Volatile Compounds Produced by the Reaction of Leucine and Valine with Glucose in Propylene Glycol

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Equimolecular amounts of amino acids (L-leucine and L-valine) and D-glucose were refluxed at 140 °C for 2 h in propylene glycol. The volatiles were isolated by simultaneous steam distillation and extraction. The extracts were separated in advance by column chromatography on silica gel with a benzene–ether gradient. The resulting fractions were analyzed by GC and GC-MS. Twenty-seven compounds, including 13 pyrazines, 6 dioxolanes, 2 aldehydes, 2 acids, and 2 pyridines, were identified. Dioxolanes, aldehydes, and pyrazines are the major volatiles, and the percentages of their total gas chromatographic peak areas are 41.13, 23.67, and 17.69 respectively. Formation pathways and sensory properties are described.

INTRODUCTION

Many model reaction systems that produce chocolate flavors have been reported. Herz and Shallenberger (1960) obtained chocolate aroma by heating leucine at 100 °C and valine at 180 °C with glucose. Hodge (1967) summarized the aromas and flavors reported in heated equimolar mixtures of glucose and amino acids. Chocolate flavor was produced by leucine at 100–150 °C, and by valine at 180 °C. Shigematsu et al. (1971) roasted D-glucose and six alkyl-α-amino acids at 200 or 250 °C in a simple two-component system and isolated a series of 2-(5-hydroxymethyl-2-formylpyrrol-1-yl) alkyl acid lactones. Isovaleric acid lactone and isocapric acid lactone formed in valine and leucine, respectively, possessed miso, soy sauce, and slight chocolate-like aromas. Lane and Nursten (1983) described the odors of more than 400 model systems drawn from 21 amino acids and eight sugars treated under different conditions. Leucine at 100 °C and valine at 180 °C reacting with sugars as well as leucine alone at 140 °C gave cocoa and chocolate aromas.

Recently, a few patents have noted chocolate flavor. Two patents were registered by Hasegawa (1980): one is a mixture of glucose, valine, leucine, and water heated at 115 °C and 100 kg/cm^2 in a N atmosphere for 3 h to obtain a chocolate flavorant; and the other is the reaction mixture of glucose, valine, leucine, and water with stirring at 120 °C for 2 h.

Pinto and Chichester (1966) studied cocoa beans and found that leucine and valine were the predominent free amino acids in the fermented cocoa beans. Voigt et al. (1994) analyzed the free amino acids in the extracts from fermented cocoa beans by HPLC and showed similar results. Rohan and Stewart (1967) roasted cocoa beans at 100–180 °C, and reported that the chocolate flavor produced from cocoa beans roasted at 140 °C was generally preferred compared with the others. Therefore, the reaction temperature 140 °C was adopted in present model reaction system.

Comprehensive studies on the volatile flavor compounds produced by the reaction of valine and glucose, or leucine and valine with glucose, in propylene glycol are not available in literature. Hartman et al. (1983–1984) reported the identification of 20 volatile com-

pounds from the reaction of leucine and glucose in propylene glycol. In the present paper, the volatile compounds resulting from the reaction of leucine and valine with glucose in propylene glycol were identified.

EXPERIMENTAL PROCEDURES

Materials. L-Leucine and L-valine were purchased from Biochemicals, Merck Company, Germany; D-glucose was from Biochemicals, BDH Chemicals Ltd., England; Propylene glycol was of analar grade and from May & Baker Ltd., England; benzene, ether, and pentane were of analar grade, and purchased from J. T. Baker Inc., Phillipsburg, NJ; and sodium sulfate anhydrous (Na$_2$SO$_4$) was a lab reagent from Fluka Company, Germany. Silica gel 60 was used for column chromatography (particle size, 0.040–0.063 mm; Merck Company, Germany). The purities of all chemicals were carefully confirmed by HPLC.

Reaction of L-Leucine, L-Valine, and D-Glucose. A mixture of 750 g of L-leucine (23.4 g, 0.1785 mol), L-valine (13.68 g, 0.1155 mol), D-glucose (52.92 g, 0.294 mol), and propylene glycol (660 g, 8.656 mol) was refluxed at 140 °C for 2 h. The reaction mixture was then cooled to room temperature.

Isolation of the Volatile Compounds. The total reaction mixture (750 g) with 2500 g of distilled water was simultaneously distilled and extracted into pentane (200 mL) with a modified Likens and Nickerson apparatus (Heinzler and Eichner, 1991) for 2 h. After distillation, 1.55 g of Na$_2$SO$_4$ was added to absorb any possible water in the extract. The pentane was then removed from the extract by distillation with a 25-cm Vigreux column. The weight of volatile concentrates obtained from 750 g of reaction mixture was 4.04 g.

