

the typical black tea character.

Cazenave et al. (1974) identified *N*-ethylsuccinimide in black tea and they speculate that this compound is derived from theanine by a Strecker degradation during tea fermentation. We identified *N*-ethylacetamide and *N*-ethylpropionamide and these compounds may be considered to originate from the same amino acid present in fresh tea in an unusually large amount. *N*-Ethylacetamide and *N*-ethylpropionamide were synthesized and found to be identical with respect to retention time and mass spectral fragmentation with the natural material. This finding further substantiates the importance of amino acids as precursors of tea volatiles (Sanderson and Graham, 1973). The mass spectrum of *N*-ethylpropionamide not previously reported in the literature is (*m/e* values of most significant ions; intensities in parentheses): 101 (100), 100 (5), 86 (15), 72 (65), 57 (50), 56 (6), 55 (5), 54 (6), 44 (75), 30 (38), 29 (46).

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Nona-2,4,6-trienal, an Unusual Component of Blended Dry Beans

Nona-2,4,6-trienal has been characterized in the vacuum steam volatile oil of some samples of blended dry red beans and of blended dry white beans using capillary gas chromatography-mass

spectrometry. This compound probably arises from an unusual oxidative breakdown of the linolenic acid in the bean.

Alka-2,4-dienals, particularly deca-2,4-dienal, have been found in a wide variety of foods. Reports of finding alkatrienals, however, have been much rarer. Deca-2,4,7-trienal was reported recently in fish oil (Meijboom and Stroink, 1972) and deca-2,4,6-trienal was reported recently in tea (Cazenave et al., 1974). Although nona-2,4,6-trienal has been indicated as possibly occurring in butter (Badings, 1970), to our knowledge, it has not been characterized previously in any food product with any degree of certainty.

EXPERIMENTAL SECTION

Materials. Dry red and white beans (*Phaseolus vulgaris*) were obtained from local retail markets and from an Oregon bean processor.

Synthesis of Nona-2,4,6-trienal. Hepta-2,4-dienal (0.6 ml; trans,trans; Aldrich Chemical Co.) was cooled to -5° in an ice-salt bath and acetaldehyde (0.4 ml; free from acetic acid) was added. Two drops of a 50% solution of potassium hydroxide in water were then added and the mixture was

stirred for 15 min at -5° and then for 1 hr at room temperature. The mixture was then taken up in ether (100 ml), washed first with cold dilute hydrochloric acid (50 ml, 3 *N*), and then with saturated sodium bicarbonate solution, dried over sodium sulfate, and the ether removed by distillation. The nona-2,4,6-trienal was purified by gas-liquid chromatography (GLC) using a 1-m long, 0.64 cm o.d. aluminum column packed with 80-100 mesh Chromosorb P coated with 10% Tween 20. The column was kept at 150° and had a helium inlet pressure of 8 psi. The nona-2,4,6-trienal formed a well-separated peak at 20 min. Calculation from GLC showed a yield of 10% based on the original hepta-2,4-dienal. Capillary GLC (500 ft \times 0.03 in. i.d., Tween-20) showed the material separated by packed column GLC to consist of three major isomers.

Isolation of the Volatile Oil. Dry beans (1.8 kg) were blended in portions in a laboratory blender with about three times their volume of water. They were then placed in a 12-l. flask. Additional water was added to make the

total volume of water about 6 l. A small amount of colloidal antifoam (GE-60) was added to prevent foaming. The vacuum steam distillation continuous extraction (Likens Head) was then carried out as described previously (Buttery et al., 1975).

Capillary GLC-Mass Spectral Analysis. This was carried out as described previously (Buttery et al., 1975) using a 150 m \times 0.75 cm stainless steel column coated with Tween 20 containing 5% Igepal CO-880. The column was programmed from 50 to 170° at 0.5°/min and held. The mass spectrometer was a modified Consolidated 21-620, cycloidal type.

