Sample 510 appears just as nondescript as the others – a spoonful of earth from the bank of the Zambezi in Africa. But the bacteria living in the sand produce agents that are currently among the most promising cancer medicines of the future: epothilones.

1985 - THE BEGINNING OF A TURBULENT CAREER FOR ONE PARTICULAR SUBSTANCE

One of the samples that made its way to Hans Reichenbach’s store room in the Department of Biology of Natural Products contains hundreds of different types of bacteria. Some of these produce a whole range of substances that enable them to combat aggressors, procure nourishment or exchange information with other bacteria. Over 100,000 of such natural products have now been identified and these are joined by approximately 2000 new ones each year. Natural products are classics in the repertoire of medicinal agents – approximately half of all medicines in use today can be traced back, directly or indirectly, to agents produced by plants or microbes. These include substances such as the antibiotics Penicillin and Cephalosporin as well as mevinolin-type hypotensive agents. Cyclosporin enabled the first organ transplants to be carried out and continues to play a vital role in that field. However, above all, natural products are a significant basis for synthetic medicines: chemists modify the structure of the natural products slightly, thus making them stronger or more selective – as well as more tolerable – than the original molecule that the organisms produce.

Bacteria need to be able to communicate with their energy, only producing those substances that they require for survival – they do not waste energy on ineffective substances. Natural products are also effective per se, but many bacteria revert back to the same basic structures, with the consequence that similar or even identical substances are often found in different bacterial isolates. In the course of their search for new, previously unknown substances and mechanisms, Hans Reichenbach’s team investigated samples for myxobacteria – a bacteria group that had been largely ignored until that time. Myxobacteria live primarily in the soil and enjoy a special position among bacteria with regard to several aspects. They stand on the threshold between single and multi-cell bacteria and have particularly large genomes. They form swarms of thousands of individual bacteria, with these using chemical messenger substances to communicate with one another. When nourishment is scarce, they join up to form fruiting bodies. Their entire lifestyle is more reminiscent of multi-cell myxomycetes than typical bacteria. From soil sample 510 from the banks of the Zambezi Hans Reichenbach isolated “So ce90”, the abbreviation for sorangium cellulosum strain 90. One of 1700 strains of this species that the scientists at the Helmholtz Center for Infection Research in Braunschweig the scientists Hans Reichenbach and Gerhard Hofe had begun specifying in research into microbial natural products 30 years previously. At that time under the name Society for Biotechnological Research, or GBF. They investigated the samples collected from nature in the hopes of discovering new agents for medicinal or plant protection purposes.

The name epothilone is derived from the structure of the new type of natural product. The compound contains so-called functional groups, the interaction of which gives the molecule its effectiveness. The names of these characteristic groups are epoxy, thiazole and ketone – together forming the name epo-thi-l-one.

E. coli primarily produces the epothilones A and B, but with traces of a further 30. In chemical processes over 200 variants that do not occur in nature were produced at the GBF alone. They display alterations on many points of their structure and manifest completely different physical and chemical properties and activities in the biological tests.

However, the use of epothilones no longer necessarily requires the use of bacteria. Following the publication of the precise structure by Gerhard Hofe, a race began amongst chemists around the world. The finish line, passed in 1996, was to produce epothilone without bacteria, in a purely chemical process. These so-called total syntheses form the basis for further epothilone medicines that are still in the developmental stage.
The fact that So-cells is a strain with unusual potential does not become apparent until two years later. In 1967, the biologist Klaus Gerth is searching through the department collection in search of strains that produce substances to counter fungus. In a screening process he tests hundreds of bacteria for their effects on harmful fungi. The strains that are particularly intensive in the metabolites of the bacteria. In order to process this, a pure bacterial strain is placed in a culture medium. In so-called shaking flasks the bacteria can enjoy good conditions for growth: ample nutrients, warmth and – thanks to the shaking – oxygen. As a culture medium, nutrient broth is formed. These substances are then extracted. The so-called organic extract is created. Most of all, the so-called organic extract of the bacteria is concentrated form. With this organic extract the scientist can conduct the initial tests, because if the strain contains a substance, then the extract as such will be effective. And this is where So-cells appears for the first time.

in a petri dish – a round, flat dish – fungi are growing on a culture medium. Only one culture medium Klaus Gerth placed small paper filter sheets, which he then impregnates with bacteria extract. In a large circle around the test dishes nothing grows – one of the many products that So-cells actually produces for its own requirements appears to be alkaloid. Do mycobacteria produce agents that could possibly be used as a pesticide or medical fungal infection? What agent in this test, how small, impregnation is responsible for the effect?

