November/December 2006

SIM NEWS

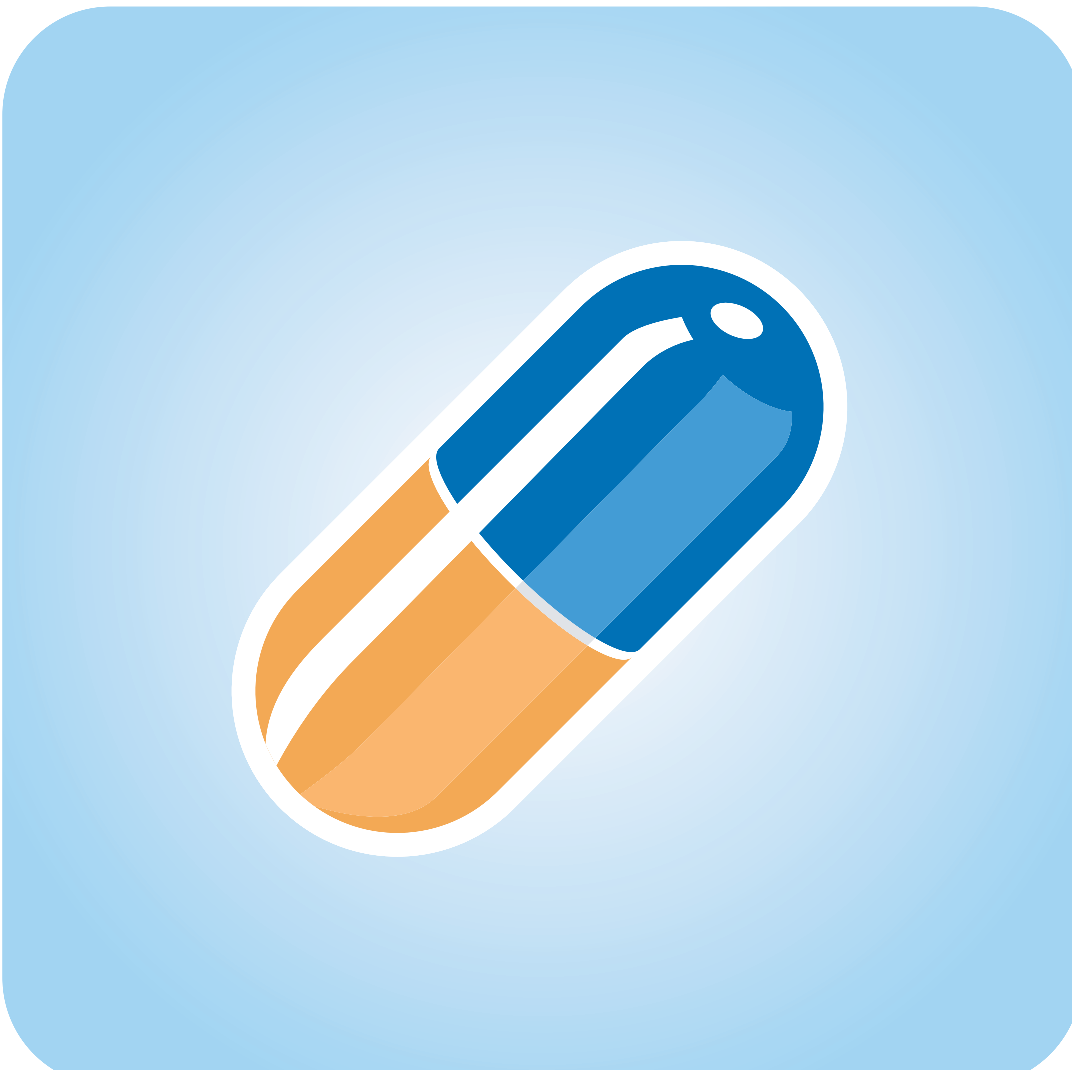
The Official News Magazine of the Society for Industrial Microbiology

Vol. 56, No. 5

Forty years of antibiotic research at Lepetit

Forty Years of Antibiotic Research at Lepetit:

A Personal Journey



By Giancarlo Lancini

In 1868 the French chemist Roberto Giorgio Lepetit established a company in northern Italy for production of tannins, dyes, and inks. The enterprise, named Ledoga, flourished thanks to some original production processes invented in the company's laboratories. Roberto Lepetit, son of Roberto Giorgio Lepetit and as talented a chemist as his father, extended the firm's research mandate to include pharmacologically active products. His principal success was the invention of nevralteine (sodium phenetidinomethanesulphonate), an analgesic antipyretic that was used for half a century, and which in 1905 became the first Italian chemical invention to receive United States patent protection. Roberto Lepetit created within Ledoga a pharmaceutical department that in 1929 became a separate company, Società Anonima Lepetit (later Lepetit S.p.A.). During the ensuing decades Lepetit grew into one of the leading pharmaceutical houses in Italy and a major innovator in the field of microbial natural products.

During Lepetit's formative years the economic and political situation in Italy was not especially supportive of new drug discovery (until 1978 Italian law forbade the granting of Italian patents to pharmaceutical products). Therefore, rather than novel drug discovery, Lepetit focused on process development. A noteworthy success was achieved in early 1949 with a new economic synthesis of chloramphenicol. As a result, Lepetit became the world leader in the manufacture of this antibiotic. Shortly thereafter, a large fermentation plant was built in Torre Annunziata near Naples for production of antibiotics, initially tetracyclines and later novobiocin. At the company's research headquarters in Milan, a Fermentation and Natural Products Department was created, charged with supporting antibiotic manufacture by improving fermentative production and downstream processing. This department was comprised of young, close-knit, motivated scientists who rapidly gained experience in disciplines as diverse as actinomycete biology, fermentation engineering, biological and physicochemical analysis of spent media, and extraction and recovery techniques. Efforts by the Fermentation and Natural Products Department, including classical strain mutagenesis and selection procedures, led to improvements in tetracycline production and the discovery of a novel microbial product, bromotetracycline (Sensi et al., 1955). Following their success with tetracycline, Lepetit's research management initiated a programme of antibiotic discovery.

Early screening

When I joined the Fermentation and Natural Products Department in February 1957, Lepetit's antibiotic screening program, under the direction of Piero Sensi, had been running for a couple of years during which time the original screening process had been streamlined and made more efficient. Screening personnel worked in contiguous laboratories on the same floor of the Lepetit building, which facilitated exchange of information between the chemistry and biology units. At this time antibiotic screening was predicated on conventional isolation and cultivation of soil streptomycetes, whereas extraction and purification of active substances from spent media was accomplished using state-of-the-art equipment, most notably a Craig countercurrent distribution apparatus (Fig. 1); this enabled a complete fractionation of >1 g of crude extract in less than 24 h. Considerable time and effort was spared by evaluating crude or partially purified extracts in animal models of infection in order to identify interesting leads and allocate precious resources to promising products.

Discovery of the rifamycins

Toward the end of 1957 an actinomycete strain was isolated from a soil sample collected near St. Raphael, France that was classified as *Streptomyces mediterranei* (subsequently reclassified as *Nocardia mediterranei* and then as *Amycolatopsis mediterranei*). This microorganism was found to produce antibacterial substances, and a crude extract of the spent medium showed interesting activities against Gram-positive bacteria and a protective effect in murine models of infection. Paper chromatography revealed an antibacterial complex comprised of four very active substances, named rifamycins A, C, D, and E, which had similar biological and chemical properties, and a fifth, less active substance, called rifamycin B, which separated cleanly from the other four rifamycins due to its acidic properties (Sensi et al., 1960b). (Though originally called "rifomycins," the name of these microbial products was changed to "rifamycins" at the advice of Lepetit's legal department due to the similarity of "rifomycin" to the name of a different antibiotic.)

Fractionation and purification of rifamycins A, C, D, and E proved very challenging due to their instability in solution. Fractionation, which could be accomplished only by countercurrent distribution, indicated that rifamycins C and D were chiefly responsible for the antibacterial activity of the spent medium, but these components could not be obtained as single pure substances in reasonable quantities. However the most serious drawback was the tissue damage and even necrosis observed in different animal species at the site of injection during tolerability tests of rifamycins C and D.

Rifamycin B, previously overlooked because of its poor bioactivity, was then reconsidered. In its favor were its easy purification and crystallization, the absence of cross-resistance with known antibiotics, and the fact that rifamycin B had an extremely low toxicity, even though it showed only a modest efficacy in experimental animal infections (Sensi et al, 1960b; Timbal, 1960).

Rifamycin B represented only 10-15% of the weight of the rifamycin complex and production of even the modest amounts of rifamycin B required for a thorough assessment of its biological properties posed

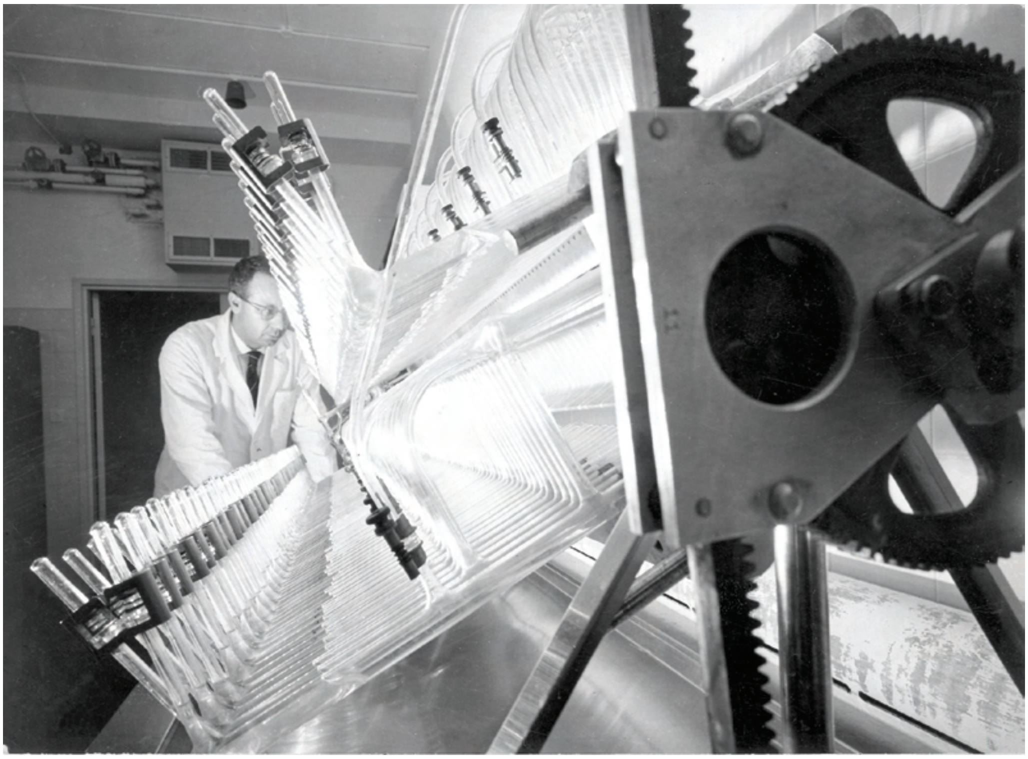


Fig. 1. Dr. Giancarlo Lancini operating the Craig countercurrent distribution apparatus (1962), which for a long time was a fundamental instrument for fractionating crude antibiotic extracts.

a serious problem. It proved serendipitous that addition to cultures of 2 g/L of barbital (diethyl barbituric acid) profoundly altered the rifamycin production profile. In the presence of 2 g/L of barbital rifamycins A, C, D, and E were no longer produced, whereas the rifamycin B yield was increased fivefold. Fortunately, in those days analyses of fermentation broths were performed by UV spectroscopy, which revealed a shift in the absorption maximum of broth extracts from fermentations performed in the presence of barbital. Had analyses been guided by bioassay alone, it would have been concluded that barbital decreased rifamycin yield. Subsequent experiments with a score of different barbiturates showed that only a few such compounds produced a similar (albeit weaker) effect on rifamycin output, and that 2 g/L of barbital were precisely the amount needed for optimal production of rifamycin B (Margalith and Pagani, 1961).

