of *C. ovandensis* because their trip efficiency was five times that of the most abundant pollinator (Table 1).

In view of the variation in the pollination ability of different visitors, the reproductive success of individual plants is a function of plant characters that determine the number and kinds of visitors that a plant attracts. The extent to which variation in plant fitness is attributable to such characters determines the potential for selection of pollinators on plants (*16*). The observed variation in pollination ability among visitors to *Calathea ovandensis* flowers may be a result of previous selection for specialization, a stage toward further specialization, or both. The demonstration of variation in pollinator ability is an important step in the assessment of how interactions between plants and their potential mutualists can influence the evolution of plant characters and mutualism specificity.

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10. Despite the specialized pollination mechanism, bagged inflorescences in the field as well as unmanipulated inflorescences in an insect-free greenhouse exhibit a low level of fruit-set.
11. The study site was a secondary forest at Laguna Encantada (altitude 350 m) near San Andres Tuxtla (see C. C. Horvitz and D. W. Schemske *Ecology*, in press).
12. Observations were made from approximately 08:30 to 13:30 on 20 days from 27 August to 7 October 1983 during the peak flowering season.
13. In calculating the chi-square statistics for fruit-set efficiency of tripped and visited flowers, all observations for Lepidoptera were grouped to minimize the number of cells with expected values less than 5 [see R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1981)].
14. Tripping probability on the *n*th visit varied only from 0.17 to 0.20 for $n = 1$ to 7.
15. Although Lepidoptera are noted as nectar robbers of *Calathea* spp. (*9*), they do not appear to have a negative effect on *C. ovandensis* at the pollinator stage. However, the larvae of several of these flower visitors have a negative impact on *C. ovandensis* at other stages. Larval *Eurybia* feed exclusively on buds, flowers, and fruits (*11*), while larval Hesperiidae feed on leaves (C. C. Horvitz, unpublished data).
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A Model Study of Fecapentaenes: Mutagens of Bacterial Origin with Alkylating Properties

Abstract. *Fecapentaene-14 and -12 are directly acting mutagens that do not require metabolic activation. Their unusual structure suggests a possible mechanism of action. A carbocation that is formed by the addition of an electrophilic species (such as a proton) to the enol ether is most probably the reactive species. A series of model enol ethers with conjugated systems of various lengths was prepared, and a correlation between mutagenicity and increasing reactivity of derived carbocations was found. The glycerol moiety does not play a crucial role in the overall reactivity of the fecapentaenes.*

The results of epidemiological studies (*1*) have indicated that human colorectal cancer is related to the diet and have led to the conclusion that a cancer-causing substance (or substances) may be present in food (*2*). During excretion in feces, this substance would be in contact with the intestinal epithelium long enough to cause neoplastic transformation. The search for such a substance has led to the discovery of four compounds, fecapentaene-14 (**1**) and three stereoisomers of fecapentaene-12 (**2**) (*3–5*) (Fig. 1). The important structural feature of all four substances is their highly unsaturated conjugated enol ether system; this bond system implies that the compounds can be hydrolyzed by an aqueous acid to an unsaturated aldehyde (**3**) and glycerol (**4**) (Fig. 1a), from which they are formally derived. The acid protonates the enol ether to give a carbocation (**5**) (Fig. 1a) that will then undergo a series of rapid rearrangements and transformations (*6, 7*). The propensity for the formation of such a cation (or cations, since several

