

Formation of Volatile Compounds from the Reaction of Leucine and D-Glucose in Propylene Glycol

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With the interest of producing inexpensive replacements for expensive natural flavorings, chemists have intensively studied flavor precursors and their sensory and chemical characteristics produced upon fermentation or heating. Through model reactions of carbohydrates, proteins and lipids, we have gained great insight as to the contribution to flavor of given precursors in a particular foodstuff.

Leucine and glucose mixtures are commonly employed as a foundation for the compounding of cocoa reaction flavors. Comprehensive studies on the volatile flavor products produced between these compounds when heated is not available in literature. Shigematsu et al. (1971) reported the production of 2-(5-hydroxymethyl-2-formylpyrrol-1-yl)-isocaproic acid, lactone upon dry reaction of leucine and glucose at 200°C. They described the flavor of this lactone to be miso, soy sauce and a little chocolate-like.

This paper reports the identification of volatile compounds resulting from the reaction of leucine

and glucose in the common food-grade solvent propylene glycol.

Experimental

Reaction of L-Leucine and D-Glucose

A mixture containing 3.5 g of L-leucine (0.03 mole), 5.0 g of D-glucose (0.028 mole) and 91.5 g of propylene glycol (1.20 mole) was refluxed for two hours at 140°C. After the reaction, 300 ml of distilled water was added and the reaction product was steam-distilled at 4mm Hg. The steam distillate was saturated with sodium chloride and extracted with 300 ml of diethyl ether. The ether extract was backwashed two times with equal portions of salt-saturated water. The ether extract was then dried over anhydrous sodium sulfate and concentrated to 100 ml on a 15-plate Oldershaw distillation column and then to 5 ml on a Kontes spinning band distillation column.

Fractionation of Reaction Mixture

The concentrate was fractionated on a Hew-

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Table I. Volatile Compounds Identified in Leucine-Glucose Model System

Fraction Number	Compound	Occurrence in Cocoa	Mass Spectral Reference, or Characteristic MS Data, m/z (rel. intensity)
1-1	3-Methyl-1-butanol	van Praag et al. (1968)	ten Noever de Brauw et al. (1980)
1-2-1	3-Methylbutanal	van Praag et al. (1968)	ten Noever de Brauw et al. (1980)
1-2-2	2,4-Dimethyl-1,3-dioxolane		MacLeod et al. (1980)
1-3	2-Methylbutanoic acid	Carlin et al. (1982)	ten Noever de Brauw et al. (1980)
2-2	2-Isobutyl-4-methyl-1,3-dioxolane		Table II
3-2	2-Methylpyrazine	Carlin et al. (1982)	Kinlin et al. (1972)
3-3	2,5-Dimethylpyrazine	Carlin et al. (1982)	Kinlin et al. (1972)
3-4-1	2,6-Dimethylpyrazine	Carlin et al. (1982)	Kinlin et al. (1972)
3-4-2	1-Methyl-2-ethylbenzene		MSDC (1974)
4-5	Trimethylpyrazine	Carlin et al. (1982)	Kinlin et al. (1972)
4-6	2-Isopropyl-5-methyl-2-hexenoic acid	van Praag et al. (1968)	Table II
5-3-1	2-Acetylfuran	Carlin et al. (1982)	ten Noever de Brauw (1980)
5-3-1	5-Methyl-2-furaldehyde	Carlin et al. (1982)	ten Noever de Brauw (1980)
5-3-3	2-Isopropyl-5-methyl-2-hexenal	van Praag et al. (1982)	Table II
5-4	2-(1-Isopropyl-4-methyl-1-pentenyl)-4-methyl-1,3-dioxolane		Table II
6-4	2-Methyl-5(or 6)isopentylpyrazine		100(100), 121(23), 39(16), 109(13), 149(12), 41(10), 42(8), 107(7); M = 164(2)
7-3-1	2,3-Dimethyl-5-isopentylpyrazine		Kitamura and Shibamoto (1981)
7-3-2	2,3,5-Trimethyl-6-isopentylpyrazine		136(100), 121(19), 149(15), 177(10), 137(10), 41(6), 53(6), 135(5); M = 192(1)
8-6	a pyridine		149(100), 204(73), 177(50), 219(42), 176(30), 150(10), 163(10), 205(10); M = 219(42)
10-3	a pyridine		148(100), 163(42), 43(23), 41(17), 149(14), 162(10), 39(9), 164(8); M = 163(42)