The relatively modest antibacterial activity of rifamycin B did not augur well for its development as a major antibiotic able to compete with the likes of penicillin, chloramphenicol, tetracycline, or erythromycin; nonetheless, its biological and chemical properties were investigated, and to everyone's surprise it was discovered that if an aqueous solution of rifamycin B were allowed to stand undisturbed, its bioactivity would gradually increase. This suggested a slow chemical transformation of rifamycin B into something more active, and it was postulated that this transformation was due either to oxidation by dissolved O_2 or to a spontaneous hydrolysis. Treatment of rifamycin B with mild oxidants resulted in its complete conversion into a substantially more bioactive product, dubbed rifamycin O (Oxidized) (Sensi et al., 1960a); however, UV spectroscopic analysis indicated that rifamycin O was not the compound that formed spontaneously from rifamycin B. When rifamycin O was subjected to mild hydrolysis, still another highly bioactive product was obtained, whose chemical properties matched those of the material formed spontaneously from rifamycin B; this second product was called rifamycin S (Self-activated). Rifamycin S proved to be a quinone with very high bioactivity, but it was also poorly soluble at neutral pH and, when injected into animals, was somewhat toxic and poorly tolerated. Examination of the site of injection revealed that the rifamycin S had become discolored, suggesting that it had been reduced to a hydroquinone by body fluids. It was surmised that the toxicity of rifamycins was related to their oxidation potential. Reduction of rifamycin S by shaking aqueous solutions with vitamin C produced the hydroquinone form, called rifamycin SV (V for "vitaminized") (Sensi et al., 1961).

Rifamycin SV had high in vitro activities against Gram-positive bacteria, *Mycobacterium tuberculosis*, and *Neisseria gonorrhoeae*, but only modest activity towards Gram-negative pathogens. Despite its high in vitro activity against *M. tuberculosis*, rifamycin SV had poor efficacy in experi-

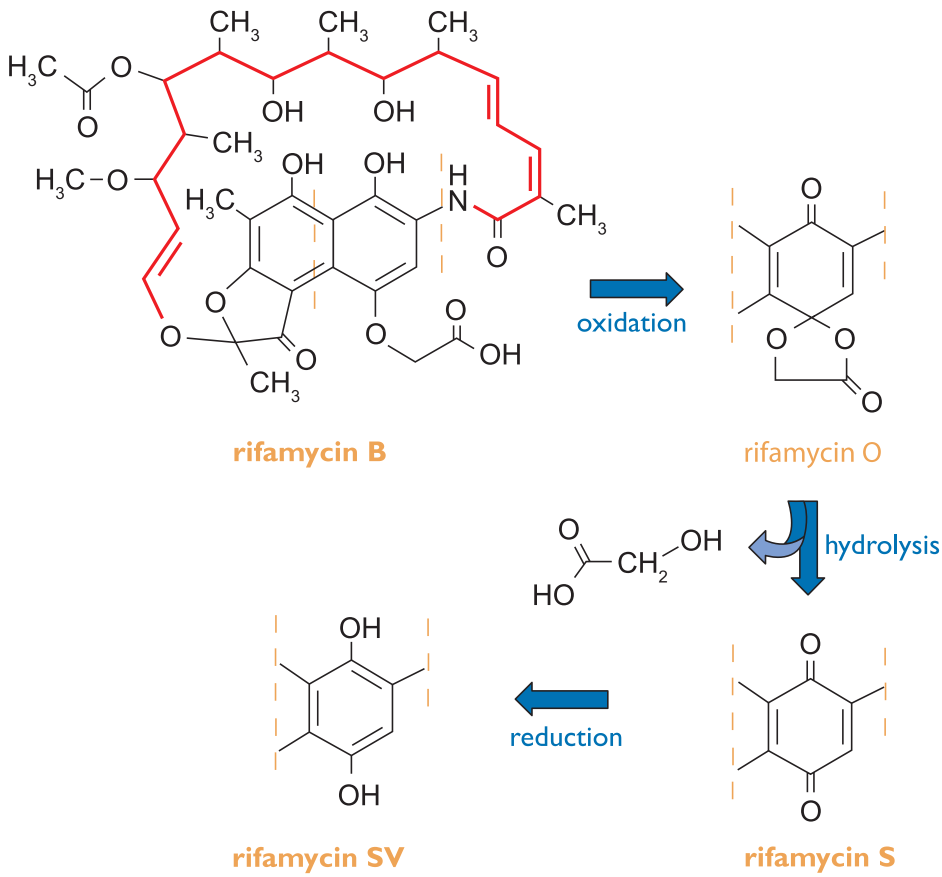


Fig. 2. Spontaneous transformation of rifamycin B into more active antibiotics. (The "ansa" chain is shown in red.)

mental models of tuberculosis, which was attributed to its short serum half-life. No cross-resistance was observed with other antibiotics, consistent with its being a new chemical class and/or having a novel target. It was effective in curing several experimental infections when administered parenterally, and had low chronic toxicity (Timbal and Brega, 1961). Rifamycin SV was not orally active because, although readily absorbed from the intestine, it was even more rapidly excreted into the bile by way of enterohepatic circulation. After injection, rifamycin S was moderately distributed throughout the body and then concentrated in the liver and bile at levels adequate to treat biliary and hepatic infections, even those caused by Gram-negative bacteria. After favorable results in clinical trials the sodium salt of rifamycin SV was marketed in 1963 in several European and South American countries as a solution in ready-to-use vials under the trade name Rifocin®.

The chemical interrelationship between rifamycins B, O, S and SV is shown in Fig. 2. The structural differences between these compounds were established on the basis of spectroscopic data and synthesis of model compounds (Gallo et al., 1962). In 1963 Prof. Dr. Vladimir Prelog at the Eidgenösische Technische Hochschule Zürich succeeded, by chemical degradation and extensive use of ^{1}H-NMR spectroscopy, in assigning to rifamycin B the structure depicted in Fig. 2 (Oppolzer et al., 1965). At that time ^{1}H-NMR was still a new technique. When Prelog presented his results to Lepetit he remarked at the end of his lecture that "Piero Sensi probably thinks that God created rifamycin B for the benefit of Lepetit, but I think that it was created to demonstrate the power of NMR in elucidating complex chemical structures." Rifamycins were the first natural compounds found to contain an aromatic moiety spanned by an aliphatic bridge; Prelog named such compounds "ansamycins" (from the Latin "ansa," meaning "handle of a basket"). At the same time a team at the University of Rome published an X-ray crystal structure of rifamycin B (Brufani et al., 1964).

Chemical modifications of rifamycins

That both natural and chemically modified rifamycins demonstrated good antibacterial activities suggested that further modifications might produce better anti-

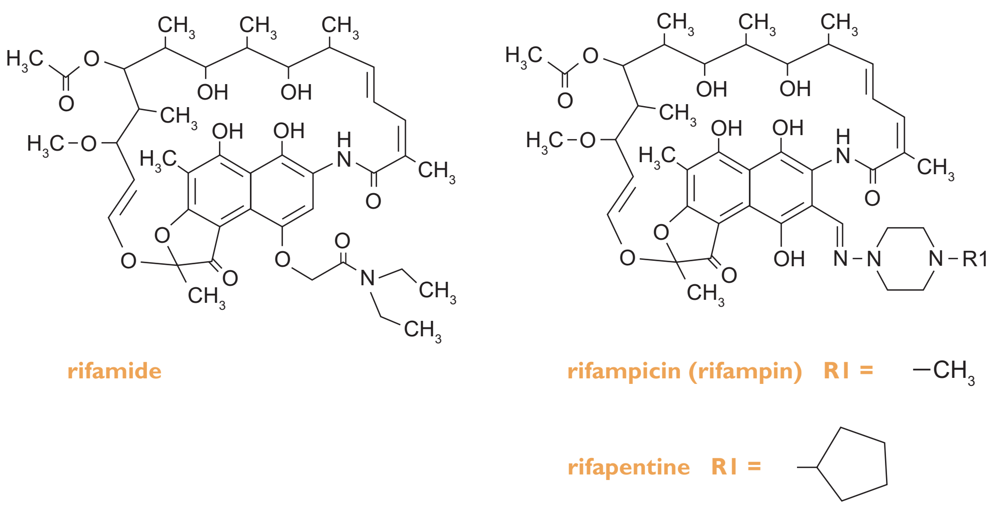


Fig. 3. Additional rifamycins which have entered clinical practice.

infective agents. This idea was met with some skepticism by Lepetit's management, since at that time (1959) several attempts to improve the properties of natural antibiotics by synthetic modification had met with only marginal success. Moreover, it was argued that naturally-occurring antibiotics, having been fine-tuned in terms of spectrum and potency through natural selection, were already "optimized." However it was pointed out that, whereas high antibacterial activity may have been selected by evolution, other therapeutically important properties such as oral bioavailability, distribution in the body, and favorable pharmacokinetics certainly were not. Lepetit's management agreed to initiate a program of further chemical modifications of rifamycins. A series of rifamycins substituted at position 3 confirmed that, indeed, rifamycins with improved properties could be achieved by semisynthesis. Rifamycin O reacted readily with amines and hydrazines; in particular the aminoguanidine derivative (rifamycin AG) was highly active in vitro against *Staphylococcus aureus* (MIC 0.001 µg/ml) and *M. tuberculosis* (MIC 0.005 µg/ml) but had poor efficacy in experimental infections, possibly due to solubility problems (Sensi et al., 1962). All naturally occurring rifamycins, with the exception of rifamycin B, had high in vitro bioactivities, and it was postulated that the free carboxylate group in rifamycin B hindered penetration of the antibiotic into bacterial cells. [What little bioactivity rifamycin B did possess was due to the presence of rifamycin B oxidation products (*vide supra*)]. However, the methyl ester of rifamycin B proved to be about as active as rifamycins A, C, D, and E. Therefore, a series of esters, amides, and hydrazides of rifamycin B were synthesized during 1961-1962, all of which demonstrated good antibacterial activities (Sensi et al., 1964). Within a series of homologous amides the most promising was the diethylamide (Fig. 3), whose properties resembled those of Rifocin® but which had a wider therapeutic window. Rifamycin B diethylamide was developed and marketed in 1964-1965 as Rifamide® in a few countries, most notably the U. K.

Beginning in 1963, following an agreement between Lepetit and CIBA (Chemical Industries of BAsel) AG, the program of rifamycin modification was pursued as a collaboration between the Swiss and Italian companies. Prelog's elucidation of the structure had enabled the establishment of structure-activity relationships for rifamycins. It was reasoned that attempts to interfere with molecular features responsible for interactions between rifamycin and its molecular target (*vide infra*) probably would be accompanied by loss of antibacterial activity, whereas modification of physicochemical features impacting predominantly on pharmacology (e.g., pKa, lipophilicity, serum protein binding) would less likely impact on rifamycin's intrinsic antibacterial potency. Thus, efforts were directed mainly towards synthesizing rifamycins with one or more of the following properties: enhanced oral bioavailability; slow rate of biliary excretion; and diminished toxicity. At the same time, some resources also were directed towards trying to increase penetration of rifamycins into Gram-negative pathogens. "Rifazine," the simplest phenazino derivative of rifamycin (Gallo et al., 1966), showed excellent oral efficacy in animal models of infections and was considered for clinical development, but ultimately was abandoned due to tolerability problems. Meanwhile, a healthy competition between Lepetit and CIBA spurred research, since the agreement between the companies stipulated that they would develop jointly the most promising product synthesized.

A series of 3-amino-substituted rifamycin SV derivatives synthesized at CIBA were evaluated; one of these, the morpholino derivative, with an ED_{50} <1 mg/kg

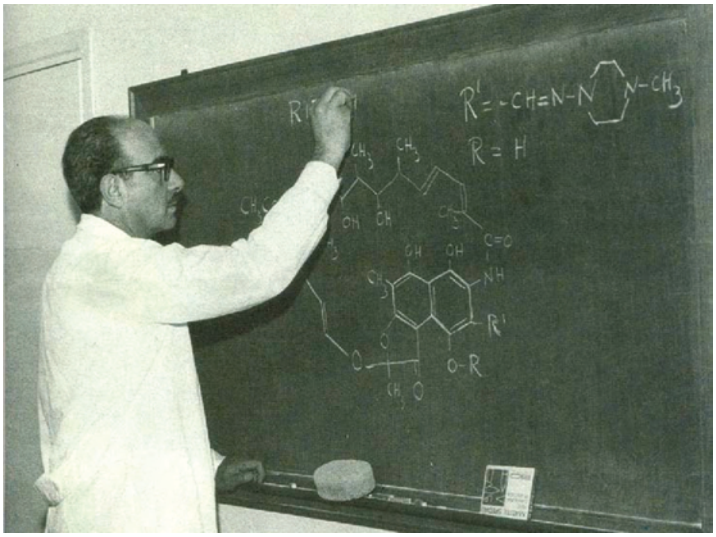


Fig. 4 Prof. Piero Sensi describing the structure of rifampicin (ca. 1965).

in an *S. aureus* murine infection model, looked particularly promising (14). At the same time, a series of 3-aminomethylrifamycins was being prepared from rifamycin S at Lepetit. These products were of moderate interest, but the observation that controlled oxidation produced 3-formylrifamycin SV or "rifamycin AF" ("Araba Fenice" = "Arabian Phoenix," so-called because of its elusive behavior) (Maggi et al., 1967) opened the route to synthesis of a very large number of important products by condensation of the 3-formyl moiety with various amines, hydroxylamines, and hydrazines.

