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6 $HC^{1}\equiv C^{2}-\underset{OH}{C^{3}H}-CH^{4}=CH^{5}-CH_2^{6}$: PMR δ 2.56 (H-1, d, J 2 Hz), 4.84 (H-3, bd, J 5 Hz), 5.62 (H-4, dd, J 15 and 5 Hz), 5.90 (H-5, dt, J 15 and 6 Hz); CMR δ 73.9 (C-1), 62.7 (C-3), 134.0 (C-4), 128.7 (C-5), 31.8 (C-6).

$CH_2^{1}-CH^{2}=CH^{3}-C^{4}\equiv C^{5}-\underset{OH}{C^{6}H}-C^{7}\equiv C^{8}-CH_2^{9}$: 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.

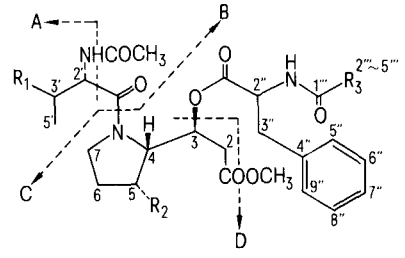
Table 2. Assignment of ^{13}C resonances of the detoxin congeners (ppm)

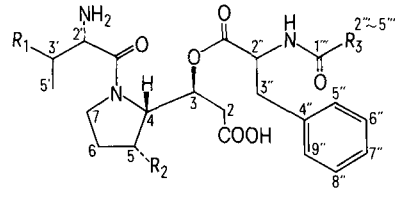
Carbons[a]	Detoxins D_1(1)	E_1(2)	C_1(3)	C_3(5)	B_1(6)	A_1(8)[c]	Valyldetoxinine (9)[c]
C-2	39.5	38.4	40.0	39.9	38.1	43.5	43.8
C-3[b]	72.1	72.4	72.1	72.0	75.1	68.3	68.5
C-4	57.5	57.0	57.6	57.5	51.5	57.5	57.4
C-5[b]	72.0	71.9	72.6	72.3	27.4	72.6	70.3
C-6	30.9	30.6	31.0	30.6	23.2	31.0	32.0
C-7	46.0	46.1	46.1	46.0	46.4	46.1	46.3
C-9	21.0	21.0	20.9	20.9		21.0	
C-2′	59.4	59.3	59.3	59.3	58.7	61.2	62.9
C-3′	30.9	37.9	31.0	31.0	31.1	30.3	30.2
C-4′	17.4	25.0	17.5	17.4	18.1	17.6	17.1
C-5′	19.4	16.0	19.5	19.9	19.9	19.5	19.4
C-6′		12.1					
C-2″	58.4	54.7	55.3	54.2	55.8		
C-3″	38.0	37.9	38.0	38.0	38.4		
C-4″	138.2	138.0	138.2	138.2	138.1		
C-5″(9″)	130.1	130.0	130.1	130.6	130.0		
C-6″(8″)	129.3	129.2	129.4	129.3	129.4		
C-7″	127.6	127.5	127.7	127.6	127.7		
C-2‴	43.3	43.8	23.3	35.8	23.3		
C-3‴	27.9	28.0		19.5			
C-4‴	17.7	17.8		19.5			
C-5‴	12.8	12.4					

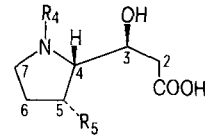
[a] Assignment of the carbonyl carbons could not be realized. [b] Assignment of this carbon pair is interchangeable. [c] Since the ^{13}C-NMR of detoxin A_1 and valyldetoxinine exhibit the doubling of resonances owing to syn-anti isomerism of amido bond, the comparison of the spectra was carried out for the major isomer i.e., signals having higher intensities.

