Figure 5. Mass spectrum of 2-ethyl-5,6,7,8-tetrahydroquinoxaline.

hydrogens to form the fulvene radical ion. The same is true with the tetrahydroquinoxalines, yielding radical ions of quinoxalines under consecutive elimination of four hydrogen atoms.

Because no high-resolution measurements of the key fragment ions have been made yet, structures and compositions given in the schemes are based on fragmentation patterns found to be identical in a series of mono-, di-, and trisubstituted bicyclic pyrazine compounds. Modifications of the fragmentation pathways can be clearly understood by the nature and the positions of the substituents. In some cases, however, high-resolution proof appears to be necessary. Thus, for example, the fragment ions m/e 120 and 119 in the mass spectrum of 2-ethyl-5,6,7,8-tetrahydroquinoxaline (Scheme IV) may result from loss of C_3H_6 from the molecular ion (m/e 162) or the $M - 1$ ion, respectively, although this appears to be unlikely from comparable examples.

The isomeres 2(or 3),5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine and 3(or 2),5-dimethyl-6,7-dihydro-5H-cyclopentapyrazine are separable by capillary GC, but their mass spectra are so similar that structural assignment was not possible. Other workers (Pittet et al., 1974) encountered the same problem in structural assignments for the two isomeric cyclopentapyrazines.

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Characterization of Some Volatile Constituents of Dry Red Beans

Ron G. Buttery,* Richard M. Seifert, and Louisa C. Ling

Dry red beans (Phaseolus vulgaris) were treated with a steam distillation continuous extraction apparatus, both under vacuum and at atmospheric pressure. The volatile oils obtained were analyzed by the direct combination of capillary gas chromatography and mass spectrometry. Major components characterized in the vacuum isolated oil included oct-1-en-3-ol, oct-cis,5-en-2-one, oct-cis,5-en-2-ol, hex-cis,3-enol, hexanol, and octa-3,5-dien-2-one. Additional major components characterized in the atmospheric pressure isolated oil included thialdine, p-vinylguaiacol, 3,5-dimethyl-1,2,4-trithiolane, 2,4,5-trimethylthiazole, 2,4-dimethyl-5-ethylthiazole, 2,5-dimethyl-4-ethylthiazole, 2-acetylthiazole, 2-isopropyl-4,5-dimethylthiazole, 2-isobutyl-4,5-dimethylthiazole, 2,4,5-trimethyl-2-thiazoline, and 2-methyl-5-ethylpyridine.

Dry beans (Phaseolus vulgaris) are becoming a more important food product. This is largely because of their high protein yield per acre and their good storage stabili-

Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710.

ty. Off-aromas and off-flavors occasionally develop during storage of dry beans, resulting in the rejection of large quantities of beans for human food purposes. This study was carried out to learn more about the compounds responsible for dried bean aroma and flavor, and to make possible better control of these flavor problems.

Studies have been made on the volatile components from soybeans (Arai et al., 1967; Mattick and Hand, 1969;

Table I. Compounds Characterized in the Vacuum Steam Volatile Oil of Dry Red Beans[a]

	Approx. rel % in oil
Aliphatic Ketones	
Oct-cis,5-en-2-one, MS, RT, (16)	5–12
Octa-3,5-dien-2-one, MS, RT, (32), (39) (2 major isomers, prob. cis,trans and trans,trans)	2–8
Aliphatic Alcohols	
2-Methylbutanol, MS, RT, (9)	
3-Methylbutanol, MS, RT, (9)	0.5–2
Hexanol, MS, RT, (17)	1–4
Hex-cis,3-enol, MS, RT, (18)	2–6
Octanol, MS, RT, (41)	0.5
Octan-3-ol, MS, RT, (22)	0.1–1
Oct-1-en-3-ol, MS, RT, (25)	16–26
Oct-cis,5-en-2-ol, MS, RT, (26)	6
Nonanol, MS, RT, (50)	1
Aromatic Oxygenated and Heterocyclic Compounds	
2,5-Dimethylpyrazine, MS, RT, (15)	0.6
Benzaldehyde, MS, RT, (31)	1
2-Phenylacetaldehyde, MS, RT, (47a)	0.5
Aromatic Hydrocarbons	
Benzene, MS, RT, (1)	0.6
Toluene, MS, RT, (4)	0.3
Xylene (2 isomers), MS, RT, (9)	1
p-Cymene, MS, RT, (13)	
Trimethylbenzene, MS, (13)	1–4
Naphthalene, MS, RT, (51)	2
1- and 2-Methylnaphthalene, MS, RT, (66), (65)	2
Dimethylnaphthalene, MS (several isomers) (71–74)	1
Terpenes	
Limonene, MS, RT, (11)	1–4
γ-Terpinene, MS, RT (12)	0.1–0.6
α-Terpineol, MS, RT (53)	1–3