Some derivatives of rifamycin AF, prepared by condensation with disubstituted hydrazines, demonstrated excellent in vitro and in vivo activities. However, by far the best results were obtained by reacting rifamycin AF with *N*-amino-*N*'-alkylpiperazines. The product of condensation with *N*-amino-*N*'-methylpiperazine met our criteria for a clinically useful antibiotic, and a thorough evaluation of its biological properties was initiated (Figs. 3 and 4).

3-[[(4-Methyl-1-piperazinyl)imino]methyl]rifamycin SV, called "rifampicin" ("rifampin" in the United States) (Maggi et al., 1966), stood apart from the other semisynthetic rifamycins by virtue of its outstanding efficacy in curing experimental infections (e.g., an oral ED_{50} of 0.1 mg/kg for *S. aureus* septicemia in mice). Antibacterial efficacy was demonstrated in various experimental infection models, including infections with *M. tuberculosis* H37Rv (Arioli et al., 1967; Pallanza et al., 1967). Clinical studies confirmed the exceptional therapeutic properties of rifampicin, particularly for tuberculosis and leprosy; today, forty years after it was invented, rifampicin remains the most effective ingredient in the combination therapy used to treat these mycobacterial diseases (Omerod, 2003). Rifampicin is also recommended to prevent meningitis in persons who have come into contact with patients infected with the meningococcus; and, in combination with other antibiotics, to treat refractory staphylococcal infections (Parenti and Lancini, 2003). Mutation rates for resistance to rifampicin in Gram-positive organisms are about 10^{-7}, so for severe infections it is preferable to use rifampicin as part of a combination therapy to preclude emergence of rifampicin-resistant mutants, though the risk of spread of rifampicin resistance through a bacterial population is modulated by the fact that it is not horizontally transferable. Whilst the mutation rate for resistance to rifampicin in *M. tuberculosis* is only about 10^{-10}, standard treatment of tuberculosis uses a combination of rifampicin plus at least two other antibiotics (standard treatment: rifampicin + isoniazid + pyrazinamide).

Rifamycin fermentation and biosynthesis

The introduction of rifamycin SV and later rifampicin into clinical practice obviously required an adequate industrial production process. A strain improvement program was initiated in 1961 at Lepetit's fermentation plant in Torre Annunziata. An unexpected success followed from the observation that a mutant strain of *A. mediterranei* resistant to an actinophage was a better rifamycin producer. Subsequent isolation of four mutant strains from phage-lysed cultures resulted in an incremental increase in rifamycin yields up to 1 g/l (Thiemann et al., 1964).

In Milan we began investigating some aspects of rifamycin production, including the mysterious action of barbital, whose effects on rifamycin B yield were presumably exerted towards the end of the biosynthetic pathway. Addition to cultures of barbital labeled in different positions with ^{14}C showed that neither barbital nor its degradation products were precursors of rifamycin (Kluepfel et al., 1965). Rifamycin B has a glycolate moiety attached to the aromatic nucleus and we surmised that the last biosynthetic step might be glycolation of rifamycin SV to rifamycin B (see Fig. 2). Indeed, cultures of *A. mediterranei* efficiently converted [^{14}C]rifamycin SV into [^{14}C]rifamycin B. Resting cells of *A. mediterranei* could convert rifamycin SV to rifamycin B, and conversion yields were significantly higher if the organism had been cultured in the presence of barbital (Lancini and Sensi, 1967). The biochemistry of this reaction was investigated as well as the origin of the glycolic moiety (Lancini et al., 1969). The findings suggested the possibility of using *A. mediterranei* mutants blocked in the glycolation of rifamycin SV to obtain the starting material for rifampicin manufacture. Mutagenesis of *A. mediterranei* mycelial fragments followed by plating the fragments on solid medium seeded with a rifamycin SV-sensitive but rifamycin B-insensitive indicator strain (*Pseudomonas reptilivora*) enabled detection of *A. mediterranei* colonies producing rifamycin SV. One isolate thus recovered produced as much rifamycin SV as the parent strain had produced rifamycin B (Lancini and Hengheller, 1969). Further experiments demonstrated that production of rifamycin SV was unaffected by barbital, confirming that barbital exerted its effect on the glycolation step. While we were unable to convince Lepetit's Production Division to commercialize our results, nowadays fermentation plants in China and other third-world countries routinely use strains blocked at the rifamycin SV stage for industrial manufacture of rifamycins.

Using our established mutagenetic protocol on mycelial fragments in conjunction with a suitable bioassay, another mutant strain of *A. mediterranei* was obtained which produced high titres of rifamycin B even in the absence of supplemental barbital (White and Lancini, 1975).

Studies were undertaken on the formation of the rifamycin skeleton. Whereas it was evident that part of the molecule originated from condensation of small acids, the "ansa" chain was interrupted by a vinylic oxygen atom (Fig. 2), and the origin of the aromatic nucleus was unclear. Attempts to elucidate the biosynthesis of rifamycins using [^{14}C]- or [^{3}H]propionate or [^{14}C]- or [^{3}H]acetate gave ambiguous results. Therefore, we resorted to ^{13}C-NMR spectroscopy, a technique which had only recently been applied to studying biosynthetic routes. By feeding cells [^{13}C]-enriched precursors we could demonstrate that the ansa chain of rifamycin B was assembled by condensation of acetate and propionate units initiated at the hydroxyaminobenzoate moiety (White et al., 1973); the vinylic oxygen was inserted into the ansa chain later (White et al., 1974). Remembering Prelog's statement about NMR and structure elucidation, we felt that possibly God also had created rifamycins to demonstrate the power of ^{13}C-NMR for solving complex biosynthetic problems.

Other details of the rifamycin biosynthesis pathway and the production of other rifamycins by mutant *A. mediterranei* strains have been reported independently by Lepetit and by CIBA scientists. Interested readers are referred to two reviews on the subject (Lancini and Grandi, 1981;Lancini and Cavalleri, 1997).

Mode of action of rifamycins

In 1967 and 1968 two short articles by Hartmann at the University of Munich and colleagues at CIBA reported that rifamycins inhibited bacterial RNA polymerase in a cell-free transcription system (Hartmann et al., 1967; Sippel and Hartmann, 1968). Shortly afterwards we verified that rifamycins specifically inhibited RNA synthesis in growing bacterial cells (Lancini and Sartori, 1968). These findings attracted the interest not only of bacteriologists but also of virologists, who had just discovered the presence of RNA polymerase in the vaccinia virus. Initial tests suggested some activity of rifamycin on the viral RNA polymerase, but later it became clear that the modest antiviral activity of some rifamycins was due to a different mechanism (Lancini et al., 1971). Meanwhile, Howard Temin and David Baltimore had each discovered the reverse transcriptase of oncogenic RNA viruses; when rifamycins were tested on this enzyme, a moderate inhibition was observed. As a result, numerous laboratories contacted us, requesting samples of different rifamycins for testing against various biological systems. Melvin Calvin, who at this time often visited our laboratory in his capacity as a consultant to Lepetit, initially was interested in these potential activities of rifamycins (Tischler et al., 1974); later, however, he remarked: "When their activity on RNA polymerase became known … rifamycins were investigated by many laboratories for almost every activity for which they could be imagined, and perhaps even for a few activities beyond imagination." Since lipophilicity appeared to enhance rifamycin activity towards transcriptases, a program aimed at synthesizing rifamycins bearing lipophilic chains was initiated at Lepetit. Shortly afterwards a contract was offered by the U. S. National Cancer Institute for a joint Lepetit-Dow Chemical program of synthesis, to be performed at Dow's laboratories in Midland, Michigan. A few hundred rifamycins later we had confirmation of what we suspected: derivatives with reasonable activity against reverse transcriptase were equally active against other enzymes (e.g., DNA polymerases, mammalian RNA polymerases), and so were not suitable candidates for clinical development (Yang et al., 1972). The results produced by studies "even beyond imagination" have been summarized in a review on structure-activity relationships of rifamycins (Lancini and Zanichelli, 1977).

Among the many rifamycins synthesized in our laboratory, however, rifapentine (formerly DL 473, see Fig. 3) was found to be about as active as rifampicin but with a better pharmacokinetic profile, thereby permitting treatment of tuberculosis by intermittent therapy (38). The product was not developed by Lepetit *per se* but by Hoechst (*vide infra*). Rifapentine has been marketed in the United States under the trade name Priftin®.

Screening for new products continues: 1960-1965

While Lepetit's chemists and medical microbiologists were focused on the synthesis and evaluation of rifamycins, the screening group continued their search for new microbial products with antibiotic activities. By 1961 we had recognized that high rates of rediscovery of known substances represented a major hurdle to identifying new products from streptomycetes (*cf.* Baltz, 2005); moreover, while the number of new molecules discovered was steadily increasing, the proportion of new compounds with clinical potential was dwindling. To increase the probability of finding new, therapeutically useful, molecules it was proposed to seek new antibiotics from untapped microbial sources, such as the oceans, caves, or niches with unconventional salinities, temperatures, etc. Alternatively, it was suggested that research be reoriented towards narrow-spectrum antibiotics active against less common pathogens, or to focus efforts on combating microorganisms of agricultural or veterinary interest. The need to screen increasingly greater and greater numbers of microorganisms for "hits" was felt acutely in our laboratories, which could not compete with large American and Japanese companies in terms of the number of new strains isolated. We decided that our best chances lay in studying non-streptomycetes similar in habitat and lifestyle to streptomycetes. This led us to collect strains belonging to new or unexplored genera of the order *Actinomycetales*, the so-called "rare actinomycetes." At about the same time a similar approach, focusing on *Micromonospora* spp., was pursued with great success at Schering/Schering-Plough in the United States, leading to the discovery of, among other drugs, gentamicin (Weinstein, 2004).

At Lepetit a program was started to isolate strains belonging to the genus *Thermoactinomyces*. A novel antibiotic, thermorubin, was soon discovered, noteworthy for its broad spectrum of activity against Gram-positive bacteria and, to a lesser extent, Gram-negative bacteria (Craveri et al., 1964). Thermorubin was found to inhibit protein synthesis by a mechanism different from that of all known antibiotics (Pirali et al., 1974). Unfortunately, thermorubin did not cure experimental infections in animals, probably due to its inactivation by serum proteins, and attempts to improve its in vivo activity were unsuccessful. It soon became apparent that the genus *Thermoactinomyces* was not particularly gifted with respect to the variety of antibiotic molecules it could produce: screening several hundred *Thermoactinomyces* strains from diverse soil samples resulted in repetitive detection of thermorubin and a handful of other products of little commercial interest. We now know that this genus belongs to the order *Bacillales* rather than the order *Actinomycetales* (Stackebrandt and Woese, 1981; Stackebrandt et al., 1983), and this experience taught us that morphological similarity to streptomycetes is not necessarily accompanied by a corresponding biosynthetic versatility.

In 1962 Lepetit's research laboratories moved to a larger building in the Bovisa district of Milan. The Department of Fermentation and Natural Products was split into three departments. One of these, Antibiotics and Natural Products, under the direction of Piero Sensi was comprised of strain isolation and fermentation laboratory, a recovery and purification laboratory, a natural products chemistry laboratory, and a microbial biochemistry laboratory; the other two departments were Medical Microbiology, focusing on in-depth evaluation of a new product's therapeutic potential, and Physical Chemistry, focusing on analytical methods and structure elucidation.

