

# Preparation and Properties of 1, 4, 5, 6-Tetrahydro-2-Acetopyridine, a Cracker-Odor Constituent of Bread Aroma<sup>1</sup>

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## ABSTRACT

A pentane extract of a reaction mixture of proline, dihydroxyacetone, and sodium bisulfite has a strong odor reminiscent of freshly baked soda crackers. The compound responsible has been found to be 1, 4, 5, 6-tetrahydro-2-acetopyridine, which was isolated by vacuum distillation. Structure of the compound was determined by means of functional group reagents and by infrared, Raman, nuclear magnetic resonance, and mass spectra. Gas-chromatographic analysis of the fresh liquid showed it to be a single substance that coincided with the aroma peak prepared from an extract of freshly baked bread.

The compound is an unstable, colorless liquid that undergoes both physical and chemical changes on standing in air. It is stable as the bisulfite complex, or when hermetically sealed under nitrogen. A pentane solution remains stable for several weeks. It has a distinct odor of crackers. As little as one part in 100,000 in an extract of fresh bread is easily detected and imparts a pleasant crusty overtone to the extract. Spraying week-old bread with an aqueous solution containing 6 p.p.m. of the sodium bisulfite complex of the tetrahydropyridine compound returned a desirable fresh-bread odor to the crust after it had dried.

One may assume that all compounds present in the aroma of freshly baked bread are probably responsible, in varying degrees, for its universal appeal. The inference is, therefore, that these components must all be separated and identified in order to gain a true insight into the problems of improvement and stabilization of bread flavor and aroma. However, successful progress on a basic bread-flavor problem involves isolation, not of all components—a most formidable task—but only of those compounds clearly recognizable as closely allied to bread flavor and aroma. The discovery of two such dominant components in a freshly baked bread extract initiated the present investigation. They were found to be crackerlike or biscuitlike in nature, depending on dilution.

Crackerlike aromas have been previously prepared, synthetically, by several investigators. Wiseblatt and Zoumut (1) reacted proline and dihydroxyacetone (DHA) to yield a cracker aroma. They did not report separation of the components responsible for the odor. Kobayasi and Fujimaki (2) roasted proline and glucose and also mixed pyrrolidine and pyruvaldehyde to obtain a bread or cracker aroma. They identified the compound responsible for the odor as N-acetyl pyrrole. Hunter *et al.* (3) reacted proline and glycerol and prepared aroma concentrates from the reaction mixture. Separation by gas chromatography gave rise to three main peaks having a crackerlike or breadlike aroma. Two of these peaks, those least volatile, were assigned tentative structures based on information available at the time.

The present paper describes the preparation, isolation, characterization, and properties of a synthetic cracker-aroma compound, prepared from a modified Wiseblatt reaction, whose structure we propose to be 1, 4, 5, 6-tetrahydro-2-acetopyridine.

<sup>1</sup>Contribution from the Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, Calif. 94710. Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

## MATERIALS AND METHODS

### Reagents

The chemicals in this study were reagent grade or better and were used as purchased without further purification. Pentane and dichloromethane were of the chromatography grade of Matheson, Coleman, and Bell. Dihydroxyacetone and pyrrolidine (research grade) were purchased from Aldrich Chemical Co. Proline (NRC grade), free of hydroxy proline, was obtained from General Biochemicals. Sodium bisulfite was from Mallinkrodt, analytical grade.

### Preparation of a Bread-Aroma Concentrate

The preparation of a bread-aroma concentrate was carried out essentially as described by Hunter *et al.* (3) except for the following modifications. A dichloromethane extract of freshly baked bread was gently shaken with 100 ml. of 5% sodium bisulfite solution. On standing, the mixture separated into two phases and the lower solvent phase was withdrawn. The upper aqueous layer was shaken with 1 g. of decolorizing charcoal and several of Celite. The mixture was then filtered, and the resulting clear filtrate evaporated to dryness at 30°C. under vacuum. The residue was pumped overnight with a mechanical vacuum pump to remove any adhering odors. The material left in the flask was powdered and extracted with several aliquots of pentane. The pentane extracts were discarded. The bisulfite salts were dissolved in twice their volume of water and cooled in an ice-bath. An equal volume of 4*N* sodium hydroxide was next added slowly. The mixture was then extracted with three aliquots of pentane, each equal in volume to the volume of water originally used to dissolve the bisulfite. The extracts were combined and gently evaporated under vacuum to a volume of approximately 100  $\mu$ liters. This concentrate was used for subsequent gas-chromatographic studies.

