dd (1.5, 10.3)	
							2.28, m	1,4,5,10		
4	6.01, s	6,10	2.22, m	2,3,5,6	6.09, s	2,5,6	5.73, m		6.02, s	2,6
			2.36, m							
5			1.94, m	19		1,2,4,6,19				
6	6.21, dd (10.0, 2.9)	4,5,7,10	1.43, m	8	2.48, m	4,8,9,10	2.35, m	7	6.23, dd (2.9, 9.9)	4,5,7
					2.37, m					
7	6.07, dd (9.7, 1.4)	5,6,8,9	1.83, m	9	1.21, m	14	1.91, m		6.08, d (9.2)	5,6,9,10,14
			1.61, m		2.76, m		1.05, m	9		
8	2.35, m	6,7,9,14	1.54, m	7,9,13	1.74, m	7,9	1.40, m	9,11	2.35, m	6,9,13,14
9	1.49, m	8,10	1.54, m	11	1.11, m	6,8	0.95, m		1.50, m	8,11
11	1.87, m	13	1.92, m		2.00, m	11,18	1.40, m	8,12,13	1.96, m	1.66,m
	1.43, m	13	1.56, m	12	1.68, m		1.69, m	9,13		
12	2.74, m		2.73, m	11,13	1.56, m		2.69, m		2.77, m	
	1.31, m	9,13,14,17,18	1.16, m		1.21, m		1.14, m	11,14,17,18	1.15, m	9,10,11,13,18
14	1.58, m	17,18	1.43, m	7,18	1.56, m	7,18	1.53, m	8,11,12,13,18	1.57, m	8,13,18
15	2.01, m		1.91, m	13,16	1.87, m	12	1.87, m		1.96, m	1.77, m 14
	1.46, m		1.68, m		0.88, m	8	1.57, m	16		
16	1.81, m	22	1.94, m	13,14,15,18	1.74, m	14	1.93, m		0.93, m	1.12, m
	1.54, m		1.68, m		1.53, m		1.22, m			
17	1.58, m	12,16,20,21	1.54, m	16	1.42, m	21	1.40, m	13,15,18,20	1.57, m	15,20,22
19	1.09, s	1,5,9,10	0.94, s	1,5,9,10,21	1.17, s	1,2,5,9,10	1.10, s	1,5,9,10	1.12, s	1,5,9,10
20	1.36, m	17,21,23	1.35, m		1.42, m	17,21	1.68, m		1.43, m	21
21	0.98, (6.5)	17,20,22	1.02,d (6.4)	17,22	1.06, d (6.3)	17,20	1.00, d (6.6)	16,17,23	1.01, d (6.8)	20
22	1.95, m	17,23	1.54, m		2.06, m		1.83, m		1.81, m	
	1.58, m		0.89, m		1.11, m		1.60, m	20	1.57, m	
23	1.62, m		1.61, m	24,25,28	2.06, m		1.98, m		1.96, m	22,25
	1.31, m	22,24	1.35, m		1.87, m	22,24,28	1.68, m	22,24,25	1.81, m	25
					2.21, m (6.8)	23,24				
26	4.87, s	24,25,27	4.90, s		1.02, d (1.5)	25,27,24	4.93, s	24,25	4.93, s	24,25
	4.91, s		4.94, s				4.89, s		4.96, s	
27	1.62, s	24,25,26	1.66, s	25,26	1.01, d (1.5)	17,25,26,28	1.65, s	24,25,26	1.65, s	24,25,26
28	1.49, m	24,29	1.61, m	24,29	4.64, s	23,24,25	1.53, m	22,24,25	1.81, m	25
	1.58, m		1.54, m		4.72, s		1.60, m		2.06, m	
29	0.77, t (7.1)	24,28	0.81, t (7.3)	24,28			0.79, t (7.4)	24,28	0.73, t (7.6)	24,28
30										
31									2.04, s	24,30

[a] Recorded at 500 MHz in $CDCl_3$

(δ_H 0.77). Placement of this assemblage at C-24 was based on HMBC correlations of H_2-23 (δ_H 1.31) to both C-24 and C-22 (δ_C 28.8), the latter of which could be followed back to the steroidal nucleus by HMBC correlations from H-21 (δ_H 0.98).

