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CAVERNICOLIN-1 AND CAVERNICOLIN-2, TWO EPIMERIC DIBROMOLACTAMS FROM THE MEDITERRANEAN SPONGE APLYSINA (VERONGIA) CAVERNICOLA

Michele D'Ambrosio,° Antonio Guerriero,° Pietro Traldi,^ and Francesco Pietra°*
(°Laboratorio di Chimica, Libera Università di Trento, 38050 Povo-Trento,
and ^Istituto di Polarografia del CNR, 35100 Padova, Italy)

Summary: Cavernicolin-1 and Cavernicolin-2, two epimeric compounds having a γ-lactam ring fused to a dibromocyclohexenone ring, have been isolated from the Mediterranean sponge Aplysina (Verongia) cavernicola.

Sponges of the genus Aplysina and Ianthella[1] have given the tyrosine-derived[2] compounds (+)-1,[3] (+)-3,[4] 5a, 5b,[5] 7[6] (A. aerophoba); 6 (A. cauliformis);[7] 4a,[2,8] 6, 8, 9, 12-14,[2] 10[1] (A. fistularis); 4b (diastereoisomeric mixture), (+)-3, 5a, 6 (A. thiona and A. spp);[9] 15 (A. lacunosa);[12] (-)-2 (I. ardis);[10] 11 (I. basta)[11] and (±)-3 (I. spp).[4] It is seen that the tyrosine side-chain has been either changed into (oximino)(N-vinyl)amide, nitrile, lactone, 2-oxazolidone, and isoxazole groupings, or it has been oxidatively removed, while the central nucleus has been either retained, rearranged, or reduced at a double bond.

We now report on what can be viewed as another metabolic change of tyrosine by marine sponges, the side chain giving a lactam and the benzene nucleus undergoing reduction at two double bonds. In fact, we have now isolated from A. cavernicola racemic, or slightly enriched by one of the enantiomers, 16 and 17. Fresh sponge (210g, dry w.), collected in July 1981 near the Ile de Riou, Marseille, was extracted with ethanol. The aqueous residue from ethanol evaporation (from here on, at reduced pressure) was partitioned between water and ethyl ether and then between water and n-butanol. The residue (11g) from evaporation of the butanolic phase was subjected to chromatography on a 5 x 60 cm 70-230 mesh Merck Kieselgel 60 column, ethyl acetate-methanol 9 : 1. Central fractions were evaporated to leave a residue (1.26g) which was chromatographed on a 2 x 40 cm Lichroprep SI 60, 15-25 μm column, ethyl ether-acetonitrile 9 : 1, 15 ml min^{-1}. The eluate at 48 min was collected (254 nm) and evaporated to leave a residue (0.14g) which was subjected to reverse-phase HPLC on a Merk 10 x 250 mm LiChrosorb RP-18, 7 μm column, 65 : 35 water-methanol, 5.5 ml min^{-1}. The eluates at 4 and 5.5 min were collected as single peaks due to, respectively, cavernicolin-1 (16) and cavernicolin-2 (17).

On evaporation, both eluates gave a 3 : 1 equilibrium, oily mixture of 16 and 17 (0.025%, on dry w.). The structures were secured as follows. The 3 : 1 mixture gave MS (VG-ZAB, EI) m/e(%) 328(2), 326(4), 324(2)(M+1), 327(6), 325(12), 323(6)(M) [calcd for $C_8H_7{}^{79}Br_2NO_3$ 322.8790; found 322.8766 ± 0.005], 246(51), 244(52)(M-Br), 229(5), 227(6)(244-OH), 218(7), 216(7)(244-CO), 204(40),

202(42)(244-ketene), 176(24), 174(24)(202-CO), 95(17)(174-Br), 94(30)(174-HBr), 43(100)($O{=}C{=}NH^{+\cdot}$); UV λ_{max}^{MeOH} 255(ε=4000), sh 315 nm ($\varepsilon\simeq$100); IR ν_{max}^{nujol} 3400-3200 str br (NH and OH), 1700 str br (enone and lactam C=O), 1620 w (C=C). The ^{1}H-NMR spectra (Table I) suggest that, in the case of 16, H-2 and H-3 are in a nearly trans diaxial relationship (large J), whilst with 17 they are in a nearly cis, skewed, diaxial position (small J). With 17, as a consequence of having the enone carbonyl in endo position, H-5 is raised towards the exo direction, thus entering a W^4 relationship with H-3 (small J). On addition of D_2O, proton couplings with NH were soon lost. Moreover, H-2 was also exchanged, more slowly with 16 than with 17 owing to the hindered, endo position of H-2 with 16. Also, every carbon for both 16 and 17 could be assigned (Table II).

