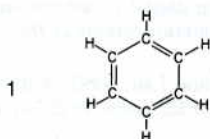# OF WHAT USE ENEDIYNES?

Roald Hoffmann

The story begins in the early seventies with that wonderful focal point of organic chemistry, benzene (*compound 1*). From this simple ring chemists' hands and nature have

1

shaped as diverse a set of molecules as adrenaline, TNT, aspirin and mescaline. To benzene—to its special aromatic stability and that perfect hexagon seemingly inconsistent with the three double bonds we draw in it—were and are drawn the minds of generations of chemists.

Benzene is stable. Rip two hydrogens off, and you have something pretty unstable, and therefore reactive, a so-called dehydrobenzene. You can (oh, so *easy* on paper!) remove two hydrogens in three different ways, to get three isomers, molecules made up of the same atoms (six carbons, four hydrogens, C_6H_4) but bonded differently (*compounds 2–4*).
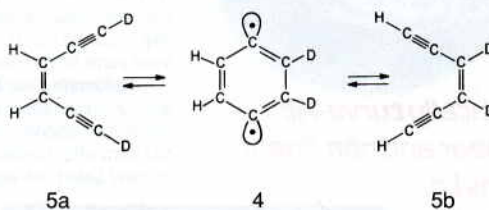

dehydrobenzenes
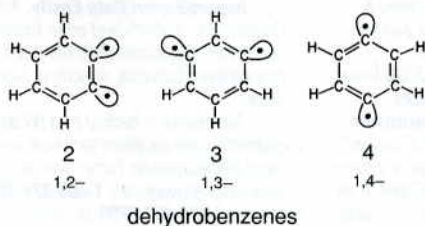
Physical organic chemists love these unstable beasts. It is a challenge to make them; they may persist for a bare microsecond, and then it is fun to get (quickly) evidence for their existence. Or to find traces of their sojourn in other ways. And knowledge of these fleeting species may turn out, in unexpected ways, to be useful for understanding the workings of different reactions. As we shall see.

Good evidence for compound 2, the 1,2-dehydrobenzene, the most stable of these isomers, has been with us since the fifties (1). The others have been a hard pull. Robert G. Bergman, a brilliant young organic chemist then at Caltech, began

work in 1971 on compound 4, the 1,4-dehydrobenzene. He had the idea that it might be generated by heating a relatively unstable precursor, the enediyne (*compound 5*).



enediyne   1,4–dehydrobenzene

5   4
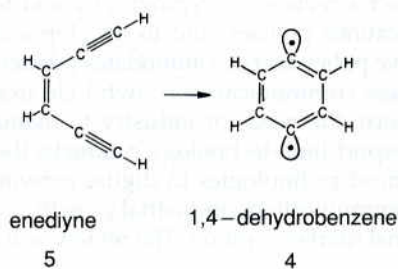
But compound 4 is not very stable, not seen; so how can we get evidence for its being there? Bergman and Jones did it in two ways (2), first by showing that an enediyne labeled with deuterium at a specific position in compound 5a equilibrates with compound 5b. No other pattern of deuterium distribution is found. This argues convincingly for the intermediacy of compound 4, even though it is not detected in the reaction, only inferred.

5a   4   5b

The Caltech group also trapped the fleeting 1,4-dehydrobenzene, with a source of hydrogen or chlorine atoms, as shown in compound 6 (3). The reactivity exhibited is typical of that of biradicals, chemical species with two unpaired electrons. And we shall see that this reactivity figures prominently in the sequel.

