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ISOLATION AND STRUCTURE ELUCIDATION OF AMPICILLIN AND AMOXICILLIN OLIGOMERS

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SUMMARY

Oligomers of ampicillin and amoxicillin were isolated by preparative chromatography on DEAE-Sephadex and purified by chromatography on Amberlite XAD-2. The purity of the oligomers was checked by thin-layer chromatography and by high-performance liquid chromatography. Proton magnetic resonance and fast atom bombardment mass spectrometry showed that ampicillin yielded mainly the dimer, trimer and tetramer, and amoxicillin the dimer, trimer and some tetramer. The corresponding penicilloates were also present. For amoxicillin an important amount of the piperazinedione was formed simultaneously.

INTRODUCTION

The preparation of ampicillin oligomers with a structure as shown in Fig. 1 was first described by Grant *et al.*[1]. The products formed in these polycondensation reactions are also called polymers. However, as the number of units is lower than ten, we prefer the name oligomers. Several authors have since described the isolation by chromatography on Sephadex G-25 of fractions containing oligomers[2–6]. The fractions contained mixtures of oligomers and the structure was therefore not elucidated, although that represented in Fig. 1 was generally assumed. The dimerization of ampicillin, amoxicillin, epicillin and cyclacillin in solution was studied in detail by Bundgaard[7–9]. Pure oligomers of ampicillin were first isolated by Bundgaard and Larsen[10]. After chromatography of a concentrated solution of ampicillin on DEAE-Sephadex

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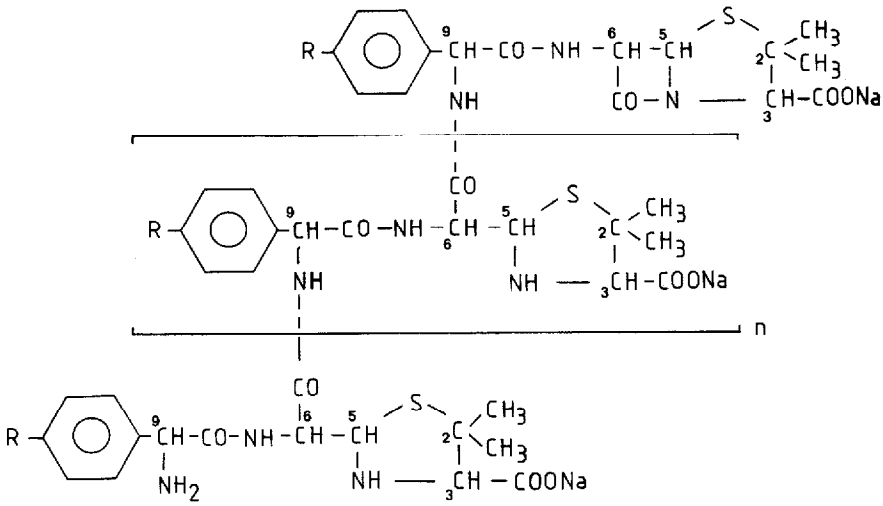

Fig. 1. Structure of oligomers of ampicillin (R = H) and amoxicillin (R = OH). Dimer, $n = 0$; trimer, $n = 1$; tetramer, $n = 2$.

A-25, three oligomers were isolated and characterized as the dimer, tetramer and hexamer (Fig. 1). Oligomers with an uneven number of ampicillin units were not isolated but were found to be present in minor amounts. The same authors described the determination of the oligomers in bulk samples of sodium ampicillin by high-performance liquid chromatography (HPLC)[11].

The rate of formation of ampicillin oligomers in solutions for parenteral use has also been briefly studied[12]. HPLC of ampicillin oligomers on TSK-Gel G 2000 SW has been described, but the separations achieved were not as good[13]. Thin-layer chromatography (TLC) of ampicillin oligomers has been reported[14].

The isolation of amoxicillin dimer (Fig. 1) was cited by De Angeli *et al.*[15]. Higher oligomers were not isolated. More recently, mixtures of oligomers have been isolated by chromatography on Sephadex G-25. The presence of dimers, trimers and tetramers was assumed from molecular weight determinations[16].

In this paper we report on the isolation, purification and structure determination of sodium salts of oligomers of ampicillin and amoxicillin.

EXPERIMENTAL

Apparatus

Preparative chromatography was carried out with a peristaltic pump (Pharmacia, Uppsala, Sweden) or with a Milton Roy Minipump (Laboratory Data Control, Riviera Beach, FL, U.S.A.) and the elution was followed with an Altex 150 B UV detector equipped with a 200-μl flow cell with low back pressure (Altex, Berkeley, CA, U.S.A.). HPLC chromatograms were obtained on a Varian LC 4200 liquid chromatograph (Varian, Palo Alto, CA, U.S.A.), equipped with a Waters U6K injector (Waters, Milford, MA, U.S.A.), a Pye Unicam LC3UV detector (Pye Unicam, Cambridge, U.K.) and a Kipp & Zonen BD40 recorder (Kipp & Zonen, Delft, The Netherlands).