lett-Packard 5840A gas chromatograph equipped with a flame ionization detector and a splitter with a split ratio of 10:1. A 10 ft. x 1/8 in. i.d. stainless steel column packed with 10% OV-351 on 60-80 mesh Chromosorb W was employed. The flow rate was 30 ml/min. The column temperature was held at 40°C for 3 minutes then increased by 5° C/min to a holding temperature of 225°C. The chromatogram was divided into ten broad fractions. Each broad fraction was accumulatively collected according to the method of Thompson et al. (1978).

Each of the ten broad fractions was subjected to a second fractionation using a 10 ft. x 1/8 in. i.d. stainless steel column packed with 10% OV-17 on 60-80 mesh Chromosorb W.

Identification of the Gas Chromatographic Fractions

Mass spectrometry was performed on a DuPont 21-490 mass spectrometer with a jet separator interfaced to a Varian Moduline 2700 gas chromatograph with an FID detector, and a 10 ft. x 1/8 in. i.d. stainless steel column packed with 10% OV-101 on 60-80 mesh Chromosorb W. The

flow rate was 30 ml/min. and the column temperature was programmed from 50° to 250°C at 10° C/min. Infrared spectra were obtained on a Beckman Acculab 4 IR spectrometer by using ultramicro sodium chloride cells of 0.1-mm light path. Nuclear magnetic resonance spectra were obtained on a Bruker UM 250 MHz Nuclear Magnetic Resonance Spectrometer.

Preparation of 2-Isopropyl-5-Methyl-2-Hexenal

45 G of 3-methylbutanal (0.52 mole) was added by drops into an agitated 11.3 ml of 1N sodium hydroxide solution at 80°C. The mixture was refluxed for one hour and the organic layer separated. The product was vacuum distilled at 0.2 mm Hg, b.p. 54-57°C.

Mass Spectrum: See Table II

IR Spectrum, cm⁻¹: 2980, 2935, 2852, 1680, 1626, 1465, 1386, 1370, 1072, 910, 758

Preparation of 2-Isopropyl-5-Methyl-2-Hexenoic Acid

It was prepared by mild oxidation of 2-isopropyl-5-methyl-2-hexenal with KMnO₄ according to the method of Volhard (1891).

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Mass Spectrum: See Table II

IR Spectrum, cm^{-1} : 3500-2400, 2958, 1682, 1628, 1462, 1413, 1279, 1260, 1181, 1164

$^1\text{H-NMR}$ (CFCl_3): δ , 0.95 (6H,d), 1.25 (6H,d), 1.76 (1H, m), 2.65 (2H, t), 2.91 (1H, m), 6.78 (1H, t), 10.67 (1H, S)

Preparation of 2-Isobutyl-4-Methyl-1,3-dioxolane

2-Isobutyl-4-methyl-1,3-dioxolane was prepared by the addition of 0.27 g of HCl (0.0075 mole) in 27 g of propylene glycol (0.35 mole) to 15 g of 3-methylbutanal (0.17 mole) and refluxing for 15 hours. The mixture was neutralized with 1 N sodium hydroxide. The product was distilled at 0.2 mm Hg, b.p. 48°C.

Mass Spectrum: See Table II

IR Spectrum: cm^{-1} : 2980, 2862, 1466, 1412, 1382, 1130, 1038, 972

Preparation of 2-(1-Isopropyl-4-Methyl)-1-Pentenyl-4-Methyl-1,3-Dioxolane

It was prepared by a combination of the same molar proportions of 2-isopropyl-5-methyl-2-hexenal, propylene glycol and HCl as in the case of 2-isobutyl-4-methyl-1,3-dioxolane preparation but the reaction mixture was refluxed for 5 days. The product was distilled at 0.01 mm Hg, b.p. 62°C.

Mass Spectrum: See Table II

IR Spectrum, cm^{-1} : 2920, 2872, 1458, 1380, 1115, 1060, 972

Results and Discussion

Volatile compounds identified from the reactions of leucine and glucose in propylene glycol are listed in Table I. Table II lists the mass spectral data of some authentic compounds synthesized.