TABLE I. ^{1}H-NMR‡ ASSIGNMENT FOR 16, 17 ($(CD_3)_2CO$), 18, 19 (C_6D_6), and 20 ($CDCl_3$, upper row; C_6D_6, lower row)

	H-2	H-3	OH	H-5	CH_2	NH	OCO-Me
16	5.16d(9.8)[a]	4.22dd(9.8,1.6)[b]	5.5br	7.44s	2.92,2.48AB(16.8)	7.6br	
17	5.28d(4.2)[c]	4.46brd(4.2)[d]	5.6br	7.33d(0.6)[e]	2.75,2.58AB(16.8)	7.6br	
18[f]	3.42d(9.0)	3.82dd(9.0,1.3)		6.90s	2.49,2.11AB(17.6)	5.6br	1.38s
19[f]	4.45d(4.1)	3.59ddd(4.1,1.0,1.0)		6.72d(1.0)	2.50,2.25AB(17.5)	5.6br	1.36s
20		4.84brs		6.52s	2.94s	6.3br	2.09s,2.29s
		4.33brs		6.26s	2.58,2.42AB(17.6)	6.2br	1.80s,1.39s

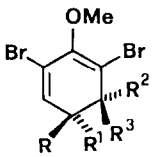
[a] It became a s on irradiation at 4.22; [b] it became either a d, $J_{H,NH}$ 1.6 on irradiation at 5.16 or a d, $J_{H,H}$ 9.8 on irradiation at 7.6; [c] it became a s on irradiation at 4.46; [d] it became a s on irradiation at 5.28 (neither the coupling with NH nor with H-5 could be discerned owing to the doublet broadness); [e] it became a s on irradiation at 4.46; [f] from a 1 : 1 mixture of 18 and 19.

TABLE II. ^{13}C-NMR‡,[a] ASSIGNMENTS

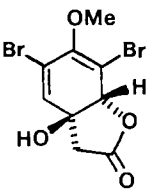
	C-1	C-2	C-3	C-4	C-5	C-6	CH_2	CONH	OCO—Me	
16(DMSO-d_6)	183.7s	57.4d	68.0d	75.5s	150.4d	119.0s	42.3t	173.9s		
17(DMSO-d_6)	183.9s	52.7d	63.4d	74.2s	149.2d	119.2s	44.1t	173.5s		
18[b](C_6D_6)	182.1	52.7	64.9	80.7	143.6	123.2	43.6	172.6	169.7	21.0
19[b](C_6D_6)	181.7	52.1	62.3	79.9	142.8	122.8	41.5	173.0	169.7	20.8
20 (C_6D_6)	140.8	113.7[c]	64.2	81.4	130.0	117.0[c]	43.4	172.9	169.0 166.4	20.6 19.7

[a] Multiplicities are from off-resonance experiments; [b] data from a 1 : 1 mixture of 18 and 19; the values can be interchanged for every carbon; [c] the values can be interchanged. ‡ In both Tables, δ values are given in ppm with respect to internal $SiMe_4$, while J values, in brackets, are given in Hz.

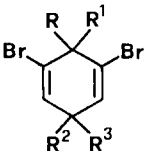
On acetylation (Ac_2O; pyridine; 0°C; overnight), a 3 : 1 mixture of 16 and 17 gave a 1 : 1 oily mixture (74%) of the alcohol acetates 18 and 19 (HPLC, as above, retention times 8 and 9 min, respectively). These gave the enol acetate 20 (71%) mp 81-82°C on acetylation at room temp. (HPLC, as above, retention time 18 min. Structures 18 and 19 are supported by the NMR spectra in the Tables and by UV spectrum similar to that of the 16-17 mixture. In particular, as in the case of



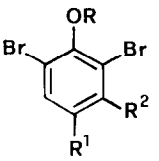
1 R=R²=OH; R¹=CH_2CN; R³=H
2 R=CH_2CN; R¹=R³=OH; R²=H



