The Occurrence of Simple Alkylpyrazines in Cocoa Butter

Seven alkyl-substituted pyrazines were isolated from a chocolate aroma complex obtained via steam distillation of cocoa butter. The pyrazines which were identified comprised a significant fraction of the odoriferous basic substances present in cocoa butter volatiles.

Davison and Wiggins (1956), Deck and Chang (1965), Dietrich *et al.* (1964), Kosuge and Kamiya (1962), Kosuge *et al.* (1962), Mason *et al.* (1966), and Viani *et al.* (1965) have presented evidence for the presence of alkylpyrazines in a wide variety of foodstuffs and food-related products. In this connection the author reports his observation of a similar series of low molecular weight alkylpyrazines in cocoa butter.

The more volatile components of cocoa butter were separated from the bulk of fatty material by steam distillation at 200° C. and 3 mm. of Hg. The isolated volatiles were dissolved in ether and the resulting solution was extracted with dilute hydrochloric acid to separate a crude basic fraction (0.05% of the original butter weight).

The complex mixture of basic components thus obtained was analyzed by a gas chromatography–mass spectrometry technique similar to one described by Watson and Biemann (1965). An F & M Model 810 gas chromatograph was employed in conjunction with an Atlas CH-4 mass spectrometer. Individual mass spectra were obtained for materials which appeared as peaks in the gas chromatogram of the cocoa butter basic fraction. A typical chromatogram showing partial resolution of the basic components is presented in Figure 1. Compound I (Figure 1) was immediately recognized as methylpyrazine by comparison of its mass spectrum with a similarly obtained spectrum of the authentic compound. The fragmentation patterns of the remaining components closely resembled I, thus suggesting the presence of a series of homologous alkylpyrazines with molecular weights ranging from 94 (methylpyrazine) to 150.

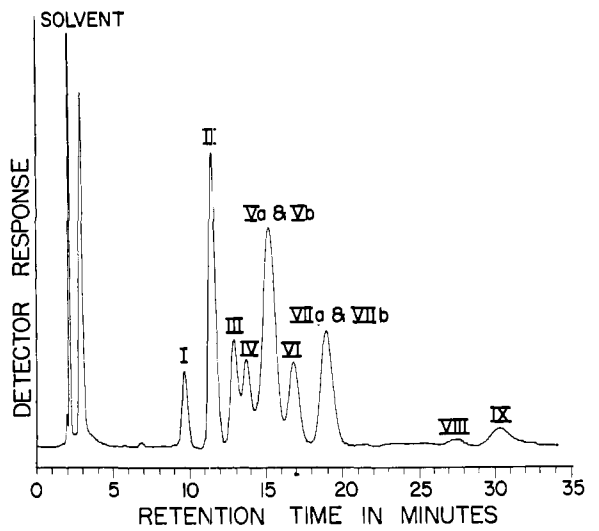

Figure 1. Gas chromatogram of the volatile basic fraction obtained from cocoa butter

610 × 0.635-cm. 18% DEGS column at 135° C.
He flow 50 ml./minute. Detector: thermal conductivity

Seven components from the mixture were isolated by repeated preparative gas chromatography on a 610 × 0.635-cm. column packed with 18% diethylene glycol succinate on silanized 60- to 80- mesh Chromosorb W and a 305 × 0.635-cm. column packed with 15% fluorinated silicone (SF-96) on silanized 60- to 80-mesh Chromosorb W.

The components identified were methylpyrazine (I), 2,3-dimethylpyrazine (III), 2-ethyl-5-methylpyrazine (IV), trimethylpyrazine (Va), 2,5-dimethyl-3-ethylpyrazine (Vb), 2,6-dimethyl-3-ethylpyrazine (VI), and tetramethylpyrazine (VIIa). Identifications were made by comparison of gas chromatography retention times and infrared spectra of the isolated compounds with those of authentic materials. Isolates VIIb, VIII, and IX were not identified but their mass spectra suggested they were higher homologs of methylpyrazine (I), having molecular weights of 136, 150, and 150, respectively. Gas chromatography and mass spectral data did not readily distinguish 2,5-dimethylpyrazine from the 2,6-isomer, but the infrared spectrum of isolate II indicated that it was probably a mixture of these two substances. The spectrum of II exhibited the unique absorption bands of 2,5-dimethylpyrazine (7.56, 9.69, 10.45, and 11.44 microns) and of 2,6-dimethylpyrazine (7.83, 7.98, 9.91, 10.71, and 14.17 microns) in addition to all the bands which both dimethyl isomers have in common.

Reference Standards

All pyrazines used as standards were either obtained commercially or synthesized via published procedures.

