BROWN & WILLIAMSON TOBACCO CORPORATION

RESEARCH, DEVELOPMENT & ENGINEERING

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FROM: Mr. K. M. Walker

DATE: May 17, 1984

SUBJECT: CHARACTERIZATION OF SUGAR/AMMONIA REACTION PRODUCTS/358

Preliminary experiments comparing the products of the reactions of glucose, fructose and ammonia run at ambient conditions (AC) and 50°C showed that, in both cases, the formation of alkylimidazoles and/or alkylpyrazoles was favored over pyrazine and alkylpyrazine formation. Chromatograms obtained using a nitrogen-phosphorus detector (NPD) showed that two hours reaction time at 50°C gave about the same amount of products as 30 hours at AC. However, these chromatograms also suggested that larger proportions of pyrazine and alkylpyrazines might have been formed at the higher temperature.

Reaction

The ambient condition (AC) and 50°C reactions were run using the same reaction mixture. This mixture was prepared using 37g of glucose and 37g of fructose dissolved in 242 mL of deionized water and 212 mL of concentrated ammonium hydroxide.

Half of the solution was placed in a 500-mL round bottom flask which contained a teflon coated stirring bar. This flask was stoppered and suspended over a magnetic stirrer. The flask did not touch the surface of the stirrer to avoid heat transfer from the stirrer motor.

The remainder of the solution was transferred to an identical round bottom flask which also contained a stirring bar. This flask was fitted with a closed, water-cooled condenser before it was immersed in a 50°C oil bath atop a magnetic stirrer.

Sampling

A 10.0 mL aliquot was withdrawn from the sugar/ammonia solution when it was prepared. This sample was immediately extracted with 3 x 5 mL portions of dichloromethane, and the organic layer was concentrated to 1.0 mL in a Kuderna-Danish concentrator.

Additional 10.0 mL samples were withdrawn from the AC and 50°C flasks at 1, 2, 4, 7.5, and 30 hours. These samples were immediately treated in the same manner as the first one. Aliquots of the extracts were placed in sealed sample vials which were stored in a freezer until they were analyzed by GC and GC/MS/DS.

At 30 hours, an additional 100 mL sample was taken from the AC reaction flask. This sample was continuously extracted for about 18 hours with dichloromethane in order to recover the more polar reaction products that might not have been extracted in the separatory funnel. The organic layer was concentrated to 10.0 mL via Kuderna-Danish concentrator, and the concentrated extract was stored in a freezer until it was analyzed.

Chromatography

GC analyses were done on a 60 m x 0.32 mm ID CP Wax 57 capillary column mounted in an HP-5880 gas chromatograph. The instrument was equipped with FID, NPD, and an autoinjector. Each sample was analyzed twice, and the NPD Area % reports were stored on magnetic tape for future manipulation. Chromatograms for the original sample and those taken at 2 hours 50°C, 30 hours AC, and the 30-hour AC continuous extract are compared in Figure 1. Relative amounts of the reaction products, taken from NPD Area % reports, are shown in Figure 2.

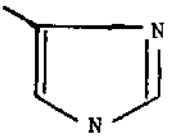
Mass Spectrometry

The 30-hour continuous extract was analyzed by gas chromatography/mass spectroscopy/data system (GC/MS/DS) to identify the reaction products. Separations were done on a 60 m x 0.32 mm ID Supelcowax-10 capillary column. This column is very similar to that used in the HP-5880 GC. Reaction product spectra were compared to those in B&W Libraries. Identified dichloromethane-soluble reaction products are labeled in Figures 1 and 2.

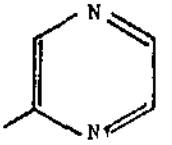
Results & Discussion

The major dichloromethane-soluble reaction product of glucose, fructose and ammonia at AC and 50°C was tentatively identified as 4 or 5-methylimidazole (peak 9 on the chromatograms), MW. 82, which eluted at 41.3 minutes, Figure 1. Two other major products, although not positively identified, are alkylimidazoles or alkylpyrazoles of MW. 110 and 124 which eluted at 33.1 and 33.6 minutes, respectively. These compounds are probably substituted at the number 1 nitrogen because they eluted well before the 4 or 5-methylimidazole despite their greater molecular weights.

