Prianicin A and B, nor-sesterterpenoid peroxide antibiotics from Red Sea sponges[1]

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Summary. The antibiotic properties of 2 acidic $C_{24}H_{40}O_4$ nor-sesterterpenoid peroxides, prianicin A (**1**) and B (**2**), against gram-positive and gram-negative bacteria, and against fungi are herein described. They are 4–10 times more effective than tetracycline against *beta hemolytic Streptococcus,* but significantly non-effective against a variety of gram-negative bacteria.

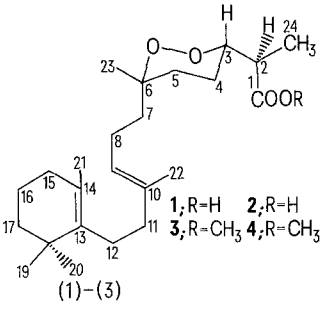
The bacterial resistance to antibiotics could be justly viewed as the most serious challenge to their long-standing value as chemotherapeutic agents providing a quest for new structures of antibiotics. As a potential source, marine organisms could rival terrestrial life forms in producing novel antibacterial and antiprotozoal compounds of therapeutic interest. To investigate organisms with this end in view, a team of researchers from this laboratory[4] undertook a few years ago a survey of sponges endogeneous to the Red Sea demonstrating antibiotic activity. As a result, *Prianos sp.*[5], sponges collected off-shore in Eilat Bay at a depth of some 30 m were selected as a possible source because of the activity they showed against gram-positive bacteria.

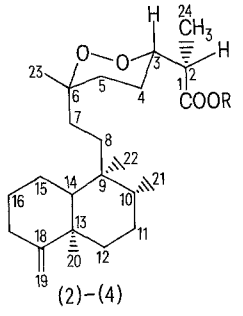
The name 'Prianicin' was given initially to the active principle which was isolated from the methanolic extract of the whole sponge, *Prianos sp.*, by chromatography over silica-gel column (using 1.2%-CH_3OH in CH_2Cl_2 as eluting solvent). Analysis of the 300 MHz NMR-spectrum of 'Prianicin' revealed that, in fact, it was a 1:4-isomeric mixture of 2 acidic $C_{24}H_{40}O_4$ nor-sesterterpenoid peroxides, which could be separated by flash chromatography[5] over an argentated silica-gel column. The major component (yellow oil) was given the name prianicin A. Study has shown that it was in fact identical to Muqubilin, reported earlier by Kashman and Rotem[7], to which we assigned structure (**1**)[8]. The minor component (yellow oil) was given the name prianicin B, and shown to be identical with the fish-toxin, sigmosceptrillin-B[9], being isolated from the *Sigmosceptrella laevis,* off the coast of Papua-New Guinea, and to be of structure (**2**)[8]. Neither (**1**) or (**2**), nor their corresponding methyl esters (**1a**) or (**2a**), could be induced to crystallize.

Sensitivity tests[10] for (**1**) and (**2**) towards various microorganisms were effected by application of agar diffusion methods and 6 mm discs. For gram-positive bacteria Brain Heart Infusion Agar (Difco) was used, Nutrient Agar (Difco) was used for gram-negative bacteria, and Sabouraud Dextrose Agar (Difco) for *Saccharomyces cerevisiae.* For determination of minimal inhibitory concentration (MIC) for *Staphylococcus aureus* and *Streptococcus sp.,* Brain Heart Infusion broth (Difco), and Medium 3 (Difco), were used, respectively. Tetracyclin-HCl (Teva Ltd) and Mycostatin (E.R. Squibb and Sons) were used as antibiotic standards.

The microorganisms used for sensitivity tests were derived from 4 sources: 1. the Central Laboratory of the Ministry of Health, Jerusalem (CLMH); 2. the Laboratory of Clinical Microbiology, Hebrew University-Hadassah Hospital, Jerusalem (LCM); 3. the Hebrew University Marine Biological Laboratory, Eilat (MBLE); 4. the American Type Culture Collection, Rockville, Maryland (ATCC).

From the table it can be seen that prianicins inhibited the growth of gram-positive bacteria quite remarkably, in-





Antibiotic properties of prianicins

Organism	Antibiotic	Extent of inhibition (in mm)	M.I.C. μg/ml
Beta hemolytic Streptococcus	Tetracycline	8	6.3
strain CLMH	Prianicins A, B[a]	> 13	1.5
	Prianicin A	13	2.5
	Prianicin B	> 13	1.0
strain LCM	Prianicin A	–[b]	10.0
	Prianicin B	–	4.0
Staphylococcus aureus	Tetracycline	7	12.5
strain CLMH	Prianicin A	9	12.0
Streptococcus faecalis	Tetracycline	13	–
ATCC 8043	Prianicin A	9	–
Corynebacterium diphteriae	Tetracycline	13	1.5
strain MBLE	Prianicin A	7	3.0
Escherichia coli	Prianicins A, B no inhibition at 1 mg per disc		
strain ATCC 8739			
Salmonella paratyphi B	Prianicins A, B no inhibition at 1 mg per disc		
strain CLMH			
Pseudomonas aeruginosa	Prianicins A, B no inhibition at 1 mg per disc		
strain CLMH			
Saccharomyces cerevisiae	Mycostatin	13	–
strain ATCC 9763	Prianicins A, B	7	–

