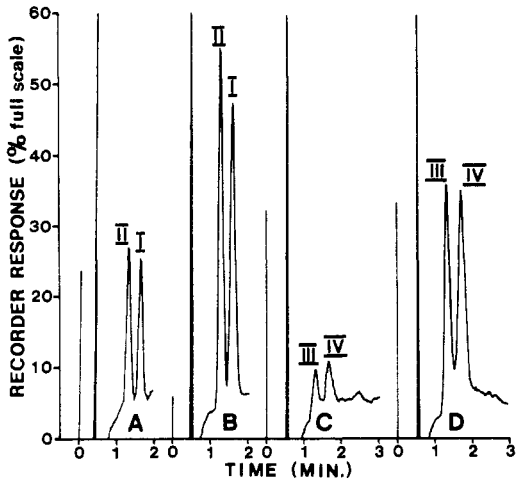

Figure 1. Gas chromatograms showing separation of: (A) 5 ng and (B) 50 ng of phorate (I) and POA (II) on OV-17; (C) 5 ng and (D) 20 ng of PSO (III) and PSO_2 (IV) on XE-60. Sensitivity: 3.2×10^{-8} AFS for (A), (C), and (D); 1.28×10^{-7} AFS for (B).

final choice was made on the basis of separation, sensitivity, and elution time (Figure 1). Conditioning of the XE-60 column at 10° above operating temperature for at least 72 hr was necessary for acceptable separation.

The use of an ice water bath during extraction is essential to prevent emulsion formation. A bath of flaked ice alone does not provide sufficient cooling.

The microcleanup column is used in the interest of speed and economy of materials. The silane-treated glass wool used in the column is necessary because untreated glass wool results in loss of significant amounts of phorate. The make of cellulose powder is also important because some products may reduce recovery of phorate.

All evaporations are done under nitrogen; exposure to an air stream, even in vacuum evaporation, results in large-scale oxidation of phorate, primarily to PSO.

Although these refinements have not yet been applied to other crops, they should provide a basis for analysis of phorate and its principal metabolites. With these few modifications, analyses were easily completed in 1 day.

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Terpenoid Ethers as Juvenile Hormone Analogs

Several simple terpenoid ethers, derived from various combinations of geraniol, nerol, γ,γ-dimethylallyl alcohol, 3-methyl-2-pentenol, and farnesol show juvenile hormone activity with *Oneopeltus fasciatus* and *Autographa californica*.

Numerous juvenile hormone (JH) analogs have appeared in the literature which simulate the effect of endogenous juvenile hormone when applied to various insect species (Slama, 1971). In order to utilize such compounds as insecticides it would seem desirable that such materials be both readily available and of minimum toxicity to higher animal species. Accordingly we have explored the use of simple terpenoid ethers, devoid of heteroatoms other than oxygen, in order to determine if additional useful compounds could be prepared. In addition, we have examined the general effect of replacing chemically reactive double bonds with less reactive ether oxygen.

Ethers, both terpenoid and nonterpenoid, have occasionally been reported as JH active, the simplest being farnesyl methyl ether (Bowers and Thompson, 1963; Schmialek, 1963) and dodecyl methyl ether (Schneiderman *et al.*, 1965). More complex ethers derived from sesamole or the methylenedioxyphenyl moiety have been reported as particularly effective, especially in the form of mixed ethers with terpenoid side chains (Bowers, 1969). Another series of ethers which has been reported is that derived from α-hydroxy acids (Slama *et al.*, 1973). Ethers derived from the addition of ethanol to terpenoid double bonds are also effective (Wakabayashi, 1969; Brieger, 1971).

The JH molecule itself can be considered as an isoprenoid chain, with functional groupings at each end. It appears that the epoxide group at one terminal is not essential, but certainly enhances the activity. An ester grouping or a related electronegative grouping such as an amide at the other terminus is important for activity (Slama, 1971).

