Hemifistularin 3: a Degraded Peptide or Biogenetic Precursor? Isolation from a Sponge of the Order Verongida from the Coral Sea or Generation from Base Treatment of 11-Oxofistularin 3

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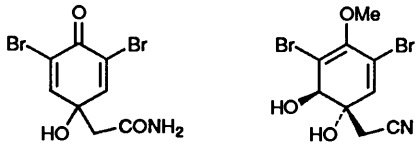
Hemifistularin 3 **10**, contained in a new species of sponge of the family Aplysinellidae, order Verongida, from the Coral Sea, corresponds to the right half of 11-oxofistularin 3 **6**, also contained in this sponge, and from which it can be obtained in a peculiar base-catalysed degradation. This raises the question of whether or not hemifistularin 3 is the first example of a biogenetically degraded peptide, or rather an elaborated biogenetic precursor, in sponges of the order Verongida. The new compounds 19-deoxyfistularin 3 **8** and 19-deoxy-11-oxofistularin 3 **9** have also been isolated from the same sponge.

Sponges belonging to the order Verongida have yielded a wide variety of tyrosine metabolite,[1a] usually based on 3,5-dibromotyrosine or less frequently 3-bromo-,[2] 3-chloro-,[3] 3,5-dichloro-,[3] or 3-bromo-5-chloro-tyrosine.[3] In many cases a single tyrosine unit is involved, as with dibromoverongiaquinol **1**[1,3] and aeroplysinin 1, either dextro- (+)-**2**[4a] or laevo-rotatory[4b] or of unspecified chirality.[4c] Other metabolites entail two or more tyrosine units through either amide bonding, as in aerothionin (+)-**3** and homoaerothionin **4** which also incorporate biogenic amines,[5] or ether bonds, as in the bastadins[2a] or fistularin 3 **5**.[6,7] *

We report here on a novel decarboxylated dipeptide, isolated from a new species of the order Verongida from the Coral Sea, which corresponds to the right half of either fistularin 3 or 11-oxofistularin 3 and which can be obtained from the latter in a peculiar base-catalysed degradation. This raises intriguing questions about the biogenesis of these metabolites. We also report on two new fistularin 3 derivatives.



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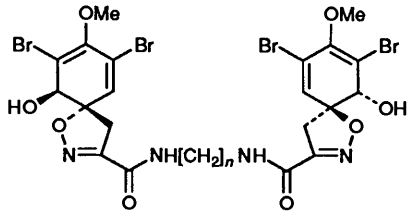
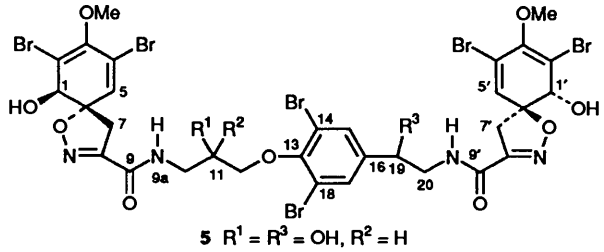
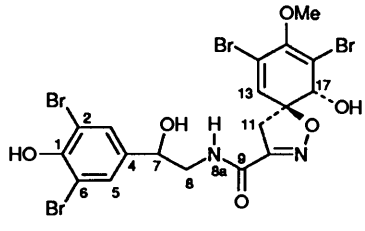
(+)-**2**



(+)-**3** $n = 4$
4 $n = 5$



5 $R^1 = R^3 = OH, R^2 = H$
6 $R^1 = R^2 = O, R^3 = OH$
7 $R^1 = R^2 = R^3 = H$
8 $R^1 = OH, R^2 = R^3 = H$
9 $R^1 = R^2 = O, R^3 = H$



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Results and Discussion

Extracts of this sponge, collected at two spots in the Coral Sea, proved to contain the long known dibromoverongiaquinol **1**,[1,3] dextro-rotatory aeroplysinin 1 (+)-**2**,[4] aerothionin (+)-**3**,[5] homoaerothionin **4**,[5a] and the recently reported 11-oxofistularin 3 **6**[7] and 11,19-dideoxyfistularin 3 **7**,[8] † besides the new 19-deoxyfistularin 3 **8** and 19-deoxy-11-oxofistularin 3 **9**.

More interestingly, our sponge proved also to contain the new compound **10**, which, corresponding to the right half of either fistularin 3 or 11-oxofistularin 3, was named hemifistularin 3.

