Aldehydes in Grain Sorghum Wax

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ABSTRACT: Improved knowledge of the properties, composition, and analysis of grain sorghum wax would assist in efforts for industrial application of this product. Wax extracted from grain sorghum, harvested in 1996 in Nebraska, using hot hexane was fractionated with silica gel column chromatography using a series of mixtures of hexane, chloroform, methanol, and acetic acid. During TLC analysis of the sorghum wax, a dark band, which did not appear in carnauba wax, was found between wax esters and TAG. This dark band fraction was the primary component, representing more than 40% of the total sorghum wax weight. The purpose of this study was to chemically characterize the dark band. The fraction containing the dark band was subjected to borohydride reduction and autoxidation by exposure to air. The borohydride reduction gave a dark band at the fatty alcohol position on TLC, whereas the oxidized sample showed a dark band at the FA position, strongly suggesting the original dark band contained aldehydes. NMR and GC–MS data confirmed that this fraction contained a saturated C_{28} aldehyde.

Paper no. J10112 in *JAOCS 79*, 529–533 (June 2002).

KEY WORDS: Aldehyde, autoxidation, borohydride reduction, grain sorghum, wax.

Production of grain sorghum in the United States fluctuated between 12 and 22 million metric tons per year during 1990–2000 (1). In 2000 alone, 13% of the crop was used to produce ethanol using a dry-grind ethanol process. A primary component remaining in the distillers' grains after ethanol production is grain sorghum wax. Since it was first noted in the 1940s and judged at that time as a potential source of natural wax with properties similar to carnauba wax, grain sorghum wax has been studied in terms of its recovery, physicochemical properties, and applications. Although sorghum wax has physical properties similar to carnauba wax, the chemistry of sorghum wax is not fully understood. Furthermore, the few studies on the chemical composition of the wax that have been published are not in agreement.

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Bunger and Kummerow (2) reported that an ethanol-insoluble fraction of sorghum wax contained 48% hydrocarbons, 19% FA (esterified and free forms), and 16% FA (esterified and free forms). Later, Cannon and Kummerow (3) suggested the wax contained wax esters, FFA, and probably free fatty alcohols. Dalton and Mitchell (4) reported that grain sorghum wax appeared to be composed of 5% hydrocarbons, 49% wax esters, and 46% free fatty alcohols. They also concluded it did not contain carbonyl compounds. Seitz (5) reported that the wax extracted from grain sorghum consisted of 4–5% hydrocarbons, 46–50% wax esters, 40–45% fatty alcohols, and about 8% other lipid components. Bianchi and his group extensively studied waxes from sorghum plants (6–9). They reported that most of the sorghum plant waxes observed contained aldehydes in large quantities as well as other traditional wax components.

The limited and conflicting information on sorghum wax chemistry has hindered attempts to develop applications for the wax. Because of this, we were encouraged to pursue the chemistry of sorghum wax further to see if the conflicting information could be resolved. In a preliminary study, a very dark band was observed on a charred TLC plate of sorghum wax. The band did not match any standard compound typically found in natural waxes. Furthermore, it represented at least 40%, by weight, of the sorghum wax. Therefore, the purpose of the present study was to identify this component and elucidate the chemistry of grain sorghum wax.

MATERIALS AND METHODS

Materials and reagents. Grain sorghum was Golden Harvest H512 from the 1996 crop in southeastern Nebraska. Carnauba wax (T-1) was obtained from Strohmeyer & Arpe Co., Ltd. (Short Hills, NJ). All the solvents and reagents were American Chemical Society grade. Silica gel (particle diameter: 2–25 μm; average pore diameter: 60Å) and silica gel TLC plates (general-purpose, 20 × 20 cm, particle size: 250 μm) were purchased from Aldrich Chemical Co. (Milwaukee, WI). *n*-Octacosane, arachidic acid, arachidyl alcohol, *cis*-13 octadecenal, and dodecanal were purchased from Sigma Chemical (St. Louis, MO). β-Sitosterol was purchased from

Acros (Somerville, NJ). Stigmasterol was purchased from ICN Biomedicals Inc. (Aurora, OH). All the other standard compounds were purchased from Nu-Chek-Prep, Inc. (Elysian, MN).

