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한미산 황색종 잎담배의 휘발성 정유성분 비교연구

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THE COMPARISONS OF VOLATILE OILS OF FLUE-CURED TOBACCO PRODUCED IN KOREA AND IN THE UNITED STATES

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ABSTRACT

Generally, the same quality tobacco may give similar concentration of each chemical component. This research investigation was studied to obtain the differences in concentrations of volatile oil compounds in physically similar tobacco produced in different environment and managements–in Korea and in the United States.

The flue-cured leaf tobacco produced in Korea and America was regraded to B3L and P3L by American grading system and analyzed for volatile oils relating to tobacco flavor and aroma. Sixty compounds of the more than 100 peaks distinguishable on the total neutral volatile oils were identified by GS-MS and quantified. Their concentrations are compared between B3L and P3L produced in Korea and in the United States.

The most volatile oil concentrations of B3L and P3L grade tobacco are higher in American than in Korean. Only a few components such as benzaldehyde, pulegone, 4, 6, 9-megastigmatriene-3-one, and coumaran are less in American.

INTRODUCTION

The tobacco quality is evaluated through physical and chemical devices. The numerous compounds isolated from tobacco and tobacco smoke continue to increase. Most flue-cured tobacco are consumed as the smoke of cigarettes. Smoke is a complex aerosol. Recently, 3875 compounds had been summed in cigarette smoke by Dube and Green (11). Of these, 1135 also occur in tobacco (11) and therefore must have transferred directly into the mainstream during smoking.

Leffingwell (21) listed 1081 compounds that had been tested as potential tobacco flavorants; three-fourths of those listed were neutral oxygenated compounds.

Currently the thrust in research relating to tobacco flavor and aroma is concerned with volatile constituents. The volatiles are obtained as essential oils by steam distillation or as an essence by solvent extraction. They can be used for evaluation of tobacco quality. Many of these are volatile and semivolatile relating to tobacco flavor and smoke aroma. They are found in the leaf and in the smoke in different concentrations.

Flue-cured tobacco produced in the southeastern United States might be the most flavorful in the world because of the favorable and suitable environments; climate, soils, and managements. Because the locations of Korea and southeastern United States are close latitudinally, it might be possible to produce good quality tobacco even though the environments in both countries are different. It is known that Korean tobacco is less flavorful than tobacco grown in the United States.

The objective of this research was to compare the concentrations of volatile oils in flue-cured tobacco between two grades produced in Korea and the United States.

MATERIALS AND METHODS

Production of Analytical Samples. Tobacco leaves (*Nicotiana tabacum,* NC 2326) produced in Eumseong in Korea in 1980 were graded to Thick 1, 2, 3, 4, 5, and Thin 1, 2, 3, 4, 5. The graded tobacco leaves were regraded to B3F (or B3L) and P3L by the method of Official Standard Grades for Flue-Cured Tobacco (Table 2). They were used to compare quality with B3L and P3L of NC 2326 produced in Clayton, NC, USA.

Separation and Identification of Volatile Oil. The selected tobacco samples were stemmed and ground to pass a 200-mesh sieve. The procedure for the collection and analysis of volatile oils is shown schematically in Figure 1.

A one-microliter of the concentrate of total fraction (F-T) was analyzed on Varian Model 3700 GC equipped with a flame ionization detector (FID) and Varian CDS 111 computer. 3.2mm × 304.8cm Ni column packed with 80/100 mesh Chromosorb WAW coated with 5% Carbowax 20M was used to separate components. The temperature of the column was programmed from 70°C to 200°C at the rate of 1°C/minute after a 5-minute delay. The final temperature was held at 200°C for 30 minutes.

F-T was separated into nonpolar (F-A) and polar (F-B) subfractions through a glass column 2 cm in diameter and 30 cm long which was packed with 12g of 100 mesh silicic acid. The following gradient methanol-in-hexane was used to elute the columns (Table 1).

Table 1. Gradient Solution and Subfractions

(1) 3% CH_2Cl_2	—	in Hexane	100 ml:	F–A
(2) 3% CH_2Cl_2	2% CH_3OH	in Hexane	75 ml:	F–A
(3) 3% CH_2Cl_2	3% CH_3OH	in Hexane	75 ml:	F–A
(4) 3% CH_2Cl_2	4% CH_3OH	in Hexane	75 ml:	F–A
(5) 3% CH_2Cl_2	5% CH_3OH	in Hexane	50 ml:	F–B

Subfractions F-A and F-B were each concentracted to volume of 1 ml in a rotary evaporator with nitrogen and analyzed by GC to determine the effectiveness of the separation.

Constituent separations and identifications were accomplished on a 5985 Hewlett Packard GC-MS systems equipped with a Carbowax 20M 0.5mm x 50m capillary column. Column temperature was programmed from 60°C to 200°C at the rate of 3°C/minute after a 5-minute delay.

Quantification of Specific Constituents. Two different chromatographic systems were involved in the quantification of particular compounds; the Varian GC with its coupled computer supplied peak areas whereas the Hewlett Packard GC-MS provided identifications. The mate of the two bits of information for particular peaks was conformed by coinjection and coelution of authentic compounds. Quantifications were based on calibration curves established with phenethyl alcohol

RESULTS AND DISCUSSION

Four samples were selected to compare components of the essential oils of flue-cured tobacco between B3F (or B3L) and P3L grades produced in Korea and U.S.A (Table 2).

The volatile oil profiles of gas chromatogram for the identification of the components in American flue-cured tobacco by GC-MS are shown in Figure 2 for the total neutral fraction (F-T), in Figure 3 for the nonpolar subfraction (F-A) and in Figure 4 for the separation of polar constituents (F-B). The profiles of GC chromatogram of the samples for this study are shown in Figures 5, 6, 7 and 8.

Table 2. Classification of Korean and American tobacco leaf by U.S.A. flue-cured grading system.

