DURAMYCINS B AND C, TWO NEW LANTHIONINE CONTAINING ANTIBIOTICS AS INHIBITORS OF PHOSPHOLIPASE A_2

STRUCTURAL REVISION OF DURAMYCIN AND CINNAMYCIN†

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Duramycins B and C, two new lanthionine containing antibiotics, have been isolated from *Streptoverticillium* strain R2075 and *Streptomyces griseoluteus* (R2107). The known antibiotics duramycin and cinnamycin were reisolated from *Streptoverticillium hachijoense* (DSM 40114) and *Streptomyces longisporoflavus* (DSM 40165). The structures of these latter two compounds should be revised by changing amino acid residue 3 to glutamine and 17 to asparagine, respectively.

Cinnamycin therefore seems to be identical to Ro 09-0198. Leucopeptin has been shown to be identical to duramycin. Physico-chemical data of these compounds provide evidence for a similar structure for all duramycin antibiotics. All compounds of this group inhibit human phospholipase A_2 at a concentration of 10^{-6} molar.

Phospholipase A_2 [EC 3.1.1.4] plays a major role in the release of arachidonic acid from phospholipids in the cell membranes[1]. Further oxidative metabolism of free arachidonic acid leads to prostaglandins and leukotrienes[2]. Several of these eicosanoids are potent mediators of diseases, such as inflammation and allergy[3]. Inhibition of the enzymic activity of phospholipase A_2 may therefore be therapeutically beneficial.

In our search for naturally occurring inhibitors of phospholipase A_2 among secondary metabolites of microorganisms two new inhibitors from the actinomycetes strains R2075 and R2107 were identified which appeared to be closely related to the known peptide antibiotic duramycin. Using a different screening assay for compounds stimulating DNA repair, we found a strain producing duramycin as well as one producing cinnamycin (W. MÄRKI and E. RÖMMELE; unpublished results). In the present communication the taxonomy of these strains and the production, isolation, physico-chemical data and phospholipase inhibition of the new compounds duramycins B and C, as well as of duramycin and cinnamycin are described. Experimental results relating to the structural characterization are presented as well.

Materials and Methods

Microorganism

DSM 40114: *Streptoverticillium hachijoense* (HOSOYA *et al.*), LOCCI, BALDACCI and PETROLINI BALDAN, 1969.

DSM 40165: *Streptomyces longisporoflavus*, WAKSMAN, 1953.

† Dedicated to Prof. Dr. HANS ZÄHNER on the occasion of his 60th birthday.

†† This work has been presented at the Festsymposium "Sekundärmetabolite aus Mikroorganismen", Abstract P18, Tübingen, Sept. 27~29, 1989.

R2075[a]: *Streptoverticillium* sp.; The strain was isolated in 1984 from a soil sample, taken from a tropical forest soil near Djilebor (Senegal).

R2107[a]: *Streptomyces griseoluteus*, UMEZAWA, HAYANA, MAEDA, OGATA and OKAMI, 1950. The strain was isolated in 1984 from a soil sample, taken from a rice-field near Djilebor (Senegal).

Fermentation

The fermentation of the 4 strains was carried out according to the following general scheme. Strain dependent variations are mentioned below.

General Fermentation Scheme

A well sporulated agar slant culture was used as a seed culture. The slant was washed with 5 ml of a 0.2-molar Soerensen phosphate buffer pH 7.

The resulting suspension was poured into a 500-ml Erlenmeyer flask with one baffle. It contained 100 ml nutrient broth of the following composition: Soybean 2%, mannitol 2%. The pH was adjusted before sterilization to 8.2 with 1 N NaOH. The pH after sterilization was 7.4. The flask was shaken at 250 rpm and 28°C for 48 hours. For a second preculture, three 2-liter Erlenmeyer flasks with four baffles, each containing 500 ml of the soybean medium mentioned above, were inoculated with 25 ml each of the culture broth from the first preculture. The incubation was carried out at 120 rpm and 28°C for 48 hours.