Extraction of wax from grain sorghum. Wax was extracted from grain sorghum using hexane or ethanol (10). Eight hundred grams of grain sorghum kernels in 800 mL hexane or ethanol was refluxed for 30 min. The filtered solution was stored at −18°C overnight. Filtration with Whatman No. 42 filter paper collected the precipitate. Residual solvent was removed from collected wax using vacuum at room temperature.

Silica column chromatography. Wax was fractionated using silica column chromatography. Silica gel was heated in a muffle furnace at 550°C overnight, cooled at room temperature, and stored in a bottle with a stopper. Just before preparing the column, 43 g silica gel was heated at 125°C for at least 5 h and cooled in a desiccator at room temperature. Water (3%) was added to the silica gel followed by shaking and standing for at least 3 h before use. The silica gel was dispersed in hexane, transferred into a glass column (15 cm length, 4.5 cm diameter) without air pockets, and topped with 10 g anhydrous sodium sulfate. The column was wrapped with two heating tapes (45–50°C). Wax (about 0.5 g) was dissolved in 10 mL hexane with heat (45–50°C) and applied on the column. The eluting solvent mixtures were applied on the column in the sequence of 50 mL hexane, 30 mL hexane/chloroform (containing 0.75% ethanol) (29:1, vol/vol), 30 mL hexane/chloroform (5:1), 60 mL hexane/chloroform (1:1), 30 mL hexane/chloroform (1:2), 180 mL chloroform, 102 mL chloroform/acetic acid (50:1), 84 mL chloroform/acetic acid (20:1), and 100 mL methanol. Solvents were heated to 45–50°C before use. The column was pressurized with nitrogen to elute at a rate of 3 drops/s. The eluate was collected in 10-mL fractions in succeeding test tubes. The eluates were concentrated to one-fourth of their original volume under nitrogen, capped, and stored at –20°C until used.

TLC. About 10 μL in each tube from column chromatography was spotted on a TLC plate. Whole sorghum wax, carnauba wax (20–200 μg), and standards (10–40 μg) also were spotted. The developing solvents were hexane/diethyl ether/acetic acid (85:15:2, by vol), hexane/diethyl ether/acetic acid (88:12:2), hexane/diethyl ether (92:8), hexane/diethyl ether (94:6), hexane/diethyl ether (98:2), hexane/diethyl ether/acetaldehyde (95:5:2), chloroform (containing 0.75% ethanol), and chloroform/acetone (98:2). Developed bands were visualized by dipping the plate in a solution of 10 g cupric sulfate dissolved in 100 mL 8% phosphoric acid for 5 s, letting it dry for 5 min, and heating it in an oven at about 150°C until the developed bands were charred (11).

Identification of unidentified component. The column chromatography fraction containing materials that appeared very dark on charred TLC plates was subjected to reactions with 2,4-dinitrophenylhydrazine (12), Benedict's test solution (13), chromic acid (14), KOH under hydrolysis conditions (15), KOH in the Cannizzaro reaction (14,16), borohydride reduction (16), and autoxidation by exposing the fraction to room temperature air for 5 d. In addition, subsamples of the dark band fraction were analyzed using NMR spectroscopy and GC–MS. Subsamples for NMR analysis were dissolved in deuterated methylene chloride (99% D). NMR spectra were acquired using an Omega-300 NMR (300.52 MHz for proton observations; General Electric NMR Instruments, Fremont, CA). The ^{1}H NMR was acquired using 32 scans, with 16 K (complex) data points. The data were processed using 0.3 Hz exponential line-broadening for apodization and signal enhancement. The ^{13}C NMR (75.56 MHz) was a result of 1024 scans, using 16 K (complex) points. The data were processed using an exponential window function (1.0 Hz), with zero-filling to 32 K complex points. The GC–MS system consisted of an HP 5890 Series GC coupled with HP 5972 Series MSD (Hewlett-Packard Co., Wilmington, DE) in a CI mode with methane as the CI gas. Chromatographic separation of subsamples was accomplished using a DB-5 capillary column (0.25 mm i.d.), with a helium gas flow rate of 12.7 mL/h. The GC temperature ramp was 100–280°C, with an initial solvent delay of 3 min, followed by a temperature ramp of 20°C/min. The final temperature (280°C) was held for 5 min to ensure that all material had eluted from the column. The MS data were scanned at a rate of 3 scans/s, with a scan range of m/z = 90–550 amu.