Korean Leaf (NC 2326)			American Leaf (NC 2326)	
Sample Name	Korean Grade	U.S.A. Grade	Sample Name	U.S.A. Grade
K-B3L	Thick 1, 2, 3	B3F	U-B3L	B3L
K-P3L	Thin 5	P3L	U-P3L	P3L

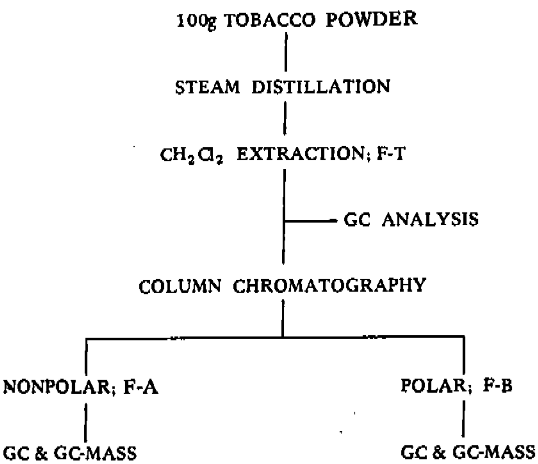

Fig. 1. Scheme of analysis for extraction, separation and identification.

Of the more than 100 peaks distinguishable on the total neutral volatile oil chromatogram (F-T, Figure 2), 60 compounds were identified by GC-MS and quantified. The compounds are grouped according to their special classification and functional groups, and concentrations of them are listed to be compared between Korean tobacco (K-B3L and K-P3L) and American tobacco (U-B3L and U-P3L) (Table 3). Particular ones associated with tobacco flavor and smoke aroma are discussed separately.

Hydrocarbones. Neophytadiene (GC Peak 33) is the predominate compound in the volatile oils of flue-cured tobaccos (2, 4, 14, 28). The compound comprised about 30% of total fraction of each sample in this research. The concentration of neophytadiene in U-B3L and U-P3L are higher by 50.4% and 140% than those in K-B3L and K-P3L, respectively (Table 3 and Figure 9). This component imparts smoothness to smoke and contributes importantly to flavor of flue-cured tobacco (1, 21, 28, 31).

Aldehydes. Furfural (Peak 4) was the most abundant range in the concentration from 6.7 to 19.4 $\mu g/g$ among the five aldehydes identified in this study (Figure 10). This compound imparts a sweet, buttery flavor to smoke (20). The concentrations of furfural in K-B3L and K-P3L are much lower than ones in U-B3L and U-P3L (Figure 10).

In Figure 11, the concentrations of phenyl acetaldehyde, 5-methyl-2-furfural, and safranal in K-B3L and K-P3L are also lower than those in American samples. Benzaldehyde has higher concentrations in Korean tobacco than in American tobacco. Benzaldehyde is related to almond and cherry character to smoke taste, and sweet and fruity to smoke aroma (21). It enhances tobacco aroma (21).

Alcohols. Of thirteen alcohols identified and quantified, linalool (Peak 9), benzyl alcohol (Peak 25), phenethyl alcohol (Peak 27), 1, 2, 3, 4-tetrahydro-2-isopropylene-8-methyl-1-naphthol (Peak 60), 1-hydroxymethyl-4, 5, 6-trimethyl-7, 8-dihydro-5, 6-naphthalene (Peaks 70 and 71), and four duvane-ols (R.T. 117, Peaks 68, 75, 76) were more abundant. The concentrations of these alcohols in U-B3L and U-P3L are higher than those in K-B3L and K-P3L as shown in Table 3 and Figure 12 and 13.

Linalool (Peak 9) imparts sweet, floral and citrus character to smoke taste and aroma (7, 24, 27). It is slightly higher in the concentration in U-B3L and U-P3L than that in K-B3L and K-P3L.

Benzyl alcohol and phenethyl alcohol impart a floral (rose) flavor to smoke (21); however at high concentration, benezyl alcohol gives a bitter taste. Both are metabolic derivatives of phenylalanine (32).

Of four duvane-ols found in the tobacco leaves, the concentrations of 4, 8, 13-duvatriene-1-ol and 13-duvane-1, 3-diol (Peak 75, 76) are significant and higher in American tobacco. They were degraded to low molecular aroma compounds such as solanone, oxysolanone, and solanol during the drying stage of curing. Therefore, they are believed to be an important precursor of flavor compounds.

Ketones. Thirty of the sixty compounds contain ketone-functions in their structures. Twenty-five structures are suggested that they may be oxygenated cleavage products of carotenoid pigments. Most ketones contribute to the flavor and aroma of smoke (21, 31).

Pulegone (Peak 14) gives herbaceous and minty character to smoke taste and aroma (21). It has much higher concentration in Korean tobacco than in Ameracan tobacco in both B3L and P3L (Figure 14).

β-Ionone (Peak 30), a derivative of carotenoids, and geranylacetone (Peak 24) contains more concentrations in U-B3L and U-P3L than in K-B3L and K-P3L (Figure 14).

As shown in Figure 15, solanone (Peak 20), damascenone (Peak 23), megastigmatriene-ones (Peaks 39, 43, 45, 51), and oxysolanone (Peak 42) are more abundant concentrations in American samples than in Korean. Solanone was isolated initially from cigarette tobacco (16) and later from smoke (6, 25). It imparts a smoothing character to smoke. Damascenone, first identified in rose oil (9), gives a burley-like flavor to tobacco smoke. The 4, 6, 8-megastigmatriene-3-ones differ in the orientation of the substituents on the side chain (8, 13, 21, 27).

Oxysolanone is an oxidative degradation product of the cembrene nucleus (17, 19) and can be an important precursor of volatile oil compounds considered as flavor components in tobacco (12).

Miscellaneous. Three alkaloids, two lactone, phenol, and indol were identified among the volatile oils of the sample. Dihydroactinodiolide (Peak 20) is abundant and gives slight cooling to smoke taste (3, 22, 24, 26, 27, 30). Indole (Peak 57) gives smoothing and floral character to smoke taste and aroma (21). The concentrations of above two compounds are higher in American tobacco.

