

TEICHOMYCIN A₂ PURE SINGLE FACTORS 1, 2, 3, 4 AND 5
AND METHOD FOR THEIR PRODUCTION

GB-A-1496386 discloses and claims Teichomycin A₂, one of the several different antibiotic substances
5 obtained by cultivating the strain Actinoplanes
Teichomyceticus nov.sp. ATCC 31121 in a culture medium containing assimilable sources of carbon, nitrogen and inorganic salts. According to the procedure described in
10 GB-A-1496386, an antibiotic mixture containing
Teichomycins A₁, A₂ and A₃ is recovered from the separated fermentation broth by extraction with a suitable water-immiscible organic solvent and precipitation from the extracting solvent, according to common procedures. Teichomycin A₂ is then separated from
15 the antibiotic mixture by column chromatography on Sephadex. Teichomycin A₂, after being further purified by passing through a sulphonated polystyrene resin, is then characterised by a series of different
chemico-physical parameters including the R_f values in a
20 set of paper and thin layer chromatographic systems where this compound behaved as a true unitary product.

It has now been found, unexpectedly, that Teichomycin A₂ actually comprises a mixture of several closely related antibiotic materials, the main factors of
25 which have been named Teichomycin A₂ factor 1, Teichomycin A₂ factor 2, Teichomycin A₂ factor 3, Teichomycin A₂ factor 4 and Teichomycin A₂ factor 5. It has also been found that these pure single factors are biologically distinguishable from Teichomycin A₂ complex
30 in that they have a higher degree of antibiotic activity against susceptible micro-organisms.

The essentially pure antibiotic compound factors are characterised as follows:

Teichomycin A₂ factor 1, a white amorphous powder that, on heating, begins to darken at about 220°C and is completely decomposed at 255°C, and which

(a) is freely soluble in water at pH > 7.0 or at pH < 2, in dimethylformamide, dimethyl sulphoxide and propylene glycol; slightly soluble in 2-methoxyethanol and glycerol; poorly soluble in methanol and ethanol; and almost insoluble in chloroform, benzene, n-hexane, acetonitrile, diethyl ether, acetone, ethyl acetate and carbon tetrachloride;

(b) has an ultraviolet absorption spectrum (see Figure 1 of the accompanying drawings) that exhibits the following three absorption maxima:

$\lambda_{\max}$ 278 nm ($E_{1\%}^{1\text{cm}} = 49.5$) in 0.1N hydrochloric acid
 $\lambda_{\max}$ 278 nm ($E_{1\%}^{1\text{cm}} = 50.0$) in phosphate buffer pH 7.4
 $\lambda_{\max}$ 297 nm ($E_{1\%}^{1\text{cm}} = 72.1$) in 0.1N sodium hydroxide;

(c) an infrared absorption spectrum (see Figure 2) in liquid paraffin with the following absorption maxima: 3700-3100,

2960-2840 (paraffin), 1645, 1590, 1510, 1460 (paraffin), 1375 (paraffin), 1305, 1230, 1180, 1155, 1060, 1025, 970, 890, 845, 815, 720 (paraffin);

(d) an average approximate elemental analysis, after drying at about 140°C under an inert atmosphere (% Δ w = 8.5): C, 56.70%; H, 4.90%; N, 6.65%; Cl, 3.80%; O (by difference) 27.95%;

(e) a retention time (t_R) of 21.2 minutes, when analysed by reverse-phase HPLC using a 5 μ m silanised silica gel column, and eluting with a linear gradient from 0 to 50% solution B in solution A in 40 minutes (solution A: 25 mM NaH₂PO₄/acetonitrile (9/1 v/v) buffered at pH 6.0 with 0.1N NaOH; solution B: 25 mM NaH₂PO₄/acetonitrile (3/7 v/v) buffered at pH 6.0 with 0.1N NaOH), with a flow rate of 2 ml/min

(internal standard: 3,5-dihydroxytoluene t_R 8.84 minutes);