RESULTS AND DISCUSSION

TLC of grain sorghum wax. TLC of grain sorghum wax showed several light (L) bands and three distinctive dark (D) bands (Fig. 1). The compositions of the waxes extracted with hexane and ethanol were almost the same when comparing TLC plates. Hereafter, references to sorghum wax pertain to the wax extracted from grain sorghum with hexane and can be inferred to relate to wax extracted with ethanol. Light bands 1, 2, and 3 in Table 1 were possibly hydrocarbons, wax esters, and wax esters/steryl esters, respectively, based on R_f values. Dark band 1, in Table 1, of the sorghum wax developed between the wax esters and the TAG in the TLC solvent system of hexane/diethyl ether/acetic acid (85:15:2) and did not match any of the tested standards using different TLC solvent systems (Fig. 1, Table 1). This band appeared very lightly in carnauba wax (Fig. 1). Dark bands 2 and 3 in Table 1 were determined to be alcohols and acids, respectively, since they always developed at almost the same distances as standards of a FA (arachidic acid) and a fatty alcohol (arachidyl alcohol) in different TLC developing solutions. R_f values of the wax components were not exactly the same as those for the standards because acids and alcohols in wax are longer-chained than the standards. One of the major components of carnauba wax was wax esters (Fig. 1).

Silica column chromatography. Over seventy 10-mL fractions were collected by silica column chromatography. Fractions 6 and 7 contained the light band 1 component of Table 1. When fractions 6 and 7 were combined and developed on TLC, one more light band appeared with a higher R_f [0.84 in hexane/diethyl ether/acetic acid (85:15:2)] than the light band

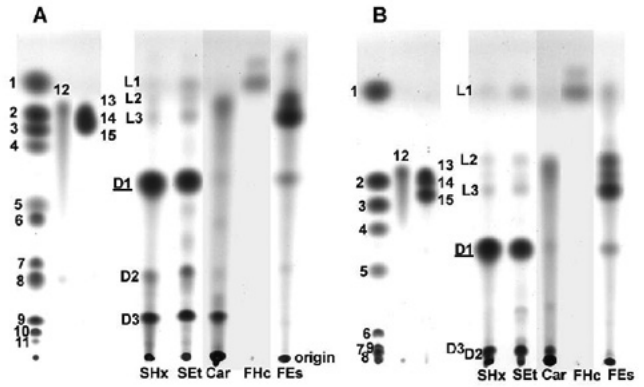

FIG. 1. TLC of waxes. (A) Developed in hexane/diethyl ether/acetic acid (85:15:2). (B) Developed in hexane/diethyl ether (94:6). SHx: grain sorghum wax extracted with hexane. SEt: grain sorghum wax extracted with ethanol. Car: carnauba wax. FHc: the 6th and 7th fractions of silica column chromatography. FEs: the 18th–21st fractions of silica column chromatography. L1, L2, L3: light bands 1, 2, and 3. D1, D2, D3: dark bands 1, 2, and 3. Standards: (1) *n*-octacosane, (2) arachidyl arachidate, (3) arachidyl linolenate and linolenyl arachidate, (4) linolenyl linolenate, (5) *cis*-13 octadecenal, (6) triarachidin, (7) trilinolenin, (8) arachidic acid, (9) arachidyl alcohol, (10) 1,3-diarachidin, (11) 1,2-diarachidin, (12) lignoceryl lignocerate, (13) cholesteryl arachidate, (14) cholesteryl palmitate, and (15) cholesteryl linoleate.