The second flask preculture (1.5 liters) served as an inoculum for the 30 liters fermentation. Conditions: 50 liters stainless steel-fermenter containing 30 liters of the soybean medium, 600 rpm, 28°C, 1 liter/liter/minute air throughput and 0.5 kp/cm^2 pressure.

Strain Dependent Variations

A) R2075 (*Streptoverticillium* sp.) and R2107 (*S. griseoluteus*): Preculture medium: Oxo Lab Lemco 0.5%, Peptone C 0.5%, Casitone 0.3%, yeast extract 0.05%, glucose 2.2%, NaCl 0.15%, pH before sterilization: 7.0, after sterilization: 6.5.

Fermentation medium for strain R2075: Fishmeal 2.5%, malt extract 2.5%, glucose 0.5%, NH_4NO_3 0.05%, $MgSO_4$ 0.1%, $CaCO_3$ 0.5%, $Ca_3(PO_4)_2$ 0.3%, 1 ml/liter trace element solution, pH before and after sterilization: 6.5~6.7.

Trace element solution: $ZnSO_4 \cdot 7H_2O$ 0.3%, $MnSO_4 \cdot 4H_2O$ 0.2%, $CuSO_4 \cdot 5H_2O$ 0.2%, $FeSO_4 \cdot 7H_2O$ 0.3%, $CoCl_2 \cdot 6H_2O$ 0.5%, $(NH_4)_6Mo_7O_{24} \cdot H_2O$ 0.2%, H_3BO_3 0.2%.

Fermentation medium for strain R2107: As with R2075 but 2.5% cotton seed meal instead of fishmeal.

Optimal production levels were reached after 168 hours for R2075 and after 132 hours for R2107.

B) DSM 40114 (*S. hachijoense*): Fermentation at 450 rpm instead of 600 rpm; optimal production titers after about 72 hours.

C) DSM 40165 (*S. longisporoflavus*): Preculture conditions: For the second preculture the same conditions as for the first one were used. The inoculum was 5%.

Fermentation conditions: 10 liters glass fermenter with 5 liters soybean medium, 28°C, 800 rpm, 1 liter/liter/minute air, no overpressure. Optimal production after about 72 hours.

Isolation Procedure

In general, aqueous solvents were removed in the following way: First the organic component was distilled *in vacuo*. The remaining solution was then lyophilized.

Isolation of Duramycin (1)

The whole culture broth was filtered, the culture solution extracted with equal volumes of ethyl acetate and the organic phase discarded. The aq phase was concentrated *in vacuo* and then adsorbed on Amberlite XAD-1180 (9 liters). The resin was washed consecutively with water (0.4 bed volumes), aq 20% methanol (1 bed volume) and aq 70% 2-propanol (2 bed volumes). The 2-propanol eluate was concentrated on a Rotavap to a dark concentrate (2 liters) which was extracted with 1 liter of butanol. The organic phase was washed with a sodium chloride solution (10%, 2 × 250 ml) and yielded 6.6 g of a brownish residue

[a] The authors wish to thank Dr. R. M. KROPPENSTEDT (DSM, Braunschweig) for the taxonomical definition of strains.

upon drying. The material was further purified by partition chromatography[4] on a Sephadex G-25 column (coarse, 9 liters), using butanol - water - acetic acid (4 : 5 : 1) as the partitioning medium. The stationary phase was saturated with 2 bed volumes of lower phase before equilibration with 1 bed volume of the upper phase (water saturated BuOH). After sample loading in 90 ml of upper phase compound **1** was eluted with the upper phase (320 ml/hour). The active fractions from 3.4 to 5.4 liters were collected to give 0.68 g of a colorless residue. This material was dissolved in 50% aq 2-propanol (2.5% w/v) and finally purified to homogeneity on a reversed phase column (Antec UPC12, 10 μm as the stationary phase; 30 × 170 mm; H_2O - MeOH - TFA (28 : 72 : 0.1); 7.5 ml/minute; back pressure 30 bar; sample load 45 mg/run; Rt 22 minutes) to yield 220 mg of pure peptide **1** on drying (25% overall recovery).

