used in calculating the total cosmogenic ^{36}Cl production rate were 3560 ± 465 atoms of ^{36}Cl per mole K per year, 2745 ± 245 atoms of ^{36}Cl per mole Ca per year and (2.64 ± 0.42) × 10^5 thermal neutrons stopped per kilogram of rock per year. These values are at the rock surface, at sea level, and at 90° geomagnetic latitude.
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17. The samples were ground to a particle size smaller than the mean grain diameter and leached with deionized water to remove meteoric or anthropogenic ^{36}Cl. They were then dissolved in hot HF and HNO_3 and the Cl was extracted as HCl by air stripping, followed by trapping in a $AgNO_3$ solution (*14*). Major and minor elements were measured by x-ray fluorescence and rare earth elements by inductively coupled plasma atomic-emission spectrometry. The Cl content was measured in a teflon diffusion cell using an ion-specific electrode technique. [P. S. Aruscavage and E. Y. Campbell, *Talanta* **30**, 745 (1983); H. N. Elsheimer, *Geostand. News.* **11**, 115 (1987)]. Values given are the mean of three to five replicate analyses. A value of 30×10^{-15} [typical for granitic rocks (*12*)] was assumed for the radiogenic $^{36}Cl/Cl$ ratio (R_0). This radiogenic ^{36}Cl background ranges from about 5% to less than 1% in relation to the cosmogenic ^{36}Cl used for dating.
18. We have considered two alternative hypotheses: (i) The boulder age distribution from the Mono Basin moraines may indicate more than one glaciation between 80 and 120 ka. Some evidence from moraine morphology supports the notion that there were multiple glaciations (*8*), although the evidence is not as strong as for the older Tahoe moraines. (ii) Even the oldest boulder dates on the Mono Basin may only be minimum and the Mono Basin glaciation could have an age of ~140 ka. Although this assumption would bring the Bloody Canyon deposits into better agreement with the marine ^{18}O record regarding the relative glacial volumes during isotope stages 6 and 5d, in the absence of positive results to support this assumption, we prefer the interpretation indicated by the maximum measured ages.
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Biomimetic Total Synthesis of *Proto*-Daphniphylline

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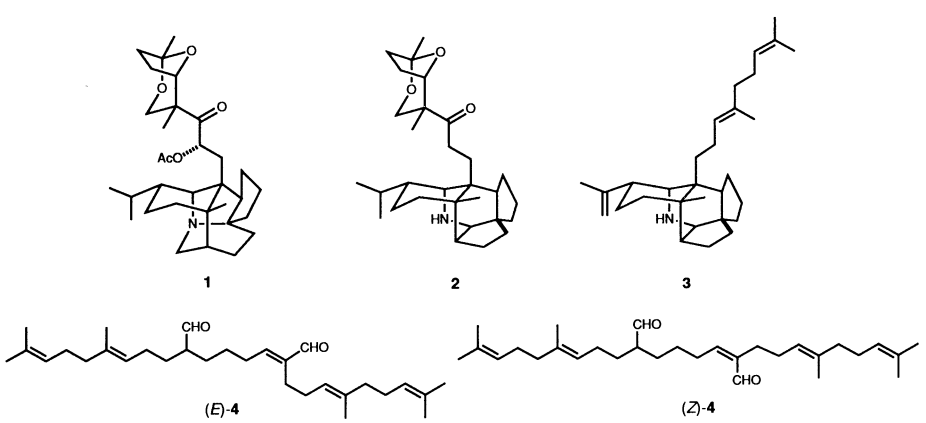
***Proto*-daphniphylline, the imputed biogenetic parent of the *Daphniphyllum* alkaloids, has been assembled in a biogenetically styled laboratory synthesis in which a pentacyclization process is the fundamental synthetic stratagem. This extraordinary transformation involves the formation of six σ-bonds under the influence of three elementary reagents—potassium hydroxide, ammonia, and acetic acid. The facility of the process adds credibility to the previous speculation that a similar process is an important step in the biosynthesis of the *Daphniphyllum* alkaloids.**

THE ORIENTAL DECIDUOUS TREE Yuzuriha (*Daphniphyllum macropodum* Miquel) contains a family of squalene-derived alkaloids, of which daphniphylline (**1**) and secodaphniphylline (**2**) are representative members (*1*). It has been suggested that these complex natural products are biosynthesized from squalene by way of the embryonic precursor **3**, *proto*-daphniphylline (*2*). In this report, we describe a remarkably simple synthesis of **3** wherein the five rings are formed in a series of three straightforward chemical transformations of the acyclic dialdehydes (*E*)-**4** or (*Z*)-**4**.