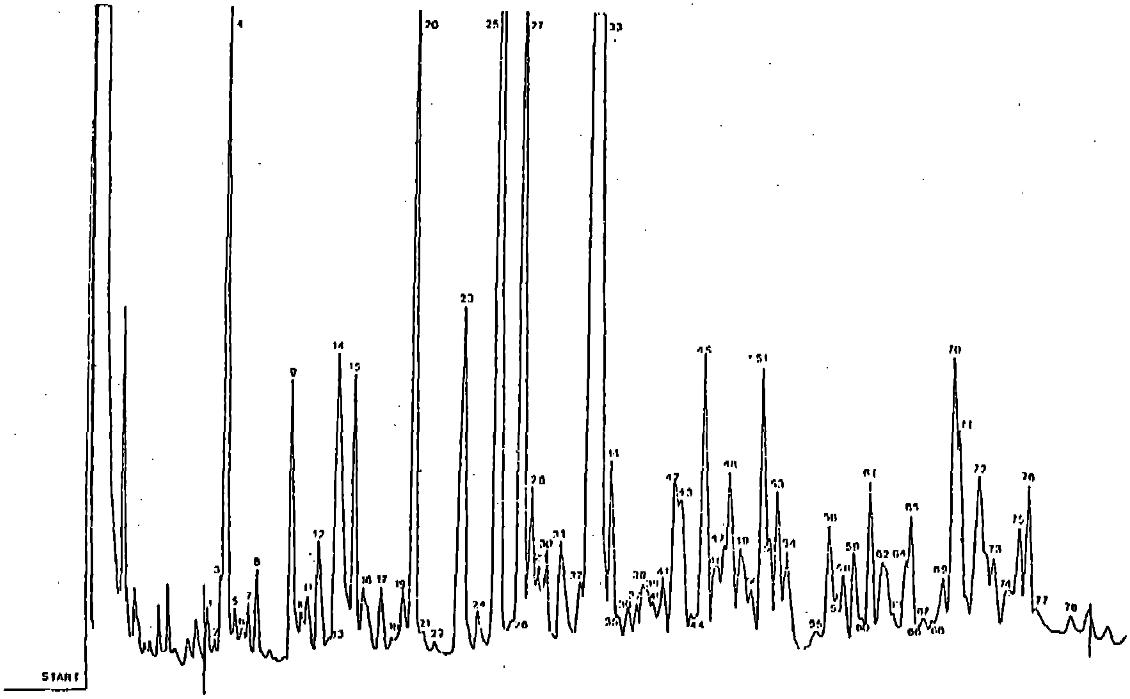

Fig. 2. GC chromatogram of total fraction (F-T) of American flue-cured tobacco.

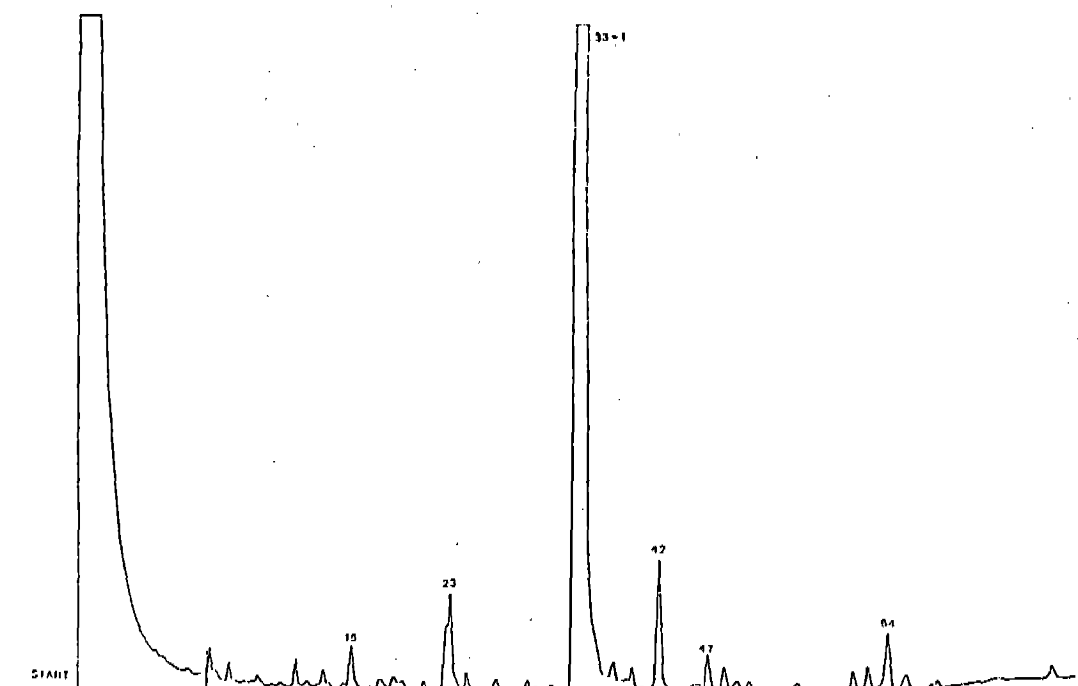

Fig. 3. GC chromatogram of nonpolar subfraction (F-A) of American flue-cured tobacco.

