Studies on Flavor Components of Roasted Barley

Part II. The Major Volatile Carbonyl Compounds

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From the volatiles of roasted barley or those formed during roasting of barley, furfural, 2-methylbutanal, 2-methylpropanal, 3-methylbutanal, 2,3-pentanedione, ethylglyoxal and pyruvaldehyde were isolated and identified as their mono- or bis-2,4-dinitrophenylhydrazones.

Removal of the carbonyl compounds from the volatiles resulted in a loss of the characteristic aroma of roasted barley.

Hrdlicka *et al.*¹⁾ reported that a number of aldehydes and ketones were present in the volatiles of toasted oats. The carbonyls certainly play an important role in the flavor of toasted or roasted foods. Roasted barley contains delicious flavor, but little has been done to identify the specific compounds associated with its typical flavor. One of the authors²⁾ have reported that 1) the most favorable aroma developed when barley was roasted at about 160°C for twenty minutes, 2) the part of aleurone was more reactive than any other parts, and 3) during the roasting process the color gradually changed to brown and carbon dioxide occurred. The present paper deals with the results on the qualitative investigations of the volatile carbonyl compounds from roasted barley.

MATERIALS AND METHODS

Raw barley. General size, produced in Saitama prefecture, Japan.

Authentic 2,4-dinitrophenylhydrazone (2,4-DNPH) of carbonyl compounds. Furfural, 2-methylpropanal, 3-methylbutanal, 2,3-pentanedione and pyruvaldehyde were obtained from commercial sources. Each compound was treated with 2,4-dinitrophenyl-

hydrazine solution prepared from 2 g of the crystalline hydrazine and 1 liter of 2N hydrochloric acid. 2,4-DNPHs of furfural, 2-methylpropanal and 3-methylbutanal were recrystallized from 80% ethanol; bis-2,4-DNPHs of 2,3-pentanedione and pyruvaldehyde were recrystallized from nitrobenzene. Authentic 2-methylbutanal was prepared as follows: the mixture of L-isoleucine and ninhydrin was heated and the resulting volatile was passed through the 2,4-dinitrophenylhydrazine solution,³⁾ and the precipitated 2,4-DNPH was recrystallized from 80% ethanol. Authentic bis-2,4-DNPH of ethylglyoxal was provided by Dr. T. Kurata.⁴⁾

Trapping method of carbonyl compounds

a) Trapping of volatile carbonyls from ground roasted barley. Fig. 1 shows the apparatus used for trapping carbonyl compounds as their 2,4-DNPH derivatives. Raw barley was roasted at about 160°C for 20 min. After cooling to room temperature, the roasted barley was finely ground and put into the round bottom flask as shown in Fig. 1. To 1 kg of roasted barley powder, 2.3 liters of distilled water was added with stirring. The sample was maintained at 45°C throughout the experiment with the aid of a muffleheater. Nitrogen was bubbled into the sample and the inner pressure was reduced to about 20 mm Hg with an aspirator. Most of the carbonyls were trapped and precipitated in trap C, small amounts of them in trap D, but trap E remained unchanged

1) J. Hrdlicka and G. Janiček, *Nature*, **201**, 1223 (1964).

2) P. S. Wang and Y. Sakurai, *J. Japanese Soc. Food and Nutrition*, (Abstract paper) **19**, 116 (1966).

3) W. S. Fones, *J. Am. Chem. Soc.*, **76**, 1377 (1954).

4) T. Kurata and Y. Sakurai, *Agr. Biol. Chem.*, **31**, 177 (1967).

FIG. 1. Trapping Apparatus Used for Ground Roasted Barley.

- A: Distillation flask with a muffle heater
 B: Buffering bottle
 C, D and E: Trapping bottle, containing 2,4-dinitrophenylhydrazine solution

throughout the experiment, indicating that the carbonyls in volatiles were completely removed. 2,4-DNPH derivatives thus obtained were filtered and washed with 2N hydrochloric acid repeatedly until yellow color disappeared in the filtrate, washed again with water and dried over calcium chloride in a vacuum desiccator. Approximately 30 mg of the 2,4-DNPH mixture was obtained from each experiment.

b) Trapping of volatile carbonyls formed during roasting barley. In order to investigate the volatile carbonyls formed during roasting of barley, the volatiles formed were bubbled directly into the 2,4-dinitrophenylhydrazine solution as shown in Fig. 2. The electric roaster was made by coiling nichrome wire around the quartz glass tube of 54 cm $\times$ 2.5 cm *i.d.* Raw barley was mealed to about 16 mesh just before use and the powder was put in the electric roaster. The inner temperature of roaster was maintained at