- (f) the following groups of signals in the 270 MHz 1H NMR spectrum (see Figure 3) registered in DMSO- d_6 with the addition of a few drops of D_2O (conc. 25 mg/0.5 ml) (TMS as internal standard $\delta = 0.00$ ppm):
0.8-1.5 (m), 1.7-2.3 (m), 2.7-4.0 (m), 4.0-4.7 (m), 4.8-5.8 (m), 6.2-8.1 (m);
 - (g) an acidic function capable of forming salts;
 - 10 (h) a salifiable basic function; and
 - (i) a molecular weight of about 1875 as determined by FAB mass spectrometry.
- Teichomycin A_2 factor 2 is a white amorphous powder that, on heating, begins to darken at 210°C and is
- 15 completely decomposed at 250°C, and which
- (a) has solubility characteristics as defined for factor 1(a);
 - (b) has an ultraviolet absorption spectrum (see Figure 4) that exhibits three respective absorption maxima, as defined for factor 1(b), of 48, 49.0 and 70.0;
 - 20 (c) an infrared absorption spectrum (see Figure 5) in liquid paraffin with the following observable absorption maxima: 3700-3100, 2960-2860 (paraffin), 1645, 1590, 1510, 1460 (paraffin), 1375 (paraffin), 1300, 1260, 1230, 1180, 1150, 1060, 1025, 970, 890, 845, 815, 720 (paraffin);
 - 25 (d) an average approximate elemental analysis, as defined for factor 1(d) (% $\Delta w = 9.8$): C, 56.15%; H, 5.15%; N, 6.30%; Cl, 3.90%; O (by difference) 28.50%;
 - 30 (e) a retention time, as defined for factor 1(e), of 22.6 minutes;
 - (f) the following groups of signals in the 270 MHz 1H NMR spectrum (see Figure 6), as defined for factor 1(f): 0.7-1.5 (m), 1.8-2.2 (m), 2.7-4.5 (m), 4.6-5.7
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(m), 6.2-8.1 (m);

- (g) an acidic function capable of forming salts;
- (h) a salifiable basic function; and
- (i) a molecular weight of about 1877 as determined by
5 FAB mass spectrometry.

Teichomycin A₂ factor 3 is a white amorphous powder that, on heating, begins to decompose at 205°C and is completely decomposed at 250°C, and which

- (a) has solubility characteristics as defined for factor
10 1(a);
- (b) an ultraviolet absorption spectrum (see Figure 7) that exhibits the three respective absorption maxima, as defined for factor 1(b), of 49.2, 50.8 and 72.7;
- 15 (c) an infrared absorption spectrum (see Figure 8) in liquid paraffin with the following observable absorption maxima: 3700-3100, 2960-2850 (paraffin), 1645, 1590, 1510, 1460 (paraffin), 1375 (paraffin), 1300, 1230, 1180, 1150, 1120, 1060, 1030, 970, 890,
20 845, 820, 800, 720 (paraffin);
- (d) an average approximate elemental analysis, as defined for factor 1(d) (%Δw = 12.0): C, 56.26%; H, 5.20%; N, 6.69%; Cl, 3.95%; O (by difference) 27.90%;
- 25 (e) a retention time, as defined for factor 1(e), of 23.3 minutes;
- (f) the following groups of signals in the 270 MHz ¹H NMR spectrum (see Figure 9), as defined in claim 1(f): 0.7-1.5 (m), 1.8-2.0 (m), 2.7-4.5 (m), 4.6-5.7
30 (m), 6.2-8.0 (m);
- (g) an acidic function capable of forming salts;
- (h) a salifiable basic function; and
- (i) a molecular weight of about 1877 as determined by
35 FAB mass spectrometry.

Teichomycin A₂ factor 4 is a white amorphous powder that, on heating, begins to darken at about 210°C and is completely decomposed at 250°C, and which

- (a) has solubility characteristics as defined for factor 1(a);
 - (b) has an ultraviolet absorption spectrum (see Figure 10) that exhibits the three respective absorption maxima, as defined for factor 1(b), of 52.5, 52.5 and 75.5;
 - (c) an infrared absorption spectrum in liquid paraffin with the following absorption maxima: 3700-3100, 2960-2840 (paraffin), 1645, 1590, 1510, 1460 (paraffin), 1375 (paraffin), 1300, 1230, 1175, 1140, 1060, 1025, 970, 890, 840, 815, 720 (paraffin);
 - (d) an average approximate elemental analysis, as defined for factor 1(d) (%Δw = 9.8): C, 56.50%; H, 5.10%; N, 6.50%; Cl, 3.80%; O (by difference) 28.10%;
 - (e) a retention time, as defined in claim 1(e), of 25.8 minutes;
 - (f) an acidic function capable of forming salts;
 - (g) a salifiable basic function; and
 - (h) a molecular weight of about 1891 as determined by FAB mass spectrometry.
- Teichomycin A₂ factor 5 is a white amorphous powder that, on heating, begins to darken at 210°C and is completely decomposed at 250°C, and which
- (a) has solubility characteristics as defined for factor 1(a);
 - (b) has an ultraviolet absorption spectrum (see Figure 12) that exhibits the three respective absorption maxima, as defined for factor 1(b), of 49.6, ~~51.8~~ 51.8 and 78.8;
 - (c) an infrared absorption spectrum (see Figure 13) in liquid paraffin with the following observable

absorption maxima: 3700-3100, 2960-2840 (paraffin), 1645, 1590, 1510, 1460 (paraffin), 1375 (paraffin), 1300, 1230, 1175, 1145, 1060, 1025, 970, 890, 840, 815, 720 (paraffin);

- 5 (d) an elemental analysis, as defined for factor 1(d) (% Δ w = 10.1): C, 56.60%; H, 5.05%; N, 6.63%; Cl, 3.85%; O (by difference) 27.87%;
- (e) a retention time, as defined for factor 1(e), of 26.4 minutes;
- 10 (f) an acidic function capable of forming salts;
- (g) a salifiable basic function; and
- (h) a molecular weight of about 1891 as determined by FAB mass spectrometry.