[a] MS, RT = mass spectral and GLC retention evidence, respectively. Evidence cited consistent with that of an authentic sample.

Wilkens and Lin, 1970) and green snap beans (Stevens et al., 1967), but very few previous reports are available (Johnson et al., 1971) on the volatiles from dry red and white beans.

EXPERIMENTAL SECTION

Materials. Dry red beans (*Phaseolus vulgaris*) were obtained from an Oregon bean processor.

Authentic samples of chemical compounds were obtained from commercial sources, or synthesized by well-established methods. Alkylthiazoles were synthesized by the condensation of the appropriate bromoketone with the appropriate thioamide, as outlined previously (Buttery et al., 1973). 2,4,5-Trimethylthiazoline was synthesized by heating 2-amino-3-bromobutane hydrobromide with thioacetamide at 150° under nitrogen for 10 min following the procedure of Gabriel and Hirsch (1896).

Octa-3,5-dien-2-one was synthesized by the condensation of pent-2-enal (1 g) with acetone (50 ml), catalyzed by 50% KOH (5 ml) as outlined by Bondarovich et al. (1967). Three major cis–trans type isomers, with almost identical mass spectra, were obtained from this synthesis. Their exact geometry has not yet been established.

Oct-cis,5-en-2-ol was synthesized in the following way. Hex-cis,3-enol was treated with PBr_3 to give 1-bromohex-cis,3-ene. The distilled bromo compound (5.4 g) was then allowed to react with magnesium (1.0 g) in ether (50 ml) to give the Grignard reagent. Acetaldehyde (2.0 ml) in ether (20 ml) was then dropped in gradually over 15 min. The mixture was stirred for another 15 min, and then dilute sulfuric acid (10%, 20 ml) was added with ice cooling. Separation of the ether layer, washing with $NaHCO_3$, drying over sodium sulfate, and removal of the ether on the steam bath gave the crude oct-cis,5-en-3-ol (4 g). The compound was purified by GLC.

Oct-cis,5-en-3-one was obtained from the above alcohol (1 g) by oxidation with CrO_3 (0.6 g) in acetic acid (20 ml) at room temperature for 30 min. This gave 0.6 g of crude oct-cis,5-en-3-one which was purified by GLC.

Isolation of the Volatile Oil. Unbroken dried beans (1.8 kg) were placed in a 12-l. flask with odor-free distilled water (ca. 6 l.) and allowed to soak overnight. A small amount (1 ml) of a colloidal antifoam (GE-60, treated using steam distillation to remove volatiles) was added to prevent foaming. The flask was then connected to a steam distillation continuous extraction apparatus (Likens head). A 250-ml flask containing purified hexane (100 ml) was attached to the solvent arm of the head. The isolation was carried out under vacuum (100 mm pressure) with the beans at a temperature of about 50°. The condensor was cooled with ice water, and the isolation carried out for 3 hr. The hexane extract was washed with $NaHCO_3$ and dried over sodium sulfate, and the hexane removed through low hold-up columns to give the vacuum steam volatile bean oil; the yield amounted to ca. 1 ppm of the original dry beans.

For obtaining the atmospheric steam volatile bean oil the procedure was essentially as described above except that atmospheric pressure was used with the beans at 100°. Freshly purified diethyl ether was used as the extracting solvent instead of hexane. The yield of atmospheric steam volatile bean oil amounted to ca. 10 ppm of the dry beans.