Now we jump to the 1980s. Fermentation products of the bacterium *Micromonospora echinospora* ssp. *calichensis* (named after its isolation

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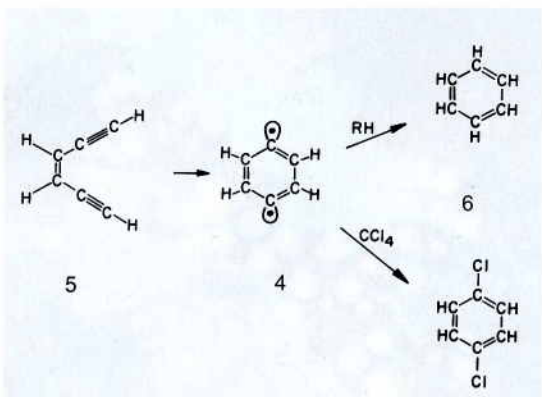
from a caliche, or chalky, Texan soil sample) were found to have remarkable antitumor activity. The scale of the fermentation work is incredible —2 to 10 milligrams of one of the products, calicheamicin γ^I_1, were isolated from 100,000 liters of fermentation broth. The pure molecule is over a thousand times more potent (when tested in model systems) than adriamycin, an antitumor drug already in use (4).

The structure of calicheamicin (there is here a group of closely related molecules; the one shown is calicheamicin γ^I_1) is indicated in compound 7. The molecule's geometry was determined in 1987. Around the same time the structures of three other potent antitumor antibiotics were solved: esperamicin (*compound 8*), dynemicin (*compound 9*), and neocarzinostatin (*compound 10*) (*4a*). Their pictures lapse into the organic chemists' usual semiotics —carbon and

hydrogen are not shown explicitly.

Look at these hairy molecules, marvelous demonstrations of the beauty in complexity that is this world! What do you see that they have in common? Some pieces of a saccharide or a sugar to be sure, an interesting trisulfide group in two of them, but first and foremost, inescapably dominating the landscape of the molecule, the enediyne unit! Never seen in nature before, here it is, in not one but several very different molecules, isolated from very different species. If the biochemical origins of the molecules be not the same, their functions might be.

K. C. Nicolaou of the Scripps Research Institute in La Jolla, California, who has played a most important role in the organic chemistry of these species, describes the way the static structure of calicheamicin translates into its mode of action:

> The molecule of calicheamicin γ^I_1 is a masterpiece of natural ingenuity. Its structure can be roughly divided into three components: a) the enediyne systems with its potential power to wreak havoc on any biological target, but cleverly locked in place until activated; b) the oligosaccharide fragment which is thought to serve as a delivery system to bring the lethal warhead to its intended target, DNA; and c) the trisulfide moiety, which acts as a triggering device allowing the initiation of the crucial chemical events which lead to the generation of highly aggressive species that cause fatal damage to the genetic material (*4b*).

The "Rambo" language I can do without (5), but the scenario is clear. The enediyne system