The antibiotic materials of the present invention

15 are prepared starting from Teichomycin A₂ which is described in GB-A-1496386, by separating the antibiotic complex into the single factors, using high-efficiency chromatographic methods, and recovering the main ones.

The terms "Teichomycin A₂", "Teichomycin A₂ complex"

20 and "antibiotic complex", as used in this specification, refer to the mixture containing the five antibiotic factors according to the invention as obtained, for example, by the procedure disclosed in GB-A-1496386 and there named Teichomycin A₂.

25 The complex may be separated into the main pure single factors by reverse-phase partition or ion-exchange chromatography. In the former case, inactivated silica gel is conveniently used as the column packing, and a gradient elution of acetonitrile/aqueous ammonium formate

30 as the developer; in the latter case, a weak gel-type anion exchanger is suitably employed as the stationary phase and aqueous buffers or mixtures of aqueous buffers and non-aqueous solvents as the eluting systems.

It is especially preferred that Teichomycin A₂ is

35 separated into the single compounds by reverse-phase

column chromatography using an octadecylsilane column and a 24:76 (v/v) mixture of acetonitrile:0.2% aqueous ammonium formate as the developer.

5 An alternative preference is that Teichomycin A₂ is separated by column chromatography using a silanised silica gel column and a gradient elution of acetonitrile in aqueous ammonium formate as the developer. More preferably, the column is developed in a linear gradient using from 10 to 20% acetonitrile in a 0.2% ammonium
10 formate solution. Most preferably, this process comprises dissolving Teichomycin A₂ complex in a 0.2% ammonium formate/acetonitrile 9/1 mixture, adjusting the pH to about 7.5 by the addition of NaOH, passing the obtained solution through the column, developing the
15 column, collecting the fractions which have the HPLC profile characteristic of Teichomycin A₂ factor 2, evaporating off the organic solvent under reduced pressure, desalting the aqueous solution left by passing it through a silanised silica gel column, washing the
20 column with water, eluting it with 50% aqueous acetonitrile, concentrating the eluate to a small volume and precipitating essentially pure Teichomycin A₂ factor 2 therefrom by the addition of a non-solvent.

Good results are also obtained by using the
25 diethylaminoethyl derivative of agarose as the stationary phase and carrying out the separation by progressive elution with buffer solutions or mixtures of buffer solutions and non-aqueous water-miscible solvents.

The separation procedure is monitored by HPLC.
30 Fractions with similar HPLC profile are combined, if desired, further purified by preparative HPLC, and desalted. From these solutions, the single factors are then recovered by evaporating the organic solvents, stripping off water to a small volume, and adding an

excess of an organic solvent in which the compounds are not soluble, to precipitate the products.

The following illustrates the separation process of the invention. All percentages are by weight, and liquid ratios by volume, unless otherwise stated or apparent.

"Ethyl ether" refers to diethyl ether and "butanol" to n-butanol. "Sephadex", "Merck", "Whatman", "Partisil", "Cellosolve", "Difco", "Nujol" and "Zorbax" are registered Trade Marks. "Methylcellosolve" represents 2-methyloxyethanol, "Nujol" represents liquid paraffin and "Zorbax" represents silanised silica gel.

Separation Process

10 g Teichomycin A₂ complex obtained by the process described in GB-A-1496386 are dissolved in 1 litre of a 0.2% ammonium formate-acetonitrile (9:1) mixture, and adjusted to pH 7.5 with 1N NaOH. This solution is passed through a column containing 500 g silanised silica gel 60 (Merck). The column is then eluted with a linear gradient of from 10 to 20% acetonitrile in a 0.2% ammonium formate solution in a total volume of 10 litres. Fractions of about 20 ml are collected and checked by HPLC.

Table I gives the retention times (t_R) for Teichomycin A₂ factors 1, 2, 3, 4 and 5 in a representative HPLC separation (the operating conditions are indicated below the Table):

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TABLE I

	Teichomycin A ₂ factor	Retention time (minutes)
5	1	21.2
	2	22.6
	3	23.3
	4	25.8
	5	26.4
10	3,5-dihydroxytoluene (internal standard)	8.84

Column: 5 μ Zorbax ODS (Du Pont)

Mobile Phase: Linear gradient from 0%B to 50%B in A in 40 minutes

- 15
- A) 25 mM NaH₂PO₄ /Acetonitrile (9:1)
buffered at pH 6.0 with 0.1N NaOH
 - B) 25 mM NaH₂PO₄ /Acetonitrile (3:7)
buffered at pH 6.0 with 0.1N NaOH

Flow: 2 ml/min.

Detector: U.V. Photometer at 254 nm.