Following our experiences with *Thermoactinomyces*, screening of streptomycetes resumed. Meanwhile, a new test for antiviral activity was added to the usual panel of antibacterial and antifungal assays. This approach bore fruit when a new compound with moderate antiviral activity but potentially interesting antitumour activity was detected (Murthy et al., 1966). The product, named alanosine [from its chemical structure, β-*N*-nitroso-hydroxylaminoalanine (Coronelli et al., 1966)], was synthesized (Lancini et al., 1966) and exploratory studies of its use against head and neck cancer were conducted at the National Institutes of Health in the United States; however, it was abandoned following poor results in clinical trials. Among its other bioactivities, alanosine was able to sterilize flies and acted as an abortifacient in animals when administered during the first days of pregnancy.

Also during this period we isolated from *Streptomyces eurocidicus* azomycin (2-nitroimidazole), an antibiotic reported previously by Umezawa and colleagues (Maeda et al., 1953). Azomycin had attracted the attention of microbiologists because of its anti-anaerobe activity, particularly against the protozoan *Trichomonas vaginalis*. Although very active in vitro, azomycin had poor efficacy in experimental infections, which led several laboratories to try to synthesize more active analogues. Since no synthetic route to 2-nitroimidazoles was known, chemists focused on preparing 5-nitroimidazoles, one of which, metronidazole, became the first nitroimidazole to be used clinically.

In the hope that 2-nitroimidazoles might prove to be better drugs than 5-nitroimidazoles, we endeavored to obtain 2-nitroimidazoles in two ways. At that time the origin of nitro groups in natural products was not known, but it was suspected that they derived from oxidation of amino groups. In accordance with this expectation, addition of [^{14}C]2-aminoimidazole to cultures of *Streptomyces* sp. CE/3342 gave [^{14}C]2-nitroimidazole in good yields (Lancini et al., 1966). A general synthesis of 2-aminoimidazoles containing alkyl substituents at various positions was devised (Lancini and Lazzari et al., 1965), and addition of 4(5)-alkyl-2-aminoimidazoles to cultures of *Streptomyces* sp. CE/3342 resulted in formation of the corresponding 4(5)-alkyl-2-nitroimidazoles. However, 1-alkyl-2-aminoimidazoles were not transformed into 1-alkyl-2-nitroimidazoles (Lancini et al., 1968).

Additionally, we devised a chemical method for converting 2-aminoimidazoles into the corresponding nitro derivatives (Lancini and Lazzari, 1968). Using alkyl derivatives of 2-aminoimidazole obtained by chemical synthesis, it was relatively simple to prepare numerous 1,4- and 1,5-dialkyl-2-nitroimidazoles, as well as other derivatives. In animal experiments some of these compounds proved superior to the 5-nitroimidazoles already in clinical

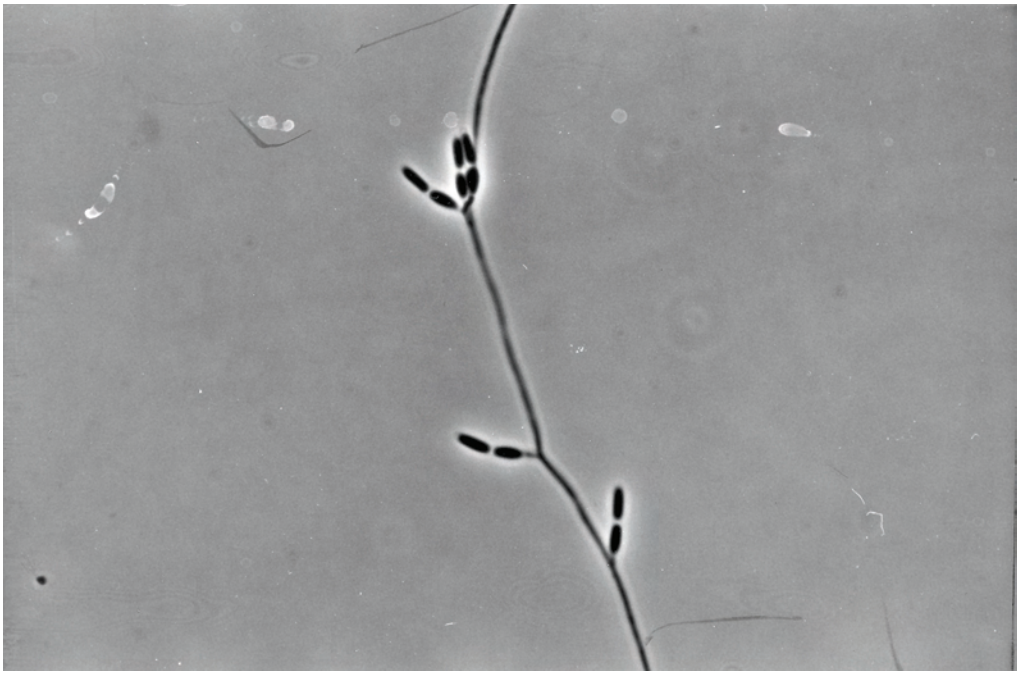


Fig. 5 Hypha of *Planobispora rosea* bearing sporangia characteristic of this genus (1000x magnification). (Photo provided by Ms. Grazia Beretta.)

use (Cavalleri et al., 1977), but none of these 2-nitroimidazoles were tested clinically due to projected development and marketing costs.

New ideas for antibiotic screening

In 1966 Piero Sensi was named Director of Lepetit Research Laboratories and I replaced him as Head of the Antibiotics and Natural Products Department.

Despite disappointing results from our *Thermoactinomyces* screening program, an interest in screening rare actinomycetes for novel antimicrobial products remained. At that time relatively few genera of rare actinomycetes had been described, and there were no efficient methods for selecting them. The protocol initially employed in our laboratories was to examine colonies on isolation plates with a microscope and use a micromanipulator to pick strains displaying unusual morphologies. This approach led to the discovery during 1966-1967 of four new genera (Thiemann et al., 1967a, b; Thiemann and Beretta., 1968; Thiemann et al., 1968) (*Dactylosporangium*, *Planomonospora*, *Microtetraspora,* and *Planobispora*) (Fig. 5), but was far too laborious for routine screening. Only a few strains belonging to each new genus were isolated and though strains of other genera of rare actinomycetes such as *Streptosporangium* were more frequently encountered, the process was too inefficient to support novel antibiotic discovery.

A method for mass isolation of uncommon actinomycetes was needed, i.e., a system capable of physically separating the pervasive streptomycetes from their much rarer actinomycete kin. One option was to exploit the fact that, unlike *Streptomyces*, some actinomycete genera produce motile spores. A very simple procedure was devised whereby soil samples were dispersed in water (containing a bacterial growth inhibitor) and subjected to low-speed centrifugation; motile spore soon migrated from the pellet into the supernatant, where they could be collected in large numbers (Pagani and Lancini, 1970). The system was particularly effective for isolating *Actinoplanes* spp., since colonies of this genus can be identified easily by shape, color, and the presence of characteristic sporangia (Fig. 6). During 1971 we isolated and cultured several thousand *Actinoplanes* strains. This enrichment technique was classified as “confidential” by Lepetit and never published by us, and I remain astonished that such a simple procedure was not rediscovered by other laboratories until years later (e.g. Makkar and Cross, 1982).

The hypothesis that rare actinomycetes would produce novel antibiotics was confirmed by our observation that none of the active *Actinoplanes* strains we examined produced an antibiotic that had been isolated previously from a streptomycete. One of the first *Actinoplanes* products studied, purpuromycin, had broad-spectrum antibacterial and antifungal activities. Its biological properties were assessed in detail, and the chemical structure elucidated (Coronelli et al., 1974), but the product did not enter clinical development because of its very narrow therapeutic window.

To identify extracts active on bacterial metabolic pathways a relatively simple method was established using labeled

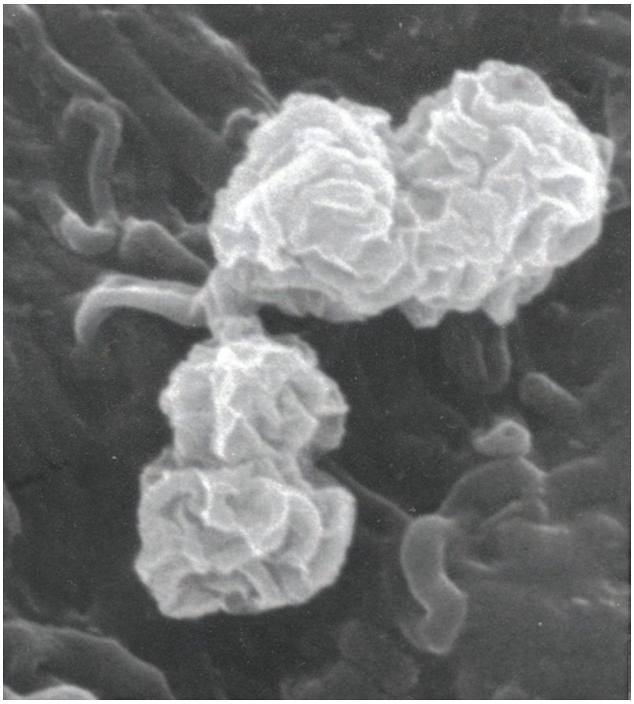


Fig. 6. SEM image of *Actinoplanes* sp. 21954 sporangia (5000x magnification). (Photo provided by Dr. Giorgio Toppo.)

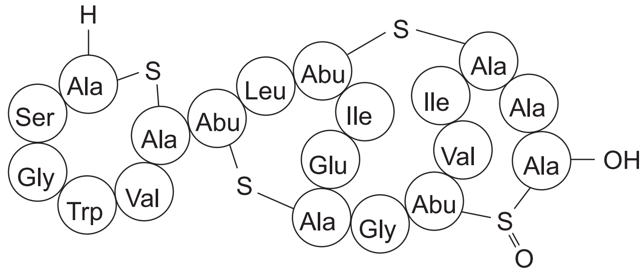
precursors to determine which of several macromolecular processes was inhibited first in growing cells (White et al., 1972). Nowadays this is a commonly employed tool for screening fermentation broths and semi-purified extracts, but in the early 1970s it represented a novel screening method. Using this procedure we identified lipiarmycin (from "leap year," the producing strain was identified on 29 February 1972), a novel inhibitor of bacterial RNA polymerase (Coronelli et al., 1975) and the first example of a new class of macrolides that inhibits transcription initiation. The activity of lipiarmycin towards Gram-positive bacteria was sensitive to the pH of the culture medium [macrolide antibiotic activity often shows some pH dependence (Sabath et al., 1968; Barry and Fuchs, 1991)], and its high activity against *Streptococcus mutans* led to its being considered for development as an anti-plaque agent, though Lepetit's management declined to pursue this market.

By now the bottleneck in our screening programs was not the number of strains that could be isolated and cultivated, but the number of culture extracts that could be analyzed for structural novelties and antibiotic properties. We needed simple, rapid tests able to provide information about the antibacterial activity in unfractionated fermentation broths to avoid working up potentially toxic substances.

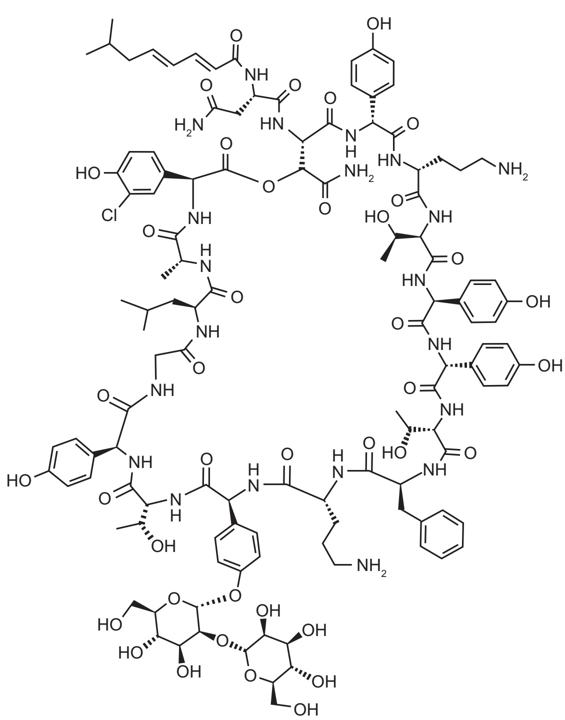
Cell wall biosynthesis was already a validated antibacterial target, and in 1965 we had devised a simple procedure involving assay of spent media for antibacterial activity in the presence and absence of cell wall fragments; broths that were active in the absence of bacterial cell wall fragments but inactive in the presence of these fragments were subject to further scrutiny. The test achieved some modest success during a streptomycete screening campaign, though only a few antibiotics among the numerous prospective inhibitors of cell wall biosynthesis could be detected in this manner. Moreover, the results of these tests were rarely straightforward and unambiguous. However, to this author's knowledge, it was the first example of what has become known as "target-oriented screening," which subsequently became an important strategy for microbial metabolite discovery.

