Identification of Components in the Essential Oil of Hybridsorgo, a Forage Sorghum

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The essential oil of Hybridsorgo, a forage sorghum, was isolated by steam distillation of the fresh grass with a yield of 0.001%. The essential oil was fractionated into five fractions, in which acid, phenolic, nonpolar, and polar fractions were analyzed by combined gas chromatography–mass spectrometry, and the basic fraction was analyzed by gas chromatography. Sixty-two compounds including 12 acids, 6 phenols, 5 aldehydes, 6 ketones, 6 alcohols, 11 esters, and 16 hydrocarbons were thus identified as steam-distilled Hybridsorgo constituents. The phenols were considered to play an important role in the aroma of Hybridsorgo.

Although it is generally believed that the aroma of forage has some connection with its palatability for domestic animals, chemical investigation on the aroma components of forage has not yet been reported. The present investigation was undertaken on the essential oil of a forage sorghum in order to clarify the nature of the aroma of the grass.

Forage sorghums which are native to Africa and India are cultivated to a great extent all over the world as feedstuffs and, according to the utility and plant form, they are classified into six agricultural species: sorgos, grass sorghums, grain sorghums, broomcorns, special purpose sorghums, and male sterile lines. Among them, sorgos and grass sorghums are suitable for forage production because of their sweet stalk, juiciness, and high green forage yield. In the southwestern warm district of Japan, where the so-called summer depression of forage production is an annual phenomenon owing to high temperatures and drought, the cultivation of sorgos is recommended for its strong drought resistance, high green yield, and high nutritive value.

EXPERIMENTAL SECTION

Materials. The grass of Hybridsorgo, one variety of the sorgo species, which was cultivated on a farm of the Faculty of Fisheries and Animal Husbandry, Hiroshima University, was harvested in October 1970 as the second crop.

Isolation of Essential Oil. The fresh crop (160 kg), after being chopped, was steam distilled in 8-kg lots under 0.5 kg/cm^2 pressure for 1 hr and the distillate was led into a series of three traps cooled with water (18°), ice water, and Dry Ice–acetone to collect, in total, about 70 l. of cloudy aqueous liquid in the first, 14.6 g of colorless aqueous liquid in the second, and 2.9 g of colorless aqueous liquid in the third trap. About 68 l. of the cloudy aqueous liquid in the water-cooled trap was saturated with sodium chloride, and 2-l. lots were then extracted twice with 300 ml of redistilled diethyl ether using a glass separatory funnel of 3-l. capacity. The ether solution (20.4 l.) was dried over anhydrous sodium sulfate and concentrated by distilling the solvent through a 30-cm long Widmer-distilling column under a water bath temperature of less than 45° to give a dark brown oil (1.88 g, η^{20}D 1.4550) which exhibited an unusual smoky odor. The essential oil thus obtained was stored in a sealed glass tube at 3° before the examination, as were the aqueous condensates from the second and third traps.

Fractionation of Essential Oil. The essential oil was successively shaken with 10% sodium carbonate (10 ml), 3% sodium hydroxide (25 ml), and 3% hydrochloric acid (25 ml) solutions, three times with each, to separate it into acid, phenolic, and basic fractions, respectively. The pH of the aqueous solution of acid and phenolic fractions was reversed, and the solution was reextracted with diethyl ether. The basic fraction was concentrated through a Claisen flask under reduced pressure at a water bath temperature of 85°, and some light brown precipitate was obtained at the bottom of a flask. The washed oil layer was further treated three times with 30% sodium bisulfite solution (25 ml) to remove the aldehydes, and the nonextracted portion was eluted through a 20 cm × 2 cm i.d. silica gel packed glass column with 200 ml of *n*-hexane and then diethyl ether to separate it into nonpolar and polar fractions. A portion of the acid fraction was converted to the methyl esters with diazomethane (Vorbeck et al., 1961).