[a] Prianicin A, B stand for a 4:1 mixture of prianicin A and prianicin B; [b] – = not tested.

terestingly, were non-effective against the gram-negative bacteria included in this study. Most spectacular is their activity against *beta hemolytic Streptococcus,* the CLMH strain of which is 4-fold more sensitive to prianicins than to tetracycline. Structurally, prianicin A and B are alike in comprising a 6-membered ring cyclic peroxide but differ from each other in the stereochemistry of the propionic acid side-chain, on the one hand, and in the carbon skeleton of the $C_{15}H_{25}$-isoprenic side-chain, on the other hand. These differences are reflected in their antibacterial potency. Thus, (**2**) being 2.5-fold more effective than (**1**) against the CLMH strain of *beta hemolytic Streptococcus.*

Of particular interest is the activity of prianicins against the yeast species *Saccharomyces cerevisiae.* The importance of this observation lies in the ability of prianicins to prevent secondary infections due to pathogenic fungi.

The data now at hand strongly suggest that (**1**) and (**2**) are of therapeutic potential, meriting further study in the sense of structure-activity relationship. A study towards this end is currently in progress in our laboratory.

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The efficacy of a novel compound, (*E*)-1-(4′-bromo-4-biphenylyl)-1-(4-chlorophenyl)-3-dimethylaminoprop-1-ene against *Trypanosoma cruzi* in mice

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Summary. The biological properties of a novel compound 353C with high activity against *Trypanosoma cruzi,* are described. The compound was about 10 times and 20 times more effective than either benznidazole or nifurtimox respectively, in producing radical cure in mice. 353C had a long half-life and showed anti-trypanosomal properties when given to mice at weekly intervals.

South American trypanosomiasis (Chagas' disease), which is caused by the protozoan *Trypanosoma cruzi,* is primarily vector-borne and is transmitted by reduviid bugs of the sub-family *Triatominae.* It has been estimated[1] that some 12 million persons are affected by the disease, which is an important cause of morbidity and mortality in endemic areas in South and Central America (particularly Brazil, Venezuela and Argentina). Current therapy of the disease is unsatisfactory[2]. Only 2 drugs (nifurtimox and benznidazole) are in general use. However both produce a high incidence of side-effects at therapeutic dose levels (10 mg kg^{-1} day^{-1} for 60–90 days, and 5–10 mg kg^{-1} day^{-1} for 30–60 days respectively). Both drugs are less effective in the chronic stages of the disease than they are in the acute phase. We report here the synthesis and biological activities of (*E*)-1-(4′-bromo-4-biphenylyl)-1-(4-chlorophenyl)-3-dimethylaminoprop-1-ene (353C) (fig.), one of a series of more than 130 1,1-diaryl-3-aminoprop-1-enes possessing high efficacy against *T. cruzi* in experimentally infected animals.

Synthesis of 353C was achieved by the Wittig reaction between 4′-bromo-4-biphenylyl 4-chlorophenyl ketone and the ylid from 2-(dimethylamino)ethyl triphenylphosphonium bromide. After separation from the accompanying (Z)-isomer, 353C was converted into the tartrate salt m.p. 163 °C.

Following conventional techniques[3], the activity of 353C (as tartrate salt) in mice (strain CDI) was determined using an oral regimen of 5 once-daily doses. The drug was shown to be highly effective in suppressing infections induced by 5 stocks of *T. cruzi,* with ED_{50}-values which ranged from 0.5 mg $kg^{-1} \times 5$ to 3.0 mg $kg^{-1} \times 5$ (as base).

In experiments designed to examine the ability of the drug to effect radical cure (sterilization) of *T. cruzi* infections, mice were infected with a variety of stocks of the parasite. When the infection was established, 353C was administered orally once daily for 30 days, then 30 days after the last dose, blood from each mouse was collected, cultured and examined for the presence of parasites[3]. The results obtained (table) indicated that 353C was about 10 times more potent than benznidazole and about 20 times more active than nifurtimox.

In mice (strain CDI) infected with a Peruvian stock of *T. cruzi,* 353C (as tartrate) appeared to be a unique trypanocide, in that it cured > 90% of infected animals when it was administered orally as 7 once-weekly doses of 25 mg kg^{-1} (as base).