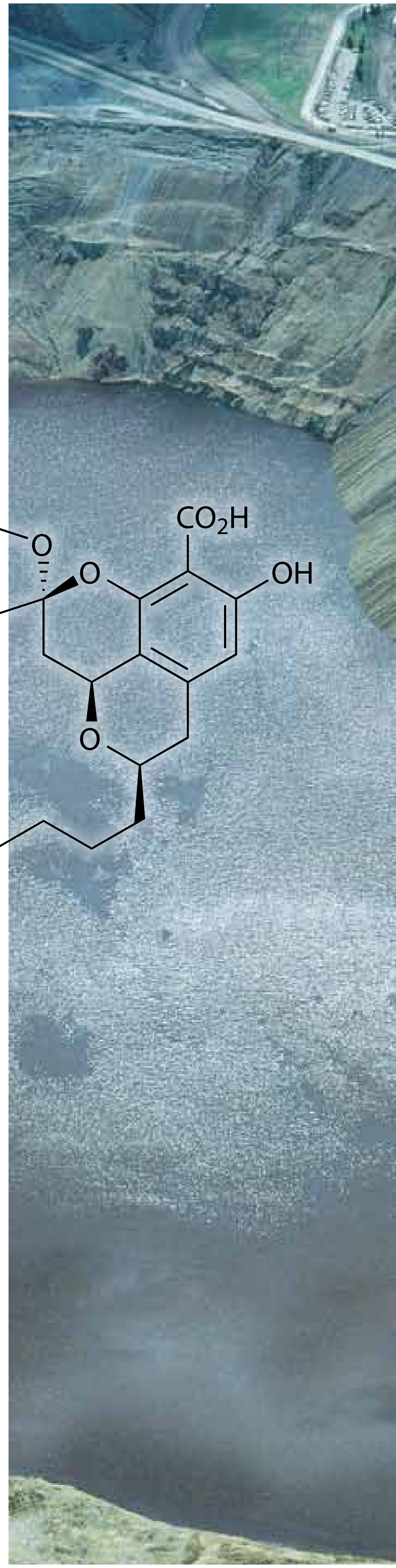
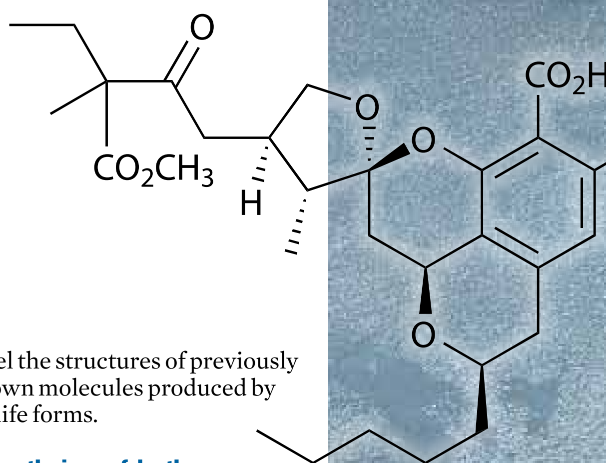
Extreme potential

The world's least hospitable environments are treasure troves for synthetic organic chemists and drug developers, reports Bea Perks



CALVIN LARSEN/SCIENCE PHOTO LIBRARY



In 1995, a flock of 300 snow geese migrating across the US state of Montana landed on a lake near the former mining town of Butte and died. Berkeley Pit lake marked the site of an abandoned open-pit copper mine that had shut down 13 years earlier. Tests on the birds revealed exposure to high concentrations of copper, cadmium and arsenic.

The appeal of the lake – 115 billion litres of acidic (pH2) metal-laden water – is not immediately obvious. But living in that toxic pool are organisms that could help treat a spectrum of illnesses, from ovarian cancer to Alzheimer's disease.

Berkeley Pit lake is clearly an extreme environment. The idea that organisms could thrive in such environments wasn't fully recognised until the 1980s and 1990s. The term 'extremophile' was first proposed in the 1970s by Robert MacElroy, a biochemist working at Nasa's Ames Research Center in Moffat Field, US. MacElroy predicted that the inhospitable environment on Mars could be one day be colonised by Earth-based life.

Biologists have been busy discovering ever more extraordinary organisms inhabiting a range of extreme environments on Earth (see 'Extreme extremophiles', p50), prompting chemists to unravel the structures of previously unknown molecules produced by these life forms.