Combined Gas Chromatography–Mass Spectrometry (GC–MS). A Hitachi K53 gas chromatograph and a Hitachi RMU-6E mass spectrometer were combined by inserting a Watson-Biemann type porous glass enricher (Watson and Biemann, 1965) between them. The gas chromatograph was operated, in connection with a U-shaped 2 m × 3 mm i.d. stainless steel column packed with 10% Carbowax 20M on 80–100 mesh Celite 545, under 1.0 kg/cm^2 flow pressure of a helium carrier gas (oven temperature, 3°/min from 60 to 220°; injection port temperature, 280°). The mass spectrometer was worked at 1800 V ion accelerating voltage, 70 eV ionization voltage, 80 μA total emission, 55 μA target emission, and 220° ion source temperature.

GC of the Basic Fraction. A small amount of precipitate of the basic fraction was transferred to a test tube of 20 ml capacity and 0.4 ml of 2 *N* sodium hydroxide solution was added. This test tube was sealed and boiled in an oil bath. The seal was broken after cooling, and 50 ml of the headspace vapor was immediately taken in a large syringe. The vapor was injected into a 10-ml gas sampler attached to a Shimadzu 3AF gas chromatograph. An FID-equipped GC was operated at a column temperature of 65° with a 17 ml/min flow rate of nitrogen carrier gas in a stainless steel spiral tube (3 m × 3 mm i.d.) packed with 20% Triethanolamine on 60–80 mesh Celite 545. The peak assignment was performed by comparison of t_R values with authentic samples.

RESULTS AND DISCUSSION

When the essential oil obtained by steam distillation of Hybridsorgo grass was subjected to GC–MS using a Carbowax 20M column without any fractionation, 58 peaks were detected, and their mass spectra were taken. However, the assignment of the mass spectra was so difficult that only 19 components, indicated by W in Table I, were identified through comparison of the spectra with those of authentic specimens and/or with authentic spectra (Stenhagen et al., 1969), because nearly all mass spectra were the superimposed spectra of two or three components. For further analysis the essential oil was fractionated to each functional group and each fraction was analyzed directly or via chemical treatment by GC–MS, GC, and TLC.

The acid fraction converted to the methyl esters was subjected to GC–MS. Although 28 peaks were detected,

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Table I. Compounds Identified from Hybridsorgo[a]

