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Mechanistic investigations of a biomimetic polycyclization process that leads to the *Daphniphyllum* alkaloids

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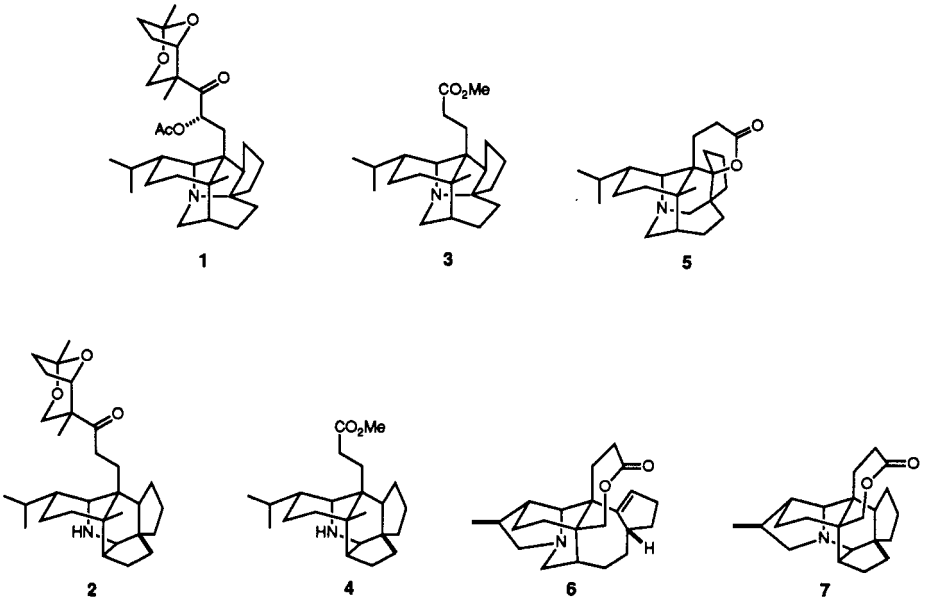
Abstract - Synthetic experiments pertaining to a postulated biosynthesis of the *Daphniphyllum* alkaloids (Fig. 2) have been carried out. The key features of this hypothesis are the postulated cyclizations of **8** to **9** and **9** to **10** (*proto*-daphniphylline). One question that arises in respect to this biosynthetic hypothesis relates to the regioselectivity of the final ring formation (Fig. 4). This question was addressed by carrying out the synthesis of *proto*-daphniphylline shown in Fig. 5. Evidence is presented that the selectivity of this ring closure derives from the greater stability of the six-membered ring product, relative to several possible five-membered ring alternatives (Fig. 7). Five-membered ring formation is so heavily disfavored that it does not occur even when the preferred mode of closure is impossible (Fig. 8). The conversion of **8** to **9** could be stepwise or concerted (Fig. 9). This question was addressed by synthesizing the bis-neryl analog **33**, which was cyclized to **34**. Thus, the conversion of **8** to **9** is concerted. Finally, *proto*-daphniphylline has been prepared in a straightforward manner from the dihydrosqualene dialdehydes (*E*)- and (*Z*)-**37** as shown in Figures 16 and 17.

The oriental deciduous tree Yuzuriha (*Daphniphyllum macropodum* Miquel) contains a family of triterpene alkaloids that now has about 35 members (ref. 1). Representative structures are depicted in Fig. 1. Although daphniphylline (**1**) is the most abundant member of the group, the minor alkaloid secodaphniphylline (**2**) is probably more primitive in a biosynthetic sense because in this molecule the nitrogen atom has been introduced without cleavage of any of the C-C bonds of the triterpene precursor. Methyl homodaphniphyllate (**3**) and methyl homoseco-



1 **3** **5**

2 **4** **6** **7**

Fig. 1. Representative *Daphniphyllum* alkaloids (compound **7** was isolated from *Sapium baccatum*).

daphniphyllate (**4**) are truncated versions of daphniphylline and secodaphniphylline, respectively. Two other members of the family, daphnilactone A (**5**) and daphnilactone B (**6**), further illustrate the structural diversity found in this group of natural products. Finally, attention is called to the heptacyclic alkaloid bukittinggine (**7**), recently isolated from *Sapium baccatum* (Roxb.) Ridley (ref. 2). It is obvious that this alkaloid is closely related to the *Daphniphyllum* alkaloids.

We have previously reported a possible biosynthetic route to these compounds (ref. 3). The proposal is summarized in Fig. 2. A key feature of this scheme is the proposed conversion of dihydropyridine **8** into tetrahydropyridine **9** and thence into the pentacyclic product **10**, a compound that we believe may well be the biogenetic parent of all of the *Daphniphyllum* alkaloids. Because of this conjectured ancestral relationship, we refer to **10** as *proto*-daphniphylline.