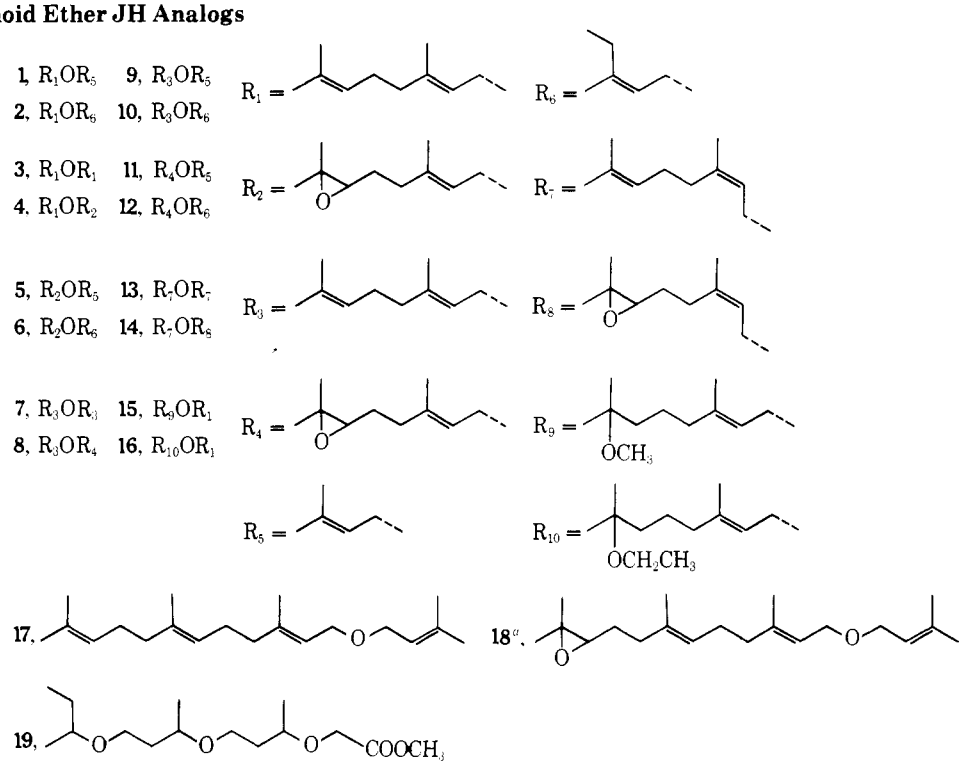
We wanted to determine whether functionality at the ends is absolutely necessary, or whether in fact a simple functional group could be located in other portions of the chain.

We chose to concentrate on ethers derived from the readily available geraniol and citronellol. As indicated in Chart I, various possible combinations of ethers which can be derived from geraniol, citronellol, γ,γ-dimethylallyl alcohol, and 3-methyl-2-pentenol were prepared. In all cases the terminal epoxides were also prepared.

MATERIALS AND METHODS

Infrared spectra were determined on a Beckman I.R-5 spectrophotometer. Nmr spectra were determined using a Varian T-60 spectrometer, using TMS as an internal standard. Gas–liquid chromatographic analyses were performed with a Varian 1200 flame ionization instrument. Generally, a 10-ft, 15% DEGS, Chromosorb W column was used. Elemental analyses (performed by Spang Microanalytical Laboratories, Ann Arbor, Mich.) and nmr spectral data used to characterize the compounds appear

Chart I. Terpenoid Ether JH Analogs



[a] Also contains some 6,7-epoxide.

in the microfilm edition of this journal (see paragraph at end of communication regarding supplementary material).

Synthesis of the reported compounds was carried out by a straightforward displacement reaction using the bromide of the smaller alcohol component (when applicable) and the sodium salt of the other alcohol in dimethylformamide (DMF).

A typical procedure is the following for geranyl-dimethyl allyl ether: 1.7 g of a 57% sodium hydride dispersion in mineral oil was added to 50 ml of dry dimethylformamide. To this slurry was added 4.6 g of geraniol, and the resulting mixture was heated at 45° for 0.5 hr. Dimethylallyl bromide (6.0 g) in 5.0 ml of dry DMF was then added dropwise over a period of 15 min. The mixture was permitted to stir at room temperature for 18 hr. Thereafter, 15 ml of water was added carefully, and the resulting mixture poured into excess water. The products were extracted with hexane washed with water, dried, and evaporated to yield 7.1 g of crude product. Glc analysis indicated a mixture of 85% ether and 10% unchanged geraniol. Purification proceeded by column chromatography on 60.0 g of silica gel, the desired material eluting with a mixture of ether–hexane (1:9). Short-path distillation (bp ~50–70°) gave 2.43 g (37%) of pure ether 1 as determined by glc, ir, and thin-layer chromatography.

Epoxidation was carried out under standard conditions (Bowers *et al.*, 1965) using *m*-chloroperbenzoic acid. A 50% yield of chromatographically pure epoxide 5 was obtained.

The ethoxy and methoxy derivatives were prepared by the oxymercuration reaction under standard conditions (Brown and Geoghegan, 1970; Brieger and Burrows, 1972).

All products were purified by column chromatography, and were analyzed for purity by gas–liquid chromatography and thin-layer chromatography. Nmr spectra (see microfilm edition) support the assigned configuration and the location of the epoxide group at the end of the chain, except for compound **18**, which is a 1:1 mixture of the 6,7- and 10,11-epoxides.