As shown in Scheme 1, the lithium enolate of *tert*-butyl (*t*-Bu) acetate was alkylated with homogeranyl iodide (**5**) (*3*) to give ester **6**, which was deprotonated with lithium diisopropylamide (LDA). The resulting enolate was alkylated with the dimethyl acetal of **4**-bromobutanal to provide ester **7**. Mild acidic hydrolysis of the acetal function afforded aldehyde **8**, which was allowed to react with another mole-equivalent of the lithium enolate derived from ester **6** to provide hydroxy-diester **9**. Dehydration of this β-hydroxy ester was accomplished by successive treatment with methanesulfonyl chloride in the presence of triethylamine and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU); diester **10** was obtained as a 10:1 mixture of *E* and *Z* isomers at the newly created double bond. The isomers were separated by chromatography on silica gel, and the major isomer was reduced by treatment with diisobutylaluminum hydride (DIBAL). The resulting diol was oxidized by the method of Swern (*4*) to obtain (6*E*,14*E*,18*E*)-10,11-dihydrosqualene-27,28-dialdehyde (**4**).

Synthesis of (*Z*)-**4** is summarized in Scheme 2. Alkylation of the lithium enolate of *tert*-butyl trimethylsilylacetate with halide **5** gave ester **11**, which was deprotonated with LDA and treated with aldehyde **8**. The diester produced in this manner [Peterson olefination reaction (*5*)] is a 7:3 mixture of *Z* and *E* double-bond isomers. The isomers were separated by chromatography and the major isomer converted in two steps to (*Z*)-**4**.

Both (*E*)- and (*Z*)-**4** were converted into *proto*-daphniphylline (**3**) by the following simple procedures (Scheme 3). Gaseous ammonia was added to a dichloromethane solution of the dialdehyde, ammonium acetate, and triethylamine hydrochloride. After 16 hours at room temperature the solvent was removed under vacuum and the residue was taken up in glacial acetic acid. After 2 hours at 80°C, compound **3** was obtained in 15 ± 2% yield. The yield of **3** was improved to 50% by the following modified three-step procedure. A benzene solution of the dialdehyde was added to a vigorously stirring solution of 50% aqueous potassium hydroxide containing 5% by mole of tetra-*n*-butyl-



1 2 3

(*E*)-**4** (*Z*)-**4**

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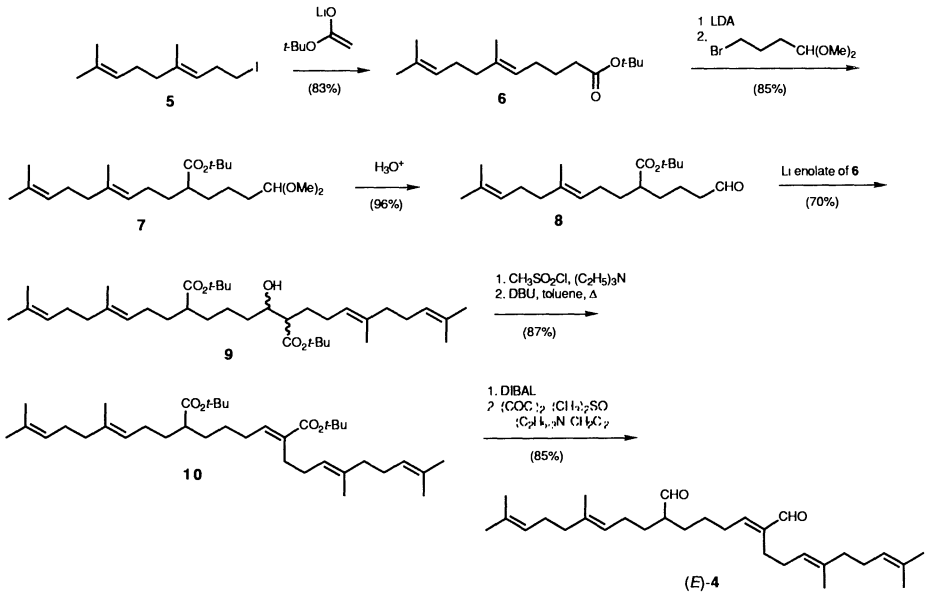
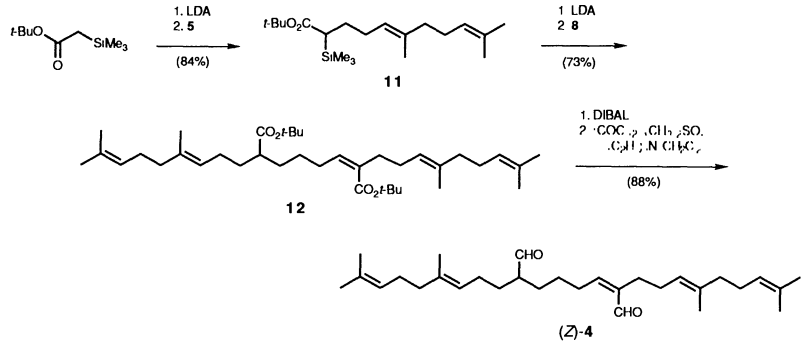
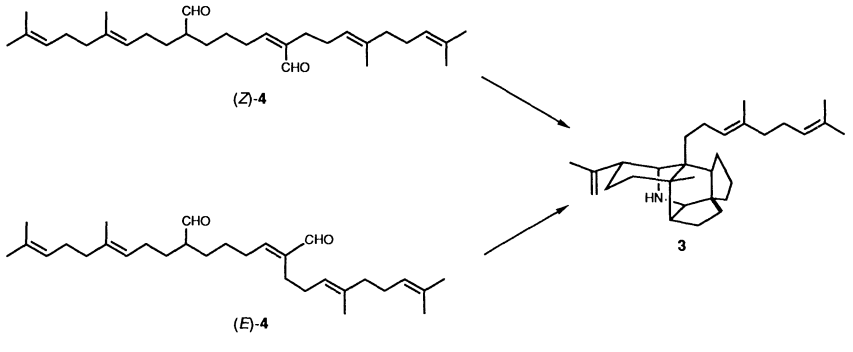