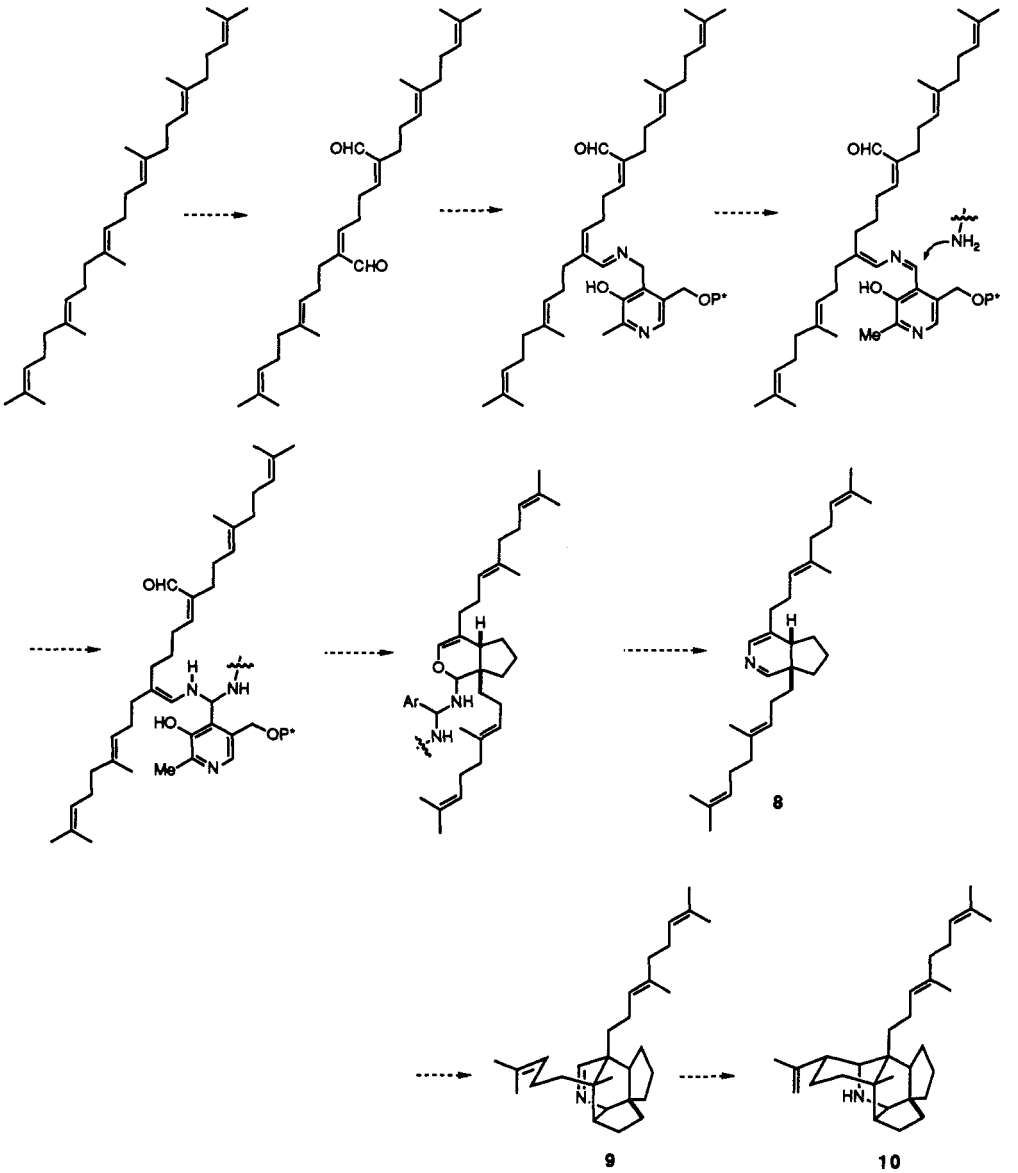


Fig. 2. Proposed Biosynthesis of the *Daphniphyllum* alkaloids.

At the outset of this work, the postulated polycyclization process had been demonstrated and employed in syntheses of methyl homosecodaphniphyllate (ref. 4) and daphnilactone A (ref. 5). The key transformations in these two syntheses are depicted in Fig. 3 (Note a).

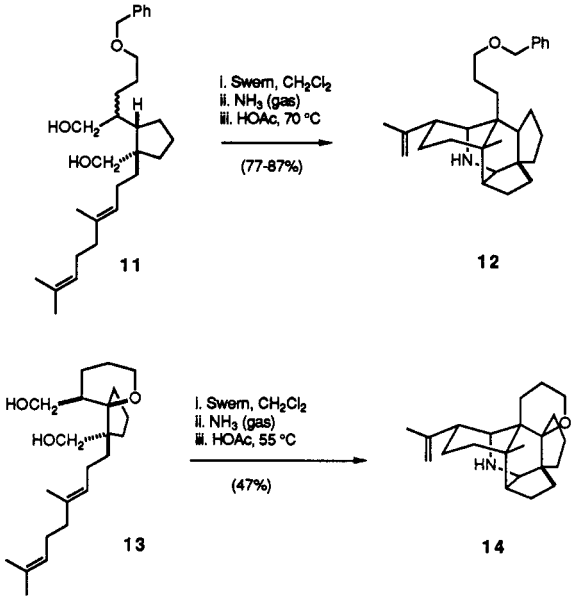


Fig. 3. Ruggeri tetracyclization reaction.