Identification of alkylimidazole and alkylpyrazole isomers based solely on mass spectra is not reliable because these spectra are very similar. Therefore, if positive identifications are necessary, the spectral data will have to be supported with the mass spectra and GC retention times of authentic compounds. Furthermore, some of the possible reaction products are not commercially available and would have to be synthesized.



Peak 9



Peak 2

The only pyrazine formed in appreciable quantities was methylpyrazine (peak 2 on the chromatograms) which eluted at 9.8 minutes. Methylpyrazine to methylimidazole peak area ratios suggested that methylpyrazine formation was favored at higher temperatures. The methylpyrazine to methylimidazole peak area ratio was 1:146 for 30 hours AC, 1:190 for the 30-hour AC continuous extract, 1:45 for 2 hours 50°C, and 1:21 for 4 hours 50°C. It should be noted, however, that these are only estimates. Although quantitative preparative techniques were used, the GC method was not calibrated to quantitate any of the products, but rather to determine the effects of reaction times and conditions on the products and to aid in determining what the products were.

K. M. W.

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Attachments

FIGURE 1

NPD CHROMATOGRAMS OF SUGAR/AMMONIA

REACTION PRODUCTS

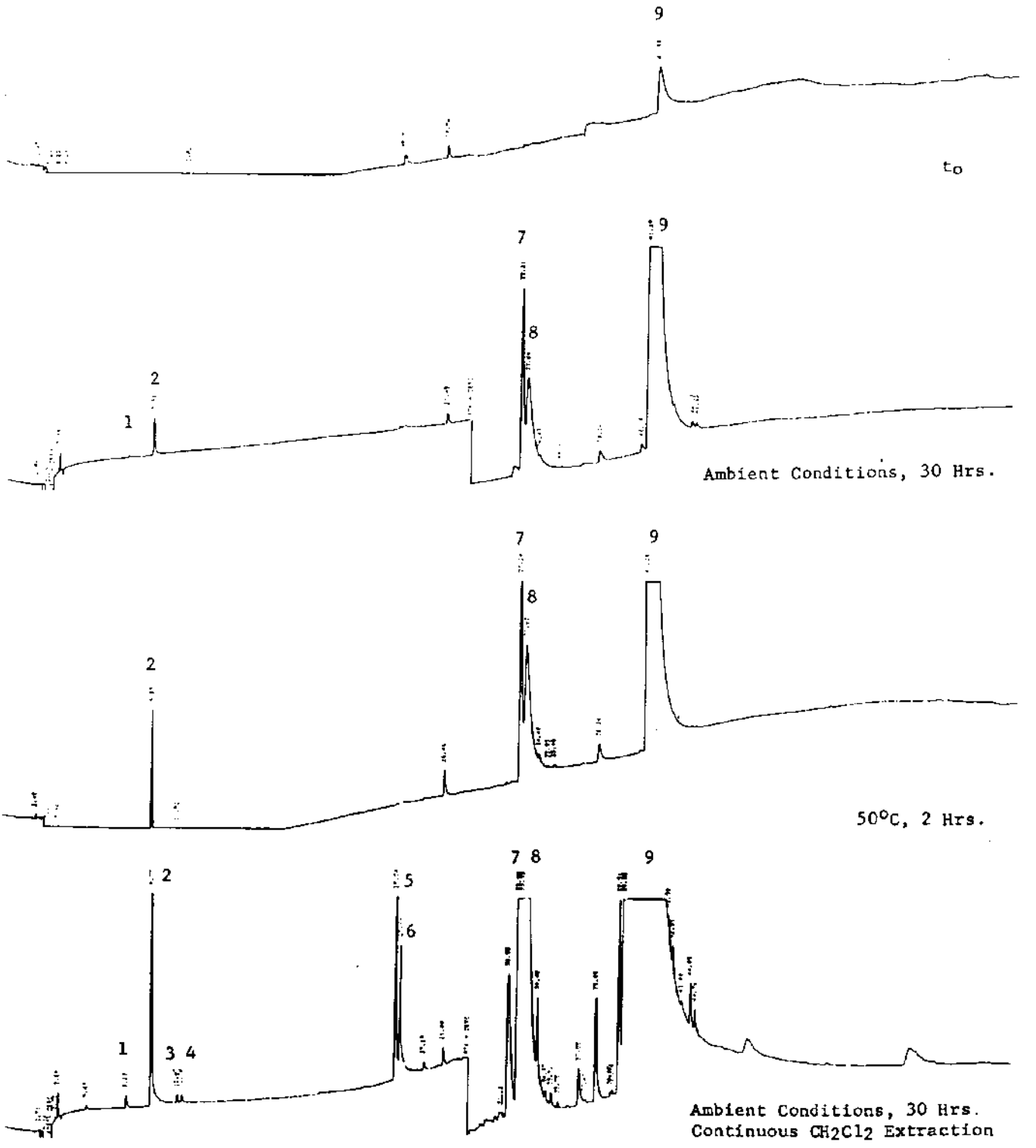

