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Received for review October 3, 1980. Revised manuscript received April 27, 1981. Accepted May 14, 1981.

Composition of the Essential Oil of Soiling Dent Corn

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The essential oil of soiling dent corn was isolated by steam distillation of the fresh grass with a yield of 0.0031%. The essential oil was analyzed by combined gas chromatography-mass spectrometry and gas chromatographic comparison with authentic specimens, and 90 compounds consisting of hydrocarbons, aldehydes, ketones, alcohols, esters, acids, phenols, and miscellaneous were identified. Quantitative analysis was further carried out on the essential oil, and soiling dent corn oil was relatively rich in hydrocarbons and aldehydes.

For clarification of the connections between the aromas of forage crops and their palatability for domestic animals, a series of studies on the aromatic constituents of forage crops have been carried out. In the present work, the essential oil of soiling dent corn was analyzed by means of combined gas chromatography-mass spectrometry and gas chromatography, following that of the previous papers (Kami, 1975, 1977, 1978) on the analyses of the essential oils of Hybridsorgo, Sudangrass, and red and Ladino white clovers.

Since the corns (*Zea mays* L.) occupy a very important role in the feedstuffs of domestic animals, they are cultivated in a great extent all over the world. In Japan, similarly, the corns are the important forage crops and they are cultivated over the whole country from old times to the present. According to the classification from the starch of grain, dent species is especially applied to silage and silage of dairy cattle, owing to the good adaptability, the high nutrition, and the abundant yield.

EXPERIMENTAL SECTION

Materials. Soiling dent corn (Nagano No. 1, early variety) was cultivated on a farm of the Faculty of Applied Biological Science, Hiroshima University, and the aerial parts were harvested in Oct 1977 by mower. The harvest time corresponded to the milky stage of the seed of dent corn.

Isolation of the Essential Oil. The fresh aerial parts (220 kg), after chopping, were steam distilled in 28-kg lots for 1 h under 0.8 kg/cm² distillation kettle pressure, and yielded about 130 L of cloudy distillate in a water-cooled trap and 7.3 and 1.0 g of colorless aqueous condensates in ice-water-cooled and dry ice-methanol-cooled traps, respectively. After the cloudy distillate of the water-cooled trap was saturated with sodium chloride, 2-L lots were extracted twice with 300 mL of distilled diethyl ether in the same way as the previous paper (Kami, 1975) to yield a dark brown oil with a sweet silage-like odor (6.783 g).

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-methanol-cooled traps.

Fractionation of the Essential Oil. A portion (2360 mg) of the essential oil was sequentially extracted to separate acidic (517 mg), phenolic (382 mg), and basic (54 mg) fractions according to the procedure previously described (Kami, 1978). The remaining neutral oil layer (1400 mg) was extracted first in *n*-pentane and then in diethyl ether with silicic acid to separate it into nonpolar (185 mg) and polar (813 mg) fractions (Kami, 1977). Among them, the acidic fraction was further converted into the methyl esters with diazomethane (Vorbeck et al., 1961).

Analysis of the Essential Oil. In the beginning, the unfractionated essential oil was analyzed by combined gas chromatography-mass spectrometry (GC-MS). A Hitachi Model RMU-6MG mass spectrometer was used. The GC column was a 0.28 mm i.d. × 50 m glass capillary column coated with PEG 20M, and the column temperature was programmed from 50 to 180 °C at 2 °C/min. The carrier gas was helium. The mass spectrometer was worked at 70-eV ionizing voltage, 3200-V ion accelerating voltage, and 200 °C ion source temperature. In the next phase, the fractions of the essential oil, except for the basic fraction, and the unfractionated essential oil were analyzed by programmed temperature gas chromatography (GC). An FID-type Yanagimoto GCG-550T gas chromatograph was used. The GC column was a U-shaped 2.5 mm i.d. × 2.2 m glass column packed with 5% PEG 20M on 80-100-mesh Chromosorb W, and the column temperature was maintained at 48 °C for the first 5 min and then increased at a rate of 4 °C/min to 230 °C (helium flow rate, 13 mL/min; injection port temperature, 280 °C). The basic fraction was heated with a 2 N solution of sodium hydroxide, and the regenerated gases of amines were analyzed by isothermal GC with a triethanolamine column at 65 °C (Kami, 1975). For the analysis of low-boiling compounds, the headspace vapors in ice-water- and dry ice cooled traps were directly chromatographed by using the same procedure as previously reported (Kami et al., 1972).