$160 \pm 5^\circ\text{C}$ and the flow rate of air was 50~60 ml per min. Soon after roasting commenced, precipitates began to appear in the first trap (trap C), and no precipitate appeared in the last trap (trap E) throughout the experiment. After roasting for 2 hr, to the condensate in buffering bottle (trap B) was added 10 volumes of the saturated solution of 2,4-dinitrophenylhydrazine in 2N hydrochloric acid, and the precipitates were combined with those in trap C and D, and then treated similarly as (a).

Fractionation of the 2,4-DNPH derivatives by column chromatography. The mixture of 2,4-DNPH derivatives obtained above was fractionated by column chromatography on silica-gel. About 220 g of silica-gel of 100~120 mesh particle range was mixed with the mixture of benzene and chloroform (3:1), and then packed into glass column of 3.5 cm *i.d.* Approximately 120 mg of the mixture of 2,4-DNPH derivatives was dissolved in 30 ml of chloroform and transferred to the top of the column, then developed with the mixture of benzene and chloroform (3:1). The colored bands of the 2,4-DNPH were collected separately on elution, then evaporated to dryness.

Thin-layer chromatography of 2,4-DNPH derivatives. Each fraction of the 2,4-DNPH derivatives obtained from column chromatography was still so contaminated that they were isolated by preparative thin-layer chromatography until a completely single band was attained, 2 or 3 times repetition being necessary for this purpose. A glass plate coated 0.25 mm in thickness with Kieselgel G (Merck) was activated for 2 hr at 110°C , on which a solution of 2,4-DNPH dissolved in chloroform or ethyl acetate

FIG. 2. Apparatus Used for Trapping Volatile Carbonyl Compounds Formed during Roasting Process.

A: An electric roaster, made by a quartz glass tube (q), nichrome wire (n), thermometer (t), and silicone rubber plug (p). The sample (s) was put in the roaster with the glass wool (g).

B: A buffering bottle

C, D and E: Trapping bottle, containing 2,4-dinitrophenylhydrazine solution

was dropped to form a thick line. Development was performed by the usual ascending method using the following solvent systems: solvent system I, benzene-chloroform, 3 : 1 (v/v); solvent system II, ethyl acetate-*n*-hexane, 1 : 3 (v/v); solvent system III, petroleum ether (bp 30~70°C) -diethyl ether, 10 : 1 (v/v); solvent system IV, nitrobenzene-chloroform-benzene, 1 : 2 : 3 (v/v); solvent system V, benzene-dioxane-ethyl acetate, 20 : 0.08 : 0.4 (v/v). After development, each component was extracted by chloroform and evaporated in a rotary evaporator. The residues were crystallized from 80% ethanol, chloroform or nitrobenzene.

Ultra-violet and visible spectrometry. The spectrum of each single component of 2,4-DNPH was obtained in chloroform solution with a EPS-3T Hitachi Recording Spectrophotometer.

Infrared spectrometry. Infrared spectra of these single components were obtained in nujol mull with a JASCO Model IR-S Infrared spectrophotometer.

Melting point determination. This was carried out on a heat-stage microscopical apparatus. Melting points were uncorrected.

Regeneration of carbonyls from their 2,4-DNPH derivatives. Regenerations were performed according to the method of Ralls⁵⁾ with a slight modification. Two to 5 mg of 2,4-DNPH derivatives were triturated with 3 times their weight of α -ketoglutaric acid and the mixtures were heated. By using a gas-tight syringe, the regenerated carbonyl vapor was taken from the hot tube immediately after regeneration and injected into the gas chromatograph.

Gas chromatography. Two ml of the regenerated carbonyl vapors were analyzed with Shimadzu Model DC-1C Gas Chromatograph equipped with a hydrogen flame detector using a 10 feet $\times$ 3 mm *i.d.* stainless steel column packed with 10% (w/w) tricresyl phosphate on Diasolid M, 40~60 mesh. Nitrogen was the carrier gas and flow rate was 35 to 40 ml per minute. Column temperature was maintained at $30 \pm 2^\circ\text{C}$.