1. This additional band must have been longer-chain hydrocarbon(s). Components of light bands 2 and 3 mostly coeluted in fractions 18–21 with light band 2 eluting a little faster than light band 3. When fractions 18–21 were combined, concentrated, and spotted on TLC (Fig. 1), part of light band 3 showed a very light pink color during charring, indicating this band contained a small amount of steryl esters. Pink coloration of a TLC plate during charring was a typical characteristic of free sterols and their esters (but not stanols and their esters) when checked with standards. These three light band components were minor materials in sorghum wax, constituting about 2% (w/w) of total wax. The alcohols and acids mostly eluted in fractions 34–48 and 52–55, respectively, representing about 32% (w/w) and 7% (w/w) of the wax. The unidentified dark band fraction mostly eluted in fractions 22–28. This was the primary component in sorghum wax, constituting about 43% (w/w).

Identification of the dark band fraction—aldehydes. The reaction of the dark band 1 fraction, obtained using the silica column chromatography, with 2,4-dinitrophenylhydrazine resulted in a color change to yellow, but no precipitation, as acetaldehyde, acetone, and a longer aldehyde standard, *cis*-13-octadecenal, showed in their positive reactions.

TABLE 1
TLC R_f Values of Standard Compounds and Major Sorghum Wax Components

	Solvent system[a]							
	A	B	C	D	E	F	G	H
Standard								
n-Octacosane	0.79	0.79	0.78	0.78	0.85	0.78	0.77	0.83
Lignoceryl lignocerate	0.73			0.54				
Arachidyl arachidate	0.72	0.65	0.63	0.51	0.38	0.60	0.71	0.80
Arachidyl linolenate & linolenyl arachidate	0.69	0.60	0.57	0.44		0.53	0.69	0.80
Linolenyl linolenate	0.65	0.54	0.51	0.37		0.46	0.67	0.80
Cholesteryl behenate	0.74					0.62		0.82
Cholesteryl arachidate	0.71			0.58				
Cholesteryl palmitate	0.69			0.57				
Cholesteryl linoleate	0.66			0.48				
cis-13 Octadecenal	0.49	0.39	0.36	0.25		0.35	0.53	0.68
Triarachidin	0.47	0.24	0.17	0.08		0.15	0.48	0.76
Trilinolenin	0.36	0.19	0.10	0.03		0.08	0.44	0.76
Arachidyl alcohol	0.16	0.07	0.06	0.02	0.03	0.04	0.16	0.31
Arachidic acid	0.34	0.21	0–0.10	0.01	0.03	0.04	0.02–0.07	0.05–0.15
1,3-Diarachidin	0.10	0.03					0.10	0.26
1,2-Diarachidin	0.06							0.19
Sterols	0.10	0.04				0.02		0.21
Sorghum wax								
Light band 1	0.79	0.80	0.79	0.78		0.79		
Light band 2	0.76	0.69	0.69	0.57		0.65		
Light band 3	0.70	0.64	0.63	0.39		0.57		
Dark band 1	0.54	0.50	0.42	0.31	0.24	0.40	0.60	0.71
Dark band 2	0.16	0.07	0.05	0.02	0.02	0.05	0.19	0.29
Dark band 3	0.37	0.21	0.01	0.01	0.02	0.05	0.02–0.06	0.01–0.13

[a]A, hexane/diethyl ether/acetic acid (85:15:2); B, hexane/diethyl ether/acetic acid (88:12:2); C, hexane/diethyl ether (92:8); D, hexane/diethyl ether (94:6); E, hexane/diethyl ether (98:2); F, hexane/diethyl ether/acetaldehyde (95:5:2); G, chloroform (containing 0.75% ethanol); H, chloroform/acetone (98:2).

