Steam Volatile Aroma Constituents of Roasted Cocoa Beans

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Steam-volatile components from cocoa beans were examined on a support-coated capillary gas chromatographic column coupled to a mass spectrometer. Of the identified compounds, the following contribute to cocoa aroma: acetaldehyde, isobutyraldehyde, isovaleraldehyde, benzaldehyde, phenylacetaldehyde, 5-methyl-2-phenyl-2-hexenal, 2-furaldehyde, methyl disulfide, 11 alkyl-substituted pyrazines, acetic acid, and isopentyl acetate. Possible precursors of the pyrazine compounds are discussed.

The chemical nature of cocoa flavor has fascinated chemists for a long time. Dietrich *et al.* (1964) identified 29 new compounds and 43 previously reported compounds in a solvent extract of cocoa beans but could not reconstitute the cocoa flavor. Rizzi (1967) characterized seven alkyl-substituted pyrazines in cocoa butter. Recently Marion *et al.* (1967) listed 126 cocoa flavor constituents, 35 of which were formerly unknown. Simultaneously, Flament *et al.* (1967) identified 62 constituents in a cocoa concentrate, 42 of which were not previously reported. Van der Wal *et al.* (1968) investigated commercial cocoa powder and found 81 constituents which had not been reported before.

In our study, gas chromatography and mass spectrometry were used to identify the compounds in a steam distillate from cocoa nibs, so that the flavor could be reproduced with synthetic compounds. ("Nibs" is that part of roasted cocoa beans utilized in the manufacture of chocolate liquor.) A cocoa nibs distillate was chosen since it contains an essential part of the cocoa flavor. The distillate lacks many flavor factors such as astringency and bitterness (Powell and Harris, 1967).

EXPERIMENTAL

A commercial blend of cocoa nibs was used. Steam distillation was carried out at atmospheric pressure (0.84 liter of distillate per 1 kg. of cocoa nibs). Basic, neutral, and acid fractions were prepared from the distillate for separate examination. In addition, a gas-liquid chromatographic (GLC) headspace analysis of the distillate was performed.

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Basic Fraction. By freeze concentration, 3800 ml. of steam distillate was concentrated to 500 ml. No significant losses of the aromatic compounds occurred during the freeze concentration, performed by placing a cold finger cooled with a dry ice–isopropyl alcohol mixture in the magnetically stirred distillate. Fifty grams of sodium chloride was dissolved in the concentrated distillate and the initial pH of 6.9 was adjusted to pH 0.7 with concentrated hydrochloric acid. The acidified distillate was extracted with purified dichloromethane (5 × 25 ml.) in a separatory funnel. The pH of the aqueous solution was increased to 8.3 with sodium hydroxide and the solution re-extracted with dichloromethane (4 × 20 ml.). The latter extract was dried with anhydrous sodium sulfate and allowed to evaporate through a Kuderna Danish column (Kontes Glass Co., Vineland, N. J.) until the volume was reduced to about 0.5 ml.

Neutral Fraction. The first dichloromethane extract (125 ml.) was treated twice with 50 ml. of a 3% sodium carbonate solution to remove the acids. The residual dichloromethane extract containing neutral compounds was dried and concentrated as described above.

Acid Fraction. Sodium chloride (250 grams) was added to 2500 ml. of steam distillate and the pH adjusted to 13.0 with 100 grams of sodium hydroxide. The alkaline solution was extracted with 5 × 150 ml. of an ethyl ether–2-methylbutane mixture (v./v., 2 to 1) to remove the neutral and basic constituents. The extracted aqueous phase was acidified with concentrated hydrochloric acid to pH 1.0. The acids and phenols were extracted with ethyl ether, and the extract was dried and concentrated. Acids were converted into methyl esters before analysis (Schlenk and Gellerman, 1960).

Headspace Analysis. Twenty milliliters of a fivefold freeze-concentrated steam distillate was pipetted into a

50-ml. vial that contained 2 grams of purified sodium sulfate. The vial was sealed with a screw cap in which a hole was drilled and in which the original liner was replaced by a rubber septum 1/8 inch thick. The vial was then heated for 10 minutes at 65° C. in a water bath, leaving only the top of the cap dry. After flushing a 5-ml. Hamilton syringe twice with the headspace vapors, 1 ml. of the vapor was withdrawn and analyzed in the same manner as the other fractions.