However, in spite of the extraordinary efficiency of the Ruggeri tetracyclization process, a significant question remained with regard to its biosynthetic relevance. In the proposed biosynthetic scheme (Fig. 2), the final ring closure, a formal ene reaction, might be complicated by competing five-membered ring formation (Fig. 4).

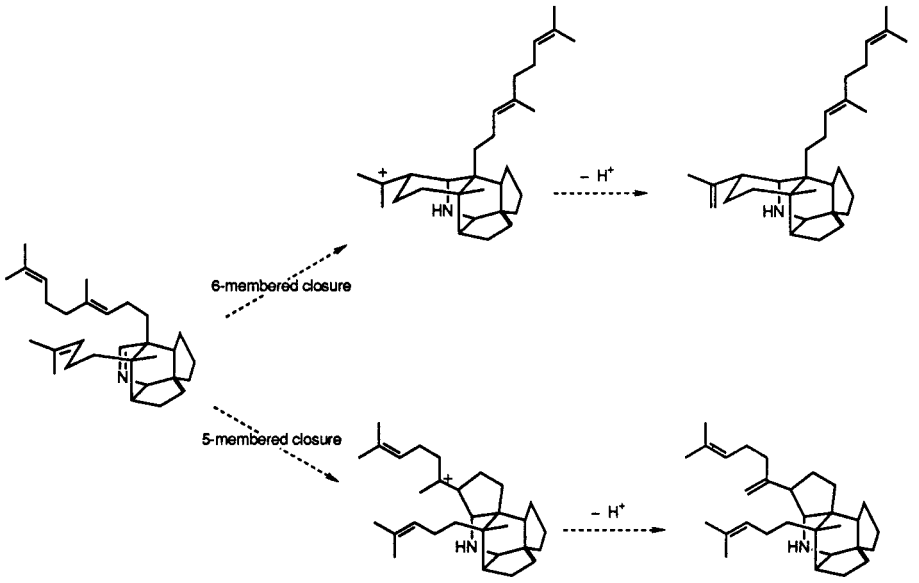


Fig. 4. Alternative closure modes for the final cyclization in the *Daphniphyllum* alkaloid biosynthetic proposal.

Note a: In recent work aimed at the total synthesis of bukittinggine (**7**), the conversion of **11** to **12** has been achieved on a 500-mg scale in 97% yield, after chromatographic purification of **12** (J. A. Stafford, unpublished work).

To answer this question we carried out a synthesis of *proto*-daphniphylline along the same general line that we had used for the methyl homosecodaphniphyllate synthesis (ref. 4). Amide **15** was prepared by alkylation of the lithium salt of N-acetylpyrrolidine with homogeranyl iodide (ref. 6) at -78 °C (87% yield). Components **15**, **16**, and **17** were merged as shown in Fig. 5. Compound **18** was obtained in 80% yield, accompanied by small amounts of the other three diastereomers. Treatment of **18** with diisobutylaluminum hydride (DIBAL) provided hydroxy amide **19** (86%), along with 8% of the corresponding amino alcohol. Amide **19** was hydrolyzed by treatment with potassium hydroxide in aqueous ethanol; lactonization occurred immediately upon acidification of the basic solution. The resulting lactone (**20**) was a 1:1 mixture of diastereomers due to epimerization at the side-chain position. Reduction of the lactone mixture with lithium aluminum hydride provided a separable mixture of diols (**21**). The diols were each oxidized by the Swern protocol (ref. 7) to give the corresponding aldehydes (**22**). A solution of the dialdehyde and 2 molar equivalents of triethylammonium hydrochloride in methylene chloride was treated with gaseous ammonia while stirring at 0 °C for one hour. Evaporation of the solvent under reduced pressure gave an oily residue that was dissolved in acetic acid containing ammonium acetate. After one hour at room temperature, tetracyclic imines **23** and **24** were isolated in 70% and 4% yields, respectively. If the acetic acid solution was heated at 75 °C for two hours before workup, *proto*-daphniphylline (**10**) was isolated in 78% yield. Treatment of tetracyclic imine **23** under the same conditions provided **10** in 90% yield.