In short

- **Temperature, pressure, toxicity and salinity extremes can sustain unusual organisms**
- **Extremophile enzymes can be more suitable for use in biotechnology than those from regular species, and their unusual metabolites are a source of potential new drugs**
- **Growing extremophiles in the lab is difficult and expensive, so synthesis is often required to fully evaluate new compounds**

Life from the jaws of death

The full potential of those molecules remains unknown, but there is plenty of evidence to suggest they will be significant. For example berkelic acid, isolated from a species of fungus living in the Berkeley Pit lake, has been shown to have selective activity against ovarian cancer.

The race is on – there is clearly a need to clean up a lake which will kill any passing wildlife. But, perversely, the lake may be harbouring life-saving agents for mankind.

And it's a race on several levels. While the Berkeley Pit lake is a dangerous manmade lake in serious need of remediation, there are naturally extreme environments that require protection in their natural state (see 'Protecting natural extremes', p51). One way or another, extremophiles are under threat. There is a growing need to devise ways of synthesising the unusual, but potentially very useful, secondary metabolites produced by these organisms.

Among the best studied molecules produced by extremophiles are enzymes, sometimes referred to as extremozymes. The enzymes

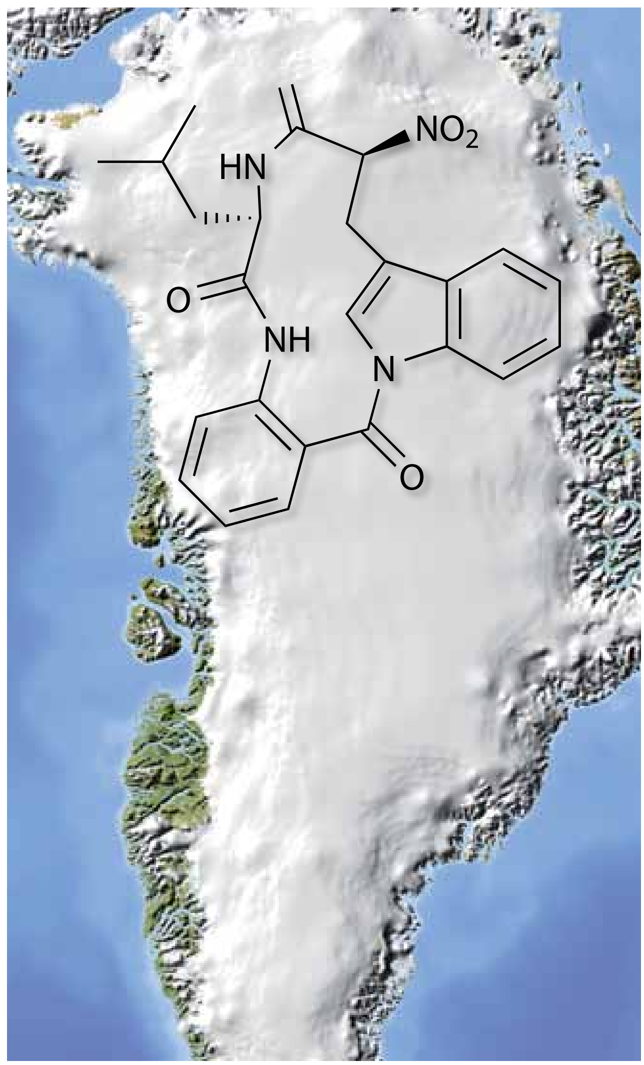
The acidic and metal-laden waters of Berkeley Pit lake in Montana, US, may seem inhospitable, but they are home to bacteria and fungi that produce unique molecules like berkelic acid

produced by organisms that inhabit extreme environments are, not surprisingly, quite unlike the enzymes you will find in a standard textbook (see *Chemistry World*, September 2005, p51). They catalyse reactions far outside the long-established temperature, pH and pressure norms, suiting them to a range of biotechnological applications, particularly in the oil industry.

