Negative chemical ionization (NCI) mass spectral analysis with CF_2Cl_2 as a reagent gas[14-16] provided additional details about the structure of the toxin. The NCl mass spectra of toxins I, II and III (table) were almost identical except for relative intensities of the peaks. Fragment-Cl-adduct ions in the spectrum corresponded to a sequential loss of 4 monosaccharide units from the molecular ion. The loss of 236 mass units from the ion at m/e 595 was attributed to the elimination of an aglycone moiety. Except for the shift in mass due to the aglycone, the fragmentation pattern of the toxin was similar to that obtained for di- and trisaccharides analyzed under similar conditions; in every case Cl-adducts of fragments corresponded to losses of monosaccharides or monosaccharides minus water.

Analysis of the hydrolysis products of the toxin (0.05 M trifluoroacetic acid at 90 °C for 1 h) indicated the presence of only 1 monosaccharide that cochromatographed in GLC (after silylation) and TLC with galactose. The identity of the aglycone(s) is not known, but the electron impact (EI) mass spectrometry provided some clues. In the EI mode, the mass spectra of the 3 isomeric toxins showed an ion at m/e 218 with further ions in the lower mass region. The ion at m/e 218 apparently originated from the aglycone moiety, 236, with a loss of 18 mass units (H_2O). From high resolution mass measurement of the ion at m/e 218 of toxin isomer II an empirical formula $C_{15}H_{24}O_2$ for the aglycone moiety was calculated.

From these data it can be concluded that the 3 toxins have the composition $C_{39}H_{64}O_{22}$, corresponding to a 4-fold galactosidation of a $C_{15}H_{24}O_2$ aglycone. Extended ^1H- and ^{13}C-NMR investigations, to be reported in detail elsewhere, confirm this conclusion and reveal in addition that the 3 isomers differ only in the position of 1 double bond in the aglycone component.

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Polyacetylenes from the sponge *Petrosia ficiformis* found in dark caves[1]

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Summary. Several high molecular weight polyacetylenes have been isolated from the sponge *Petrosia ficiformis* found in dark caves. These compounds are related to, but different from, the polyacetylenes isolated from the same sponge living in its usual habitat.

Usually the mediterranean sponge *Petrosia ficiformis* displays a red-brown colour due to the presence of the symbiotic alga *Aphanocapsa feldmanni*[3]; however *P.ficiformis* found in dark caves lacks this symbiotic alga and therefore appears white. In the course of a study[4] on the secondary metabolites of the sponge *P.ficiformis* (red-brown) and of its predator, the nudibranch *Peltodoris atromaculata*, we have recently isolated[5] the high molecular weight polyacetylene mixtures **1** and **2** from both invertebrates.

We wish to report now the isolation of related compounds from *P.ficiformis* (white) which also provokes a positive food response by the nudibranch[4].

The ether-soluble fraction from the acetone extracts of the sponge was separated into 2 main fractions containing mixtures of acetylenic compounds, by chromatography on silica gel. The less polar mixture was further divided into 2 fractions (**a** and **b**) by preparative HPLC (μBondapak C_{18}; $CH_3OH \cdot H_2O$, 9:1).

Fraction a (0.033% dry weight of the sponge). Inspection of

$$HC{\equiv}C{-}\underset{\displaystyle OH}{\underset{|}{CH}}{-}CH{=}CH{-}CH_2{-}R^1{-}CH_2{-}CH{=}CH{-}C{\equiv}C{-}\underset{\displaystyle OH}{\underset{|}{CH}}{-}C{\equiv}C{-}CH_2{-}R^2{-}CH_2{-}CH{=}CH{-}\underset{\displaystyle OH}{\underset{|}{CH}}{-}C{\equiv}CH$$

1, $R^1+R^2=C_nH_{2n-6}$; n = 25, 28 **2**, $R^1+R^2=C_nH_{2n-4}$; n = 28, 31, 34

the NMR-data exhibited by fraction **a** allowed the conclusion that the main structural features of this fraction[6] are similar to those of the compounds **1** and **2**[5]. In addition the presence in the molecule of a terminal cis-enyne was evident (partial structure I).

$$\overset{5}{C}H_2-\overset{4}{C}H=\overset{c\ 3}{C}H-\overset{2}{C}\equiv\overset{1}{C}H$$

I

Partial structure I displays: PMR δ 3.04 (H-1, d, J 2Hz), 5.45 (H-3, dd, J 10 and 2Hz), 6.0 (H-4, dt, J 10 and 6Hz); CMR δ 81.1 (C-1), 107.9 (C-3), 146.1 (C-4), 30.2 (C-5).

Thus fraction **a** differs from the previously isolated compounds **1** and **2** in having an enyne function at the end of the molecule.

In addition in the PMR- and CMR-spectra of this fraction there are signals for 2 isolated double bonds (PMR δ 5.36, 4H; CMR δ 128.1, 129.6, 130.1, 130.8) whose stereochemistry must be cis on the same grounds as for the isolated double bonds of **1** and **2**[5].