APPARATUS AND METHODS

The cocoa flavor fractions were analyzed on an Aerograph 1520 gas chromatograph coupled through a Biemann separator (Watson and Biemann, 1964) to a Hitachi Perkin-Elmer RMU 6E mass spectrometer. The support-coated capillary Carbowax 20M column (50 feet long, 0.02-inch i.d.) was programmed from 30° to 175° C. at 2° C. per minute. The effluent from the gas chromatographic column (flow rate 5 ml. of helium per minute) was split so that 80% passed into the Biemann separator and hence to the mass spectrometer, and 20% to a flame ionization detector. Approximately 10% of the ion beam was collected on the total ion monitor, so that a dual chromatographic record was obtained.

RESULTS AND DISCUSSION

The compounds identified in the various fractions obtained are listed in Table I. Identities of all compounds were confirmed by mass spectrometry. Each of the fractions isolated from the steam distillate contributes to the total aroma. Chemical characterization of these fractions has enabled us to collect data for the reproduction of natural cocoa flavor.

The basic fraction contributed a nutlike odor which could be attributed to alkyl-substituted pyrazines. The identity of those pyrazines not commercially available was confirmed by synthesis and subsequent comparison of mass spectra and relative retention time indices calculated as I_E values. Under temperature-programmed conditions I_E values are calculated by linear interpolation of the retention time of the unknown between retention times of a series of ethyl esters of normal carboxylic acids used as internal standards. Arbitrarily the I_E value of each marker is given the value of the carbon number of the acid of the ester—i.e., ethyl hexanoate has an I_E value of 6.0 (van den Dool and Kratz, 1963). Two pyrazine compounds not previously reported in cocoa aroma were identified: 2-ethylpyrazine and 2-ethyl-5,6-dimethylpyrazine.

The presence of pyrazine compounds in roasted foodstuffs such as cocoa, coffee, and peanut has been reported (Bondarovich *et al.*, 1967; Goldman *et al.*, 1967; Mason *et al.*, 1966). Dawes and Edwards (1966) identified methyl-substituted pyrazines in the volatile reaction products of a heated aqueous mixture of amino acids with D-fructose. We reacted neutral amino acids such as glycine, serine, leucine, isoleucine, valine, and alanine with D-fructose and observed the formation of a similar series of pyrazines, regardless of the amino acid used. These results agreed with those published by Newell *et al.* (1967). We assumed that ammonia was an intermediate and that the composition of the pyrazine mixture did not depend upon the amino acid involved. The same series of pyrazine compounds could be isolated from a reaction mixture of fructose or glucose and ammonia in our laboratories. There are several reports of the presence of alkyl-substituted pyrazines in sugar-ammonia reaction mixtures (Brandes and Stoehr, 1896; Jezo and Luzak, 1966).