Table 3. Diagonistic fragment ions in the mass spectra of N-acetylmethyl ester derivatives of detoxin congeners

	R_1	R_2	R_3	Fragment ions (m/z) A	B	C	D
N-Acetylmethyl ester of:							
D_1(14)	CH_3	$OCOCH_3$	$CH(CH_3)C_2H_5$	114	142	462	355
E_1(10)	C_2H_5	$OCOCH_3$	$CH(CH_3)C_2H_5$	128	156	462	369
C_1(11)	CH_3	$OCOCH_3$	CH_3	114	142	420	355
C_2(12)	CH_3	$OCOCH_3$	C_2H_5	114	142	434	355
C_3(13)	CH_3	$OCOCH_3$	$CH(CH_3)_2$	114	142	448	355
B_1(15)	CH_3	H	CH_3	114	142	362	297
B_3(16)	CH_3	H	$CH(CH_3)_2$	114	142	390	297







		R_1	R_2	R_3
Detoxin	D_1 (1)	CH_3	$OCOCH_3$	$CH(CH_3)C_2H_5$
	E_1 (2)	C_2H_5	$OCOCH_3$	$CH(CH_3)C_2H_5$
	C_1 (3)	CH_3	$OCOCH_3$	CH_3
	C_2 (4)	CH_3	$OCOCH_3$	C_2H_5
	C_3 (5)	CH_3	$OCOCH_3$	$CH(CH_3)_2$
	B_1 (6)	CH_3	H	CH_3
	B_3 (7)	CH_3	H	$CH(CH_3)_2$

	R_4	R_5
Detoxin A_1 (8)	Valyl	$OCOCH_3$
Valyldetoxinine (9)	Valyl	H
Detoxinine	H	H

The structures of detoxin congeners.

The structures of **2**, **3**, **4** and **5** were established by comparison of their ^{13}C-NMR-spectra with that of **1** as well as by the mass spectral evidence for the corresponding N-acetylmethyl ester derivatives (**10**, **11**, **12** and **13**, respectively).

For example, the structure of **2** was established as follows: comparison of the ^{13}C-NMR-spectra of **2** and **1** showed almost superimposable identity as shown in table 2, except for the signals due to the valine residue which is replaced by isoleucine in **2**; this result is strongly supported by the mass spectral evidence, i.e., the fragment ions A, B and D in the mass spectrum of **10** showed a shift by 14 mass unit compared with those of the N-acetylmethyl ester of detoxin D_1 (**14**) respectively.

Similarly, the structural differences within the detoxin C group were shown to be located at the fatty acids, hence the structures of **3**, **4** and **5**, were thus established as shown in the figure.

The structures of detoxin B_1(6) and B_3(7). The structural alteration of the detoxin B group from the known congeners was ascribed to the presence of a new amino acid in place of detoxinine. Acid hydrolysis of detoxins B_1(**6**) and

B_3(7) yielded a new spot which showed a slightly higher R_f-value than that of detoxinine. The ^{13}C-NMR-spectrum of **6** showed a close similarity to that of **3** except for the new appearance of a methylenic carbon at δ27.4 ppm in the former; this is consistent with the mass spectral data for the N-acetylmethyl ester of 6(**15**), viz., the fragment ions derived from C and D in the mass spectrum of **15** showed a shift by 58 mass units compared with those of **11**. Based on these results, the structure of **6** was established as a new congener, having the deoxydetoxinine nucleus, in the detoxin family.

Next, the fragmentation pattern in the mass spectrum of the N-acetylmethyl ester of detoxin B_3(**16**) showed a close resemblance to that of **15**, the difference being a shift by 28 mass units (ion C) in the latter. This result, along with the detection of isobutyric acid in the acid hydrolysate, gave the evidence that the structure of **7** is as depicted in the figure.