RESULTS AND DISCUSSION

The precipitates of 2,4-DNPH derivatives obtained by the trapping procedure described above were examined preliminarily with thin-layer chromatography before their separation by column chromatography. Thin-layer chromatogram of the mixture of 2,4-DNPH ob-

tained from the volatiles of roasted barley with solvent system I showed seven main spots. As shown in Fig. 3, 2,4-DNPH from the volatiles formed during roasting barley also showed qualitatively the same pattern. Accordingly, the main carbonyls of roasted barley and those formed during roasting barley were considered to be qualitatively similar. Upon spraying the ethanolic solution of potassium hydroxide, spots 1~3 gave deep brown color, indicating that they were 2,4-DNPH from monocarbonyls. Chromatography on a column of silica-gel with solvent system I yielded seven main bands which were collected separately and termed fractions 1~7, fraction 1 being eluted first. Thin-layer chromatography of these fractions showed that each fraction contained more or less impurities and fractions 1 and 7 contained two or more components. Fraction 1 was separated into two fractions, 1A and 1B, by preparative thin-layer chromatography with solvent system II

FIG. 3. The Thin-Layer Chromatogram of the Mixture of 2,4-DNPH Developed with Solvent System I.

A: 2,4-DNPH of carbonyls from ground roasted barley

B: 2,4-DNPH of carbonyls formed during roasting barley

5) J. W. Ralls, *Anal. Chem.*, **32**, 332 (1960).

FIG. 4. The Thin-Layer Chromatograms of Fraction 1 in Comparison with Authentic 2,4-DNPHs of Carbonyl Compounds with Solvent System II (A) and Solvent System III (B).

TABLE I. IDENTIFICATION DATA ON 2,4-DNPH DERIVATIVES OF CARBONYL COMPOUNDS ISOLATED FROM THE VOLATILES OF ROASTED BARLEY

Compounds	$Rf^*\lambda_{\max}$ ($m\mu$)		Mp (°C)	Mixed mp (°C)	Elementary analyses**			
					C(%)	H(%)	N(%)	
Fraction 1Aa	1.28	358	115~117	122 ~123	{	—	—	—
2-Methylbutanal 2,4-DNPH	1.28	358	125			—	—	—
Fraction 1Ab	1.28	358	114~116	118 ~119	{	—	—	—
3-Methylbutanal 2,4-DNPH	1.28	358	120			—	—	—
Fraction 1B	1.28	357	177~178	178 ~179	{	47.31	4.48	22.02
2-Methylpropanal 2,4-DNPH	1.28	357	179			(47.62)	(4.80)	(22.22)
Fraction 2	1.13	374	211~212	211.5~212	{	47.32	3.12	20.12
Furfural 2,4-DNPH (<i>syn</i>)	1.13	374	212			(47.83)	(2.92)	(20.29)
Fraction 3	1.00	388	222~223	224 ~225	{	47.15	3.04	19.90
Furfural 2,4-DNPH (<i>anti</i>)	1.00	388	225			(47.83)	(2.92)	(20.29)
Fraction 4	0.78	396	276~277	277.5~278	{	44.17	3.25	24.25
2,3-Pentanedione bis-2,4-DNPH	0.78	440(s)	278			(44.34)	(3.47)	(24.34)
	440(s)							
Fraction 5 (Unidentified)	0.70	398 450(s)	—	—	—	—	—	
Fraction 6	0.60	396	241~242	241.5~242	{	—	—	—
Ethylglyoxal bis-2,4-DNPH	0.60	440(s)	242			—	—	—
	440(s)							
Fraction 7A	0.51	395	299~300	299.5~300	{	42.25	2.83	25.47
Pyruvaldehyde bis-2,4-DNPH	0.51	440(s)	300			(41.66)	(2.78)	(25.92)
	440(s)							
Fraction 7B (Unidentified)	0.51	412 450(s)	—	—	—	—	—	

* Rf : Rf of 2,4-DNPH of component/ Rf of 2,4-DNPH of furfural (*anti*) with benzene-chloroform 3:1.

** (): Calculated value

(Fig. 4A). Fraction 1B was a single component which yielded yellow needles on crystallization from hot 80% ethanol. It was identified as 2,4-DNPH of 2-methylpropanal, by thin-layer chromatography, ultra-violet and infra-red absorption spectra (Fig. 5-I), mixture melting point, and elementary analysis (Table I). Similarly, fraction 2 was identified as the

syn form of furfural 2,4-DNPH, and fraction 3 as the *anti* form of furfural 2,4-DNPH (Table I, Figs. 5-II and 5-III). Fraction 1A gave only one spot on thin-layer chromatogram developed with solvent system I or II. However, on development with solvent system III, fraction 1A was separated into two fractions, 1Aa and 1Ab (Fig. 4-B). Both fractions 1Aa

FIG. 5. Infrared Spectra of Fractions 1~7 in Nujol Mull.