Capillary GLC–Mass Spectral Analysis. Several studies were made with both the vacuum and atmospheric steam isolated oil using different columns and different temperature programming conditions. The main studies, however, were carried out with a 150 m × 0.075 cm i.d. stainless steel capillary column coated with Tween 20 containing 5% Igepal CO-880. This column was programmed from 50 to 170° at 0.5°/min and held at the upper limit. Helium, at 8 psi inlet pressure, was the carrier gas. The column was coupled to a modified Consolidated 21-620 cycloidal type mass spectrometer using a silicone membrane molecular separator.

Infrared Absorption (Ir) Spectra. Packed column GLC separation was carried out using a 2 m long × 0.64 cm o.d. aluminum column, packed with Chromosorb P coated with 15% Amine 220. Samples were collected in 150 × 3 mm borosilicate tubes, which were immediately sealed off after collection, and stored at −20°. Infrared spectra were recorded with a Perkin-Elmer 237 instrument using ultramicrocavity cells and a reflecting beam condensor.

RESULTS AND DISCUSSION

Table I lists the components characterized in the vacuum steam volatile bean oil. These would be expected to represent medium boiling volatiles in the uncooked beans. Figure 1 shows the capillary GLC analysis of the oil. The peak numbers in Figure 1 are shown in parentheses in Table I alongside the corresponding identified component. The amount of oil obtained was only about 1 ppm of the dry beans. Some idea of typical relative percentages found in vacuum steam volatile bean oil samples also may be obtained from the data given in Table I. Several samples
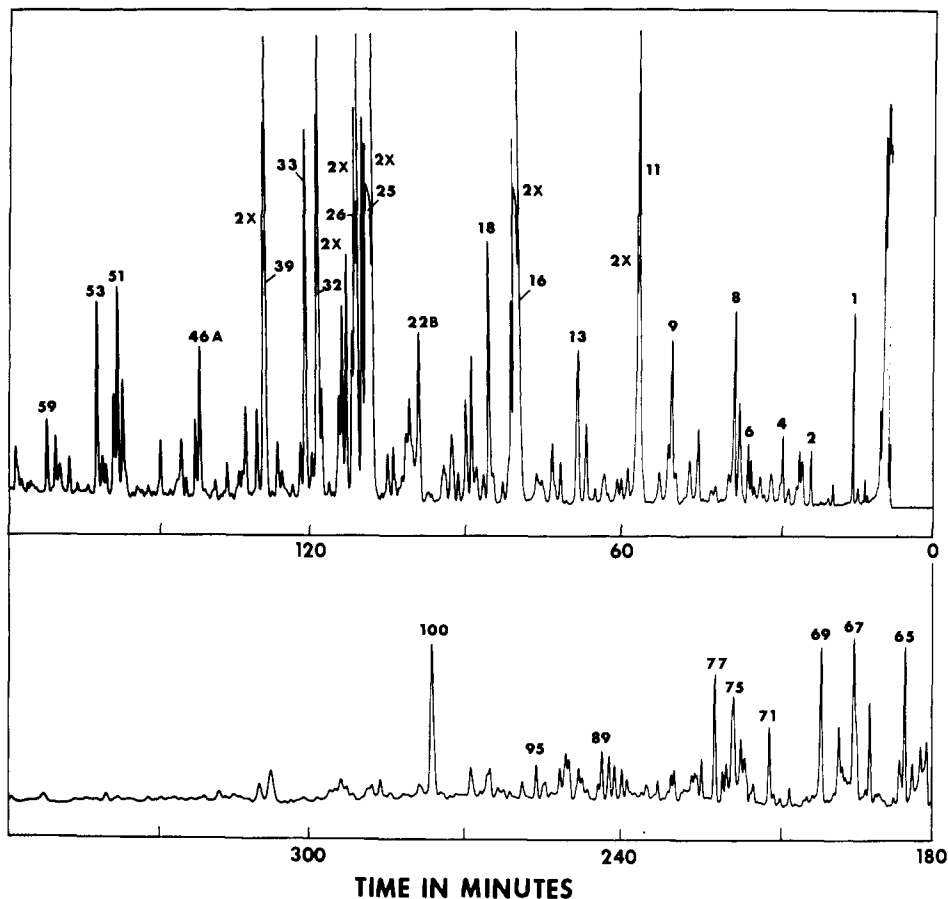
Figure 1. Capillary GLC analysis of the volatile oil obtained from dry red beans by vacuum steam distillation continuous extraction. The capillary column was 150 m long × 0.075 cm i.d. stainless steel coated with Tween 20 containing 5% Igepal CO-880. The column was temperature programmed from 50 to 170° and held at the upper limit. Flame ionization detection.

