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

complete solution for the fractionation of volatile flavor compounds, particularly those with high polarity or high boiling points. Polak (1968) pointed out that certain stationary phases may hold back some volatile components. For example, phenylacetaldehyde, phenyl alcohol, and benzyl alcohol would not elute from a Ucon-13 coated capillary column. Therefore, there is an obvious need for other fractionation instruments which do not require the high temperature of gas chromatography.

The present investigation is an attempt to ascertain whether high-pressure liquid chromatography can be used to fractionate less volatile and perhaps highly polar compounds which cannot be successfully eluted from gas chromatographic columns. Its intention is not to use high-pressure liquid chromatography to replace gas chromatography for the fractionation of volatile flavor compounds. The flavor compounds isolated from a food should be separated into more volatile and less volatile fractions. The more volatile fractions evidently should be fractionated by gas chromatography. Only the less volatile fractions which cannot be handled by gas chromatography should be studied by high-pressure liquid chromatography.

EXPERIMENTAL SECTION

Material Used. The eight known compounds used in this investigation were purified with a Varian 202 gas chromatograph (Varian Instruments, Inc., Walnut Creek, Calif.) equipped with a thermal conductivity detector and a linear temperature programmer. The eight known compounds were selected from the list of compounds identified by Hirai *et al.* in boiled beef (1973). Columns used were stainless steel, ⅛ in. i.d., 20 ft in length, packed with 60–70 mesh Anakrom ABS (Analabs, Inc., Hamden, Conn.). One column had 10% Carbowax 20M as the stationary phase and the other 10% methyl silicone, SE-30.

High-Pressure Liquid Chromatography. The instrument was assembled from components made by different companies. The micro bore glass columns of inner diameters 1, 2, and 3 mm were made by Chromatronix, Inc. (Berkeley, Calif.). Connections and injectors were made of Teflon. A Chromatronix Model 183A on column septum injector was used. The solvent was delivered into the column by using a Model CMP-2 pulseless pump from Chromatronix, Inc. The effluent from the column was constantly passed through a Model 200 uv detector, manufactured by Chromatronix, Inc. This detector had a cell capacity of only 8 μl. The absorption at 254 mμ was continuously recorded by a Beckman linear 10-in. recorder of 10 mV. The effluent from the detector was then collected by a Buchler automatic fractional collector (Buchler Instruments, Fort Lee, N.J.).

Detector Response. A 1% solution of each of the known compounds in ethyl ether was prepared. Each solution, 5 μl, was then injected into a 1 mm × 1000 mm glass column containing SIL-X (Nester/Faust Mfg. Corp., Newark, Del.) as the adsorbent. The compound was then eluted with methanol at a flow rate of 24 ml/hr. The amount of the compound required to give a 25% recorder response at the peak where it was eluted was noted.

Solvent Gradient Elution. A solvent gradient elution, changing from *n*-hexane to chloroform, was accomplished by using a simple laboratory-made device. A 125-ml erlenmeyer flask, containing 24 ml of *n*-hexane, was used as the solvent reservoir. Chloroform was added into the flask by gravity through a needle valve stopcock at a rate of 48 ml/hr. The solvent in the flask was mixed with a magnetic stirrer. The mixture was then delivered into the column by the pump at a rate of 24 ml/hr.

Infrared Spectroscopy. The aqueous solution of a fraction eluted by reverse phase liquid chromatography was first saturated with reagent grade sodium chloride and then extracted four times with an equivolume of redistilled spectrograde *n*-pentane. The *n*-pentane extracts were concentrated to a volume of approximately 1 ml. It was then evaporated to dryness under nitrogen. The solvent-free samples were then dissolved in 5 μl of CCl_4 and the infrared spectra were obtained with a Beckman IR-8 spectrophotometer equipped with a beam condenser.

Table I. Responses of Chromatronix Model 200 Uv Photometer

Compound	Amt required for 25% recorder response, μl
Undecane	0.02
Butyl acetate	0.015
Nonyl alcohol	0.02
3-Heptanone	0.006
3-Decanone	0.007
Butylbenzene	0.0004
γ-Valerolactone	0.01
Phenyl ether	0.0005

Table II. Amount of Samples Appliable to a SIL-X Column (1 mm i.d. × 1 m and 3 in. Length)

Compound	μl	Compound	μl
Undecane	0.48	3-Decanone	0.36
Butyl acetate	0.30	Butylbenzene	0.08
Nonyl alcohol	0.36	γ-Valerolactone	0.30
3-Heptanone	0.36	Phenyl ether	0.04

RESULTS AND DISCUSSION

Instrumentation. The use of an apparatus assembled from components made by different companies has three advantages. First, it can utilize the unique, desirable designs of several companies. Second, it is easier to modify and repair, and third, it is more economical. Glass columns and a Teflon injector were used instead of metal parts to prevent possible adsorptions and side effects.

