Components Contributing to Beef Flavor

Isolation of 4-Hydroxy-5-methyl-3(2*H*)-furanone and Its 2,5-Dimethyl Homolog from Beef Broth

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A beef flavor concentrate was prepared by continuous extraction of a clear beef broth with diethyl ether, followed by concentration of the ethereal solution. Fractionation of this concentrate by gas-liquid chromatography revealed two components with characteristic odors, one reminiscent of caramel and the other of roasted chicory root. By repeated gas-liquid chromatography over another stationary phase the odorous compounds were isolated in a pure state. They were identified by comparison of their ultraviolet absorption spectra and their mass and infrared spectra with those of synthetically prepared samples. The component with the caramel odor proved to be 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, while the smell of roasted chicory root was due to 4-hydroxy-5-methyl-3(2*H*)-furanone.

Many studies, reviewed by Hornstein and Crowe (1964) and by Macy (1966), have been devoted to the identification of the volatile flavor compounds of meat. These investigations can be classified according to the type of meat studied—for example, beef, pork, lamb, chicken, etc. The flavor volatiles of cooked beef are reported by Bender and Ballance (1961), Hornstein *et al.* (1960), Kramlich and Pearson (1960), Sanderson *et al.* (1966), and Yueh and Strong (1960). The volatile compounds identified by these authors are: methanal, ethanal, propanal, 2-methylpropanal, butanal, 2-methylbutanal, propanone, butanone, butane-2,3-dione, ammonia, methylamine, methanol, ethanol, hydrogen sulfide, methanethiol, ethanethiol, dimethyl sulfide, formic acid, acetic acid, propionic acid, isobutyric acid, and butyric acid.

Wood and Bender (1957) and Bender *et al.* (1958) have discussed the nonvolatile flavor components of meat extract.

Despite all this research, much still remains to be learned about the compounds contributing to the flavor of beef. The investigations described in this article concern the isolation from beef broth of two such compounds and the subsequent elucidation of their structures. One was characterized by a caramel odor and the other by a roasted chicory root odor.

EXPERIMENTAL

Preparation of Beef Broth. A beef broth was prepared by simmering 2 kg. of minced lean shin of beef with 2 kg. of distilled water for $2^1/_2$ hours. After the product had been cooled to room temperature, the meat and the solidified fat were removed by suction filtration. The resulting clear broth, which had an intense meaty odor, was slightly yellow and had a pH of about 6.

Preparation of Flavor Concentrate. The clear beef broth was extracted continuously with diethyl ether (C.P., distilled just before use) for at least 18 hours. To avoid emulsification, 0.1 gram of sodium lauryl sulfate (reagent grade) was added to the aqueous phase. The ether extract was dried with anhydrous Na_2SO_4 and concentrated to a volume of 50 ml. by distillation over a Widmer column. After a second treatment with anhydrous Na_2SO_4 the extract was further concentrated to a volume of 2 ml. The flavor concentrate thus obtained had a typical meaty odor and showed an ultraviolet absorption maximum at 289 mμ.

Gas-Liquid Chromatography (GLC). The GLC analyses were performed on an F & M Model 402 gas chromatograph provided with an all-glass T-shaped effluent splitter. This splitter was proportioned in such a way that 10% of the effluent was fed to a hydrogen flame ionization detector, whereas the other 90% was led through a heated all-glass liner to an exhaust. This arrangement permitted the gas chromatogram to be recorded while the odor of the eluent was being assessed at the exhaust. In addition it was possible to trap components with interesting odors. This was done in glass traps (length 11 cm., diameter 0.4 cm.) filled with either Diatoport S 60/80 or Diatoport S 60/80 coated with 10% Apiezon L and 1% Carbowax 20 M. The former was used when the trapped material was to be investigated spectrometrically. The latter was used for collecting peak material that had to be purified further by a second GLC fractionation, in which

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case the trap was inserted between the injection port and the column used for the second purification.

The ether extract was first fractionated over a 2-meter 10% Carbowax 20 M column with an internal diameter of 0.4 cm. Peak material was trapped and subjected to renewed chromatography over a 3-meter Apiezon L–Carbowax 20 M (10%/1%) column with an internal diameter of 0.4 cm. In both cases the solid support was Diatoport S 60/80 and the oven temperature was programmed from 50° to 230° C. at a rate of 3° C. per minute. Injection temperature was 50° C., detector temperature 250° C., and exhaust temperature 250° C. The pure components isolated by this procedure were further analyzed by spectrometric methods.