### Preparation of a Synthetic Cracker-Aroma Compound

Sixty grams of sodium bisulfite, 40 g. of proline, and 20 g. of dihydroxyacetone were ground intimately in a mortar. The resultant powder was transferred to a Teflon-lined 1-lb. capacity baking pan and heated in an oven at 92°C. for 30 min. During the baking period the mixture fused, gradually rose to fill the container, and dried to a porous cake. On cooling, the resulting cake was a yellow, friable sinter that had an odor somewhat reminiscent of baked bread or crackers. The sinter, after powdering, could be stored indefinitely in a dry closed container without deterioration. It was used as a convenient starting material for subsequent separation of the aroma constituent.

Fifty grams of the sinter was dissolved in 100 ml. of water and then cooled in an ice-bath, and 100 ml. of 4*N* NaOH was then added slowly, with stirring. The mixture was extracted with three 100-ml. portions of pentane. The extracts were combined, boiled for several min. with 1 g. of decolorizing charcoal, and filtered through Celite on a Büchner funnel. The resulting sparkling-clear, faintly yellow solution was distilled on a water bath to remove excess solvent. An aliquot of the light-amber residue that remained was transferred to a short-path microdistillation apparatus (Kontes Catalog No. K-28450). The sample was distilled under vacuum while being heated to 85° - 90°C. The material distilled over at 65° - 67°C. at 3 mm. pressure and was collected in a miniature receiver immersed in an ice-water bath. The distillate was rapidly transferred to small capillary glass tubes, flushed well with nitrogen, and sealed under vacuum. A sample was taken for analysis.

Results were as follows: Anal. Found: C, 67.3; H, 8.71. Calcd. for  $C_7H_{11}NO$ : C, 67.2; H, 8.8.

#### Gas-Chromatographic Separation of Aroma Concentrates

The gas-chromatographic equipment, techniques, and column used in this study were essentially the same as those described by Hunter *et al.* (3), except for the following modifications. Flow rate of nitrogen passing through the column was 60 ml./min. at 10 p.s.i.g. Chart speed was 7.5 in./hr. Heating of the column was programmed during the separation (see Fig. 1 for program details). All separations were preceded by a conditioning injection of 3  $\mu$ l. of a 10% solution of pyrrolidine in pentane. The pyrrolidine treatment covered acidic sites present in the separating column so as to allow the aroma compounds to pass through the column and be detected by smell, as well as by the recording potentiometer.

Aliquots of the bread-aroma concentrate and of the synthetic cracker odor were analyzed. Figure 1 is a typical chromatogram obtained under the conditions described.

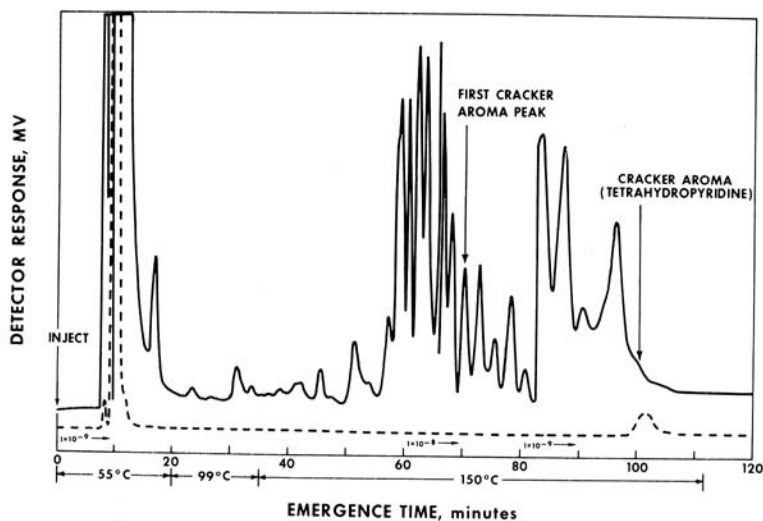


Fig. 1. Gas chromatogram of a pentane extract concentrate from a single 1-lb. loaf of freshly baked bread, and a pentane extract of the synthetic tetrahydropyridine (dotted line). Injection sample size 50  $\mu$ l. Recorder range 5 mv, chart speed 7½ in./hr.