— Isolated
 ----- Authentic

- I: 2-Methylpropanal 2,4-DNPH (fraction 1B)
- II: Furfural 2,4-DNPH, *syn* form (fraction 2)
- III: Furfural 2,4-DNPH, *anti* form (fraction 3)
- IV: 2-Methylbutanal 2,4-DNPH (fraction 1Aa)
- V: 3-Methylbutanal 2,4-DNPH (fraction 1Ab)
- VI: 2,3-Pentanedione bis-2,4-DNPH (fraction 4)
- VII: Ethylglyoxal bis-2,4-DNPH (fraction 6)
- VIII: Pyruvaldehyde bis-2,4-DNPH (fraction 7A)

and 1Ab yielded yellow crystals from 80% ethanol. The behavior on thin-layer chromatography, and ultra-violet and infrared absorption spectra of fraction 1Aa (Table I, Fig. 5-IV) were identical with those of 2-methylbutanal 2,4-DNPH. Similarly, fraction 1Ab was identical with 3-methylbutanal 2,4-DNPH. Further evidence was given by gas chromatography. The retention times of the regenerated vapors of fractions 1Aa and 1Ab were same with those of the authentic 2-methylbutanal and 3-methylbutanal, respectively.

On spraying the ethanolic potassium hydroxide, fractions 4~7 gave violet color, being characteristic of the bis-2,4-DNPH derivatives of compounds having adjacent carbonyl groups.⁶⁾ In order to remove the impurities contained in fractions 4~7, fractions 4 and 5 were thin-layer chromatographed with solvent system IV, then crystallized from nitrobenzene, and fraction 6 with solvent system I, then crystallized from chloroform. Fraction 7 was separated into two fractions, 7A and 7B by thin-layer chromatography with solvent system V. Both 7A and 7B were crystallized from nitrobenzene. Fractions 4, 6 and 7A yielded orange-colored crystals and were identified by thin-layer chromatography, ultra-violet and infrared absorption spectra, and mixture melting point, as bis-2,4-DNPHs of 2,3-pentanedione, ethylglyoxal and pyruvaldehyde, respectively. (Table I, Figs. 5-VI, 5-VII and 5-VIII). The crystals obtained from fractions 5 and 7B were very small in amounts and identification could not be attained.

These components identified are the major carbonyls produced when barley are roasted under the conditions described. However, other components are also present, because the mixture of 2,4-DNPH derivatives obtained above was not completely soluble in chloroform, benzene or ethyl acetate. The com-

ponents, which were present in much lower concentrations and ran faster than the 2,4-DNPH derivatives of the C5-aldehydes with solvent system I, may be longer-chain carbonyls.

Among the carbonyls identified above, the amounts of monocarbonyls were much more than those of the dicarbonyls and furfural was most abundant.

Furfural, 2,3-pentanedione, ethylglyoxal and pyruvaldehyde are probably the degradation products of the carbohydrates of barley. 2-Methylpropanal, 2-methylbutanal and 3-methylbutanal could be derived from valine, isoleucine and leucine, respectively, by Strecker degradation.⁷⁾ Among the identified carbonyl compounds, 2,3-pentanedione and ethylglyoxal have been identified for the first time in this paper in the volatiles of cooked or roasted foods.

All the carbonyls detected in the volatile of roasted barley have pungent odors when each of them exists alone and in concentrated state. But when they are mixed and in some low concentrations, they give a somewhat favorable odor. However, on removing the carbonyl compounds by passing the volatiles from roasted barley through the 2,4-dinitrophenylhydrazine solution, the characteristic delicious aroma of roasted barley was lost and changed to somewhat unfavorable odor. This fact indicates that the carbonyl compounds play an important role in the flavor of roasted barley. After removal of carbonyl compounds from volatiles of roasted barley there remains sulfur compounds and others which may also play some roles in the flavor of roasted barley, and these will be elucidated in the following study.

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6) C. Neuberg and E. Strauss, *Arch. Biochem.*, **7**, 211 (1945).

7) A. Schonberg and R. Moubacher, *Chem. Revs.*, **50**, 267 (1952).