ROESY data supported configurational assignments of the relationships in the steroidal nucleus of norselic acid A (**1**). For example, correlations between H_3-19, H-11β, and H-8 (see Figure 2) secured the *trans*-relationship of the B/C ring junction and placed the C-19 methyl group on the same face of the steroid ring as H-8. The C/D ring junction was assigned on the basis of ROESY correlations between H-12α and H-14 with concomitant correlations of both H_3-21 and H-20 to H-12β. No correlations were observed between H-8 and -14. The protons on C-9 and C-17 overlap, so the configuration could not be securely assigned on the basis of the ROESY data, but the correlations described above are consistent with the *trans*/*trans* B/C/D relationship. The C-24 configuration required alternative methods. We initially attempted lanthanide shift studies based on a method recently published[5] but found the results inconsistent. Because the tertiary hydroxy group was recalcitrant toward esterification, we grew a crystal of **1** for X-ray analysis (Figure 2). The X-ray structure confirmed results from the ROESY analysis and defined C-24 as *R**, relative to the known configuration of the steroid nucleus, thereby completing the structural assignment of norselic acid A.

Norselic acids B (**2**) and D (**4**) were found to be isomeric by their HRESIMS spectra (*m*/*z* 457.3312 for **2**, *m*/*z* 457.3306 for **4**, $[M + H]^+$), which demonstrated both to be four mass units larger than norselic acid A (**1**). Their ^{1}H NMR spectra differed from **1** in displaying a much simpler olefinic region, integration of which could demonstrate the additional mass units had resulted from reduction of two olefins from **1**, a result corroborated by the ^{13}C NMR spectrum, which displayed only four olefinic carbon resonances. The carbonyl carbon shifted significantly downfield, from δ_C 186.8 in **1** to δ_C 200.4 and δ_C 200.0 in **2** and **4**, respectively, indicative of a change in the cross conjugation found in **1**. 2D NMR data (Table 1) supported the observation that the A and B rings were both reduced relative to **1**: in **2**, olefinic protons H-1 (δ_H 7.11) and H-2 (δ_H 5.86) were coupled to one another, based on the COSY spectrum, and H-1 displayed HMBC correlations to C-2 (δ_C 127.7), C-3 (δ_C 200.4), and C-5 (δ_C 44.5), the latter of which was a methine, requiring the C-4/C-5 olefin of **1** to have been reduced. Only three olefinic protons were observed in the ^{1}H NMR spectrum of **4**; besides the terminal methylene protons of the C-25/C-26 olefin, a singlet at δ_H 5.73 (H-4) was the lone olefinic resonance in the steroid nucleus of **4**. While H-4 displayed few HMBC correlations, the A ring was deduced by HMBC correlations of H_2-1 (a, δ_H 1.98; b, δ_H 1.68) and H_2-2 (a, δ_H 2.35; b, δ_H 2.28) to C-3 (δ_C 200.0), H-1a and H-2b to C-5 (δ_C 171.6), and H-1b and H-2b to C-10 (δ_C 38.8), securing the lone olefin in the A ring of **4** to the C-4/C-5 position. Taken with C-6/C-7 being saturated, and given the correspondence of the remaining data (Table 1, Table 2) with **1**, norselic acids B and D were assigned structures **2** and **4**.

Norselic acid C (**3**) differed in carbon count relative to the other norselic acids, evidenced by a HRESIMS determination of $C_{28}H_{40}O_3$ (*m*/*z* 425.3082, $[M + H]^+$). Both the ^{1}H and ^{13}C NMR spectra displayed fewer olenific resonances relative to norselic acid A (**1**), and especially notable was the absence of an oxygen-bearing sp^3 carbon atom. The DEPT spectrum of **3** revealed six quaternary, nine methine, nine methylene, and four methyl carbons. HMBC data established an A ring identical to that of **1** but a reduced B