Ultraviolet Absorption Spectra. The components with the chicory and caramel odors were trapped on solid support after GLC purification over the two columns. The volatile material was washed from the trap with 3 ml. of ether (C.P., distilled just before use). The ether was evaporated and the residue dissolved in 3 ml. of water. The ultraviolet spectra of these solutions were recorded on a Unicam SP 800 spectrophotometer in microcells with an optical path length of 4 cm. The component with a chicory odor showed an absorption maximum at 287 mμ and that with the caramel odor at 289 mμ. If the solutions were made alkaline, the characteristic odors disappeared and the ultraviolet absorption maxima shifted to 333 and 337 mμ, respectively. Upon subsequent neutralization, however, the original spectra reappeared.

Infrared Spectra. The method of Copier and Van der Maas (1967) was followed to obtain the infrared spectra of the trapped components. The spectra were recorded on a Perkin-Elmer Model 257 infrared spectrophotometer.

Mass Spectra. The volatile components were introduced directly from the traps into the ionization source of an A.E.I. MS-9 mass spectrometer. The transfer technique used was developed by de Bruyn and Soeting (1967) and permitted the high resolution mass measurement of selected *m/e* values.

Synthesis of Reference Compounds. The synthesis of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (melting point 79.5–80.5° C.) was performed in this laboratory according to the method described by Hodge (1960). The compound 4-hydroxy-5-methyl-3(2*H*)-furanone (melting point 126.5–27.5° C.) was synthesized by two different methods developed by Peer and Van den Ouweland (1968) and by Peer *et al.* (1968).

Nuclear Magnetic Resonance (NMR). The NMR spectra of the reference compounds were recorded on a Varian Type A 60 NMR spectrometer of the Unilever Research Laboratory, Vlaardingen, The Netherlands. Tetramethylsilane was used as an internal standard. Solutions were made in $CDCl_3$.

RESULTS

The gas chromatogram of the flavor concentrate on the Carbowax 20M column is given in Figure 1.

The majority of the components were identified easily from their mass spectra and the retention data on other stationary phases—i.e., Tween 80, free fatty acid phase (Aerograph). The peaks numbered 2 to 11 in the gas chromatogram correspond to the following compounds:

Acetoine 2	Valeric acid (trace) 7
Acetic acid 3	Isocaproic acid (trace) 8
Propionic acid 4	Caproic acid 9
Isobutyric acid 5	Lauryl alcohol 10
Butyric acid 6	Lactic acid 11

Most of these compounds are well-known constituents of meat flavor. Lauryl alcohol was an artifact, however, being formed by hydrolysis of the lauryl sulfate used as antiemulsifying agent.

The most interesting odors, however, observed at the

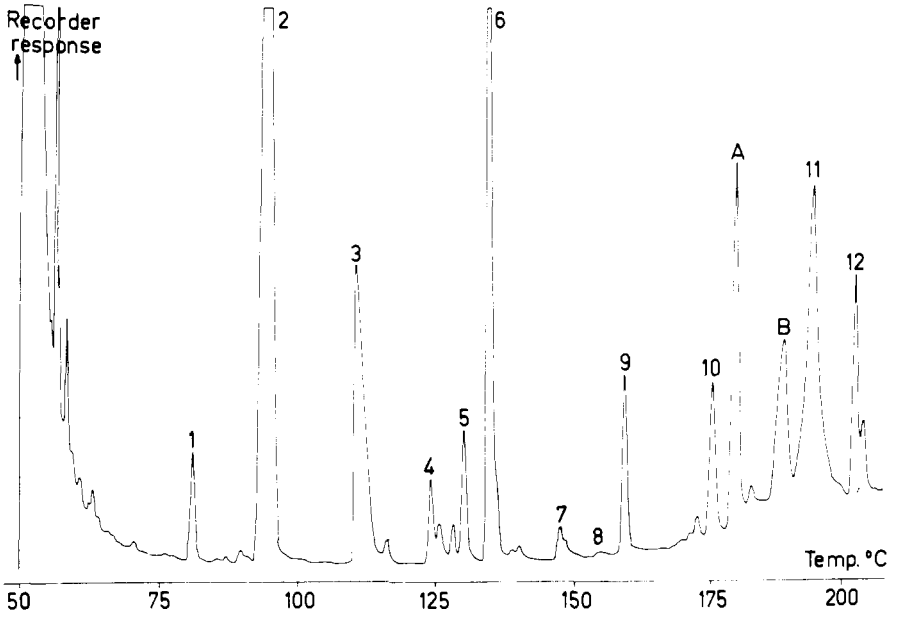


Figure 1. Gas chromatogram showing separation of a flavor concentrate obtained from beef broth

exhaust of the gas chromatograph were associated with peaks *A* and *B* in Figure 1. They were reminiscent of caramel and roasted chicory root, respectively. The peak material was condensed on the coated traps and subjected to renewed GLC purification over the Apiezon column. The resulting chromatograms are shown in Figure 2 for fractions *A* and *B*.