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FIGURE 2

SUGAR/AMMONIA REACTION PRODUCTS

NPD DATA

REACTION PRODUCT	RETENTION TIME	PEAK AREAS, AC 30 HOUR CE	PEAK AREAS, AC 30 HOUR	PEAK AREAS, 50°C 2 HOUR	PEAK AREAS, 50°C 4 HOUR	PEAK AREA t_o
	5.69	3.05	ND	ND	ND	ND
1. Pyrazine	8.23	7.05	ND	ND	ND	ND
2. Methylpyrazine	9.83	310.2	23.90	82.90	642.3	ND
3. 2, 5-Dimethylpyrazine	11.47	3.08	ND	ND	12.73	ND
4. 2, 6-Dimethylpyrazine	11.76	4.29	ND	ND	6.05	ND
	12.23	Trace	ND	ND	0.85	ND
5. Acetamide	25.34	191.8	Trace	ND	Trace	ND
6. Formamide	25.64	108.3	Trace	ND	Trace	ND
	27.19	5.40	ND	ND	Trace	ND
	28.40	13.81	9.24	19.54	ND	ND
	32.18	0.79	ND	ND	ND	ND
	32.53	281.2	Trace	261.2	Trace	ND
7. 1-Ethyl-(?)-methylimidazole (tent.)	33.24	1930	281.3	ND	1428	ND
8. A-C_4-substituted imidazole (tent.)	33.63	3330	351.5	440.0	2846	ND
	34.40	89.17	20.15	19.02	69.48	ND
	34.97	3.58	ND	0.45	ND	ND
	35.31	7.40	ND	1.17	ND	ND
	35.75	2.94	0.74	ND	0.27	ND
	37.05	41.56	Trace	ND	11.91	ND
	37.47	0.92	ND	ND	0.46	ND
	38.09	116.1	ND	24.55	ND	ND
	39.07	1.92	ND	ND	ND	ND
	39.16	5.00	ND	ND	ND	ND
	39.51	180.6	10.04	ND	6.64	ND
	39.80	491.6	ND	ND	ND	ND
9. 4 or 5-Methylimidazole (tent.)	41.28	58990	3497	3528	13380	53.53
	42.58	204.0	ND	ND	ND	ND
	42.89	251.5	Trace	ND	ND	ND
	43.38	69.60	ND	ND	ND	ND
	44.05	50.39	12.06	ND	3.77	ND
	44.36	18.87	7.12	ND	2.18	ND

ND= None Detected

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