Table 2. ^{13}C and DEPT NMR Spectroscopic Data for Norselic Acid A−E (**1**−**5**)[a]

pos	A (**1**)	B (**2**)	C (**3**)	D (**4**)	E (**5**)
1	153.4, CH	158.4, CH	156.6, CH	35.8, CH_2	153.2, CH
2	128.3, CH	127.7, CH	127.8, CH	32.9, CH_2	128.3, CH
3	186.8, qC	200.4, qC	186.4, qC	200.0, qC	186.7, qC
4	123.9, CH	41.2, CH_2	124.2, CH	124.0, CH	123.9, CH
5	163.1, qC	44.5, CH	169.2, qC	171.6, qC	162.9, qC
6	127.9, CH	27.7, CH_2	32.9, CH_2	34.1, CH_2	127.9, CH
7	138.3, CH	31.3, CH_2	36.5, CH_2	31.9, CH_2	138.0, CH
8	40.3, CH	37.8, CH	37.0, CH	36.2, CH	40.2, CH
9	48.4, CH	50.1, CH	52.3, CH	53.9, CH	48.4, CH
10	41.5, qC	39.2, qC	43.7, qC	38.8, qC	41.5, qC
11	24.0, CH_2	23.4, CH_2	29.1, CH_2	23.1, CH_2	23.9, CH_2
12	36.1, CH_2	37.8, CH_2	34.7, CH_2	36.5, CH_2	36.4, CH_2
13	57.3, qC	57.1, qC	57.2, qC	56.7, qC	57.3, qC
14	54.6, CH	57.0, CH	56.5, CH	56.7, CH	54.6, CH
15	24.6, CH_2	23.4, CH_2	25.1, CH_2	28.7, CH_2	28.4, CH_2
16	36.5, CH_2	28.5, CH_2	24.9, CH_2	28.3, CH_2	28.8, CH_2
17	56.4, CH_2	56.7, CH	56.2, CH	56.4, CH	56.0, CH
18	178.6, qC	177.9, qC	179.6, qC	171.6, qC	178.8, qC
19	20.8, CH_3	13.2, CH_3	18.8, CH_3	17.5, CH_3	20.8, CH_3
20	36.4, CH	36.5, CH	36.8, CH	37.4, CH	36.1, CH
21	19.0, CH_3	19.1, CH_3	18.6, CH_3	19.1, CH_3	19.3, CH_3
22	28.8, CH_2	28.8, CH_2	33.6, CH_2	24.9, CH_2	24.6, CH_2
23	36.5, CH_2	36.0, CH_2	30.9, CH_2	36.0, CH_2	30.5, CH_2
24	78.8, qC	78.5, qC	156.6, qC	78.6, qC	89.0, qC
25	147.6, qC	148.0, qC	34.1, CH	147.8, qC	144.5, qC
26	111.4, CH_2	111.2, CH_2	22.1, CH_3	111.2, CH_2	112.5, CH_2
27	19.9, CH_3	19.9, CH_3	22.2, CH_3	19.9, CH_3	19.1, CH_3
28	32.5, CH_2	32.5, CH_2	106.4, CH_2	32.5, CH_2	27.1, CH_2
29	7.6, CH_3	7.6, CH_3		7.6, CH_3	7.7, CH_3
30					170.1, qC
31					22.1, CH_3

[a] Recorded at 125 MHz in $CDCl_3$.

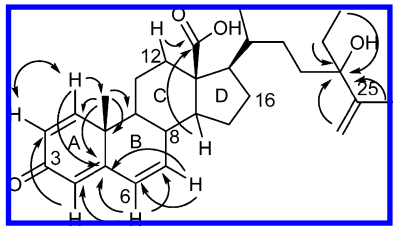

Figure 1. Key HMBC (→) and COSY (↔) correlations observed for norselic acid A (**1**).