Earlier researchers, Dalton and Mitchell (4) and the Bianchi group (17), reported contradictory observations. The former stated, "Attempts to obtain a reaction between the crude wax and 2,4-dinitrophenylhydrazine were unsuccessful, and it was concluded that the wax did not contain a ketone." The latter described, "TLC detection of aldehydes was readily accomplished using 2,4-dinitrophenylhydrazine, previously heating in a test tube an ethanolic solution of the reagent with the wax. The 2,4-dinitrophenylhydrazones formed were clearly visible as yellow spots on developed plates." From our observation it was difficult to confirm either previous finding so more testing was completed.

Benedict's solution is supposed to react with aldehydes (but not with ketones), converting cupric oxide to cuprous oxide to produce a green precipitate and then a red one (13). The reaction did not give positive results for *cis*-13-octadecenal and the dark band 1 fraction. In a chromic acid reaction, the original orange color was supposed to disappear and a green or blue-green precipitate form in the presence of aldehydes (14). The reaction with dodecanal produced a quick green precipitate, a slower green precipitate with *cis*-13-octadecenal, and a brown precipitate with dark band 1 fraction that turned green with time; a blank test gave a green color with time. Again it was hard to draw a solid conclusion from the above two tests due to their vague results.

Dark band 1 fraction was reacted with KOH under hydrolysis conditions. Acids and alcohols were expected to be produced if the fraction contained esters. However, it should be noted that an aldehyde can also be converted to acid and alcohol by reaction with KOH in similar conditions in the Cannizzaro reaction (14,16). The reaction of the dark band 1 fraction with KOH under either hydrolysis or Cannizzaro conditions produced numerous bands on the TLC plate including alcohol and acid bands, as did dodecanal and *cis*-13-octadecenal.

Aldehydes can be reduced to alcohols by borohydride (16). This reaction with dark band 1 fraction was successful. Dark band 1 became pale, and the alcohol band became much darker (Fig. 2). The fraction was also exposed to room temperature air for 5 d and applied on a TLC plate, resulting in a very dark acid band (Fig. 2). These results from borohydride reduction and autoxidation (18) suggested that the dark band was aldehydes, supporting the observations of the Bianchi group (6,7).

The unsuccessful reactions for aldehyde identification with 2,4-dinitrophenylhydrazine, Benedict's solution, and chromic acid mentioned above might have been attributable to the test solutions being aqueous (in more polar solvents), retarding the contact of nonpolar long-chain aldehydes with the reacting compounds.

NMR spectroscopy and the GC–MS analysis were both consistent with a mixture of long-chain aldehydes, with the primary component being C_{28}. The NMR spectra of these samples were consistent with this structure, both in observed chemical shifts (δ) and integrated intensities (^{1}H NMR). Examination of the ^{1}H NMR data showed the following signals:

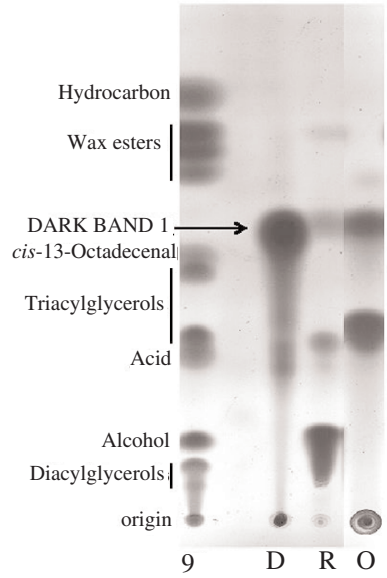

FIG. 2. TLC of borohydride reduction and autoxidation of dark band 1 fraction (Fig. 1) of sorghum wax from silica gel column chromatography. Developing solvent was hexane/diethyl ether/acetic acid (85:15:2). S: standard mixture; D: dark band 1 fraction in Table 1; R: borohydride reduction product of the dark band fraction; O: autoxidation product of the dark band faction when exposed to air for 5 d at room temperature.