Optionally, aliquots of the TFA salt were converted to the acetate salt by ion exchange chromatography on DE-52 (Whatman; AcO^-) using 0.05 M ammonium acetate buffer at pH 4.5.

Isolation of Cinnamycin (**2**)

The whole culture broth (5 liters) was filtered and the culture solution extracted with ethyl acetate. The aq phase was concentrated *in vacuo* and adsorbed on Amberlite XAD-1180 as described above for the isolation of duramycin. The resin was washed stepwise with water, aq methanol (20%) and 2-propanol (70%), yielding 2.5 g of a colored lyophilizate. The powder was dissolved in aq AcOH (0.1 N, 100 ml) and 100 ml of BuOH. The aq phase was extracted twice with the same volume of BuOH. The combined organic phases were concentrated *in vacuo* and lyophilized (880 mg). The material was fractionated by partition chromatography on Sephadex G-25 (fine, 2.5 × 70 cm). The elution was carried out with the upper phase (3 bed volumes) of BuOH - H_2O - AcOH (4 : 5 : 1) as described for duramycin (sample load in 3 ml of upper phase; flow rate 0.3 ml/minute; 4 ml fractions). Fractions No. 45 ~ 65 were pooled, concentrated and lyophilized, yielding 190 mg of a colorless residue. The final purification with 100 mg of this material was carried out by reversed phase chromatography (Antec UP C12, 10 μm; 30 × 170 mm; 8.0 ml/minute; 35 bar; 224 nm; 50 mg/run; Rt 25 minutes). The elution was performed by a step gradient of H_2O - MeOH - TFA from 35 : 65 : 0.1 to 28 : 72 : 0.1 after 18 minutes, yielding 50 mg of the pure lyophilizate (18% overall recovery).

Isolation of Duramycin B (**3**)

The whole culture broth was filtered, concentrated *in vacuo* and then lyophilized yielding 371 g of a solid residue. The powder was triturated three times with methanol (11 liters total) and the solvent discarded leaving 100 g of a brownish residue. An aliquot (10.9 g) was suspended in water (200 ml), acidified with TCA (30 ml of a 30% solution) at 0°C, stirred for 10 minutes and centrifuged (10 minutes, 10,000 rpm). The clear supernatant was adjusted to pH 3 (1 N NaOH) and applied on a reversed-phase column (LiChroprep RP-18, 15 ~ 25 μm; 40 × 250 mm; gradient elution with water - acetonitrile - TFA (100 : 0 : 0.1 to 60 : 40 : 0.1) in 30 minutes; 30 ml/minute; Rt 38 minutes; 220 nm) to give **3** as a colorless powder (342 mg, 29% overall recovery).

Isolation of Duramycin C (**4**)

The whole culture broth of two 30 liters fermenters was filtered yielding 42 liters of filtrate. This was applied on a column with Amberlite XAD-1180 (20 ~ 50 mesh; 15 × 135 cm; 25 liters; 37 liters/hour). Fraction 1 (acetonitrile - water (20 : 80); 50 liters; 50 liters/hour) contained almost no active compound and was discarded. Fraction 2 (acetonitrile - water (30 : 70): 50 liters; 25 liters/hour) was concentrated *in vacuo* to 3 liters and extracted three times with butanol (400 ml) whereby the organic phase was discarded. The aq phase was then lyophilized to yield 10.5 g of a brown powder. The lyophilizate was suspended in water (200 ml) and centrifuged (5 minutes, 12,000 rpm). The brown precipitate (3.51 g) contained the desired material in 75% purity. Additional material was obtained by addition of TCA (16 ml of a 30% solution) to the supernatant and centrifugation (5 minutes, 12,000 rpm) to give a second precipitate (1.87 g; 20% purity). Both solids were finally chromatographed on a preparative reversed-phase column (LiChroprep RP-18, 15 ~ 25 μm; conically shaped Büchi column, 300 ml; solvent A: water - TFA (100 : 0.1); solvent B: acetonitrile - water - TFA (80 : 20 : 0.07); gradient from 10% B to 40% B in 4 minutes then to 50% B in 30 minutes; 30 ml/minute; sample dissolved in 17 ml H_2O - acetonitrile (90 : 10) and adjusted to pH 8 with