Methylpyrazine and 2,5-dimethylpyrazine were obtained from the Aldrich Chemical Co., Inc., Milwaukee, Wis. Tetramethylpyrazine and 2,6-dimethylpyrazine were purchased from K and K Laboratories, Inc., Plainview, N. Y. 2,3-Dimethylpyrazine was synthesized by a two-step procedure starting with ethylenediamine and 2,3-butanedione. These substances were allowed to react by the method of Ishiguro and Matsumura (1958) to form 2,3-dimethyl-5,6-dihydropyrazine. This intermediate was then oxidized with $HgCl_2$ to yield 2,3-dimethylpyrazine according to Gabriel and

Table I. Gas Chromatography and Infrared Data on Pyrazines Isolated from Cocoa Butter

Isolate[a]	GC Column[b]	Rel. Ret. Times[c] Isolate	Known	Known Pyrazine	Characteristic Infrared Bands (Expressed in Microns)[d]
I	A	1.25	1.27	Methyl	7.31 (broad), 7.73, 8.05, 8.52, 8.66, 9.47, 9.81,
I	B	1.35	1.34	Methyl	10.31 (broad), 12.11, 12.41
II	A	1.00	1.00	2,5-Dimethyl	7.29, 7.56, 8.59, 9.69, 10.45, 11.44, 13.59
II	B	0.98	1.00	2,5-Dimethyl	
II	A	1.00	1.00	2,6-Dimethyl	7.28, 7.83, 7.98, 8.61, 9.29, 10.71, 11.65, 13.45,
II	B	0.98	1.00	2,6-Dimethyl	14.17
III	D	1.06	1.05	2,3-Dimethyl	7.35, 8.06, 8.61, 9.91, 10.19, 10.36, 11.36, 11.91,
III	E	1.17	1.15	2,3-Dimethyl	14.01
IV	C	1.20	1.20	2-Ethyl-5-methyl	7.27, 7.41, 7.64, 8.01, 8.61, 9.69, 10.31, 11.26,
IV	E	1.24	1.23	2-Ethyl-5-methyl	12.73
IV	F	0.76[e]	0.76[e]	2-Ethyl-5-methyl	
Va	E	1.37	1.37	Trimethyl	7.30, 7.39, 7.86, 8.00, 8.54, 8.62, 9.92, 10.05, 10.32, 10.65
Va	D	1.65	1.62	Trimethyl	11.24, 13.43, 14.27
Vb	D	2.54	2.58	2,5-Dimethyl-3-ethyl	7.36, 7.61, 7.89, 8.02, 8.53, 8.63, 8.80, 9.56, 9.76, 9.93, 10.42, 11.20, 12.50, 13.59, 14.38
Vb	E	1.44	1.45	2,5-Dimethyl-3-ethyl	
VI	E	1.59	1.61	2,6-Dimethyl-3-ethyl	6.48, 6.81, 7.17, 7.85, 8.05, 8.53, 8.83, 10.07, 10.32, 11.07, 13.51, 14.35
VI	D	2.71	2.62	2,6-Dimethyl-3-ethyl	
VIIa	E	1.80	1.76	Tetramethyl	7.32 (broad), 7.42, 8.22, 8.39, 8.48, 10.10, 12.52,
VIIa	G	1.80	1.78	Tetramethyl	14.32

[a] Compare with Figure 1.
[b] Refer to Table II.
[c] Ratios of retention times (R_t) to the R_t of 2,5-dimethylpyrazine (internal standard, added to each isolate and known material just prior to analysis).
[d] All spectra except those of cocoa isolate VI and 2,6-dimethyl-3-ethylpyrazine were determined in CS_2 solution. These two materials were run as liquid films. Apparatus: Perkin-Elmer Model 137, Infracord spectrophotometer.
[e] Internal standard was 2,5-diethylpyrazine.

Table II. Gas Chromatographic Operating Conditions

Code[a]	Column Dimensions,[b] Cm.	Stationary Phase	Carrier Gas	Flow Rate, Ml./Minute	Temp., ° C.
A	305 × 0.318	10% neopentyl glycol succinate	N_2	20	104
B	305 × 0.318	15% Carbowax 20M	N_2	20	92
C	610 × 0.635	18% diethylene glycol succinate	He	50	140
D	305 × 0.318	15% SF-96	N_2	20	112
E	Column A		N_2	20	113
F	Column A		N_2	20	82
G	Column B		N_2	20	112

[a] Refer to Table I.
[b] All columns were stainless steel; solid support used in every case was 60- to 80-mesh Chromosorb W (acid-washed and silicone-treated).

Pinkus (1893). Trimethylpyrazine and 2,5-dimethyl-3-ethylpyrazine were prepared by allowing 2,5-dimethylpyrazine to react with methyl- and ethyllithium, respectively, according to directions provided by Klein and Spoerri (1951).