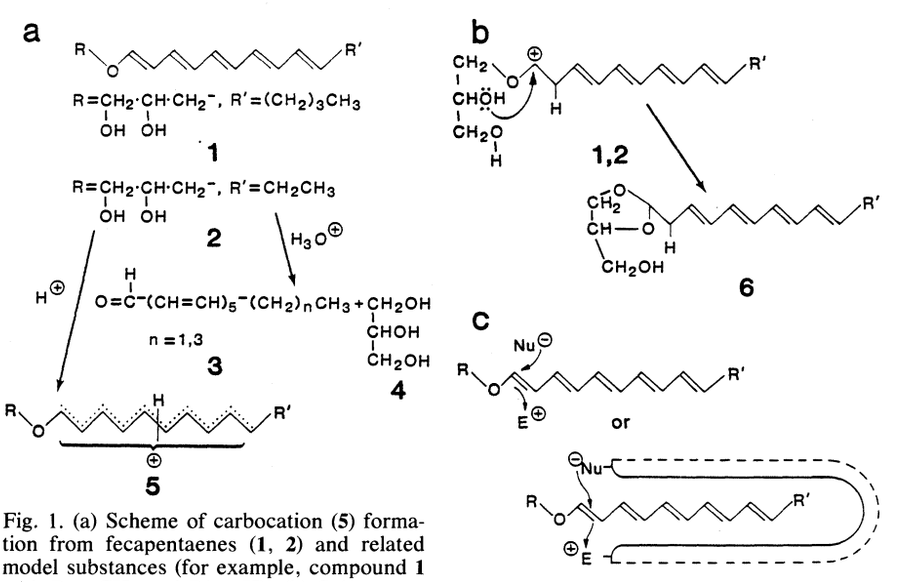

Fig. 1. (a) Scheme of carbocation (**5**) formation from fecapentaenes (**1**, **2**) and related model substances (for example, compound **1** with R = CH_3 and R′ = H). (b) Subsequent acetal (**6**) formation [see (*18*)]. (c) A more general depiction of (a) in which $E^{\oplus}$ and $Nu^{\ominus}$ can be parts of the same molecule (for example, a nucleic acid or a protein), resulting in intramolecular linking. The formulas have no configurational or conformational implications and may represent several combinations of *cis-trans* geometries [see (*3*)].

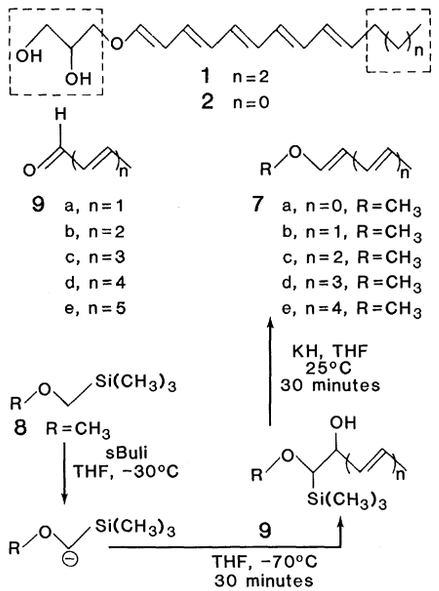

Fig. 2. Scheme of preparation of fecapentaene analogs. The fecapentaene molecule (**1**, **2**) was simplified by substituting the boxed areas with methyl groups. Note that any aldehyde (**9**) will give an ether (**7**) with one additional carbon-carbon double bond (for example, compound **9b** gives compound **7b**). The formulas have no configurational or conformational implications and may represent several combinations of *cis-trans* geometries.

rearranged structures may be present at the same time) increases with extended conjugation, because an increasingly conjugated system can be expected to delocalize the charge more effectively (*7*); this chain of events would provide the driving force for the cation formation.

Carbocations of type **5** are highly reactive, seeking a nucleophile with which to react and neutralize the charge. Homologated polyenylic cations have been shown to become increasingly reactive in a variety of reactions (*6*). Since most chemical carcinogens and mutagens are described as electrophilic reagents (*8*), the increasing reactivity of progressively conjugated polyenylic carbocations would be expected to be shown by a stronger activity in a bacterial assay of mutagenicity (*9*), provided that the cation **5** functions as the ultimate mutagen. The glycerol moiety would therefore play no substantial role in this mechanism, although it may modulate the mutagenic activity, perhaps by formation of an acetal (**6**) (Fig. 1b) or by influencing the transportability of **1** and **2** across membranes.

We synthesized a series of methyl enol ethers of the general formula **7** (n = 0 to 4) (Fig. 2) to investigate whether increasing mutagenicity of the more highly unsaturated homologs would give evidence of the involvement of the carbocation of type **5** in the molecular mechanism of fecapentaene mutagenicity. The model substances **7a** to **7e** were prepared by a modification (*10*) of the Peterson reaction (*11*) (Fig. 2) with the use of (methoxymethyl)trimethylsilane (**8**) (*12*, *13*) [similar to the procedure used to synthesize **1** (*14*)]. The aldehydes **9c**, **9d**, and **9e** were prepared (*15*, *16*), and **9a** and **9b** were purified, commercial products (*17*).

Unactivated *Salmonella typhimurium* strain TA-100 (*9*) was used in the assay of fecapentaene mutagenicity. Although precautions were taken to avoid acid hydrolysis of the enol ethers, in order to exclude the possibility that the observed mutagenicity was due to the corresponding aldehydes formed by such hydrolysis (for example, **7e** would hydrolyze to **9d**, **7d** to **9c**), we assayed the mutagenicity of these aldehydes with the same system. Saturated aliphatic aldehydes of the general formula $CH_3(CH_2)_nCHO$ (n = 0 to 4) were used as another set of controls and showed no mutagenicity.