UV spectra were obtained on a Beckman Model 25 spectrophotometer (Beckman, Fullerton, CA, U.S.A.), IR spectra on a Perkin-Elmer 197 spectrophotometer (Perkin-Elmer, Norwalk, CT, U.S.A.), ^{1}H NMR spectra on a Jeol FX-90 spectrometer (Jeol, Tokyo, Japan) or a Varian XL-200 spectrometer and electron impact mass spectra on an AEI MS-12 apparatus (AEI, Manchester, U.K.). Fast atom bombardment mass spectra were obtained on a Kratos MS-50 instrument (Kratos, Manchester, U.K.) fitted with an Ion Tech fast atom gun (Ion Tech, Teddington, U.K.) and a high field magnet. Samples of oligomers dissolved in glycerol were applied to the copper probe tip and bombarded with 6-keV xenon atoms. All evaporations were carried out on a rotary evaporator, *in vacuo* and at room temperature (Büchi, Flawil, Switzerland) using a cooler at −30°C (Lauda, Königshofen, F.R.G.).

Chemicals

Sodium ampicillin was obtained from Bristol-Myers (Syracuse, NY, U.S.A.) and sodium amoxicillin from Beecham (Heppignies, Belgium). DEAE-Sephadex A-25 (40–120 μm) was purchased from Pharmacia and Amberlite XAD-2 (300–1000 μm) from Serva (Heidelberg, F.R.G.). Organic solvents were of reagent grade (Janssen Chimica, Beerse, Belgium) and were distilled before use. Water was twice distilled. Other reagents were of pro analysi quality (Merck, Darmstadt, F.R.G.).

Chromatographic methods

Thin-layer chromatography (TLC) was performed on ready made silica gel plates, *viz.*, Stratochrom SiF254 (Carlo Erba, Milan, Italy) with ethyl acetate–acetic acid–water (7:2:1, v/v) (ampicillin) or (3:1:1, v/v) (amoxicillin) as the mobile phase, using iodine vapour for detection. HPLC was performed on a 25 × 0.46 cm I.D. column kept at 30°C and packed with Zorbax C_8, 7-μm (Du Pont, Wilmington, DE, U.S.A.). Gradient elution was performed with mobile phases A and B, comprising methanol–0.2 *M* potassium phosphate buffer pH 7.0–water (5:5:90, v/v) for A and (50:5:45, v/v) for B. For ampicillin (detection at 254 nm; 0.08 a.u.f.s.) the gradient elution was as follows: 40% of mobile phase B for 5 min, increased at a rate of 2% of B per min to 80% of B, held at 80% of B for 20 min, decreased at a rate of 8% of B per min to 40% of B. For amoxicillin (detection at 274 nm; 0.08 a.u.f.s.) the gradient elution was as follows: 5% of mobile phase B for 5 min, increased at a rate of 2% of B per min to 65% of B, held at 65% of B for 30 min, decreased at a rate of 8% of B per min to 5% of B.

Preparation and isolation of crude ampicillin oligomers

The oligomers were prepared in essentially the same way as described before[10]. A 10-ml portion of a 3-day old 20% (w/v) solution of sodium ampicillin, adjusted to pH 8.5, was chromatographed on a 45 × 3 cm I.D. column of DEAE-Sephadex A-25. The flow-rate was about 60 ml/h. The gradient and the elution pattern were as before[10]. The fractions from peaks corresponding to the oligomers with an intact β-lactam were collected. Each of these peaks was followed by a smaller one, containing the corresponding penicilloic acids or oligomeroic acids, *i.e.*, the oligomers with an opened β-lactam ring. No attempt was made to isolate these latter compounds. For the sake of clarity, the eluted oligomers with intact β-lactam rings will be referred to as dimer, trimer or tetramer, to correspond with their structures as

shown in this study, and not dimer, tetramer and hexamer as was proposed before[10].

Ampicillin dimer. The pooled fractions were cooled in ice and neutralized to pH 5.7 with cold 2 *M* hydrochloric acid. The solution was concentrated to about 15 ml, cooled in ice and acidified to pH 3.1 with cold 2 *M* hydrochloric acid. The precipitate was collected and washed with a small volume of cold water. It was suspended in 5 ml of cold water and dissolved by adding 1 *M* sodium hydroxide solution to a final pH of 6.8. The solution was evaporated to dryness. The residue was taken up in 20 ml of acetonitrile and evaporated again to dryness to give the crude dimer.

Ampicillin trimer. The pooled fractions were cooled in ice, 10 ml of ethyl acetate were added and the pH was brought to 3.0 with cold 2 *M* hydrochloric acid. The precipitate was treated further as described above for the dimer.

Ampicillin tetramer. The pooled fractions were treated as described for the trimer.

