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Composition of the Essential Oils of Sudangrass and Hybridsorgo, Forage Sorghums

Takayasu Kami

The essential oil of Sudangrass, a grass sorghum, was isolated by steam distillation of the fresh grass with a yield of 0.0006%. The essential oil was fractionated to each functional group, and the fractions were analyzed by gas chromatographic comparison with authentic samples using polar and nonpolar columns. Thus, 62 compounds including 13 acids, 6 phenols, 5 aldehydes, 6 ketones, 6 alcohols, 10 esters, and 16 hydrocarbons were tentatively identified as steam-distilled Sudangrass constituents. Almost all the compounds, however, have been detected in the essential oil of Hybridsorgo. In order to clarify the odor difference, quantitative analyses were further carried out on both essential oils of Sudangrass and Hybridsorgo: Sudangrass was rich in carbonyl compounds, while Hybridsorgo was rich in phenols.

In a previous investigation, the essential oil of Hybridsorgo had been fully analyzed by means of combined gas chromatography–mass spectrometry (GC–MS) to identify the aromatic constituents (Kami, 1975). The present work deals with the quantitative analyses of the essential oils of Sudangrass and Hybridsorgo by means of gas chromatography (GC).

Forage sorghums are classified to six agricultural species, and many varieties in each species are cultivated all over the world as feedstuffs of domestic animals. Among them, Hybridsorgo and Sudangrass are especially cultivated in the southwestern warm district of Japan as roughages of dairy cattle, because of their strong drought resistance, strong regrowth, and higher forage yield than other forage grasses. Of the two, Hybridsorgo is superior in yield (leaf size, leaf number, stalk diameter, and plant height), moisture percent, and sugar content, while Sudangrass is superior in offshoot (Harada et al., 1966). In Japan, Hybridsorgo is generally believed to be inferior in its palatability for dairy cattle.

EXPERIMENTAL SECTION

Materials. Sudangrass (Piper) was cultivated on a farm of the Faculty of Fisheries and Animal Husbandry, Hiroshima University, and the grass was harvested by mower in Oct 1970 as the second crop.

In the gas chromatographic analysis of Hybridsorgo, the essential oil obtained in a previous experiment was used (Kami, 1975).

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Isolation of Essential Oil from Sudangrass. The fresh crop (160 kg) was chopped and steam distilled according to a procedure previously described (Kami, 1975) to give about 72 L of a cloudy distillate in a water-cooled trap, and 3.4 g and 0.8 g of colorless aqueous condensates in ice–water-cooled and dry ice–acetone-cooled traps, respectively. The cloudy distillate of the water-cooled trap was saturated with sodium chloride, and 2-L lots were then extracted twice with 300 mL of redistilled diethyl ether to yield a dark brown oil with a sweet silage-like odor (0.96 g, η^{20}_{D} 1.4470, pH 3.2). The essential oil was stored in a sealed glass tube at 3 °C, as were the aqueous condensates from the ice–water- and dry ice-cooled traps.

Fractionation of the Essential Oil. A portion (680 mg) of the essential oil was sequentially extracted (Kami, 1975) to separate acid (brown viscous, 42 mg), phenolic (light brown viscous, 31 mg), and basic (drab viscous, 22 mg) fractions. The remaining neutral oil layer (302 mg) was transferred to 100 mL of *n*-pentane in a 200-mL beaker and stirred with 10 g of Mallinckrodt 100-mesh silicic acid (freshly activated at 125 °C for 3 h just before use) at room temperature for 2 h on a magnetic stirrer. The silicic acid, after being filtered out on a filter paper, was stirred with 100 mL of diethyl ether at room temperature for 2 h to dissolve the adsorbed polar components. The ethereal solution and the pentane filtrate thus obtained were each dried over anhydrous sodium sulfate, filtered, and evaporated on a rotary evaporator at room temperature, yielding polar (yellow brown liquid, 150 mg) and nonpolar (white crystal, 23 mg) fractions.