Structural assignments of the fistularin 3 derivatives **6**–**9** were based on NMR data‡ and on FABMS data for their products of spirooxazolidine ring opening. In the case of compounds **6** and **7** this serves now only to confirm the assigned structures, spectral data being in accord with those already published.[7,8] The assignments for compounds **8** and **9** were based on the observations that ^1H NMR signals for the 19-CHOH and 11-

* No absolute configurational meaning is implied by the structural formulae in this paper, except for aeroplysinin 1 (+)-**2**[4a,b] and aerothionin (+)-**3**.[5b]

† Compounds **6** and **7** were reported[7,8] when we had already completed their structural assignment and were long awaiting biological screening from external laboratories.

‡ Including ^1H–^1H COSY experiments[9] for compounds **6**–**9** and hemifistularin 3 **10**, ^1H–^{13}C COSY experiments[10a] for compounds **6** and **7**, and the long-range variant of the latter[10b] for compounds **6**–**10**.

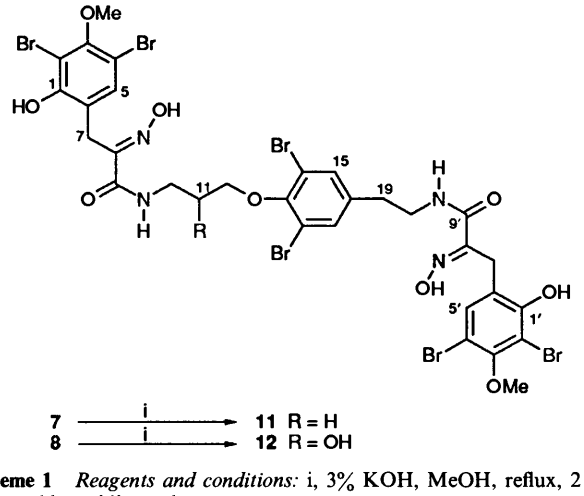
Table 1 ^{13}C and ^{1}H NMR data for 19-deoxyfistularin 3 **8** and 19-deoxy-11-oxofistularin 3 **9** in $(CD_3)_2CO$

Atom position	**8**		**9**	
	^{13}C	^{1}H[a]	^{13}C	^{1}H[b]
1, 1′	75.09, 75.20 (d)	4.23, 4.18 (dd, 8.1, 0.9)	75.11, 75.18 (d)	4.23, 4.18 (dd, 8.1, 0.9)
2, 2′	122.10 (s)		122.11 (s)	
3, 3′	148.75 (s)		148.73, 148.74 (s)	
4, 4′	113.78 (s)		113.85 (s)	
5, 5′	132.28 (d)	6.56, 6.52 (d, $J_{5,1} = J_{5',1} = 0.9$)	132.27, 132.28 (d)	6.57 and 6.52 (d, $J_{5,1} = J_{5',1} = 0.9$)
6, 6′	91.56, 91.76 (s)		91.95, 91.59 (s)	
7, 7′	40.03, 40.08 (t)	3.86 and 3.22, 3.84 and 3.18 (d, J_{gem} 18.1)	39.87, 40.09 (t)	3.86 and 3.22, 3.84 and 3.16 (d, J_{gem} 18.1)
8, 8′	155.14, 155.16 (s)		154.79, 155.16 (s)	
9, 9′	160.00, 160.41 (s)		160.24, 160.03 (s)	
10	43.49 (t)	3.50, 3.70 (m)	47.46 (t)	4.60 (d, 6.0)
11	69.68 (d)	4.25 (m)	200.87 (s)	
12	75.79 (t)	4.03 (m)	76.48 (t)	4.75 (s)
13	151.97 (s)		151.23 (s)	
14, 18	118.31 (s)		118.07 (s)	
15, 17	134.14 (d)	7.53 (s)	134.21 (d)	7.57 (s)
16	139.88 (s)		140.53 (s)	
19	34.71 (t)	2.88 (t, $J_{19,20}$ 7.0)	34.74 (t)	2.91 (t, $J_{19,20}$ 7.0)
20	40.91 (t)	3.58 (td, 7.0, 6.0)	40.85 (t)	3.58 (q, $J_{20,19} \approx J_{20,HN} = 6.9$)
3-, 3′-MeO	60.19 (q)	3.78 (s), 3.73 (s)	60.19 (q)	3.74 (s), 3.72 (s)

[a] 1-HO 5.46 (d, 8.1), 1′-HO 5.44 (d, 8.1), 9a-H 7.65 (br t, 6.1), 9′a-H 7.77 (br t, 6.0), 11-HO 4.77 (d, 5.2). [b] 1-HO 5.49 (d, 8.1), 1′-HO 5.44 (d, 8.1), 9a-H 7.86 (br t, 6.0), 9′a-H 7.77 (br t, 6.9).