At this point a more convenient assay to identify inhibitors of cell wall biosynthesis in fermentation broths was developed (S. Somma, unpublished), which involved determining antibacterial activity in parallel towards a *S. aureus* strain and an isogenic L-form [see Gumpert, 2005]. Broths active against the normal strain but inactive against the L-form were considered to contain an inhibitor of some step in cell wall biosynthesis. The combination of screening *Actinoplanes* strains (*vide supra*) and identifying natural products inhibiting cell wall biosynthesis led to the discovery of some interesting antibacterial agents: gardimycin in 1971, the teichomycins in 1972, and ramoplanin in 1975 (Figs. 7 and 8).

Gardimycin (later called "actagardine") was the first known example of a new group of lantibiotics characterized by their relatively small size and their mechanism of action (Coronelli et al., 1976; Zerilli et al., 1977) [the second example, mersacidin, was described nearly 20 years later (Chatterjee et al., 1992)]. Gardimycin is a very narrow-spectrum antibiotic produced by *Actinoplanes garbadinensis*; its activity is restricted largely to streptococci, though it proved surprisingly more effective at curing experimental infections than was predicted from its in vitro activity (Arioli et al., 1976). Gardimycin inhibits peptidoglycan biosynthesis by binding to lipid II (Somma et al., 1977). Years later a few semisynthetic derivatives of gardimycin were found to have an extended spectrum of activity, including moderate activity towards enterococci; again, the therapeutic efficacies of these derivatives were greater than expected on the basis of in vitro tests (Malabarba et al., 1990). None of these lantibiotics transited into commercial development due to lack of activity towards *S. aureus*, regarded as an essential Gram-positive bacterial target. Given their excellent tolerability, however, some semisynthetic lantibiotics belonging to the gardimycin class still



actagardine (gardimycin)



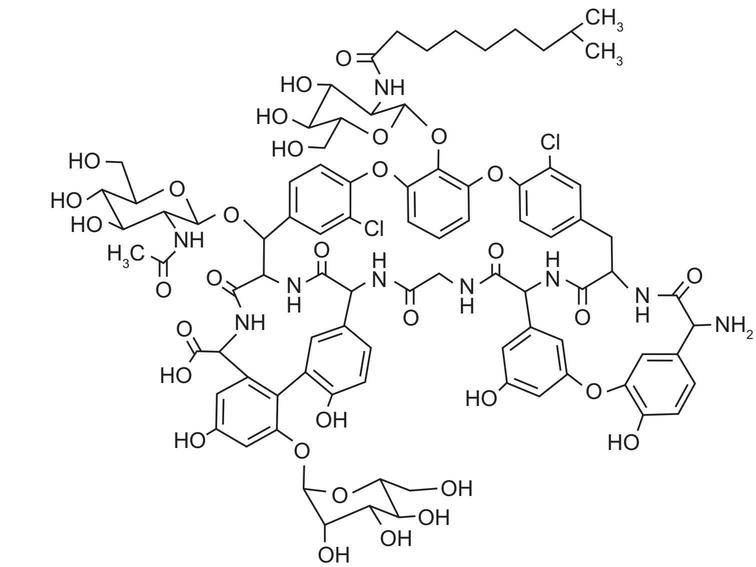
ramoplanin (main component)

Fig. 7. Gardimycin and ramoplanin, two antibiotics produced by *Actinoplanes* strains.

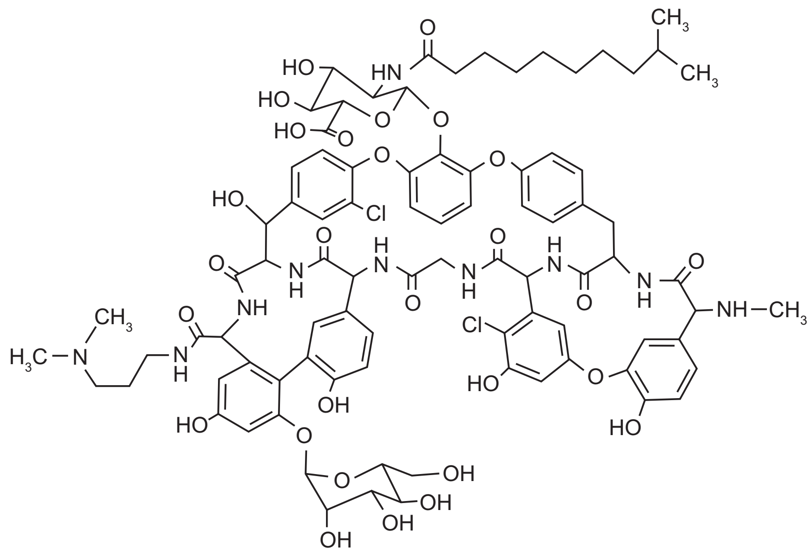
have a potential clinical role as highly efficacious, albeit extremely narrow-spectrum, antibiotics to treat infections caused by vancomycin-resistant enterococci.

Actinoplanes teichomyceticus produces several antibiotics, of which teichomycin A_2 (now called "teicoplanin") was of particular interest. Teicoplanin is a member of an antibiotic complex whose five main components differ with respect to their fatty acid moieties (Bardone et al., 1978; Borghi et al., 1984). It has a similar spectrum, properties, and mechanism of action as vancomycin (Pallanza et al., 1983; Somm et al., 1984); however, it showed better pharmacokinetics, being therapeutically effective when administered i.v. once daily, in contrast to vancomycin which had to be administered i.v. every six hours. Teicoplanin was introduced into clinical practice in several countries (Parenti et al., 2000), though its initial Food and Drug Administration (FDA) application was rejected and Hoechst Marion Roussel (the successor of Lepetit; *vide infra*) declined subsequent invitations by the FDA to resubmit its application. Hundreds of derivatives of teicoplanin have been synthesized, among which one in particular, mideplanin (formerly MDL 62,873) (Berti et al., 1992), seemed worthy of development. With the appearance of very active semisynthetic derivatives of another glycopeptide, MDL 62,476 (known also as A 40926), produced by an *Actinomadura* sp. (*vide infra*), efforts to develop mideplanin were abandoned.

Ramoplanin, produced by *Actinoplanes* sp. ATCC 33076 (Cavalleri et al., 1984), is a complex of three components, each of which consists of a large cyclic peptide bearing an acyl chain (Ciabatti et al., 1989). Ramoplanin originally attracted the attention of medical bacteriologists because of its excellent activity against Gram-positive organisms, including strains resistant to all antibiotics then available (Pallanza et al., 1984). Ramoplanin proved highly active against anaerobes and was effective in curing experimental infections. It soon



teicoplanin (main component)



dalbavancin (main component)

Fig. 8. Clinically effective glycopeptides: teicoplanin and dalbavancin

became evident that ramoplanin was not orally available and could not be administered parenterally due to severe toxicity at the site of injection. Subsequent clinical experiments indicated efficacy for ramoplanin in the oral treatment of *Clostridium difficile* pseudomembranous colitis, and the drug is currently undergoing Phase 3 clinical trials for this occasional side effect of conventional antibiotic therapy.

Activities: 1975-1983

In December 1974 Piero Sensi was promoted to Director of Research and Development at Lepetit, and I was appointed Director of the Research Laboratories, which included, beside the Department of Antibiotics and Natural Products, the Departments of Organic Synthesis, Pharmacology, Toxicology, Medical Microbiology, and Physical Chemistry. Franco Parenti, who had led the strain isolation and fermentation laboratory since 1972, was chosen to head the Antibiotics and Natural Products Department while retaining his other position.

Screening

During this period different screening approaches were devised in an effort to increase the probability of identifying novel microbial products with antibiotic activity. Since a medical need was perceived for agents to treat sexually transmitted diseases, specific assays were implemented to reveal activities against neisseriae and chlamydiae. The assays were laborious and not always very informative; for technical and safety reasons preliminary tests were performed using model microorganisms, and promising hits were retested on the pathogens of interest. No worthwhile products were discovered, and this screening program was terminated.

A miniaturized assay for detecting antitumor agents, based on the ability of culture filtrates to inhibit thymidine uptake by tumor cell lines [*cf.* (Miyazaki et al., 1992)], was devised. Several inhibitors were detected, but no novel products with chemotherapeutic value were identified.

Screening for cell wall biosynthesis inhibitors using L-forms continued (*vide supra*), and a very effective test for identifying glycopeptide antibiotics was introduced based on the ability of glycopeptides to bind to the **D**-Ala-**D**-Ala terminus of peptidoglycan precursors. A selective adsorbent for affinity chromatography of glycopeptides, **D**-Ala-**D**-Ala-agarose, was prepared (Corti and Cassani, 1985) and fermentation broths applied to small columns of this matrix; elution with ammonia solutions afforded semi-purified glycopeptide antibiotics. From about 2,000 strains surveyed, over 70 (>3.5%) were found to produce glycopeptides, most of which turned out to be known compounds (Cassani, 1989). However, an interesting new product, dubbed A 40926, which was as active as teicoplanin, was produced by an *Actinomadura* (later reclassified as a *Nonomuraea*) strain (Goldstein et al., 1987). A 40926 turned out to be a complex of several components, each containing different fatty acids moieties (Waltho et al., 1987). Though this glycopeptide was not considered worthy of development, it was used successfully as a starting material for the semisynthesis of many new active products.

Origin of the acyl moieties of glycopeptide antibiotics

One of the functions of the fermentation laboratory was to improve production processes for antibiotics selected for clinical development. For example, in connection with the industrial production of teicoplanin it was necessary to establish how different factors affected the ratios of the various components in the teichomycin complex. Studies on the origin of the acyl moieties indicated that the amount of each component was determined by the availability of the fatty acid constituting its acyl moiety. Moreover, these fatty acids did not arise by *de novo* biosynthesis; rather, linear acyl moieties derived from β-oxidation of linear C_{18} acids present in the medium, whereas branched acyl moieties derived from β-oxidation of branched-chain fatty acids in cell membrane lipids (teicoplanin contains an 8-methylnonanoyl moiety). On the basis of these results it became possible to increase (to some extent) the amount of any particular component of the teichomycin complex by adding to the medium either the specific precursor of a given branched fatty acid or a linear C_{18} saturated or unsaturated fatty acid (Borghi et al., 1991).

We discovered that this mode of biosynthesis of acyl moieties was shared by ramoplanin (Lancini et al., 1988) and A 40926 (Zerilli et al., 1992). We suggested that this is a general mechanism for the biosynthesis of the medium-size acyl moieties present in many antibiotics.

1983: End of Lepetit's research laboratories in Milan

Since 1964 Gruppo Lepetit S.p.A. had been a subsidiary of the Dow Chemical Company of Midland, Michigan. However, Lepetit's ownership by the American chemical giant had little impact on antibiotic research, since Dow's management, lacking experience in the pharmaceutical business, preferred to let Lepetit set their own research programs and priorities. In 1980, after Dow acquired Merrell Pharmaceuticals, Inc. of Cincinnati, Ohio, the center of gravity for decision making relating to pharmaceuticals started shifting towards the United States. In 1983 Dow decided to shut down Lepetit's research laboratories in Milan in order to concentrate pharmaceutical research at the Merrell site. The only pharmaceutical research Dow permitted to continue in Italy was antibiotic discovery. The antibiotic screening staffs were offered an opportunity to work in a new facility to be built in Gerenzano, 20 miles north of Milan. Because of bureaucratic delays the new facility was not completed until mid-1986. In the interim no new antibiotic screening was performed, enabling scientists to catch up on their paperwork, complete internal reports, and prepare manuscripts for publication. During this transition period I resigned my managerial position and returned to research as a Senior Scientist at the Gerenzano Centre.

