zoate, inhibits DNA synthesis[23]. Micronuclei formation leads to a loss of genetic material and is a true mutagenic effect[24]. There is a strong correlation between chromosome aberrations and mutagenicity[25]. Although benzoate and sulphite are not mutagenic in *Drosophila* and *Salmonella*, the fact that they cause chromosome aberrations leading to a loss of genetic material, inhibit DNA synthesis, and might interact with DNA and bases, calls for a closer look at their genetic toxicological effects.

1 Present address: Institute of Animal Genetics, University of Edinburgh, Edinburgh, Scotland.
2 Reprint requests should be addressed to: Department of Botany, University of Nairobi, P.O. Box 30197, Nairobi, Kenya.
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Nosiheptide, a sulfur-containing peptide antibiotic isolated from *Streptomyces actuosus* 40037

F. Benazet, M. Cartier, J. Florent, C. Godard, G. Jung, J. Lunel, D. Mancy, C. Pascal, J. Renaut, P. Tarridec, J. Theilleux, R. Tissier, M. Dubost and L. Ninet

Rhône-Poulenc Industries, Recherche et Développement, Centre Nicolas Grillet, 13 quai Jules Guesde, F-94400 Vitry-sur-Seine (France), 17 July 1979

Summary. Nosiheptide (9671 R.P.) isolated from *Streptomyces actuosus* 40037 (NRRL 2954) is a sulfur-containing polypeptidic antibiotic, quite different from all the other members of this family. Very active in vitro against gram-positive bacteria, it is inactive in vivo in experimentally infected mice. Not toxic, even at high dose, it may be used as a feed additive for chickens and pigs and it shows a favourable effect on the growth and conversion index.

In the course of a study on the production of antimicrobial agents by microorganisms, an antibiotic, nosiheptide (also known as 9671 R.P.), was discovered in the culture broths of *Streptomyces actuosus* 40037 (NRRL 2954)[1]. The main features of this strain have been reported by Shirling and Gottlieb[2].

Nosiheptide is obtained by culture of *Streptomyces actuosus* 40037 on aerated and stirred media as follows: the strain, stored as a spore-soil mixture, is grown in test tubes on Bennett's agar medium[3] for 3 weeks at 26 °C. It is brought to a suitable development by 2 successive transfers, first into 250 ml of liquid medium (composition in g/l: corn-steep liquor 20, saccharose 30, ammonium sulfate 2, calcium carbonate 7.5) in a 2-l flask, incubated for 48 h at 27 °C on a rotary shaker, then into 500 l of the following medium (in g/l): peptone 10, yeast extract 5, glucose monohydrate 10, agar 2, in a 800-l stainless steel fermenter in which the culture is agitated and aerated for 24 h at 27 °C. The final culture is obtained by seeding 50 l of the previous culture into a 800-l stainless steel fermenter containing 500 l of the following medium (in g/l): soybean flour 40, distillers' solubles 5, soybean oil 20, sodium chloride 5, heptahydrated magnesium sulfate 0.5, monohydrated manganese sulfate 0.3, heptahydrated ferrous sulfate 0.2, pentahydrated copper sulfate 0.02, hexahydrated cobalt chloride 0.02. The culture is agitated, aerated and kept at

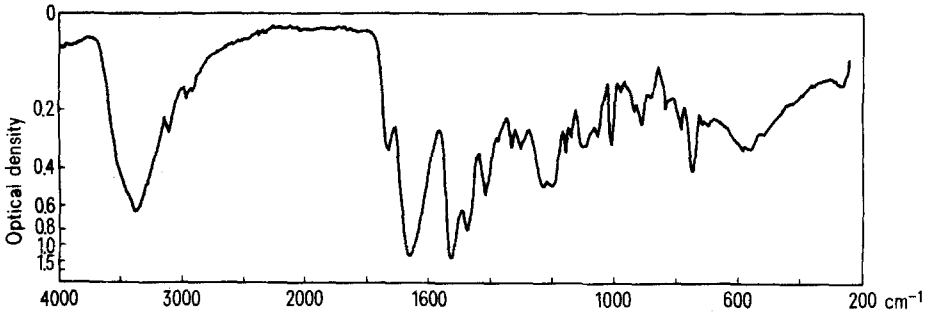

Fig. 1. IR-absorption spectrum of nosiheptide (KBr pellet).

27 °C. The production of nosiheptide reaches 600 µg/ml after 6 days. Assays are performed according to Pascal et al.[4].

The antibiotic is essentially associated with the mycelium which is first collected by filtration at pH 5 with a filter-aid, Clarcel DIC. Nosiheptide is extracted from the mycelium cake by a volume of methylene chloride-isopropanol (67:33, v/v) equal to 75% of the initial broth. After filtration, the cake is washed by the same mixture and the spent mycelium is discarded. Filtrate and wash are combined; after decantation and elimination of the aqueous phase, the distillation under reduced pressure of the solvent phase to 15 l affords a crude precipitate containing about 70% of the antibiotic.