Compound	Fraction detected	Mol ion (rel intens., %)	Five strongest fragment ions (rel intens., %)
Acids			
Acetic acid	W, O	60 (56)	43 (100), 45 (94), 60 (56), 29 (33), 41 (29)
2-Methylbutyric acid	W	102 (3)	41 (100), 29 (70), 57 (51), 74(46), 60(40)
Valeric acid	W	102 (2)	60 (100), 43 (98), 41 (97), 27 (73), 39 (67)
Caproic acid	A_2	116 (0)	60 (100), 73 (46), 41 (33), 43 (29), 27 (24)
Oenanthic acid	A_2	130 (0)	60 (100), 73 (99), 41 (74), 43 (64), 55 (50)
Caprylic acid	A_1, A_2	144 (3)	60 (100), 73 (64), 43 (61), 41 (51), 55 (38)
Pelargonic acid	A_2	158 (5)	60 (100), 73 (80), 43 (74), 41 (70), 57 (69)
Capric acid	A_2	172 (8)	60 (100), 73 (90), 43 (83), 41 (73), 55 (69)
Lauric acid	W, A_2	200 (16)	73 (100), 60 (96), 43 (90), 41 (81), 55 (68)
Myristic acid	A_2	228 (22)	73 (100), 60 (88), 43 (87), 41 (86), 55 (76)
Benzoic acid	A_1, A_2	122 (78)	105 (100), 77 (82), 122 (78), 51 (47), 50 (28)
Phenylacetic acid	A_1	150 (25)*	91 (100), 39 (28), 150 (25), 65 (18), 51 (10)*
Anisic acid (+)	A_1	166 (37)*	135 (100), 166 (37), 77 (28), 43 (19), 92 (18)*
Veratric acid (+)	A_1	196 (99)*	165 (100), 196 (99), 79 (32), 51 (29), 59 (27)*
Phenols			
Guaiacol	P	124 (78)	109 (100), 124 (78), 81 (62), 27 (34), 39 (31)
Phenol	W, P	94 (100)	94 (100), 39 (37), 66 (34), 65 (27), 40 (19)
o-Cresol	W, P	108 (100)	108 (100), 107 (79), 79 (47), 77 (43), 90 (28)
p-Cresol	W, P	108 (85)	107 (100), 108 (85), 77 (29), 79 (25), 39 (24)
p-Ethylphenol	P	122 (37)	107 (100), 122 (37), 77 (22), 39 (18), 27 (16)
3,4-Xylenol	P	122 (68)	107 (100), 122 (68), 121 (60), 39 (32), 77 (26)
Aldehydes			
Acetaldehyde	W, T	44 (65)	29 (100), 44 (65), 43 (45), 42 (16), 41 (12)
Furfural	W	96 (100)	96 (100), 95 (93), 39 (72), 29 (63), 38 (46)
Phenylacetaldehyde	W, T	120 (22)	91 (100), 65 (31), 92 (26), 120 (22), 89 (7)
Anisaldehyde	A_1	136 (66)	135 (100), 136 (66), 77 (45), 107 (27), 92 (22)
Veratraldehyde	A_1	166 (100)	166 (100), 165 (58), 95 (51), 77 (44), 51 (35)
Ketones			
Acetone	O, T, H	58 (29)	43 (100), 58 (29), 27 (18), 42 (12), 26 (11)
Methyl ethyl ketone	O, T, H	72 (17)	43 (100), 29 (40), 27 (23), 72 (17), 42 (9)
Methyl *n*-butyl ketone	N, O, T	100 (5)	43 (100), 58 (34), 29 (23), 41 (20), 27 (17)
Methyl *n*-hexyl ketone	O	128 (2)	43 (100), 58 (30), 41 (21), 29 (18), 27 (18)
Methyl *n*-heptyl ketone	O	142 (2)	43 (100), 29 (40), 41 (37), 58 (35), 57 (28)
2,3-Pentanedione	O	100 (12)	43 (100), 29 (54), 57 (54), 27 (36), 71 (31)
6,10,14-Trimethyl-pentadecan-2-one (+)	W, N, O	268 (0)	43 (100), 58 (57), 41 (36), 55 (30), 57 (30)
Alcohols			
Ethanol	W, A_1, O, H	46 (18)	31 (100), 45 (41), 27 (26), 29 (24), 43 (21)
3-Methyl-3-pentanol	N, O	102 (0)	73 (100), 43 (62), 55 (59), 45 (44), 29 (32)
Linalool oxide (five membered) (+)	O	170 (0)	59 (100), 43 (50), 68 (33), 94 (28), 83 (28)
Benzyl alcohol	W, O	108 (66)	79 (100), 108 (66), 77 (64), 91 (57), 107 (55)
2-Phenylethanol	W, O	122 (23)	91 (100), 92 (48), 65 (27), 122 (23), 51 (23)
Isophytol	N, O	296 (0)	71 (100), 43 (48), 57 (34), 55 (31), 41 (28)
Phytol	N	296 (0)	71 (100), 43 (50), 57 (34), 55 (27), 41 (26)
Esters			
Ethyl formate	W, H	74 (12)	31 (100), 28 (80), 29 (65), 27 (49), 45 (33)
Ethyl acetate	W, O, H	88 (4)	43 (100), 29 (27), 27 (16), 45 (16), 61 (10)
Ethyl propionate	O, H	102 (8)	29 (100), 57 (66), 28 (41), 27 (39), 45 (24)
n-Hexyl acetate	O	144 (0)	43 (100), 56 (35), 41 (31), 55 (29), 29 (28)
n-Octyl acetate	N, O	172 (0)	43 (100), 56 (33), 41 (32), 55 (30), 70 (28)
Benzyl acetate	O	150 (31)	108 (100), 91 (91), 60 (67), 79 (51), 90 (50)
Cetyl acetate	N	284 (0)	43 (100), 57 (82), 55 (56), 41 (54), 69 (49)
Ethyl myristate	O	256 (9)	88 (100), 43 (75), 41 (58), 101 (55), 73 (48)
Methyl palmitate	N	270 (5)	74 (100), 87 (60), 43 (35), 41 (21), 55 (20)
Ethyl palmitate	W, O	284 (11)	88 (100), 43 (75), 41 (56), 101 (53), 55 (48)
Phytyl acetate	N	338 (0)	43 (100), 57 (86), 55 (73), 41 (60), 69 (59)
Hydrocarbons			
p-Xylene	N	106 (54)	91 (100), 106 (54), 39 (27), 105 (24), 51 (16)
n-Pentadecane	N	212 (5)	57 (100), 43 (99), 71 (54), 41 (47), 85 (36)
n-Hexadecane	N	226 (5)	57 (100), 43 (100), 71 (59), 41 (46), 85 (37)