The results (Table 1 and Fig. 3) corroborated the original hypothesis that a carbocation of type **5** formed by protonation is most likely the ultimate reactive species. The unsaturated aldehydes were less mutagenic than the corresponding enol ethers (compare **7e** to **9d**, **7d** to **9c**). The mutagenicity of the five-double-bond model system (pentaene, **7e**) was comparable to that of the fecapentaene-14 and -12. This confirms that neither the glycerol moiety nor the saturated portion of the enol ether in the fecapentaenes is crucial for mutagenicity and suggests that their presence in **1** and **2** may have a modulating effect, as can be seen in the higher toxicity of **7e** compared to that of **1**. The higher toxicity of these compounds might reduce their apparent mutagenicities (see Fig. 1). Thus, we propose that fecapentaenes act as alkylating agents through the intermediacy of carbocations of type **5** (Fig. 1, a and b). Moreover, if the cation is formed by an attack of an electrophilic species located on a macromolecule instead of a proton, then the carbocation would be quenched by a nucleophilic species on the same or another macromolecule, giving rise to intra- or intermolecular linking (Fig. 1c).

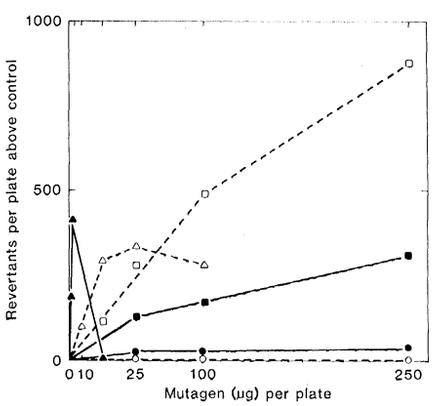

Fig. 3. Effect of extended conjugation of compounds **7** and **9** on mutagenicity in *Salmonella typhimurium* strain TA-100. (●) Compound **7c**; (■) compound **7d**; (▲) compound **7e**; (○) compound **9c**; (□) compound **9d**; (△) compound **9e** (see Table 1).

Table 1. Mutagenic activities of fecapentaene-related compounds assayed with *Salmonella typhimurium* (*9*). Appropriately concentrated solutions of tested compounds were made in acid-free dimethyl sulfoxide (DMSO). A 0.1-ml sample of such a solution and 0.1 ml of fresh *S. typhimurium* TA-100 cultured overnight were mixed with 2 ml of top agar containing L-histidine (0.05 m*M*) and biotin (0.05 m*M*) at 45°C and poured onto a minimal agar plate. Positive [*N*-methyl-*N*-nitroso-*N'*-nitroguanidine (MNNG)] and negative (DMSO) controls were included in each assay. The plates were incubated for 48 hours at 37°C and counted; the number of spontaneous revertants (mean = 209) has been subtracted from the experimental values to give the values shown.

Compound	Number of carbon-carbon double bonds*	Amount per plate (mg)	Revertants†
9a	1	1000	0
9b	2	1000	0
9c	3	500	0
9d	4	250	280
9e	5	25	299
7a	1	1000	0
7b	2	1000	0
7c	3	500	92
7d	4	250	304
7e	5	2.5	411
1	5	1.7	2067
MNNG		2.0	1865

*Note that all forms of compounds **9** also have one carbon-oxygen double bond in addition to the carbon-carbon double bonds shown. †Mutagenicity was measured by the number of revertants found per plate.

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Voltage-Dependent Sodium Channels in an Invertebrate Striated Muscle

Abstract. *Striated skeletal muscles from the planktonic arrowworm* Sagitta elegans *(phylum Chaetognatha) were voltage-clamped. The muscles displayed classical voltage-dependent sodium channels that (i) showed peak transient currents when the membrane was depolarized 90 millivolts from rest, (ii) opened rapidly with peak currents flowing within 0.4 milliseconds at 4°C, (iii) showed voltage-dependent inactivation with 50 percent inactivation at +25 millivolts from rest, and (iv) were blocked by 500 nanomolar tetrodotoxin.*