The antibiotic may be purified by several techniques, mainly by preparative high pressure chromatography. For example, a stainless steel column (h = 1 m; diameter = 5 cm) is packed with 1 kg of silicagel, mixed with chloroform (minimal volume); washing is carried out under pressure by 10 l of flowing chloroform-ethanol-water mixture (95:5:0.25, v/v). 6 g of the crude nosiheptide, dissolved in 150 ml of chloroform-ethanol-water (80:20:0.25, v/v) is then poured in at the top of the column. Elution is achieved by 50 l of chloroform-ethanol-water mixture (95:5:0.25, v/v) under an inlet pressure of 1150 psi and a flow rate of 5.5 l/h. The eluate is collected in 2.5-l fractions. After TLC analysis (silicagel Merck F 254 plate activated at 110 °C for 1 h; solvent: toluene-methanol-pyridine-acetic acid, 70:15:15:2, v/v), fractions containing nosiheptide are combined, concentrated under reduced pressure and dried at 40 °C for 15 h (5 mm/Hg). Pure nosiheptide (3.6 g) is obtained as a yellow microcrystalline powder [m.p. 310–320 °C with decomposition, $[\alpha]_D^{20} = +38°$ (c = 1 in pyridine)]. It is soluble in pyridine and dimethylformamide as well as in methylene chloride-isopropanol (70:30, v/v), very slightly soluble in chloroform and acetone and practically insoluble in water, methanol, 95% ethanol and petroleum ether.

In vitro bacteriostatic activity of nosiheptide

Test organism	Minimal inhibitory concentration (µg/ml)
Staphylococcus aureus (strain 209 P - ATCC 6538 P)	0.0009
S. aureus (strain 133, Institut Pasteur)	0.0019
S. aureus (strain B_3, resistant to streptomycin and penicillin)	0.003
S. aureus (strain Hb, resistant to tetracyclin and penicillin)	0.003
Micrococcus citreus (ATCC 8411)	0.0038
M. lysodeikticus (ATCC 4698)	0.003
Sarcina lutea (ATCC 9341)	0.0011
Streptococcus faecalis (ATCC 9790)	0.0007
S. viridans (Institut Pasteur)	0.0065
S. pyogenes hemolyticus (strain Dig 7, Inst. Pasteur)	0.00028
Diplococcus pneumoniae (strain Til, Institut Pasteur)	0.00015
Neisseria catarrhalis (Faculté de Pharmacie, Paris)	0.0017
Lactobacillus casei (ATCC 7469)	0.0007
Bacillus subtilis (ATCC 6633)	0.003
B. cereus (ATCC 6630)	0.0071
Mycobacterium smegmatis (ATCC 607)	> 138
Escherichia coli (ATCC 9637)	> 138
Salmonella typhimurium (Institut Pasteur)	> 138
Aerobacter aerogenes (ATCC 8308)	> 138
Proteus vulgaris (Faculté de Pharmacie, Paris)	> 138
Klebsiella pneumoniae (ATCC 10031)	> 138
Serratia marcescens (A 476, Lausanne)	> 138
Pseudomonas aeruginosa (strain Bass, Institut Pasteur)	> 138
Brucella bronchiseptica (CN 387, Wellcome Institute)	> 138
Pasteurella multocida (A 125, Institut Pasteur)	0.0024

The IR-spectrum (KBr pellet) is given in figure 1. The UV-spectrum of a solution of nosiheptide in methanol does not present any characteristic UV-absorption peak. Nosiheptide presents a typical fluorescence-spectrum which is given in figure 2.

Elemental composition: C 49.6%, H 4.0%, N 14.4%, O 16.7%, S 15.8% agrees with the molecular formula $C_{51}H_{43}N_{13}O_{12}S_6$ resulting from different studies conducted by Prange et al.[5], Pascard et al.[6] and Depaire et al.[7]. The mol.wt is 1222.37. The structure is given in figure 3.

Numerous antibiotics exhibiting a high sulfur content, thiazole rings and polypeptidic chains with threonyl, alanyl and dealanyl units are described in the literature and present structural analogies with nosiheptide. The main ones are: A 59[8], actinotiocin[9], althiomycin[10] identical to matamycin[11], micrococcin P[12], pepthiomycin[13], pumilin[14], saramycetin or X-5079C[15], siomycin A[16] identical to mutabilicin[17] and sporangiomycin[18], sulfactin[19], sulfomycins[20], taitomycins[21], thermothiocin[22], thiocillins[23], thiopeptins[24], thiostrepton[7,16] identical to bryamycin[25] and thiactin[26] and antibiotic compound 46192[27]. All of them differ from nosiheptide in some characteristics: sulfur or nitrogen content, parts of structure, toxicity. Recently multhiomycin[28] has been shown to be identical to nosiheptide by Endo and Yonehara[29].