Table I *(Continued)*

Compound	Fraction detected	Mol ion (rel intens., %)	Five strongest fragment ions (rel intens., %)
n-Heptadecane	N	240 (4)	57 (100), 43 (90), 71 (58), 41 (44), 85 (34)
n-Octadecane	N	254 (4)	57 (100), 43 (98), 71 (58), 41 (45), 85 (34)
n-Nonadecane	N	268 (4)	57 (100), 43 (88), 71 (57), 41 (46), 85 (36)
n-Eicosane	N	282 (3)	57 (100), 43 (93), 71 (57), 41 (48), 55 (40)
n-Heneicosane	N	296 (0)	43 (100), 57 (53), 71 (39), 41 (38), 55 (34)
n-Docosane	N	310 (3)	43 (100), 57 (98), 71 (58), 41 (54), 55 (49)
n-Tricosane	W, N	324 (2)	57 (100), 43 (85), 71 (59), 85 (36), 41 (35)
n-Tetracosane	N	338 (0)	57 (100), 43 (86), 71 (62), 85 (41), 41 (37)
n-Pentacosane	N	352 (0)	43 (100), 57 (79), 71 (50), 55 (44), 41 (42)
n-Hexacosane	N	366 (3)	57 (100), 43 (88), 71 (68), 55 (41), 85 (40)
n-Heptacosane	N	380 (3)	57 (100), 43 (88), 71 (71), 85 (46), 55 (30)
n-Octacosane	N	394 (4)	57 (100), 43 (83), 71 (66), 55 (42), 85 (37)
n-Nonacosane	N	408 (2)	57 (100), 43 (76), 71 (63), 85 (40), 55 (25)
Amines			
Trimethylamine (T)	B	t_R 4.1 min	
Triethylamine (T)	B	t_R 7.7 min	
Dimethylamine (T)	B	t_R 11.0 min	
Diethylamine (T)	B	t_R 13.7 min	
Isopropylamine (T)	B	t_R 15.4 min	
Ethylamine (T)	B	t_R 17.3 min	
n-Propylamine (T)	B	t_R 31.3 min	
Miscellaneous			
Chloroform (+)	W, A_2, N, O	118 (4)	83 (100), 85 (64), 47 (45), 48 (20), 35 (19)

[a] W, whole essential oil; A_1, acid fraction via methylation; A_2, acid fraction without methylation; P, phenolic fraction; N, nonpolar fraction; O, polar fraction; T, TLC of 2,4-DNPHs; H, headspace vapors of ice water and Dry Ice cooled traps; B, basic fraction; (+), probable artifact; (T), tentative; *, mass spectra of methyl esters of each acid.