#### Physical-Measurement Instrumentation

The following instruments were used in making the physical measurements described in the text. An infrared spectrometer (Cary Model 90); a Raman spectrometer (Perkin Elmer LR-1) with 8 mW 6328 Å He-Ne laser; a mass spectrometer (Consolidated Electrodynamics Model 21-110); and a high-resolution NMR spectrometer (Varian Associates Model HR-100) with internal field-frequency lock.

## RESULTS AND DISCUSSION

#### Modification of the Wisblatt Reaction

The proline:DHA reaction, though it yielded relatively large quantities of crack-

er aroma, also produced side-products that were colored and had undesirable odors. These latter interfering constituents were apparently formed from reactions occurring between proline and compounds arising from DHA in the following manner. DHA crystallizes as a dimer, m.p.  $80^{\circ}\text{C}$ ., and the solid is quite stable. In solution, however, it becomes monomeric and is easily converted by means of an internal oxidation-reduction mechanism to two extremely reactive compounds, glyceraldehyde and pyruvaldehyde (4). Neither of these two aldehydes when reacted with proline would yield a cracker aroma, but both did give rise to colored compounds and off-odors. To suppress the action of these interfering aldehydes, the proline:DHA reaction was carried out in the presence of excess sodium bisulfite. The resulting crude product was lighter-colored and had fewer off-odors than the crude product obtained by reacting proline and DHA alone.

Gas-chromatographic analysis of the purified product from the above reaction indicated that it was a single substance. It had a penetrating odor recognizable as crackerlike or popcornlike when sufficiently diluted in air. On standing exposed to air, the substance underwent a color change, turning dark amber after several hours. Concurrently, aroma and solubility characteristics of the material changed. Whereas the original material was completely soluble in pentane, the polymerized dark product that formed on standing was insoluble. Although no recognizable change in aroma could be detected by smelling—submicrogram quantities had a strong cracker aroma—the gas-chromatographic peak produced by the substance was observed to diminish with time.

The above-mentioned degradative changes could be eliminated or minimized by the following techniques: 1) Hermetically sealing the aroma substance under nitrogen extended its life indefinitely. 2) Preparation of a derivative, the bisulfite com-

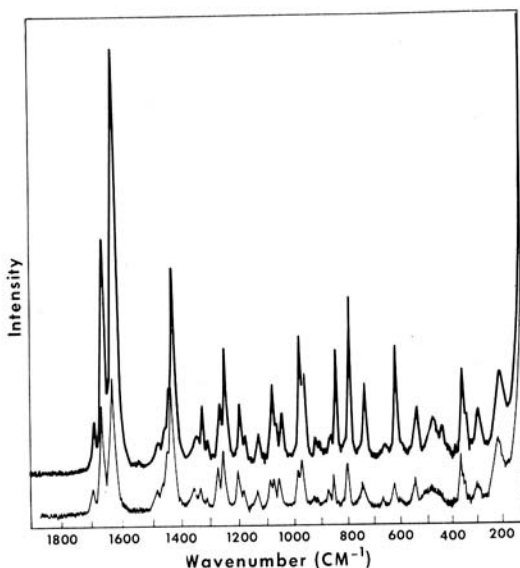


Fig. 2. Raman spectrum of approximately  $0.3 \mu\text{l}$ . of liquid sample. Slit width at exciting time  $\sim 5 \text{ cm}^{-1}$ . Scan rate  $\sim 0.96 \text{ cm}^{-1}/\text{sec}$ . Time constant, 0.8 sec. Laser power  $\sim 80 \text{ mW}$ . Upper trace: laser polarization  $\perp$ ; lower trace; laser polarization  $\parallel$ .