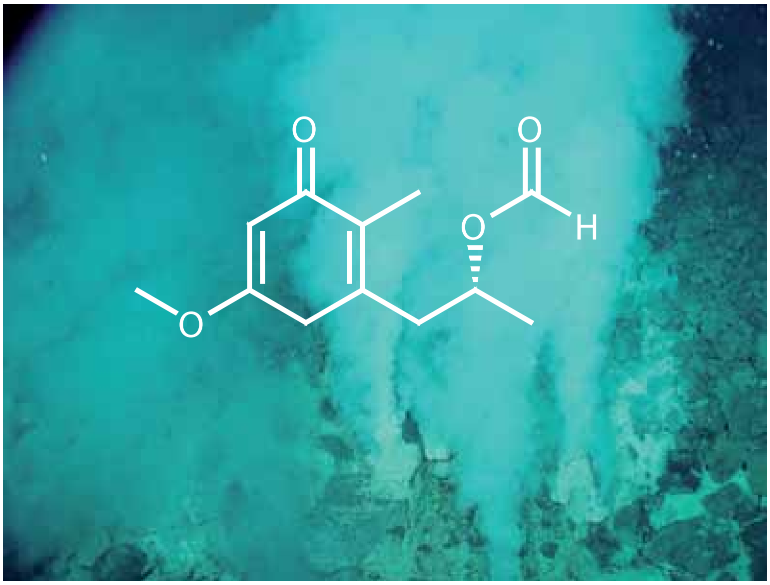
More recently, the natural products produced by extremophiles have been attracting attention. These are usually secondary metabolites – molecules that are not directly required to keep the organism alive, but perform some kind of helpful function, such as defence mechanisms and colourings.

Hot and cold

Several secondary metabolites isolated from thermophilic sources (organisms that thrive in the heat) have been shown to have antibacterial activity. For example, one species of the thermophilic



Psychrophilin D was isolated from a cold-loving fungus in Greenland



(+)-Formylanserinone B was isolated from fungi living 1335m below sea level, but shows promise for treating leukaemia

'It's a really toxic environment, there are unique microbes and they're producing some really unusual metabolites'

fungus *Streptomyces thermoviolaceus* was shown to be a source not only of a previously isolated antibiotic molecule called granaticin, but also a source of a previously unknown biosynthetic precursor of that antibiotic called dihydrogranaticin.

Among the secondary metabolites isolated from psychrophilic sources (organisms that thrive in the cold), three cyclic peptides – mixirins A, B and C – collected from a *Bacillus* strain living in the Arctic, were shown to inhibit the growth of human colon cancer cells. A species of cold-loving fungus, *Penicillium algidum* – which was found under a redcurrant bush in Greenland – produces the previously unknown compound psychrophilin D, which shows some activity against a mouse leukaemia cell line.

Deep, deep down

Down at the bottom of the ocean are the piezophiles (or barophiles; organisms that thrive under high pressure). These organisms normally have to be able to withstand extreme cold as well as high pressure, except for the few that live near hot vents. Secondary metabolite production by piezophiles is particularly well studied. A mixture of two *Penicillium corylophilum* strains collected 1335m below sea level produced five previously unknown compounds. Among these was (+)-formylanserinone B, which was shown to selectively inhibit growth in mouse leukaemia cells.

Apart from compounds with antitumour activity, secondary metabolites produced by piezophiles have been linked to a range of applications: from a previously unknown violet pigment isolated from a species of bacteria (*Shewanella violacea*), living 5110m down in an ocean trench off southern Japan; to a group of potential antifoulants capable of fending off barnacle attachment, produced by the bacteria *Streptomyces fungicidicus* discovered 5000m deep in the Pacific.

Sturdy old salts

The halophiles, organisms that thrive in high salt conditions, counter the osmotic stress of their surroundings either by accumulating or maintaining concentrations of osmolytes such as KCl close to that of their surroundings. Relatively little is known about the secondary metabolites produced by these organisms, and rather than offering cures for intractable human diseases, some of them seem more likely to make us ill. A halophilic Gram-negative bacteria, *Vibrio parahaemolyticus*, was first isolated in 1950 as the cause of seafood poisoning.

Even less is known about secondary metabolites produced by organisms that thrive at high pH, the alkaliphiles. The enzymes from these organisms have attracted more attention though, particularly for their use in biological detergents.

From lake to lab

The secondary metabolites produced by organisms that thrive at low pH, the acidophiles, are a different matter, taking us back to the Berkeley Pit lake. Over 40 different bacteria, fungi, algae and protozoans, have been discovered in this one polluted, manmade lake. Despite the ecological disaster that the lake represents, over 20 previously unknown natural products have been isolated from its resident microbe population.