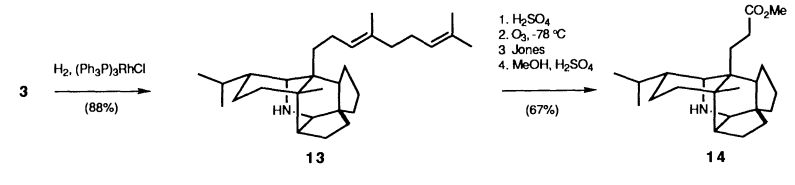
Scheme 1. Synthesis of acyclic dialdehyde (*E*)-**4**; Me, methyl; Δ, heat; see text for other abbreviations.



Scheme 2. Synthesis of acyclic dialdehyde (*Z*)-**4**.



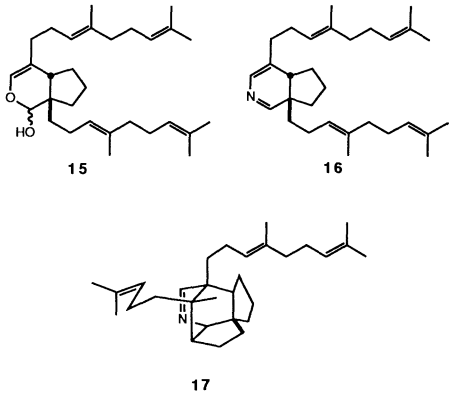
Scheme 3. Synthesis of *proto*-daphniphylline **3**.



Scheme 4. Structure determination of **3**.

ammonium bisulfate at room temperature. After 10 min, the layers were separated and the benzene removed under vacuum. The residue was taken up in dimethyl sulfoxide containing ammonium acetate. The solution was saturated with gaseous ammonia, sealed in a pressure bottle, and heated at 80°C. After 4 hours, glacial acetic acid was added and heating was continued at 80°C for an additional 2 hours. After workup, *proto*-daphniphylline was obtained in 50% yield.

The structure of **3** was rigorously determined by the three-step conversion to (±)-methyl homosecodaphniphyllate (**14**) shown in Scheme 4. Careful catalytic hydrogenation in the presence of the soluble catalyst tris(triphenylphosphine)-rhodium-(*I*) chloride saturated the less substituted double bond in **3**, giving **13**. A methanol solution of the sulfuric acid salt of **13** was treated with ozone at −78°C. Treatment of the crude product with 8 M chromic acid in acetone [Jones reagent (*6*)] gave an amino acid that was esterified by reaction with methanolic sulfuric acid. The product, obtained in 60% overall yield, was (±)-methyl homosecodaphniphyllate, identical by ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectrometry with an authentic sample.



Compounds **15** through **17** are believed to be intermediates in the remarkable pentacyclization process depicted in Scheme 3. Treatment of (*Z*)- or (*E*)-**4** with KOH provides the hydroxydihydropyran **15** as a 2:1 mixture of anomers. The reaction leading from **4** to **15** may be viewed as an intramolecular Michael reaction, and appears to afford the cis-fused 2-oxabicyclo[4.3.0]nonane skeleton with high stereoselectivity, as no trans-fused isomers have been detected in the reaction mixture. Reaction of **15** with ammonia is believed to afford the intermediate 2-aza-1,3-diene **16** (*7*), which undergoes an acid-catalyzed intramolecular Diels-Alder reaction to provide imine **17**. The latter substance has been isolated and converted into **3** under the reaction conditions.