20 Fractions with similar HPLC profile are combined and the organic solvent is evaporated under reduced pressure. The aqueous solutions left, are passed through a column containing 10 grams of silanised silica gel (60) (Merck). The column is washed with distilled water in order to
25 eliminate the ammonium formate and then eluted with 50% aqueous acetonitrile.

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The eluate is concentrated to a small volume by adding butanol to facilitate the evaporation of the water and then precipitated with a 1:1 acetone-ethyl ether mixture. Pure Teichomycin A₂ factor 1 (410 mg) and factor 2 (770 5 mg) are obtained by the above procedure.

Teichomycin A₂ factor 3 as a 1:1 mixture with Teichomycin A₂ factor 2 is further purified by HPLC on a semipreparative column at the following operating conditions.

Column: Whatman Partisil ODS M 9 10/50

10 Mobile Phase: 0.2% ammonium formate in H₂O/acetonitrile (76:24).

Flow: 4.5 ml/min.

Detector: U.V. Photometer at 254 nm,

Load: 20 mg

15 Also in this case purification is monitored by checking each fractions by HPLC.

Fractions containing pure Teichomycin A₂ factor 2 as well as fractions containing pure Teichomycin A₂ factor 3 are combined, desalted and precipitated as previously described. 20 (Yield: 510 mg of Teichomycin A₂ factor 2 and 520 mg of Teichomycin A₂ factor 3).

Fractions containing factors 4 and 5 in 1:1 proportion (about 500 mg) obtained from the first column, are combined with another pool of fractions containing a 25 mixture of factors 4 and 5 (about 490 mg) obtained in the same manner from a second parallel separation and are separated by semipreparative HPLC using the same

operating conditions as above for the purification of Teichomycin A₂ factor 3, yielding 350 mg of Teichomycin A₂ factor 4 and 300 mg of Teichomycin A₂ factor 5.

The present invention includes the pharmaceutically acceptable salts of the individual factors.

The alkali metal, alkaline earth metal and pharmaceutically acceptable ammonium salts of Teichomycin A₂ factors 1, 2, 3, 4 and 5 are preferred. Representative alkali metal and alkaline earth metal salts include sodium, potassium, lithium, calcium and magnesium salts; ammonium salts include ammonium and primary, secondary or tertiary (C₁-C₄) alkylammonium and hydroxy-(C₁-C₄) alkylammonium salts.

The alkali and alkaline earth metal salts are prepared according to the usual procedures commonly employed for preparing metal salts. As an example, Teichomycin A₂ factor 1, 2, 3, 4 or 5 in the free acidic form is dissolved into a suitable solvent such as propylene glycol and an approximately stoichiometric amount of a suitable selected mineral base is gradually added to the resultant solution. The alkali or alkaline earth metal salt which forms is then recovered by precipitation with a non-solvent and filtration.

Alternatively, these salts can be prepared in a substantially anhydrous form, by lyophilisation; in this

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case aqueous solutions containing the desired salts, resulting from the salification of the free acidic form with a suitably selected alkali or alkaline earth metal carbonate or hydroxide in such a quantity as to obtain a 5 pH between 7 and 8 are filtered from any insoluble and lyophilized.

The organic ammonium salts can be prepared either by adding the properly selected amine to a solution of the free acidic form of Teichomycin A₂ factors 1,2,3,4 and 5 10 in a suitable solvent such as propylene glycol, and then evaporating off the solvent and the excess of the amine reagent or alternatively contacting the two reagents in the minimum amount of water and then precipitating the obtained salts by the addition of a non-solvent..

15 As stated before, each of Teichomycin A₂ factors 1,2,3,4 and 5 contains also a basic function capable of being salified. Their pharmaceutically acceptable acid addition salts, prepared as known per se in the art, by contacting the pure single factors with a rather strong acid, 20 preferably with a mineral acid, represent a further specific object of the present invention.

Preparation of Teichomycin A₂ factor 2 sodium salt

An aqueous solution of Teichomycin A₂ factor 2 (150 mg, 15 ml) is brought to pH 8.0 by the dropwise addition of

0.1N NaOH. The obtained solution is filtered, transferred into the chamber of a freeze-drying system and frozen. After freezing is complete, the chamber is evacuated to 0.1 Torr and ice is sublimated by bringing the heating plate to 0°C. The process continues until the product is almost dry (about 1% moisture). Titration of a solution of Teichomycin A₂ factor 2 sodium salt thus obtained in 25 ml methylcellosolve/H₂O 3/1 with 0.1N HCl showed the presence of two titratable functions characterized by the following pK: 7.03 and 4.78.

By following the procedure described above but starting from Teichomycin A₂ factors 1,3,4 and 5 the corresponding sodium salts are obtained. Determination of the amount of sodium in the end salts confirms the formation of a monosodium salt.