Percentage Composition of the Essential Oil. The relative peak areas in GC of the unfractionated essential oil and the fractions of the essential oil were calculated

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Table I. Compounds Identified from the Essential Oil of Soiling Dent Corn and Their Percentage Compositions

compound	peak no. ^a	% ^b	compound	peak no. ^a	% ^b
hydrocarbons (16)			alcohols (14)		
limonene	12	0.1	ethanol	5	0.8
<i>p</i> -cymene	18	Tr	3-methylbutanol	13	0.1
<i>n</i> -tetradecane	29	Tr	1-pentanol	17	0.1
<i>n</i> -pentadecane	35	Tr	1-hexanol	25	0.4
<i>n</i> -hexadecane	42	Tr	<i>trans</i> -3-hexenol	26	0.1
<i>n</i> -heptadecane	47	Tr	<i>cis</i> -3-hexenol	27	1.3
α -selinene }	48	1.6	<i>trans</i> -2-hexenol	29	0.1
β -selinene }			38	0.4	
β -bisabolene	50	0.2	linalool	39	0.1
<i>n</i> -octadecane	52	0.1	1-octanol	44	0.1
<i>n</i> -nonadecane	60	0.1	furfuryl alcohol	47	0.8
neophytadiene	65	1.0	α -terpineol	58	0.9
<i>n</i> -tricosane	80	0.8	benzyl alcohol	59	0.8
<i>n</i> -hexacosane	91	0.5	2-phenylethanol	68	0.4
<i>n</i> -heptacosane	95	2.3	nerolidol		
<i>n</i> -nonacosane	100	4.1	esters (15)		
aldehydes (16)			ethyl formate	2	1.3
3-methylbutanal	4	0.2	ethyl acetate	3	0.9
1-pentanal	6	0.1	<i>n</i> -hexyl formate	15	0.1
1-hexanal	7	0.5	ethyl caproate	16	0.1
1-heptanal	11	0.2	<i>cis</i> -3-hexenyl formate	19	0.2
<i>trans</i> -2-hexenal	14	Tr	<i>n</i> -hexyl acetate	20	0.1
1-octanal	21	0.2	<i>cis</i> -3-hexenyl acetate	23	0.3
1-nonanal	28	0.5	ethyl oenanthatate	24	0.1
furfural	32	0.5	ethyl pelargonate	37	0.2
2,4-heptadienal	34	0.4	ethyl benzoate	44	Tr
1-decanal	35	0.1	methyl salicylate	51	0.2
benzaldehyde	36	4.1	ethyl myristate	70	0.6
5-methylfurfural	40	1.1	ethyl pentadecanoate	73	0.3
phenylacetaldehyde	44	0.2	ethyl palmitate	78	0.7
2,4-decadienal	53	0.1	benzyl benzoate	91	0.6
vanillin	89	9.6	acids (10)		
<i>p</i> -hydroxybenzaldehyde	99	0.5	acetic acid	30	0.4
ketones (13)			isovaleric acid	45	1.1
heptan-2-one	10	Tr	2-methylbutyric acid }		
octan-2-one	20	Tr	caproic acid	56	0.5
2-methyl-2-hepten-6-one	24	Tr	oenanthic acid	62	0.5
nonan-2-one	27	Tr	caprylic acid	69	0.4
2,6,6-trimethyl-2-cyclohexen-1-one	41	0.2	pelargonic acid	73	0.4
2-methyl-2,4-heptadien-6-one	42	0.3	lauric acid	86	1.7
undecan-2-one	43	0.3	myristic acid	94	2.6
acetophenone	44	0.1	palmitic acid	99	11.8
2,6,6-trimethyl-2-cyclohexen-1,4-dione	46	0.2	phenols (3)		
tridecan-2-one	54	0.2	guaiacol	56	0.8
β -damascenone	55	0.2	4-vinylguaiacol	74	1.2
β -ionone	61	0.7	4-vinylphenol	82	1.5
6,10,14-trimethylpentadecan-2-one	72	5.0	miscellaneous (3)		
			2-acetylthiazole	44	0.2
			ethylmethylmaleimide	77	0.8
			dihydroactinidiolide	81	1.3

^a Peak numbers were given in the order of appearance in the programmed temperature GC of the unfractionated essential oil. ^b Percentages were calculated from (the peak area percents of the unfractionated essential oil) \times 0.97. The percentages of the overlapped compounds of same peak number were calculated from the ratio of the relative peak areas of fractions.

from the tracing paper cutout weights of the peaks in each chromatogram by using a chemical balance. The relative peak areas of the unfractionated essential oil were then multiplied by 0.97 (derived from the residual 97%; 3% of the yield is assumed to be the basic fraction, because amines could not be detected by GC of the unfractionated essential oil).

RESULTS AND DISCUSSION

Identification of the Components in the Essential Oil. The fresh crop of soiling dent corn was distilled with steam and then extracted with diethyl ether to collect the essential oil. The essential oil was first analyzed by GC-MS without any fractionation. Among 177 peaks in all, 90 compounds consisting of 16 hydrocarbons, 16 aldehydes, 13 ketones, 14 alcohols, 15 esters, 10 acids, 3 phenols, and 3 miscellaneous, which are indicated in Table I, were

identified through comparison of mass spectra with those of authentic specimens and/or with authentic spectra (Stenhagen et al., 1969). The other peaks could not be identified because their mass spectra were those of mixed state or indistinct state.