calicheamicin γ^I_1

7

esperamicin A$_1$

8

dynemicin A

9

neocarzinostatin

10
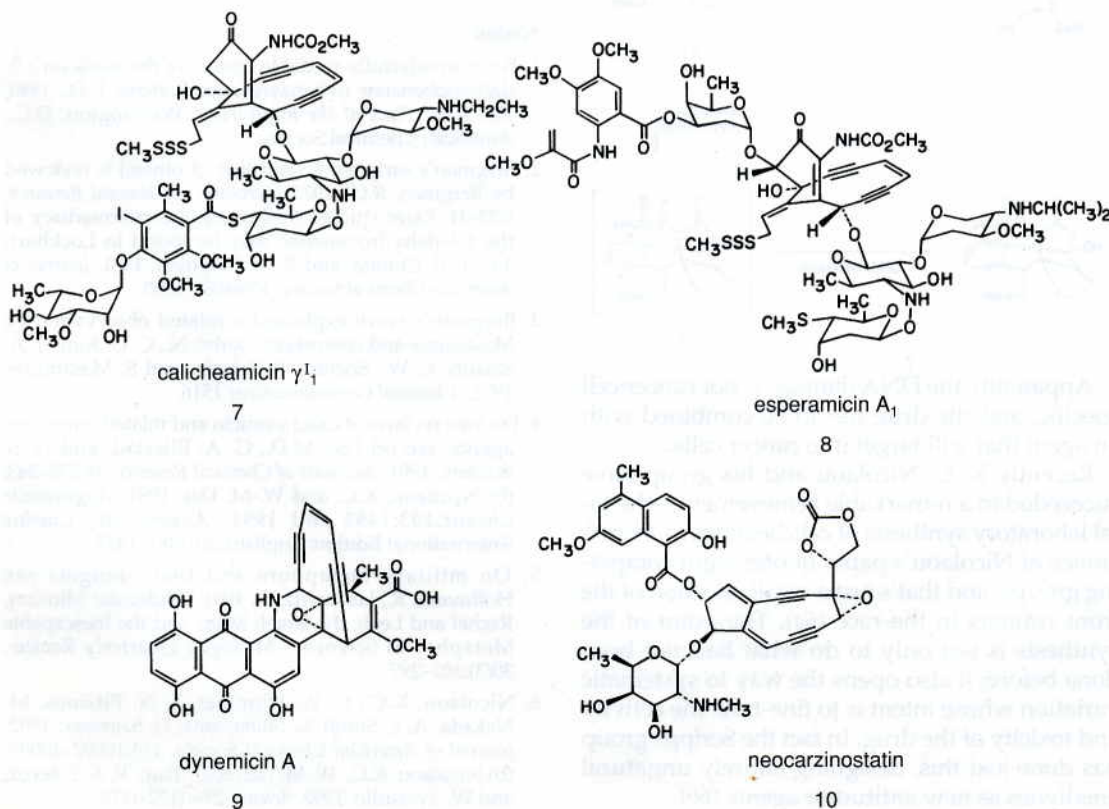
(compound 10 contains a little variant) is likely to be the common antitumor motif, and the details of its action have been worked out in some detail.

First it was shown that calicheamicin cuts DNA strands. Not just anywhere, but at certain quite specific base sequences (TCCT and CTCT; C=cytosine, T=thymine). The molecule binds in the so-called minor groove of DNA; the sugary tail of calicheamicin plays an important role in this binding.

What then? There follows a molecular Rube Goldberg (in England—Heath Robinson; both date me) sequence, a typical chemical mechanism. Let me quote Nicolaou again:

> A nucleophile (e.g., glutathione), probably activated by an internal basic nitrogen, then attacks the central sulfur atom of the trisulfide group, causing the formation of a thiolate (bioreduction) which finds itself in a perfect position, due to the proper geometry of the allylic double bond, to attack intramolecularly the α,β-unsaturated ketone embedded in the adjacent six-membered ring to give [compound 11]. This reaction... paves the way for a Bergman cyclization reaction, leading to the benzenoid diradical [compound 12]... [which] is capable, and well positioned, to abstract two hydrogen atoms, one from the C5′ position of deoxycytidine (C) and the other from a ribose position of the opposing strand. The DNA radicals so generated then proceed to react with molecular oxygen leading to double strand cleavages (4b)... .

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Bergman cyclization

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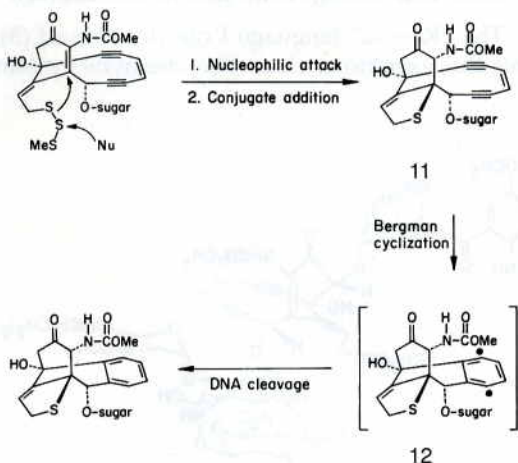
Apparently the DNA damage is not cancer-cell specific, and the drug has to be combined with an agent that will target it to cancer cells.