Living in this toxic soup are life forms that researchers predict could help treat migraine, high blood pressure, ovarian cancer and lung cancer. One team is beginning to investigate the possibility that there might also be compounds to help treat neurological disorders like Alzheimer's disease.

'It's a really toxic environment, there are some pretty unique microorganisms and they're producing some really unusual secondary metabolites,' says Margaret Brimble, professor of organic and medicinal chemistry at the University of Auckland in New Zealand. 'We're looking at making some of those metabolites because they've got some really unusual structures that we haven't seen before in the chemical world.'

Brimble's group was the first to synthesise berkeleyamide A, a secondary metabolite isolated from

Extreme extremophiles

Extremophiles turn up in surprising places. We now expect to find extraordinary organisms on the sea floor, in hydrothermal vents, or in geysers and hot pools, but researchers were surprised recently to find heat-loving thermophiles in the Arctic. Casey Hubert, a geomicrobiologist at the University of Newcastle, UK, found thermophiles lying dormant in arctic marine sediments, where it is 50°C colder than they would need to grow. Hubert put them in a thermophile-friendly incubator, and they sprang back to life. Importantly, he showed a stable supply of thermophiles to the Arctic sea bed – arriving by some kind of passive transport mechanism. He is now figuring out why they are there, and what this might tell us about, for example, the presence of oil reserves deep below the sea bed.

And when they're not turning up in the 'wrong' place, extremophiles can turn up in just odd places. A couple of years ago a Japanese research team discovered a new species of extremophile, *Microbacterium hatanonis*, living in hairspray. The bacteria grows best at 30°C, pH7.

JOEL E KOSTKA

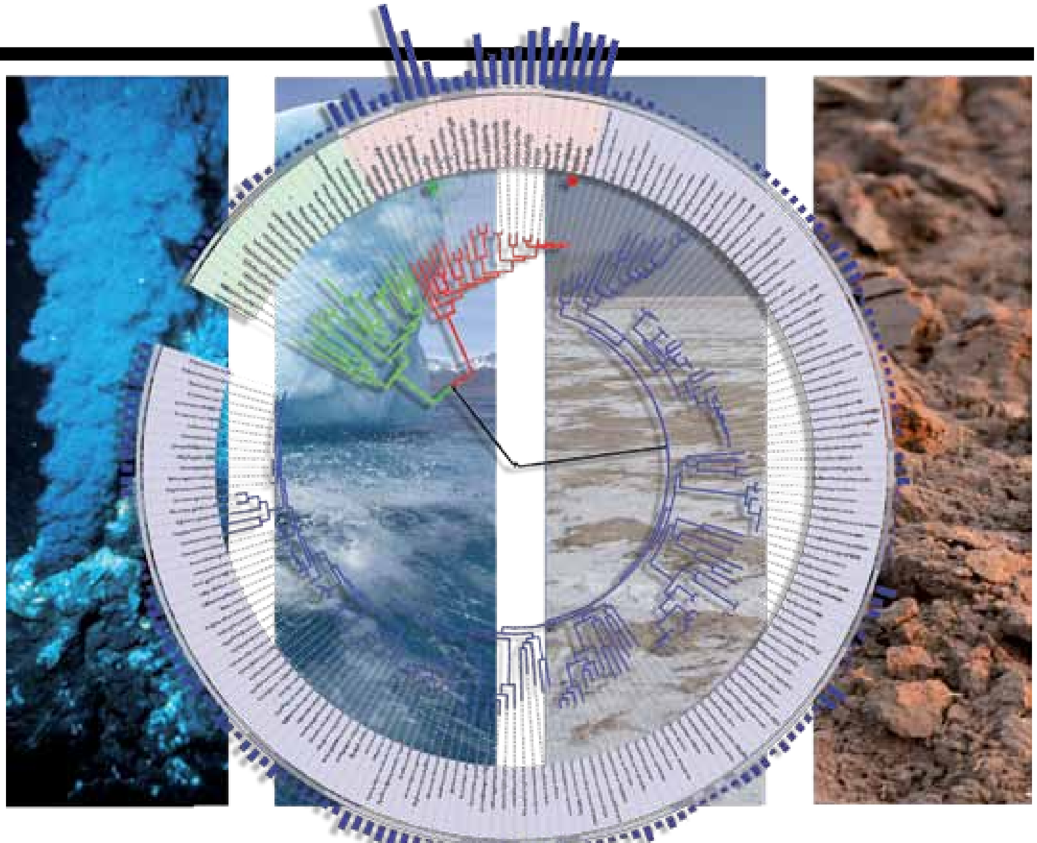
Casey Hubert has found heat-loving thermophiles in the Arctic

a *Penicillium rubrum* Stoll species living in the lake. Considering that the natural source of berkelyamide wouldn't be available once the lake was cleaned up, a total synthesis of the molecule became a pressing need after it was found to have anti-inflammatory and potential antitumour activity. It inhibits the proteases caspase-1 and matrix metalloproteinase 3 (MMP3), which are implicated in the mechanisms by which some cancers spread.