only 8 components including 5 carboxylic acids and 3 nonesters were successfully assigned (A_1 in Table I). On the other hand, the acid fraction was directly analyzed by GC–MS without converting to the methyl esters, and a series of normal monocarboxylic acids from C_6 to C_{14}, except for undecanoic and tridecanoic acids, was identified together with benzoic acid and chloroform (A_2 in Table I). Among these, benzoic acid was the major constituent of this fraction (17.5%). Anisic and veratric acids were thought to be artifacts on the basis of the fact that anisaldehyde and veratraldehyde were detected in the same fraction.

By GC–MS of the phenolic fraction, 6 phenolic compounds were identified (P in Table I). Phenol and *o*-cresol appeared as an unseparable peak and they were the major constituents of this fraction (39.8%). The phenolic compounds amounted to 34.8% of the essential oil. The phenolic fraction does indeed exhibit a predominant phenolic odor and the essential oil has a smoky odor. Therefore, the phenols were considered to play an important role in thc aroma of Hybridsorgo.

In GC–MS of the nonpolar fraction, a series of normal alkanes (C_{15}–C_{29}), including the odd- and even-numbered carbons, was identified together with 11 other compounds such as acetates, allylic alcohols, ketones, and methyl esters (N in Table I). Among these, heneicosane, docosane, and pentacosane were detected as overlapped peaks with 6,10,14-trimethylpentadecan-2-one, methyl palmitate, and phytol, respectively. In the hydrocarbon series, odd-numbered carbons predominated over even-numbered carbons in the region above docosane just as in surface waxes of higher plants, and nonacosane was the major constituent of this fraction (13.0%).

In the polar fraction, 22 compounds were identified (O in Table I). The terpene-like constituents (Gautschi et al., 1967) such as linalool oxide (Bondarovich et al., 1967) and 6,10,14-trimethylpentadecan-2-one (Stoll et al., 1967) were detected, and 6,10,14-trimethylpentadecan-2-one was the major constituent of this fraction (16.0%). These compounds were thought to be artifacts yielded from the thermal breakdown of such higher terpenes as cafestol, kahweol, and squalene during the steam distillation (Kaufmann and Sen Gupta, 1964).

The amines were regenerated by heating the basic fraction with alkali and examined by isothermal GC only. Thus, seven amines were tentatively identified (B in Table I). Diethylamine was the major constituent of this fraction (24.3%).

Although some carbonyl compounds could be identified by GC–MS of the various fractions, further analysis was carried out by TLC of 2,4-dinitrophenylhydrazones which were prepared by adding a 2,4-dinitrophenylhydrazine solution to 2 l. of cloudy aqueous liquid in the water-cooled trap (Kami et al., 1972). Five carbonyl compounds were assigned through comparison of the R_f values with those of authentic samples and all the compounds were those identified by GC–MS (T in Table I).

Both headspace vapors of the ice water and Dry Ice cooled traps were also examined by isothermal GC to clarify low-boiling constituents of the essential oil (Kami et al., 1972). Six compounds were assigned through comparison of the t_R values with those of authentic samples and all the compounds were those identified by GC–MS (H in Table I). Ethyl formate was the major constituent of both traps (64.2%).

CONCLUSION

As a result of this study, 67 compounds have been identified by GC–MS, and among these, 5 compounds, anisic acid, veratric acid, linalool oxide, 6,10,14-trimethylpentadecan-2-one, and chloroform, are thought to be artifacts. In addition, 7 amines have been tentatively identified by GC alone. All these compounds are reported for the first time as steam-distilled Hybridsorgo constituents. Many of the components identified were considered to contribute to the

overall aroma of Hybridsorgo and, especially the phenols, to have the typical volatile character.