Table I. Compounds Identified in Various Fractions of Cocoa Nibs Distillate

Compound	I_E Value
Basic Fraction	
2-Methylpyrazine	6.1
3-Hydroxy-2-butanone	6.2
2,5-Dimethylpyrazine	6.6
2,6-Dimethylpyrazine	6.7
2-Ethylpyrazine[a]	6.7–6.8
2,3-Dimethylpyrazine	6.9
2-Ethyl-5-methylpyrazine	7.3–7.4
2,3,5-Trimethylpyrazine	7.5
2-Ethyl-3,6-dimethylpyrazine	7.9
2-Ethyl-5,6-dimethylpyrazine[a]	8.0–8.1
2,3,5,6-Tetramethylpyrazine	8.2
2-Ethyl-3,5,6-trimethylpyrazine	8.6
Furfuryl alcohol	9.6–9.7
Phenethyl alcohol	12.0
Methyl pyrrol-2-yl ketone	12.6
Pyrrole-2-carboxaldehyde	13.0
Neutral Fraction and Headspace	
Acetaldehyde[b]	0.4
Methyl sulfide	0.6
Isobutyraldehyde[b]	0.9
Ethyl acetate	2.0
Tetrahydro-2-methylfuran	2.0
2-Butanone	2.2
Isovaleraldehyde[b]	2.5
2,3-Butanedione[b]	2.7
Isobutyl acetate[b]	3.2
2,3-Pentanedione[b]	3.6
Methyl disulfide[b]	4.0
2-Pentyl acetate[b]	4.1
2-Pentyl alcohol[b]	4.3
trans-3-Penten-2-one	4.4
Isopentyl acetate[b]	4.6
Isopentyl alcohol	5.3
Tetrahydro-2-methyl-3(2H)-furanone	5.9
3-Hydroxy-2-butanone[c]	6.2
2-Isopropyl-5-methyl-2-hexenal[a]	7.1
Methyl trisulfide[a]	7.3
2-Furaldehyde	7.9
Methional[a]	7.9
2-Furyl methyl ketone	8.2
Benzaldehyde[b]	8.5
3,7-Dimethyl-1,6-octadien-3-ol	8.8
5-Methyl-2-furaldehyde	8.9
Dihydro-2(3H)-furanone	9.3
Phenylacetaldehyde	9.5
Acetophenone	9.6
α-Methylbenzyl alcohol	11.0
Phenethyl acetate	11.1
o-Methoxyphenol	11.4
Isopentyl benzoate[a]	11.5
Phenethyl alcohol	12.0
2-Phenyl-2-butenal[a]	12.3
Methyl pyrrol-2-yl ketone[c]	12.6
4-Methyl-2-phenyl-2-pentenal[a]	12.6
Phenol	12.9
Pyrrole-2-carboxaldehyde[c]	13.0
5-Methyl-2-phenyl-2-hexenal[a]	13.6

[a] Not previously reported present in cocoa flavor. [b] Identified by headspace analysis. [c] Also found in basic fraction.

From the known pathways of the Maillard reaction, it can be assumed that an addition product between glucose and ammonia loses water to form glucosylamine, which yields 1-amino-1-deoxy-2-fructose through an Amadori rearrangement. Progressive 2,3-enolization with loss of the amino group gives a methyl-α-dicarbonyl intermediate. This can undergo further hydrolytic cleavage, yielding fragments such as glycolaldehyde, 1-hydroxy-2-propanone, pyruvaldehyde, 2,3-butanedione, and 3-hydroxy-2-butanone (Hodge, 1967). These fragments could condense with NH_3 to form methyl-substituted pyrazines. We obtained considerable yield of 2,3,5,6-tetramethylpyrazine from a 3-hydroxy-2-butanone–ammonia reaction. From the reaction of rhamnose with ammonia, a mixture of methyl- and ethyl-substituted pyrazines was isolated in about 0.5% yield. Here 2-oxobutyraldehyde and 2,3-pentanedione must form as hydrolytic cleavage fragments in addition to those mentioned above. The composition of the pyrazine mixtures obtained from reactions between glucose and rhamnose with ammonia are listed in Table II. Although the difference between refluxing sugars in aqueous ammonia and roasting cocoa beans is considerable, the similarity in composition of the pyrazine mixtures (Table II) and the basic fraction (Table I) is remarkable.

A characteristic part of cocoa aroma resided in the neutral fraction. After standing for 2 weeks, its cocoa character diminished and many peaks of low I_E value disappeared, suggesting the importance of highly volatile substances to the over-all aroma. Headspace analysis furnished data concerning this volatile topnote of the cocoa nibs distillate. The neutral fraction was also fractionated on a packed 6-foot × 1/4-inch o.d. Carbowax 20M column and the effluent sniffed. A distinct cocoa odor could not be associated with any single gas chromatographic peak. However, a cocoa-like odor appeared at I_E about 3. A mixture of isovaleraldehyde and methyl disulfide eluted at this point. This observation is consistent with the work of Rohan (1967), who measured the potential chocolate strength of cocoa beans from the height of