CHOH functions of fistularin 3 **5**[6] are replaced in compound **9** by, respectively, the signals for a methylene group (δ_H 2.91, as a tt for coupling to 20-H_2) and a keto group (δ_C 200.87, s), whilst in compound **8** the signals for 19-CHOH are replaced by the signals for a methylene group (δ_H 2.88) (Table 1). The structure of hemifistularin 3 **10** was based on the NMR detection of single dibromospirocyclohexadienyl ring and trisubstituted phenolic moieties. For the new compounds **8**–**10** a *trans* relationship between the OH group and the isoxazolidine oxygen atom was established by comparison of NMR data (Table 1) with those of synthetic *cis*, *cis*-aerothionin.[11]

These structural assignments were further substantiated by MS data. While hemifistularin 3 **10** smoothly gave the molecular ion in FABMS (see Experimental section), metabolites **6**–**9** did not. Therefore, following literature indications for aerothionin **3** and fistularin 3 **5**,[12] 11,19-dideoxyfistularin 3 **7** and 19-deoxyfistularin 3 **8** were subjected to treatment with 3% methanolic KOH at reflux, followed by acidic work-up. This gave compounds **11** and **12**, respectively, in the oximino *E* form (Scheme 1). Both these compounds gave the respective molecular ions in FABMS (see Experimental section). However,

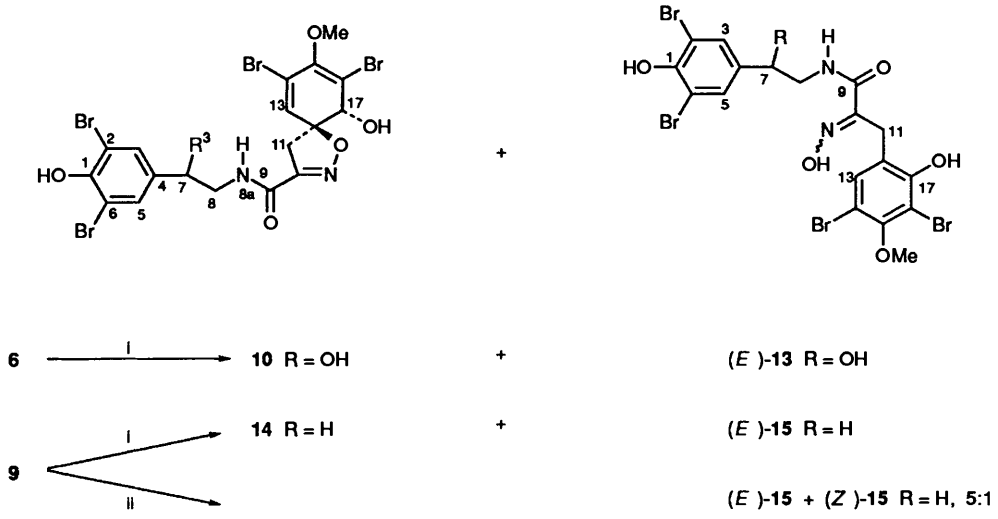


Scheme 1 *Reagents and conditions:* i, 3% KOH, MeOH, reflux, 2 h; followed by acidic work-up.

when either 11-oxofistularin 3 **6** or 19-deoxy-11-oxofistularin 3 **9** was subjected to the same alkaline treatment, O–C(12) bond breaking unexpectedly occurred. This is indicated in Scheme 2 where it is seen that base-treatment of compound **6** at room temperature, followed by acidic work-up, led to hemifistularin 3 **10** and its oxime (*E*)-**13**. The latter structure was established by 1D NMR data (see Experimental section), ^{1}H–^{1}H COSY and long-range ^{1}H–^{13}C COSY experiments. Compound **9** gave a mixture of the spirooxazolidine **14** and oxime (*E*)-**15** on similar alkaline treatment, while changing to reflux conditions followed by neutral work-up gave products (*E*)-**15** and (*Z*)-**15** only (5:1).* These results show that base-induced O–C(12) bond breaking with 11-oxofistularins 3 is an easier process than is spirooxazolidine ring opening.

It is interesting that the spirooxazolidine product of base-catalysed degradation of 11-oxofistularin 3 **6** corresponds to hemifistularin 3 **10**. As both compounds are present in our sponge, the question is raised as to if hemifistularin 3 is a product of biogenetic degradation of 11-oxofistularin 3 or rather an elaborated biogenetic precursor of it. In either case these are uncommon observations for tyrosine metabolites of sponges of the order Verongida. Viewing compound **10** as a biogenetic precursor, the O–C(12) bond might have been established *via* nucleophilic displacement of phosphorylated serine's hydroxy group followed by decarboxylation. Viewing compound **10** alternatively as a degradation product, its generation from 11-oxofistularin 3 may be imagined in analogy

