

board-like notes include 2-(*n*-pentyl)furan, 2-octenal, 3-octen-2-one, 3,5-octadien-2-one, and 3,5-undecadien-2-one. These compounds contribute to the flavor of PBPM and pet foods containing this ingredient.

Lipids appear to be the primary source of flavor in PBPM. This is supported by the fact that the majority of the compounds isolated in this study have been identified in literature reports to be products of lipid (specific fatty acid) oxidation reactions. This is further supported by the fact that PBPM has a high fat content (usually greater than 14%) which when rendered can accelerate autoxidation. A literature report has also demonstrated storage instability of fatty acids such as linoleic acid in PBPM.

This study identified 41 volatile compounds in PBPM. Future studies will center on identifying more of the volatiles, many of which need further concentration before assigning structures and organoleptic importance to them.

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## Methyl 3,4-Dimethyl-5,6-dihydro- $\alpha$ -pyran-6-carboxylate in Roast Beef Volatiles: Identification and Synthesis

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Methyl 3,4-dimethyl-5,6-dihydro- $\alpha$ -pyran-6-carboxylate was identified as a compound in the volatiles isolated from roast beef. This compound was synthesized by Diels-Alder reaction between methyl glyoxylate and 2,3-dimethyl-1,3-butadiene. The structure of the synthesized compound was established by infrared, nuclear magnetic resonance, and mass spectroscopy. The identification of this compound in the volatiles of roast beef was confirmed by comparing the mass spectrum with that of the authentic sample.

The volatile flavor components of meats have been reviewed by Herz and Chang (1970) and by Dwivedi (1975). Up to now ~600 components are mentioned in the literature or have been identified in meat flavor (Flament et al., 1978).

The present paper reports the isolation, identification, and synthesis of a new heterocyclic compound, methyl 3,4-dimethyl-5,6-dihydro- $\alpha$ -pyran-6-carboxylate (MDDPC) in the volatiles of roast beef.

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#### EXPERIMENTAL SECTION

##### Isolation of Volatile Flavor Compounds (VFC).

Good quality, "medium rare" roast beef of top round was purchased from a local delicatessen. The meat was trimmed of excess fat and cut into 1/2-in. chunks. The VFC were isolated by using a specially designed apparatus employing headspace gas flushing with an inert carrier gas (Chang et al., 1977). This apparatus could be used to isolate VFC from 22 lb of roast beef in each batch. The roast beef was maintained at 86 °C by circulating water in the jacket of the cylinder which held the roast beef on a perforated bottom. The VFC were condensed in a series of cold traps cooled with a dry ice-acetone slurry. The VFC from 160 lb of roast beef were isolated.

The flavor isolated was extracted with ethyl ether and the extract dried over anhydrous sodium sulfate. Approximately 4000 mL of ether solution was obtained. The roast beef flavor solution was then preconcentrated to 150 mL by using a 30-plate Oldershaw distillation column and

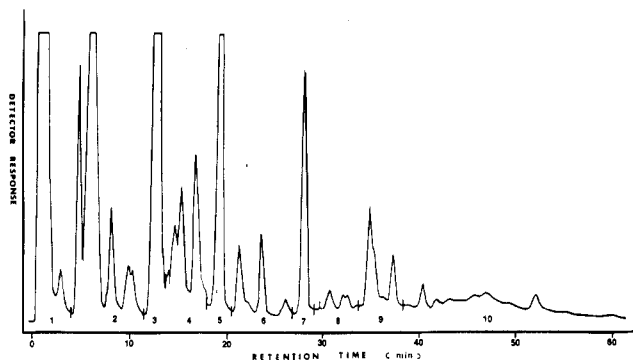


Figure 1. Broad gas chromatographic fractions of roasted beef flavor.

then further concentrated to 3 mL with a spinning band distillation apparatus.

**Fractionation and Identification of the Flavor Isolate.** The concentrated VFC were fractionated by using a gas chromatograph (Hewlett-Packard 5840 A) with a 12 ft by  $\frac{1}{8}$  in. stainless steel column packed with 10% SP-1000 on 80–100-mesh Chromosorb W. The chromatogram (Figure 1) was divided into 10 broad fractions, and each fraction was accumulatively collected according to the method of Thompson et al. (1978). Each fraction was sensory evaluated by two experienced flavorists. Three fractions (5, 7, and 9) which were judged to contain the most interesting flavors were chromatographed for a second time by the use of a 12 ft  $\times$   $\frac{1}{8}$  in. stainless steel column packed with 10% OV-17 on 80–100-mesh Chromosorb W. Each of the subfractions was collected and identified with the use of a Du Pont Model 21-490 mass spectrometer interfaced with a Varian Moduline 2700 gas chromatograph.