In the course of this work, two new pyrazines were prepared whose syntheses and properties are described below. Corresponding NMR data were obtained using a Varian high resolution HR 100 instrument operating at 100 MHz. Samples were analyzed in CCl_4 solution (5%) and all chemical shifts expressed in τ-units were measured relative to tetramethylsilane (internal reference standard).

2-Ethyl-5-methylpyrazine. 2-Ethyl-5-methylpyrazine was prepared by the general method of Strem (1965). Finely pulverized sodamide (0.779 gram, 0.0198 mole) was added to 50 ml. of refluxing, anhydrous liquid ammonia. The slurry which formed was stirred and treated dropwise with 2.16 grams (0.020 mole) of freshly distilled, anhydrous 2,5-dimethylpyrazine over the course of 5 minutes. During this time the formation of a bright red precipitate of 2-methyl-5-pyrazylmethylsodium was observed. The reaction mixture was then treated dropwise with 1.5 ml. (0.0228 mole) of reagent grade methyl iodide, and subsequently (10 minutes) most of the ammonia was allowed to boil off

spontaneously. Reaction products were isolated by adding saturated brine to the residual solid in the flask and extracting the resulting solution with ether. Evaporation of the dried ($MgSO_4$) ether solution yielded 2.27 grams of crude product which was vacuum-distilled. In this way, 1.98 grams of an oil, b.p. 57–68° C. at 11 mm. Hg, were obtained, which was shown by gas chromatographic (GC) analysis on a 305 × 0.635 cm.-column packed with 15% neopentyl glycol succinate on silanized 60- to 80-mesh Chromosorb W to consist of 2-ethyl-5-methylpyrazine (77%) and 2,5-dimethylpyrazine (16%). In addition, two minor products totaling 7% were observed, which were not identified. Analytically pure specimens of both major products were obtained by condensing their vapors from the effluent GC gas stream (He) at −78° C. 2,5-Dimethylpyrazine was immediately characterized by comparison of infrared and retention time data with similar data obtained from the authentic substance.

2-Ethyl-5-methylpyrazine obtained by GC collection was a colorless oil at 25° C. A sample of this material was analyzed.

Calcd. for $C_7H_{10}N_2$: C, 68.82; H, 8.25; N, 22.93. Found: C, 69.08, H, 7.88; N, 23.08.

The NMR spectrum indicated absorptions at: 1.82 τ, singlet, 2 ring protons; 7.26 τ, quartet (J = 8 Hz.), 2 methylene protons; 7.55 τ, singlet, ring methyl; 8.72 τ, triplet (J = 8 Hz.), 3 terminal, ethyl protons.

2,6-Dimethyl-3-ethylpyrazine. The trialkylpyrazine synthesis of Klein and Spoerri (1951) was used to prepare 2,6-dimethyl-3-ethylpyrazine. To 10 ml. of anhydrous ether, previously cooled to 0° C., were added 2.50 ml. (0.0056 mole) of a 2.22M solution of ethyllithium in benzene (Alpha Inorganics, Inc., Beverly, Mass.). The ethereal solution was stirred and maintained at 0° C. while another solution containing 0.600 gram (0.0056 mole) of 2,6-dimethylpyrazine in 5 ml. of ether was added dropwise. The red suspension which formed was stirred for 1 hour at 0° C. and 1 hour at 25° C. Subsequently the reaction mixture was cooled again to 0° C., and water was added carefully to decompose the red salt. Reaction products were isolated from the resulting homogeneous aqueous solution via continuous ether extraction (16 hours). Concentration of the dried ($MgSO_4$) ether phase yielded 0.617 gram of crude product which was evaporatively vacuum distilled. Thus there was obtained 0.409 gram of oily distillate, which was shown by GC analysis (see above preparation) to consist of 2,6-dimethylpyrazine (54%) and 2,6-dimethyl-3-ethylpyrazine (46%). As in the case of 2-ethyl-5-methylpyrazine, both major reaction products were isolated by preparative gas chromatography. 2,6-Dimethylpyrazine was identified by comparison of its infrared spectrum and GC retention time with similar data obtained from the authentic substance.

2,6-Dimethyl-3-ethylpyrazine isolated by GC was a colorless oil at 25° C.

Calcd. for $C_8H_{12}N_2$: C, 70.55; H, 8.88; N, 20.57. Found: C, 69.98; H, 8.81; N, 20.11.

The NMR spectrum of 2,6-dimethyl-3-ethylpyrazine showed absorptions at: 1.95 τ, singlet, 1 ring proton; 7.30 τ, quartet (J = 8 Hz.), 2 methylene protons; 7.58 τ, singlet, ring methyl; 7.62 τ, singlet, ring methyl; 8.76 τ, triplet (J = 8 Hz.), 3 terminal, ethyl protons.

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Received for review December 22, 1966. Accepted April 3, 1967.