COMMUNICATION TO THE EDITOR

Composition of Surface Wax from Sorghum Grain

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To the Editor:

Sorghum grain wax has been isolated but the chemical analysis was limited to incomplete identification of the major classes of components (Cannon and Kummerow 1957, Dalton and Mitchell 1959, Hubbard et al 1950, Seitz 1977).

Considering the enormous importance of sorghum grain as food and feed, we decided to investigate the chemical composition of the waxy material covering the kernels of sorghum. In addition, because the preference of birds for the various sorghum varieties has been attributed to the tannin content of sorghum (McMillian et al 1972), further knowledge of the detailed composition of the surface wax of grain might be relevant.

This communication reports the results of a study of the wax obtained from dried grain of sorghum variety SD-102.

MATERIALS AND METHODS

The grain of sorghum variety SD-102 (*Sorghum vulgare*) was obtained from plants grown in fields near Rome in 1976. Three hundred grams of intact, clean, and bright kernels were subjected to two extractions. In the first extraction, the seeds were covered with chloroform for about 60 sec at room temperature. The same grain was extracted a second time using hot benzene (~ 60° C) for the same time.

Evaporation of the solvents on a rotary evaporator under reduced pressure produced the wax samples. The yields of wax obtained by the two extractions were 210 and 450 mg, respectively.

Column separations were done by gradient elution on silica gel type H (Merck). Carbon tetrachloride eluted *n*-alkanes, esters, and aldehydes in that order; chloroform eluted alcohols; and chloroform containing 1% of acetic acid eluted free acids.

For gas chromatography (GC), free acids were transformed into the corresponding methyl esters using diazomethane (Vogel 1961). GC analyses were performed by using 2 m × 3 mm glass columns packed with OV1 1% on Chromosorb PS, 100–120 mesh, on a Carlo Erba Model Fractovap 2400 T GC with flame ionization detectors. Isothermal and programmed chromatograms were run, using column temperatures from 160–280° C as required; nitrogen, hydrogen, and air streams were adjusted to yield optimum sensitivity.

Authentic samples (Fluka) of each class of compounds were used as standards for GC.

Infrared spectra were obtained using KBr disks (0.5 mg of sample per 100 mg of KBr) with a Perkin Elmer Model 257 spectrophotometer.

The mass spectra were run on a Du Pont 21492 B mass spectrometer at 70 eV of filament energy, an ion source temperature of 230° C, an accelerating voltage of 1400 V, and a direct inlet system at 100° C.

RESULTS AND DISCUSSION

The extraction with hot benzene gave nearly twice the amount of waxy material as was obtained with chloroform. The overall yield of wax was 0.22%. The composition of the compounds in the two extracts was similar; consequently the analyses were performed on the combined materials. The percentages of each class of compound comprising the wax are given in Table I. The wax contains *n*-alkanes, esters, aldehydes, free alcohols, and free fatty acids. These findings do not entirely agree with those of Dalton and Mitchell (1959) and Seitz (1977), who reported the following averaged composition for the sorghum grain varieties examined: *n*-alkanes, 4–5%; esters, 46–50%; free alcohols, 40–46%; and about 8% other lipid components. The components shown in the present study that differ from the previous data are aldehydes and free acids (Table I).

The homologous composition profiles of *n*-alkanes, aldehydes, alcohols, and acids are presented in Table II. Structural and functional confirmation of all classes of compounds were obtained by mass spectrometry (MS) and infrared spectroscopy (IR).

The *n*-alkanes were straight chain compounds, principally heptacosane and nonacosane. The IR spectrum had strong bands at 2,960, 2,920, 2,855, 1,475, 1,465, 1,380, 730, and 720 cm^{-1}. In the MS of alkanes, the most prominent series of ionized fragments consisted of singly charged alkyl ions of the empirical formula $[C_nH_{2n+1}]^+$.