RESULTS AND DISCUSSION

The main study was carried out on the volatile oil obtained from the vacuum steam distillation continuous extraction of blended dry red beans. Similar results also were obtained with blended dry white beans. The study was repeated with several different batches of beans. The volatile oil was analyzed by the direct combination of capillary gas chromatography (GLC) and mass spectrometry. The main components of the vacuum steam volatile oil were similar to those reported previously for the unbroken beans (Buttery et al., 1975). Several additional compounds were detected, however. One prominent component (and an additional minor one, apparently an isomer) had a molecular weight of 136 and a mass spectrum which showed some features similar to that of deca-2,4,6-trienal.

The synthesis of nona-2,4,6-trienal had been reported by Bohlmann and Jastrow (1962). The present authors synthesized this compound in a convenient and rapid way by the aldol condensation of *trans,trans*-hepta-2,4-dienal and acetaldehyde. It also was synthesized from pent-2-enal and 1-methoxybut-1-en-3-yne following the method of synthesis of alka-2,4-dienals described by Pippen and Nonaka (1959).

There are eight possible *cis-trans* types of isomers of nona-2,4,6-trienal. Three major isomers were obtained from the aldol condensation synthesis. The synthesis from *trans,trans*-hepta-2,4-dienal would be expected to give the *trans,trans,trans*-nona-2,4,6-trienal as a major product (aldol condensations usually lead to the *trans*-2-enal). The major isomer obtained (probably *trans,trans,trans*) formed 75% of the total. A second isomer, 17% of the total, is probably the *trans,trans,cis*. A third isomer, 8% of the total, may be the *trans,cis,cis*. These would be probably the most energetically favored isomers. Their mass spectra were essentially identical. The mass spectrum of the major isomer (two most intense ions every 14 mass units above *m/e* 34, intensities in parentheses, molecular ion in boldface type) was as follows: 39 (100), 41 (69); 51 (39), 53 (49); 66 (38), 68 (34); 77 (71), 79 (98); 91 (44), 93 (18); 105 (10), 107 (36); 121 (15), 122 (5); 136 (44), 137 (5).

The proton magnetic resonance spectrum was consistent with the nona-2,4,6-trienal structure, i.e. (100 MHz, CDCl₃) δ 1.07 (t, 3, $J = 7$ Hz, CH₂CH₃), 2.2 (m, 2,

=CHCH₂CH₃), 5.9-7.6 (complex m, 6, (CH=CH)₃), 9.56 (d, 1, $J = 8$ Hz, CHO).

The mass spectrum and GLC retention time of the major isomer in the beans were consistent with the major *trans,trans,trans*-nona-2,4,6-trienal from the synthesis. The minor component from the beans had a GLC retention time (and mass spectrum) consistent with the suspected *trans,trans,cis* form.

Mechanism of Formation in Beans. It is reasonable to visualize that the nona-2,4,6-trienal is formed from the linolenic acid which occurs in high (>50%) concentration in the fatty acids of the bean. However, the mechanism of formation must be somewhat different from that usually accepted for the formation of other unsaturated aldehydes from fatty acid autoxidation.

The nona-2,4,6-trienal was found in all samples of beans that were blended before carrying out the vacuum steam distillation continuous extraction. It also was found in some samples of unbroken beans that were treated whole in the vacuum steam distillation continuous extraction. These generally were beans which were considered to have off-flavors. Blending, of course, is known to enhance considerably the enzyme-catalyzed autoxidation of lipids in a food. In the whole beans, similar conditions might possibly be brought about by frost damage, although such damage may not be recognized easily from the appearance of the bean.

In addition to the nona-2,4,6-trienal, other lipid autoxidation products characterized (mass spectra and retention time consistent with authentic sample) in the blended beans included hexanal, hex-2-enal, 2-pentylfuran, hept-2-enal, hepta-2,4-dienal (two isomers), oct-2-enal, non-2-enal, nona-2,4-dienal, and deca-2,4-dienal (two isomers).

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