1 N NH_3; Rt 24 minutes; 220 nm; 15 runs) to give **4** as an almost colorless powder (2.81 g, 47% overall recovery).

Determination of the Chirality of the Amino Acids

The hydrolyzates (6 N HCl, 12 hours at 105°C) of compounds **1** to **4** were esterified with 2-propanol (2 hours at 105°C; acidified with 1.2 N HCl) and then acetylated with trifluoroacetic anhydride (2 hours, room temperature). The derivatized amino acids were resolved by capillary gas chromatography on a chiral stationary phase (Chirasil-Val)[5]. The usual amino acids except arginine were identified by comparison with commercial standards. *meso*-Lanthionine was recognized as the main peak in a commercial mixture of the D-, L- and *meso*-form[6]. DL-*threo*-2-Hydroxyaspartic acid (Vega Biochemicals, Tucson, Az.) racemizes partially during the derivatization procedure and elutes in four peaks: The *threo* isomers before the *erythro* and the D-form earlier than the L (a generally accepted rule for this stationary phase). The derivatized amino acids of compounds **1** to **4** gave a peak coeluting with L-*erythro*-2-hydroxyaspartic acid.

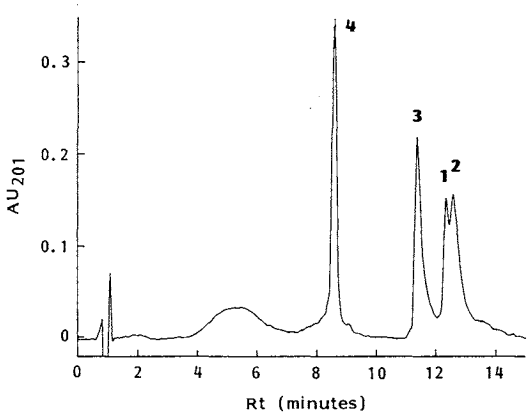
The chirality of arginine in **2** and **3** was established by separating the two enantiomers on Crownpak CR (Daicel, Tokyo, Japan), a HPLC column containing a chiral crown ether. The amino acids of **2** and **3** (hydrolyzed with 6 N HCl, 12 hours at 105°C) showed a strong peak coeluting with L-arginine, but none at the Rt of the D-form (4 × 150 mm, aq 0.01 N $HClO_4$, pH = 2.05; temperature 5°C; 0.4 ml/minute; monitored at 200 nm; 10 μg injection; 5.7 minutes (D-Arg), 11.6 minutes (L-Arg)). This finding was corroborated by identifying arginine in the collected peak by amino acid analysis (100 μg injection).

HPLC Analysis

The following equipment was used: An LCD/Milton-Roy HPLC pump (model Constametric III) furnished with a Kratos low pressure gradient-mixer (Spectroflow-430), an injection valve Rheodyne 7020 and a LKB diode array detector (model 2140) linked to an IBM-AT personal computer (WSEG software, version 1.0, LKB).

The separation of all four lantibiotics[7] is shown in Fig. 1. It is noteworthy that a very low concentration of acetonitrile is essential at the beginning of the gradient profile to separate **1**, **2** and **3**. This is an effect frequently observed with peptides and is probably due to conformational changes of the molecule on the hydrophobic stationary phase which are influenced by the solvent polarity.

Fig. 1. HPLC trace of duramycin (**1**), cinnamycin (**2**), duramycins B (**3**) and C (**4**).



The broad hump around 5 minutes is due to the gradient used. Experimental conditions: Analytical reversed-phase column (Nucleosil 5-C18; 100 Å; 4 × 120 mm); solvent A: water - TFA (100 : 0.1); solvent B: water - acetonitrile - TFA (20 : 80 : 0.075) gradient elution from 10% solvent B to 40% in 2 minutes, then to 55% in 15 minutes; 1.5 ml/minute; monitored at 201 nm; 10 μg each injected.