The chain length ranges of aldehydes and free alcohols were similar, with major C_{28} and C_{30} chains. The carbonyl band of aldehydes was at 1,715 cm^{-1}; the alcohols spectrum had the

TABLE I
Chemical Composition of Surface Wax of Dried Grain of Sorghum Variety SD-102

Component	Percent[a]
n-Alkanes	1.3
Aldehydes	31.9
Alcohols	33.7
Esters[b]	4.0
Acids	24.4
Unidentified	4.7

[a] Composition was determined by silica gel column chromatography of combined benzene and chloroform extracts.
[b] Contaminated by unidentified material.

TABLE II
Composition (%) of Fractions from Wax of Sorghum Grain[a]

Carbon Chain Length	*n*-Alkanes	Aldehydes	Free Alcohols	Free Acids
16	...	...	...	9.0
18:1	...	...	...	25.0
18:2	...	...	...	20.0
21	...	...	tr[b]	...
22	...	...	5.8	tr
23	tr	...	tr	...
24	tr	0.1	11.4	1.0
25	2.5	tr	1.1	0.2
26	4.2	2.5	12.7	2.4
27	34.0	0.1	1.6	0.6
28	4.2	46.7	44.6	20.6
29	53.2	0.3	0.7	0.6
30	0.4	49.9	22.1	20.6
31	1.5	tr	...	...
32	tr	0.4	...	...

[a] Composition was obtained by gas chromatography of material from combined benzene and chloroform extracts.
[b] tr = Trace, less than 0.1%

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CH_2-O-H bands at 3,300 and 1,060 cm^{-1}. In the mass spectra of alcohols, the molecule-ions were observed, but the highest peaks in the high mass range were $m/e = M^+ - 18$ and $M^+ - 20$, corresponding to ions formed from the molecule-ion with loss of water and water plus two hydrogens, respectively. The following characteristic ions were observed for aldehydes: $m/e = M^+ - 18$, $M^+ - 44$, and $M^+ - 46$.

In addition to C_{28} and C_{30} chains, free acids contained an unusually high amount of oleic acid (25%) and linoleic acid (20%). The IR spectrum of this fraction had a broad band in the 3,600–2,500 cm^{-1} region and a strong band at 1,710 cm^{-1}. The acids were transformed in the corresponding methyl esters and the mass spectra recorded. The peaks of molecule-ion and those at $m/e = M^+ - 29$, $M^+ - 31$, and $M^+ - 43$ were evidence for the structure of the saturated esters. In the case of methyl oleate, the diagnostic peaks $m/e = M^+$, $M^+ - 32$, and $M^+ - 74$ were observed, as were $e/m = M^+$ and $M^+ - 31$ for methyl linoleate.

Because the amount of esters available is small, this class of compounds was analyzed only by MS. The molecule-ions M^+ of C_{32} to C_{48} saturated homologues with major C_{40} to C_{46} were observed. The ions $RCO_2H_2^+$, and RCO^+ from the acid moiety and $R^+ - 1$ from the alcohols moiety showed that the esters were derived from C_{16} to C_{26} acids and C_{20} to C_{28} alcohols. However, unidentified peaks also were present in the mass spectrum. The MS fragmentation patterns we report agree with those of Ryhage and Stenhagen (1960) and Budzikiewicz et al (1964). The IR spectrum of esters had a strong band at 1,740 cm^{-1}. The unidentified material (4.7%) was gradually eluted after aldehydes. No single major component is likely to be present in this unidentified fraction.

The epicuticular wax of SD-102 plants has already been analyzed (Bianchi et al 1978). The occurrence of the same two major chain lengths, namely C_{28} and C_{30}, in both grain and epicuticular waxes of the sorghum plant is of considerable interest from a biosynthetic point of view. The grain surface wax contains higher percentages of aldehydes and free alcohols but a lower percentage of esters than does epicuticular wax. Free acids of grain wax contain unstaurated acids (18:1, 18:2) that are absent in epicuticular wax of the plant.

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