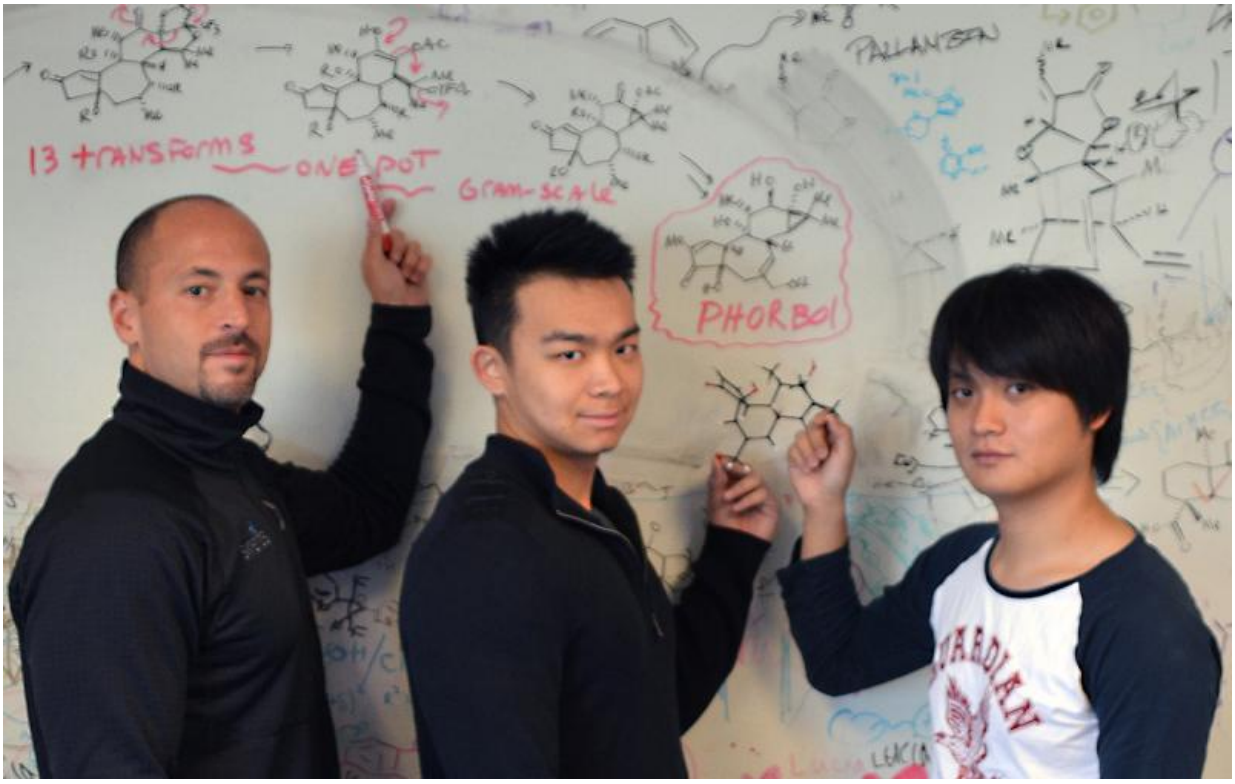
Chemists find a way to synthesize complex plant molecule phorbol and its derivatives

March 23 2016



Authors of the new study included (left to right) Phil Baran, Hang Chu and Shuhei Kawamura, pictured here at a whiteboard showing the team's 19-step synthesis of phorbol, including the incorporation a dramatic cascade transformation of 13 different chemical events occurring in one flask. Credit: The Scripps Research Institute.

In a landmark feat of chemical synthesis, scientists at The Scripps

Research Institute (TSRI) have developed a 19-step process for making the naturally occurring compound phorbol in the laboratory, in quantities that are useful for pharmaceutical research.

Phorbol has long been considered a sort of "Holy Grail" for synthetic chemists, due to the inherent difficulty of assembling its large and complex structure and to its potential value as a basis for therapies. Phorbol-related compounds extracted from trees and shrubs such as the croton oil plant (*Croton tiglium*) are known as tiglianes and have powerful biological activity. They have been used by traditional societies for applications ranging from arrow poisons and purgatives to salves against skin cancer.