The in vitro antibacterial activity of Teichomycin A₂ factors 1,2,3,4 and 5, which showed to be mainly active against Gram-positive bacteria, was determined against clinical isolates of staphylococci and streptococci using two-fold dilution method in microtiter system. Penassay broth (Difco) and Todd-Hewitt broth (Difco) were used for staphylococci and streptococci respectively. Overnight broth cultures were diluted so that the final inoculum was about 10³ colony forming units/ml. Minimal inhibitory concentration (MIC) was read as the lowest concentration

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which showed no visible growth after 18-24 h incubation at 37°C. The obtained results are summarized in TABLE II below:

TABLE II

Microorganism	No. of tested strains	M.I.C. (μ g/ml)				
		factor 1	factor 2	factor 3	factor 4	factor 5
<u>Staphylococcus aureus</u>	5	0.8-1.6	0.8-1.6	0.4-0.8	0.2-0.8	0.2-0.8
<u>Staphylococcus epidermidis</u>	4	0.2-1.6	0.1-1.6	0.2-0.8	0.2-0.8	0.2-0.8
<u>Streptococcus pyogenes</u>	7	0.05-0.1	0.025-0.1	0.025-0.05	0.006-0.05	0.006-0.05
<u>Streptococcus pneumoniae</u>	6	0.1-0.2	0.05-0.1	0.05-0.1	0.05-0.1	0.05-0.1
<u>Streptococcus faecalis</u>	5	0.2-0.4	0.1-0.4	0.1-0.2	0.1-0.4	0.1-0.4
<u>Streptococcus mitis</u>	1	0.025	0.025	0.0125	0.025	0.025
<u>Streptococcus salivarius</u>	1	0.2	0.2	0.1	0.05	0.05
<u>Streptococcus sanguis</u>	1	0.1	0.1	0.1	0.05	0.05
<u>Streptococcus bovis</u>	1	0.4	0.4	0.2	0.4	0.4
<u>Streptococcus agalactiae</u>	1	0.1	0.1	0.05	0.1	0.1

The relationship of microbiological potency of the individual factors 1,2,3,4 and 5 was determined by the agar diffusion method using S.aureus ATCC 6538 as germ test, and Teichomycin A₂ complex as standard.

5 In particular an adequate amount of Teichomycin A₂ factor 1, Teichomycin A₂ factor 2, Teichomycin A₂ factor 3, Teichomycin A₂ factor 4, Teichomycin A₂ factor 5 and Teichomycin A₂ complex used as the standard is dissolved in dimethylformamide at a concentration of 2000 µg/ml.

10 These solutions are further diluted using phosphate buffer 0.067 M pH 7.4 supplemented with 1% bovine serum in order to provide the following concentrations: 2.5, 5, 10, and 20 µg/ml.

Filter paper discs are then soaked into the sample

15 solutions and placed at regular intervals on the surface of agar plates seeded with a suspension of the test microorganism. The plates are incubated at 37°C for 18 hours and then the diameter of the inhibition zones are measured. The data obtained are fed into a computer to
20 calculate the potency of the individual factors relative to the complex.

The results obtained are reported herein below

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	Teichomycin A ₂ factor 1	841 U/mg
	Teichomycin A ₂ factor 2	1086 U/mg
	Teichomycin A ₂ factor 3	1131 U/mg
	Teichomycin A ₂ factor 4	1066 U/mg
5	Teichomycin A ₂ factor 5	954 U/mg
	Teichomycin A ₂ complex	1000 U/mg

Teichomycin A₂ factors 2,3,4 and 5 were also tested in the experimental infections caused by S. pneumoniae and by S. pyogenes in mice. The experiments were carried out in comparison with Teichomycin A₂ complex. The results obtained are reported in Table III below

TABLE III
In vivo antibacterial activity

Compound	ED ₅₀ (mg/kg/day) s.c.	
	<u>S.pneumoniae L 44</u>	<u>S.pyogenes L 49</u>
Teichomycin A ₂ factor 2	0.28 (0.22-0.34)	0.15 (0.13-0.18)
Teichomycin A ₂ factor 3	0.27 (0.23-0.32)	0.13 (0.11-0.16)
Teichomycin A ₂ factor 4	0.12 (0.098-0.14)	0.098- (0.073-0.11)
Teichomycin A ₂ factor 5	0.13 (0.10-0.15)	0.10 (0.098-0.13)
Teichomycin A ₂ complex	0.35 (0.28-0.44)	0.18 (-)

The approximate acute toxicity in mice (i.p.) for Teichomycin A₂ factors 1,2,3,4 and 5 is as shown in Table IV below:

TABLE IV
Acute toxicity in mice (i.p.)