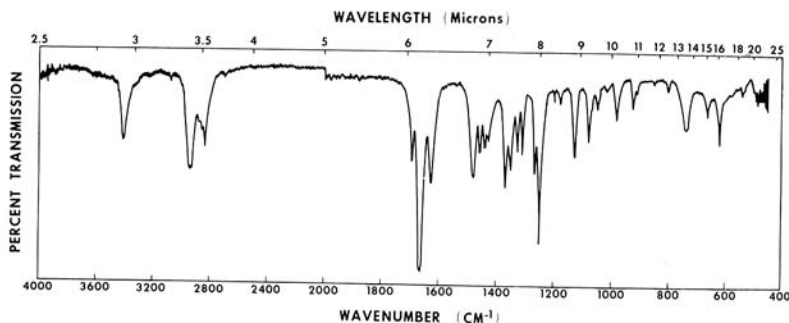


Fig. 3. Infrared. Capillary film between KBr plates.  $2 \text{ cm.}^{-1}$  constant band width. Scan rate  $1 \text{ cm.}^{-1}/\text{sec}$ . Period 3 sec.

plex, gave a stable product that could be treated to regenerate the aroma. 3) Solutions of the aroma material in pentane were stable, even after several months' storage at room temperature.

Although the cracker-odor isolate was unstable, the rate of decomposition was slow enough so that the impurities formed did not seriously interfere with the physical and chemical analyses necessary for structural identification.

#### Determination of Structure

Carbon and hydrogen determinations, as well as mass spectroscopy, established the unknown as having the empirical formula  $\text{C}_7\text{H}_{11}\text{NO}$  and molecular weight 125.

Preliminary chemical tests, such as formation of a hydrochloride salt, indicated an amine-type compound. Reduction of the compound with sodium borohydride, with an attendant loss of odor, indicated the presence of a carbonyl grouping. Reaction with sodium bisulfite indicated that the carbonyl was probably an aldehyde, methyl ketone, or cyclic ketone. The compound decolorized an alkaline potassium permanganate solution and discharged the color of bromine in carbon tetrachloride solution to indicate the presence of unsaturation.

Infrared and nuclear magnetic resonance (NMR) spectra helped clarify ambiguities that arose from a study of the chemical tests and permitted the assignment of a molecular structure.

#### Interpretation of Infrared Spectra

Infrared and Raman spectra (see illustration Figs. 2, 3) gave the following information. The compound exhibited a strong absorption band at  $3,410 \text{ cm.}^{-1}$ . Spectra that were run in  $\text{CCl}_4$  solution at concentrations of 100 mg./ml. showed no shift (within  $4 \text{ cm.}^{-1}$ ) of this band in the pure liquid. The band was assigned to the NH stretch of an aryl-alkyl amine and was confirmed by spectra of the hydrochloride salt. The material also showed a band at  $1,480 \text{ cm.}^{-1}$  which is attributed to the  $-\text{CH}_2-$  scissors mode of the  $-\text{CH}_2-\text{N}$  group. This was confirmed by a shift of this band to  $1,420 \text{ cm.}^{-1}$  in the hydrochloride. Methyl, methylene, and ethylenic hydrogens were detected by the presence of absorption bands at 2,950 and 2,870, 2,925 and 2,840, and 3,070  $\text{cm.}^{-1}$  respectively. The absorption band at 1,352

$\text{cm.}^{-1}$  was assigned the methyl umbrella mode next to a carbonyl group,  $\text{CH}_3-\overset{\text{O}}{\parallel}{\text{C}}-$ . The infrared band at  $1,665 \text{ cm.}^{-1}$  was assigned to a C=O stretch conjugated with a

C=C group. Raman spectra of the material showed a very strong  $1,627\text{ cm}^{-1}$  line and a weaker  $1,665\text{ cm}^{-1}$  line, confirming the presence of a C=C-C=O group. Observation of the strong Raman line at this high frequency ruled out a five-membered cyclene. Consistent with a band at  $1,250\text{ cm}^{-1}$  was the presence of an alpha, beta unsaturated carbonyl. Infrared spectra of different samples showed various amounts of an unidentified impurity which had a strong carbonyl absorption at  $1,690\text{ cm}^{-1}$ . The impurity was presumed to be due to sample decomposition after collection by GLC. Efforts made to obtain spectra free of the impurity were not successful.