**Synthesis of Methyl 3,4-Dimethyl-5,6-dihydro- $\alpha$ -pyran-6-carboxylate (MDDPC).** A mixture of 8 g (0.10 mol) of methyl glyoxylate [prepared according to the method described by Kelly et al. (1972)], 7 g (0.09 mol) of 2,3-dimethyl-1,3-butadiene, 15 mL of acetonitrile, and a trace of hydroquinone was heated in a glass pressure reaction vessel at 140 °C for 10 h. The reaction mixture was fractionated on a Beckman GC-5 gas chromatograph using a 12 ft  $\times$   $\frac{1}{8}$  in. stainless steel column packed with 10% OV-17 on 70–80-mesh Anakrom ABS. The major product was MDDPC.

**Identification of MDDPC.** The mass spectrum was obtained on a Du Pont 21-490 mass spectrometer. The nuclear magnetic resonance spectrum was obtained on a Varian Associates Model T-60 NMR spectrometer. The infrared spectrum was obtained on a Beckman Acculab 4 IR spectrometer using ultramicro sodium chloride cells of 0.1-mm light path.

## RESULTS AND DISCUSSION

**Isolation and Fractionation of VFC from Roast Beef.** Roast beef flavor is a very complex mixture of compounds and some of the key compounds are present in extremely small amounts. In order to identify such compounds, we started with a relatively large amount (160 lb of roast beef) of isolated volatiles with a genuine flavor of the original roast beef.

A preliminary gas chromatogram of the VFC (on an SP-1000 column) is shown in Figure 1. The chromatogram was divided into 10 fractions. Each fraction was sensory evaluated by two expert flavorists. Three of the fractions were determined as being the key flavor of roast beef. Fraction 5 had a fatty meat aroma, fraction 7 had a savory, beeflike aroma with rich, very pleasant, tallowy overtones, and fraction 9 had a red meat aroma reminiscent of the

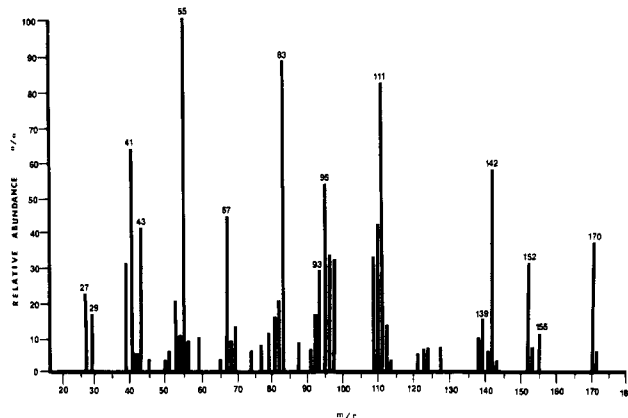


Figure 2. Mass spectrum of methyl 3,4-dimethyl-5,6-dihydro- $\alpha$ -pyran-6-carboxylate.

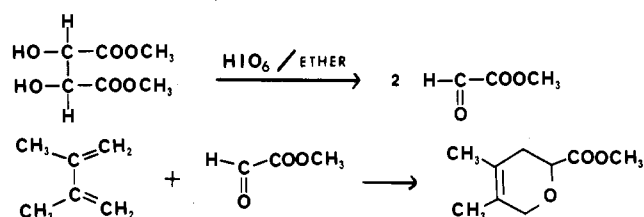


Figure 3. Synthetic scheme of methyl 3,4-dimethyl-5,6-dihydro- $\alpha$ -pyran-6-carboxylate.

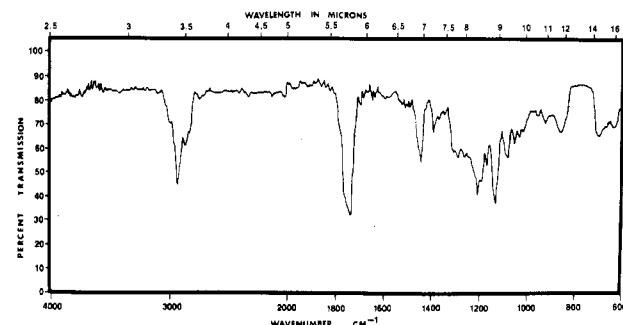


Figure 4. Infrared absorption spectrum of methyl 3,4-dimethyl-5,6-dihydro- $\alpha$ -pyran-6-carboxylate.

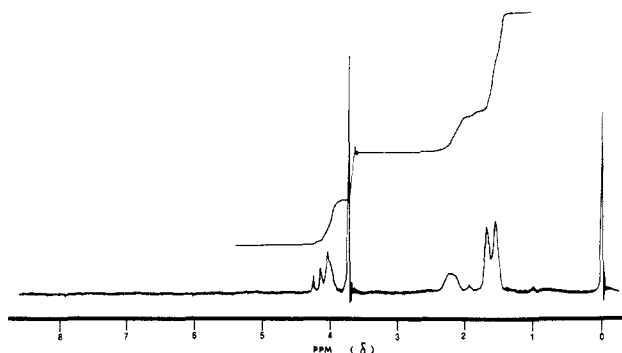
center of a cooked roast beef. Only these three fractions were subjected to further fractionation.