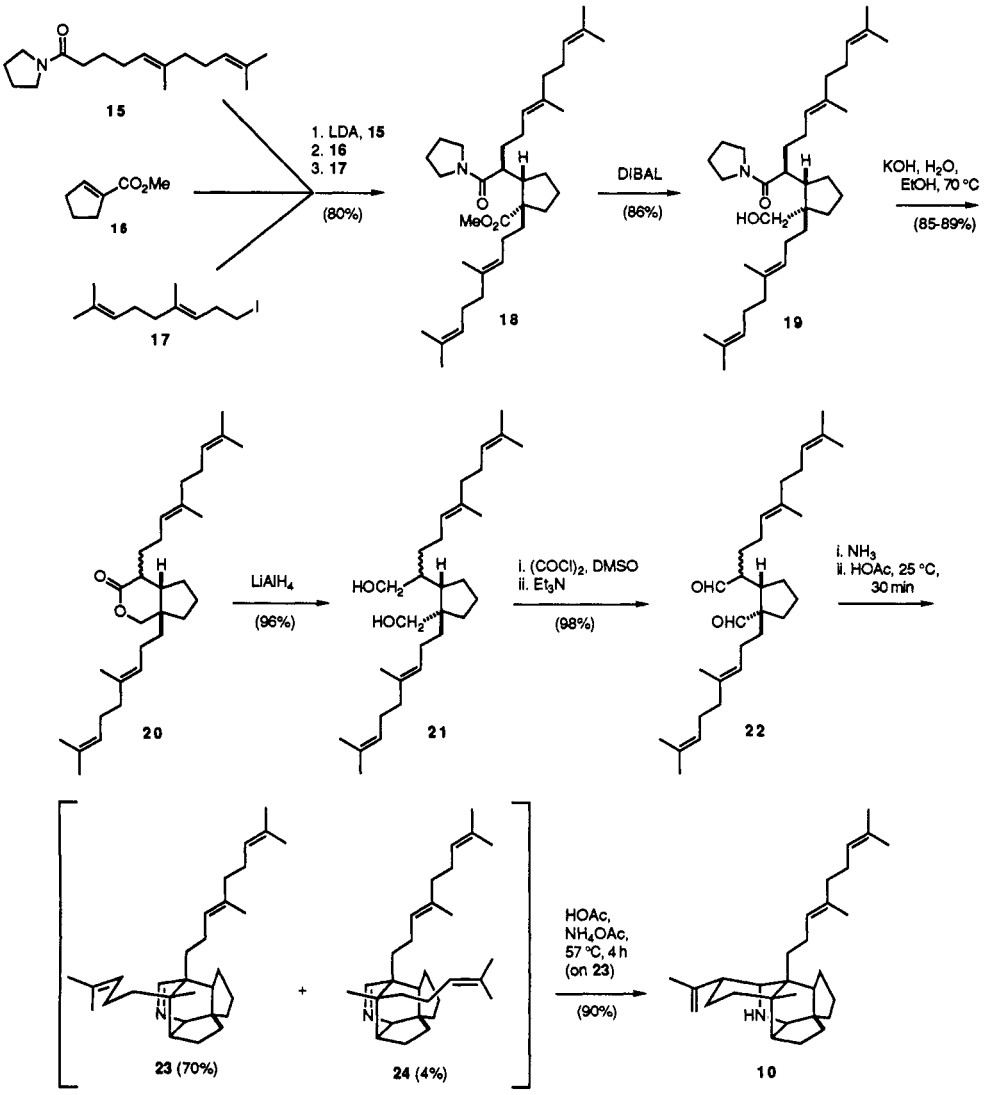


Fig. 5. Convergent synthesis of *proto*-daphniphylline (**10**).

The structure of the final tetracyclization product was firmly established by its conversion into methyl homosecodaphniphyllate as shown in Fig. 6. Catalytic hydrogenation of the isopropenyl double bond gave **25**, which was degraded as indicated to the racemic alkaloid.

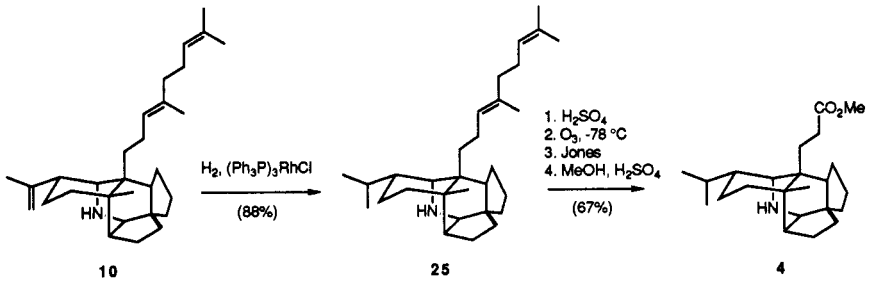


Fig. 6. Proof of structure of *proto*-daphniphylline; conversion into (±)-methyl homosecodaphniphyllate.

The synthesis outlined in Fig. 5 answered the question of double bond selectivity, but left the question of why. This question was evaluated with molecular mechanics (ref. 8). The five skeletons depicted in Fig. 7 were evaluated; side-chains were abbreviated for simplicity of calculation. It was found that the *proto*-daphniphylline skeleton is 9-15 Kcal $mole^{-1}$ more stable than the four stereoisomeric five-membered cyclization products. This difference in stability is, then, the probable reason for the high regioselectivity observed in the final cyclization.