After the second GLC purification the peak material with the specific odor was trapped in a tube containing solid support. The isolated pure components I and II were then investigated by spectrometric methods. The spectra obtained are represented in Figures 3, 4, and 5. Spectrometric evidence, combined with data from the literature, permitted the identification of the two odorous compounds.

Component I. The ultraviolet absorption spectrum indicated that the molecule contained a conjugated system. The bathochromic shift of the absorption maximum toward 337 mμ in alkaline solution indicated that the compound could function as an acid-base system possibly originating in an enol group. A similar shift is shown for example by maltol which has λ_{max} = 278 mμ in a neutral medium, whereas in alkaline media λ_{max} = 320 mμ.

High resolution mass spectrometry revealed a molecular weight of 128.047, which is consistent only with a molecular formula $C_6H_8O_3$ (calculated mass 128.0473). The high intensity of the parent peak led to the assumption that the structure was cyclic. These results drew our attention to a compound isolated from pineapples by Rodin *et al.* (1965) which was identified as 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone. The same compound has been described by Hodge (1960), Hodge *et al.* (1963), Hofmann and Eugster (1966), and Willhalm *et al.* (1965). According to Hodge, it has a caramel-like odor, whereas Rodin *et al.* described its odor as "burnt pineapple." The ultraviolet absorption spectrum in neutral solution and the molecular formula given in these earlier publications were identical with those found for component I, while the mass spectra showed fair agreement, differing only slightly in the relative intensities of the peaks. To confirm the identity of component I, its infrared spectrum was compared with the spectrum given by Rodin *et al.* (1965) (Table I).

Table I. Comparison between Infrared Adsorptions of Component I and of 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone

Component I, Cm.$^{-1}$	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone, Cm.$^{-1}$[a]
1705	1705
1625	1625
1455	1458
1405	...
1377	1377
1313	1312
1200	1203
1150	1152
1125	...
1100	1100
1075	1075
1045	1045
1004	1010
930	932
878	878
760	760

[a] Values estimated from spectrum published by Rodin *et al.* (1965).

The close similarity of the two spectra strongly suggests that component I is identical to the compound isolated from pineapples. Final proof of the structure was obtained by synthesis of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone according to Hodge (1960). Spectra of the synthetic and the isolated compound were identical.

The NMR spectrum of the synthesized compound (in $CDCl_3$, tetramethylsilane as an internal reference) showed a doublet at δ = 1.40 p.p.m., a doublet (*j* = 1.0 c.p.s.) at