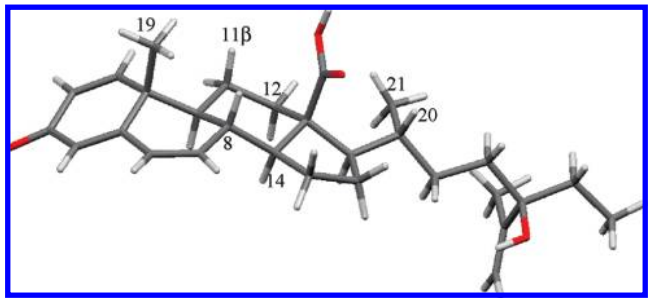

Figure 2. Norselic acid A (**1**) as determined by X-ray analysis.

ring (Table 1). The missing carbon could be accounted for on the side chain, where the HMBC showed the olefinic methylene to reside on C-24 rather than C-25, as found in the other norselic acids. For example, the coincident methyl groups, H_3-26/H_3-27, correlated to, besides the methine carbon C-25 (δ_C 34.1), an olefinic quaternary carbon at δ_C 156.6 (C-24), and olefinic proton δ_H 4.64 (H-28a) correlated to the same two carbons (C-24 and C-25), as well as C-23 (δ_C 30.9). As with **1**, C-23 was connected back to the steroid nucleus via HMBC and COSY correlations (Table 1), resulting in norselic acid C being assigned structure **3**.

The HRESIMS spectrum of norselic acid E (**5**) established a formula of $C_{31}H_{42}O_5$ (*m*/*z* 495.3124, $[M + H]^+$), which represented two additional carbons and one additional oxygen relative to norselic acid A (**1**). The correspondence of both 1D and 2D NMR spectroscopic data (Table 1) between **1** and **5** pointed to C-24 as the sole significant difference between the two. In the ^{13}C NMR spectrum in particular, C-24 of **1** was found at δ_C 78.8, while that of **5** at δ_C 89.0. A 3H singlet at δ_H 2.04, which correlated in the HMBC spectrum to a carbonyl at δ_C 170.1, secured **5** as the C-24 acetate derivative of **1**.

The highly oxidized steroids represented by the norselic acids A−E (**1**−**5**) lack the potent cytotoxicity of their related dimers, the crellastatins,[2−4] though they displayed modest anti-infective properties. Norselic acid A had broad-spectrum antibiotic activity (Table 3), including a 9 mm (hazy) zone of inhibition (ZOI), at 200 μg/disk, toward methicillin-resistant *Staphylococcus aureus* (MRSA). However, **1** was not selective for the drug-resistant *S. aureus* strains, being equally active (8 mm ZOI) toward a drug-sensitive strain, methicillin-sensitive *S. aureus* (MSSA). Toward vancomycin-resistant *Enterococcus faecium*, **1** displayed an 8 mm (hazy) ZOI, and *Candida albicans* an 8 mm zone. The only Gram-negative bacteria studied, *Escherichia coli*, was insensitive to norselic acid A. All of the norselic acids displayed activity against the Leishmania parasite, with low micromolar activity among the series A: (**1**), 2.5 μM; B (**2**), 2.4 μM; C (**3**), 2.6 μM; D (**4**), 2.0 μM; E (**5**), 3.6 μM.

At tissue level concentrations estimated as "natural", norselic acid A (**1**) reduced consumption of artificial foods by the amphipod *Gondogeneia antarctica* by 9%, although this was not statistically significant ($p = 0.285$). However, the "natural" concentration was calculated assuming that 100% of the norselic acid A present in the original sponge tissue was recovered and purified, which clearly underestimates the true tissue-level concentration. When the experiment was repeated with 3× the "natural" concentration of norselic acid A, consumption relative to the solvent control decreased by 18%, which was statistically significant ($p = 0.013$). Feeding by the sea star *Odontaster validus* was not deterred by 3× the "natural" concentration of norselic acid A (8% of pellets rejected vs 0% of the solvent control; $p = 0.545$). Norselic acid A did significantly deter sea star feeding at 10× the "natural" concentration (35% of pellets rejected vs 0% of the solvent control; $p = 0.0418$).