The amount of each of the eight known compounds required to give a 25% recorder response at the peak where it was eluted from the column is shown in Table I. Theoretically, undecane should not show an absorption at 254 mμ. The response might be due to the change of the solvent front in the detector. In general, the results appeared to be satisfactory, as the ones with even the lower sensitivities corresponded to the sensitivity of a Varian 202 gas chromatograph set at an attenuation of 2. This kind of sensitivity should be sufficient for the fractionation of most of the flavor compounds isolated from foods.

The maximum amount of samples appliable to a SIL-X column, 1 mm i.d. and 1 m in length, is shown in Table II. This is the amount which can be injected into the column without any significant loss in resolution. These results also appeared satisfactory because the amount the 1 mm i.d. column can handle is significantly higher than the minimum sensitivity of the detector. Furthermore, this amount is also more than enough for the determination of the infrared and mass spectra of these compounds.

Application of the Separation of Eight Known Flavor Compounds. When a SIL-X column was applied to separate a mixture of the eight known compounds, only five distinct peaks were observed in the chromatogram. It was therefore evident that the separation could not be accomplished by just using one column. A scheme, using repeated chromatography by the combination of different columns to separate these eight known compounds, was therefore designed and carried out, as shown in Figure 1.

The mixture of the eight known compounds was first

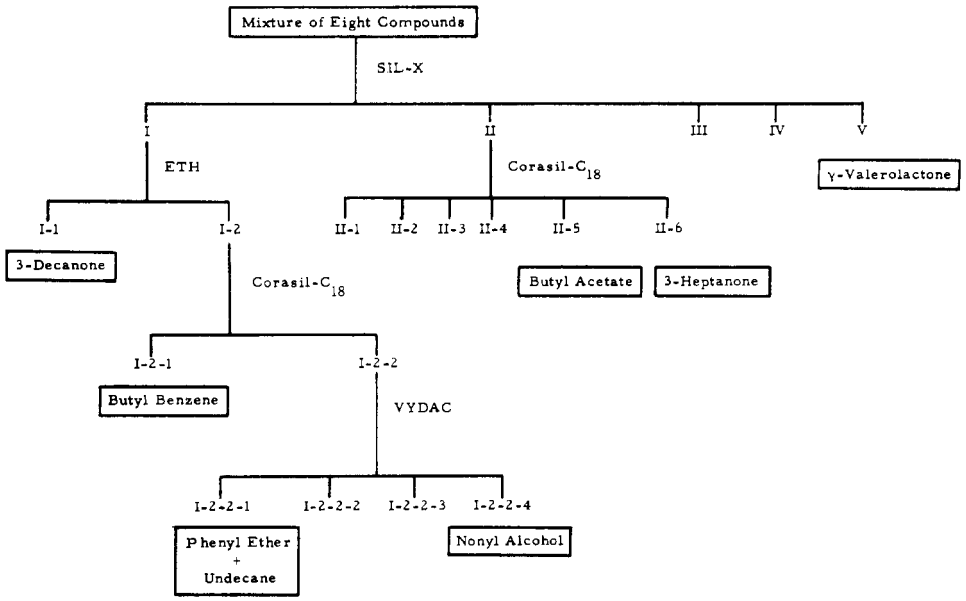

Figure 1. Separation of a mixture of eight known flavor compounds by repeated high-pressure liquid chromatography, using different columns.

separated by SIL-X adsorption chromatography, using gradient elution with hexane to chloroform as eluents. Among the five peaks obtained, only peak 5 was a pure compound, γ-valerolactone. Fraction I, obtained from the SIL-X column, was separated into two fractions by reverse phase partition chromatography, using ETH (a silicone base stationary phase, permanently bound on porous glass beads, manufactured by DuPont, Inc., Wilmington, Del.), and 49% ethanol in water as the eluent. The first fraction was identified as 3-decanone. The second fraction was further separated by reverse phase partition chromatography, using Corasil-C_{18} (an octadecyltrichlorosilane, permanently bound on porous glass beads produced by Water Associates, Inc., Framingham, Mass.), and 48% methanol in water as the eluent. Two fractions were obtained. One was butylbenzene. The other fraction was finally separated by adsorption chromatography, using Vydac (specially activated silica coated on inert support, produced by the Separation Group, Berkeley, Calif.) as the adsorbent and hexane as the eluent. Among the four fractions obtained, one was a mixture of phenyl ether and undecane, and another was nonyl alcohol.

The second fraction obtained from the SIL-X column was further fractionated by Corasil-C_{18} reverse phase partition chromatography, using 3% methanol in water as the eluent. One fraction thus obtained was pure butyl acetate and the other was 3-heptanone.

It therefore appeared that the mixture of eight known compounds which were previously identified in boiled meat could be successfully fractionated by this scheme of repeated high-pressure liquid chromatography (Figure 1). However, the procedure is long and tedious. Whether it can be successfully adopted to fractionate complex mixtures of flavor compounds isolated from foods is quite questionable.

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