δ = 0.9 ppm (triplet, 3H), corresponding to the terminal–CH_3; δ = 1.3 ppm (multiplet, 52H), corresponding to approximately 26–CH_2–groups; δ = 2.4 ppm (triplet of doublets, 2H), corresponding to –CH_2–next to –CH=O; and δ = 9.7 ppm (triplet, 1H), corresponding to –CH=O. Additional signals, corresponding to minor components of the sample mixture, appeared between 6.0 and 8.0 ppm, indicating the presence of some unsaturated species, which would account for the minor (early eluting) components visible in the GC–MS chromatographic data. The ^{13}C NMR data showed: δ = 14.2 ppm, corresponding to –CH_3; δ = 22.4, 23.1, 29.5, 29.7, 29.8, 29.9, 30.0, and 32.3 ppm, all corresponding to –CH_2–; δ = 44.2 ppm, corresponding to –CH_2–CH=O; and δ = 203.1 ppm, corresponding to –CH=O. Signals were also present in the ^{13}C NMR at δ = 126.7, 127.9, 128.8, and 128.9 ppm, consistent with a trace of unsaturated materials in the sample.

In the GC–MS data, the total ion current trace showed a series of low M.W. components eluting between 10 and 13 min, with the primary (high M.W.) component eluting at 19.5 min. The MS data of the primary fraction were: *m/z* = 408 [M of $CH_3(CH_2)_{26}CHO$]; abundance 5%); 409 (M + 1; abund. 25%); 410 (M + 2; abund. 7%); 407 (M – 1; abund. 16%); 391 (M – 17; abund. 4%); 390 (M – 18; abund. 9%); 389 (M – 19; abund. 20%); 437 (M + 29; abund. 7%; an adduct observed in methane CI mode); and a lot of cascading fragments differing by 14 amu due to successive loss of CH_2, indicating that the prominent, M.W. compound in the dark band fraction was a saturated C_{28} aldehyde. The minor unsaturated materials observed in the NMR spectra, which most likely arose from the low M.W. fractions that eluted in <13 min in the GC

trace, might result from the impurities included during the column chromatography.

It was concluded that the published results of the Bianchi group (6,7) were the most reliable regarding grain sorghum wax chemistry. They reported that grain sorghum kernel wax had 1.3% hydrocarbons, 4% wax esters, 34% fatty alcohols, 24% FA, and 32% aldehydes, or 7% hydrocarbons, 13% wax esters, 32% fatty alcohols, 27% FA, and 21% aldehydes (6,7). At first glance, these composition values seemed to be different from our observations described above. However, the difference in the values might have resulted from the use of different sorghum varieties, different extraction methods, and different analytical methods. The Bianchi group also reported compositions of individual sorghum wax classes, stating that aldehydes, alcohols, and acids in sorghum wax were mainly saturated C_{28} and C_{30} (6,7).

Implication. Aldehydes in sorghum wax are easily oxidized to form acids when exposed to the air during storage and transportation, and even after application of the wax on a surface. Oxidation of aldehydes in the wax may alter the physical and functional properties of the wax. Autoxidation of aldehydes to acids in sorghum wax is different from the traditional autoxidation in highly unsaturated lipids, which typically affects flavors of lipid-containing foods. Since aldehydes in the wax are mostly long-chained, the converted acids do not affect flavors. Also, since the wax most likely consists of saturated compounds, the traditional autoxidation, usually taking place around double bonds, is not expected.

ACKNOWLEDGMENT

The authors wish to thank Dr. Patrick H. Dussault in the Department of Chemistry at University of Nebraska–Lincoln for his assistance with our work. This article is recorded as Journal Series No. 13509, Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska–Lincoln.