Purification of ampicillin oligomers

A glass column (40 × 2 cm I.D.) was filled with Amberlite XAD-2. The column was washed with dichloromethane, methanol and distilled water. The solvents used for elution were degassed by sonication. The flow-rate was about 3 ml/min. The crude ampicillin dimer was dissolved in 20 ml of water and the solution was applied to the column. Salt and polar impurities were washed from the column with water (about 200 ml). Ampicillin dimer was eluted with 95% ethanol–water (30:70, v/v) (about 150 ml). The fractions containing the pure dimer were evaporated to dryness, the residue was taken up in 5 ml of acetonitrile and evaporated again. The purified sodium ampicillin dimer was dried *in vacuo* over P_2O_5 (yield: about 0.30 g or 15%).

The crude ampicillin trimer was purified in an analogous way, using water and acetonitrile–water (20:80, v/v) as the mobile phases (yield: 0.15 g or 7.5%). The crude ampicillin tetramer was purified as described for the trimer, using water and acetonitrile–water (30:70, v/v) as the mobile phases (yield: 0.10 g or 5%).

Preparation and isolation of crude amoxicillin oligomers

Preparative chromatography on DEAE-Sephadex A-25 was performed as mentioned above for ampicillin, except that the solution to be chromatophed was

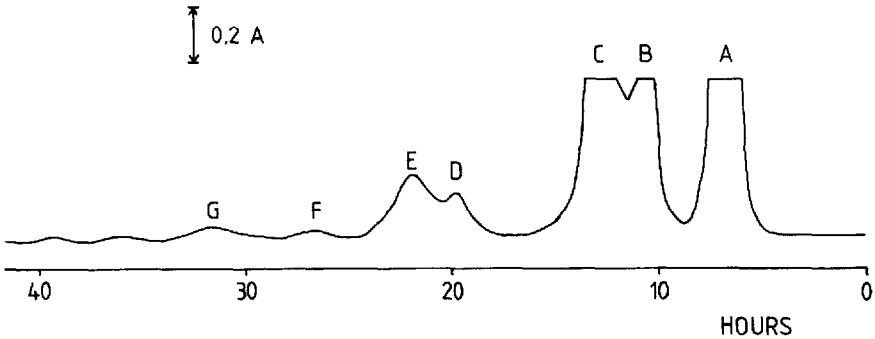

Fig. 2. Chromatogram of a 8-h old 20% (w/v) solution of sodium amoxicillin on DEAE-Sephadex A-25. See Experimental for chromatographic conditions. Peaks: A = amoxicillin and amoxicilloic acid; B = amoxicillin piperazine-2,5-dione; C = dimer and dimeroate (the penicilloate, with open β-lactam, corresponding to the dimer); D = trimeroate; E = trimer; F = possibly tetrameroate; G = possibly tetramer.

only 8 h old and that the mobile phase was at pH 7.7. The elution pattern is shown in Fig. 2.

Amoxicillin dimer. The fractions corresponding to the second half of peak C were pooled, neutralized to pH 6.0 with 2 *M* hydrochloric acid and concentrated to about 50 ml. Ethyl acetate (20 ml) was added and the solution was cooled in ice and acidified to pH 3.0 with cold 2 *M* hydrochloric acid. The precipitate was collected and washed with a small volume of cold water. It was suspended in 5 ml of cold water and dissolved by adding 1 *M* sodium hydroxide solution to a final pH of 6.8. The solution was evaporated to dryness. The residue was taken up in 20 ml of acetonitrile and evaporated again to dryness to give the crude dimer.

Amoxicillin trimer. The fractions corresponding to peak E were treated in a similar way as described for the dimer to give crude trimer.

Purification of amoxicillin oligomers

Preparative chromatography on Amberlite XAD-2 was performed in an analogous way as mentioned above for ampicillin. The crude amoxicillin dimer was purified with water (about 200 ml) and 95% ethanol–water (5:95) (about 200 ml) and (10:90) (about 250 ml) as the mobile phases. The pure product was further isolated in an analogous way as described for ampicillin dimer (yield: about 0.16 g or 8%). The crude amoxicillin trimer was purified as described for the dimer (yield: about 0.04 g or 2%).