Table II. Pyrazine Compounds Formed in the Reaction of Glucose and Rhamnose with Ammonia[a]

Compound	Glucose + Ammonia, Parts by Weight	Rhamnose + Ammonia, Parts by Weight
Pyrazine	0.9	1.6
2-Methylpyrazine	78.7	30.0
2,5-Dimethylpyrazine	2.4	16.0
2,6-Dimethylpyrazine	13.5	25.4
2,3-Dimethylpyrazine	2.7	
2,3,5-Trimethylpyrazine	0.9	
2-Ethylpyrazine		5.7
2-Ethyl-5-methylpyrazine		1.8
2-Ethyl-3-methylpyrazine		10.8
2-Ethyl-3,5-dimethylpyrazine		1.1
2-Ethyl-3,6-dimethylpyrazine		1.2
2-Ethyl-3,5,6-trimethylpyrazine		0.8

[a] D-glucose or L-rhamnose (100 grams), ammonium hydroxide (28%; 40 ml.), and water (100 grams) boiled under reflux for 2 hours. Distillate (100 ml.) extracted with dichloromethane yielded 0.4 to 0.5 gram of pyrazine compounds. Other extractable compounds not observed.

Table III. Mass Spectral Data of Alkyl Substituted Pyrazines and 2-Phenyl-2-Alkenals Isolated from Cocoa Nibs Distillate as Compared with Standard Compounds

2-Ethylpyrazine			2-Ethyl-5-methylpyrazine			2-Ethyl-5,6-dimethylpyrazine			2-Ethyl-3,5,6-trimethylpyrazine			2-Phenyl-2-butenal			4-Methyl-2-phenyl-2-pentenal			5-Methyl-2-phenyl-2-hexenal		
m/e	Isolated	Syn.	m/e	Isolated	Syn.	m/e	Isolated	Syn.	m/e	Isolated	Syn.	m/e	Isolated	Syn.	m/e	Isolated	Syn.	m/e	Isolated	Syn.
108	80.2	84.5	122	82.2	81.0	136	81.0	80.3	150	92.0	94.9	146	86.7	90.0	174	100.0	100.0	188	74.5	88.1
107	100.0	100.0	121	100.0	100.0	135	100.0	100.0	149	100.0	100.0	118	23.3	24.8	159	30.0	40.5	132	36.9	33.3
81	13.6	14.3	107[a]	5.4	5.2	108	19.0	19.3	122	24.3	20.5	117	100.0	100.0	145	29.5	32.8	117	100.0	100.0
80	24.4	26.0	94	17.8	18.2	80	7.1	7.3	67	17.1	19.6	116	32.2	33.0	131	53.3	52.0	116	38.2	34.0
53	21.6	23.4	56	22.5	24.3	56	16.0	8.5	56	16.7	17.8	115	70.0	70.6	117	30.0	32.8	115	66.5	69.2
52	18.2	21.4	54	17.1	12.2	54	30.2	33.8	54	27.4	28.0	91	47.8	44.0	115	39.4	36.6	104	77.0	67.1
51	10.2	11.7	53	11.6	10.1	53	19.8	21.1	53	34.2	38.3	78	17.8	19.3	103	57.6	59.7	103	39.0	32.0
42	11.4	15.5	52	10.1	9.4	52	13.5	13.6	42	35.0	32.2	63	21.1	18.4	91	52.0	48.1	91	53.7	51.6
40	18.2	15.6	42	14.7	13.5	42	35.7	40.8	41	17.5	17.7	51	25.5	20.1	77	30.0	28.8	43	45.0	43.1
39	27.9	25.4	40	13.2	11.5	39	33.6	31.0	39	25.8	26.6	39	31.1	25.7	51	28.0	21.2	41	43.0	33.3
			39	38.8	35.7															

[a] m/e 107 included, since it is a characteristic fragment of this isomer.

a so-called "cocoa peak." Another peak having an I_E value of 13.6 could be associated with cocoa flavor. This compound was identified as 5-methyl-2-phenyl-2-hexenal. Mass spectral analysis showed the presence of homologous compounds with molecular weights of 146, 174, and 188. Two distinct compounds of molecular weight 188 had similar spectra, suggesting the presence of cis and trans isomers. Since cocoa aroma contains large quantities of acetaldehyde, isobutyraldehyde, isovaleraldehyde, and phenylacetaldehyde, it was postulated that these unknowns were dehydrated aldol condensation products between phenylacetaldehyde and the other aldehydes. Subsequent synthesis of these condensation products and comparison of their chromatographic and mass spectral properties with those of the substances obtained from cocoa confirmed this assumption.