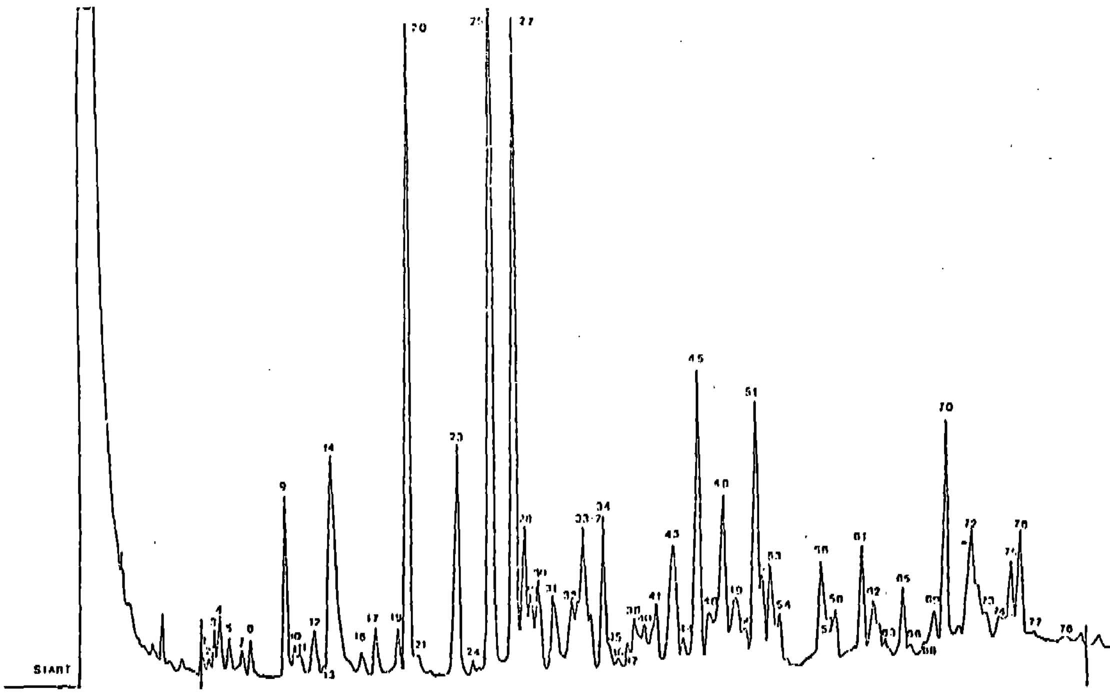

Fig. 4. GC chromatogram of polar subfraction (F-B) of American flue-cured tobacco.

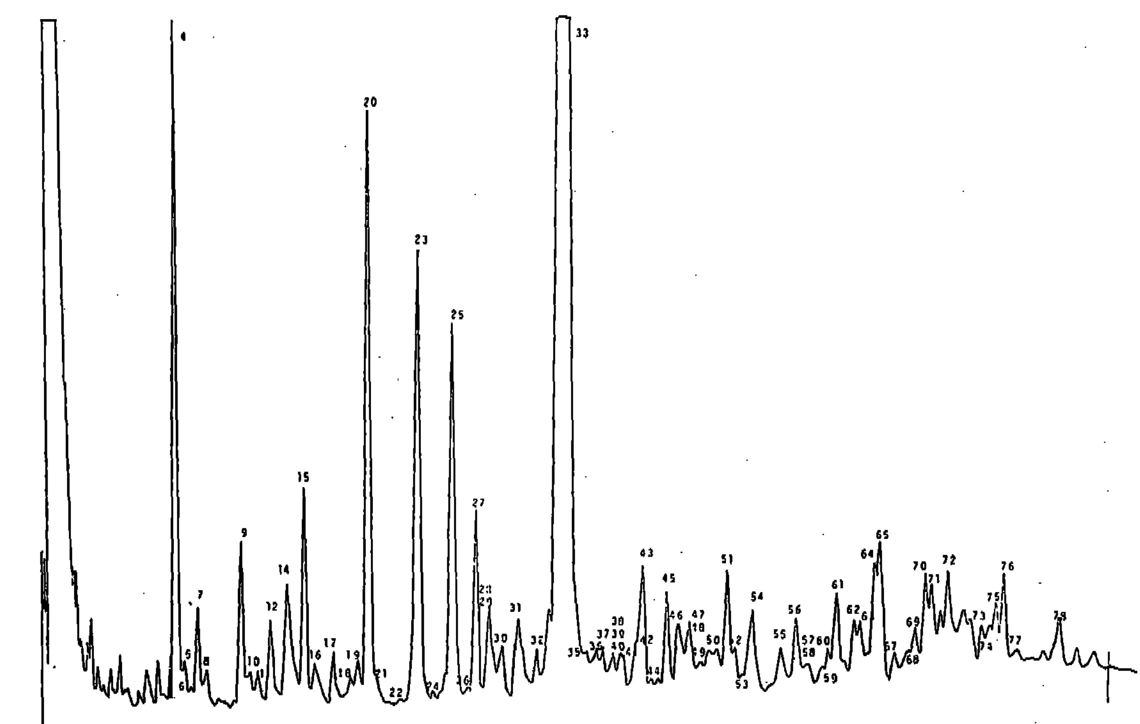

Fig. 5. GC chromatogram of total fraction (F-T) of K-B3L.

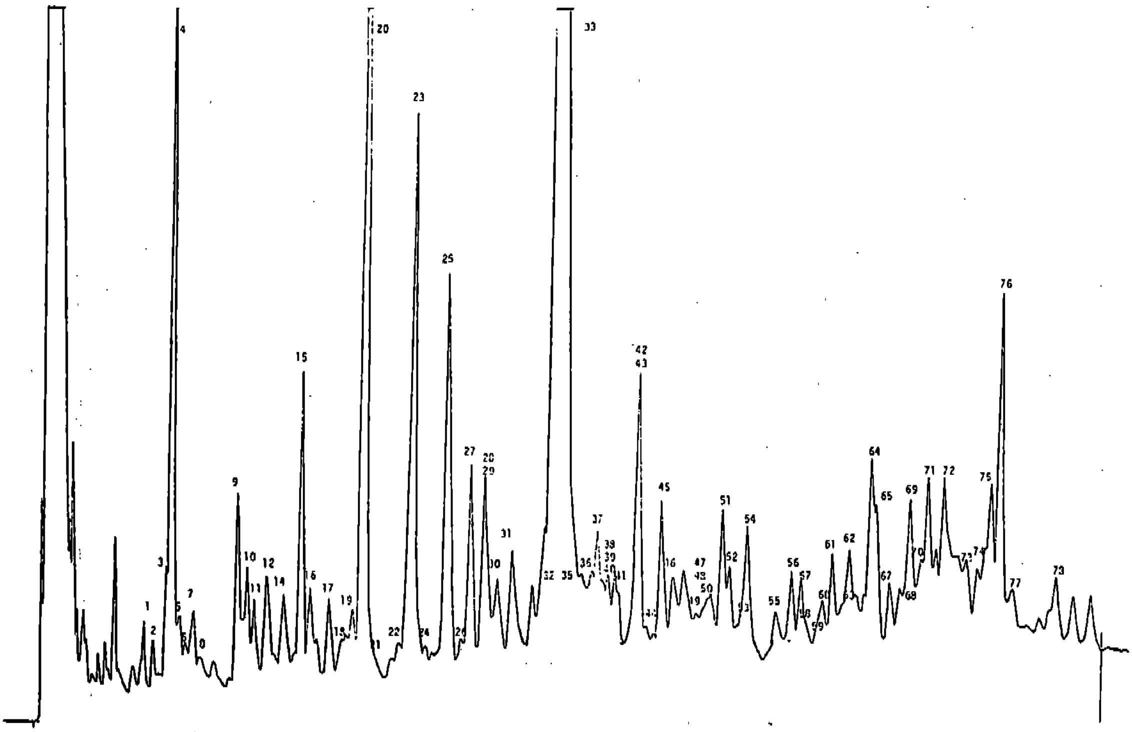

Fig. 6. GC chromatogram of total fraction (F-T) of U-B3L.