Trisulfoxide of Duramycin B (**5**)

A solution of duramycin B (100 mg) and H_2O_2 (30 μl; 30%) in 0.5 ml acetic acid was stirred for 90 minutes at room temperature, then kept at 4°C overnight. The mixture was diluted with 0.5 ml H_2O, lyophilized and the residue applied on a preparative HPLC column (Nucleosil 5-C18, 100 Å; 16 × 250 mm; gradient after 2 minutes from acetonitrile - water - TFA (21.6 : 78.4 : 0.1 to 40.5 : 59.5 : 0.09) in 20 minutes; 6 ml/minute; Rt (product) 20 minutes; Rt (duramycin B) 25 minutes; 222 nm) to give **5** as a colorless powder (27.8 mg, 27% yield).

Physical Data: FAB-MS (M + H): 2,055, UV $\lambda_{max}^{H_2O}$ nm 193 (146,000), CD $\lambda_{extreme}^{H_2O}$ nm (θ) 203 (−213,000).

Assay for Inhibition of Phospholipase A_2 Activity

Phospholipase A_2 was extracted from human polymorphonuclear (PMN) leukocytes by treatment with acid and high salt concentration, followed by dialysis, as described previously[8]. Human synovial fluid obtained by biopsy was centrifuged and the enzyme in the cell-free supernatant was used without further

treatment. Both enzymes were assayed as described[8] by incubation for 1 hour at 37°C in the presence of 0.1 M Tris-HCl buffer pH 7.0, 1.0 mM $CaCl_2$ and 2.5×10^8 [1-^{14}C]oleate-labeled autoclaved *Escherichia coli* (5 nmol phospholipid) as substrate. Enzyme concentrations were adjusted to produce a hydrolysis of the substrate of 10~15%, which is within the linear range of the assay. The reaction was stopped by extraction with Dole reagent. Released [1-^{14}C]oleic acid was separated from substrate on silicic acid columns and determined by radiometry. IC_{50} values were determined graphically from plots of percent inhibition versus log concentration of inhibitors. The values reported are the mean ± standard error of 3~5 independent assays.

Results and Discussion

Structure and Physico-chemical Properties

Duramycin, cinnamycin and several other antibiotics of this family have been known for thirty years[9]. They contain the unusual amino acids lanthionine (abbreviation: ala-S-ala) and methyllanthionine (abbreviation: abu-S-ala) with a stable thioether bridge. Although a partial structure of duramycin was already published by GROSS *et al.* in 1976[10], the first complete structure of a member of this class, namely ancovenin, was presented by SHIBA's group only 4 years ago[11]. Amino acid analysis of the four metabolites presented in this paper also showed the presence of these unusual amino acids suggesting that they belong to the growing family of lantibiotics.

The product isolated from *S. hachijoense* (DSM 40114) was shown to be identical to duramycin by the following results: 1) Acid hydrolysis gave the same amino acid composition as published earlier[10] (Table 1). 2) The FAB-MS of the isolated material **1** and duramycin from our antibiotics collection† were identical. 3) The two compounds were esterified with methanol and gave again a matching *m/z* in the

Table 1. Amino acid composition of **1** to **4** and ancovenin.

	Duramycin (**1**) (=leucopeptin)	Cinnamycin (**2**)	Duramycin B (**3**)	Duramycin C (**4**)	Ancovenin
L-Asp/Asn[a]	0/1	0/1	0/1	0/2	1/1
L-Gln[a]	1	1	1	0	1
L-Ser			0	1	1
L-Gly	2	2	2	2	2
L-Ala			0	1	
L-Leu			1	1	1
L-Val	1	1	1	0	1
L-Pro	1	1	1	1	1
L-Phe	3	3	2	0	1
L-Tyr			0	1	
L-Trp[b]			0	1	1
L-Arg	0	1	1	0	0
L-Lys	1	0	0	0	1
L-*erythro*-3-OH-Asp	1	1	1	1	0
meso-Lanthionine	1	1	1	1	1
3-Methyllanthionine[c]	2	2	2	2	2
Lysinoalanine[c]	1	1	1	1	0
2,3-Didehydroalanine					1
Total	15	15	15	15	16

[a] Identified by gas phase sequencing.
[b] Identified by ^{1}H NMR.
[c] Identified by GC-MS of the *N*-TFA-isopropyl ester derivative.