Compound	Approximate LD ₅₀ (mg/kg)
Teichomycin A ₂ factor 1	> 1500 < 2000
Teichomycin A ₂ factor 2	> 1500 < 2000
Teichomycin A ₂ factor 3	> 1000 < 1500
Teichomycin A ₂ factor 4	> 500 < 1000
Teichomycin A ₂ factor 5	> 500 < 1000

In view of the above, the compounds of the present invention, Teichomycin A₂ factors 1, Teichomycin A₂ factor 2, Teichomycin A₂ factor 3, Teichomycin A₂ factor 4 and Teichomycin A₂ factor 5 can effectively be employed as the active ingredient of antimicrobial preparations used in human and veterinary medicine for the prevention and treatment of infectious diseases caused by pathogenic bacteria which are susceptible to the active ingredients. In such treatments these compounds may be employed as such or used as single individual factors or, considering the similarity of their activity pattern, also in the form of mixtures of two or more of the five factors in any proportion.

The compounds of the present invention can be administered orally, topically or parenterally wherein however parenteral administration is most preferred. Depending on the route of administration, these compounds can be formulated into various dosage forms. Preparations for oral administration may be in the form of capsules, tablets, liquid solutions or suspension. As known in the art the capsules and tablets may contain in addition to the active ingredient, conventional excipients such as diluents, e.g. lactose, calcium phosphate and sorbitol, lubricants, e.g. magnesium stearate, talc, polyethylene glycol, binding agents, e.g. polyvinylpyrrolidone, gelatin, sorbitol, tragacanth, acacia, flavoring agents, and acceptable disintegrating and wetting agents. The liquid preparations generally in the form of aqueous or oily solutions or suspensions, may contain conventional additives such as suspending agents. For topical use the compounds of the present invention may also be prepared in suitable forms for absorption through the skin, the mucous membranes of the nose and throat or the bronchial tissues and may conveniently take the form of liquid sprays or inhalants, lozenges, or throat paints. For medication of the eyes or ears, the preparation may be presented in liquid or semi-liquid form. Topical applications may be formulated in hydrophobic or hydrophilic bases as ointments, creams, lotions, paints, or powders.

Compositions for injection may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

5 Alternatively, the active ingredient may be in powder form for reconstitution, at the time of delivery, with a suitable vehicle, such as sterile water.

The amount of active principle to be administered depends on various factors such as the size and condition of the
10 subject to be treated, the route and frequency of administration, and the causative agent involved.

Teichomycin A₂ factors 1, 2, 3, 4 and 5 are generally effective at a daily dosage comprised between ~~about~~ 0.1 and ~~about~~ 20 mg of active ingredient per Kg of body
15 weight, optionally divided in 2 administrations per day.

Particularly desirable compositions are those prepared in the form of dosage units containing from ~~about~~ 50 to ~~about~~ 250 mg per unit.

Representative examples of preparation of pharmaceutical
20 compositions are as follows:

A parenteral solution is prepared with

100 mg of Teichomycin A₂ factor 2 sodium salt
dissolved in 2 ml of sterile water for injection

A parenteral solution is prepared with
250 mg of Teichomycin A₂ factor 3 sodium salt
dissolved in 3 ml of sterile water for injection

A topical ointment is prepared with
5 200 mg of Teichomycin A₂ factor 2
 600 mg of polyethylene glycol 4000 U.S.P.
 1.2 g of polyethylene glycol 400 U.S.P.

Besides their activity as medicaments, the compounds of
the present invention can be used as animal growth
10 promoters.

For this purpose, one or more of the compounds of the
invention is administered orally in a suitable feed. The
exact concentration employed is that which is required to
provide for the active agent in a growth promotant
15 effective amount when normal amounts of feed are
consumed.

The addition of the active compounds of the invention to
animal feed is preferably accomplished by preparing an
appropriate feed premix containing the active compounds
20 in an effective amount and incorporating the premix into
the complete ration.

Alternatively, an intermediate concentrate or feed
supplement containing the active ingredient can be
blended into the feed.

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The way in which such feed premixes and complete rations can be prepared and administered are described in reference books (such as "Applied Animal Nutrition", W.H. Freedman and Co., S. Francisco, USA, 1969 or "Livestock
5 Feeds and Feeding", O and B Books, Corvallis, Oregon, USA, 1977). ~~and are incorporated herein by reference.~~