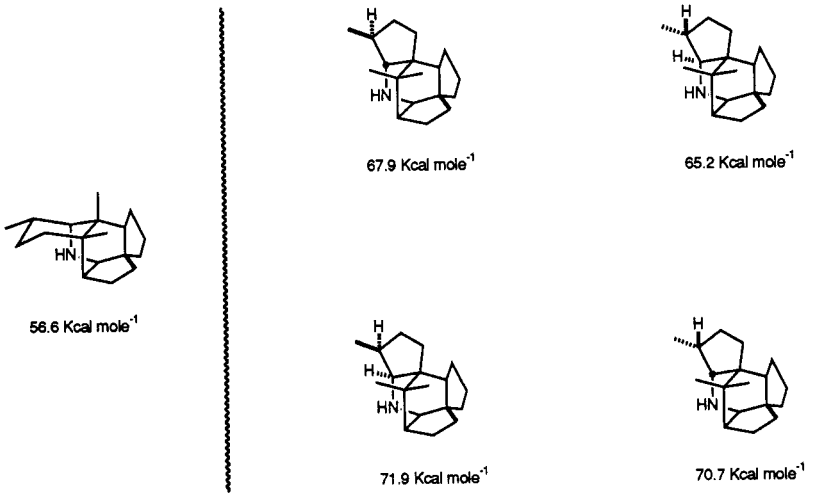


Fig. 7. Molecular mechanics evaluations of alternative pentacyclic skeletons.

To further evaluate the possibility of formation of an alternative five-membered cyclization product, we prepared the dialdehyde **26** by a route completely analogous to that shown in Fig. 5 except that homoprenyl iodide was substituted for homogeranyl iodide both in synthesis of the original amide and in the convergent step. Treatment of this material sequentially with ammonia and acetic acid gave the tetracyclic model **27** (Fig. 8). This material was recovered unchanged after being heated in refluxing acetic acid for 16 hours. Cyclization also did not occur when **27** was refluxed with trifluoroacetic acid for 12 hours. The latter conditions, however, did appear to hydrate the C-C double bond.

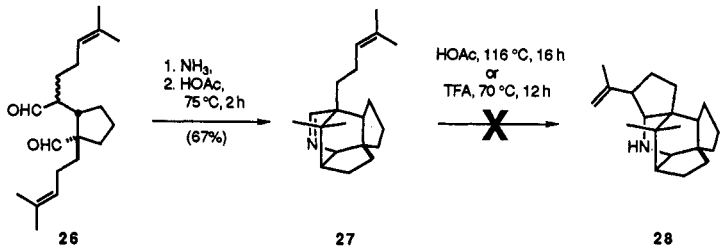


Fig. 8. Failure of a simplified tetracyclic model compound to undergo five-membered ring formation.

Another question with regard to the tetracyclization process used in the synthesis of the *Daphniphyllum* alkaloid skeleton is whether the formation of the third and fourth rings is concerted or stepwise (Fig. 9).

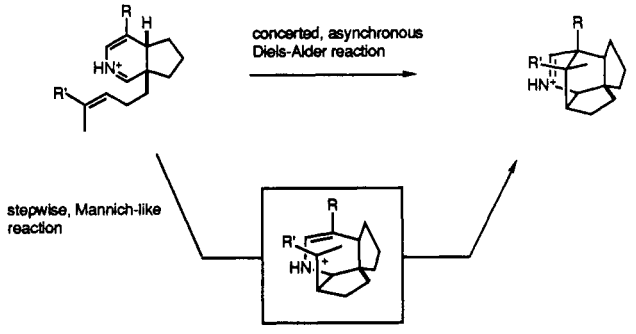


Fig. 9. Alternative Mechanisms for the Ruggeri cyclization reaction.

In order to evaluate the mechanism of this step, we prepared the bis-homoneryl substrate as shown in Fig. 10. The nerol used in the production of amide **29** and iodide **30** contained 3% of geraniol, so diol **33** was a 94:3:3 mixture of the (*ZZ*), (*ZE*), and (*EZ*) isomers. In the event, the tricyclization process provided in 79% yield the tetracyclic imine **34**. Also isolated was