As it was impossible to get interpretable mass spectra of these molecules, the molecular weights were determined by reduction[5] to the saturated straight chain hydrocarbons $C_{46}H_{94}$ (20%) and $C_{49}H_{100}$ (80%), identified by GLC data, thus establishing that fraction **a** is constituted of homologues of general formula **3** having n = 26 and 29 respectively.

On oxidation with MnO_2[5], fraction **a** yielded the corresponding diketoderivative **4** which displays spectral properties similar to those of the oxidation products of **1** and **2**[5].

From these data the general formula **3** can be inferred for fraction **a**[7].

The more polar mixture of acetylenic acid compounds was directly treated with CH_2N_2 and the resulting esters purified on silica gel.

The presence of a terminal cis-enyne was inferred from PMR (δ 3.04, d, J 2Hz; 5.40, dd, J 10 and 2Hz; 5.97, dt, J 10 and 7Hz), CMR (δ 81.1, 107.9, 146.1, 30.2) and IR (3290 cm^{-1}) data. In addition the molecules contain a methyl ester conjugated with an acetylene function (CMR δ 19.0, $CH_2-C\equiv C-COOCH_3$ and 52.4 $-COOCH_3$; PMR δ 2.30 and 3.72 respectively; IR 2200 and 1690 cm^{-1}).

The UV spectrum (λ_{max} 212_{infl}, 220 and 230_{infl} nm) is in agreement with the presence of both the enyne and the conjugated carboxylic ester functions in the molecule.

Finally the presence of a cis[5] isolated double bond was evident (PMR δ 5.32, 2H; CMR $\delta \sim 129.9$, olefinic carbons; 27.6 and 27.2, allylic carbons).

The mass spectrum of the mixture gives 2 molecular ions at m/z 412 and 384 indicating that the mixture was constituted of 2 compounds (**7** and **8**).

$$HC\equiv C-CH\overset{c}{=}CH-CH_2-R-CH_2-C\equiv C-COOCH_3$$

$R = C_nH_{2n-2}$

7, n = 16

8, n = 18

Catalytic reduction (H_2, Pd/C) afforded the mixture of the 2 corresponding straight chain fatty acid methyl esters (C-25 and C-27; circa 2:1) identified by GLC (SE-30 3% on Gas-chrom Q, 100–120 mesh, 192 °C).

It is worth noting that the same sponge living in different habitats contains similar acetylenic compounds but not the same ones. The polyacetylenes isolated from the specimens living in the dark are absent in the sponge living in its usual habitat and vice versa. However, among the secondary metabolites this diversity is restricted to the polyacetylenes. All the specimens examined contain the same sterolic pattern, including the unusual sterol petrosterol[9], which is the major component.

$$HC\equiv C-\underset{OH}{CH}-CH=\overset{t}{C}H-CH_2-R^1-CH_2-CH=\overset{t}{C}H-C\equiv C-\underset{OH}{CH}-C\equiv C-CH_2-R^2-CH_2-CH=\overset{c}{C}H-C\equiv CH$$

3

$$HC\equiv C-\underset{O}{\overset{\|}{C}}-CH=CH-CH_2-R^1-CH_2-CH=CH-C\equiv C-\underset{O}{\overset{\|}{C}}-C\equiv C-CH_2-R^2-CH_2-CH=CH-C\equiv CH$$

4

3,4, $R^1 + R^2 = C_nH_{2n-4}$; n = 26, 29

A confirmation of the relative arrangement of the functions as in **3** and **4** came from an oxidation experiment on fraction **a** which accidentally[8] produced in good yield the monoketoderivative **5**, PMR δ 2.55 (H-1, d, J 2Hz), 4.82 (H-2, bd, J 5Hz), 5.58 (H-3, dd, J 15 and 5Hz), 5.90 (H-4, dt, J 15 and 6Hz), 6.54 (H-5, dt, J 15 and 6Hz), 5.60 (H-6, d, J 15Hz) and 3.04 (H-7, d, J 2Hz); ν_{max}1610 cm^{-1}; λ_{max}224, 230, 286 and 298 nm.

$$\overset{1}{HC}\equiv\overset{2}{C}-\underset{OH}{\overset{3}{CH}}-\overset{4}{CH}=CH-CH_2-R^1-CH_2-\overset{5}{CH}=\overset{6}{CH}-C\equiv C-\underset{O}{\overset{\|}{C}}-C\equiv C-CH_2-R^2-CH_2-CH=CH-C\equiv\overset{7}{C}H$$

5, $R^1 + R^2 = C_nH_{2n-4}$; n = 26, 29

Fraction ***b*** (0.07% dry weight of the sponge) differs from fraction **a** in having at the end of the molecule an acetylenic instead of an enynic function (CMR δ 68.1, $-CH_2-C\equiv CH$; 18.4, $-CH_2-C\equiv CH$); the remaining structural features are the same as those of fraction **a**. On reduction, the mixture of straight chain hydrocarbons $C_{46}H_{94}$ (5%), $C_{49}H_{100}$ (43%), $C_{52}H_{106}$ (52%) was obtained. The general formula for fraction **b** is therefore **6**[7].