The essential oil was next fractionated into acidic, phenolic, basic, nonpolar, and polar fractions by the usual chemical procedure. The acidic fraction, after conversion into the methyl esters, and the phenolic, nonpolar, and polar fractions were directly analyzed by programmed-temperature GC of the PEG 20M column. The basic fraction was heated with alkali, and the regenerated amines were analyzed by isothermal GC of a triethanolamine column. These peak assignments were based on the coincidence of t_R with those of the authentic samples. In the GC analysis of the nonpolar fraction, a series of normal

alkanes from C₁₃ to C₃₁, selinene, bisabolene, and neophytadiene were detected. *n*-Nonacosane was the major constituent of the nonpolar fraction (relative peak area, 11.6%). In the acidic fraction, the methyl esters of normal carboxylic acids from C₈ to C₂₀, oleic acid, linolic acid, and linolenic acid were detected. Methyl palmitate was the major constituent of the acidic fraction (46.8%). From the phenolic and basic fractions, phenol and *sec*-amylamine were additionally detected. The GC patterns of these fractions were the small peaks on the whole. The GC pattern of the polar fraction resembled to that of the unfractionated essential oil, but the absence or the decrease of the peaks corresponding to acids, phenols, and hydrocarbons was observed. 6,10,14-Trimethylpentadecan-2-one was the major constituent of the polar fraction (20.3%). In the GC of the headspace vapors of ice-water- and dry ice cooled traps, six compounds of methyl formate, ethyl formate, ethyl acetate, 3-methylbutanal, 1-pentanal, and ethanol were detected.

From the above results, 90 compounds in all were identified as the essential oil constituents of soiling dent corn. Among these, many terpenoid and terpenoid-like compounds are included, and these compounds are considered to contribute to the characteristic aroma of the essential oil. Linalool and β -ionone are derived from primary oxidation of carotenoid compounds, and dihydroactinidiolide and 2,6,6-trimethyl-2-cyclohexen-1-one are derived from secondary oxidation of carotenoid compounds (Sanderson et al., 1971). The unsaturated C₆ alcohols and their esters are also important aroma constituents in production of the green note. However, further investigation of the unknown compounds is necessary.

Percentage Composition of the Essential Oil. The percentage composition of the essential oil of soiling dent corn was subsequently calculated to clarify the aroma character. The result is listed in Table I. The compounds containing more than 3% of essential oil were palmitic acid, vanillin, 6,10,14-trimethylpentadecan-2-one, *n*-nonacosane, and benzaldehyde, in the order of abundant percentage. In the total amounts by functional groups, hydrocarbons, aldehydes, ketones, alcohols, esters, acids, phenols, and miscellaneous were ca. 11, 18, 7, 6, 6, 19, 4, and 2%, respectively, in the essential oil. In comparison with the previous results (Kami, 1978), the essential oil of soiling dent corn was relatively rich in hydrocarbons and aldehydes, while esters and amines were less abundant. Besides this, the total amounts of terpenoid and terpenoid-like compounds were ~7% in the essential oil, and those of C₆ unsaturated alcohols and their esters were ~2%.

In Japan it is generally believed that soiling dent corn is superior to sorghums of the same *Gramineae* in its palatability for dairy cattle. Among sorghums, Hybrid-

sorgo oil was relatively rich in phenols, and Sudangrass oil was relatively rich in aldehydes and ketones (Kami, 1977). From the assumption that the qualitative and quantitative differences of the aromatic constituents of forage crops might have some connection with the palatability of forage crops for domestic animals, it may be said that soiling dent corn is superior to sorghums in its palatability for dairy cattle. However, further investigation of the relation between the aroma of soiling dent corn and its palatability for dairy cattle is necessary.

CONCLUSION

From the essential oil of soiling dent corn, 90 compounds including 16 hydrocarbons, 16 aldehydes, 13 ketones, 14 alcohols, 15 esters, 10 acids, 3 phenols, and 3 miscellaneous were identified by GC-MS and GC. In addition, 22 compounds including 9 normal alkanes (C₁₃, C₂₀, C₂₁, C₂₂, C₂₄, C₂₅, C₂₈, C₃₀, and C₃₁), methyl formate, 10 normal carboxylic acids (C₁₀, C₁₁, C₁₃, C₁₅, C₁₇, C₁₈, C_{18:1}, C_{18:2}, C_{18:3}, and C₂₀), phenol, and *sec*-amylamine were detected by GC alone. Many terpenoid and terpenoid-like compounds were included in the essential oil. In the quantitative analysis by functional groups, the essential oil was relatively rich in hydrocarbons and aldehydes. All the compounds detected are considered to contribute to the overall aroma of soiling dent corn, but especially the terpenoid and terpenoid-like compounds seem to give the characteristic aroma.

ACKNOWLEDGMENT

The author is deeply indebted to S. Hayashi of the Department of Chemistry, Faculty of Science, Okayama University of Science, for his helpful advice and for guidance of the work. The author is very grateful to K. Hayashi and T. Yanai of Kawasaki Research Laboratories, T. Hasegawa Co., Ltd., for their kind determination of the GC-MS data.

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Received for review February 9, 1981. Revised manuscript received April 30, 1981. Accepted June 15, 1981. This paper is part IV in the series Aromatic Constituents of Forage Crops.