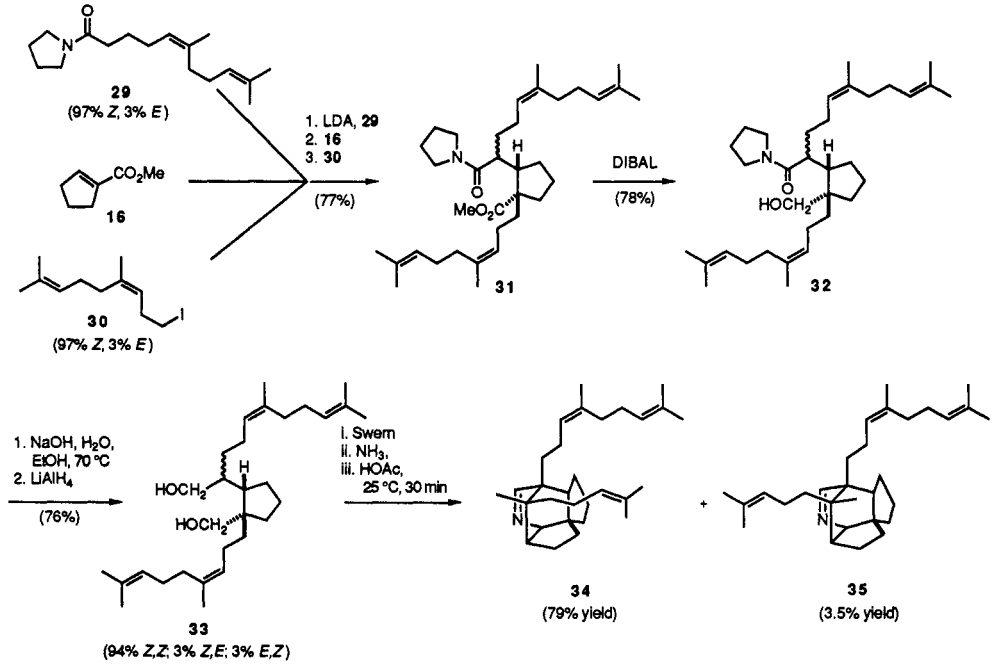
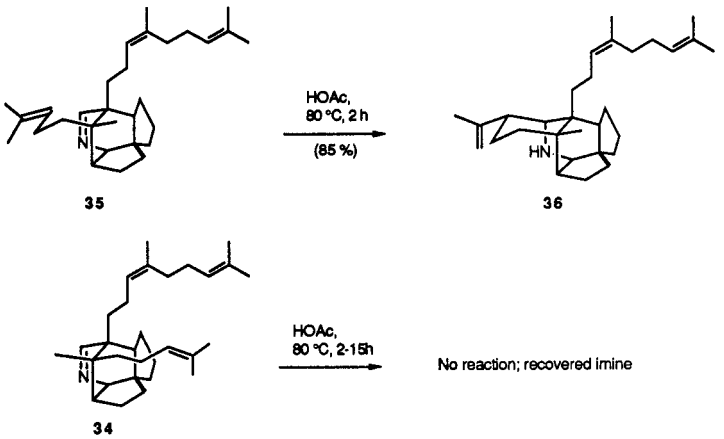


Fig. 10. Synthesis and cyclization of the homoneryl analog; a mechanistic test.

imine **35**, in 3.5% yield. This experiment shows that the formation of the third and fourth rings is probably a concerted process; it may be viewed as a reverse-electron-demand Diels-Alder reaction.

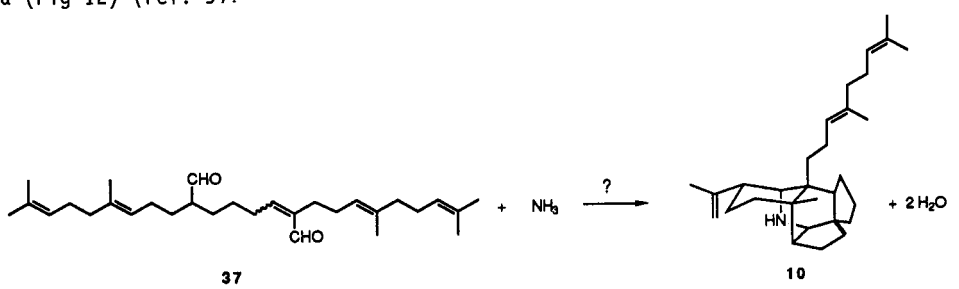
The products of the tricyclization reaction were further treated with acetic acid at 80 °C (Fig. 11). The minor product, **35**, was converted into a pentacyclic double bond isomer of *proto*-daphniphylline (**36**). The major isomer, **34**, was recovered unchanged after 15 hours. The latter experiment shows that the acid-catalyzed Diels-Alder reaction leading to **34** (and also, presumably, that leading to **23** in Fig. 5) is not reversible.



35 **36** **34**

Fig. 11. Illustrating the cyclization of **35** and lack of cyclization of imine **34**.

The biosynthetic proposal summarized in Fig. 2 stimulated us to consider the possibility of forming all five of *proto*-daphniphylline's rings from a dihydrosqualene dialdehyde and ammonia (Fig 12) (ref. 9).



37 **10**

Fig. 12. Possible biomimetic synthesis of *proto*-daphniphylline by pentacyclization of a squalene derivative.

A straightforward retrosynthetic analysis for the required dialdehyde is presented in Fig. 13.

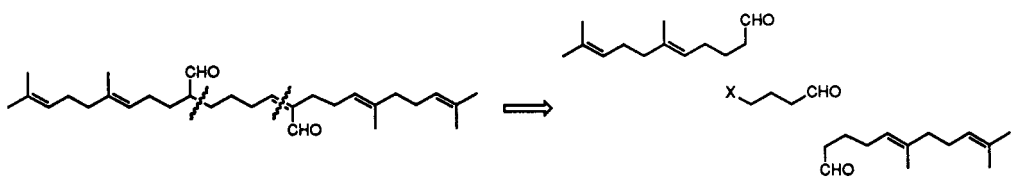


Fig. 13. Retrosynthetic analysis of the required dihydrosqualene dialdehyde.

The preparation of (6*E*,14*E*,18*E*)-10,11-dihydrosqualene-27,28-dialdehyde ((*E*)-**37**) is shown in Fig. 14. Alkylation of *t*-butyl lithioacetate with iodide **17** provided an ester (**38**) which was further deprotonated and alkylated with the dimethyl acetal of 4-bromobutanal. The resulting acetal (**39**) was hydrolyzed and used in an aldol addition reaction with the lithium enolate of **38** to obtain β-hydroxy esters **41**. Base-mediated elimination of the derived methanesulfonates gave **42** as a 10:1 mixture of *E* and *Z* isomers. These were separated by silica gel chromatography and the major isomer converted into (*E*)-**37** by a standard two-step process involving sequential reduction with diisobutylaluminum hydride and Swern oxidation.