Fractionation of Volatile Concentrates. The volatile concentrates (4.04 g) obtained from the isolation of volatile compounds were separated by partition column chromatography (Shigematsu et al., 1972). The concentrates were charged on the column (3 cm o.d. × 30 cm) of silica gel 60 that had been packed in benzene, and then the concentrates were successively developed with benzene, a benzene–ether mixture in which the ether content was increased 10% for every 200 mL each, and finally ether. The eluates were collected in 11 fractions of 200 mL each and concentrated under reduced pressure. All fractions were analyzed by gas chromatography (GC) and GC-mass spectrometry (MS).

GC Analysis. The GC analyses were done on a Hewlett-Packard 5890 with a flame ionization detector (FID) fitted with a 0.32 mm i.d. × 25 M fused silica capillary column (Uuadrex Corporation) coated with methyl silicone. The column oven

Table 1. Volatile Compounds Identified from the Reaction of Leucine, Valine, and Glucose

peak no.	retention time (min)	compound	MW	relative peak area (%)	MS refs
1	4.59	*cis*-2-isopropyl-4-methyl-1,3-dioxolane	130	17.4	a
2	4.749	*trans*-2-isopropyl-4-methyl-1,3-dioxolane	130	4.43	a
3	6.956	2,5-dimethylpyrazine	108	<0.1	b, c, d
4	7.937	*cis*-2-isobutyl-4-methyl-1,3-dioxolane	144	14.35	b, e
5	8.031	1-ethyl-2-methylbenzene	120	0.71	b
6	8.25	*trans*-2-isobutyl-4-methyl-1,3-dioxolane	144	4.07	b, e
7	8.274	1-ethyl-4-methylbenzene	120	<0.1	b
8	9.399	2-ethyl-6-methylpyrazine	122	2.18	b, d, f
9	9.646	2-ethyl-5-methylpyrazine	122	0.36	b, f
10	9.827	trimethylpyrazine	122	0.11	b, c, d
11	14.067	(*Z*)-2-isopropyl-5-methyl-2-hexenal	154	18.52	b, e
12	14.343	(*E*)-2-isopropyl-5-methyl-2-hexenal	154	5.15	b, e
13	15.151	2-isobutyl-5-methylpyrazine	150	0.53	f, g
14	16.227	2-isobutyl-6-methylpyrazine	150	1.27	f, g
15	17.418	2,3-dimethyl-5-isobutylpyrazine	164	2.74	b, h
16	17.712	2,5-dimethyl-3-isobutylpyrazine	164	<0.1	b, h
17	18.967	(*Z*)-2-isopropyl-5-methyl-2-hexenoic acid	170	2.47	e
18	19.159	(*E*)-2-isopropyl-5-methyl-2-hexenoic acid	170	0.71	e
19	19.261	2-methyl-5 (or 6)-isopentylpyrazine	164	1.69	d, e
20	20.046	*cis*-2-(1-isopropyl-4-methyl-1-pentenyl)-4-methyl-1,3-dioxolane	212	0.88	e
21	20.887	3,5-diisopropylpyridine	163	<0.1	b, e
22	21.102	*trans*-2-(1-isopropyl-4-methyl-1-pentenyl)-4-methyl-1,3-dioxolane	212	<0.1	e
23	21.388	2,5-dimethyl-3-isopentylpyrazine	178	6.72	b
24	21.771	2,3-dimethyl-5-isopentylpyrazine	178	0.57	h
25	22.588	2,3,5-trimethyl-6-isopentylpyrazine	192	1.52	e, f
26	22.784	2-isobutyl-3-methyl-6-ethylpyrazine	178	<0.1	f
27	26.028	an alkylpyridine	219	0.37	e
				total peak area 86.75	

[a] MacLeod et al. (1980). [b] Budde et al. (1989). [c] Kato et al. (1973). [d] Kinlin et al. (1972). [e] Hartman et al. (1983–1984). [f] Vernin et al. (1992). [g] Baltes and Bochmann (1987). [h] Masuda et al. (1980).

temperature was programmed from 70 to 250 °C at a rate of 3 °C/min. The helium carrier gas flow was 1 mL/min, the injector temperature was 100 °C, and the detector temperature was 280 °C.