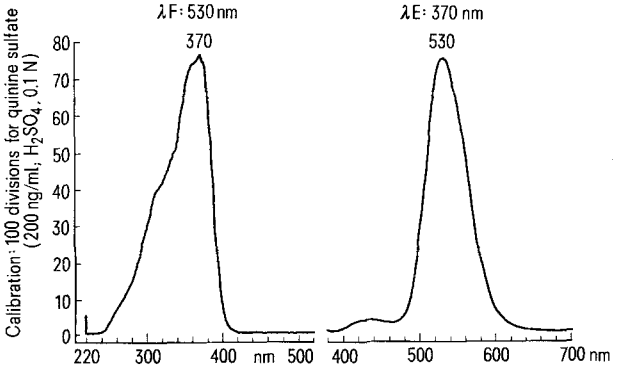

Fig. 2. Fluorescence spectrum of nosiheptide (solvent: chloroform).

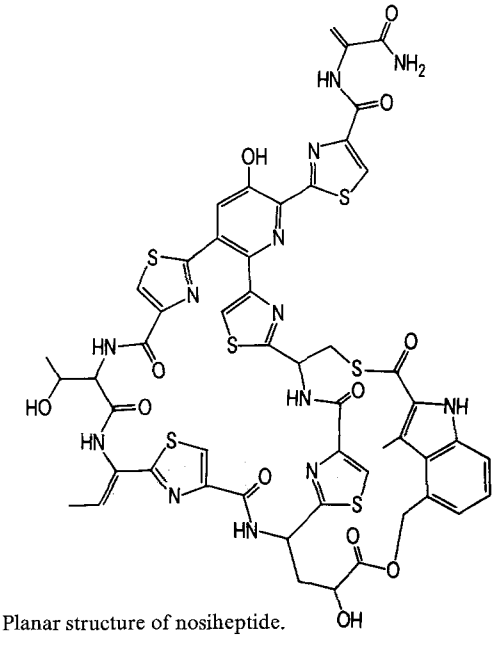

Fig. 3. Planar structure of nosiheptide.

Nosiheptide exhibits no toxicity when administered to the mouse by the oral, s.c. and i.p. routes at the dose of 2.5 g/kg. The bacteriostatic activity of nosiheptide against some microorganisms is shown in the table. The minimum inhibitory concentration (MIC) determinations were carried out by the dilution method in the appropriate medium for each microorganism and after incubation for 18 h at 37 °C. Nosiheptide is mainly active in vitro against gram-positive cocci and bacilli, including staphylococcal strains resistant to penicillin, streptomycin, tetracyclin and numerous other antibiotics such as chloramphenicol, novobiocin, spiramycin and erythromycin. It is also active against certain gram-negative bacteria such as *Neisseria catarrhalis* and *Pasteurella multocida*. In vitro, nosiheptide reveals an activity against staphylococci and streptococci which is similar or superior to that of penicillin G. In vivo, nosiheptide does not exhibit any systemic activity either p.o. or s.c. in mice infected experimentally with staphylococci or streptococci. However, in mice with a s.c. or i.p. infection by staphylococci, it is active when injected immediately after the germs into the site of inoculation.

Used as a feed additive on the proportion of 2.5–10 g per ton, nosiheptide shows a favourable effect on the growth and conversion index in chickens[30,31] and pigs[32]; it is not detected in the flesh of chicks maintained during 8 weeks on a diet containing 200 g/t[33].

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Polypeptides of the lens fibre cell intracellular matrix[1]

M. Katar, W.-K. Lo, M. Nagpal and H. Maisel

Department of Anatomy, Wayne State University School of Medicine, Detroit (Michigan 48201, USA), 26 June 1979

Summary. The cytoskeletal proteins of the vertebrate lens fibre cell comprise polypeptides ranging in mol.wt from 43,000 to 250,000 daltons. The main intermediate filament polypeptide of the pickerel, Northern frog, chick, bovine, and human lens has a mol.wt of 54,000 daltons. Peptide analysis revealed that the chick 54,000 dalton protein was more similar to the bovine protein than to the pickerel protein.

The water-insoluble protein of the lens can be fractionated by 8 M urea into urea-soluble and urea-insoluble components[2]: The urea-insoluble fraction is enriched in membrane, while the urea-soluble fraction contains lens crystallins (water-soluble protein) as well as the noncrystallin proteins of the fibre cell matrix or cytoskeleton[2].

This study presents a comparative electrophoretic analysis of the urea-soluble protein of lenses of different species. The isolation and peptide analysis of one of the noncrystallin polypeptides is also reported.

Lenses were obtained from the following mature animals immediately on sacrifice of the animal: bovine *(Bos taurus)*, chick *(Gallus domesticus)*, Northern frog *(Rana pipiens)*, and fish (pickerel; *Esox vermiculatus*). Human lenses obtained from 2 newborns (set A and B) were removed within 24 h after death. The cortex fibre cell mass was dissected from each lens and homogenized in standard salt buffer (designated SM) consisting of 0.1 M KCl, 0.005 M $MgCl_2$, 0.006 M sodium phosphate buffer pH 7.2, to which 0.001 M 2-mercaptoethanol was added.