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CLAIMS

1. An essentially pure antibiotic compound, named Teichomycin A₂ factor 1, which is a white amorphous powder that, on heating, begins to darken at about 220°C and is completely decomposed at 255°C, and which
- (a) is freely soluble in water at pH > 7.0 or at pH < 2, in dimethylformamide, dimethyl sulphoxide and propylene glycol; slightly soluble in 2-methoxyethanol and glycerol; poorly soluble in methanol and ethanol; and almost insoluble in chloroform, benzene, n-hexane, acetonitrile, diethyl ether, acetone, ethyl acetate and carbon tetrachloride;
- (b) has an ultraviolet absorption spectrum that exhibits the following three absorption maxima:
- $\lambda_{\max}$ 278 nm ($E_{1\%}^{1\text{cm}} = 49.5$) in 0.1N hydrochloric acid
 $\lambda_{\max}$ 278 nm ($E_{1\%}^{1\text{cm}} = 50.0$) in phosphate buffer pH 7.4
 $\lambda_{\max}$ 297 nm ($E_{1\%}^{1\text{cm}} = 72.1$) in 0.1N sodium hydroxide;
- (c) an infrared absorption spectrum in liquid paraffin with the following absorption maxima: 3700-3100, 2960-2840 (paraffin), 1645, 1590, 1510, 1460 (paraffin), 1375 (paraffin), 1305, 1230, 1180, 1155, 1060, 1025, 970, 890, 845, 815, 720 (paraffin);
- (d) an average approximate elemental analysis, after drying at about 140°C under an inert atmosphere (% Δw = 8.5): C, 56.70%; H, 4.90%; N, 6.65%; Cl, 3.80%; O (by difference) 27.95%;
- (e) a retention time (t_R) of 21.2 minutes, when analysed by reverse-phase HPLC using a 5 μ m silanised silica gel column, and eluting with a linear gradient from 0 to 50% solution B in solution A in 40 minutes
solution A: 25 mM NaH₂PO₄/acetonitrile (9/1 v/v) buffered at pH 6.0 with 0.1N NaOH; solution B: 25 mM NaH₂PO₄/acetonitrile (3/7 v/v) buffered at pH 6.0 with 0.1N NaOH), with a flow rate of 2 ml/min

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(internal standard: 3,5-dihydroxytoluene t_R 8.84 minutes);

(f) the following groups of signals in the 270 MHz 1H NMR spectrum registered in DMSO- d_6 with the addition of a few drops of D_2O (conc. 25 mg/0.5 ml) (TMS as internal standard $\delta = 0.00$ ppm): 0.8-1.5 (m), 1.7-2.3 (m), 2.7-4.0 (m), 4.0-4.7 (m), 4.8-5.8 (m), 6.2-8.1 (m);

(g) an acidic function capable of forming salts;

(h) a salifiable basic function; and

(i) a molecular weight of about 1875 as determined by FAB mass spectrometry;

or a pharmaceutically acceptable salt thereof.

2. An essentially pure antibiotic compound, named Teichomycin A_2 factor 2, which is a white amorphous powder that, on heating, begins to darken at $210^\circ C$ and is completely decomposed at $250^\circ C$, and which

(a) has solubility characteristics as defined in claim 1(a);

(b) has an ultraviolet absorption spectrum that exhibits three respective absorption maxima, as defined in claim 1(b), of 48, 49.0 and 70.0;

(c) an infrared absorption spectrum in liquid paraffin with the following observable absorption maxima:

3700-3100, 2960-2860 (paraffin), 1645, 1590, 1510, 1460 (paraffin), 1375 (paraffin), 1300, 1260, 1230, 1180, 1150, 1060, 1025, 970, 890, 845, 815, 720 (paraffin);

(d) an average approximate elemental analysis, as defined in claim 1(d) ($\%Aw = 9.8$): C, 56.15%; H, 5.15%; N, 6.30%; Cl, 3.90%; O (by difference) 28.50%;

(e) a retention time, as defined in claim 1(e), of 22.6 minutes;

(f) the following groups of signals in the 270 MHz 1H



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NMR spectrum, as defined in claim 1(f): 0.7-1.5 (m),
1.8-2.2 (m), 2.7-4.5 (m), 4.6-5.7 (m), 6.2-8.1 (m);

- (g) an acidic function capable of forming salts;
- (h) a salifiable basic function; and
- 5 (i) a molecular weight of about 1877 as determined by
FAB mass spectrometry;

or a pharmaceutically acceptable salt thereof.

3. An essentially pure antibiotic compound, named
Teichomycin A₂ factor 3, which is a white amorphous
10 powder that, on heating, begins to decompose at 205°C and
is completely decomposed at 250°C, and which

- (a) has solubility characteristics as defined in claim
1(a);
- (b) an ultraviolet absorption spectrum that exhibits the
15 three respective absorption maxima, as defined in
claim 1(b), of 49.2, 50.8 and 72.7;
- (c) an infrared absorption spectrum in liquid paraffin
with the following observable absorption maxima:
20 3700-3100, 2960-2850 (paraffin), 1645, 1590, 1510,
1460 (paraffin), 1375 (paraffin), 1300, 1230, 1180,
1150, 1120, 1060, 1030, 970, 890, 845, 820, 800, 720
(paraffin);
- (d) an average approximate elemental analysis, as
defined in claim 1(d) (%Aw = 12.0): C, 56.26%; H,
25 5.20%; N, 6.69%; Cl, 3.95%; O (by difference)
27.90%;
- (e) a retention time, as defined in claim 1(e), of 23.3
minutes;
- (f) the following groups of signals in the 270 MHz ¹H
30 NMR spectrum, as defined in claim 1(f): 0.7-1.5 (m),
1.8-2.0 (m), 2.7-4.5 (m), 4.6-5.7 (m), 6.2-8.0 (m);
- (g) an acidic function capable of forming salts;
- (h) a salifiable basic function; and
- (i) a molecular weight of about 1877 as determined by
35 FAB mass spectrometry;



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or a pharmaceutically acceptable salt thereof.