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Ethoxyquin Nitroxide

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Ethoxyquin (EQ), a widely used antioxidant, is easily oxidized to a stable free radical, ethoxyquin nitroxide (EQN). This paper describes the synthesis, isolation, and characterization of EQN. When squalene containing EQ is oxidized in air, EQN is an identifiable intermediate; rapid oxidation does not proceed until the EQN electron paramagnetic resonance (EPR) signal disappears. EQN is stable in methyl laurate but the EPR signal decreases in unsaturated lipid substrates, even in the absence of oxygen. At temperatures used in gas chromatography, EQN was detected as a single peak whereas EQ was changed into several components. In limited tests, EQN was slightly superior to EQ as an antioxidant in unsaturated lipids.

The antioxidant ethoxyquin (2,2,4-trimethyl-1,2-dihydro-6-ethoxyquinoline) (EQ) was first used in rubber formulations and later adapted to use in feeds, particularly on dehydrated alfalfa as a protective agent for carotenoids (Thompson, 1950; Van der Veen and Olcott, 1964; Knowles et al., 1968), in fish meals (Wessels, 1971; Atkinson et al., 1972), and in foods (Parke et al., 1973). EQ is an excellent antioxidant in squalene and fish oils (Olcott, 1958; Weil et al., 1968).

The concept that antioxidants may operate by being converted to free radicals capable of neutralizing substrate free radicals and thus inhibiting oxidation was suggested first by others with nonlipid substrates (Thomas and Tolman, 1962; Adamic et al., 1969). Harris and Olcott (1966) found that trioctylamine was converted to the free radical, dioctyl nitroxide, in an oxidizing lipid system, and Weil et al. (1968) showed that some stable synthetic nitroxides were considerably more effective antioxidants than EQ in squalene. Evidence was obtained that proline was converted to its nitroxide in an oxidizing system (Van der Veen et al., 1970). Recently proline nitroxide has been isolated and shown to have antioxidant activity (Lin et al., 1974a). These combined observations indicated to us that the mechanism of action of EQ should involve the free radical, ethoxyquin nitroxide (EQN). When solutions of EQ were exposed to air and light or mixed with oxidizing unsaturated lipids, an electron paramagnetic resonance spectrum (EPR) indicating the presence of EQN was readily obtained. In this paper we describe the synthesis, isolation, and some properties of EQN.

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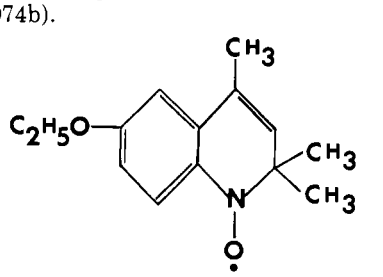
EXPERIMENTAL SECTION

Materials and Methods. Reddish brown samples of technical grade ethoxyquin (Santoquin, Monsanto) were purified by silicic acid column chromatography (SilicAR CC7, 200–325 mesh, Mallinckrodt, column o.d. 25 mm, height 500 mm) with chloroform (Mallinckrodt) as eluent. Silica gel thin-layer (Eastman) chromatography with chloroform and gas–liquid chromatography (Hewlett-Packard, Model 810 with a 6 ft × 0.25 in. i.d. glass column packed with 10% diethylene glycol adipate (DEGA) on Gas-Chrom Q) were used to demonstrate the homogeneity of the EQN. EPR, uv, and ir spectra were obtained with a Varian E-3 x-band spectrometer and Cary Model 15 and Perkin-Elmer Model 137 instruments, respectively. A Finnigan GC Model 9500 (3% Carbowax 20M on Chromosorb G, i.d. 2 mm, 5 ft glass column) interfaced to Finnigan MS Model 3200 equipped with electron impact source at 70 eV and Finnigan Computer Data System Model 6000 were used for gas chromatography–mass spectral (GC–MS) measurements. The methods used for simultaneous evaluation of nitroxide radical content and lipid oxidation were those described by Lin et al. (1974b).



ETHOXYQUIN NITROXIDE