* NMR data for compounds **6** and **7** are also reported (see Experimental section), since they are available from the literature,[7,8] but in other solvents, which does not allow safe structural comparisons. The *E*/*Z* stereochemistry of the oxime was established by comparison of NMR data with those reported for the oximes of brominated tyrosine metabolites.[2c,13,14] Diagnostically important is δ_C for the benzylic C-11 in compounds (*E*)-**13** and (*E*)-**15** (25.56 t and 25.71 t, respectively) and for C-7 and C-7′ in compounds **11** and **12** (25.72 t and 25.67 t, respectively) in comparison with the corresponding values for the *Z* isomer (37.5 t).[13] Our observation that in $(CD_3)_2CO$ both the benzylic protons and 13-H for compound (*Z*)-**15** resonate at higher field than do the corresponding protons for the *E* isomer is in accord with NMR observations in $CDCl_3$–CD_3OD mixtures.[13] It is also relevant that for the *Z* isomer there is concentration dependence of δ_H for OH groups, very likely reflecting H-bonding between the oxime OH and carbonyl oxygen.



Scheme 2 Numbering is for convenience. *Reagents and conditions:* i, 3% KOH, MeOH, room temp., overnight; followed by acidic work-up; ii, 3% KOH, MeOH, reflux, 2 h; followed by neutral work-up.

with that of β-naphthol on treatment of its phenacyl ethers with 5% ethanolic alkali.[15,*]

In cytotoxicity screenings, the most active of the metabolites of these sponges proved to be 11-oxofistularin 3 **6** with 100% inhibition of KB cells in culture at 7 μg cm^{-3}. Antiviral activity of this compound was also reported.[7]

Experimental

General.—All evaporations were carried out at reduced pressure. M.p.s were measured out with a Kofler hot-stage microscope and are uncorrected. Flash chromatography (FC) was performed on Merck Si-60, 15–25 μm; TLC on Merck Kieselgel 60 PF_{254} plates; and reversed-phase HPLC on 25 × 1 cm columns filled with either Merck LiChrosorb RP18 (7 μm) or Merck LiChrosorb RP-8 (7 μm), with UV monitoring at λ 254 nm, and solvent flux 5 cm^3 min^{-1}. UV spectra were recorded on a Perkin-Elmer Lambda-3 spectrophotometer. IR spectra were taken on a Perkin-Elmer 337 spectrometer. NMR spectra were recorded in $(CD_3)_2CO$ with a Varian XL-300 spectrometer, ^1H at 299.94 MHz, ^{13}C at 75.43 MHz; δ-values are reported with respect to internal $SiMe_4$ (δ 0) and *J*-values in Hz; multiplicities from DEPT;[16] assignments confirmed by ^1H–^{13}C COSY.[10] Optical rotation data were obtained with a JASCO-DP-181 polarimeter; [α]-values are given in 10^{-1} deg cm^2 g^{-1}. Mass spectra (EI, unless otherwise stated) were taken with a Kratos MS80 mass spectrometer with home-built data system. FAB spectra were taken with the same apparatus equipped with a Vacumetrics DIP gun.

Collection, Taxonomy and Isolation.—The sponge was first collected by SCUBA diving on 20 March 1988 at a depth of 53 m on the external part of the south New Caledonian Grand Récif (collection R193/504M, 3 kg fresh weight, 390 g freeze-dried powder) and then on 11 March 1989 at the Récif de Nokanhui, Ile des Pins (collection R246/552M, 3 kg fresh weight, 500 g freeze-dried powder). The two collections proved to contain the same secondary metabolites, although in different relative proportions. Sample R193/504M was examined by Prof. C. Lévi, who retains a voucher specimen, and classified as a new species, formerly considered as a *Verongia* sp. [= *Aplysina* = *Verongula*]. The sponge, unlike typical *Verongia*, revealed a fully dendritic fibrous skeleton and therefore must be classified in the family Aplysinellidae Bergquist, order Verongida. The sponge was immediately freeze-dried and freeze-dried powder (310 g) was then extracted (MeOH) and the extract was evaporated to give a residue (16.1 g), which was extracted (EtOAc) to give a residue (7.15 g). This was subjected to FC, first with hexane–Et_2O (7:3), then hexane–EtOAc (gradient elution), with fractions (100 cm^3) collected. Fractions 18–20 were evaporated to give a residue (0.39 g), which was subjected to RP-8 HPLC with MeCN–water (55:45) to give compounds **10** (t_R 5.5 min, 24 mg), **6** (t_R 9.4 min, 37 mg), **8** (t_R 13.5 min, 2 mg), **9** (t_R 14.5 min, 11 mg) and **7** (t_R 18.4 min, 43 mg). Fractions eluted with EtOAc were evaporated to give a residue (1.59 g), which was subjected to further FC. The eluates with hexane–Et_2O (1:1) were evaporated to give a residue (1.15 g), which was subjected to RP-8 HPLC with MeCN–water (1:1) to give compounds (+)-**2** (t_R 3.5 min), further purified on elution with MeCN-water (3:7) (t_R 8.1 min, 3.0 mg), **4** (t_R 6.8 min, 4.0 mg), (+)-**3** (t_R 10.4 min, 3.5 mg). Head fractions from the last process were combined, evaporated, and subjected to RP-8 HPLC with MeCN–water (15:85) to give compound **1** (t_R 10.0 min, 34 mg).