† Duramycin, leucopeptin and cinnamycin are from our antibiotics collection.

Table 2. Physico-chemical properties of **1**~**4**.

	Duramycin (**1**)	Cinnamycin (**2**)	Duramycin B (**3**)	Duramycin C (**4**)
Formula	$C_{89}H_{125}N_{23}O_{25}S_3$	$C_{89}H_{125}N_{25}O_{25}S_3$	$C_{86}H_{127}N_{25}O_{25}S_3$	$C_{82}H_{115}N_{23}O_{27}S_3$
Form	TFA salt	TFA salt	TFA salt	TFA salt
Analysis found	—	—	C 43.01, H 5.22, N 14.19, F 7.81, S 4.79	C 44.78, H 5.77, N 13.59, F 5.14, S 4.05
Purity[a] (%)	80	62	83	78
FAB-MS $(M+H)^+$	2,014	2,042	2,008	1,951
FAB-MS of methyl ester	2,042	2,070	2,036	1,979
Residues found by Edman degradation[b]	?-Lys-Gln-?-?-?-Phe-Gly-Pro-Phe-?-Phe-Val-?-?-?-Asn	?-Arg-Gln-?-?-?-Phe-Gly-Pro-Phe-?-?-Val-?-?-?-Asn	?-Arg-Gln-?-?-?-Phe-Gly-Pro-Leu-?-?-Val-?-?-?-Asn	?-Ala-Asn-?-?-?-Tyr-Gly-Pro-Leu-?-Trp-Ser-?-?-?-Asn
Chemical nature	Amphoteric, basic	Amphoteric, basic	Amphoteric, basic	Acidic
Decomposition point	—	—	290°	280°
$[\alpha]_D^{25}$	−65° (*c* 0.25, H_2O)[c] −53° (*c* 0.5, 0.1 N HCl)	—	−57° (*c* 0.57, 0.1 N HCl)	−94° (*c* 0.5, H_2O) −61° (*c* 0.5, 0.1 N HCl)
CD $\lambda_{extreme}^{H_2O}$ nm (θ)	208 (−173,000)	208 (−150,000)	208 (−190,000)	204 (−158,000), 236 (+4,900), 268 (−1,100)
IR (KBr) cm^{-1}	3410, 3080, 2940, 1680, 1500, 1400, 1380	3380 (br), 2920, 2860, 1680, 1520, 1430	3420 (br), 2960, 2920, 1650, 1520, 1450, 1440	3400 (br), 2960, 2920, 1660, 1520, 1450
UV $\lambda_{max}^{H_2O}$ nm (ε)	196 (94,300), 257 (1,590), 264 (sh)	196 (91,300), 258 (1,320), 264 (1,190)	196 (90,800), 258 (710), 264 (610)	198 (100,000), 220 (sh), 277 (8,650), 288 (sh)

[a] According to amino acid analysis of the salt free peptide.

[b] Sequencing yields after step 10 are very weak. Assignments are based on the known amino acid composition and are consistent with the published sequences of Ro 09-0198[14] and ancovenin[11]. Asn can clearly be distinguished from Asp.

[c] Although the published value for duramycin is much lower (−6.5°, *c* 3.9, water)[9], all other duramycin antibiotics have $[\alpha]_D$ values in a similar range (*e.g.* Ro 09-0198 $[\alpha]_D^{25}$ −72° (*c* 1, 0.1 N HCl)[16]) including our own value for duramycin.