Preparation of piperazine-2,5-dione derivatives of ampicillin and of amoxicillin

The ampicillin piperazine-2,5-dione (I) was prepared according to a literature method[17]. The amoxicillin derivative (II) was prepared in a similar way. Yield: about 50%. Structures are represented in Fig. 3. 2-[6′-(*p*-Hydroxyphenyl)piperazine-2′,5′-dion-3′-yl]-5,5-dimethylthiazoline-4-carboxylic acid (II), m.p. 218°C (with decomposition). IR (KBr):ν_{max} (cm^{-1}) at 3420, 3200–2900, 1735, 1660, 1610, 1595, 1518, 1450, 1270, 1195, 830. ^1H NMR [in $(C^2H_3)_2SO$, tetramethylsilane (TMS) as reference]; δ 1.20 (s, CH_3), 1.54 (s, CH_3), 3.55 (s, 4-H), 3.80 (br, 3′-H), 4.78 (br, 6′-H), 5.04 (d, J = 3.5 Hz, 2-H), 6.70, 7.04 (d, J = 8 Hz, *para*-substituted phenyl ring), 7.55 (br, 4′-H), 8.38 (br, 1′-H). On addition of ^2H_2O the signals at δ 7.55 and 8.38 ppm disappear. On irradiation of the signal at δ 8.38 ppm the signal at δ 4.78 ppm becomes a doublet, J = 1 Hz; on irradiation of the signal at δ 7.55 ppm the signal at 3.80 ppm becomes a doublet, J = 1.5 Hz. Mass spectrum: M^+ at m/z 393 (for the sample treated with CH_2N_2, giving the 3N-CH_3 and $COOCH_3$ derivatives).

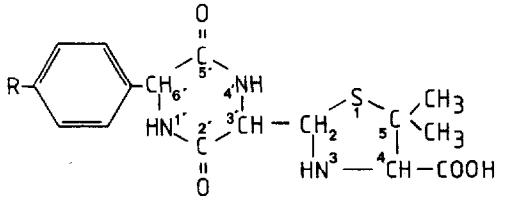

Fig. 3. Piperazine-2,5-dione derivative of ampicillin (I, R = H) and of amoxicillin (II, R = OH).

RESULTS AND DISCUSSION

The method described above for the isolation of ampicillin oligomers yields the sodium salts which were found to be more stable than the acids isolated in the method originally described by Bundgaard and Larsen[10]. The supplementary chromatography on XAD-2 eliminates residual salt and other polar impurities. The chromatography on DEAE-Sephadex was followed with HPLC and IR spectroscopy. The elution pattern of amoxicillin oligomers on DEAE-Sephadex differs from that obtained for ampicillin mainly in that the amoxicilloate and oligomeroates (penicilloates of the oligomers) are eluted right before, and not after, the amoxicillin and corresponding oligomers. This is illustrated in Fig. 2, which shows that the piperazine-2,5-dione II is an important product in amoxicillin oligomer production. During ampicillin polymerization only very small amounts of the corresponding piperazine-2,5-dione I are formed. The product isolated from peak B from the DEAE-Sephadex column proved to be identical with the piperazine-2,5-dione II obtained as described in Experimental.

Amoxicillin oligomers are much more soluble in water than the corresponding ampicillin derivatives and are therefore more difficult to isolate in good yield. Figs. 4 and 5 show chromatograms obtained with aged 20% (w/v) solutions of respectively sodium ampicillin and sodium amoxicillin. Fig. 4 corresponds well to a similar chromatogram published earlier by Larsen and Bundgaard[11]. The oligomeroates indicated in Figs. 4 and 5 were also prepared in solution by treating the corresponding pure polymer with dilute sodium hydroxide solution. The formation of several peaks was observed and the mixture became more complex upon standing. This can be explained by epimerization, mainly at C-5, and less at C-6 which has been reported before for penicilloates[18–21]. The figures show that amoxicillin polymerizes faster than ampicillin and also that amoxicillin gives appreciable amounts of piperazine-2,5-

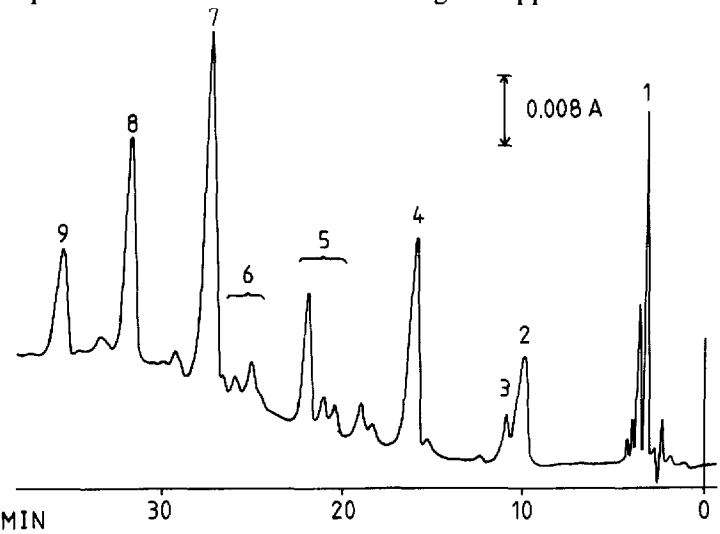

Fig. 4. HPLC chromatogram of an 8-days old 20% (w/v) solution of sodium ampicillin. Amount injected: 25 µl of a 1:20 dilution. See experimental for chromatographic conditions. Peaks: 1 = ampicilloates; 2 = dimeroates; 3 = piperazine-2,5-dione I; 4 = ampicillin; 5 = trimeroates; 6 = tetrameroates; 7 = dimer; 8 = trimer; 9 = tetramer.