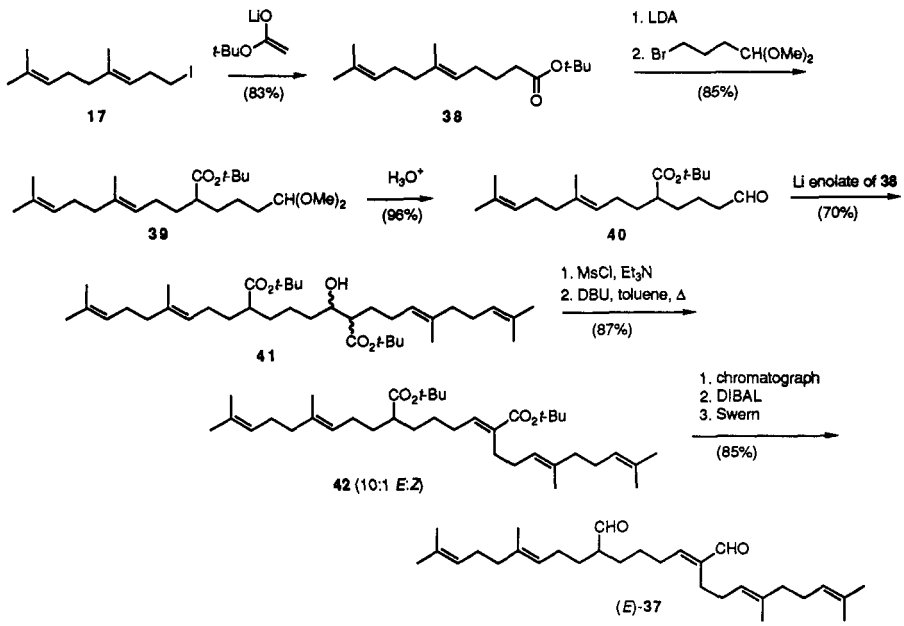


Fig. 14. Preparation of (6*E*, 14*E*, 18*E*)-10,11-dihydrosqualene-27,28-dialdehyde.

The *Z* isomer of **37** was obtained as shown in Fig. 15. Alkylation of *t*-butyl lithio(trimethylsilyl)acetate afforded the α-trimethylsilyl derivative of **38**, compound **43**. The lithiated derivative of this material was used in a Petersen olefination reaction (ref. 10) to obtain the α,β-unsaturated ester **42** as a 7:3 mixture of *Z* and *E* isomers. These were separated by chromatography and the major isomer converted into (*Z*)-**37**.

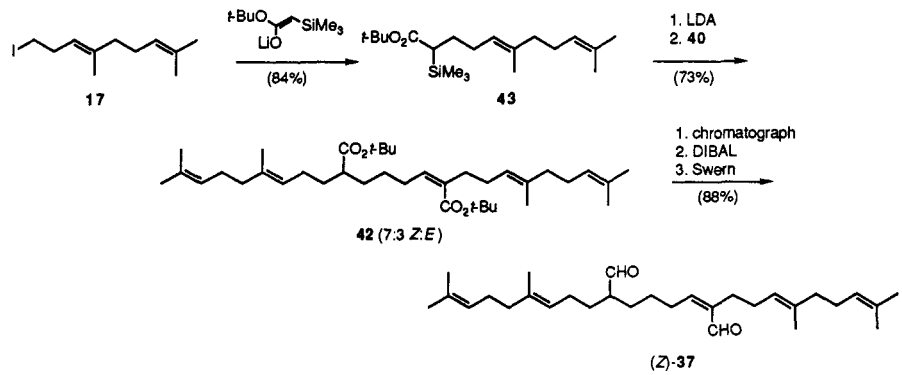


Fig. 15. Preparation of (6*E*, 14*Z*, 18*E*)-10,11-dihydrosqualene-27,28-dialdehyde.

Both of the isomeric dialdehydes are somewhat labile and react upon silica gel chromatography. In particular, (*Z*)-**37** was isomerized to the *E* isomer under these conditions.

The high-water mark of the project was reached on February 17, 1989, when dialdehydes **37** were treated successively with ammonia and acetic acid (Fig. 16). *Proto*-daphniphylline (**10**) was found in the reaction product in approximately 15% yield! A large amount of less polar material was also isolated from the reaction product. Nuclear magnetic resonance spectrometry showed that this material was a mixture of compounds having homogeranyl units. This material is believed to be oligomers of the starting dialdehydes that result from competing processes, such as Michael and aldol reactions.

Although the yield in this pentacyclization process is not high, it is actually not bad, considering that six new σ-bonds and all five of *proto*-daphniphylline's rings are formed in a simple process that requires only the most elementary reagents and mild reaction conditions.