Many cells, notably nerves and muscles, are termed excitable because they generate and propagate action potentials. Most invertebrate axons (*1*) as well as vertebrate axons (*2*) and skeletal muscles (*3*) rely on sodium ions as the predominant inward charge carrier during depolarization. In contrast, invertebrate muscles investigated so far seem to use calcium ions to generate action potentials (*4, 5*). Voltage-dependent calcium channels seem to be more evolutionarily primitive than sodium channels in that they are present in unicellular organisms (*6*) and are the first channels to appear during the embryonic development of excitable cells (*7*). At some point during evolution there must have been selective pressures favoring the expression of sodium channels not only in neurons but also in muscle membranes as well. Because of the large size of their skeletal muscles, most invertebrate preparations examined to date are protostomates (annelids, arthropods, or mollusks), which are not considered to have evolved along the same path as that giving rise to the chordates (*8*). Therefore, using a new patch-clamp technique that allows voltage clamping of small cells, we have examined the ionic basis of the action potential in the muscles of a primitive deuterostome, the arrowworm *Sagitta elegans* (phylum Chaetognatha) (*9*). We now report that in this species, sodium constitutes the predominant inward charge carrier during depolarization.

Groups of five to ten intact *Sagitta* (*10*) were placed in seawater or artificial seawater (ASW) and shocked with bipolar extracellular electrodes, which always elicited vigorous twitches. Removing calcium from the ASW did not prevent the animals from twitching in response to electrical stimulation during the 20-minute test period. In contrast, removing the sodium resulted in paralysis within 60 seconds. Animals remained flaccid and unresponsive to electrical stimulation until sodium was returned, at which point they regained the ability to swim. Addition of the specific sodium channel blocker tetrodotoxin (500 n*M* TTX) (*11*) to the medium resulted in rapid paralysis. These data suggest that sodium, but possibly not calcium, is required in the normal chain of neuromuscular events that ultimately lead to contraction.

We next examined the electrophysiological properties of the longitudinal muscles (*12*). The shape and kinetics of an action potential often reflects the ionic mechanisms underlying its generation. Figure 1 shows examples of action potentials obtained from a frog (*Rana temporaria*), an insect (*Manduca sexta* Lepidoptera), and *Sagitta*. The insect action potential (Fig. 1A) is mediated by calcium (*5*), as is suggested by the slower time course and broader spike than the sodium-generated action potential of the frog (Fig. 1B). The action potential recorded from *Sagitta* (Fig. 1C) both rises and falls rapidly and lacks the characteristic calcium plateau on the falling phase. The shape of the *Sagitta* action potential suggests that calcium is not the predominant inward charge carrier.

To better examine the ionic mechanisms underlying the action potential in *Sagitta*, the muscle membrane was voltage-clamped (*13–15*). Figure 2A shows typical current responses seen when the membrane was depolarized by 10 to 120 mV in steps of 10 mV. There was a fast inward current which inactivated and a slower outward current, both of which were voltage-dependent. The fast kinetics and apparent inactivation of the inward current resemble those of TTX-sensitive sodium channels observed in other cells (*16*). Inactivation was examined in more detail by measuring the fractional amplitude of the inward cur-

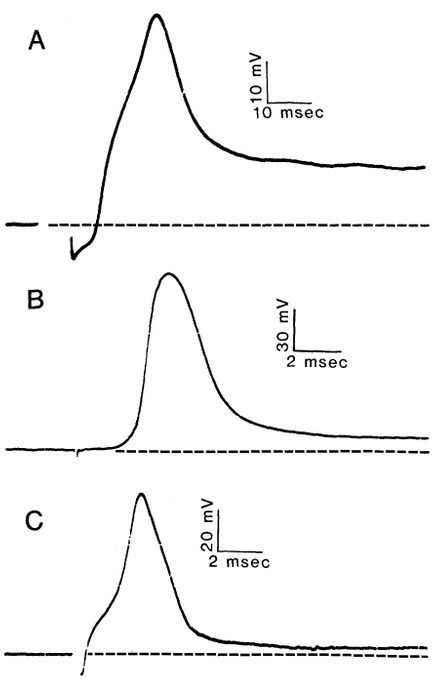

Fig. 1. Comparison of (A) insect (*Manduca sexta*), (B) frog (*Rana temporaria*), and (C) chaetognath (*Sagitta elegans*) action potentials. The insect action potential is generated by an influx of calcium (*5*), whereas that of the frog is due to the entry of sodium (*3*). The shape and kinetics of the *Sagitta* action potential are similar to that of frog. (A) The falling phase of the action potential took approximately 500 msec to return to baseline. The dashed line represents a resting potential for this fiber of −50 mV. (B) The dashed line represents a resting potential for this fiber of −90 mV. (C) The dashed line represents a resting potential for this fiber of −72 mV.