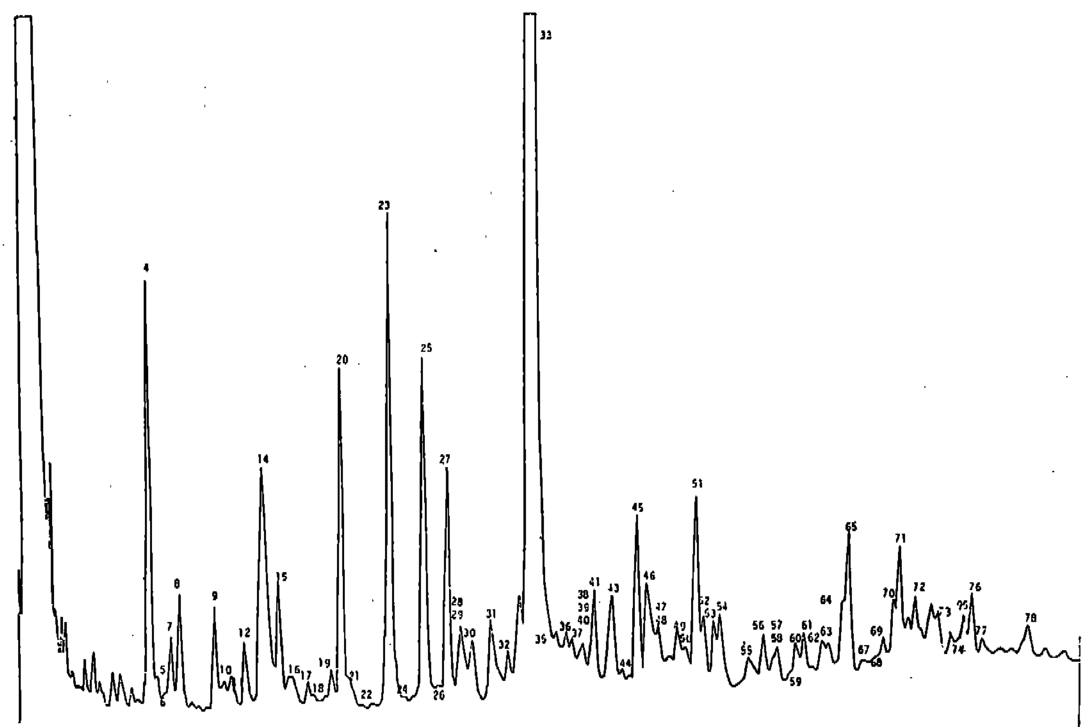

Fig. 7. GC chromatogram of total fraction (F-T) of K-P3L.

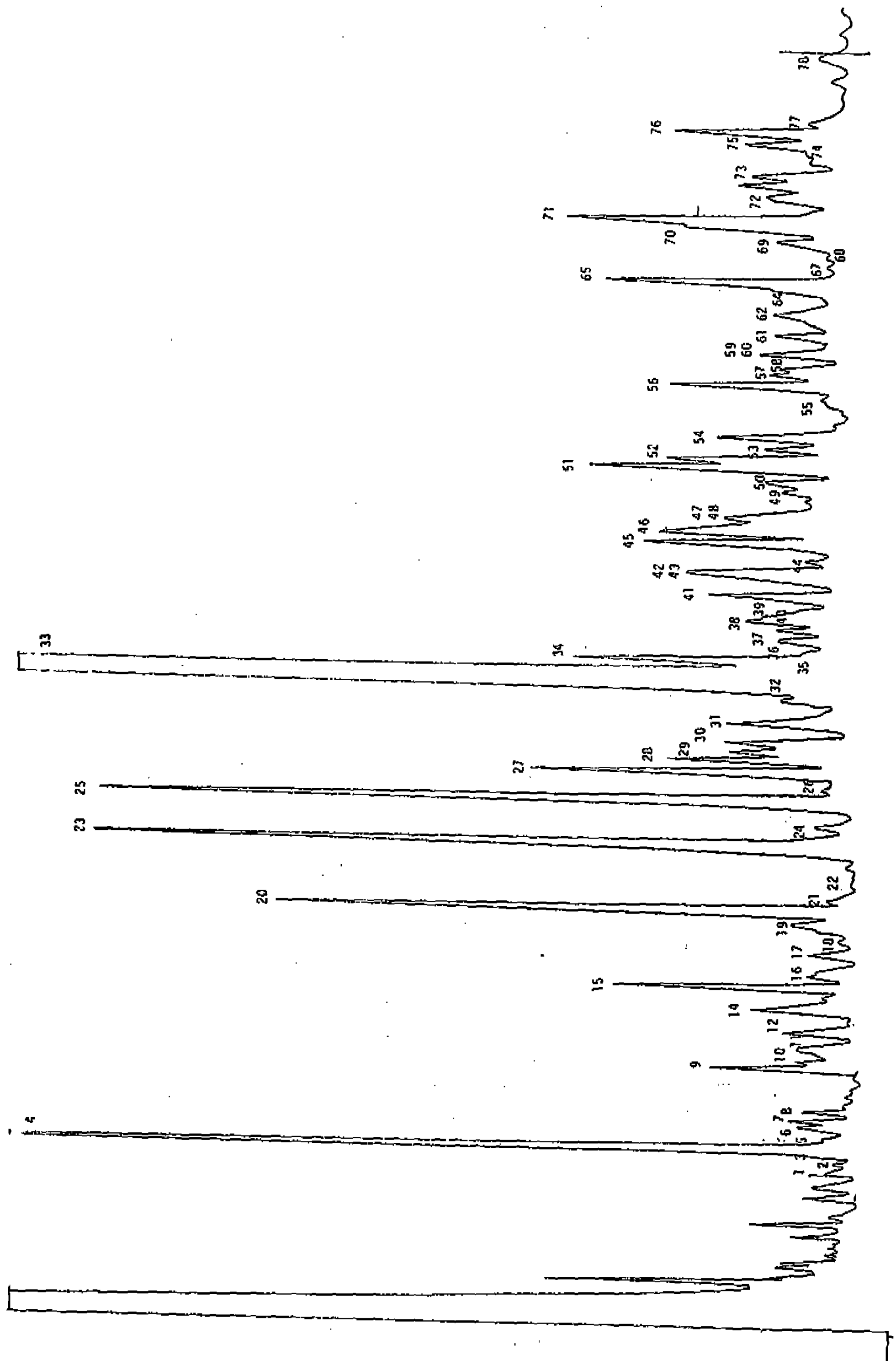

Fig. 8. GC chromatogram of total fraction (F-T) of U-P3L.