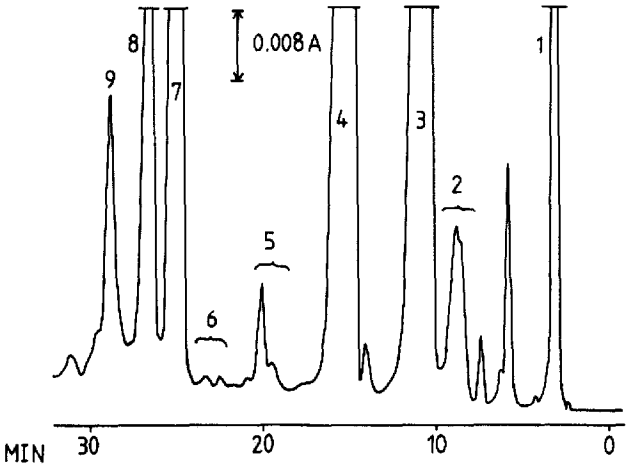

Fig. 5. HPLC chromatogram of a 6-h old 20% m/V solution of sodium amoxicillin. Amount injected: 25 μl of a 1:10 dilution. See experimental for chromatographic conditions. Peaks: 1 = amoxicilloates; 2 = dimeroates; 3 = amoxicillin; 4 = piperazine-2,5-dione II; 5 = trimeroates; 6 = possibly tetrameroates; 7 = dimer; 8 = trimer; 9 = possibly tetramer.

dione. Therefore it is advisable to use less aged amoxicillin solutions for polymer production. They also contain less of the higher oligomers, which, together with the higher solubility of the amoxicillin oligomers, explains why no amoxicillin tetramer was isolated.

The purity of the oligomers was checked by TLC and HPLC. The TLC results are summarized in Table I. The products showed only small secondary spots. The HPLC chromatograms of the purified oligomers are shown in Figs. 6 and 7.

TABLE I

TLC R_F VALUES OF AMPICILLIN AND AMOXICILLIN DERIVATIVES

Sample	*Ampicillin; mobile phase: ethyl acetate–acetic acid–water (7:2:1)*	*Amoxicillin; mobile phase: ethyl acetate–acetic acid–water (3:1:1)*
Monomer	0.32	0.39
Dimer	0.43	0.44
Trimer	0.56	0.46
Tetramer	0.59	0.48*
Piperazine-2,5-dione	0.82	0.81

* This oligomer was not isolated.

UV spectra of the oligomers were taken using water as the solvent. The piperazine-2,5-dione (Fig. 3, II) was dissolved in 0.01 *M* phosphate buffer pH 7.0. The spectra do not differ significantly from those obtained with the parent penicillin. Ampicillin and derivatives show weak maxima at 257, 262 and 269 nm. Amoxicillin and derivatives show a maximum at 274 nm.

TABLE II

PROTON MAGNETIC RESONANCE DATA (δ VALUES) FOR AMPICILLIN AND ITS OLIGOMERS

Solvent: ^2H_2O. Internal standard: sodium 3-(trimethylsilyl)-1-propanesulphonate (DSSA). Signal multiplicity: singlet (s), doublet (d), multiplet (m), AB spin system (AB). Coupling constants (J) are the measured peak spacings expressed in Hz. *a*, *b*, *c* and *d* indicate the polymer subunits; *a* always contains the β-lactam; the subunit mentioned last contains the free amino function.

	2-CH₃				*3-H*				*5- and 6-H*				*9-H*				*Phenyl*			
	a	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*				
Monomer	1.43 s 1.49 s				4.17 s				5.45 s				4.68 s				7.42 s			
Dimer	1.43 s 1.47 s	1.12 s 1.35 s			4.17 s	3.20 s			5.44 d $J = 4$ 5.48 d $J = 6$	4.52 4.86			5.46 s	5.14 s			7.43 s	7.51 s		
Trimer	1.43 s 1.47 s	1.18 s 1.47 s	1.12 s 1.36 s		4.16 s	3.34 s	3.19 s		5.39 d $J = 4$ 5.42 AB $J = 6$	4.50 4.76	4.50 4.92 d $J = 6$		5.48 s	5.48 s	5.12 s		7.42 s	7.42 s	7.50 s	
Tetramer	1.42 s 1.46 s	1.19 s 1.46 s	1.12 s 1.42 s	1.12 s 1.35 s	4.16 s	3.36 s	3.28 s	3.18 s	5.40 d $J = 4$ 5.40 m	4.38 4.62 3d	4.41 4.80 $J = 6$	4.44 4.90	5.50 s	5.46 s	5.46 s	5.14 s	7.40 s	7.43 s	7.43 s	7.50 s