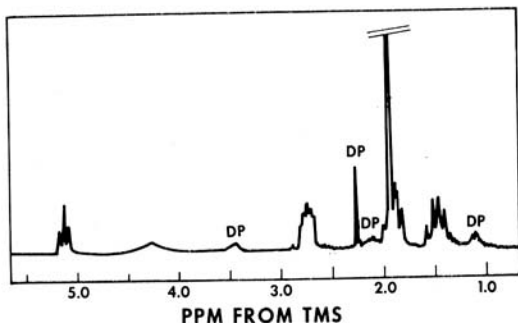
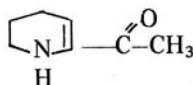


Fig. 4 100  $\mu$ Hz PMR spectrum of the synthetic aroma compound in  $d_6$ -benzene.

#### Interpretation of NMR Spectra

Figure 4 shows the proton magnetic resonance of the synthetic aroma compound in deuterated benzene at 100 mHz. The triplet centered at 5.12 p.p.m. ( $^1\text{H}$ ;  $J = 4.5\text{ Hz}$ ) can be assigned to one proton on a double bond adjacent to a methylene group. The absence of a second olefinic proton and of a *cis* or *trans* coupling, which typically falls within the range of 10 to 15 Hz, shows that a substituted double bond is present. The broad peak (1H) centered at 4.26 p.p.m. arises from an exchangeable proton, since it disappeared when the solution was shaken with  $\text{D}_2\text{O}$ . On the basis of chemical and IR evidence it can be assigned to an amino proton. The complex multiplet centered at 2.74 p.p.m. (2H) can be assigned to the protons of a methylene group adjacent to a nitrogen. The sharp singlet at 1.94 p.p.m. (3H) can be assigned to the protons of an acetyl group on the basis of position and shape. The remaining peaks in the area of the methyl resonance represent two protons and can most reasonably be assigned to an allylic methylene group. The approximate quintet ( $^2\text{H}$ ;  $J = 5.5\text{ Hz}$ ) centered at 1.46 p.p.m. is quite characteristic of the resonance from the protons of a cyclic methylene group adjacent to methylene. The remaining peaks in the spectrum, indicated in Fig. 4 by "DP," varied widely in intensity in different samples and are, therefore, assigned to decomposition products of the aroma compound. In none of the samples examined by NMR were these peaks ever completely absent.

The only structure consistent with the chemical, NMR, IR, and MS data of the aroma compound is:



1,4,5,6-tetrahydro-2-acetopyridine

The compound isolated is apparently just one of a family of compounds that have a cracker odor. Five synthetic compounds having a cracker odor have been detected by us. Three arise from the reaction of proline and glycerol (3) and two from the destructive distillation of the calcium salts of proline and acetic or propionic acid. This rather unusual phenomenon has been assigned the term "iso-osmic" to describe a series of different compounds having the same odor.

To determine the feasibility of enriching or enhancing the aroma of freshly baked bread, preliminary tests were made concerning the effect of adding synthetic cracker aroma to an extract of fresh bread. One part of the synthetic, when added to 100,000 parts of the extract, was easily detected and imparted an enhanced and desirable crusty overtone. Spraying week-old bread with an aqueous solution containing 6 p.p.m. of the sodium bisulfite complex of the tetrahydropyridine compound, after air-drying, returned a desirable fresh-bread odor to the crust after it had dried.

Loss in bread aroma with time is a commonly observed phenomenon. If we assume that the cracker-odor compounds are an integral part of the bread-aroma complex, the unstable nature of the isolated cracker-odor component is a clue as to how the aroma of freshly baked bread deteriorates. Significantly, since there are at least two, or possibly more, cracker-odor components in the bread-aroma complex, it is quite possible that differences in stability exist between the compound isolated and the other cracker-odor compounds present. If the latter are presumed to be stable, then their lack of strong influence could well be attributed to amounts in which they exist in bread.

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[Received March 15, 1968.]