FAB-MS. 4) Both antibiotics eluted at the same Rt in the HPLC (see Fig. 1). Using the same method it was demonstrated that strain DSM 40165 (*S. longisporoflavus*) produces cinnamycin[12].

Moreover another previously unidentified antibiotic namely leucopeptin†, a peptide isolated by KONDO *et al.* in 1964[13], was proven to be identical to duramycin by the same procedures described above.

In Table 1 the amino acid compositions of some duramycin type antibiotics are compared. Cinnamycin (**2**) has the same amino acid composition as Ro 09-0198[14], also called lanthiopeptin[15]. In the first patent application[16] this antibiotic was reported to contain D-phenylalanine residues. However, in a more recent publication, the presence of the L-form of this amino acid could be unambiguously established[17].

In duramycin B (**3**) one of the three phenylalanines found in **2** is replaced by leucine. Preliminary structural data on leucinamycin[18] indicate a close homology to **3**. Leucinamycin appears to contain four glutamic acids, whereas **3** has only a single glutamine.

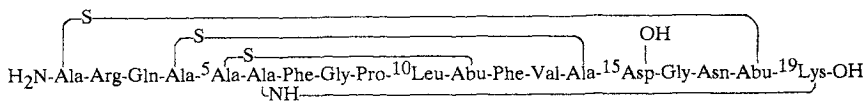
In contrast to compounds **1** to **3** duramycin C (**4**) has no basic amino acid residue, but it contains tyrosine and tryptophane which give rise to the characteristic UV spectrum of **4**. Therefore, its amino acid composition is similar to ancovenin. But important differences are seen in the unusual amino acids. In ancovenin the formation of the lysinoalanine ring from 2,3-didehydroalanine and lysine has not taken place. The close structural resemblance between duramycin C and ancovenin suggests a common biosynthetic pathway with ring closure at a very late stage. Formation of lysinoalanine during workup of **4** can be excluded, since the HPLC retention time of **4** in the crude fermentation broth and as a pure compound is identical.

The physico-chemical properties of compounds **1** to **4** are compiled in Table 2. For the first time we present FAB-MS data of duramycin and cinnamycin. The mass difference of m/z 28 between the methyl esters and the parent compounds suggests that they all have only two acidic functions. Therefore the structures of duramycin and cinnamycin showing four acidic groups as reported by GROSS[12] have to be revised. Sequencing data of duramycin and cinnamycin reveal glutamine (and not glutamic acid) in position 3 and asparagine instead of aspartic acid in step 17. Based on all our results cinnamycin **2** is most likely identical to Ro 09-0198. The proposed structures of the new compounds **3** and **4** are shown in Fig. 2.

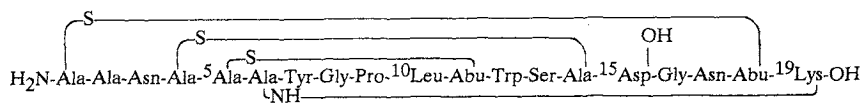
Fig. 3 presents a general structural skeleton for all duramycin type antibiotics. The thioether bridges as assigned for ancovenin[11] and Ro 09-0198[14,15] are assumed to be common for all. Further work on the structure of compounds **1** to **4** is in progress.

The CD spectra of all duramycin antibiotics look very similar showing a characteristic negative

Fig. 2. Proposed structure of duramycins B (**3**) and C (**4**).