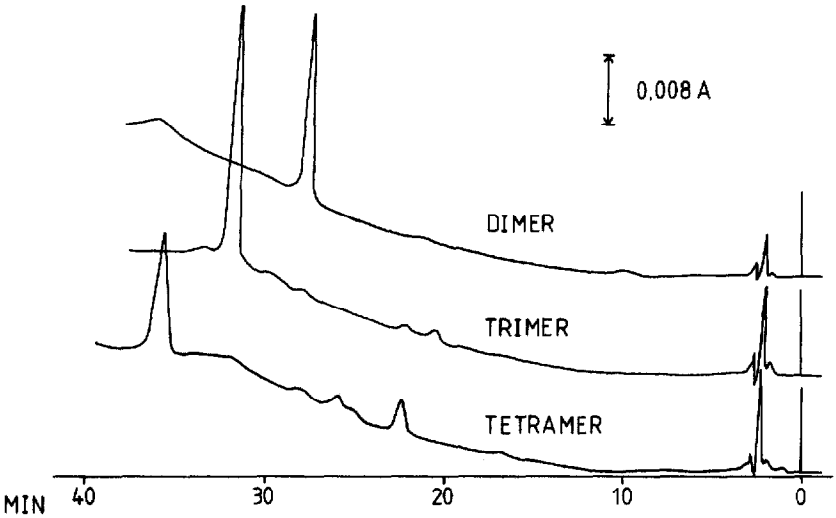

Fig. 6. Chromatogram of the purified oligomers of ampicillin. Amount injected: 25 μl of a 0.1% w/v solution. See Experimental for chromatographic conditions.

IR spectra were obtained using potassium bromide discs. The spectra of the oligomers differ from those obtained with the parent penicillin mainly in that the intensity of the β-lactam absorption band decreases as the degree of polymerization increases. Sodium ampicillin and oligomers: ν_{max} (cm^{-1}) at 1760 (β-lactam), 1660,

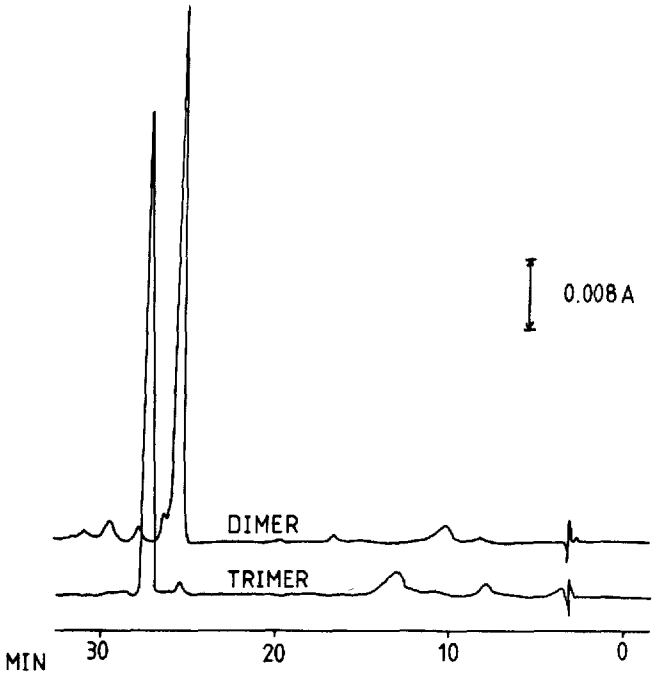

Fig. 7. Chromatograms of the purified oligomers of amoxicillin. Amount injected: 10 μl of a 0.1% w/v solution. See Experimental for chromatographic conditions.

TABLE III

PROTON MAGNETIC RESONANCE DATA (δ VALUES) FOR AMOXICILLIN AND ITS OLIGOMERS

	2-CH_3				*3-H*				*5- and 6-H*				*9-H*				*Phenyl*		
	a	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*			
Monomer	1.46				4.18				5.45				4.60				6.89		
	s				s				s				s				7.31		
	1.50																d		
	s																$J = 9$		
Dimer	1.44	1.15			4.20	3.27			5.45	4.58			5.41	5.16			6.94	7.00	
	s	s			s	s			5.41	4.93			s	s			7.33	7.45	
	1.48	1.36							AB	d							d	d	
	s	s							$J = 3.7$	$J = 6$							$J = 9$	$J = 9$	
Trimer	1.44	1.21	1.15		4.20	3.42	3.25		5.38	4.53	4.54		5.42	5.42	5.17		6.93	6.93	7.00
	s	s	s		s	s	s		5.49	4.81	5.03		s	s	s		7.32	7.34	7.44
	1.47	1.47	1.38						AB	2 AB							d	d	d
	s	s	s						$J = 3.7$	$J = 7$							$J = 9$	$J = 9$	$J = 9$

1520 (amide), 1600, 1400 (carboxylate), 700 (monosubstituted phenyl). Sodium amoxicillin and oligomers: ν_{max} (cm^{-1}) at 1762 (β-lactam), 1680, 1520 (amide), 1600, 1390 (carboxylate), 840 (1,4-disubstituted phenyl).

