CHEMISTRY

Frog Venom Cocktail Yields A One-Handed Painkiller

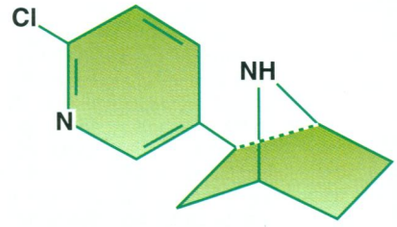
CAMBRIDGE, U.K.—For generations, Ecuadorian Indians have used the venom of the frog *Epipedobates tricolor* as a powerful weapon, even calling the animal the poison arrow frog. Last year, however, a team led by pharmacologist John Daly at the National Institutes of Health discovered that one compound in the cocktail of chemicals the frog secretes from glands on its back could be a weapon of a different kind: In tests on mice, it proved to be 200 times as effective as morphine in blocking pain. What's more, the chemical seemed to work in a completely different way from morphine and other opiates, because its painkilling power was undiminished when it was administered along with naloxone, an opiate blocker.

Those findings touched off a furious race to synthesize the chemical, known as epibatidine, as a possible first step toward producing a long-sought drug: a powerful nonsedating, nonopioid painkiller. First past the post were teams led by Chris Broka of Syntex Discovery, a Palo Alto, California-based company, and Tsung-Ying Shen at the University of Virginia. They independently published papers recently describing the synthesis of epibatidine (*Tetrahedron Lett.*, p. 3251 and 4477, 1993). But the prize may go to a group led by Stephen Fletcher of the drug company Merck Sharp and Dohme (MSD) in Harlow, Essex, which published earlier this month a synthesis that produces the compound in the exact configuration in which it is made by the poison arrow frog (*J. Chem. Soc., Chem. Commun.*, p. 1216, 1993).

This achievement means that researchers will now be able to work with large quantities of the compound to try to figure out how it works. Researchers believe that it may act through an as yet undiscovered receptor, and they may be able to produce analogs that have a similar shape but with slightly different side chains that might affect how the molecule fits into this putative receptor. And, although tests on mice suggest that epibatidine itself is probably too toxic to use as a human painkiller, "a modern drug discovery team should be able to remove toxicity [by adding or changing chemical groups on the molecule to make an analog] while optimizing the analgesic activity," says Fletcher.

The race to synthesize epibatidine took place over a difficult course. Although the compound looks fairly uncomplicated compared with other naturally occurring molecules, such as polypeptides, it has some unusual features. It is the first natural product found to contain a nitrogen-bridged six-membered carbon ring (an azabicycloheptane unit) and, surprisingly, a chlorine-bearing pyridine ring—chloropyridines are very rare in the animal world. This chloropyridyl is attached to the azabicycloheptane close to the nitrogen bridge—the "exo" configuration. The researchers believe this orientation could affect the molecule's activity, so replicating the structure exactly was crucial.

All three groups used a technique called retrosynthetic analysis, pioneered by Nobel chemist Elias J. Corey in the 1960s, to synthesize epibatidine. The approach is like breaking up a molecular jigsaw puzzle to a point where it can be seen how the pieces, be they simple laboratory reagents or previously solved puzzles, might fit together again. Once they know what pieces they need, chemists can start trying to synthesize the chemical, but the way the pieces fit together, and how readily they do so, depends on what reactive chemical groups are added to the pieces before the synthesis is begun.



TOM MCHUGH/PHOTO RESEARCHERS

Natural replica. Chemists have synthesized epibatidine *(top)*, a powerful, though toxic, painkiller produced by the poison arrow frog.

Each group started with different materials and reagents. Broka published a complete synthesis first and showed that Daly's analysis of the structure was correct. Hot on his heels was Shen's team, which took the synthesis one step further by adding an additional reaction scheme involving an auxiliary molecule with two "handed" forms, or enantiomers. The two forms are mirror images of each other—like a pair of hands, they cannot be superimposed. Depending on which enantiomer of the auxiliary molecule they used, this resulted in one of the two possible enantiomers of epibatidine (Broka's synthesis produced a mixture of the two enantiomers).

The production of single enantiomers was a potentially important step because different enantiomers, although chemically identical, can have very different effects in the body. The reason: Biological receptors and enzymes can also have left- and right-handed forms. A tragic example of this is the tranquilizer thalidomide: One enantiomer produces sedation while the other interferes with fetal development. Indeed, "the efficient synthesis of single enantiomers is the major preoccupation of today's synthetic organic chemist," says natural products chemist Nick Lawrence of the University of Manchester Institute of Science and Technology.

The MSD team also produced both enantiomers separately. But, instead of using an auxiliary molecule as Shen had done, they ran two parallel reaction schemes using the different enantiomers of one of their precursor compounds. They then went on to show which of these two synthetic enantiomers is the natural form by comparing them with a 5-microgram sample of natural epibatidine provided by Daly using high-performance liquid chromatography. Once they knew which synthetic form was the right one, they used x-ray crystallography to find the exact structure and arrangement of the atoms in the molecule. And all without laying a finger on a frog.

The achievement demonstrates the power of modern chemistry. But to Lawrence it has a different message: "The synthesis of epibatidine clearly illustrates that, despite the incredible advances of medicinal chemistry, nature can still be a source of potential drugs." Now that epibatidine can be produced in large quantities, the race is on to turn that potential into reality.

–David Bradley

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