Table 3. Concentrations ($\mu g/g$) of the components in the volatile oils of flue-cured tobacco isolated by gas chromatography and identified by capillary GC-MS. NC 2326.

Groups	GC No.	R.T. (Min.)	Name	Concentration ($\mu g/g$)				Variation*				References
				K-B3L	U-B3L	K-P3L	U-P3L	#1	#2	#3	#4	
Hydro-carbons	3	20.3	1,1,6-trimethyl tetralin	0.3	0.9	–	–	–		+	+	
	33-1	75.1	Neophytadiene	116.2	174.8	84.6	202.7	–	–	+	–	2,14,22,27,28,30
	–	95.0	3,3-Dimethyl biphenyl	–	–	–	–					
	47	98.6	Isomer of Peak 3	0.8	3.2	0.9	3.4	–	–	–	–	
Aldehydes	4	20.9	Furfural	9.1	17.3	6.7	19.4	–	–	+	–	10,15,31
	8	25.5	Benzaldehyde	0.7	0.6	1.8	0.8	+	+	–	–	7,22,24,27,31
	12	34.6	5-Methyl-2-furfural	2.2	3.0	1.5	1.6	–	–	+	+	22,30
	16	41.2	Phenyl. Acetaldehyde	1.4	2.1	1.2	1.6	–	–	+	+	22,30
	17	43.7	Safranal	1.2	1.8	0.5	1.0	–	–	+	+	24,26
Alcohols	9	30.5	Linalool	3.2	3.7	1.8	2.0	–	–	+	+	7,24,27
	19	47.0	α-Terpineol	1.1	1.8	0.8	2.0	–	–	+	–	7,24
	25	60.6	Benzyl Alcohol	12.6	16.8	9.0	22.6	–	–	+	–	7,22,24,27,31,32,
	–	61.0	Solanol	–	–	–	–					7,30
	27	64.0	Phenethyl Alcohol	4.7	5.3	4.3	7.0	–	–	+	–	7,22,24,27
	60 & 61	113.5	1,2,3,4-Tetrahydro-2-isopropylene-8-methyl-1-naphthol (2 isomers)	4.1	4.7	2.1	3.6	–	–	+	+	
	–	117.0	3,8,13-Duvatriene-1,5-diol	–	–	–	–					28,31
	68	123.8	13-Duvane-1,3-diol	–	1.6	–	0.4					
	70 & 71	126.9	1-Hydroxymethyl-4,5,6-trimethyl-7,8-dihydro-5,6-naphthalene	5.8	9.0	3.3	13.9	–	–	+	–	
	74	135.1	6,7-Dimethyl-5-hydroxy Benzofuran	1.3	1.7	1.2	1.4	–	–	+	+	
	75	136.6	4,8,13-Duvatriene-1-ol	3.0	5.8	1.5	2.2	–	–	+	+	
	76	138.0	13-Duvane-1,3-diol	2.6	8.1	1.6	3.5	–	–	+	+	

Table 3 (continued)

Groups	GC No.	R.T. (Min.)	Name	Concentration (μg/g)				Variation*				References
				K-B3L	U-B3L	K-P3L	U-P3L	#1	#2	#3	#4	
Ketones	–	18.0	6-Methyl-5-hepten-2-one	–	–	–	–					
	14	37.3	Pulegone	4.4	1.3	11.5	1.6	+	+	–	–	23
	15	39.8	3,4,7-Trimethyl Indanone	3.9	7.7	0.8	3.0	–	–	+	+	
	20	48.5	Solanone	19.2	43.5	7.9	13.5	–	–	+	+	6,16,25,27,30,31
	21	49.8	2 (Propane-2-one)-3-isopropyl Tetrahydrofuran	–	–	–	–					27
	–	51.6	2-Methyl-5-isopropyl-6 (propane-2-one) Tetrahydrofuran	–	–	–	–					
	23	55.7	Damascenone	17.4	23.7	15.3	25.9	–	–	+	–	9,22,24,26
	24	58.0	Geranylacetone	0.4	0.7	0.2	0.9	–	–	+	–	22,27,30
	–	35.1	2(2-Butene-4-one)-3, 3-dimethyl Cyclohexanone	–	–	–	–					
	30	67.6	β-Ionone	1.6	3.1	1.4	2.8	–	–	+	+	7,22,26,30
	31	69.6	2-Furfuryl Methyl Ketone	2.6	5.3	0.9	3.5	–	–	+	+	
	33-2	75.1	3-Methyl-4-(1-buteneone)-5,5-dimethyl-6-hydrobenzene	–	–	–	–					
	34	77.1	3,4,5,6-Tetrahydro-4,4,7-trimethyl-2-naphthalenone	–	–	–	–					
	–	78.0	4-Isopropyl Benzyl Lactone	–	–	–	–					
	38-51	82.2-99.1	4,6,8-Megastigmatriene-3-one (4 isomers)	9.0	24.0	10.1	20.8	–	–	–	+	13
	–	85.0	Hexahydrofarnesyl Acetone	–	–	–	–					22,24,30
	42	86.4	Oxysolanone	4.9	13.8	2.3	6.9	–	–	+	+	
	47 & 48	94.4	3-Ethene-4-methyl Succinimide	0.8	3.2	0.9	3.4	–	–	–	–	29
	49	96.0	4,6,9-Megastigmatriene-3-one	1.3	1.0	1.6	1.4	+	+	–	–	13