(different lots) were studied and considerable quantitative variation was noticed. Some idea of this variation is also shown in Table I. It should be noted that compounds with boiling points lower or near that of hexane are not isolated by the method used in this study which was aimed at medium boiling compounds.

Most of the compounds in Table I commonly occur in foods. Oct-1-en-3-ol, the major component, had also been found in snap beans (Stevens et al., 1967). The other C_8 unsaturated alcohol in Table I, oct-*cis*,5-en-2-ol, on the other hand was quite unusual, not being characterized previously in nature or synthesized previously, insofar as the authors can determine. The related oct-*cis*,5-en-2-one had been synthesized by Harper and Smith (1955), but also had not been found previously in nature. Ketones, and the corresponding secondary alcohols, are often associated in foods. Foods frequently contain both oxidizing and reducing systems which could bring about conversion of one to the other. Another related compound, octa-3,5-dien-2-one, had also been found in tea (Bondarovich et al., 1967; Cazenave et al., 1974).

The double bond in oct-*cis*,5-en-2-one is three carbon atoms from the saturated end and is cis. One can, thus, see the relation of this compound to linolenic acid and to many of the compounds that are formed from oxidative degradation of linolenic acid, such as hex-*cis*,3-enol and hept-*cis*,4-enal. It is not difficult to see how a linolenic acid peroxide could break down to oct-*cis*,5-en-2-one. However, there must be some conditions (catalysts?) associated with the bean which favor this particular pathway.

Considerable variation of the concentration of these unusual C_8 unsaturated compounds was noticed with different bean samples; there may, therefore, be some relation to variation in flavor. However, the compounds had only pleasant, moderate odors and, in themselves, are probably of only minor importance to the total odor.

Compounds characterized in the atmospheric steam distilled oil (ca. 10 ppm of the dry bean) are listed in Table II. These would be expected to represent components present in the cooked beans. The amount of oil obtained at atmospheric pressure was about 10 times that obtained under vacuum. Most of the components are compounds formed by heat. By far the major component is thialdine (2,4,6-trimethylperhydro-1,3,5-dithiazine), a compound previously found in beef broth (Brinkman et al., 1972). It was pointed out by these authors (Brinkman et al., 1972) that this compound is readily formed from hydrogen sulfide, acetaldehyde, and ammonia. These precursors are undoubtedly present in the cooked beans. Self et al. (1963) characterized hydrogen sulfide and acetaldehyde in cooked beans. Another compound in Table II, known to be formed from sulfur, acetaldehyde, and hydrogen sulfide (3,5-dimethyl-1,2,4-trithiolane) has been found in a number of cooked food products, including boiled beef (Brinkman et al., 1972). A similar condensation of acetaldehyde and ammonia probably leads to the 2-methyl-5-ethylpyridine present. The condensation of acetaldehyde and ammonia is the basis of a commercial process for making 2-methyl-5-ethylpyridine.

Another major component is vinylguaiacol previously characterized in cooked soybeans (Wilkens and Lin, 1970).

An interesting group of compounds is the alkylthiazoles; small amounts of the corresponding 2-thiazoline seem to accompany each thiazole. 2,4,5-Trimethylthiazole and 2-acetylthiazole had been found in cooked beef (Wilson et al., 1973). Two of us (Buttery and Ling, 1974) had found

Table II. Compounds Characterized in the Atmospheric Steam Volatile Oil of Dry Red Beans in Addition to Those Listed in Table I[a]