The yields for the other compounds, and boiling points when available, are recorded in Table I. The scoring system adopted for the biological evaluation of the analogs is as follows: for *Oncopeltus fasciatus*, with an application of 100 μg/1 μl of acetone, 0 = perfect adult; 1 = adult with nymphal coloration on abdomen or curled wings; 2 = adult-nymphal intermediate; 3 = perfect supernumerary nymph. The maximum score for ten insects would then be 30/test. The scale for *Autographa californica* is as follows: 0 = perfect adult; 1 = moth with curled or defective wings; 2 = moth failing to enclose completely; 3 = dead pupa. The compounds were applied topically in 1000 μg/1 μl of acetone doses to each larva. The maximum score would be 30/10 insects used for each test.

Table I

Compd	Yield, %	Bp, °C (*P*, mm)	Compd	Yield, %	Bp, °C (*P*, mm)
2	75	90–110 (0.15)	**11**	85	
3	72	125–127 (0.15)	12	85	
4	50		13	60	116–118 (0.13)
5	50		14	60	
6	70		15	40	
7	72	125–130 (0.2)	16	50	
8	60		17	57	
9	75		18	70	
10	80				

The last-instar milkweed bugs were less than 24 hr old when used. Last instar alfalfa looper larvae, aged 14–17 days, were used, the latter deriving from a starter colony kindly provided by Professor H. Shorey, Department of Entomology, University of California, Riverside (Brieger and Grau, 1970).

RESULTS AND DISCUSSION

Table II presents the results of the bioassays conducted. It shows that several simple ethers and their epoxides **1**, **2**, **10**, and **12** are quite active with the hemipteran *Oncop-*

Table II. Juvenile Hormone Activity[a]

Compd	*O. fasciatus*	*A. californica*	Compd	*O. fasciatus*	*A. californica*
1	12	4	10	16	10
2	30	0	11	21	3
3	0	0	12	24	11
4	15	1	13	3	3
5	9	8	14	0	6
6	17	4	15	3	3
7	0	3	16	1	8
8	0	0	17	1	11
9	5	11	18	21	15

[a] See Materials and Methods section for scoring system.

eltus fasciatus. Some activity is also shown with the alfalfa looper, *Autographa californica*. The differential activity can be quite large, such as for compounds **2**, **11**, and **17**, and cautions against extrapolation of general activity from a limited choice of test species. Chain lengths of 12–17 atoms are effective as seen with **1** and **18**. The general preference for trans geometry of double bonds (Slama, 1971) is confirmed with compounds **4** and **14**. It is of interest to note that, other than the ether linkage, no functional group is required at either terminus as in **2** and **10**. The carbalkoxy group found in many JH analogs, a possible target for deactivation by esterases (Slade and Wilkinson, 1973), is here circumvented.

The methoxy and ethoxy ethers **15** and **16** did not appear to be as effective as the corresponding epoxide **4**.

Compounds **10**, **11**, and **12** also showed some activity with *Musca domestica* in a standard assay (Henrick, 1973).

A question of considerable interest was whether all the multiple bonds in JH analogs could be replaced by ether oxygens. Accordingly, the polyether **19** was prepared (Brieger and Burrows, 1971). Surprisingly, it showed no activity at the levels tested.

In summary, it appears that functionality is not absolutely necessary at the termini of a JH-like chain in order to show activity. No effort has been made here to determine the minimum effective dosages as it seems of more interest to determine the critical structural parameters which determine JH activity.

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Supplementary Material Available. A listing of elemental analytical and nmr spectral data will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N. W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JAFC-75-335.

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Use of High-Pressure Liquid Chromatography for the Fractionation of Less Volatile Flavor Compounds

Gas chromatography is not a complete solution to the fractionation of flavor compounds isolated from foods. Some of the flavor compounds may be liable to heat and others may be too polar to be eluted. An attempt was therefore made to fractionate the less volatile flavor compounds by high-pressure liquid chromatography. Both the sensitivity of a continuous uv detector and the capacity of a liquid chromatographic column were found to be satisfactory for the fractionation of flavor compounds. A scheme of repeated chromatography with a combination of various absorbants, stationary phases, and eluents was successfully applied to separate a mixture of eight known compounds selected from a list of flavor components of boiled beef. However, the procedure is long and tedious. It is questionable whether the scheme could be adopted for the fractionation of complex mixtures of less volatile flavor compounds isolated from foods.

The identification of volatile compounds which are responsible for the flavor of foods is a subject of great interest to many investigators. The commonly used method to accomplish this is to isolate the volatile flavor compounds from the foods, fractionate the isolated volatiles by gas–liquid chromatography, and identify the gas chromatographic fractions by a combination of infrared and mass spectrometry. However, gas chromatography is not the