GC of the Unfractionated Essential Oil and the Acid, Phenolic, Polar, and Nonpolar Fractions. An FID-type Yanagimoto GCG-550T apparatus was operated

Table I. Analysis of the Essential Oil of Sudangrass[a]

Compound	Fraction detected	Retention time, min: Carbowax 20M	Silicone SF-96	Carbowax 1500	Tween 20	Triethanolamine
Acids						
Acetic acid	O, W	19.5*	5.9*			
Caproic acid	A	5.8	13.3			
Oenanthic acid	A	10.8	17.7			
Caprylic acid	A	19.1	22.3			
Pelargonic acid	A	25.0	25.7			
Capric acid	A	29.3	29.3			
Undecanoic acid	A	33.2	33.1			
Lauric acid	A	36.4	36.0			
Myristic acid	A	42.3	42.3			
Palmitic acid	A	47.5	48.2			
Stearic acid	A	52.3	56.5			
Benzoic acid	A	29.0	20.5			
Phenylacetic acid	A	33.8	23.6			
p-Anisic acid (+)	A	42.9	30.5			
Veratric acid (+)	A	50.9	37.5			
Phenols						
Guaiacol	P, W	36.2	20.9			
Phenol	P, W	39.7	16.4			
o-Cresol	P, W		21.1			
p-Cresol	P, W	41.9	20.8			
p-Ethylphenol	P, W	44.2	24.5			
3,4-Xylenol	P, W	45.3	25.5			
Aldehydes						
Acetaldehyde	H, T			3.5	5.2	
Furfural	O, W	23.2	9.0			
Phenylacetaldehyde	O, T, W	28.8	18.7			
p-Anisaldehyde	A, O, W	40.5	27.6			
Veratraldehyde	A, O, W	50.2	34.8			
Ketones						
Acetone	H, T			6.3	7.9	
Methyl ethyl ketone	H, T			10.1	11.1	
Methyl *n*-butyl ketone	O, T, W	4.1	7.0			
Methyl *n*-hexyl ketone	O, W	15.1	17.2			
Methyl *n*-heptyl ketone	O, W	21.6	21.5			
2,3-Pentanedione	O, W	3.3	4.4			
6,10,14-Trimethylpentadecan-2-one (+)	O, N, W	44.8	47.3			
Alcohols						
Ethanol	H			15.7	11.3	
3-Methyl-3-pentanol	O, W	4.7	7.9			
Benzyl alcohol	O, W	36.5	18.7			
2-Phenylethanol	O, W	37.3	21.9			
Isophytol	O, N, W	48.8	50.9			
Phytol	O, W	55.5	58.2			
Esters						
Ethyl formate	H			4.0	6.9	
Ethyl acetate	H			8.6	9.5	
Ethyl propionate	O, H, W	2.0	4.8	12.4	12.9	
n-Hexyl acetate	O, W	15.1	17.8			
n-Octyl acetate	O, W	25.7	26.0			
Benzyl acetate	O, W	32.0	24.0			
Ethyl myristate	O, W	43.3	45.5			
Methyl palmitate	O, N, W	46.8	49.8			
Ethyl palmitate	O, W	47.9	52.1			
Phytyl acetate	O, N, W	54.2	65.2			
Hydrocarbons						
p-Xylene	N, W	5.9	11.5			
n-Pentadecane	N, W	28.2	35.6			
n-Hexadecane	N, W	31.5	38.9			
n-Heptadecane	N, W	34.5	41.9			
n-Octadecane	N, W	37.3	44.8			
n-Nonadecane	N, W	40.2	47.6			
n-Eicosane	N, W	42.7	51.0			
n-Heneicosane	N, W	45.1	55.6			
n-Docosane	N, W	47.5	61.7			
n-Tricosane	N, W	50.2	70.0			
n-Tetracosane	N, W	52.7	80.8			
n-Pentacosane	N, W	55.3	95.9			
n-Hexacosane	N, W	58.0	115.2			
n-Heptacosane	N, W	62.6	146.0			
n-Octacosane	N, W	68.0				
n-Nonacosane	N, W	75.7				

Table I (*Continued*)