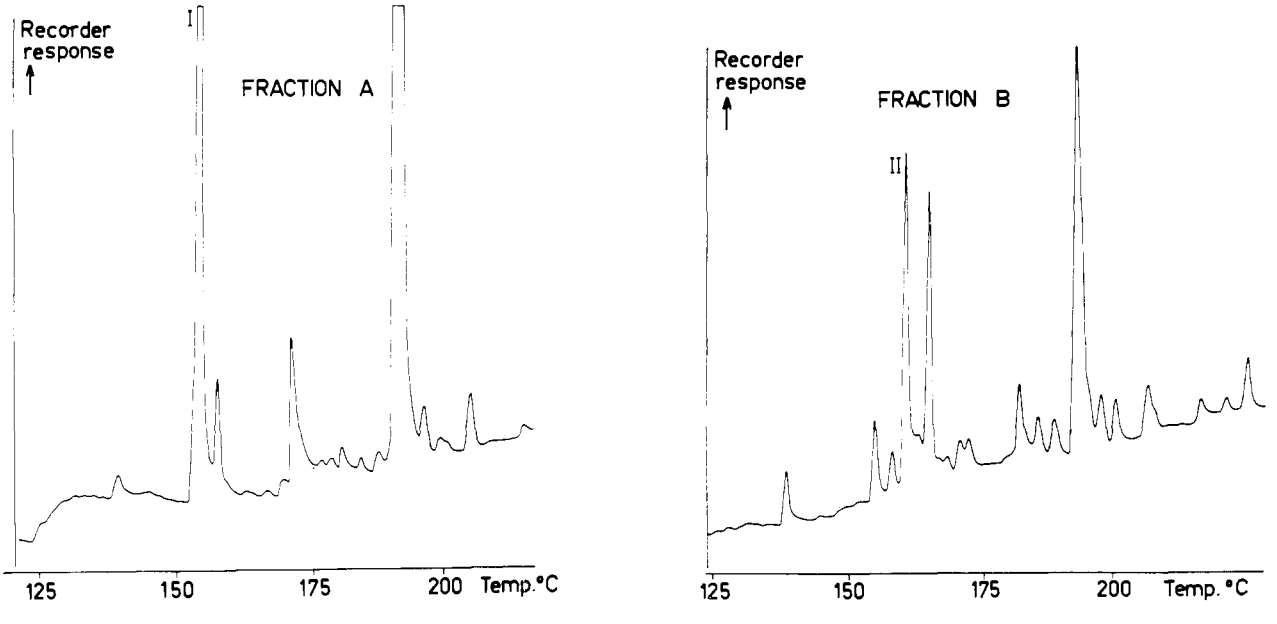


Figure 2. Gas chromatograms showing purification of trapped fractions

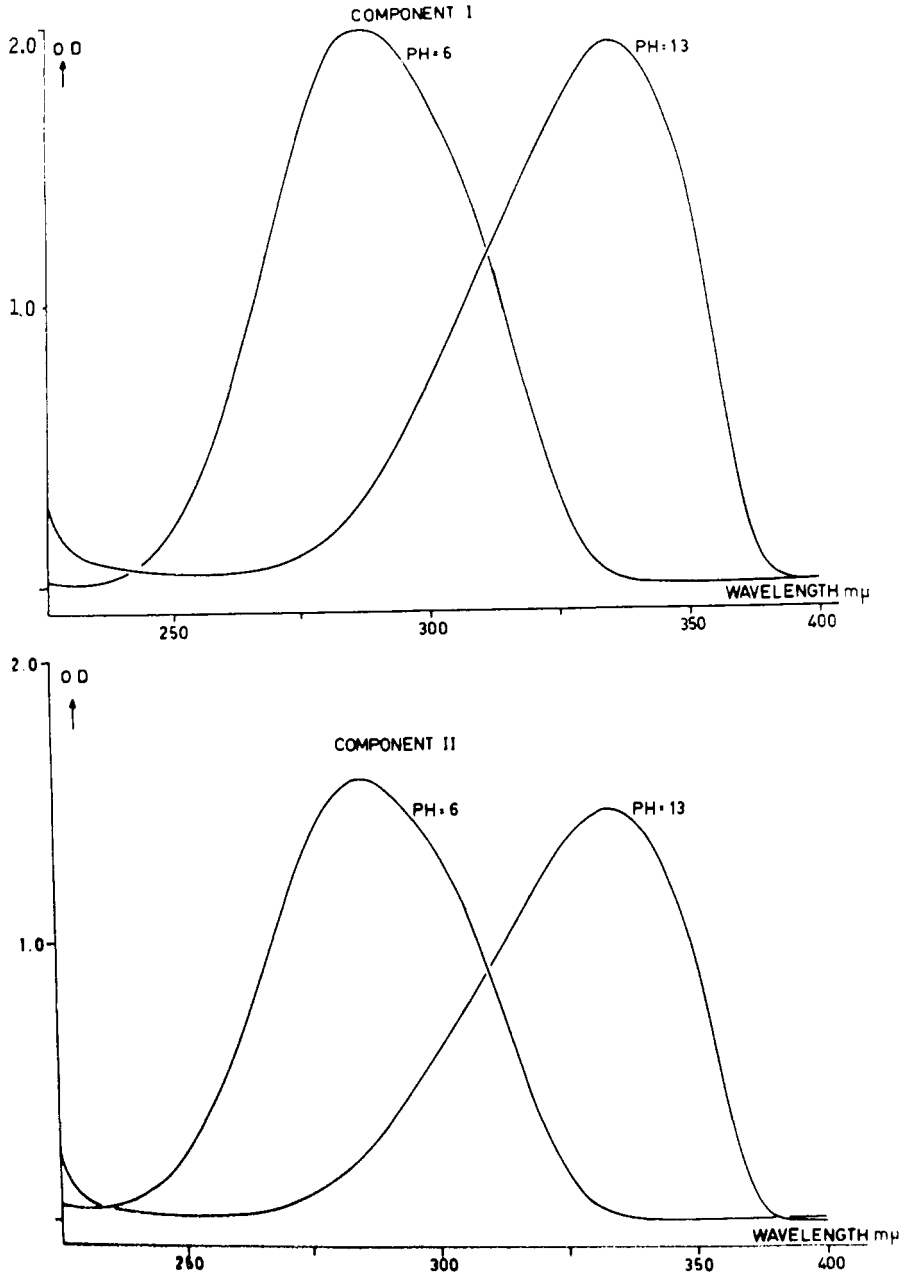
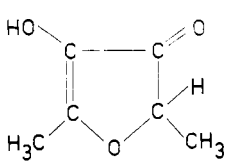