1986-1995: Lepetit Research Centre in Gerenzano (LRCG)

Continuity between Lepetit's laboratories in Milan and its new research centre in Gerenzano was ensured by the appointment of Franco Parenti as Director and by the transfer to Gerenzano of most of the personnel already working in antibiotic research. While the medical microbiology group moved virtually intact from Milan to Gerenzano, most of the scientists responsible for strain isolation and fermentation left Lepetit and were replaced by young microbiologists. Changes in the orientation of research were reflected in the hiring of biochemists and molecular geneticists. The LRCG was provided with a modern integrated information system and a pilot plant for process scale-up, including large fermentation facilities, modern downstream processing equipment, and state-of-the-art chemical synthesis equipment.

Screening

Antibiotic screening at the LRCG focused on new targets, so considerable effort was invested in devising new tests for detecting antibiotic activity and implementing these in high-throughput formats. There was a reduced emphasis on isolating novel microorganisms, on the assumption that new targets would, in any case, reveal new products (*vide infra*). Even so, actinomycete isolation activities continued, and a program for retrieving soil fungi was added, with an emphasis on genera other than *Penicillium* and *Aspergillus*.

One of the new protocols introduced at Gerenzano was a screen for inhibitors of elongation factor EF-Tu, which tested whether a crude fermentation broth's antibiotic activity could be reversed by addition of exogenous EF-Tu. In this manner a novel antibiotic, GE2270, was identified. GE2270, a thiazolyl cyclic peptide, had excellent activity against Gram-positive organisms, anaerobes, and *M. tuberculosis* (Selva et al., 1991), and was very effective at curing experimental infections. However, attempts to formulate an acceptable injectable preparation of GE2270 were unsuccessful, precluding clinical development.

A method was devised to screen for inhibitors of HIV protease using a recombinant protein mimicking the natural protease substrate, cleavage of which could be detected by ELISA. Several streptomycetes producing the peptidic aldehyde α-MAPI (microbial alkaline protease inhibitor) were found (Stella et al., 1991), as well as a strain producing two novel MAPI analogues (Stefanelli et al., 1995).

Several tests based on enzyme inhibition or utilizing cell-free systems could not be applied directly to fermentation broths due to the frequent occurrence of interfering substances or enzymes (*e.g.*, proteases or RNAses) in the broths. Therefore, spent media were treated with resins, followed by elution and solvent evaporation. This procedure was streamlined, miniaturized, and automated so that by the early 1990s crude fermentation broths were no longer tested. All screening was performed on extracts, with robots handling most of the operations required for biochemical or microbiological assays.

Screening for inhibitors of bacterial RNA polymerase identified a new cyclic peptide, GE23077, produced by an *Actinomadura* strain (Ciciliato et al., 2004). While this compound was very active in cell-free systems, it was practically inactive towards all bacterial strains examined.

During this period the LRCG also functioned as a screening center for other therapeutic areas within Dow-Merrell (later Marion Merrell Dow), and screens for non-antibiotic substances were introduced. One such screen, measuring the enzymic release of phosphate from β-glycerolphosphate, aimed at detecting inhibitors of **L**-*myo*-inositol-1-phosphate phosphatase, an enzyme thought to be involved in bipolar disorder. Out of 12,000 strains surveyed only six extracts, derived from strains of the *Stachybotrys/Memnionella* group of fungi, demonstrated the desired inhibitory activity, which turned out to be due to previously-described sesquiterpene metabolites (Stefanelli et al., 1966). In another screening campaign over 30,000 microbial extracts were tested for inhibitors of type-1 interleukin-1 receptor using a displacement assay with soluble interleukin-1 receptor. Two active compounds were found, identified as niphimycin and flavipin (Stefanelli et al., 1997).

Our results raised serious questions about the validity of the supposition that novel assays would uncover novel metabolites from highly exploited sources, since for the most part we were discovering novel (and often dubious) mechanisms of action for old compounds. A possible exception to this was novel compounds active in cell-free systems but inactive against whole cells, probably due to permeation problems, which had been overlooked in previous antimicrobial screens utilizing whole cells.

Molecular genetics

The small group of molecular geneticists at LRCG was interested mainly in identifying the biosynthetic gene clusters encoding our promising antibiotics, with the aim of genetically manipulating the biosynthetic pathways to improve yields and possibly obtain new products by combinatorial biosynthesis. Probing the non-ribosomal peptide synthetase (NRPS) genes of GE2270-producer *Planobispora rosea* demonstrated that this strain possessed many more NRPS genes than supposedly were needed for GE2270 biosynthesis. Extension of this search to other actinomycetes revealed that most strains contained NRPS genes far in excess of the number presumably required for biosynthesis of the peptide antibiotics they were known to produce (Sosio et al., 2000a). This finding has been confirmed by whole genome sequencing of *Streptomyces* strains, whose genetic information for encoding secondary metabolites is greater than the number of secondary metabolites that they presently are known to produce (Ikeda et al., 2003).

A serious limitation to working with rare actinomycetes was the lack of efficient methods for genetic manipulation. To overcome this hurdle, artificial chromosomes were developed which could be shuttled between *Escherichia coli* and a streptomycete host amenable to genetic manipulation. Over 100 kb could be mobilized into *Streptomyces lividans* using ESAC vectors (Sosio et al., 2000b). However, heterologous expression of entire gene clusters was not pursued further at LRCG.

Antibiotic modifications

A program devoted to chemical modification of teicoplanin resulted in several derivatives with marginally improved properties, none of which were deemed worthy of clinical development. Some glycopeptide modifications, such as deacylation or demannosylation, were achieved by biological rather than chemical means. Although only a few derivatives were prepared by precursor-directed biosynthesis, this technique appears to be of general utility for obtaining novel teicoplanins (and other glycopeptides) bearing novel acyl functionalities (Lazzarini et al, 1997) (*vide supra*).

A program devoted to the chemical modification of glycopeptide A 40926 yielded a product initially called AA-1, then BI 397, which was more active then either teicoplanin or vancomycin (Malabarba et al., 1995), and which had a better pharmacokinetic profile; it was developed subsequently by other companies under the name "dalbavancin" (Fig. 8). Phase 3 clinical trials of dalbavancin have been completed, and the product is awaiting final FDA approval.

1996-1997: The LRCG is closed, Biosearch Italia is born

In May 1995 the Frankfurt pharmaceutical giant Hoechst AG acquired all of the pharmaceutical assets of Dow Chemical, including the LRCG. The next year Hoechst decided that the LRCG was not of strategic importance, since Hoechst already had two centers dedicated to natural products research (in Frankfurt, Germany and Mulund, India) and one center (in Romainville, France) dedicated to anti-infectives. Eventually a management buyout of the LRCG was agreed upon, and in January 1997 a new company, Biosearch Italia, was established. With the creation of Biosearch Italia, the Lepetit Research Laboratories formally ceased to exist, whereas Lepetit S.p.A. remained as a subsidiary of Hoechst.

Several characteristics of the old Lepetit laboratories survived in Biosearch Italia. Franco Parenti, who had left the LRCG in 1990 to assume managerial positions in Italy and elsewhere in Europe, returned to Gerenzano and joined Biosearch Italia to guide its research and business activities. Screening efforts were refocused on antimicrobial targets and identifying inhibitors of bacterial transcription, translation, and cell wall biosynthesis. The search for new actinomycete genera, an approach which Lepetit had pioneered and pursued for many years, was resumed at Biosearch Italia, in which the latest techniques of molecular biology were applied to strain characterization and analysis of DNA recovered from soil samples. As a result of these efforts several new genera have been described (Donadio et al., 2005). In 2003 Biosearch Italia merged with the American firm Versicor, Inc. to become Vicuron Pharmaceuticals. In June 2005 Vicuron was acquired by Pfizer, Inc. in a deal valued at close to US$2 billion. The future of the Gerenzano research laboratories remains unclear, though it is to be hoped that this treasure trove of knowledge and experience will not become dispersed and irretrievably lost.

Acknowledgements

Special thanks go to Stefano Donadio for his relevant suggestions and for his revision of the draft manuscript. The assistance of Dr. Stuart Shapiro of Basilea Pharmaceutica AG (Basel, Switzerland; shapiro.stuart@basileapharma.com) in the preparation of this article is gratefully acknowledged.

References

Note: In the text the research programs are attributed to the periods during which they were in effect. Publication of results normally occurred some years later.

Arioli, V., R. Pallanza, S. Fürész,, and G. Carniti, 1967. Rifampicin: a new rifamycin. I. Bacteriological studies. Arzneimittelforschung 17: 523-529.

Arioli, V., M. Berti, and. L.G. Silvestri, 1976. Gardimycin, a new antibiotic from *Actinoplanes*. III. Biological properties. J Antibiot 29: 511-515.

Arioli, V., M. Berti, G. Carniti, E, Randisi, E. Rossi, and R. Scotti, 1981. Antibacterial activity of DL 473, a new semisynthetic rifamycin derivative. J Antibiot 34: 1026-1032.

Baltz, R. H. 2005. Antibiotic discovery from actinomycetes: will a renaissance follow the decline and fall? SIM News 55: 186-196.

Bardone, M. R., M. Paternoster, and C. Coronelli. 1978. Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* nov. sp. II. Extraction and chemical characterization. J Antibiot 31: 170-177.

Barry, A. L. and P. C. Fuchs. 1991. Influence of the test medium on azithromycin and erythromycin regression statistics. Eur J Clin Microbiol Infect Dis 10: 846-849.

Berti, M., G. Candiani, M. Borgonovi, P. Landini, F. Ripamonti, R. Scotti, L. Cavenaghi, M. Denaro, and B. P. Goldstein. 1992. Antimicrobial activity of MDL 62,873, a semisynthetic derivative of teicoplanin, in vitro and in experimental infections. Antimicrob Ag Chemother 36: 446-452.

Borghi, A., C. Coronelli, L. Faniuolo, G. Allievi, R. Pallanza, and G. G. Gallo. 1984. Teichomycins, new antibiotics from *Actinoplanes teichomyceticus*. Separation and characterization of the components of teichomycin (teicoplanin). J Antibiot 37: 615-620.

Borghi, A., D. Edwards, L. F. Zerilli, and G. C. Lancini. 1991. Factors affecting the normal and branched-chain acyl moieties of teicoplanin components produced by *Actinoplanes teichomyceticus*. J Gen Microbiol 137: 587-592.

Brufani, M., W. Fedeli, G. Giacomello, and A. Vaciago. 1964. The X-ray analysis of the structure of rifamycin B. Experientia 20: 339-340.

Cassani, G.. 1989. Glycopeptides: antibiotic discovery and mechanism of action. Progr Indus Microbiol 27: 221-235.

Cavalleri, B., G. C. Volpe, V. Arioli, and G. C. Lancini. 1977. Synthesis and biological activity of two metabolites of 1-methyl-5-(1-methylethyl)-2-nitro-1*H*-imidazole, an antiprotozoal agent. J Med. Chem 20: 1522-1525.

Cavalleri, B., H. Pagani, G. Volpe, E. Selva, and F. Parenti. 1984. A-16686, a new antibiotic from *Actinoplanes*. I. Fermentation, isolation and preliminary physico-chemical characteristics. J Antibiot 37: 309-317.

Chatterjee, S., S. Chatterjee, S. J. Lad, M. S. Phansalkar, R. H. Rupp, B. N. Ganguli, H. W. Fehlhaber, and H. Kogler.1992. Mersacidin, a new antibiotic from *Bacillus*. Fermentation, isolation, purification and chemical characterization. J. Antibiot 45: 832-838.

Ciabatti, R., J. K. Kettenring, G. Winters, G. Tuan, L.F. Zerilli, and B. Cavalleri. 1989. Ramoplanin (A-16686), a new glycolipodepsipeptide antibiotic. III. Structure elucidation. J Antibiot 42: 254-267.

Ciciliato, I., E. Corti, E. Sarubbi, S. Stefanelli, L. Gastaldo, N. Montanini, M. Kurz, D. Losi, F. Marinelli, and E. Selva. 2004. Antibiotics GE23077, novel inhibitors of bacterial RNA polymerase. I. Taxonomy, isolation and characterization. J Antibiot 57: 210-217.

Coronelli, C., C. R. Pasqualucci, G. Tamoni, and G. G. Gallo. 1966. Isolation and structure of alanosine, a new antibiotic. Farmaco Ed Sci 21: 269-277.

Coronelli, C., H. Pagani, M. R. Bardone, and G. C. Lancini. 1974. Purpuromycin, a new antibiotic isolated from *Actinoplanes ianthinogenes*. J Antibiot 27: 161-167.