11-*Oxofistularin* 3 **6**. Powder, $[\alpha]_D^{20}$ +156 (*c* 0.7, acetone) (lit.,[7] +130, *c* 0.1, MeOH); δ_C 75.29 (d, C-1 and 1′), 122.11 (s, C-2 and 2′), 148.82 (s, C-3 and 3′), 113.85 (s, C-4 and 4′), 132.34 and 132.23 (d, C-5 and 5′), 92.04 and 91.86 (s, C-6 and 6′), 39.99 and 39.89 (t, C-7 and 7′), 154.80 and 155.16 (s, C-8 and 8′), 160.29 and 160.48 (s, C-9 and 9′), 47.52 (t, C-10), 200.82 (s, C-11), 76.52 (t, C-12), 151.93 (s, C-13), 118.11 (s, C-14 and -18), 131.55 (d, C-15 and -17), 143.93 (s, C-16), 71.46 (d, C-19), 47.60 (t, C-20) and 60.23 (q, 3- and 3′-MeO); δ_H 4.23 and 4.18 (2 dd, $J_{1,OH}$ 8.1, $J_{1,5}$ 0.9, $J_{1'OH}$ 8.1, $J_{1'5}$ 0.9, 1- and 1′-H), 6.56 and 6.52 (2d, $J_{5,1} = J_{5',1'} = 0.9$, 5- and 5′-H), 3.86–3.22 and 3.84–3.18 (2 couples of d, J_{gem} 18.1, 7- and 7′-H_2), 4.60 (d, $J_{10,HN}$ 5.8, 10-H_2), 4.76 (s, 12-H_2), 7.68 (s, 15- and 17-H), 4.92 (td, $J_{19,20}$ 7.2, $J_{19,OH}$ 4.3, 19-H), 3.61 and 3.49 (m, 20-H_2), 5.44 and 5.47 (two d, $J_{OH,1} = J_{OH,1'} = 8.1$, 1- and 1′-OH), 3.72 (s, 3- and 3′-MeO), 7.58 and 7.69 (2 br t, $J_{HN,10} = J_{HN,20} = 5.8$, 9a- and 9′a-H) and 5.08 (d, $J_{OH,19}$ 4.3, 19-OH).

11,19-*Dideoxyfistularin* 3 **7**. Powder, m.p. 146 °C (from hexane–Et_2O); $[\alpha]_D^{20}$ +150 (*c* 2.0, acetone) (lit.,[8] +98.5, *c* 0.1); δ_C 75.28 (d, C-1), 75.33 (s, C-1′), 122.07 (C-2 and -2′), 148.77 (s, C-3 and -3′), 113.81 (s, C-4 and -4′), 132.37 (d, C-5), 132.30 (d, C-5′), 91.67 (s, C-6 and -6′), 40.13 (t, C-7), 40.09 (t,

* Possibly these processes are initiated by deprotonation of the activated methylene group (12-H_2 in 11-oxofistularins 3 **6**) followed by departure of phenolate to leave a carbonyl-stabilized carbene. However, no carboxylic ester from the expected Wolff rearrangement was isolated.

C-7'), 155.24 (s, C-8), 155.14 (s, C-8'), 160.07 (s, C-9), 160.01 (s, C-9'), 37.47 (t, C-10), 30.55 (t, C-11), 72.16 (s, C-12), 152.30 (s, C-13), 118.48 (s, C-14 and -18), 134.06 (d, C-15 and -17), 139.63 (s, C-16), 34.75 (t, C-19), 40.96 (t, C-20) and 60.21 (q, 3- and 3'-MeO); δ_H (coupling patterns are reported only if they differ from those for compound **6**) 4.17 and 4.18 (dd, $J_{1,OH}$ 8.1, $J_{1,5}$ 0.9, $J_{1'OH}$ 8.1, $J_{1',5}$ 0.9, 1- and 1'-H), 6.50 and 6.52 (5- and 5'-H), 3.84–3.18 and 3.82–3.16 (7- and 7'-H), 3.60 (q, $J_{10,11} \approx J_{10,HN}$ = 6.1, 10-H_2), 2.13 (quint, $J_{11,10} = J_{11,12}$ = 6.1, 11-H_2), 4.08 (t, $J_{12,11}$ 6.1, 12-H_2), 7.51 (15- and 17-H), 2.86 (t, $J_{19,20}$ 7.0, 19-H_2), 3.58 (td, $J_{20,19}$ 7.0, $J_{20,HN}$ 6.0, 20-H_2), 5.44 (1- and 1'-OH), 3.72 (s, 3- and 3'-MeO), 7.77 (br t, $J_{HN,10}$ 6.1, 9a-H) and 7.72 (br t, $J_{HN,2}$ 6.0, 9'a-H).