Figure 3. Ultraviolet absorption spectra of isolated components

2.21 p.p.m. and a double quartet at $\delta = 4.45$ p.p.m., which confirmed the structure:



Component II. The striking similarity of the ultraviolet absorption characteristics of components I and II suggests that the compounds are homologs. The mass spectrum of component II showed a parent peak of high intensity, again indicating a cyclic structure. High resolution mass spectrometry gave a molecular weight of 114.032, which is compatible only with a molecular formula $C_5H_6O_3$ (calculated mass 114.0317).

Comparison of the infrared spectra obtained for components I and II supported the idea that both components were homologs. The presence of a carbonyl group was indicated by the absorption at 1695 cm.$^{-1}$ This low frequency pointed to conjugation with a carbon-carbon double bond (1640 cm.$^{-1}$) and excluded a five-membered lactone ring structure. The absorption at 1195 cm.$^{-1}$ was indicative of a hydroxyl group, probably attached to one of the double-bonded carbon atoms. (The C—OH stretching vibration in phenols absorbs at about 1200 cm.$^{-1}$) The presence of a methyl group was deduced from the umbrella vibration at 1370 cm.$^{-1}$

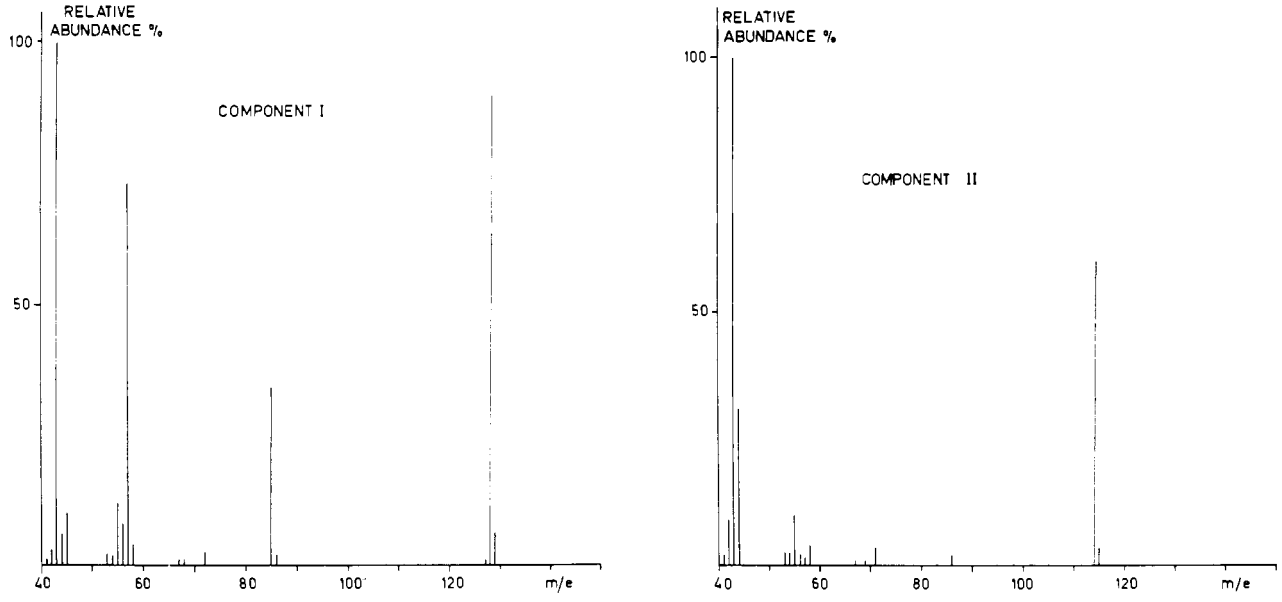


Figure 4. Mass spectra of isolated components

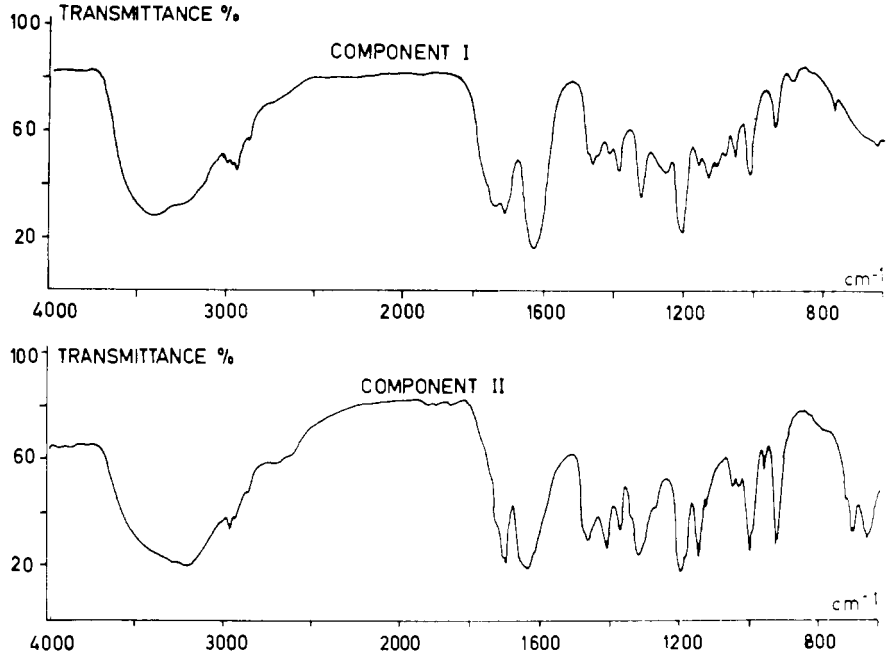
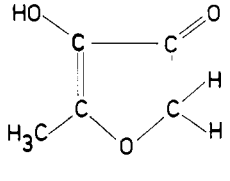


Figure 5. Infrared spectra of isolated components

The information obtained led us to assume the following structure for component II (or one of its other tautomeric forms):