4. An essentially pure antibiotic compound, named Teichomycin A₂ factor 4, which is a white amorphous powder that, on heating, begins to darken at about 210°C and is completely decomposed at 250°C, and which

- (a) has solubility characteristics as defined in claim 1(a);
- (b) has an ultraviolet absorption spectrum that exhibits the three respective absorption maxima, as defined in claim 1(b), of 52.5, 52.5 and 75.5;
- (c) an infrared absorption spectrum in liquid paraffin with the following absorption maxima: 3700-3100, 2960-2840 (paraffin), 1645, 1590, 1510, 1460 (paraffin), 1375 (paraffin), 1300, 1230, 1175, 1140, 1060, 1025, 970, 890, 840, 815, 720 (paraffin);
- (d) an average approximate elemental analysis, as defined in claim 1(d) (%Aw = 9.8): C, 56.50%; H, 5.10%; N, 6.50%; Cl, 3.80%; O (by difference) 28.10%;
- (e) a retention time, as defined in claim 1(e), of 25.8 minutes;
- (f) an acidic function capable of forming salts;
- (g) a salifiable basic function; and
- (h) a molecular weight of about 1891 as determined by FAB mass spectrometry;

or a pharmaceutically acceptable salt thereof.

5. An essentially pure antibiotic compound, named Teichomycin A₂ factor 5, which is a white amorphous powder that, on heating, begins to darken at 210°C and is completely decomposed at 250°C, and which

- (a) has solubility characteristics as defined in claim 1(a);
- (b) has an ultraviolet absorption spectrum that exhibits the three respective absorption maxima, as defined in claim 1(b), of 49.6, ^{51.8}~~51.8~~ and 78.8;



- (c) an infrared absorption spectrum in liquid paraffin with the following observable absorption maxima:
3700-3100, 2960-2840 (paraffin), 1645, 1590, 1510, 1460 (paraffin), 1375 (paraffin), 1300, 1230, 1175, 1145, 1060, 1025, 970, 890, 840, 815, 720 (paraffin);
- (d) an elemental analysis, as defined in claim 1(d) (% Δ w = 10.1): C, 56.60%; H, 5.05%; N, 6.63%; Cl, 3.85%; O (by difference) 27.87%;
- (e) a retention time, as defined in claim 1(e). of 26.4 minutes;
- (f) an acidic function capable of forming salts;
- (g) a salifiable basic function; and
- (h) a molecular weight of about 1891 as determined by FAB mass spectrometry;
- or a pharmaceutically acceptable salt thereof.
6. An alkali metal, alkaline earth metal or pharmaceutically acceptable ammonium salt of any of the compounds of claims 1 to 5.
7. A composition comprising a mixture of two or more of the compounds or salts according to any preceding claim, other than the composition described as Teichomycin A₂ in GB-A-1496386.
8. A compound or composition according to any preceding claim, for anti-bacterial use.
9. A method of producing a compound or salt according to any preceding claim, which comprises separating Teichomycin A₂ into the single factors by reverse-phase partition or ion-exchange chromatography and, if desired, converting the compounds thus obtained into the corresponding pharmaceutically acceptable salt.
10. A method according to claim 9, wherein Teichomycin A₂ is separated into the single compounds by reverse-phase column chromatography using an octadecylsilane column and a 24:76 (v/v) mixture of

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acetonitrile:0.2% aqueous ammonium formate as the developer.

11. A method according to claim 9, wherein Teichomycin A₂ is separated by column chromatography using a silanised silica gel column and a gradient elution of acetonitrile in aqueous ammonium formate as the developer.

12. A method according to claim 11, wherein the column is developed in a linear gradient using from 10 to 20% acetonitrile in a 0.2% ammonium formate solution.

13. A method according to claim 12, for producing the compound of claim 2, which comprises dissolving Teichomycin A₂ complex in a 0.2% ammonium formate/acetonitrile 9/1 mixture, adjusting the pH to about 7.5 by the addition of NaOH, passing the obtained solution through the column, developing the column, collecting the fractions which have the HPLC profile characteristic of Teichomycin A₂ factor 2, evaporating off the organic solvent under reduced pressure, desalting the aqueous solution left by passing it through a silanised silica gel column, washing the column with water, eluting it with 50% aqueous acetonitrile, concentrating the eluate to a small volume and precipitating essentially pure Teichomycin A₂ factor 2 therefrom by the addition of a non-solvent.

14. A method according to claim 9, wherein separation is achieved by column chromatography using the diethylaminoethyl derivative of agarose as the stationary phase and buffer solutions or mixtures of buffer solutions and non-aqueous water-miscible solvents as the eluents.