19-*Deoxyfistularin* 3 **8**. Powder; TLC (Et_2O) R_f 0.45; $[\alpha]_D^{20}$ + 155 (*c* 0.17, acetone) (Found: C, 34.1; H, 2.8; N, 5.2. $C_{31}H_{30}Br_6N_4O_{10}$ requires C, 33.9; H, 2.7; N, 5.1%); λ_{max}(MeOH)/nm 283 (ε/dm^3 mol^{-1} cm^{-1} 10 400), 235 (22 000) and 206 (60 400); ν_{max}(Nujol)/cm^{-1} 3400 br, 1650, 1590, 1535, 1450 and 1050; *m/z* 351, 349, 347 (3, 6, 3%), 336, 334, 332 (2, 5, 2), 323, 321, 319 (1, 2, 1), 308, 306, 304 (3, 6, 3), 307, 305, 303 (6, 12, 6) and 267, 265, 263 (2, 3, 2).

19-*Deoxy*-11-*oxofistularin* 3 **9**. Powder; TLC (Et_2O) R_f 0.65; $[\alpha]_D^{20}$ +136 (*c* 0.2, acetone) (Found: C, 33.8; H, 2.5; N, 5.2. $C_{31}H_{28}Br_6N_4O_{10}$ requires C, 34.0; H, 2.6; N, 5.1%); λ_{max}(MeOH)/nm 280 (ε/dm^3 mol^{-1} cm^{-1} 3400) and 205 (18 500); ν(Nujol)/cm^{-1} 3400 br, 1725, 1650, 1590 and 1530; *m/z* 351, 349, 347 (2, 4, 2%), 336, 334, 332 (2, 4, 2), 323, 321, 319 (4, 9, 5), 308, 306, 304 (6, 12, 6) and 267, 265, 263 (6, 12, 6).

Hemifistularin 3 **10**. Powder; m.p. 73–75 °C (from hexane/Et_2O); $[\alpha]_D^{20}$ +110 (*c* 0.2, acetone) (Found: C, 31.9; H, 2.5; N, 4.2. $C_{18}H_{16}Br_4N_2O_6$ requires C, 31.9; H, 2.4; N, 4.1%); λ_{max}(MeOH)/nm 281 (ε/dm^3 mol^{-1} cm^{-1} 6900) and 207 (39 200); ν(Nujol)/cm^{-1} 3350 br, 1660, 1590, 1540, 1270, 1220 and 1095; δ_C 150.72 (s, C-1), 111.38 (s, C-2 and -6), 130.90 (d, C-3 and -5), 138.50 (s, C-4), 71.43 (d, C-7), 47.71 (t, C-8), 160.42 (s, C-9), 155.14 (s, C-10), 40.08 (t, C-11), 91.84 (s, C-12), 132.27 (d, C-13), 113.90 (s, C-14), 148.78 (s, C-15), 122.15 (s, C-16), 75.21 (d, C-17) and 60.22 (q, MeO); δ_H 7.57 (s, 3- and 5-H), 4.84 (ddd, *J* 7.3, 6.4 and 3.8, 7-H), 3.58 and 3.45 (2 ddd, *J* 13.5, 6.4 and 5.8 and *J* 13.5, 7.3, 5.8, 8-H_2), 3.83 and 3.17 (2d, J_{gem} 18.2, 11-H_2), 6.52 (d, $J_{13,17}$ 0.9, 13-H), 4.18 (dd, $J_{17,OH}$ 7.8, $J_{17,13}$ 0.9, 17-H), 3.79 (s, 1-OH), 5.02 (d, $J_{OH,7}$ 3.8, 7-OH), 7.69 (br t, $J_{HN,8}$ 5.8, HN), 3.72 (s, OMe) and 5.48 (d, $J_{OH,17}$ 7.8, 17-OH); *m/z* (glycerol–MeOH–H^+, FAB) 676.8 (MH^+, 0.2%, as the centre of a cluster of ions); (3-nitrobenzyl alcohol; FAB) 698.6 (MNa^+, 1.0% as the centre of a cluster of ions) and 658.6 ($MH^+ - H_2O$, 1.1% as a quintet).