This compound had not been mentioned until Severin and Seilmeier (1967) reported its formation from a reaction of pentoses and primary amines. In our laboratory Peer and Van den Ouweland (1968) and Peer *et al.* (1968) synthesized the compound starting from ribose or ribose-5-phosphate. The spectra obtained from the synthetic sample and from the component isolated from beef broth were identical. Final proof of the molecular structure was obtained from the NMR spectrum of the synthesized compound. Absorptions were found at $\delta = 2.26$ p.p.m. (CH_3) and at $\delta = 4.51$ p.p.m. (CH_2). From the long-range coupling between CH_2 and CH_3 ($j = 1.0$ c.p.s.) it was concluded that, in $CDCl_3$ at least, $C_5H_6O_3$ exists predominantly in the tautomeric structure indicated.

DISCUSSION

Hornstein and Crowe (1960) described the isolation from beef of a fraction with a meaty aroma. This fraction exhibited a strong ultraviolet absorption at wavelengths of from 290 to 295 mμ. However, neither the component(s) responsible for the aroma nor the component(s) causing the ultraviolet absorption were further investigated. In the light of our results, probably either one or both of the identified dihydrofuranones were present in the fraction isolated by Hornstein and Crowe.

Of the two odorous dihydrofuranones, both have been identified in beef broth while the 2,5-dimethyl derivative is present in pineapples. They probably will also occur in other meat products and in various fruits and, if so, they will contribute to the composite flavor.

Our investigations into the chemical identity of the precursors of these flavor compounds will be published later.

ACKNOWLEDGMENT

The aid of Bernard L. Schram in determining and interpreting the mass spectra of the isolated compounds is gratefully acknowledged. The contribution of Wouter Heinen in the determination and interpretation of the infrared spectra is appreciated.

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Received for review May 13, 1968. Accepted July 1, 1968.