GC-MS Analysis. A Hewlett-Packard 5890 A mass spectrometer was used for MS identification of the GC components (Hewlett-Packard 5890 Series II) under the following conditions: fused silica capillary column (0.32 mm i.d. × 25 M) coated with methyl silicone; oven temperature, programmed from 70 to 250 °C at a rate of 2 °C/min; carrier gas, helium; flow rate, 1 mL/min; electron ionization, 70 eV; ion source temperature, 200 °C.

Identification of the Volatile Compounds. Identification of the volatile compounds in the fractions was mostly based on the GC-MS results and confirmed by at least one reference mass spectrum (Budde et al., 1989) or by previously published literature (Kinlin et al., 1972; Kato et al., 1973; MacLeod et al., 1980; Masuda et al., 1980; Hartman et al., 1983–1984; Baltes and Bochmann, 1987; Vernin et al., 1992).

RESULTS AND DISCUSSION

Major Volatile Compounds Generated from the Reaction. The reaction mixture was assessed by three experienced flavorists. It smelled sweet, soy sauce-like, chocolate-like and roasted. Volatile compounds isolated from the reaction mixture of leucine and valine with glucose in propylene glycol are listed in Table 1 in order of elution from the GC column (a typical gas chromatogram is shown in Figure 1). The main constituents of the volatiles were dioxolanes, hexenals, and pyrazines. The percentages of their total GC peak areas were 41.13, 23.67, and 17.69, respectively.
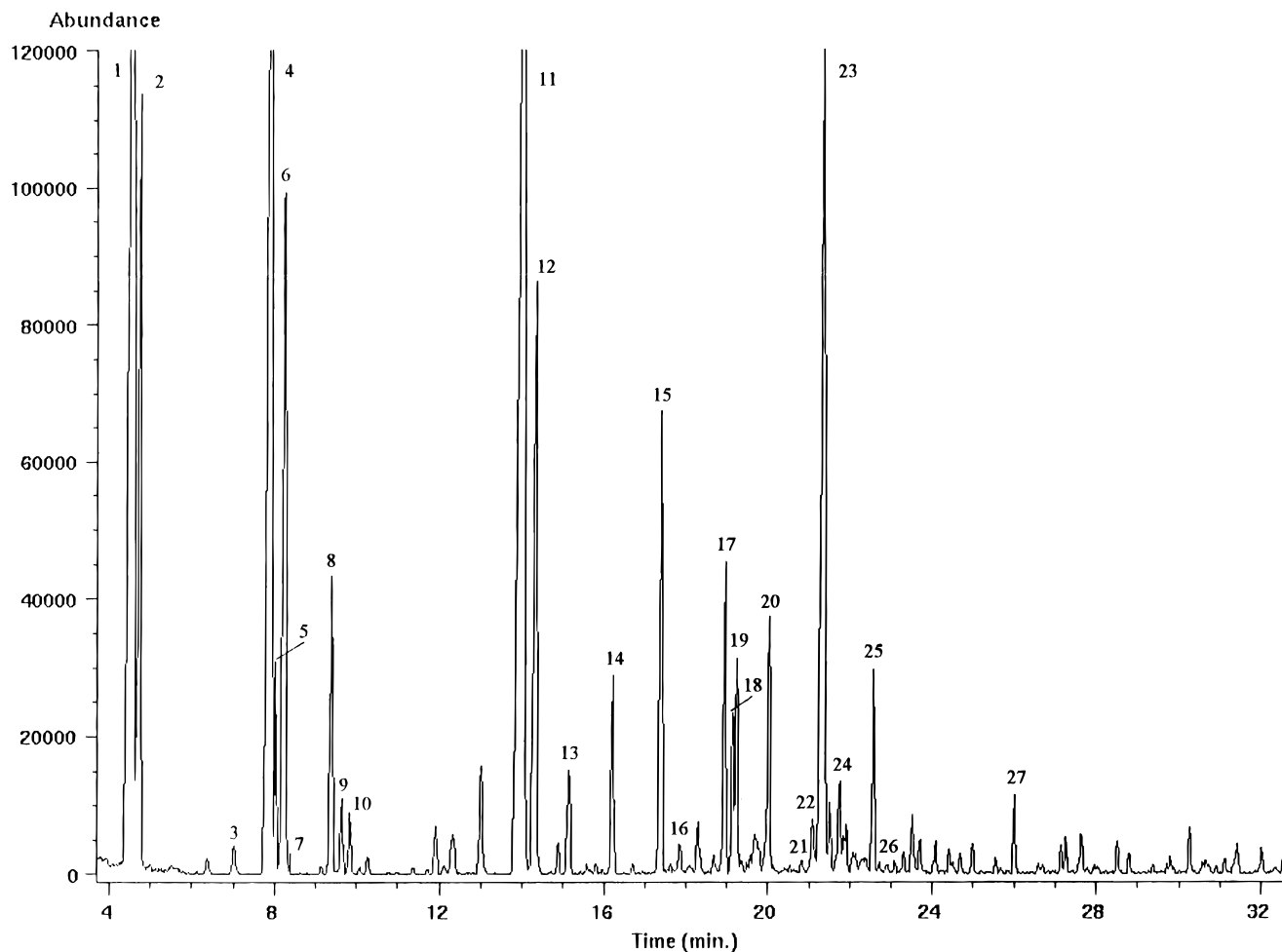
Dioxolanes. Because the MS data were collected only after 4 min, the lower boiling point compounds, such as alcohols, aldehydes, and furans, would be lost. Six dioxolanes were identified from volatile compounds. These acetals [2-isopropyl-4-methyl-1,3-dioxolane (**1** and **2**), 2-isobutyl-4-methyl-1,3-dioxolane (**4** and **6**), and 2-(1-isopropyl-4-methyl-1-pentenyl)-4-methyl-1,3-dioxolane