Although phorbol and a few other tiglianes can be reliably obtained in large quantities from plant material, most compounds in this family have been essentially unobtainable by any means. The TSRI scientists' achievement, published in *Nature* on March 23, 2016, enables pharmaceutical researchers for the first time to make such tiglianes in the laboratory and evaluate them for possible development into drugs.

"Ten years ago, synthetic chemists were thought to have no chance of making these compounds in useful quantities," said principal investigator Phil S. Baran, the Darlene Shiley Chair in Chemistry at TSRI.

Oxygen Atoms as Bar Code

Other laboratories have previously succeeded in synthesizing phorbol, but via very burdensome and low-yield routes, which require between 40 and 52 steps and deliver the useless, structural mirror-image version of phorbol mixed with the desired form. These existing synthetic routes also cannot be modified easily to generate phorbol analogs (other structurally similar molecules). Pharmaceutical researchers rarely pursue the development of a promising compound if they have no way to look

for better versions.

The properties that make phorbol so hard to synthesize—its large, 20-carbon backbone structure and its six reactive oxygen atoms—are essentially the same ones that underlie its biological potency and therapeutic promise. Phorbol and other tiglianes interact with key signaling pathways in human cells and their patterns of oxygen atoms are in principle "tunable" to optimize them for a particular effect.

"We like to think of the oxygen atoms that adorn these molecules as forming a sort of bar code," said Baran. "Their precise arrangement largely determines the molecule's function, so being able to make molecules with the desired arrangement is pretty important."

Mimicking Nature

One key to the new synthesis was a relatively simple strategy shift, which Baran and his colleagues first demonstrated in a study published in 2009, also in *Nature*. The targets then were eudesmanes, compounds that, like tiglianes, belong to the large class of chemicals known as terpenes. Baran's "two-phase terpene synthesis" approach involved broadly mimicking nature's ancient and flexible strategy by first building the carbon backbone, then adding the oxygen atoms.

"Nature makes thousands of these terpenes in plant and animal cells, and within a family such as eudesmanes or phorbols the compounds don't differ much in their carbon skeleton—they're like the same car but with a different paint job and different wheels each time," said Baran.

After the success with eudesmanes, Baran and his colleagues used the new approach again to find a short route to synthesizing ingenol, head of a family of more complex terpenes called ingenanes. That feat was reported in *Science* in 2013. One ingenane whose synthesis was made

possible by the new route is currently being developed into a drug—and seems to have better properties than a plant-derived ingenane now used for treating precancerous skin lesions.

Denmark-based LEO Pharmaceuticals, for which Baran did the ingenane work, later approached him for help concerning an even harder target: phorbol and the tiglianes. The company had identified a plant-derived tigliane as a promising skin cancer treatment, but had no way to generate improved analogs.

Thinking Differently

Over 10 months, Baran and his team, including Research Associate Shuhei Kawamura, the new study's first author, used their two-phase, carbons-then-oxygens strategy to come up with the 19-step route to phorbol.

The greatest difficulty involved the opening of a large part of the intermediate structure and installing of two separate oxygen-containing groups at one end of the molecule—a notorious challenge for would-be phorbol synthesizers.

"If that reaction didn't work, we couldn't make the molecule," said Kawamura.

The solution features one key step in which 13 different reactions occur in the same flask before yielding the desired intermediate product. "That transformation is the chemical equivalent of a Cirque du Soleil show," Baran said.

As was the case for ingenol and the ingenanes, the new route can be modified easily to generate previously unobtainable tiglianes, in quantities sufficient for initial laboratory investigations. If needed, LEO

Pharma could scale up the process to generate enough for preclinical tests in animals. In fact, the new route effectively starts from one of the intermediate products in the ingenol route, which the company is now able to supply in relatively large quantities.

Baran emphasized that the new synthetic route to phorbol didn't require the invention of any novel reaction—all the reactions used had been previously described in the chemistry literature before 1980. "The whole pathway was enabled by just thinking differently about the overall strategy," he said.

More information: Nineteen-step total synthesis of (+) - phorbol, *Nature* , DOI: 10.1038/nature17153

Provided by The Scripps Research Institute