**Identification of MDDPC in Fraction 7.** Fraction 7 was further gas chromatographed on an OV-17 column. The mass spectra were obtained for each subfraction. The mass spectrum of fraction 7-5 (Figure 2) was interpreted as MDDPC. For confirmation of the structure, the authentic compound was synthesized.

**Synthesis of MDDPC.** The MDDPC was synthesized in two steps as shown in Figure 3. Methyl glyoxylate was prepared by the cleavage of dimethyl tartrate by ethereal periodic acid according to the method described by Kelly et al. (1972). The MDDPC was synthesized by Diels-Alder reaction. Warming an acetonitrile solution of 2,3-dimethyl-1,3-butadiene and methyl glyoxylate with a trace of hydroquinone to 140 °C for 10 h afforded the adduct a good yield. The 1,4 cycloaddition reactions between dienes and carbonyls have been described previously (Ruden and Bonjouklian, 1975; Hamer and Turner, 1967).

**Confirmation of the Structure by Mass, IR, and NMR Spectra.** The structure of MDDPC was confirmed by mass, IR, and NMR spectrometric analysis.

The mass spectrum of MDDPC was consistent with the mass spectrum of fraction 7-5 (Figure 2). The molecular ion peak at  $m/e$  170 confirmed the molecular formula,  $C_9H_{14}O_3$ . The peaks at  $m/e$  139 (loss of  $-OCH_3$ ) and  $m/e$



**Figure 5.** Nuclear magnetic resonance spectrum of methyl 3,4-dimethyl-5,6-dihydro- $\alpha$ -pyran-6-carboxylate.

111 (loss of  $-\text{COOCH}_3$ ) are characteristic peaks of the  $-\text{COOCH}_3$  functional group.

The infrared spectrum of MDDPC is shown in Figure 4. The absorption band at 1740 and 1130  $\text{cm}^{-1}$  clearly indicates the presence of an ester group.

In the NMR spectrum of MDDPC (Figure 5), the two singlets at  $\delta$  1.56 and 1.67 are due to methyl groups at C-3 and C-4, respectively. The broad singlet at  $\delta$  2.23 is due to two protons at C-2. The sharp singlet at  $\delta$  3.74 is the absorption of the methyl group in  $-\text{COOCH}_3$ . The peak between  $\delta$  3.85 and  $\delta$  4.28 is due to protons at C<sub>5</sub> and C<sub>6</sub>.

**Sensory Characteristics of MDDPC.** Unfortunately, the synthesized MDDPC did not possess an odor which

could be described as beeflike. Instead, it was described as an interesting green note. Nevertheless, MDDPC represents a new class of heterocyclic compounds in food aroma and may have an application in food flavorings.

The identification of other compounds in these three fractions of roast beef flavor will be reported in the future.

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## Concentration and Fractionation of Aromas on Reverse-Phase Adsorbents

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A new procedure for concentration and fractionation of volatile aroma chemicals has been devised. The dilute aroma-bearing aqueous solution is passed over a reverse-phase packing and concentration of the volatiles is achieved. A solvent gradient is generated and the aroma chemicals are desorbed. Retention of the aromatics is proportional to the number and type of hydrocarbon groups present; thus fractionation occurs. Fractions are obtained which may be subjected to organoleptic analysis, GC, HPLC, or GC-MS. Recoveries are generally over 80%. Experimental details are discussed, and the technique is applied to a homologous series of methyl esters, a mixture of flavor chemicals, a peppermint oil, and a carbonated beverage.

The volatile chemicals in a food are important since they are responsible for the aroma of the food. These aromatic chemicals are normally present at exceedingly low levels and are frequently present as complex mixtures. Numerous works have been published on techniques for isolating and concentrating these volatile chemicals prior to gas chromatographic analysis (Bemelmans, 1979; Weurman, 1969). The isolation procedures generally fall into one of two classes. In one case, the aromatic chemicals are trapped on a porous polymer (e.g., Tenax or Porapak) and subsequently desorbed by heat into a gas chromatograph. This procedure was the subject of a recent ACS symposium (Charalambous, 1978). The other common technique involves distillation at atmospheric or reduced pressure to produce an aqueous distillate free of nonvolatile materials. The organics are then concentrated by some form of solvent extraction, often by continuous liquid-liquid ex-

traction. Frequently, the Likens-Nickerson apparatus (Likens and Nickerson, 1966) or some more efficient modification (Schultz et al., 1977) is used to concurrently distill and concentrate the aromatics. The essence may then be analyzed by gas chromatography.

Often, such concentrates are still too complex for gas chromatographic separation and further fractionation is required. Preparative scale gas chromatography has been used for this purpose (Merritt, 1971). Column chromatography on silica or alumina can be used to produce fractions based on polarity; vacuum distillation can be used to yield fractions based on boiling points.

The purpose of this paper is to describe a new technique which permits both the concentration and subsequent fractionation of volatile aromatic chemicals from dilute aqueous solutions. The concentration and fractionation are both accomplished using reverse-phase packing materials.

Trace enrichment of the organics from dilute aqueous solution on reverse-phase adsorbents was first proposed by Kirkland (1974) and has since been used in analysis of

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