Chemical shift values observed in the ^1H NMR spectra of ampicillin and amoxicillin oligomers are given in Tables II and III. The three ampicillin oligomers (Table II) showed sets of two, three or four signals (singlets or multiplets) for *gem*-dimethyl, C-3, -5, -6, -9 and phenyl protons (Fig. 1). This suggests the existence of di-, tri- and tetramers but not di-, tetra- and hexamers as reported by Bundgaard and Larsen[10]. δ Values given under the heading *a* are almost identical to those observed for ampicillin and are assigned to the β-lactam-containing terminal unit of the oligomers. Exceptions are the 9-H singlets. The δ value observed for the monomer (4.68 ppm) was not found in the spectra of the oligomers. The protons showed a downfield shift (from 4.68 to 5.45–5.50 ppm) which indicates that the amino functions are involved in oligomer formation (acylation of amino by β-lactam carbonyl). Signals under the headings *a* for the dimer, *c* for the trimer and *d* for the tetramer are also identical and are due to the terminal units which do not contain a β-lactam. δ Values given under *b* for the trimer are also found for the tetramer and can be assigned to the second structural subunit, linked to the β-lactam-containing terminal unit. A similar pattern, observed for the amoxicillin oligomers (Table III), is evidence for the formation of di- and trimers.

Further evidence as to the structure of the oligomers reported in this study was provided by mass spectrometry. Samples of the purified oligomers of ampicillin and amoxicillin were subjected to fast atom bombardment mass spectrometry (FABMS)[22]: all the compounds gave pseudomolecular ions in both the positive and negative ion modes and these confirmed the structures deduced from the ^1H NMR data. Although the oligomers were all analysed as their sodium salts, the spectra usually showed the protonated or deprotonated free acid as the most intense molecular weight-determining peak. The predominant pseudomolecular ions observed for

TABLE IV

PREDOMINANT MOLECULAR WEIGHT-DETERMINING PEAKS OF AMOXICILLIN AND AMPICILLIN OLIGOMERS

Oligomer	*Molecular weight-determining peaks (m/z)*	
	MH^+	$M - H^-$
Amoxicillin		
Monomer	366	364
Dimer	731	729
Trimer	1118*	1094
Ampicillin		
Monomer	350	348
Dimer	699	697
Trimer	1048	1046**
Tetramer	1397	1395***

* MNa^+.

** Additionally, doubly charged ions corresponding to $(M - 2H^+)^{2-}$ and $(MNA^+ - 3H^+)^{2-}$ were observed at *m/z* 522.5 and 563.5 respectively.

*** A doubly charged ion corresponding to $(M - 2H^+)^{2-}$ was observed at *m/z* 697.

each compound are listed in Table IV: they demonstrate beyond doubt that amoxicillin and ampicillin form dimers, trimers and tetramers rather than dimers, tetramers and hexamers as had been suggested[10].

In general, the positive ion mode gave poorer signal-to-background ratios for the pseudomolecular ions MH^+ than the negative ion mode gave for $M - H^-$ ions. This contrast was particularly evident for the amoxicillin oligomers for which the poor signal-to-background ratios in the positive ion spectra prevented observation of any fragment ions. In all other spectra, relatively abundant fragment ions were observed and these could be rationalized on the basis of the generalized structure shown in Fig. 1. Many of the fragmentations occurred around the amide bonds between the monomer units. Fragmentations of this type, which were common to all the oligomers except the ampicillin dimer, are shown in Fig. 8 as A, A', B, C and D for an antibiotic trimer. Fragmentations A and A' may, or may not, both occur; they cannot be distinguished with the data presently available since they involve the same mass loss. The arrows in Fig. 8 mark the direction of hydrogen atom transfer (if any) occurring upon fragmentation.

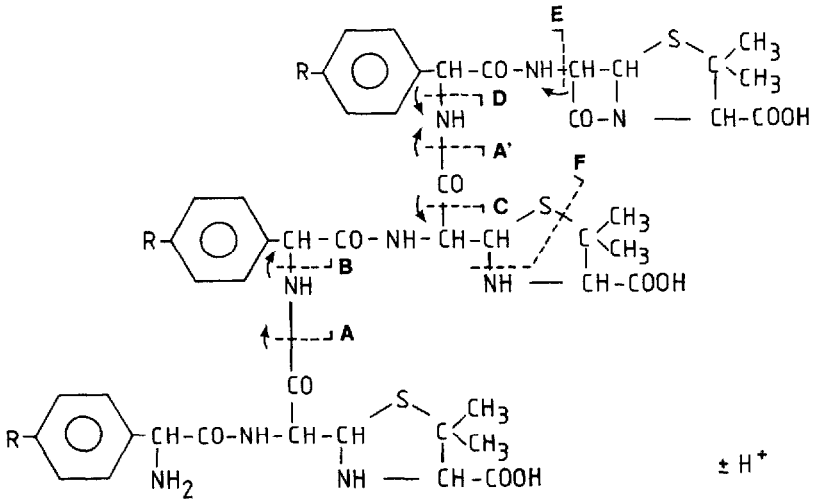

Fig. 8. Some of the fragmentations occurring in the FAB mass spectra of the ampicillin (R = H) and amoxicillin (R = OH) trimers.