Similar bioassays were conducted with both lipophilic (1:1 CH_2Cl_2/MeOH) and hydrophilic (1:1 MeOH/H_2O) extracts from 12 randomly selected sponge species at Palmer Station.[6] Only two of the 12 species had extracts that deterred feeding by *Gondogeneia antarctica*, but *Crella* sp. was one of the two species. Both the lipophilic and hydrophilic crude extracts of *Crella* sp. were deterrent. Since norselic acid A would mainly or entirely have extracted into the lipophilic fraction (which was the first made), it is likely that additional amphipod-deterrent metabolites are also elaborated by *Crella* sp. Nonetheless, amphipods are a major benthic consumer in communities along the western Antarctic Peninsula,[7,8] and the fact that relatively few sponge species in this community appear to elaborate chemical defenses against them[6] highlights the ecological importance of the identification of norselic acid A as a defense against amphipod predation. Conversely, nearly 80% of sponge species in this community are unpalatable to the sea star *Odontaster validus,* and in all species in which crude extracts were bioassayed, this unpalatability could be explained by chemical defenses present in the extracts.[9] Both inner and outer tissues of *Crella* sp. were highly unpalatable to *O. validus* (100% rejection in both vs 0% rejection of controls), but extract bioassays were not performed with *Crella* sp.[9] It is likely that norselic acid A could play a role in this strong unpalatability, but the fact that it was completely nondeterrent at 3× the "natural" concentration suggests that it may play a minor role as a sea star feeding deterrent.

Experimental Section

General Experimental Procedures. Optical rotations were measured on an Autopol IV automatic polarimeter using a Na lamp at 25 °C

Table 3. Antibiotic Activity of Norselic Acid A (**1**) and Known Antibiotics[a]

test organism	NAA200	OXA1U	KM30	VA30	AMC30	RA5	FOX30	NY100U
S. aureus 375 (MSSA)	8 VH	18:22H	15	19	30:41H	nt	nt	nt
S. aureus 310 (MRSA)	9H	0	0	15	15	nt	nt	nt
E. faecium 379 (VREF)	8H	0	10	0	14	nt	nt	nt
E. coli 442	0	0	nt	nt	nt	6	18:26H	nt
E. coli 389 (imp)	0	8H	21:37H	17:25H	24:30H	20	22:26H	nt
C. albicans 54	8	0	0	0	0	0	0	15

[a] NAA200: norselic acid A, 200 μg; OXA1U: oxacillin, 1 unit; KM30: kanamycin, 30 μg; VA30: vancomycin, 30 μg; AMC30: amoxicillin + clavulanic acid, 30 μg; RA5: rifampin, 5 μg; FOX30, cefoxitin, 30 μg; NY100U: nystatin, 100 units.

with a 10 cm cell. UV−visible data were obtained on a Perkin-Elmer Lambda 900 UV/vis/NIR spectrometer. Infrared spectra were obtained with a Nicolet Avatar 320 FT-IR spectrometer. ^{1}H, ^{13}C, DEPT 135, ^{1}H−^{1}H COSY, HSQC, and HMBC spectra were obtained on a Varian Inova 500 instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. HPLC was performed with a Shimadzu LC-8A multisolvent delivery system connected to a Shimadzu SPD-10A UV−vis tunable absorbance detector using a YMC Pack ODS-AQ C-18 analytical column and YMC Pack CN preparative column (250 × 20 mm) or Phenomenex Sphereclone (250 × 10 mm) for normal phase. EM Science silica gel 60 of 230−400 mesh was used in flash column chromatography. Mass spectroscopic data were obtained from Agilent Technologies LC/MSD TOF. The crystal structure measurements were performed using a Bruker Smart APEX CCD diffractometer using graphite-monochromated Cu Kα radiation (λ = 1.54178 Å) with a hemisphere scan mode at 100(2) K in the range $3.21° < \theta < 65.13°$. The structure was solved by direct methods with SHELXS-97. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were placed in calculated positions with fixed thermal parameters riding on the parent atoms. The final refinement was performed by full-matrix least-squares methods on F^2 by SHELXL-97.

Biomass Collection. *Crella* sp. was typically collected from 30 to 40 m depth at Norsel Point, Gamage Point, Janus Island, and Hermit Island, Antarctica, in 2001, 2003, and 2004. A voucher specimen was identified by Dr. Robert Van Soest, University of Amsterdam, where the voucher is held as ZMA17112.