The Strecker aldehyde of leucine, 3-methylbutanal, was observed to play a major role in the production of volatile compounds identified. 3-Methyl-1-butanol and 3-methylbutanoic acid may result from the reduction or oxidation of 3-methylbutanal. 2-Isopropyl-5-methyl-2-hexenal and 2-isopropyl-5-methyl-2-hexenoic acid result from Aldol condensation of 3-methylbutanal and subsequent oxidation in the case of the acid. The aroma characteristics of 3-methylbutanal have been reported to be malty (Keeney and Day, 1957). The aroma of the synthesized 2-isopropyl-5-methyl-2-hexenal was described as sharp, cocoa powder-like.

Solvent (propylene glycol) interaction with 3-methylbutanal and 2-isopropyl-5-methyl-2-hexenal was quite significant. Acetal formation between propylene glycol and aldehydes formed

Table II. Mass Spectral Data of Synthetic Authentic Compounds

Compound	Mass Spectral Data ^a , m/z (relative intensity)
2-Isopropyl-5-methyl-2-hexenal	26(25), 28(15), 41(58), 43(100), 55(25), 56(17), 67(8), 69(28), 81(8), 83(10), 97(24), 99(27), 111(14), 112(7), 121(6), 124(2), 139(13), 154(4); M = 154(4)
2-Isopropyl-5-methyl-2-hexenoic acid	27(40), 29(24), 41(100), 43(90), 56(73), 59(51), 67(35), 69(56), 81(20), 83(68), 95(42), 97(11), 113(78), 115(41), 127(11), 128(40), 137(31), 139(5), 155(25), 156(3), 170(36), 171(3); M = 170(36)
2-Isobutyl-4-methyl-1,3-dioxolane	27(10), 31(30), 41(32), 43(10), 57(7), 59(43), 69(2), 71(4), 85(11), 87(100), 99(2), 100(2), 143(2); M = 144(0)
2-(1-Isopropyl-4-methyl-1-pentenyl)-4-methyl-1,3-dioxolane	27(10), 31(14), 41(43), 43(18), 55(33), 59(41), 67(8), 69(20), 83(10), 87(76), 95(4), 97(9), 111(21), 113(11), 125(5), 127(3), 139(2), 141(2), 153(3), 155(14), 169(100), 170(12), 197(3), 211(10), 212(1); M = 212(1)

a. The two most intense ions every 14 mass units above 20 are listed.

or used in commercial flavoring has been recognized (Heydaneck and Min, 1976; MacLeod et al., 1980). 2-Isobutyl-4-methyl-1,3-dioxolane synthesized was evaluated to have a fruity, apricot and melon-like aroma. 2-(1-isopropyl-4-methyl-1-pentenyl)-4-methyl-1,3-dioxolane was characterized as having a fruity, painty and apple-like aroma.

Well-recognized caramelization products, 2-acetylfuran and 5-methyl-2-furaldehyde, have been identified in heated glucose (Walter and Fagerson, 1968). Again the interaction of propylene glycol with the breakdown products of heated glucose, acetaldehyde (Heynes et al., 1966), may have resulted in the formation of 2,4-dimethyl-1,3-dioxolane.

Interactions between leucine and glucose are undoubtedly responsible for the formation of the pyrazines observed. 2-Methylpyrazine, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine were reported to be formed upon heating glucose and ammonia (van Praag et al. 1968). Isopentylpyrazines, which were observed, may be due to the interaction of the Strecker aldehyde, isopentanal, with dihydropyrazine intermediates in the manner described by Masuda et al. (1980).

In summary, the thermal interaction between leucine and glucose in propylene glycol results in the formation of a cocoa-like aroma. Analysis of the volatile fraction resulted in the identification of the Strecker aldehyde of leucine, its oxidation and reduction products, aldol condensation product and oxidized aldol condensation product. Acetal formation between aldehydes produced and the solvent propylene glycol was quite significant. Furans, commonly identified glucose degradation products, and pyrazines, well-known interaction products of glucose and amino acid or ammonia, were also identified.

Results obtained should give insight as to the products responsible for the elicitation of commercial cocoa flavors and differences when compared with naturally occurring components.

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