Compound	Fraction detected	Retention time, min: Carbowax 20M	Silicone SF-96	Carbowax 1500	Tween 20	Triethanolamine
Amines						
Trimethylamine (T)	B					2.8
Triethylamine (T)	B					4.8
Dimethylamine (T)	B					6.6
Diethylamine (T)	B					8.0
Isopropylamine (T)	B					9.3
Ethylamine (T)	B					10.8
tert-Butylamine (T)	B					14.3
n-Propylamine (T)	B					21.4
Miscellaneous						
Chloroform (+)	O, N, W	2.6	2.7			

[a] A, acid fraction via methylation; P, phenolic fraction; O, polar fraction; N, nonpolar fraction; B, basic fraction; H, headspace vapors of ice-water- and dry ice-cooled traps; T, TLC of 2,4-DNPHs; W, whole essential oil; (+), probable artifact; (T), tentative; *, t_R of the acid without methylation.

with U-shaped 2.25 m × 3 mm i.d. stainless steel columns packed with either 5% Carbowax 20M on 60–80 mesh Celite 545 (polar column) or 10% Silicone SF-96 on 60–80 mesh Chromosorb W (nonpolar column). The carrier gas was helium. For the Carbowax 20M column, the oven temperature was maintained at 68 °C for the first 9 min and then increased at a rate of 4 °C/min to 218 °C (flow rate, 18 mL/min; injection port temperature, 280 °C). For the Silicone SF-96 column, the oven temperature was raised at 4 °C/min from 58 to 226 °C (flow rate, 14 mL/min; injection port temperature, 280 °C). Samples were dissolved and applied in diethyl ether, except for the nonpolar fraction, which was dissolved in *n*-pentane. The acid fraction, after being converted to the methyl esters, was dissolved in diethyl ether (Vorbeck et al., 1961). Approximately 10 μL of each solution was injected.

GC of the Basic Fraction and of the Headspace Vapors from Ice–Water- and Dry Ice-Cooled Traps, and Thin-Layer Chromatography (TLC) of Carbonyl Compounds Separated from the Condensate of the Water-Cooled Trap. The basic fraction was treated with 2 N sodium hydroxide solution and the headspace vapor was chromatographed on a 20% Triethanolamine on 60–80 mesh Celite 545 column, as previously reported (Kami, 1975). The headspace vapors of the ice–water- and dry ice-cooled traps were chromatographed on isothermal GC using columns of 10% Carbowax 1500 on 60–80 mesh Diasolid L and 15% Tween 20 on 100–120 mesh Chromosorb W (Kami et al., 1972). TLC of the carbonyl compounds was also carried out on 2,4-dinitrophenylhydrazones (2,4-DNPHs) obtained from the condensate of the water-cooled trap (Kami et al., 1972).

Peak Assignment in GC and Percentage Composition of the Essential Oil. The assignment of peaks in GC depended upon the coincidence of retention times (t_R) with those of the authentic samples that were available from Poly Science Corporation (Evanston, Ill.). The results of previous GC–MS analysis of Hybridsorgo were also used for peak assignment.

Percentage compositions of the essential oils of Sudangrass and Hybridsorgo were calculated from the relative peak areas in each chromatogram: the silhouette of the GC was traced on a sheet of uniform tracing paper, and peaks were cut out and weighed on a chemical balance. The relative areas of peaks thus obtained were then multiplied by the yield percent of each fraction, which was 16, 8, and 76%, respectively, for the acid, basic, and the remaining fractions of Sudangrass, while 30, 10, and 60%, respectively, for the corresponding fractions of Hybridsorgo. The relative amounts of low-boiling compounds were calculated on the basis of the relative areas of ethyl propionate peaks in analyses of the contents of ice–water-cooled traps. These were 0.7 and 0.4%, respectively, in the essential oils of Sudangrass and Hybridsorgo.

RESULTS AND DISCUSSION

Identification of the Compounds in the Essential Oil of Sudangrass. From the second crop of Sudangrass, the essential oil was obtained by steam distillation with a yield of 0.0006% and was separated into five fractions: acidic, phenolic, basic, polar, and nonpolar. The acid fraction, after being converted to the methyl esters, and the phenolic, polar, and nonpolar fractions were analyzed by programmed temperature GC using two kinds of columns, Carbowax 20M and Silicone SF-96. The basic fraction was heated with alkali, and the regenerated amines were examined by isothermal GC on a Triethanolamine column. Peak assignment was based on the coincidence of t_R with those of authentic samples, and the results are listed in Table I.