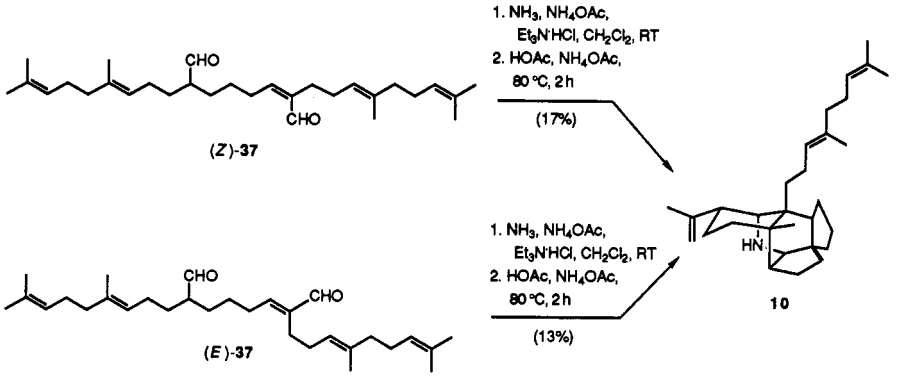


Fig. 16. A cascade of reactions forming six σ bonds and five rings.

It is obvious from a comparison of Figures 5 and 16 that the low yield in the conversion of **37** to *proto*-daphniphylline is due to inefficiency in the first ring formation. That is, the yield of the conversion of **22** to **10** (Fig. 5) is 78%. In related systems (for example, in the conversion of **11** to **12** shown in Fig. 3) the yield is as high as 97%. Thus, if we could find a better way to carry out this first step, which amounts to no more than an intramolecular Michael addition, we might be able to accomplish the pentacyclization in much higher overall yield. A considerable amount of effort was expended in optimizing this process. It soon became clear that aldehydes **37** are converted into the hydroxydihydropyran **44** under a number of conditions (basic alumina, tetra-*n*-butylammonium hydroxide in methanol, 1,8-diazabicyclo-[5.4.0]undec-7-ene). Ultimately, we found that the best yield of product (50-57%) is obtained under phase-transfer conditions, using 50% aqueous potassium hydroxide/benzene with a catalytic amount of tetra-*n*-butylammonium bisulfate as catalyst. The two anomers are separable by chromatography, but we were not able to ascertain the relative stereochemistry at the hemiacetal carbon.

A typical complete transformation of (*E*)- or (*Z*)-**37** into *proto*-daphniphylline (Fig. 17) was as follows. The starting dialdehyde was cyclized over a ten-minute period to **44** by the phase-transfer method and the crude material was worked up and dried. The crude **44** were dissolved in dimethylsulfoxide along with six molar equivalents of ammonium acetate and the resulting solution placed in a pressure bottle. The mixture was saturated with gaseous ammonia for three minutes. The bottle was closed and the reaction mixture heated at 80 °C for three hours. After brief cooling, the bottle was opened, an excess of acetic acid was introduced, and heating was continued for two-and-one-half hours. Usual workup and chromatography of the brown reaction product gave *proto*-daphniphylline (**10**) in an overall yield of 49.4%. A workup is absolutely required after the first operation as water seems to have a very deleterious effect on the subsequent tetracyclization (the overall yield dropped to about 20% if the workup was omitted).

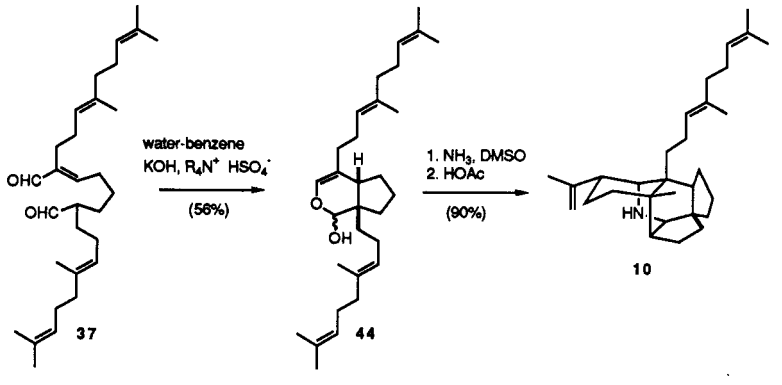


Fig. 17. The optimized pentacyclization process; formation of *proto*-daphniphylline in 50% overall yield.

We were also able to develop a one-pot version of the pentacyclization process. The reaction of **37** and one molar equivalent of tetra-*n*-butylammonium hydroxide (10% w/w in water) in dimethylsulfoxide gave a mixture of products containing only 17% of **44**. The use of one molar equivalent of powdered potassium hydroxide (ref. 11) (commercial grade, 15% water) gave **44** in 35% yield. When powdered sodium hydroxide (commercial grade, 2% water) was used, compound **44** was obtained in 44% yield. The last experiment was repeated on a scale of 132 mg of **37**. After three-and-one-half hours at room temperature, ammonium acetate and an excess of gaseous ammonia were added and the remaining process was continued as before. Workup and purification of the product of this one-pot pentacyclization process furnished *proto*-daphniphylline in 44% overall yield.

Acknowledgement

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