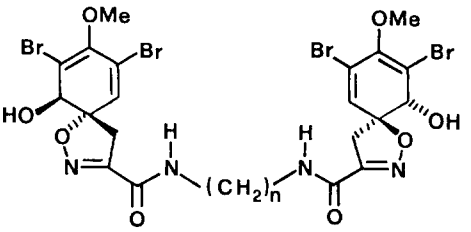
(+)-3



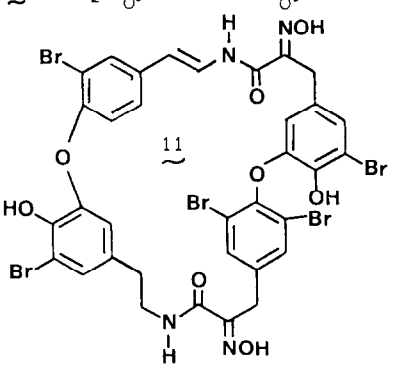
4a R=R¹=OMe; R²=OH; R³=CH_2CONH_2
4b R=OMe; R¹=OEt; R²=OH; R³=CH_2CONH_2
6 R,R¹=O; R²=OH; R³=CH_2CONH_2
12 R,R¹=O; R²,R³=O



7 R=Me; R¹=CH_2CONH_2; R²=OH
8 R=H; R¹=CH_2CN; R²=H
9 R=H; R¹=CH_2CONH_2; R²=H
10 R=R²=H; R¹=$CH_2CH_2\overset{\oplus}{N}Me_3$
13a R=R²=H; R¹=OH
13b R=Me; R¹=OMe; R²=H
14 R=H; R¹=OH; R²=CH_2CONH_2
15 R=CH_2  ; R¹=  ; R²=H



5a (n=4); 5b (n=5)



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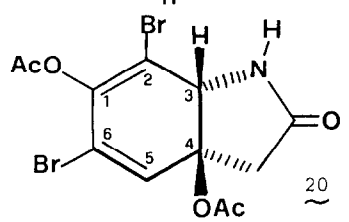

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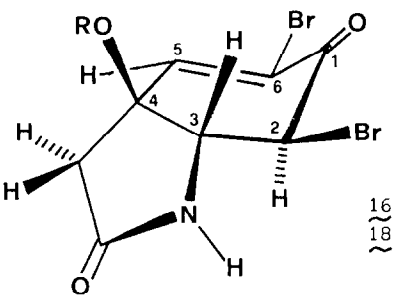
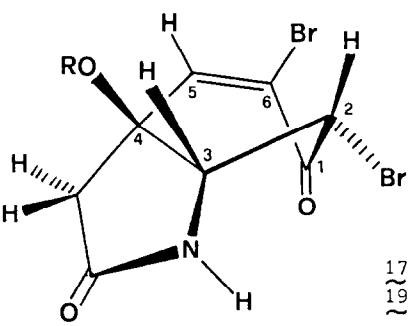


16 R=H
18 R=$COCH_3$



17 R=H
19 R=$COCH_3$

17, only the endo-carbonyl epimer (19) shows H-3 - H-5 coupling (W). Structure 20 is supported by the NMR data in the Tables and by the UV spectrum, λ_{max}^{MeOH} 268 nm.

Both the 3 : 1 mixture of 16 and 17 and the 1 : 1 mixture of 18 and 19 gave very weak, negative optical rotations at all wavelengths, from 589 to 365 nm. With 20 (0.006 g ml^{-1}; cell path 10 cm; MeOH) : α = -0.006, -0.005, -0.033, and -0.042° at respectively, 589, 546, 435, and 365 nm. On addition of the chiral reagent shift Yb(HFBC)$_3$ (C. Erba) to 20 in C_6D_6, we observed the ^{1}H-NMR

spectra (80 MHz) for the two diastereoisomeric complexes with the 20 enantiomers in approximately 1 : 1 ratio. Although natural cyclohexadienols have sometimes been isolated as racemates,[4, 13] probably the small optical rotations observed above are due to slight enantiomeric excesses, difficult to appreciate by FT NMR, rather than to optically active impurities.

Both cavernicolins may be formally viewed as products of intramolecular conjugate addition of 6 in a masked-amide form, having nucleophilic nitrogen. In fact, we have isolated 6 in large amounts (0.3%, on dry w.) from A. cavernicola. However, the intermediacy of an arene oxide[9, 13] is also conceivable.

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