	Approx rel % in oil
Furans	
2-Pentylfuran, MS, RT	0.3
2-Methyl-3-ketotetrahydrofuran, MS, RT	0.1
Furfural, MS, RT	0.1
Pyrazines and Pyridines	
2,6-Dimethylpyrazine, MS, RT ⎱	0.2[b]
2,3-Dimethylpyrazine, MS, RT ⎰	
Pyridine, MS, RT	0.1
2-Methylpyridine, MS, RT	0.3
2-Methyl-5-ethylpyridine, MS, RT, ir	2
2-Acetylpyridine, MS, RT	0.3
Thiazoles and Thiazolines	
2,4,5-Trimethylthiazole, MS, RT, ir	4
2,5-Dimethyl-4-ethylthiazole, MS, RT	0.2
2,4-Dimethyl-5-ethylthiazole, MS, RT, ir	1
2-Isopropyl-4,5-dimethylthiazole, MS, RT	0.2
2-Isobutyl-4,5-dimethylthiazole, MS, RT	0.1
2-Acetylthiazole, MS, RT	0.4
2,4,5-Trimethyl-2-thiazoline (2 isomers), MS, RT	0.2
Other Sulfur Heterocyclics	
Thialdine (2,4,6-Trimethylperhydro-1,3,5-dithiazine) MS, ir, PMR, RT	64
3,5-Dimethyl-1,2,4-trithiolane (two isomers), MS, ir, RT	3
Benzene Derivatives	
p-Vinylguaiacol (2-methoxy-4-vinylphenol) MS, ir, RT	11
Benzyl alcohol, MS, RT	0.4

[a] MS, RT, ir, PMR = mass spectral, GLC retention, ir absorption, and proton magnetic resonance evidence, respectively. Evidence cited consistent with that of an authentic sample. [b] Percent includes 2,5-dimethylpyrazine.

2-isopropyl-4,5-dimethylthiazole and 2-isobutyl-4,5-dimethylthiazole in boiled potatoes. The dimethylethylthiazoles were recently found in coffee by Vitzthum and Werkhoff (1974). The 2,4,5-trimethyl-2-thiazoline does not seem to have been found in foods before. There has been some speculation on how alkylthiazoles are formed in foods (Schutte, 1974). The occurrence of 2-methyl-, 2-isopropyl-, and 2-isobutylthiazoles indicates that an amino acid may be involved in the formation of the part of the thiazole ring around the 2 position.

The mass spectra of most of the compounds listed in Tables I and II are well enough known, or have been reported by the authors in previous publications, except for three compounds whose mass spectra are listed below (two most intense ions every 14 mass units above m/e 34, intensities in parentheses, molecular ion in boldface type): oct-*cis*,5-en-2-ol, 41 (85), 45 (100); 55 (46), 58 (29); 68 (83), 69 (26); 81 (63), 82 (5); 95 (27), 96 (3); 110 (16), 111 (1); **128** (2), 129 (0.5); oct-*cis*,5-en-2-one, 41 (32), 43 (100); 55 (16), 58 (9); 68 (35), 69 (12); 83 (3), 84 (1); 93 (0.5), 97 (7); 108 (2), 111 (1); **126** (3), 127 (0.5); 2,4,5-trimethyl-2-thiazoline (cis, both cis and trans very similar) 42 (57), 45 (24); 55 (68), 59 (100); 68 (24), 69 (92); 88 (72), 89 (5); 114 (0.3); **129** (19), 130 (1).

A study, similar to that reported here for red beans, was also carried out on dry white beans (*Phaseolus vulgaris*). The compounds characterized were qualitatively, in general, essentially the same as shown in Tables I and II, especially for the atmospheric steam volatile oil. There were some differences in the relative amounts of components for the vacuum isolated oil.

Importance of the Components to Bean Aroma. Based on results obtained so far, and on previous odor threshold studies carried out by the authors and other workers, two of the components most important to the aroma of uncooked beans probably are oct-1-en-3-ol and hex-*cis*,3-enol. With the cooked beans, in the authors' opinion, some of the most important components to the aroma probably are thialdine, *p*-vinylguaiacol, 2,4-dimethyl-5-ethylthiazole, and 2-acetylthiazole. Measurements of odor thresholds, and qualitative odor studies, are in progress. The results of these studies will be reported when all the information is collected and analyzed.

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