The extract of So-cells is transferred to the Department of Natural Products. The chemist Norbert Bedorf breaks the extract down into its components in order to discover which substances and which fungus. In a series of chromatographic partitions he tracks down the biological effect of the extract. At the end of the search is a pure substance with a strong anti-fungal effect. Less than one percent of the organic extract consists of this substance and, after months of the partial, the complete composition of the substance is apparent: 27 carbon atoms, 41 hydrogen atoms, 1 oxygen atom and one atom of nitrogen and sulfur. With the aid of NMR spectrometry, Norbert Bedorf manages to clarify the composition of the atoms and their spatial alignment with a new structural analysis. And the characteristic composition of the new discovered class of substances is a new class of bacteria.

1994 – THE SETBACK

Epothilone was effective against fungus, perhaps it would also counter other things? The biologist Florence Sasse examines the spectrum of biological properties and discovers that epothilone also kills animal cell cultures. In 1995 the chemical group Ciba-Geigy tests epothilone – partly a routine measure, the group has no specific interest in epothilone and is investigating a whole series of substances for a specific application. When its effects on cancer cells and the immune system appear not interesting and specific enough, epothilone is effective in the greenhouse against phytophthora infestans, an agricultural harmful fungus, against which there is an yet not effective remedy, and which has already been responsible for severe cases of famine.

Epothilone returns to production – the biotechnologists at the Society for Biotechnological Research are testing at full speed, producing large quantities of the material for the first time. and Heinrich Steinmetz works on the expansion of the individual components. From the large quantity of organic extract he extracts twenty grams of a mixture of the two epothilones A and B, a small portion of this A and B, for a small test trial to be carried out. The goal of this is for the successes to be confirmed in the greenhouse.

1995 – EPOTHILONE: A CANCER MEDICINE AGAIN ALL?

At the Society for Biotechnological Research the epothilone lines were being placed on the shelves, independently from the research carried out in Braunschweig the US company, Merck Sharp & Dohme is in search of a substance with a similar effect for the familiar anti-cancer agent Paclitaxel, a successfull cancer medicine under the trade brand. The first series they use 350,000 extracts of plants and micro-organisms – including a series of extracts from sorangium cellulosum. And just one of these extracts demonstrates the sought after effect on cancer cells in a so-called tulob test.

Convinced that there is a very special substance on their hands, the scientists patented the epothilones in 1991, carrying it up production and in the process beginning to alter the molecule chemically in order to optimize the effect. Despite this, the efforts lead to a dead-end. The fatal fault in Ciba-Geigy: epothilone not only has an effect on fungi, it also damages the plants. This heralds the end of its utilization as a pesticide. And although the US National Cancer Institute demonstrates good results in standard tests against cancer cells, the interest in epothilone fades. Nobody is interested in how the effect against cancer cells is triggered and So-cells is returned to the cooling chamber – plans to obtain an international patent are shelved in 1994. Ahls would still have been interested in the mechanism of action of epothilone. Because under the circumstances? dismisses professor Gerhard Hilf, head of the Department of Chemistry of Natural Products.

Just how hardworking the natural product research is down to a fact: a single chemical structure consists of this substance and, after months of the partial, the complete composition of the substance is apparent: 27 carbon atoms, 41 hydrogen atoms, 1 oxygen atom and one atom of nitrogen and sulfur. With the aid of NMR spectrometry, Norbert Bedorf manages to clarify the composition of the atoms and their spatial alignment with a new structural analysis. And the characteristic composition of the new discovered class of substances is a new class of bacteria.

**Epothilone: a basis for a new chemical scale – the production of new epothilone compounds.**

This is the point where research and production are carried out. From the natural epothilones and synthetically produced epothilone derivatives, the research institute has a practicable procedure for the production of epothilone.

Lupin and chemical. Only around 1% of the bacteria contain the process and the majority of the metabolism in place. The Department of Natural Products increases production between two and three-fold. In 1991 and 1992, the facility within a fermentation period of 3 weeks to the, the organic extract of 100 gram quantities. The time for which the biomass for the extraction is needed was reduced from a total of 120,000 to just 20,000. This procedural innovation was therefore a complete success. The production of medicines requires 200 kg / 20 kg within 14 days. A cutting story then: This is achieved. With the goal of producing a strain that is much more productive than the wild strain, the biotechnological product-oriented race begins by treating the bacteria with unforeseen adverse effects of many natural epothilones and synthetically produced epothilone derivatives, examining their mechanism of action using fluorescence microscopy in particular.