Her group is now working on improving the synthesis of the more complex berkelic acid, a highly competitive area worldwide. 'Everyone's trying to make it ... it's a bit like the new taxol,' says Brimble.

Berkelic acid, and berkeleyamide A, were both first isolated by Donald and Andrea Stierle at the University of Montana, US. Berkelic acid has a very unusual tetracyclic structure. It too inhibits both caspase-1 and MMP-3, and it shows selective activity against an ovarian cancer cell line.

As well as the anti-cancer activity of secondary metabolites discovered in the lake, Stierle's team is interested in targeting their antimicrobial potential. 'Of particular interest are compounds with antifungal activity, as opportunistic mycoses are a growing problem in immunocompromised individuals, including Aids patients and patients on immunosuppressive therapy,' says Donald Stierle.



HAMISH KIDD

Metagenomic analysis of bulk DNA from extreme locations can turn up new enzymes that could be employed industrially

Metabolite mining

Despite their obvious potential, relatively little is known about the secondary metabolites produced by extremophiles. Most of what is known about extremophiles relates to their biology and ecology. It is one thing to study these life forms at great depths, or near hydrothermal vents and other extreme environments, but taking the organisms back to the lab is a different matter. Recreating these extreme environments in vitro, in order to study the molecules produced by these extraordinary organisms, is both difficult and prohibitively expensive.

One way to get round this, suggest Stierle and Brimble, is to go down a 'metagenomic' route. Metagenomics is the sequencing of bulk genetic material from whole environmental samples rather than from individual organisms. Genomics techniques are used to study communities of microbial organisms where they live, rather than having to cultivate individual species back in the lab.

There is already a collection of metagenomes from extreme environments, which have been used to study extremozymes. Brimble hopes that these extremophile DNA libraries could be transferred into host organisms, which would then be tested to see whether they produce interesting secondary metabolites.

Currently, Brimble is working with a biotech firm looking at the DNA from extremophiles and using it as a template 'to produce the peptides normally produced by those microorganisms'. A library can be constructed using those peptides, which can then be screened against different drug targets. 'It's a new approach,' says Brimble, 'looking back at the DNA level of unusual organisms and then creating the proteome from that.'

Brimble's team is making analogues of the compounds discovered in the Berkeley Pit lake and recently teamed up with another research group at the University of Auckland working on neurological assays. 'We're going to screen these compounds in neurological assays, which hasn't been done before,' she says. 'People tend to focus on cancer and antiviral activity. We're going to take these extremophile-derived molecules and put them into assays that involve the brain.'

Protecting natural extremes

Natural extreme environments – from deep inside desert rocks to hydrothermal vents on the ocean floor – are threatened by human activities and changing climate. In April this year, the European Commission's international coordination action for research on life in extreme environments (Carex) project launched a roadmap identifying the most important questions for extreme environment researchers. Research priorities include: life's response to climate and environmental change; its adaptation methods; understanding biodiversity and interactions within extreme environments; and finding limits of habitability which could inform the search for extraterrestrial life.

Mary Voytek, senior scientist for Nasa's astrobiology programme, says this is the first effort by the European Commission to mine its community of extreme environment researchers to identify the best topics to fund. The report's findings are not dissimilar to those identified by Nasa, the US Department of Energy, and the US National Science Foundation, she says, but the roadmap will be useful to those agencies as well.

'There's a heightened payoff scientifically of having different groups work together,' says Voytek. A prime example is hydrothermal vents, which were originally discovered by geologists and then capitalised on by microbiologists, astrobiologists, and synthetic organic chemists among others. 'No one group has claimed them for itself.'

Further reading

Z E Wilson and M A Brimble, *Nat. Prod. Rep.*, 2009, **26**, 44