Table 3 (continued)

Groups	GC No.	R.T. (Min.)	Name	Concentration (μg/g)				Variation*				References
				K-B3L	U-B3L	K-P3L	U-P3L	#1	#2	#3	#4	
Ketones (cont.)	50	97.5	Solanascone	1.4	3.5	0.9	1.9	–	–	+	+	
	–	104.0	Farnesyl Acetone	–	–	–	–					
	56	108.7	1-(4-Butane-2-one)-2,3,6-trimethylbenzene	2.2	2.2	1.1	3.5	–	–	+	–	
	59	111.4	?	1.1	2.0	0.9	2.1	–	–	+	–	
	65	120.7	4-Hydroxy-β-damascone	–	–	–	–					13,18
	69	125.4	1-Hydroxy-3-keto-4,9-dimethyl-6-isopropylene-tetrahydro-1,4-naphthadiene	–	–	–	–					
	72	130.7	2-Keto-5-isopropyl-8-methyl-12-methylene-3,8,13-pentadeca triene	1.8	8.6	0.5	0.9	–	–	+	+	
	73	132.9	3-Methyl-keto-3,3'-pentenedifuran	1.5	2.8	1.3	2.0	–	–	+	+	
Alkaloids	38,39,40	81.7	3-Hydroxymethyl Pyridine	1.3	2.0	1.4	4.0	–	–	–	–	
	52	100.0	α-Nicotyrine	0.9	2.2	1.3	3.3	–	–	–	–	31,33
	–	86.9	?	–	–	–	–					
Furans	28,29	66.4	5-Propylbenzodioxyfuran	1.4	6.9	0.2	2.5	–	–	+	+	
	55	107.0	Coumaran	1.6	1.7	1.0	0.9	–	+	+	+	
Lactone	54	102.7	Dihydroactinodiolide	3.0	4.9	1.7	3.0	–	–	+	+	3,22,24,26,27,30
Phenol	44	88.7	2-Methoxy-4-vinylphenol	0.3	–	0.5	0.9	+	–	–	–	
Indole	57	110.0	Indole	0.9	2.5	1.1	2.6	–	–	–	–	

* Variation #1 : + : K-B3L > U-B3L ; – : K-B3L < U-B3L
#2 : + : K-P3L > U-P3L ; – : K-P3L < U-P3L
#3 : + : K-B3L > K-P3L ; – : K-B3L < K-P3L
#4 : + : U-B3L > U-P3L ; – : U-B3L < U-P3L

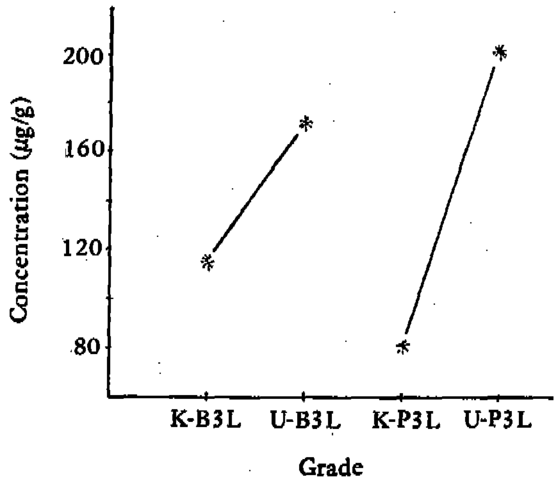

Fig. 9. Comparisons of the concentrations of neophytadiene in flue-cured tobacco between B3L and P3L grades produced in Korea and in the United States.

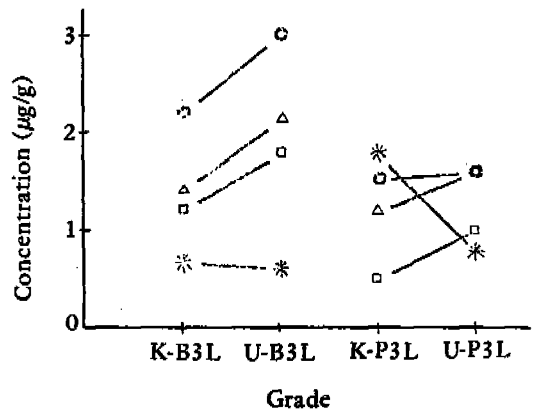

Fig. 11. Comparisons of the concentrations of benzaldehyde (*—*), phenyl acetaldehyde (Δ—Δ), 5-methyl-2-furfural (O—O), and safranal (□—□) in flue-cured tobacco between B3L and P3L grades produced in Korea and in the United States.

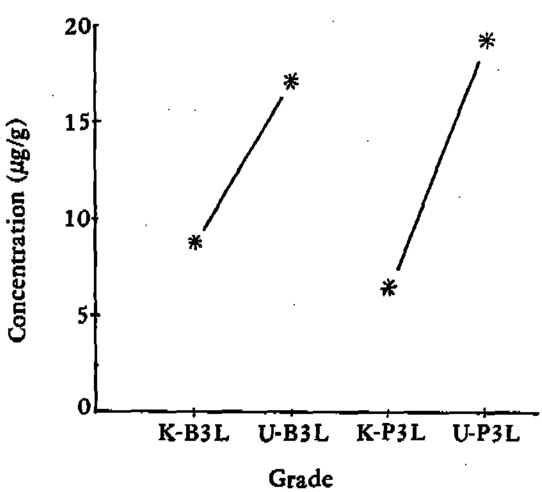

Fig. 10. Comparisons of the concentrations of furfural in flue-cured tobacco between B3L and P3L grades groduced in Korea and in the United States.