Dr. Florian Herrmann

Following a long search – fruitless in Germany and a number of neighboring countries – the scientists are able to generate new interest in epothilones in the form of Brazil-Mycin Sales GmbH. In the course of this search, and later in collaboration with the pharmaceutical company, the teams in Braunschweig research methods of producing epothilones in large quantities. The original wild strain of bacteria produces just tiny amounts of epothilones, but if the medicine is to be marketed or even merely tested for its scalability as a cancer medicine, then large amounts of it are required. Consequently, over a period of three years – from 1995 to 1998 – the scientists cultivate the most productive bacterial strains, searching for the optimal conditions for production and cultivation. Herbert Initis works on a technically practicable procedure for the production of epothilone.

The wild strain so-cells produced just 1% of epothilones per litre of broth broth. Start at the Department of Natural Products increases production between two and three-fold. In 1991 and 1992, the facility within a fermentation period of 3 weeks to the, the organic extract of 100 gram quantities. The time for which the biomass for the extraction is needed was reduced from a total of 120,000 to just 20,000. This procedural innovation was therefore a complete success. The production of medicines requires 200 kg / 20 kg within 14 days. A cutting story then: This is achieved. With the goal of producing a strain that is much more productive than the wild strain, the biotechnological product-oriented race begins by treating the bacteria with unforeseen adverse effects of many natural epothilones and synthetically produced epothilone derivatives, examining their mechanism of action using fluorescence microscopy in particular.

Dr. Florian Herrmann

Following the discovery of these conditions, the scientists transferred the strains to the BMD in 1998. For the procedural trials and the development of the production process, a total of 14 fermentation operations were performed. These trials led to the change of 13 fermentation principles. From which 18% of pure epothilone were produced. This led to a milestone for the strain’s industrial development in the medium. The production procedure now produces a strain, whose pharmacological potential can be examined. To this end, the strain was subjected to an extensive review. A series of Please contact us if you need more information on the production process. A series of Please contact us if you need more information on the production process. A series of Please contact us if you need more information on the production process. Please contact us if you need more information on the production process.
When the yield is large enough, the bioreactors at the Society for Biotechnological Research are in operation around the clock and Heinrich Steinmetz - aided by the staff of the Department of Chemistry of Natural Products - is able to produce epothilone A and B on a large scale.

Two candidates show promise. One originates from the laboratories at the Society for Biotechnological Research, produced by Nicole Horstmann, the other from BMS. Tests are conducted to determine the effects and side-effects of the two epothilone candidates 21-Aminoepothilone B and Ixabepilone. Parallel to this, the company develops the manufacturing process of this derivative until it is suitable for industrial application, followed by the commencement of exhaustive clinical studies. Both derivatives show promise in the initial studies.

Each of them appears to be suitable for the treatment of breast cancer; however, cost reasons dictate that only one of the two can be developed further: Ixabepilone. The years of global clinical trials are a nail-biting time: does the medicine really help breast cancer patients? Are there previously unseen side-effects?

Then the all-clear - Ixabepilone, developed from epothilone B, passes all tests. The American FDA (Food and Drug Administration), the approval authority for medicines, gives the go-ahead. On 16 October 2007 it receives approval as a breast cancer medicine in the US. "Clinical studies had already shown that other forms of cancer can be treated with epothilone," Professor Hans Reichenbach, then head of the Department of Biology of Natural Products, views the future with optimism. In November Bristol-Myers Squibb launched the medicine on the market under the name ixabepilone. A special success for the Society for Biotechnological Research - now the Helmholtz Center for Infection Research - as here it was and is possible to carry out research over extensive periods. The overcoming of obstacles is a part of that process. Persistence, creativity and an element of luck - these are the ingredients for successful pure research to find its way to application. "Above all, it is impossible to plan," says Prof. Hans Reichenbach. "It is possible to search for zytostatic substances, but not to say where you should look and when you will find something."

Scientists involved
Dr. Norbert Bedorf | Dr. Klaus Gerth
Dr. Ingo Hardt | Dr. Nicole Horstmann
Prof. Dr. Gerhard Höfle | Dr. Herbert Irschik
Dr. Thorsten Jahn | Dr. Larissa Jordt
Dr. Dmitri Kachin | Dr. Usama Karama
Dr. Michael Kiffe | Dr. Thomas Leibold
Prof. Dr. Hans Reichenbach | Dr. Florenz Sasse
Prof. Dr. Dietmar Schomburg
Dr. Detmar Schummel | Dr. Michael Seifow
Heinrich Steinmetz | Dr. Roman Vetter

"I discovered the epothilones and co-patented them. I optimised both the strain and production, produced epothilone in 100 gram quantities and transferred the know-how to BMS. Studies of biosynthesis enabled me to target the optimisation of the strain. I discovered and investigated new producer strains."
Dr. Klaus Gerth

"My involvement in the development of epothilone was the isolation of the pure, biologically-active substances from fermenting broths of So ce90 cultures and the clarification of the chemical structures of epothilone A and B and further compounds, later known as spriangin."
Dr. Norbert Bedorf