The goal of current research in organic synthesis is to find ways to approach or surpass the finesse shown by nature in the assembly of complex organic molecules. In this work, we have come about as close to achieving this goal as is possible at present. The complete synthesis of the pentacyclic alkaloid **3** requires about ten operations starting with the known, readily available geraniol derivative **5**; the overall chemical yield is 18%. The key transformation, conversion of (*E*)- or (*Z*)-**4** to **3**, utilizes cheap, "low-tech" reagents (potassium hydroxide, ammonia, and acetic acid) and results in the formation of six σ-bonds and five rings (*8*).

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Regulation of the Timing of Transposable Element Excision During Maize Development

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The ability of transposable elements (TEs) to insert into or excise out of a genetic locus can be regulated by genetic, environmental, and developmental factors. Tissue- or organ-specific activity of TEs is a frequent and well-characterized example of spatial, developmental regulation. Regulation of the timing of TE activity during ontogeny is less well understood. To analyze timing, TE-induced variegation was quantified in the aleurone of maize kernels, a tissue composed of only a single layer of cells, and sector sizes were assigned to specific cell divisions in aleurone development. Three TE families, *Mu*, *Spm*, and *Ac/Ds*, were studied at two genetic loci. It was found that the frequency of transposon excision changes drastically (up to 30-fold increase or equivalent decrease) during the proliferation of the aleurone. Moreover, these changes occur at the same cell divisions in all three TE families. These results suggest that the timing of TE excision during maize development can be controlled by the host.

TRANSPOSABLE GENETIC ELEMENTS (TEs) were first discovered in maize (*1*). Since then, they have been found in all the organisms in which they were sought (*2*). Their ability to generate mutations, by inserting into or excising from a locus, has been exploited for gene analysis and cloning by means of transposon tagging (*3*). In addition, the properties of TEs, such as excision from a reporter gene, have been shown to be under genetic (*1*), environmental (*4*), and developmental (*5, 6*) control. Tissue specificity is the most frequent type of developmental regulation observed with TEs, and the best characterized example of this is the P element of *Drosophila* (*6*). This element is active only in the germ line, not in the soma, as a result of tissue-specific intron splicing (*7*). Similarly, tissue specificity is found for the *Tc* element of *Caenorhabditis elegans* (*8*). In maize, the *Spm* family is more active in the side branches than in the main stalk of the plant (*9*).

A second important aspect of developmental regulation is timing. That is, when during tissue development are TEs most active? Except for the *Mutator* TE family of maize, which has been shown to become germinally (*10*) and somatically (*11*) active late in the development, timing has rarely been investigated, and it is often assumed that TE excision is a stochastic event. One difficulty has been that the cell lineage within a tissue must be known so that TE-induced sectoring can be assigned to specific cell divisions during ontogeny. In this regard, the aleurone of maize is ideal, because the tissue is composed of a single layer of cells whose ontogeny has been elucidated (*12–14*), and this tissue can accumulate anthocyanin pigment. We have monitored the size and appearance of purple sectors produced by excision of three different TE families at two loci in this tissue to calculate the frequency of excision at each cell division of the aleurone. This analysis led to two conclusions: (i) there are changes in excision frequency during development, and (ii) timing appears to be determined by the host rather than by unique properties of the transposable element.

The aleurone is the single-cell epidermal layer of the starchy endosperm of maize kernels and is the site of anthocyanin pigment deposition (*14*). Insertion of a TE in a gene required for anthocyanin synthesis can suppress this purple pigmentation, while excision of the TE can restore it. The size of a revertant sector indicates the number of anticlinal cell divisions that follow the TE excision event, because the presence or absence of this nonvital pigment has no impact on tissue development. We have examined revertant (purple) sectors from three mutable alleles in genetic backgrounds in which somatic instability is maintained. Insertions of *Mu1* (*15*) and *Ds2* (*16*) TEs in the coding region of the *Bz2* locus generated *bz2::mu1* and *bz2::Ds2*, respectively; *c2::Spm* (or *c2-m1*) originated from insertion of an *Spm* TE in the *C2* locus (*17*). *Bz2* and *C2* are loci necessary for the biosynthesis of anthocyanin pigment in most tissues of maize (*18*). Excision of the TEs from these loci can restore gene expression resulting in a purple revertant sector, or spot, on a bronze (*bz2*) or white (*c2*) background in the aleurone.

We have monitored excision events at each stage of aleurone development using video imaging. Developmental stages were defined as the number of divisions at the periphery of the endosperm that contribute to surface growth (anticlinal divisions) and hence to aleurone formation. We defined the stages by the number of cells present using an exponential (powers of two) model of proliferation at the periphery of the endosperm (*12*) (Fig. 1), which allowed assignment of any purple sector to a developmental stage by counting the number of cells in that sector. Our staging system is supported by the model of endosperm development (*12–14*). The maize endosperm is triploid, receiving two haploid nuclei from the maternal parent plus one sperm nucleus. The

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