Alkaline Treatment of 11-*Oxofistularin* 3 **6**.—A solution of 11-oxofistularin 3 **6** (11 mg) in 3% methanolic KOH (2 cm^3)–water (0.5 cm^3) was stirred at room temp. until complete disappearance (overnight, TLC monitoring). The mixture was then acidified, evaporated and the residue was extracted (EtOAc), and the extract was evaporated to give a residue, which was subjected to RP-8 HPLC with MeCN–water (55:45) to give compound **10** (t_R 5.8 min, 2.8 mg) (identical with the natural product) and (*E*)-**13** (t_R 10.0 min, 2.3 mg) as a powder.

Data of oxime (*E*)-**13**: $[\alpha]_D^{20}$ +20, $[\alpha]_{365}^{20}$ +71 (*c* 0.05, acetone); δ_C 150.66 (s, C-1), 111.24 (s, C-2 and -6), 130.79 (d, C-3 and -5), 138.11 (s, C-4), 70.85 (d, C-7), 47.79 (t, C-8), 166.57 (s, C-9), 154.54 (s, C-10), 25.56 (t, C-11), 121.79 (s, C-12), 134.89 (d, C-13), 108.81 (s, C-14), 154.74 (s, C-15), 106.76 (s, C-16), 151.05 (s, C-17) and 60.54 (q, MeO); δ_H 7.57 (s, 3- and 5-H), 4.88 (dd, $J_{7,8a} = J_{7,5} = J_{7,8b}$ = 3.3, 7-H), 3.64 and 3.48 (2 ddd, *J* 13.5, 6.2, 7.5 and *J* 13.5, 6.3, 3.3, 8-H_2), 3.81 (s, 11-H_2), 7.59 (s, 13-H), 3.80 (s, OMe) and 8.00 (br t, 6.2, HN); *m/z* 598, 596 (1, 1%), 584, 582 (1, 1), 568, 566 (0.5, 0.5), 351, 349, 347 (29, 60, 31), 336, 334, 332 (28, 54, 27), 295, 293, 291 (14, 26, 14), 283, 281, 279 (3, 6, 4) and 267, 265, 263, (7, 12, 7).

Alkaline Treatment of 11,19-*Dideoxyfistularin* 3 **7**.—A solution of compound **7** (10 mg) in 3% methanolic KOH (2 cm^3)–water (0.5 cm^3) was heated at reflux for 2 h, with monitoring by TLC. The solvent was evaporated off and the residue was acidified, then extracted (EtOAc). The extract was evaporated and the residue was subjected to RP-8 HPLC with MeCN–water (7:3), 6 cm^3 min^{-1}, to give compound **11** (t_R 14.4 min, 7.3 mg) as a powder, m.p. 100–102 °C (from MeOH) (lit.,[8] 90–93 °C); δ_C 151.18 (s, C-1 and -1'), 106.72 (s, C-2 and -2'), 154.65 (s, C-3 and -3'), 108.70 (s, C-4 and -4'), 134.99 (d, C-5 and -5'), 121.91 (s, C-6 and -6'), 25.72 (t, C-7 and -7'), 154.60 (s, C-8 and -8'), 166.55 (s, C-9 and 9'), 37.98 (t, C-10), 30.55 (t, C-11), 71.97 (t, C-12), 151.18 (s, C-13), 118.47 (s, C-14 and -18), 134.04 (d, C-15 and -17), 139.47 (s, C-16), 34.43 (t, C-19) and 41.34 (t, C-20); δ_H 7.58 and 7.56 (s, 5- and 5'-H), 3.80 (s, 7- and 7'-H_2), 3.64 (td, $J_{10,11}$ 6.9, $J_{10,HN}$ 6.0, 10-H_2), 2.11 (quint, $J_{11,10} = J_{11,12}$ = 6.9, 11-H_2), 4.03 (t, $J_{12,11}$ 6.9, 12-H_2), 7.48 (s, 15- and 17-H), 2.89 (t, $J_{19,20}$ 6.9, 19-H_2), 3.62 (td, $J_{20,19}$ 6.9, $J_{20,HN\text{-}9'a}$ 6.0, 20-H_2), 3.78 and 3.82 (s, 3- and 3'-MeO), 11.05 and 11.62 (br s, 8- and 8'-NOH) and 8.19 and 8.16 (br t, $J_{HN,10}$ 6.0, 9a-H; and br t, $J_{HN,20}$ 6.0, 9'a-H); *m/z* (3-nitrobenzyl alcohol, FAB) 1082.8 (MH^+, 1.0% as the centre of a cluster of ions).