Coronelli, C., R. J. White, G. C. Lancini, and F. Parenti.1975. Lipiarmycin, a new antibiotic from *Actinoplanes*. Isolation, chemical, biological and biochemical characterization. J Antibiot 28: 253-259.

Coronelli, C., G. Tamoni, and G. C. Lancini. 1976. Gardimycin, a new antibiotic from *Actinoplanes*. II. Isolation and preliminary characterization. J Antibiot 29: 507-510.

Corti, A., and G. Cassani. 1985. Synthesis and characterization of D-alanyl-D-alanyl-agarose: a new bioselective absorbent for affinity chromatography of glycopeptide antibiotics. Appl Biochem Biotechnol 11: 101-109.

Craveri, R., C. Coronelli, H. Pagani, and P. Sensi. 1964. Thermorubin, a new antibiotic from a thermoactinomycete. Clin Med 71: 511-521.

Donadio, S., E. Busti, P. Monciardini, R. Bamonte, P. Mazza, M. Sosio, and L. Cavaletti. 2005. Sources of polyketides and nonribosomal peptides. *In* Biocombinatorial Approaches for Drug Finding (Ernst Schering Research Foundation Workshop) (W. Wohlleben, T. Spelling, and B. F. Müller-Tiemann, Eds.). Springer-Verlag, Berlin, pp. 19-41.

Gallo, G. G., C. R. Pasqualucci, and P. Sensi. 1962. Spirodiossalanoarenoni. Nota 1. Sintesi di 1,4-diossaspiro-[4,5]-deca-6,9-dien-2,8-dioni alogenati. Ann Chim 52: 902-910.

Gallo, G. G., C. R. Pasqualucci, N. Maggi, R. Ballotta, and P. Sensi. 1966. Rifamycins. XLII. Rifazine, a new phenazinic derivative of rifamycins. Farmaco Ed Sci 21: 68-75.

Goldstein, B. P., E. Selva, L. Gastaldo, M. Berti, R. Pallanza, F. Ripamonti, P. Ferrari, M. Denaro, V. Arioli, and G. Cassani. 1987. A 40926, a new glycopeptide antibiotic with anti-*Neisseria* activity. Antimicrob Ag Chemother 31: 1961-1966.

Gumpert, J. 2005. (Z)IMET/HKI: a half-century of world-class biotechnological research in Jena. SIM News 55: 141-154.

Hartmann, G., K. O. Honikel, F. Knusel, and J. Nuesch. 1967. The specific inhibition of the DNA-directed RNA synthesis by rifamycin. Biochim Biophys Acta 145: 843-844.

Ikeda H., J. Ishikawa, A. Hanamoto, M. Shinose, H. Kikuchi, T. Shiba, Y. Sakaki, M. Hattori, and S. Ōmura. 2003. Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. Nat Biotechnol21: 526-531.

Kluepfel, D., G. C. Lancini, and G. Sartori. 1965. Metabolism of barbital by *Streptomyces mediterranei*. Appl Microbiol 13: 600-604.

Kradolfer, F., L. Neipp, and W. Sackmann. 1966. Chemotherapeutic activity of new derivatives of rifamycin. Antimicrob Ag Chemother 6: 359-364.

Lancini, G. C., D. Kluepfel, E. Lazzari, and G. Sartori. 1966. Origin of the nitro group of azomycin. Biochim Biophys Acta 130: 37-44.

Lancini, G. C. and E. Lazzari. 1965. The synthesis of azomycin (2-nitroimidazole). Experientia 21: 83.

Lancini, G. C., A. Diena, and E. Lazzari. 1966. The synthesis of alanosine [L-2-amino-3-(*N*-nitrosohydroxylamino)propionic acid]. Tetrah Lett 16: 1769-1772.

Lancini, G. C. & E. Lazzari. A new synthesis of alkyl and aryl 2-aminoimidazoles. J. Heterocyclic Chem 3: 152-154 (1966).

Lancini, G. C. and P. Sensi. 1967. Studies on the final steps of rifamycins biosynthesis. *In* Proc. 5th Int. Congr. Chemother. (K. H. Spitzy and H. Hasceck, Eds.). Verlag der Wiener Medizinischen Akademie, Vienna, vol. 1., pp. 41-47.

Lancini, G. C., E. Lazzari, and G. Sartori. 1968. Microbial oxidation of aminoimidazoles to nitroimidazoles. J Antibiot. 21: 387-392.

Lancini, G. C. and G. Sartori. 1968. Rifamycins LXI: *in vivo* inhibition of RNA synthesis by rifamycins. Experientia 24: 1105-1106.

Lancini G. C., G. G.Gallo, G. Sartori, and P. Sensi. 1969. Isolation and structure of rifamycin L and its biogenetic relationship with other rifamycins. J. Antibiot 22: 369-377.

Lancini, G. C. and C. Hengheller. 1969. Isolation of rifamycin SV from a mutant *Streptomyces mediterranei* strain. J Antibiot 22: 637-638.

Lancini, G. C., R. Cricchio, and L. Thiry. 1971. Antiviral activity of rifamycins and *N*-aminopiperazines. J Antibiot. 24: 64-66.

Lancini G. C. and W. Zanichelli. 1977. Structure-activity relationships in rifamycins. *In* Structure-Activity Relationships among the Semisynthetic Antibiotics (D. Perlman, Ed.). Academic Press, New York, pp. 531-596.

Lancini G. C. and M. Grandi.1981. Biosynthesis of ansamycins. *In* Antibiotics IV - Biosynthesis (J. W. Corcoran, Ed.). Springer-Verlag, New York, pp. 12-40.

Lancini, G. C., P. Antonini, and A. Borghi. 1988. Method for selectively increasing the ratio of single major components of antibiotic A/16686. European Patent 0259780 (A2), published 16 March 1988.

Lancini G. C. and B. Cavalleri.1997. Rifamycins. *In* Biotechnology of Antibiotics (W. R. Strohl, Ed.). Marcel Dekker, Inc., New York, pp. 521-549.98.

Lazzarini, A., A. Borghi, L. F. Zerilli, P. Ferrari, L. Colombo, and G. C. Lancini. 1997. Novel teicoplanins by directed biosynthesis. J Antibiot 50: 180-183.

Maeda, K., T. Osato, and H. Umezawa. 1953. A new antibiotic, azomycin. J Antibiotic 6: 182.

Maggi, N., C. R. Pasqualucci, R. Ballotta, and P Sensi . 1966. Rifampicin: a new orally active rifamycin. Chemotherapy 11: 285-292.

Maggi, N., G. G. Gallo, and P. Sensi. 1967. Synthesis of 3-formylrifamycin SV. Farmaco Ed Sci 22: 316-325.

Makkar, N. S. and T. Cross. 1982. Actinoplanetes in soil and on plant litter from freshwater habitats. J Appl Bacteriol 52: 209-218.

Malabarba, A., R. Pallanza, M. Berti, and B. Cavalleri. 1990. Synthesis and biological activity of some amide derivatives of the lantibiotic actagardine. J Antibiot 43: 1089-1097.

Malabarba, A., R. Ciabatti, R. Scotti, B. P. Goldstein, P. Ferrari, M. Kurz, B. P. Andreini, and M. Denaro. 1995. New semisynthetic glycopeptides MDL 63,246 and MDL 63,042, and other amide derivatives of antibiotic A-40,926 active against highly glycopeptide-resistant VanA enterococci. J Antibiot 48: 869-883.

Margalith, P. and H. Pagani. 1961. Rifomycin.

XIV. Production of rifomycin B. Appl Microbiol 9: 325-334.

Miyazaki, N., H. Ohkura, N. Kajimura, and N. Okazaki. 1992. A rapid anticancer drug screening assay by [^{14}C] thymidine uptake in cultured human cancer cells. Eur. J Cancer. 28: 1419-1422.

Murthy Y. K. S., J. E. Thiemann, C. Coronelli, and P. Sensi. 1966. Alanosine, a new antiviral and antitumour agent isolated from a *Streptomyces*. Nature 211: 1198-1199.

Oppolzer, W., V. Prelog, and P. Sensi. 1964. Konstitution des Rifamycins B und verwandter Rifamycine. Experientia 20: 336-339.

Ormerod L.P. 2003. Other mycobacterial infections. *In* Antibiotic and Chemotherapy, 8th Edn. (R. G. Finch, D. Greenwood, S. R. Norrby, and R. J. Whitley, Eds.). Churchill-Livingstone, London, pp. 817-832.

Pagani, H. and G. C. Lancini. 1970. A new technique for the isolation of microorganisms belonging to the genus *Actinoplanes*. Department of Antibiotics and Natural Products, Lepetit Report N° 44, 22 December 1970, pp. 1-13.

Pallanza, R., V. Arioli, S. Fürész, and G. Bolzoni. 1967. Rifampicin: a new rifamycin. II. Laboratory studies on the antituberculous activity and preliminary clinical observations. Arzneimittelforschung 17: 529-534.

Pallanza, R., M. Berti, B. P. Goldstein, E. Mapelli, E. Randisi, R. Scotti, and V. Arioli.1983. Teichomycin: *in-vitro* and *in-vivo* evaluation in comparison with other antibiotics. J Antimicrob Chemother. 11: 419-425.

Pallanza, R., M. Berti, R. Scotti, R. Randisi, and V. Arioli. 1984. A-16686, a new antibiotic from *Actinoplanes*. II. Biological properties. J Antibiot 37: 318-324.

Parenti, F., G. C. Schito, and P. Courvalin. 2000. Teicoplanin chemistry and microbiology. J Chemother 12(Suppl. 5): 5-14.

Parenti, F. and G. C. Lancini. 2003. Rifamycins. *In* Antibiotic and Chemotherapy, 8th Edn. (R. G. Finch, D. Greenwood, S. R. Norrby, and R. J. Whitley, Eds.). Churchill-Livingstone, London, pp. 374-381.

Pirali, G., S. Somma, G. C. Lancini, and F. Sala. 1974. Inhibition of peptide chain initiation in *Escherichia coli* by thermorubin. Biochim Biophys Acta 366: 310-318.

Sabath, L. D., V. Lorian, D. Gerstein, P. B. Loder, and M. Finland. 1968. Enhancing effect on alkalinization of the medium on the activity of erythromycin against Gram-negative bacteria. Appl Microbiol. 16: 1288-1292.

Selva, E., G. Beretta, N. Montanini, G.S. Saddler, L. Gastaldo, P. Ferrari, R. Lorenzetti, P., Landini, F. Ripamonti, B. P. Goldstein, M. Berti, L. Montanaro, and M. Denaro. 1991. Antibiotic GE2270 A: a novel inhibitor of bacterial protein synthesis. I. Isolation and characterization. J Antibiot. 44: 693-701.

Sensi, P., G. A. De Ferrari, G. C. Gallo, and G. Rolland. 1955. Un nuovo antibiotico: la bromotetraciclina. I. Isolamento e caratteristiche chimico fisiche. Farmaco Ed Sci. 10: 337-345.

Sensi, P., R. Ballotta, and M. Greco. 1960a. Rifomycin. V. Rifomycin O, a new antibiotic of the rifomycin family. Farmaco Ed Sci 15: 228-234.

Sensi, P., A. M. Greco, and R. Ballotta. Rifomycin. 1960b. I. Isolation and properties of rifomycin B and rifomycin complex. Antibiot. Annu. (1959-1960) 7: 262-270.

Sensi, P., R. Ballotta, A. M. Greco, and G. G. Gallo. Rifomycin. 1961. XV. Activation of rifomycin B and rifomycin O. Production and properties of rifomycin S and rifomycin SV. Farmaco Ed Sci 16: 165-180.

Sensi, P., M. T. Timbal, and A. M. Greco. 1962. Rifamycin XXVI. Derivatives of rifamycin O with high antimicrobial activity. Antibiot Chemother 12: 488-494

Sensi, P., N. Maggi, R. Ballotta, S. Fürész, R. Pallanza, and V. Arioli. 1964. Rifamycins. XXXV. Amides and hydrazides of rifamycin B. J Med Chem 7: 596-602.

Sippel, A., and G. Hartmann.1968. Mode of action of rifamycin on the RNA polymerase reaction. Biochim Biophys Acta 157: 218-219.

Somma, S., W. Merati, and F. Parenti. 1977. Gardimycin, a new antibiotic inhibiting peptidoglycan synthesis. Antimicrob Ag Chemother 11: 396-401.

Somma, S., L. Gastaldo, and A. Corti. 1984. Teicoplanin, a new antibiotic from *Actinoplanes teichomyceticus*. Antimicrob Ag Chemother 26: 917-923.