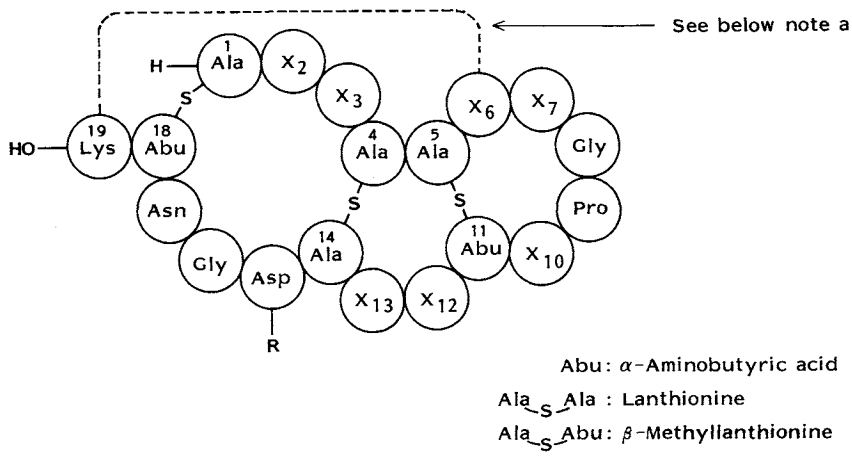
3



4

† See p. 1407.

Fig. 3. Proposed general structure of duramycin type antibiotics.



	Duramycin (1) (=leucopeptin)	Cinnamycin (2) (Ro 09-0198)	Duramycin B (3)	Duramycin C (4)	Ancovenin
X_2	Lys	Arg	Arg	Ala	Val
X_3	Gln	Gln	Gln	Asn	Gln
X_6	Ala[a]	Ala[a]	Ala[a]	Ala[a]	Dha[b]
X_7	Phe	Phe	Phe	Tyr	Phe
X_{10}	Phe	Phe	Leu	Leu	Leu
X_{12}	Phe	Phe	Phe	Trp	Trp
X_{13}	Val	Val	Val	Ser	Ser
R	OH	OH	OH	OH	H

[a] Alanine, linked to ^{19}Lys as lysinoalanine.
[b] Dehydroalanine, not bridged to ^{19}Lys.

Table 3. Inhibition of human phospholipase A_2 by duramycins, cinnamycin and 5.

Phospholipase A_2 from	IC_{50} (μM)				
	Duramycin (1)	Cinnamycin (2)	Duramycin B (3)	Duramycin C (4)	Trisulfoxide (5)
Human PMN leukocytes	1.1±0.1	0.9±0.1	1.5±0.1	0.8±0.1	>30
Human synovial fluid	0.6±0.1	0.6±0.1	1.0±0.1	1.0±0.1	>30

ellipticity at 208 nm. The strong molar ellipticity suggests a very rigid conformation. The pattern displays no similarity to the reference spectra of proteins in α-helix, β-sheet or random coil conformation[19].

To get some insight into the structure-activity relationship the trisulfoxide of duramycin B (5) was prepared by peracetic acid oxidation. The identity of the product was established by FAB-MS. According to ^{1}H NMR it consisted of a mixture of diastereoisomers.

Biological Activity

Duramycins B and C are potent inhibitors of human phospholipase A_2. As shown in Table 3 both compounds inhibited the enzyme from PMN leukocytes and synovial fluid with IC_{50} in the micromolar range. Similar activity was observed with the chemically related antibiotics duramycin and cinnamycin. Surprisingly, the trisulfoxide of duramycin B (5) did not inhibit at concentrations up to

30 μmol/liter indicating that at least one of the thioether groups is essential for biological activity.

Further studies on the mechanism of action and the effect of these compounds on other phospholipases are in progress and will be reported elsewhere.

As shown in Table 4, duramycins B and C exhibit only a weak antibacterial activity against *Bacillus subtilis*. Their antimicrobial spectrum is therefore very similar to Ro 09-0198, which was also only active against the same highly sensitive Gram-positive test strain (MIC 0.78 μg/ml)[16].

Table 4. Antimicrobial activity spectra of duramycins B and C.

Test organism	Inhibition zone (mm) with 20 μg/2 μg antibiotic per disc	
	Duramycin B (3)	Duramycin C (4)
Bacillus subtilis ATCC 6633	13/10	10/0
Staphylococcus aureus ETH 2070	0/0	0/0
Streptococcus mitis BHT 51	0/0	0/0
Escherichia coli ETH 2018	0/0	0/0
Klebsiella pneumoniae K 1080	0/0	0/0
Proteus vulgaris ATCC 9484	0/0	0/0
Candida albicans ETH 6370	0/0	0/0

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