$$HC\equiv C-\underset{OH}{CH}-CH=\overset{t}{C}H-CH_2-R^1-CH_2-CH=\overset{t}{C}H-C\equiv C-\underset{OH}{CH}-C\equiv C-CH_2-R^2-CH_2-C\equiv CH$$

6, $R^1 + R^2 = C_nH_{2n-4}$; n = 28, 31, 34

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$CH_2^{1}-CH^{2}=CH^{3}-C^{4}\equiv C^{5}-\underset{OH}{C^{6}H}-C^{7}\equiv C^{8}-CH_2^{9}$: PMR δ 6.20 (H-2, dt, J 15 and 6 Hz), 5.40 (H-3, dd, J 15 and 2 Hz), 5.20 (H-6, bs); CMR δ 33.2 (C-1), 145.5 (C-2), 109.0 (C-3), 52.8 (C-6), 18.7 (C-9).

7 Obviously the position of the terminal functions can be reversed.

8 In several runs fraction **a** yielded the expected diketoderivative **4**. In one experiment only the central alcohol function was oxidated, probably in consequence of a fortuitous poisoning of the catalyst.

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The structures of minor congeners of detoxin complex, the selective antagonist of blasticidin S[1]

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Summary. The structures of the minor congeners of detoxin complex, viz., detoxins E_1, C_1, C_2, C_3, B_1, B_3 and A_1 have been established on the basis of spectral and degradative evidence.

Detoxin complex[2], a group of metabolites produced by *Streptomyces caespitosus* var. *detoxicus* 7072 GC_1, is a selective antagonist of blasticidin S[3]. A noticeable feature of its biological activity is that the complex brings about remarkable detoxification of blasticidin S both in animal and plant cells. In the light of this interesting biological activity, the structure-activity relationship of detoxin compounds is of great interest and structural studies of the minor congeners in it have been undertaken.

Earlier chemical studies on the detoxin complex revealed that it comprises a number of closely related active principles[4], and hitherto the structures of detoxin D_1 (**1**)[5] have been established, as a new class of depsipeptide consisting of L-valine, detoxinine[6], L-phenylalanine and (S)-(+)-2-methylbutyric acid. The structures of minor components of the detoxin D group have also been established by the GC-MS procedure[7].

In this report, we describe the structural elucidation of 7 congeners of the detoxin complex, viz., detoxins E_1(**2**), C_1(**3**), C_2(**4**), C_3(**5**), B_1(**6**), B_3(**7**) and A_1(**8**).

The separation and isolation of individual compounds was accomplished by a combination of chromatographic methods, including the use of ion exchange resin (Dowex 50WX2, pyridine-AcOH type), silica gel (Wako gel, n-BuOH saturated with H_2O) and Sephadex G-10 and LH-20; as a result, 60 mg of **2**, 60 mg of **3**, 3 mg of **4**, 80 mg of **5**, 30 mg of **6**, 3 mg of **7** and 20 mg of **8** were isolated in pure form from 2 tons of the culture filtrates.

The structural elucidation was carried out by, a) degradative studies (table 1), b) comparison of the ^{13}C-NMR-spectra of these minor components with those of **1**[8] and valyl-detoxinine (**9**)[5] (table 2), and c) mass spectral analyses of the corresponding N-acetylmethyl ester derivatives (table 3).

Since the ^{13}C-NMR-spectra of **4** and **7** could not be obtained due to the small amounts of sample available, the structures of these 2 congeners were established exclusively by mass spectral evidence. The molecular fomulae of these congeners are summarized in table 1.

The structures of detoxin E_1(2) and detoxin C group (3-5).

Table 1. Molecular formulae and structural components of detoxins

	Molecular formulae[a]	Acid hydrolysate[b] Amino acid	Fatty acid
Detoxin E_1(**2**)	$C_{29}H_{43}N_3O_8$	Isoleucine Detoxinine Phenylalanine	Acetic acid 2-Methyl-butyric acid
Detoxin C_1(**3**)	$C_{25}H_{35}N_3O_8$	Valine Detoxinine Phenylalanine	Acetic acid
Detoxin C_2(**4**)	$C_{26}H_{37}N_3O_8$	Valine Detoxinine Phenylalanine	Acetic acid Propionic acid
Detoxin C_3(**5**)	$C_{27}H_{39}N_3O_8$	Valine Detoxinine Phenylalanine	Acetic acid Isobutyric acid
Detoxin B_1(**6**)	$C_{23}H_{33}N_3O_6$	Valine Deoxydetoxinine Phenylalanine	Acetic acid
Detoxin B_3(**7**)	$C_{25}H_{37}N_3O_6$	Valine Deoxydetoxinine Phenylalanine	Isobutyric acid
Detoxin A_1(**8**)	$C_{14}H_{24}N_2O_6$	Valine Detoxinine	Acetic acid

[a]Molecular formulae of detoxins were determined by the high resolution mass spectrometry of the corresponding N-acetylmethyl esters of these congeners. [b]Amino acids and fatty acids were identified by TLC (BuOH:AcOH:H_2O = 4:1:2) or amino acid analysis, and GLC, respectively.