Recently K. C. Nicolaou and his group have succeeded in a remarkable achievement—the total laboratory synthesis of calicheamicin. One reference of Nicolaou's paper quotes eight competing groups, and that's just a small selection of the front runners in the race (6a). The point of the synthesis is not only to do what had not been done before; it also opens the way to systematic variation whose intent is to fine-tune the activity and toxicity of the drug. In fact the Scripps group has done just this, designing entirely unnatural enediynes as new antitumor agents (6b).
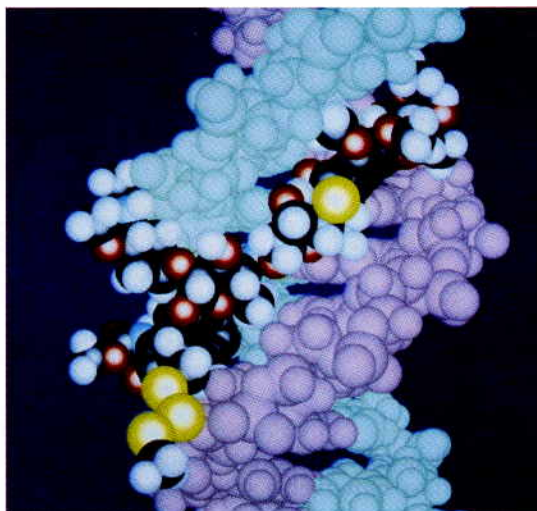
Computer-generated model of DNA-bound compound 7. (Photograph courtesy of K. C. Nicolaou, reproduced by permission from *Angewandte Chemie*, 1991, 103:1453.)

What I think is beautiful in this story is that the reaction Bergman elucidated, in magnificent detail, while chasing an unseen molecule, should be found to be precisely the one operating when a part of nature (the bacterial fermentation product) is used by us to bind and inactivate another part of nature (the human tumor). Some would view this as a justification of basic research—that a use, however remote, will eventually be found for the results of pure chemistry. That is so. But to me the beauty of this story is simply in the conjunctions and relationships, molecular and human, that make this world such an interesting place.

Notes

1. For a wonderfully readable review of the work on 1,2-dehydrobenzene (o-benzyne), see Roberts, J. D., 1990, *The Right Place at the Right Time*, Washington, D.C.: American Chemical Society.

2. Bergman's early work (and that of others) is reviewed by Bergman, R.G., 1973, *Accounts of Chemical Research*, 6:25-31. Later studies confirming the intermediacy of the 1,4-dehydrobenzene may be found in Lockhart, T.P., P. B. Comita, and R. G. Bergman, 1981, *Journal of American Chemical Society*, 103:4082-4090.

3. Bergman's work explained a related observation by Masamune and coworkers. Darby, N., C. U. Kim, J. A. Salaun, K. W. Shelton, S. Takada, and S. Masamune. 1972. *Chemical Communications* 1516.

4. For two reviews of calicheamicin and related antitumor agents, see (a) Lee, M.D., G. A. Ellestad, and D. B. Borders, 1991, *Accounts of Chemical Research* 24:235–243; (b) Nicolaou, K.C. and W.-M. Dai, 1991, *Angewandte Chemie*,103:1453 and 1991. *Angewandte Chemie* (International Edition: English), 30:1387–1415.

5. On military metaphors and their dangers see Hoffmann, R., Leibowitz, S., 1991 "Molecular Mimicry, Rachel and Leah, the Israeli Male, and the Inescapable Metaphor in Science," *Michigan Quarterly Review*, 30(3):382–297.

6. Nicolaou, K.C., C. W. Hummel, E. N. Pitsinos, M. Nakada, A. L. Smith, K. Shibayama, H. Saimoto, 1992. *Journal of American Chemical Society*. 114:10082–10085. (b) Nicolaou, K.C., W.-M. Dai, S.-C. Tsay, V. A. Estevez, and W. Wrasidlo. 1992. *Science* 256:1172-1178.