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Inter-office Memorandum

Subject: Amino Acid Analyses of Ammoniated Tobaccos

Date: October 18, 1978

To: Dr. W. M. Henley

From: J. P. Dickerson

Selected competitive brands and tobaccos which had been exposed to ammonia were subjected to amino acid analysis.[1] A major objective of this study was to determine the effect of ammonia on the concentration of amino sugars in tobacco. It was anticipated that the reaction of ammonia with sugars would lead to a high amino sugar concentration in ammoniated tobaccos. A secondary objective was to identify amino acid differences between brands and between tobacco types.

Amino acid analyses of WINSTON, Marlboro and CAMEL FILTER are shown in Table I. As was expected, the total amino sugar content of the two brands which contained ammoniated tobaccos, Marlboro and CAMEL FILTER, was higher than that of the non-ammoniated WINSTON. The amino sugar content of two ammoniated sheet materials, G7A and Marlboro reconstituted sheet (Table II), was higher than that of regular G7. Similarly, ammoniated CF flue-cured blend, denicotinized CF blend which had been exposed to ammonia[2] and Marlboro puffed tobacco contained more amino sugars than CF blend (Table III). The Marlboro data offer further evidence which indicates that Philip Morris uses ammonia in its puffing processes.

All of the ammoniated tobaccos contained more amino sugars than comparable non-ammoniated tobaccos. The increase in amino sugars was primarily due to an increase in glucosamine and mannosamine. The reaction of fructose and/or glucose, the major monosaccharides in flue-cured tobacco, with ammonia is the probable source of these amino sugars. Formation of glucosamine from ammonia and fructose through a Heyns Rearrangement[3] has been reported. This type of reaction, shown in Figure 1, might also produce mannosamine, which is the 2-epimer of glucosamine. Under alkaline conditions, glucose isomerizes to form fructose through the Lobry de Brun-Alberda van Ekenstein Transformation[3] (Figure 2). Consequently, glucose may be converted to fructose which is then converted to glucosamine and mannosamine.

As shown in Figure 2, both glucose and fructose can be converted to D-psicose under alkaline conditions. This ketose could react with ammonia to form allosamine and/or altrosamine. Neither of these amino sugars has been identified in the ammoniated tobacco. However, the possibility that these sugars are among unidentified peaks in the amino acid curves can not be excluded.

Increases in galactosamine content of the ammoniated tobacco were small in comparison to those of glucosamine and mannosamine. This is not surprising. Galactose is the 4-epimer of glucose. It is doubtful that glucose or fructose would isomerize under alkaline conditions to give galactose. Any increase in galactosamine due to ammoniation would probably arise from the reaction of ammonia with the small amount of naturally-occurring galactose in tobacco.

Ammoniation increases the quantity of amino acids in tobacco. In particular, proline and glycine are generally higher in tobaccos that have been treated with ammonia. This trend can be seen in the comparison of WINSTON, Marlboro and CAMEL FILTER shown in Table I. The WINSTON, which contains no ammoniated tobaccos, had the lowest amino acid content of the three brands.

Some inconsistencies between the brand analyses and reconstituted sheet analyses (Table II) were noted. Although the CAMEL FILTER amino acid analyses were high in comparison to those of WINSTON, the differences in amino acid content of G7 and G7A were relatively small. Proline and glycine analyses of G7A were higher than those of G7, but total amino acids were slightly lower.

Marlboro reconstituted sheet (Table II) had an unusually high proline content. In contrast to other ammoniated products, glycine content of this material was relatively low. Aspartic acid and asparagine content of the Marlboro sheet was higher than that of RJR G7. These amino acids are generally high in burley tobaccos. On the other hand, proline is the major amino acid in flue-cured tobaccos.

The effect of ammoniation on amino acids in flue-cured tobacco is shown in Table III. The CF data were obtained from a controlled experiment in which flue-cured tobacco (CF-2) was ammoniated and also treated under denicotinization conditions. Both of the treated samples, ammoniated and denicotinized, showed increases in proline and glycine. The increases in proline and glycine appear to be characteristic of the reaction of ammonia with flue-cured tobacco.

The reason for the increase in proline during ammoniation is not clear. It is doubtful that this compound, shown in Figure 3, is actually synthesized by the reaction of ammonia with some tobacco constituent. A more likely explanation is that the proline is produced by protein hydrolysis or the hydrolysis of prolinofructose.

The synthesis of glycine from the reaction of ammonia and some tobacco constituent would appear to be more likely than in the case of proline. Glycine (Figure 3) is the simplest of the amino acids and precursors for this compound may be present in tobacco. As is the case with proline, the glycine may arise from the hydrolysis of protein or an Amadori compound. The production of glycine and proline is difficult to rationalize totally in terms of protein hydrolysis. One would expect other amino acids to increase dramatically if protein hydrolysis were the source of these compounds. Although ammoniation increases some of the other amino acids, the largest increase is in glycine and proline.