In addition, fragmentations occurred within a single monomer unit of the antibiotic oligomers. Many of these fragmentations could be correlated with those described by Barber *et al.*[23] for ampicillin and amoxicillin free acid monomers, although not all of the losses noted by those workers were observed in this study of the oligomers. In addition, losses which could be rationalized as fragmentations E (−199 daltons) and F (−115 daltons) in Fig. 8 were common, as was loss of CO_2 (−44 daltons) from the pseudomolecular ion. All compounds showed dimers in their FAB mass spectra, for example, the dimeric anion of the ampicillin tetramer at m/z 2791; these dimers were generally most easily seen in the negative ion spectra.

It can be concluded that during the polycondensation reaction, occurring in solutions of sodium ampicillin or sodium amoxicillin, oligomers with the structure

given in Fig. 2 are formed. Oligomers with an even and uneven number of subunits are formed; there is no limitation to even numbers as was reported previously for ampicillin oligomers[10].

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REFERENCES

1 N. H. Grant, D. E. Clark and H. E. Alburn, *U.S. Pat.* 3,351,586 (1967).
2 H. Smith, J. M. Dewdney and A. W. Wheeler, *Immunology*, 21 (1971) 527.
3 B. T. Butcher, M. K. Stanfield, G. T. Stewart and R. Zemelman, *Mol. Cryst. Liquid Cryst.*, 12 (1971) 321.
4 S. Shaltiel, R. Mizrahi and M. Sela, *Proc. R. Soc. London, Ser. B*, 179 (1971) 411.
5 S. Ahlstedt, A. Kristofferson, P. O. Svärd, L. Thor and B. Örtengren, *Int. Arch. Allergy Appl. Immunol.*, 51 (1976) 131.
6 M. K. Stanfield, B. T. Butcher and G. T. Stewart, *Anal. Biochem.*, 89 (1978) 1.
7 H. Bungaard, *Acta Pharm. Suecica*, 13 (1976) 9.
8 H. Bungaard, *Acta Pharm. Suecica*, 14 (1977) 47.
9 H. Bundgaard, *Acta Pharm. Suecica*, 14 (1977) 67.
10 H. Bundgaard and C. Larsen, *J. Chromatogr.*, 132 (1977) 51.
11 C. Larsen and H. Bungaard, *J. Chromatogr.*, 147 (1978) 143.
12 E. Cavatorta, V. Springolo, S. Tedeschi and E. Cingolani, *Il Farmaco Ed. Pr.*, 35 (1980) 273.
13 H. Ueno, M. Nishikawa, M. Muranaka and Y. Horiuchi, *J. Chromatogr.*, 207 (1981) 425.
14 C. Larsen and M. Johansen, *J. Chromatogr.*, 246 (1982) 360.
15 M. G. De Angeli, G. Mercandalli, F. Minoja, S. Tedeschi and E. Cingolani, *Farmaco, Ed. Pr.*, 35 (1980) 100.
16 S. Takagi, Y. Nobuhara and Y. Nakanishi, *J. Chromatogr.*, 258 (1983) 262.
17 H. Bundgaard and C. Larsen, *Int. J. Pharm.*, 3 (1979) 1.
18 R. Busson, P. J. Claes and H. Vanderhaeghe, *J. Org. Chem.*, 41 (1976) 2556.
19 R. D. Carroll, S. Jung and C. G. Sklavounos, *J. Heterocycl. Chem.*, 14 (1977) 503.
20 J. P. Degelaen, S. L. Loukas, J. Feeney, G. C. K. Roberts and A. S. V. Burgen, *J. Chem. Soc., Perkin Trans. II*, (1979) 86.
21 A. E. Bird, E. A. Cutmore, K. R. Jennings and A. C. Marshall, *J. Pharm. Pharmacol.*, 35 (1983) 138.
22 M. Barber, R. S. Bordoli, R. D. Sedgwick and A. N. Tyler, *J. Chem. Soc., Chem. Commun.*, (1981) 325.
23 M. Barber, R. S. Bordoli, R. D. Sedgwick, A. N. Tyler, B. N. Green, V. C. Parr and J. L. Gower, *Biomed. Mass Spectrom.*, 9 (1982) 11.