In the acid fraction, 16 compounds were tentatively identified as normal monocarboxylic acids from C_6 to C_{12} and of C_{14}, C_{16}, and C_{18}, 4 aromatic acids, and 2 aromatic aldehydes (A in Table I). Of these, undecanoic, palmitic, and stearic acids had not been identified in the essential oil of Hybridsorgo by GC–MS. From the phenolic fraction, 6 phenolic compounds including phenol and *o*-cresol, which appeared as overlapping peaks in GC with the Carbowax 20M column and as separate peaks in the case of the Silicone SF-96 column, were tentatively identified (P in Table I). In the previous paper on the analysis of Hybridsorgo, the neutral portion of the essential oil had been treated with 30% solution of sodium bisulfite to remove the aldehydes. In this experiment, however, the neutral portion of Sudangrass oil was immediately treated with silicic acid in *n*-pentane to adsorb the polar compounds, and then the compounds were eluted from the silicic adsorbent into diethyl ether. Thus 24 compounds, consisting of 4 aldehydes, 5 ketones, 5 alcohols, 8 esters, acetic acid, and chloroform were tentatively identified from the polar fraction (O in Table I). From the nonpolar fraction, which was not adsorbed in the silicic treatment, a series of normal alkanes from C_{15} to C_{29} was tentatively identified together with 6 other compounds (N in Table I). The unfractionated essential oil was similarly analyzed by GC, and all the compounds detected in each fraction, except for acids, amines, and low-boiling compounds, were also detected (W in Table I). In addition, 8 amines (B in Table I), 7 low-boiling compounds (H in Table I), and 5 carbonyl compounds (T in Table I) were detected in GC of the regenerated gas from the basic fraction, in GC of the

Table II. Percentage Composition of the Essential Oils of Sudangrass and Hybridsorgo[a]

Compound	Sudan-grass	Hybrid-sorgo	Compound	Sudan-grass	Hybrid-grass
Acetaldehyde	Tr	Tr	*n*-Heneicosane		
Ethyl formate	0.7	6.7	Methyl palmitate	0.5	0.2
Acetone	0.8	2.2	*n*-Docosane	0.1	0.2
Ethyl acetate	Tr	0.2	Ethyl palmitate	5.4	1.7
Methyl ethyl ketone	0.2	0.4	Isophytol	0.9	0.4
Ethyl propionate	0.7	0.4	Veratraldehyde	1.9	1.1
Ethanol	0.2	0.4	*n*-Tricosane	0.5	0.5
Chloroform	2.2	1.4	*n*-Tetracosane	0.2	0.2
2,3-Pentanedione	1.2	0.5	Phytyl acetate	1.1	0.7
Methyl *n*-butyl ketone	Tr	0.2	Phytol	0.5	0.9
3-Methyl-3-pentanol	0.1	Tr	*n*-Pentacosane	0.6	0.6
p-Xylene	0.5	0.1	*n*-Hexacosane	0.1	0.1
n-Hexyl acetate	0.8	0.7	*n*-Heptacosane	1.2	1.1
Methyl *n*-hexyl ketone			*n*-Octacosane	Tr	Tr
Acetic acid	1.5	0.5	*n*-Nonacosane	1.0	1.2
Methyl *n*-heptyl ketone	0.4	0.2	Caproic acid	0.1	0.4
Furfural	1.5	0.6	Oenanthic acid	0.1	0.5
n-Octyl acetate	0.6	0.2	Caprylic acid	0.8	1.5
n-Pentadecane	0.1	0.2	Pelargonic acid	1.4	1.7
Phenylacetaldehyde	15.2	7.7	Benzoic acid	2.6	5.2
n-Hexadecane	0.2	0.2	Capric acid	Tr	Tr
Benzyl acetate	1.2	0.8	Undecanoic acid	0.2	0.4
n-Heptadecane	Tr	Tr	Phenylacetic acid	0.3	0.6
Guaiacol	1.4	2.6	Lauric acid	1.8	2.0
Benzyl alcohol	1.9	1.8	Myristic acid	0.6	1.6
2-Phenylethanol	2.5	1.3	*p*-Anisic acid	0.7	1.5
n-Octadecane			Palmitic acid	0.4	0.8
Phenol	0.5	11.5	Veratric acid	1.0	1.9
o-Cresol			Stearic acid	0.3	0.7
p-Anisaldehyde	2.5	1.1	Trimethylamine	0.1	0.1
n-Nonadecane			Triethylamine	1.3	0.5
p-Cresol	2.4	6.8	Dimethylamine	0.1	0.3
n-Eicosane	0.2	0.2	Diethylamine	Tr	2.4
Ethyl myristate	0.9	0.3	Isopropylamine	1.0	2.1
p-Ethylphenol	0.1	2.1	Ethylamine	0.7	1.9
6,10,14-Trimethylpentadecah-2-one	8.0	2.0	*tert*-Butylamine	3.6	1.9
3,4-Xylenol	1.0	0.7	*n*-Propylamine	1.1	0.7