Sosio, M., E. Bossi, A. Bianchi, and S. Donadio. 2000a. Multiple peptide synthetase gene clusters in actinomycetes. Mol Gen Genet 264: 213-221.

Sosio, M., F. Giusino, C. Cappellano, E. Bossi, A. M. Puglia, and S. Donadio. 2000b. Artificial chromosomes for antibiotic-producing actinomycetes. Nat Biotechnol 18: 343-345.

Stackebrandt, E. and C. R.Woese. 1981. Towards a phylogeny of the actinomycetes and related organisms. Curr Microbiol 5: 197-202.

Stackebrandt, E., W. Ludwig, E. Seewaldt, and K.H. Schleifer. 1983. Phylogeny of spore-forming members of the order *Actinomycetales*. Int J Syst Bacteriol 33: 173-180.

Stefanelli, S., L. Cavaletti, E. Sarubbi, E. Ragg, L. Colombo, and E. Selva. 1995. GE20372 factor A and B. New HIV-1 protease inhibitors, produced by *Streptomyces* sp. ATCC 55925. J Antibiot 48: 332-334.

Stefanelli, S., F. Sponga, P. Ferrari, C. Sottani, E. Corti, C. Brunati, and K. Islam. 1996. Inhibitors of *myo*-inositol monophosphatase, ATCC 20928 factors A and C. Isolation, physico-chemical characterization and biological properties. J Antibiot 49: 611-616.

Stefanelli, S., E. Corti, N. Montanini, M. Denaro, and E. Sarubbi. 1997. Inhibitors of type-I interleukin-1 receptor from microbial metabolites. J Antibiot 50: 484-489.

Stella, S., G. Saddler, E. Sarubbi, L. Colombo, S. Stefanelli, M. Denaro, and E. Selva. 1991. Isolation of α-MAPI from fermentation broths during a screening program for HIV-1 protease inhibitors. J Antibiot 44: 1019-1022.

Thiemann, J. E., C. Hengheller, A. Virgilio, O. Buelli, and G. Licciardello. 1964. Rifamycin. XXXIII. Isolation of actinophages active on *Streptomyces mediterranei* and characteristics of phage-resistant strains. Appl Microbiol 12: 261-268.

Thiemann, J. E., H. Pagani, and G. Beretta. 1967a. A new genus of *Actinoplanaceae*: *Dactylosporangium*, gen. nov. Arch

Mikrobiol 58: 42-52.

Thiemann, J. E., H. Pagani, and G. Beretta. 1967b. A new genus of *Actinoplanaceae*: *Planomonospora*, gen. nov. G Microbiol 15: 27-38.

Thiemann, J. E. and G. Beretta. 1968. A new genus of the *Actinoplanaceae*: *Planobispora*, gen. nov. Arch Microbiol 62: 157-166.

Thiemann, J. E., H. Pagani, and G. Beretta. 1968. A new genus of the A*ctinomycetales*: *Microtetraspora*, gen. nov. J Gen. Microbiol 50: 295-303.

Timbal, M. T. 1960. Rifomycin. II. Antibacterial activity of rifomycin B. Antibiot Annu (1959-1960) 7: 271-276.

Timbal, M. T. and A. Brega. 1961. Rifomycin XVII. Rifomycin SV treatment of experimental infections. Farmaco Ed Sci 16: 191-199.

Tischler, A. N., F. M.Thomspon, L. J. Libertini, and M. Calvin. 1974. Rifamycin derivatives as inhibitors of a ribonucleic acid instructed deoxyribonucleic acid polymerase function. Effect of lipofilicity. J Med Chem 17: 948-952.

Waltho, J. P., D. H. Williams, E. Selva, and P. Ferrari. 1987. Structure elucidation of the glycopeptide antibiotic complex A40926. J Chem Soc, Perkin Trans I, 2103-2107.

Weinstein, M.P. 2004. *Micromonospora* antibiotic discovery at Schering/Schering-Plough (1961-1973): a personal reminiscence. SIM News 54: 56-66.

White, R. J., G. Sartori, and G. C. Lancini. 1992. A system for studying the mechanism of action of new antibiotics. Department of Antibiotics and Natural Products, Lepetit Report N° 58, 03 January 1972, pp. 1-22.

White, R. J., E. Martinelli, G. G. Gallo, G. Lancini, and P. Beynon.1973. Rifamycin biosynthesis studied with ^{13}C-enriched precursors and carbon magnetic resonance. Nature 243: 273-277.

White, R. J., E. Martinelli, and G. Lancini. 1974. Ansamycin biogenesis: studies on a novel rifamycin isolated from a mutant strain of *Nocardia mediterranei*. Proc Nat Acad Sci USA 71: 3260-3264.

White, R. J. and G. C. Lancini. 1975. Production of rifamycin B. USP 3,871,965, issued 18 March 1975.

Yang, S. S., F. M. Herrera, R. G. Smith, M. S. Reitz, G. Lancini, R. C. Ting, and R. C. Gallo. 1972. Rifamycins antibiotics: inhibitors of Rauscher murine leukemia virus reverse trancriptase and of purified DNA polymerases from human normal and leukemic lymphoblasts. J Nat Cancer Inst 49: 7-25.

Zerilli, L. F., G. Tuan, M. Turconi, and C. Coronelli. 1977. Mass spectra of lanthionine and β-methyllanthionine isolated from gardimycin. Ann Chim 67: 691-697.

Zerilli, L. F., D. M. F. Edwards, A. Borghi, G. G. Gallo, E. Selva, M. Denaro, and G. C. Lancini. 1992. Determination of the acyl moities of the antibiotic complex A 40926 and their relation with the membrane lipids of the producing strain. Rapid Comm Mass Spectrom 6: 109-114.

Brief biographies of some persons mentioned in the text

Melvin Calvin (1910-1997) American chemist. Nobel laureate. After receiving a Ph. D. in chemistry from the University of Minnesota in 1935, Calvin spent two years at the University of Manchester (U.K.). In 1937 he began his academic career at the University of California, Berkeley, and in 1946 he was appointed director of the bio-organic chemistry group at the Lawrence Radiation Laboratory in Berkeley. The bioorganic chemistry group became the Laboratory of Chemical Biodynamics in 1960; in 1980, upon Calvin's retirement, the laboratory was renamed the Melvin Calvin Laboratory. Calvin's scientific interests focused on theoretical aspects of organic chemistry, particularly metal chelate compounds, though his major accomplishment, for which he was awarded the Nobel Prize in Chemistry in 1961, was the elucidation of the pathway of CO_2 assimilation during photosynthesis.

Roberto Giorgio Lepetit (1842-1907). French chemist (Italian citizen since 1886). After studying under Théophile-Jules Pelouze in Paris, Lepetit moved to Basel where, working at the Dolfuss Chemical Company, he synthesized two new, important dyes. In 1868, Lepetit established the firm "Legoda" (Lepetit + Dolfuss + Gansser) in northern Italy to produce tannins, dyes, inks, and other chemicals. Original production processes developed at Ledoga led to its becoming a leading Italian chemical company. In 1875 Lepetit published "Manuale del Tintore" ("Dyer's Manual"). He devised an innovative process for extracting high quality tannins from chestnut wood, which led to their use in the tanning industry.

Roberto Lepetit (1865-1928) Italian chemist, son of Roberto Giorgio Lepetit. Lepetit graduated from the ETH (Zürich) in 1885 and gained professional experience in various European chemical companies before joining Ledoga as "Head Chemist" in 1893, eventually becoming Scientific Director. In addition to Ledoga's traditional product line, Lepetit pursued research on medicinal chemistry and established a Pharmaceutical Department that was the precursor of the pharmaceutical company Lepetit S.p.A. Lepetit was a discoverer of the "Bucherer-Lepetit reaction" (interconversion of β-naphthol and β-naphthylamine), used widely for the synthesis of dyes. Lepetit was a founder and first president of the Italian Association of Chemists and Tanners, and in 1923 was appointed Professor of Medicinal Chemistry at the University of Pavia.

Francesco Parenti (1940-) Italian biologist. A 1963 graduate of the University of Milan, Parenti began his academic career at the same university, then worked at the Smithsonian Institution (Washington, D. C) and Yale University before being appointed an associate professor at the University of Bari. In 1972 he joined Lepetit as head of the fermentation laboratory, and in 1974 became head of the Antibiotics and Natural Products Department. Parenti made essential contributions to the development and commercialisation of teicoplanin. In 1985 he was named Director of the LGRC; in 1990 he left research to become General Director of Lepetit S.p.A. and in 1992 was appointed General Director of Marion Merrell Dow Europe Inc. In 1995 Parenti returned to Gerenzano as an Executive Officer and Research Director of Biosearch Italia. Although formally retired since 2005, he remains active as a consultant to important financial enterprises.

Vladimir Prelog (1906-1998). Swiss chemist. Born in Sarajevo (then part of the Austro-Hungarian Emprie), Prelog attended a gymnasium in Zagreb, then studied chemistry at the Czech Institute of Technology in Prague, from which he graduated in 1929. Prelog worked in the small chemical laboratory of the firm G. J. Dríza in Prague until 1935, when he became a lecturer at the University of Zagreb. During the German occupation of Yugoslavia his situation became untenable, and in 1941 he was invited by Prof. Dr. Leopold (Lavoslav) Ružička (Nobel Prize in Chemistry, 1939) to join the Organic Chemistry Laboratory at the ETH in Zürich. Starting as assistant professor, Prelog was promoted to full professor in 1952 and Head of the Laboratory in 1957. In 1959 Prelog became a Swiss citizen but spoke of himself as "a Swiss patriot and a world citizen." While pursuing research on natural products, stereochemical problems emerged continuously, which aroused his interest in chirality and its relevance to enzyme-catalyzed reactions. Prelog was awarded the Nobel Prize in Chemistry in 1975 "for his research into the stereochemistry of organic molecules and reactions."

Piero Sensi (1920-) Italian chemist. Graduated from the University of Naples in 1944 with a degree in physical chemistry. His first working experience involved synthesis of new dyes at the Italian chemical company Montecatini. In 1950 Sensi joined Lepetit, where he established the Physical Chemistry Laboratory, which at the time was equipped with the most modern spectroscopic tools. Sensi devised industrial processes for the recovery and purification of tetracycline and novobiocin from fermentation broths, and helped organize the antibiotic screening program; in 1956 he was appointed Head of the Antibiotics and Natural Products Department. Sensi's international reputation was established with the discovery of the rifamycins. In 1966 he was named Director of Lepetit's Research Laboratories which, under his leadership, grew in size and scientific excellence. In 1974 he was appointed Director of Research and Development at Lepetit, during which time he continued his scientific activities, giving lectures and contributing to important treatises on medicinal chemistry. Sensi taught Industrial Microbiology at the University of Milan, and upon his retirement from Lepetit in 1978 he was appointed to a professorship at that university.

About the author

Giancarlo Lancini graduated from the University of Pavia in 1956 with a degree in Chemistry. In 1969 he earned the title "Professor" ("Libero Docente") in Pharmaceutical and Toxicological Chemistry. In 1957 Lancini joined Lepetit, where in 1966 he became head of the Department of Antibiotics and Natural Products, and in 1974 Director of the Research Laboratories. His research activities span several aspects of the antibiotic field: screening, biosynthesis, mechanisms of action, synthesis, semisynthesis, and microbial transformation. Besides his work at Lepetit, Lancini taught courses on the Chemistry of Fermentation at the University of Pavia (1971-1982) and the Biotechnology of Fermentations at the University of Milan (2000-present). He formally retired from Lepetit in 1994, working thereafter as a consultant with important responsibilities at the LGRC and subsequebntly at Biosearch Italia. Lancini has participated as an invited speaker or session chairman at various international symposia and congresses. He is an emeritus member of the *Journal of Antibiotics* Editorial Board and of the International Commission of Genetics of Industrial Microorganisms; he is also a member of the Quarter Century Club of the SIM, as well as a member of the Council of the Federation of European Microbiological Societies. Lancini has authored over 100 scientific papers and two books, one of which (*Antibiotics: An Integrated View*, published in English by Springer-Verlag in July 1982) has been translated into Italian, Russian, and Chinese.

Contact Information:

Dr. Giancarlo Lancini
Via Forcella 9
20100 Milano, Italy
Tel: ++39 02 89407498
E-mail: gclan@ibero.it