REFERENCES

1. USDA, Agricultural Statistics, National Agricultural Statistics Service (http://www.usda.gov/nass/pubs/agstats.htm), The United States Department of Agriculture, Lanham. MD, August 27, 2001.
2. Bunger, W.B., and F.A. Kummerow, A Comparison of Several Methods for the Separation of Unsaponifiable Material from Carnauba and Grain Sorghum Waxes, *J. Am. Oil Chem. Soc. 28*:121–123 (1951).
3. Cannon, C., and F.A. Kummerow, A Comparison of Plant and Grain Wax from Two Varieties of Sorghum, *Ibid. 34*:519–520 (1957).
4. Dalton, J.L., and H.L. Mitchell, Fractionation of Grain Sorghum Wax, *J. Agric. Food Chem. 7*:570–573 (1959).
5. Seitz, L.M., Composition of Sorghum Grain Wax, *Cereal Foods World 22*:472 (1977).
6. Bianchi, G., P. Avato, and G. Mariani, Composition of Surface Wax from Sorghum Grain, *Cereal Chem. 56*:491–492 (1979).
7. Avato, P., G. Bianchi, and C. Murelli, Aliphatic and Cyclic Lipid Components of Sorghum Plant Organs, *Phytochemistry 29*:1073–1078 (1990).
8. Avato, P., G. Bianchi, and G. Mariani, Epicuticular Waxes of Sorghum and Some Compositional Changes with Plant Age, *Ibid. 23*:2843–2846 (1984).
9. Bianchi, G., P. Avato, P. Bertorelli, and G. Mariani, Epicuticular Waxes of Two Sorghum Varieties, *Ibid. 17*:999–1001 (1978).
10. Weller, C.L., S.L. Cuppett, L.C. Hua, K.R. Lochte-Watson, C. Gaudoin, J. Arrault, C.M. Hubbard, and J.P. Mua, Solvent Influence on Yield and Fractions of Grain Sorghum Wax, Paper No. 00-6134, American Society of Agricultural Engineers, St. Joseph, MI, 2000.
11. Bitman, J., and D.L. Wood, An Improved Copper Reagent for Quantitative Densitometric Thin-Layer Chromatography of Lipids, *J. Liq. Chromatogr. 5*:1155–1162 (1982).
12. Linstromberg, W.W., and H.E. Baumgarten, Identification of an Unknown Carbonyl Compound, in *Organic Experiments*, 3rd edn., D.C. Heath and Co., Lexington, MA, 1974, pp. 129–133.
13. Linstromberg, W.W., and H.E. Baumgarten, Reactions of Aldehydes and Ketones, in *Organic Experiments*, 3rd edn., D.C. Heath and Co., Lexington, MA, 1974, pp. 119–128.
14. Roberts, R.M., J.C. Gilbert, L.B. Rodewald, and A.S. Wingrove, Oxidation Reactions of Alcohols and Carbonyl Compounds, in *Modern Experimental Organic Chemistry*, 3rd edn., Holt, Rinehart and Winston, New York, 1979, pp. 277–289.
15. Christie, W.W., The Preparation of Derivatives of Lipids, in *Lipid Analysis*, 2nd edn., Pergamon Press, Oxford, 1982, pp. 51–61.
16. Durst, H.D., and G.W. Gokel, The Carbonyl Group, in *Experimental Organic Chemistry*, McGraw-Hill, New York, 1980, pp. 552–578.
17. Bianchi, G., P. Avato, and F. Salamini, Glossy Mutants of Maize. VI. Chemical Constituents of *glossy-2* Epicuticular Waxes, *Maydica 20*:165–173 (1975).
18. Streitwieser, A., Jr., and C.H. Heathcock, Aldehydes and Ketones, in *Introduction to Organic Chemistry*, 2nd edn., Macmillan, New York, 1981, pp. 357–411.

[Received October 1, 2001; accepted February 20, 2002]