[a] Percentage compositions of low-boiling compounds, acids, amines, and remaining compounds were calculated from GC of Carbowax 1500, Carbowax 20M, Triethanolamine, and Carbowax 20M columns, respectively.

headspace vapors of ice–water- and dry ice-cooled traps, and in TLC of 2,4-DNPHs prepared from the condensate of the water-cooled trap.

Composition of the Essential Oils of Sudangrass and Hybridsorgo. In the above-mentioned GC analysis, it became evident that the constituents of the essential oil of Sudangrass are very similar to those of Hybridsorgo. For this reason, a quantitative comparison of the two essential oils was attempted to clarify the difference in the aromas. GC of each unfractionated essential oil was carried out on Carbowax 20M column to determine the relative areas of all the peaks. However, it is known that acids and amines cannot be analyzed by this method, nor can low-boiling compounds be measured under these conditions. Therefore, the compositions of both essential oils were calculated by multiplying the relative area of each compound detected in GC of the unfractionated essential oils, acid fractions (after methylation), and basic fractions (after regeneration of amines) by the yield percent of these fractions. The compositions of the low-boiling compounds in both essential oils were calculated from the relative area of ethyl propionate detected in GC of the contents of ice–water-cooled traps. The results for both essential oils are listed in Table II.

In both essential oils, the total amount of hydrocarbons (ca. 5%) was similar, but the total amounts of oxygen-containing compounds, phenols, acids, and amines were ca. 49, 5, 12, and 8%, respectively, in the essential oil of Sudangrass, and ca. 33, 24, 19, and 10%, respectively, in that of Hybridsorgo. The difference in the two essential oils was especially obvious in their contents of oxygen-containing compounds and phenols. Among the oxygen-containing compounds, furthermore, the total amounts of ketones, aldehydes, esters, and alcohols were ca. 11, 21, 11, and 6%, respectively, in the essential oil of Sudangrass, and ca. 6, 10, 12, and 5%, respectively, in that of Hybridsorgo: the difference in the amounts of oxygen-containing compounds was especially pronounced for the carbonyl compounds. Accordingly, it can be said that the essential oil of Sudangrass is relatively rich in carbonyl compounds, while the essential oil of Hybridsorgo is relatively rich in phenols. The result agrees with the observation that the essential oil of Sudangrass had a silage-like odor, while that of Hybridsorgo had a smoky odor. The phenolic fraction of Hybridsorgo had an especially strong phenolic odor. Such discrepancy of the essential oils of two forages, which were obtained from the same soil, the same climate, and the same harvest time, seems to have some connection with their palatability for dairy cattle. However, further investigation on the relation between the odor of forages and its palatability for domestic animals is necessary.

CONCLUSION

From the essential oil of Sudangrass, 74 compounds in all, consisting of 15 acids, 6 phenols, 5 aldehydes, 7 ketones, 6 alcohols, 10 esters, 16 hydrocarbons, 8 amines, and chloroform, were detected by GC. Qualitatively, they were

nearly the same as the components of the essential oil of Hybridsorgo. With respect to the total amounts of carbonyl compounds and phenols, however, a remarkable difference was recognized between these essential oils. Accordingly, it was concluded that the odor difference between Sudangrass and Hybridsorgo does not depend upon the kinds of compounds present, but rather upon their relative amounts.