According to organoleptic evaluation, 5-methyl-2-phenyl-2-hexenal possesses a deep bitter persistent cocoa note. Odor assessment of the observed peaks was of great help in identifying methyl sulfide, 2,3-butanedione, and methional. The compounds identified are listed in Table I. From quantitative information based on gas chromatography, acetaldehyde, isobutyraldehyde, isovaleraldehyde, benzaldehyde, phenylacetaldehdye, 5-methyl-2-phenyl-2-hexenal, 2-furaldehyde, methyl disulfide, and isopentyl acetate were considered to contribute to cocoa aroma. Compounds identified in the headspace fraction are indicated as such. The acid fraction contributed to the cocoa taste characteristics, but cannot be associated with cocoa aroma, as it possessed a slightly phenolic type odor.

The identity of the acids was confirmed by comparing the mass spectra of their methyl esters with those of commercially available samples. The following acids were identified: acetic, propionic, isobutyric, isovaleric, valeric, 4-methylvaleric, hexanoic, heptanoic, octanoic, nonanoic, decanoic, dodecanoic, tetradecanoic, hexadecanoic, benzoic, phenylacetic, and crotonic. In addition, 2,3-dimethylphenol was found in the acid fraction.

IDENTIFICATIONS

Pyrazines. Mass spectral data of natural compounds were compared with mass spectral data of synthetic compounds obtained under similar conditions. Compounds not commercially available were synthesized by known procedures (Flament and Stoll, 1967; Marion, 1967; Rizzi, 1968).

All but two of the pyrazine compounds listed in Table I were previously reported in cocoa aroma. The presence of 2-ethyl-5-methyl-pyrazine reported by Rizzi (1967) was not confirmed by Marion *et al.* (1967), the latter presenting evidence for the presence of 2-ethyl-6-methyl-pyrazine. Mass spectral data (10 most intense peaks) of the isolated and synthesized pyrazines not reported by either of the above-mentioned authors are compared in Table III. The presence of 2-ethyl-3,5,6-trimethyl-pyrazine has been reported by Flament *et al.* (1967).

2-Phenyl-2-alkenals. The 2-phenyl-2-alkenals were prepared from phenylacetaldehyde with acetaldehyde, isobutyraldehyde, and isovaleraldehyde, respectively, by base-catalyzed aldol condensation followed by dehydration (Nielsen and Houlihan, 1968). The structures of the pure 2-phenyl-2-alkenals, separated by preparative gas chromatography, were confirmed by infrared and NMR-spectroscopy.

2-Phenyl-2-butenal is a pungent liquid having the following physical properties: Infrared 2800, 2700, 1680, 1630, 730, and 700 cm.$^{-1}$ NMR τ = 0.50(1), 2.77(5, multiplet), 3.35(1, quartet), and 8.12 (3, doublet).

4-Methyl-2-phenyl-2-pentenal is a liquid having the following physical properties: Infrared 2800, 2700, 1675, 725, and 708 cm.$^{-1}$ NMR τ = 0.27(1), 2.78(5, multiplet), 3.62(1, doublet), 7.30 (1, multiplet), and 8.95(6, doublet.

5-Methyl-2-phenyl-2-hexenal is a liquid having the following physical properties: Infrared 2800, 2700, 1675, 1630, 725, and 700 cm.$^{-1}$ NMR τ = 0.43(1), 2.80(5, multiplet), 3.41(1, triplet), 7.80(2, triplet), 8.30(1, multiplet), and 9.12(6, doublet).

Mass spectral data of the isolated and synthesized compounds are compared in Table III.

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