As shown in Table II, the amino acid and amino sugar content of Marlboro puffed tobacco and denicotinized CF are similar. This is further evidence that Philip Morris is puffing flue-cured tobacco. If burley tobacco were being puffed, a higher aspartic acid and asparagine concentration would be expected.

An investigation of the effect of amino acids (particularly glycine and proline) and amino sugars on smoke quality might be desirable. It is possible that some of the effects of ammoniation on smoke quality may be due to these compounds.

The amino acid analyses is a potentially valuable research tool. With proper reference data this analysis might be used to differentiate tobacco types and to identify ammoniated materials. An amino acid analysis of puffed tobacco from the Basic brand should establish whether this tobacco is puffed with ammonia.

J. P. Dickerson

J. P. Dickerson

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Xc: Dr. R. A. Lloyd
Dr. M. E. Stowe
Mr. C. W. Miller
Mr. W. D. Young
Ms. E. H. Villegas

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References:

1. Amino acid analyses performed by Ms. Elizabeth H. Villegas.

2. Analyses obtained from C. W. Miller.

3. Kort, M. J., Reaction of Free Sugars with Aqueous Ammonia, Advances in Carbohydrate Chemistry and Biochemistry 25, 311-349 (1970).

TABLE I

COMPARISON OF AMINO ACID ANALYSES OF WINSTON, MARLBORO AND CAMEL FILTERS

Amino Acid, μg/g	WINSTON	Marlboro	CAMEL F.	WINSTON / Marlboro	CAMEL F. / Marlboro
Aspartic Acid	464	801	528	0.58	0.66
Asparagine	908	1190	886	0.76	0.74
Glutamic Acid	146	222	171	0.66	0.77
Glycine	89	174	161	0.51	0.93
Proline	2185	3725	3619	0.59	0.97
Alanine	128	152	159	0.84	1.05
Valine	29	38	37	0.76	0.97
Methionine	9	10	9	0.90	0.90
Isoleucine	12	14	12	0.86	0.86
Leucine	11	15	12	0.73	0.80
Tyrosine	15	19	18	0.79	0.95
Phenylalanine	68	90	85	0.76	0.94
γ-Aminobutyric Acid	25	38	30	0.66	0.79
Lysine	10	27	22	0.37	0.81
Histidine	16	38	28	0.42	0.74
Arginine	17	27	24	0.63	0.89
Glucosamine	124	228	263	0.54	1.15
Mannosamine	27	108	76	0.25	0.70
Galactosamine	24	32	42	0.75	1.31
Total Amino Acids[a]	4132	6580	5801	0.63	0.88
Total Amino Sugars[a]	175	368	381	0.48	1.04

[a]Total of amino acids and amino sugars analyzed

TABLE II

AMINO ACID CONTENT OF SHEET MATERIAL

Amino Acids, μg/g	G7	G7A	Marlboro Reconstituted Sheet[b]
Aspartic Acid	504	239	826
Asparagine	428	494	1190
Glutamic Acid	213	60	298
Glycine	79	172	23
Proline	1876	2010	8272
Alanine	123	102	217
Valine	26	28	29
Methionine	7	8	14
Isoleucine	13	11	16
Leucine	12	12	15
Tyrosine	16	16	13
Phenylalanine	41	35	41
γ-Aminobutyric Acid	14	16	31
Lysine	10	+	+
Histidine	20	+	11
Arginine	10	+	11
Glucosamine	124	232	375
Mannosamine	27	53	200
Galactosamine	24	+	--
Total Amino Acids[a]	3392	3203	11007
Total Amino Sugars[a]	175	285	575

[a]Total of measured values

[b]Combination of two types of sheet

+ = Compound detected but not quantitated

- = Compound not detected

TABLE III

AMINO ACID ANALYSIS OF FLUE-CURED TOBACCOS

Amino Acids, µg/g	CF-1	CF-2[a]	CF Ave.	Ammoniated[a] CF	Denicotinized[a] CF	Mar. Puff
Aspartic Acid	237	178	208	186	239	264
Asparagine	779	916	848	684	799	930
Glutamic Acid	99	121	110	119	113	125
Glycine	63	67	65	347	182	182
Proline	1357	1844	1600	5318	2877	2180
Alanine	146	181	164	196	223	199
Valine	37	37	37	40	28	44
Methionine	18	19	19	26	17	23
Isoleucine	17	17	17	14	14	18
Leucine	13	14	14	15	14	18
Tyrosine	24	20	22	23	26	31
Phenylalanine	78	77	78	70	79	68
γ-Aminobutyric Acid	33	39	36	48	66	48
Lysine	8	9	9	14	19	15
Histidine	21	24	23	35	24	28
Arginine	10	15	13	10	9	11
Total Amino Acids[b]	2940	3578	3259	7144	4729	4184
Glucosamine	109	70	90	461	185	216
Mannosamine	20	15	18	154	82	92
Galactosamine	7	20	14	+[c]	19	48
Total Amino Sugars[b]	136	105	122	615	286	356