Extraction and Isolation. The freeze-dried biomass (193 g) was extracted by MeOH/CH_2Cl_2 (1:1 × 3 1 L). The resulting extract (34.0 g, only 13.2 g was used) was partitioned between EtOAc and H_2O. The EtOAc partition (5.7 g) was concentrated *in vacuo* and was loaded on a silica gel flash column. The EtOAc residue was fractionated with a step gradient from 100% hexane to 100% EtOAc to 100% MeOH using the silica gel flash column. The 70% EtOAc/hexane fraction (792 mg) containing crude **1** was concentrated *in vacuo*, followed by the purification by recrystallization in 1:1 CH_2Cl_2 and hexane for three days to allow slow crystal formation of **1** (310 mg). Crude **2**−**5** (426 mg) eluted with 60% EtOAc/hexane from the flash column. The fraction was loaded on the second silica gel flash column. The 50% EtOAc and hexane fraction (245 mg) was subjected to an isocratic elution on analytical NP HPLC with 40% EtOAc/hexane to afford pure **2** (32 mg). The 40% EtOAc/hexane fraction (120 mg) eluted from the second flash column was further purified by preparative CN HPLC to afford **3** (4 mg), **4** (7.5 mg), and **5** (8.3 mg). Additional **3** (2 mg) was obtained after further analytical RP HPLC using 80% aqueous methanol.

Norselic Acid A (1): white, crystalline needles (from 1:1 hexane/CH_2Cl_2); mp 157−158 °C; $[\alpha]^{20}_D$ +53 (*c* 0.2 $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 299 (3.83) nm; IR (thin film) ν_{max} 3500, 1706, 1648 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m/z* 453.3000 [M + H]$^+$(calcd for $C_{29}H_{41}O_4$, 453.3005).

Single-Crystal X-ray Crystallographic Data of Norselic Acid A (1). Formula $C_{29}H_{40}O_4$; MW = 452.61; orthorhombic $P2_12_12_1$; a = 6.646(2) Å, b = 17.261(5) Å, c = 22.793(6) Å, V = 2614.6(13) Å^3, Z = 4, density(calculated) = 1.150 mg m^{-3}; crystal size 0.45 × 0.16 × 0.12 mm^3. The total number of reflections collected was 7788, of which 3305 independent reflections were measured with an R(int) = 0.0665. The final indices were R1 = 0.0629, wR2 = 0.1586, S = 1.056. Crystallographic data have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 736592). These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44 1223 336033.

Norselic Acid B (2): yellow, crystalline solid; mp 201 °C; $[\alpha]^{20}_D$ +42 (*c* 0.2 $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 277 (2.51) nm; IR (thin film) ν_{max} 3462, 1653, 1671 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m/z* 457.3312 [M + H]$^+$ (calcd 457.3318 for $C_{29}H_{45}O_4$); *m/z* 439.3203 [M + H − H_2O]$^+$ (calcd for $C_{29}H_{43}O_3$, 439.3212).

Norselic Acid C (3): white, amorphous solid; $[\alpha]^{20}_D$ +43 (*c* 0.2 $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 267 (3.78) nm; IR (thin film) ν_{max} 1675, 1653; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m/z* 425.3082 [M + H]$^+$ (calcd for $C_{28}H_{41}O_3$, 425.3056).

Norselic Acid D (4): green, glassy solid; $[\alpha]^{20}_D$ +54 (*c* 0.1 $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 242 (4.00) nm; IR (thin film) ν_{max} 3290, 1670, 1655 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m/z* 457.3306 [M + H]$^+$ (calcd for $C_{29}H_{45}O_4$, 457.3318).

Norselic Acid E (5): yellow, glassy solid; $[\alpha]^{20}_D$ +57 (*c* 0.1 $CHCl_3$); UV (CH_3OH) λ_{max} (log ε) 299 (3.70) nm; IR (thin film) ν_{max} 3450, 1622, 1615; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS *m/z* 495.3124 [M + H]$^+$ (calcd for $C_{31}H_{43}O_5$, 495.3110).