(**20** and **22**)] might be formed by interaction of propylene glycol (solvent) with isobutanal (the Strecker aldehyde of valine), isopentenal (the Strecker aldehyde of leucine), and 2-isopropyl-5-methyl-2-hexenal (the aldolized product of isopentanal), respectively.

Dioxolanes have not been reported as volatile flavor compounds of cocoa beans, but a few have been identified in some foods of plant origin (e.g., grapes, wines, cranberries, bilberries, and tomatoes; Macleod et al., 1980; Hartman et al., 1983–1984). 2-Iospropyl-4-methyl-1,3-dioxolane smelled etherish, menthol-like, and fruity. 2-Isobutyl-4-methyl-1,3-dioxolane was evaluated to have a fruity, apricot-like, and melon-like aroma, and 2-(1-isopropyl-4-methyl-1-pentenyl)-4-1,3-dioxolane was characterized as possessing a fruity, painty, and apple-like aroma.

Aldehydes. The Strecker aldehydes of valine and leucine, 2-methylpropanal and 3-methylbutanal, respectively, were observed to play a major role in the production of the volatile compounds identified. The formation of dioxolanes might result from the condensation of propylene glycol and the Strecker aldehydes of valine and leucine. 2-Isopropyl-5-methyl-2-hexenal and 2-isopropyl-5-methyl-2-hexenoic acid resulted from aldol condensation of 3-methylbutanal and subsequent oxidation for the acid. The aroma of 3-methylbutanal has been reported to be malty and the aroma of 2-isopropyl-5-methyl-2-hexenal has been described as a sharp, cocoa powder-like (Hartman et al., 1983–1984).

Pyrazines. Thirteen pyrazines were detected in the volatiles. These pyrazines have been isolated from natural cocoa products (Hoskin and Dimick, 1984) and identified in many model systems of sugar and amino compounds (Takayuki, 1983). Interaction between leucine, valine, and glucose was undoubtedly responsible for the formation of the pyrazines observed. It is surprising that nine isobutyl and isopentylpyrazines were observed. The isobutylpyrazines and isopen-

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Figure 1. Gas chromatogram of volatiles formed by the reaction of L-leucine and L-valine with glucose in propylene glycol.

tylpyrazines were <25.7% and 59.4%, respectively, of the total pyrazine peak area.

The formation of isobutyl and isopentylpyrazines, which were observed, may be due to the interaction of the Strecker aldehydes, isobutanal, and isopentanal with dihydropyrazine intermediates in the manner reported by Masuda et al. (1980). The isobutylpyrazines were described as sweet, coffee-like, and caramel-like, and the isopentylpyrazines smelled sweet and like roasted nut (Fors, 1983).

LITERATURE CITED

Baltes, W.; Bochmann, G. Model reactions on roast aroma formation: 1. Reaction of serine and threonine with sucrose under the conditions of coffee roasting and identification of new coffee aroma compounds. *J. Agric. Food Chem.* **1987**, *35*, 340−346.

Budde, W. L.; Lias, S. G.; Heller, S. R.; Milne, G. W. A. In *The Wiley/NBS Registry of Mass Spectral Data*; McLafferty, F. W., Stauffer, D. B., Eds.; Wiley: Canada, 1989.