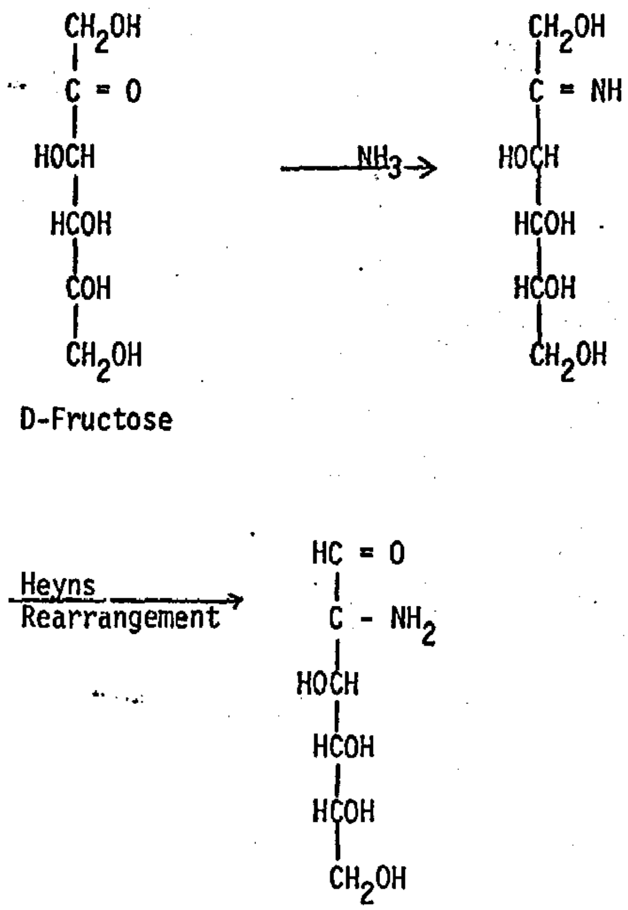
[a]Data from C. W. Miller

[b]Total of measured analytical values

[c]Detectable but not calculable

FIGURE 1

CONVERSION OF FRUCTOSE TO GLUCOSAMINE



Glucosamine (2-Amino-D-glucose)

FIGURE 2

LOBRY DE BRUYN-ALBERDA VAN EKENSTEIN TRANSFORMATION

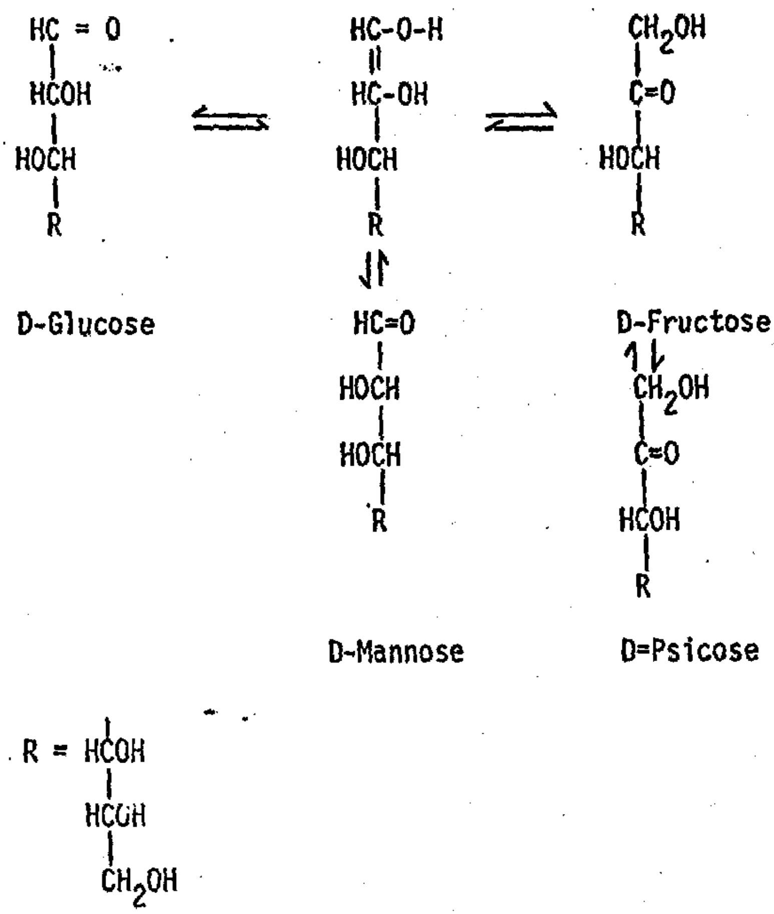
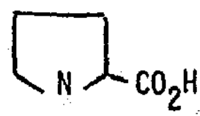


FIGURE 3

AMINO ACIDS IN TOBACCO



Proline

$H_2NCH_2CO_2H$

Glycine