Antimicrobial Assays. *In vitro* antimicrobial activities against methicillin-sensitive (MSSA, strain 375) and -resistant (MRSA, strain 310) *Staphylococcus aureus*, vancomycin-resistant *Enterococci faecium* (VREF, strain 379), *E. coli* (strain 442), *E. coli imp* (strain 389, a mutant strain with increased permeability to large molecular weight compounds), and *Candida albicans* (strain 54) were determined by an agar diffusion method. Media used were Difco nutrient agar (pH 6.8) for *S. aureus*, LB (Luria−Bertani) agar for *E. faecium* and *E. coli*, and YM (yeast maltose) agar for *C. albicans*. Assay plates (9 in. × 9 in., Sumilon) were prepared by pouring a 125 mL volume of agar medium (tempered at 50 °C) inoculated with an overnight broth culture of the test organisms (adjusted to approximately 10^6 cells per mL). Sample concentrations of 200 μg in 10 μL aliquots were spotted onto the agar surface, and the plates were incubated at 37 °C for 18 h. The zone of growth inhibition was measured using a hand-held digital caliper and recorded in mm (digits) of either clear zone (reported with no caveat), almost clear (AC) or resistant colonies, when the test material results in quick resistance colonies, hazy (H), usually used when the test material is bacteriostatic and does not kill 100% of the cells, or very hazy (VH), when the test material shows a hint of inhibition, which may occur when the test material is highly nonpolar and insoluble.

Field Bioassay Procedures: Artificial Food Preparation.[13] Artificial foods consisted of 2% ground, freeze-dried Antarctic krill (*Euphausia superba*) in a 2% alginate matrix. Norselic acid A (**1**) was dissolved in a minimum volume of CH_2Cl_2 and dried onto the freeze-dried krill under reduced pressure such that the final artificial food pellets would represent the "natural" concentration of norselic acid A present in the sponge (assuming 100% extraction and recovery) on a wet weight basis as well as 3× and 10× the "natural" concentrations. Solvent controls were prepared with an equal volume of CH_2Cl_2 alone on the dried krill. The resulting artificial foods were approximately 2 mm thick by 1 cm diameter disks for amphipod bioassays or blocks approximately 2 mm by 4 mm by 4 mm for sea star bioassays.

Amphipod Bioassays.[6,13] Individuals of *Gondogeneia antarctica,* a very common omnivorous amphipod species in the study region, were maintained in flow-through seawater aquaria. Maintenance diet consisted of control artificial food pellets and palatable macroalgae. For bioassays, 20 randomly selected amphipods were placed into each of ten 250 mL plastic bottles with preweighed wet, paired norselic acid A and control alginate food pellets (experimentals). Paired norselic acid A (**1**) and control alginate food pellets cut adjacent to experimental pellets were placed into 10 paired 250 mL plastic bottles as autogenic controls. Amphipods were allowed to feed on the artificial foods for 72 h, and water was replaced every 24 h. Experimental and autogenic control pellets were then reweighed wet. Consumption was calculated as the difference between the experimental norselic acid A and control pellets

with their initial weights corrected by the ratio of the final-to-initial wet weight ratio of the paired autogenic control pellets. Statistical comparisons were performed with Wilcoxon signed rank tests using SPSS software (SPSS Inc.).

Sea Star Bioassays.[9,13] Individuals of *Odontaster validus*, a very common, omnivorous sea star species in the study region, were maintained in flow-through seawater for at least several days before use. Maintenance diet consisted of control artificial food pellets. *O. validus* individuals routinely climb the aquarium walls to the air−water interface and then extend one or more of their arms along the interface, thus exposing their chemosensory tube feet. In bioassays, a single artificial food block containing either norselic acid A (**1**) or solvent control was placed on chemosensory tube feet in the ambulacral groove equidistant between the mouth and arm tip with acceptance and rejection determined as previously described.[9,13] Sample sizes per treatment ranged from 10 to 17 individuals that accepted satiation controls.[9,13] Statistical comparisons were performed with a one-tailed Fisher's exact test using VassarStats (http://faculty.vassar.edu/lowry/VassarStats.html).

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Supporting Information Available: ^{1}H, ^{13}C, DEPT 135, gCOSY, gHSQC, gHMBC, and ROESY NMR spectra of **1**−**5** and the single-crystal X-ray crystallographic data tables for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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