Fors, S. Sensory properties of volatiles Maillard reaction productions and related compounds: a literature review. In *The Maillard Reaction in Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; American Chemical Society: Washington, DC, 1983; pp 212−231.

Hartman, G. J.; Scheide, J. D.; Ho, C.-T. Formation of volatile compounds from the reaction of leucine and D-glucose in propylene glycol. *Perfum. Flavor* **1983−1984**, *8*, 81−86.

Hasegawa, T., Co., Ltd. Chocolate flavourant. Tokyo Koho (Patent) 80 11, 299 and 80 11, 300, 1980.

Heinzler, M.; Eichner, K. The role of Amadori compounds during cocoa processing. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 445−450.

Herz, W. J.; Shallenberger, R. S. Some aromas produced by simple amino acid and sugar reaction. *Food Res.* **1960**, *25*, 491−496.

Hodge, J. E. Nonenzymic browning reactions. In *The Chemistry and Physiology of Flavors*; Schultz, H. W., Ed.; AVI Publishing: Westport, CT, 1967; pp 465−491.

Hoskin, J. C.; Dimick, P. S. Role of nonenzymatic browning during the processing of chocolate: a review. *Process Biochem.* **1984**, *19*, 92−102.

Kato, S.; Kurata, T.; Fujimaki, M. Volatile compounds produced by the reaction of L-cysteine or L-cystine with carbonyl compounds. *Agric. Biol. Chem.* **1973**, *37*, 339−544.

Kinlin, T. E.; Muralidhara, R.; Pittet, A. O.; Sanderson, A.; Walradt, J. P. Volatile compounds of roasted filberts. *J. Agric. Food Chem.* **1972**, *20*, 1021−1028.

Lane, M. J.; Nursten, H. E. The variety of odors produced in Maillard model systems and how they are influenced by reaction conditions. In *The Maillard Reaction in Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; American Chemical Society: Washington, DC, 1983; pp 141−158.

MacLeod, G.; Mehdi, S.-A.; MacLeod, A. J. Substituted 4-methyl-1,3-dioxolanes: solvent interaction products in some commercial beef flavorings. *J. Agric. Food Chem.* **1980**, *28*, 441−446.

Masuda, H.; Tanaka, M.; Akiyama, T.; Shibamoto, T. Preparation of 5-substituted 2,3-dimethylpyrazines from the reaction of 2,3-dimethyl-5,6-dihydropyrazine and aldehydes or ketones. *J. Agric. Food Chem.* **1980**, *28*, 244−246.

Pinto, A.; Chichester, C. O. Changes in the content of free amino acids during roasting of cocoa beans. *J. Food Sci.* **1966**, *31*, 716−732.

Rohan, T. A.; Stewart, T. Precursors of chocolate aroma: studies in the degradation of amino acids during the roasting of Accra cocoa beans. *J. Food Sci.* **1967**, *32*, 625−629.

Shigematsu, H.; Kurata, T.; Kato, H.; Fujimaki, M. Formation of 2-(5-hydroxymethyl-2-formylpyrrol-1-yl)-alkyl acid lactones on roasting alkyl-α-amino acid with D-glucose. *Agric. Biol. Chem.* **1971**, *35*, 2097−2105.

Shigematsu, H.; Kurata, T.; Kato, H.; Fujimaki, M. Volatile compounds formed on roasting DL-α-alanine with D-glucose. *Agric. Biol. Chem.* **1972**, *36*, 1631−37.

Takayuki, S. Heterocyclic compounds in browning and browning/nitrite model systems: occurrence, formation mechanisms, flavor characteristics and mutagenic activity. In *Instrumental Analysis of Foods*; Charalambous, G., Inglett, G., Eds.; Academic: New York, 1983; pp 229−278.

Vernin, G.; Debrauwer, L.; Vernin, G. M. F.; Zamkotsian, R.-M.; Metzger, J.; Larice, J. L.; Parkanyi, C. Heterocycles by thermal degradation of Amadori intermediates. In *Off-flavors in Foods and Beverages*; Charalambous, G., Ed.; Elsevier Science: Amsterdam, 1992; pp 567−623.

Voigt, T.; Biehl, B.; Heinrichs, H.; Kamaruddin, S.; Marsoner, G. G.; Hugi, A. In-vitro formation of cocoa-specific aroma precursors: aroma-related peptides generated from cocoa-seed protein by co-operation of an aspartic endoprotease and a carboxypeptidase. *Food Chem.* **1994**, *49*, 173−180.