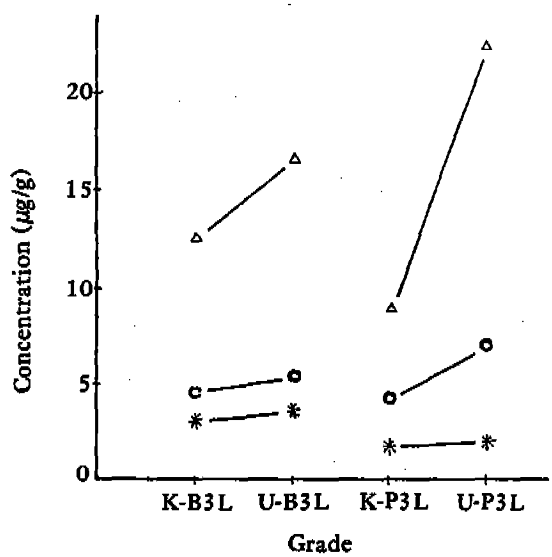

Fig. 12. Comparisons of the concentrations of linalool (*—*), benzyl alcohol (Δ—Δ), and phenethyl alcohol (O—O), in flue-cured tobacco between B3L and P3L grades oroduced in Korea and in the United States.

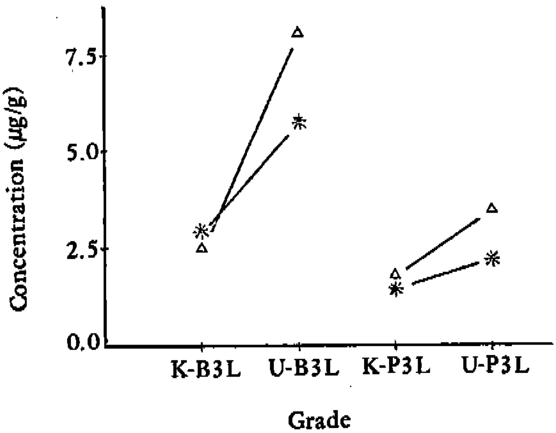

Fig. 13. Comparisons of the concentrations of 4,8,13-duvatriene-1-o1 (*—*) and 13-duvane-1, 3-diol (Δ—Δ) in flue-cured tobacco between B3L and P3L grades produced in Korea and in the United States.

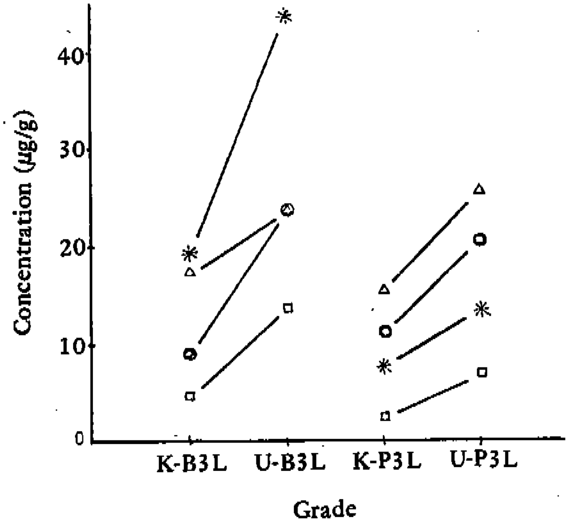

Fig. 15. Comparisons of the concentrations of solanone (*—*), damascenone (Δ-Δ), 4,6,8-megastigmatriene-3-ones (4 isomers) (O-O), and oxysolanone (□—□) in flue-cured tobacco between B3L and P3L grades produced in Korea and in the United States.

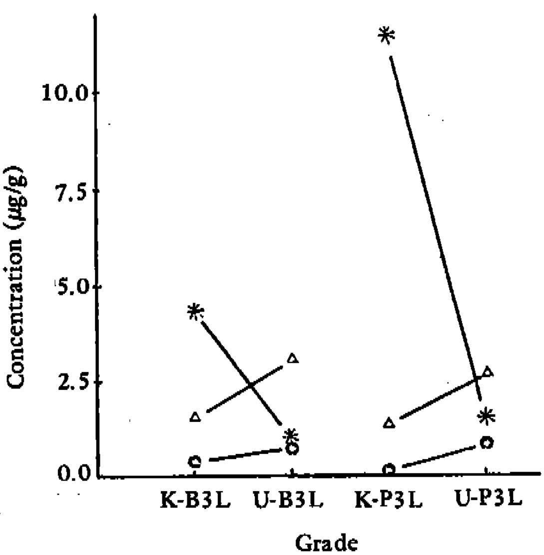

Fig. 14. Comparisons of the concentrations of pulegone (*—*), β-ionone (Δ—Δ), and geranylacetone (O-O) in flue-cured tobacco between B3L and P3L grades produced in Korea and in the United States.

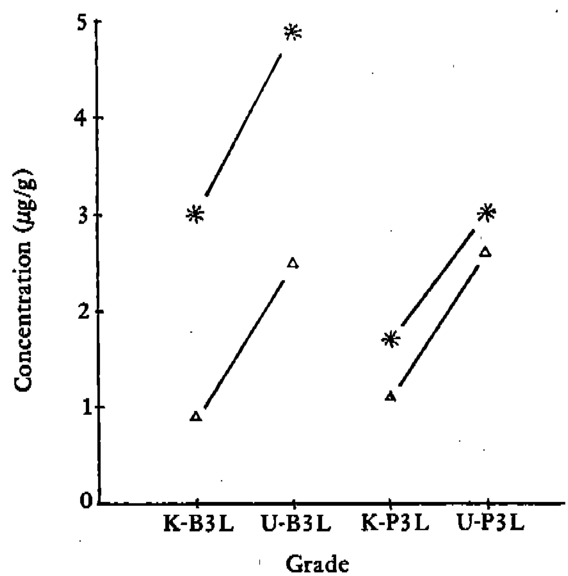

Fig. 16. Comparisons of the concentrations of dihydroactinodiolide (*—*) and indole (Δ—Δ) in flue-cured tobacco between B3L and P3L grades produced in Korea and in the United States.

CONCLUSIONS

The concentrations of volatile oils in flue-cured tobacco were compared between B3L and P3L grades produced in Eumseong of Korea and in North Carolina State of the United States.

Sixty compounds were identified and quantified through GC and GC-MS. The following conclusions were obtained:

1. The concentrations of most volatile oils in B3L and P3L grade tobacco are higher in American than in Korean. It is suggested that Korean tobacco is less flavorful than American.

2. Only a few components such as benzaldehyde, pulegone, 4,6,9-megastigmatriene-3-one, and coumaran are less in American.

3. Between two different grades (B3L and P3L) produced in Korea or in the United States, the changes of the content of each volatile oil are fluctuating.

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