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Digestibility of Delignified Forage Cell Walls

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The effect of lignin on the digestion of plant cell walls by rumen microorganisms was examined. Intact cell walls of Coastal bermudagrass (*Cynodon dactylon* (L.) Pers.) and Ky-31 Tall fescue (*Festuca arundenacea* Schreb.) were delignified with potassium permanganate in an acetic acid buffer. The delignified cell walls and untreated controls were incubated with rumen microorganisms and the relative ease and extent of digestion observed with the scanning electron microscope. Sclerenchyma tissue was more susceptible to the permanganate treatment than the inner bundle sheath. The rate of digestion of all tissues in the cell walls was increased in the treated samples. The vascular bundle of the delignified sample (as determined histochemically) was not digested. These data suggest that while lignin does impede the rate and extent of digestion, the plant cell wall polysaccharides still show marked differences in their rate and extent of digestion when lignin is not a barrier. The relationship of anatomical characteristics to digestibility should include considerations of the association of cell wall polysaccharides with lignin as well as of the type, site, and extent of lignification.

Lignin content has been correlated with decreased forage digestibility and more specifically with decreased digestibility of particular cell wall polysaccharides (Wilkins, 1969, 1972; Smith et al., 1972; Duble et al., 1971; Waldo et al., 1972; Akin et al., 1975; Barton et al., 1976). Kamstra et al. (1958) also showed that cellulose and hemicellulose isolated as holocellulose (Phillips et al., 1960) from orchardgrass and alfalfa were digested in vitro more completely than intact plant cellulose in situ. Addition of lignin to in vitro samples isolated by the procedure of Patton (1943) did not depress in vitro digestibility of these forages. Cross et al. (1974) showed that cellulose digestibility was increased from 66 to 91% by chemical removal of lignin with sodium chlorite. Although lignin has been related to decreased forage digestibility, no definitive evidence has been found to show the nature of its effect on the digestion of forage tissue by rumen microorganisms.

Morrison (1975) and Bailey and Pickmere (1975) showed that the amount and type of hemicellulose isolated from various plant species depended on whether the plant material is delignified before hemicellulose extraction. Lignin–carbohydrate complexes isolated from birchwood (Bolker and Wang, 1969) and ryegrass (Morrison, 1973; Hartley, 1972) yielded upon hydrolysis monosaccharides which correspond to the plant hemicellulose fraction. Therefore, the plant polysaccharides apparently are not simply encrusted by lignin, but are probably covalently bonded as suggested by Harkin (1973). The type and extent of lignin–polysaccharide bonding could affect digestion more than the amount of lignin per se.

All the above authors examined the relationship of lignin to cell walls and digestibility, but only Akin et al., (1975) did so by direct observation of intact cells using scanning electron microscopy. They observed differential rates of digestion for various plant tissues in fescue and bermudagrass and found that the lignified cells were indigestible.

In this study the scanning electron microscope (SEM) was used to observe intact cell walls digested in vitro by rumen microorganisms before and after chemical treatment to remove lignin. The objective of the study was to visualize the effect of lignin and lignified tissue on the digestion of intact cell walls, i.e., compared to a properly fixed cross section of a grass leaf blade the tissues in the treated sections appear to be undisturbed, swelled, or distorted. Any distortion noted in the treated samples must then be attributed to the treatment. The samples were treated as leaf sections and were not ground.

EXPERIMENTAL SECTION

Preparation of Grass Samples. Samples of Coastal (CBG) and Coast-cross-1 (CX-1) bermudagrass (*Cynodon dactylon* (L.) Pers.) and Kentucky-31 (Ky-31) tall fescue (*Festuca arundinacea* Schreb.) and its annual ryegrass hybrid Kenhy (KHY) were harvested after 4 weeks of summer regrowth, immediately frozen with dry ice, and maintained at −30 °C until used. Sections of leaf blades,

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