*The structure of detoxin A_1(**8**).* Comparison of the ^{13}C-NMR-spectra of **8** and valyldetoxinine (**9**) showed that they had a close resemblance to each other except for 2 signals assigned to C_5 and C_6 (table 2), respectively. In addition, the peracetate of **8** which is identical to that of **9** indicated that **8** is the C_5 monoacetate of **9** on the basis of the ^{13}C-NMR-data, viz., an acyl shift (about 2.3 ppm) in C_5 is associated with a β-shift (about 1.0 ppm upfield) of C_6 in **8**. Accordingly, the structure of **8** was determined as shown in the figure.

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HPLC of cephalosporins and their oxa-derivatives

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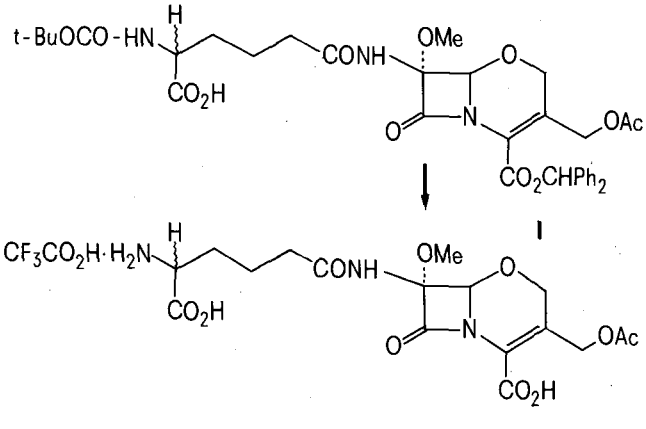
Summary. The presence of the oxygen atom in place of the sulfur atom has significant impact on the polarity of the β-lactam derivative. This has been illustrated by direct comparison of HPLC data of 4 different cephalosporin derivatives and their oxa analogues.

In connection with our investigations[3] on different metabolites in fermentation broths we were interested in differences in retention times between cephalosporins and their oxa-derivatives[4]. The oxa-β-lactam derivatives were provided as fully blocked compounds[5] and were hydrolyzed to the zwitterionic species prior to the chromatography. This procedure was carried out on small aliquots of the samples and reactions were done in flamed glassware under a nitrogen atmosphere. The following example illustrates the process of deblocking: 16 mg of N-tert-butyloxy-carbonyl-4-benzhydro-derivative **I** (mol. wt 695.7) (0.023 mmole) were added to 0.15 ml anisole in an ice bath.



Trifluoroacetic acid was chilled and 0.45 ml was slowly added to the reaction vessel. The reaction proceeded for 25 min and was confirmed to be complete by TLC on silica developed with 5% acetic acid in ethyl acetate. The reaction mixture was evaporated on a Büchi rotoevaporator, triturated with ether (2-3 ml), filtered and washed with hexane. The product was then dried in high vacuum over P_2O_5/KOH for 1.5 h. The yield of the TFA salt was 10 mg (82% theory). We have chosen 2 well-characterized chromatographic systems to compare these nuclear isomers, and these data are summarized in figures 1 and 2. The 1st system was a reversed phase paired with ion mobile phase on microBondapak C18 (Waters) (fig. 1). The solvent mixture used was prepared by mixing an aqueous solution of 0.005 M tetrabutyl ammonium hydroxide, 1% ammonium phosphate at pH 7 and 1% acetonitrile. The use of the column 4×300 mm with a flow rate of 2 ml/min led to a pressure of 2000 psi. The 2nd system consisted of an ion-exchange stationary phase, microBondapak NH_2 with a mobile phase of acetic acid, methanol, acetonitrile, and water (2/4/7.5/86.5) (fig.2). Using a column 4×300 mm with a flow rate of 3 ml/min created a pressure of 3000 psi. All chromatograms were obtained using Waters M6000A pump, U6K septumless injector (Waters Assoc., Milford, Mass.) with Schoeffel Model 770 UV detector (Schoeffel Inst., Westwood, N.J.) at 254 mm (0.2 Aufs.) and Fisher omniscribe recorder (Fisher Scientific, Cincinnati, Ohio). Solvents used were all of the spectral purity type; solvents