Alkaline Treatment of 19-*Deoxyfistularin* 3 **8**.—On treatment as above for compound **7**, followed by purification *via* RP-18 HPLC with MeCN–water (3:2), compound **12** (t_R 22.4 min) was obtained in 72% yield as a powder, m.p. 114–118 °C (from MeOH); $[\alpha]_D^{20}$ +2.2, $[\alpha]_{365}^{20}$ +17.2 (*c* 0.18, acetone); δ_C 151.16 and 151.12 (s, C-1 and -1'), 106.64 (s, C-2 and -2'), 154.71 (s, C-3 and -3'), 108.78 (s, C-4 and -4'), 134.93 (d, C-5 and -5'), 121.84 (s, C-6 and -6'), 25.67 (t, C-7 and -7'), 154.61 (s, C-8 and -8'), 166.60 (s, C-9 and -9'), 43.60 (t, C-10), 69.18 (d, C-11), 75.47 (t, C-12), 151.16 (s, C-13), 118.26 (s, C-14 and -18), 134.04 (d, C-15 and -17), 139.52 (s, C-16), 34.34 (t, C-19), 41.27 (t, C-20) and 60.52 (q, MeO); δ_H 7.58 and 7.60 (s, 5- and 5'-H), 3.82 (s, 7- and 7'-H_2), 3.55 and 3.75 (m, 10-H_2), 4.25 (m, 11-H), 4.00 (m, 12-H_2), 7.50 (s, 15- and 17-H), 2.89 (t, $J_{19,20}$ 6.9, 19-H_2), 3.62 (td, $J_{20,19}$ 6.9, $J_{20,HN}$ 6.0, 20-H_2), 3.79 (s, 3-and 3'-OMe), 11.69 and 10.63 (br s, 8- and 8'-NOH) and 8.18 and 7.99 (br t, $J_{20,HN\text{-}9'a}$ 6.0 and br t, $J_{10,HN\text{-}9a}$ 6.0, 9a- and 9'a-H); *m/z* (3-nitrobenzyl alcohol, FAB) 1098.9 (MH^+, as centre of a cluster of ions).

Alkaline Treatment of 19-*Deoxy*-11-*oxofistularin* 3 **9**.—19-Deoxy-11-oxofistularin 3 **9** (6 mg, 0.005 mmol), on treatment identical with that for compound **6** above, followed by RP-8 HPLC with MeCN–water (3:2), gave compound **14** (t_R 8.9 min) and oxime (*E*)-**15** (t_R 12.2 min). In another experiment, compound **9** was stirred in 3% methanolic KOH at reflux for 2 h, the mixture was then neutralized, evaporated, and extracted (EtOH) and the residue obtained on evaporation was subjected to HPLC under the same conditions as above to give oxime (*Z*)-**15** (t_R 11.0 min) and oxime (*E*)-**15** in 1:5 molar ratio.

Data of compound **14**: δ_H 7.60 (s, 3- and 5-H), 2.90 (t, $J_{7,8}$ 7.0, 7-H_2), 3.40 (m, 8-H_2), 3.83 and 3.16 (2 d, J_{gem} 18.0, 11-H_2), 6.53 (br s, 13-H), 4.16 (br d, $J_{17,OH}$ 8.0, 17-H), 8.12 (br s, 1-OH), 7.66 (br t, $J_{HN,8}$ 6.0, 8a-H), 3.73 (s, MeO) and 5.45 (d, $J_{OH,17}$ 8.0, 17-OH).

Data of oxime (*E*)-**15** [data for (*Z*)-**15** within square brackets]: δ_C 149.98 (s, C-1), 111.32 (s, C-2 and -6), 133.41 (d, C-3 and -5), 134.53 (s, C-4), 34.14 (t, C-7), 41.58 (t, C-8), 166.59 (s, C-9), 154.40 (s, C-10), 25.71 (t, C-11), 121.86 (s, C-12), 134.99 (d, C-13), 108.83 (s, C-14), 154.39 (s, C-15), 106.70 (s, C-16), 151.21 (s, C-17) and 60.54 (q, MeO); δ_H 7.41 [7.41] (s, 3- and 5-H), 2.82 [2.80] (t, $J_{7,8}$ 6.9, 7-H_2), 3.56 [3.52] (q, $J_{8,7} \approx J_{8,HN}$ = 6.9,

8-H_2), 3.80 [3.78] (s, 11-H_2), 7.53 [7.45] (s, 13-H), 8.02 [7.87] (m, HN) and 3.79 [3.77] (s, MeO); *m/z* (glycerol–thioglycerol, FAB) 660.7 [660.8] (MH^+, 8 [1]).

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