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LITERATURE CITED

Bills, D. D., Hildrum, K. I., Scanlan, R. A., Libbey, L. M., *J. Agric. Food Chem.* **21**, 876 (1973).
Brooks, J., Haines, R. B., Moran, T., Pace, J., Department of Science and Industrial Research, Food Investigative Special Report, No. 49, 1940.
"Canadian Food and Drug Regulations", Department of Health and Welfare, published by Information Canada, Ottawa, Canada, 1974.
Cho, I. C., Bratzler, L. J., *J. Food Sci.* **35**, 668 (1970).
Cort, W. M., *J. Am. Oil Chem. Soc.* **51**, 321 (1974).
Crosby, N. T., Foreman, J. K., Palframan, J. F., Sawyer, R., *Nature (London)* **238**, 342 (1972).
"CRC Handbook of Food Additives", 2nd ed, Furia, T. E., Ed., CRC Press, Cleveland, Ohio, 1972, p 214.
Druckrey, H., Preussmann, R., Ivankovic, S., Schmaehl, D., *Z. Krebsforsch.* **69**, 103 (1967).
Fazio, T., White, R. H., Dusold, L. R., Howard, J. W., *J. Assoc. Off. Anal. Chem.* **56**, 919 (1973).
Fiddler, W., Pensabene, J. W., Fagan, J. C., Thorne, E. J., Piotrowski, E. G., Wasserman, A. E., *J. Food Sci.* **39**, 1070 (1974).
Fiddler, W., Pensabene, J. W., Piotrowski, E. G., Doerr, R. C., Wasserman, A. E., *J. Food Sci.* **38**, 1084 (1973).
Gough, T. A., Webb, K. S., *J. Chromatogr.* **64**, 201 (1972).
Gough, T. A., Webb, K. S., *J. Chromatogr.* **79**, 57 (1973).
Greenberg, R. A., *Proc. Meat Ind. Res. Conf.*, 25 (March 23–24, 1972).
Herring, H. K., *Proc. Meat Ind. Res. Conf.*, 47 (1973).
Huxel, E. T., Scanlan, R. A., Libbey, L. M., *J. Agric. Food Chem.* **22**, 698 (1974).
Kamm, L., McKeown, G. G., Smith, D. M., *J. Assoc. Off. Agric. Chem.* **48**, 892 (1965).
Lijinsky, W., Epstein, S. S., *Nature (London)* **225**, 21 (1970).
Lijinsky, W., Tomatis, L., Wenyon, C. E., *Proc. Soc. Exp. Biol. Med.* **130**, 945 (1969).
Magee, P. N., Barnes, J., *Br. J. Cancer* **10**, 114 (1956).
Mirvish, S. S., "N-Nitroso Compounds: Analysis and Formation", International Agency for Research on Cancer Monograph, Lyon, France, 1972, p 104.
Panalaks, T., Iyengar, J. R., Donaldson, B. A., Miles, W. F., Sen, N. P., *J. Assoc. Off. Anal. Chem.* **57**, 806 (1974).
Parr, A. A., Henrickson, R. L., *Food Technol.* **24**, 118 (1970).
Pensabene, J. W., Fiddler, W., Gates, R. A., Fagan, J. C., Wasserman, A. E., *J. Food Sci.* **39**, 314 (1974).
Perigo, J. A., Roberts, T. A., *J. Food Technol.* **3**, 91 (1968).
Pivnick, H., Chang, P-C., *Proc. Int. Symp. Nitrite Meat Prod.*, 111 (1973).
Pivnick, H., Rubin, L. J., Barnett, H. W., Nordin, H. R., Ferguson, P. A., Perrin, C. H., *Food Technol.* **21**, 100 (1967).
Sen, N. P., *Food Cosmet. Toxicol.* **10**, 219 (1972).
Sen, N. P., Donaldson, B., Charbonneau, C., Miles, W. F., *J. Agric. Food Chem.* **22**, 1125 (1974).
Sen, N. P., Donaldson, B., Iyengar, J. R., Panalaks, T., *Nature (London)* **241**, 473 (1973).
Telling, G. M., Bryce, T. A., Althorpe, J., *J. Agric. Food Chem.* **19**, 937 (1971).
"U.S. Code of Federal Regulations", 1971, Chapter III, Section 318.7, Title 9.

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An Investigation of the Surface Lipids of the Glabrous Cotton (*Gossypium hirsutum* L.) Strain, Bayou SM1

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The surface lipids of the glabrous cotton (*Gossypium hirsutum* L.) strain, Bayou SM1, were analyzed with an integrated gas chromatography–mass spectrometry system. *n*-Alkanes, C_{27}–C_{38}, account for 49.9% of the total wax, with *n*-nonacosane ($C_{29}H_{60}$) as the major wax constituent (28.7%). *n*-Primary alcohols C_{26}, C_{27}, and C_{28} account for 5.5% of the wax, with *n*-octacosanol ($C_{28}H_{58}O$) predominating (4.4%). Nineteen sterols and triterpenoids were detected and identities for nine are proposed: cholesterol (0.7%), 24ξ-methyl-$\Delta^{5,22}$-cholestadien-3β-ol (0.4%), stigmasterol (2.7%), fucosterol (4.5%), 24-methylenelophenol (3.4%), 4,4,14α-trimethyl-$\Delta^{7,9(11),24}$-cholestatrien-3β-ol (0.8%), 24-ethylidenelophenol (3.6%), 24-methylenecycloartanol (0.8%), and 24-methylcycloartanol (1.0%). The sterol and triterpenoid fraction accounts for 44.6% of the total wax, with an unidentified $C_{29}H_{48}O$ (M^+ 412) as the major constituent (6.5%).

Surface lipids (wax) of the glabrous cotton (*Gossypium hirsutum* L.) strain Bayou SM1 were examined by gas–liquid chromatography/mass spectrometry (GLC–MS) to investigate a possible chemical basis for the reported nonpreference of glabrous cottons by certain cotton insects (Lukefahr et al., 1968, 1970, 1971; Davis et al., 1973). The information may also be helpful in light of the report that cotton cuticular lipids are a potential factor in boll rot resistance (Wang and Pinckard, 1973).

All previous studies of cotton wax constituents have involved the extraction of ground plant tissue, and therefore were not concerned solely with lipids of surface origin (Power and Chesnut, 1925, 1926; Chibnall et al., 1934; Sadykov et al., 1963; Sadykov and Padkudina, 1964; Sadykov, 1965). Power and Chesnut (1925, 1926) reported the isolation of *n*-alkanes C_{31} and C_{35} from ground cotton foliage, squares (flower buds), and flowers, and based their identification on melting point and elemental analysis; however, Chibnall et al. (1934), using crystal spacing data, later showed that both solids were mixtures of paraffins.

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Chibnall et al. (1934) further identified *n*-primary alcohols C_{24}, C_{25}, C_{28}, C_{30}, C_{32}, and C_{34} and *n*-fatty acids C_{24}, C_{26}, C_{28}, C_{30}, C_{32}, and C_{34} from ground cotton leaves, squares, and flowers. In 1961, Waldron et al. reexamined some of the work of Chibnall et al. (1934) by mass spectrometry and presented evidence which cast doubt on its general validity; however, cotton wax was not one of the waxes reexamined.

Sadykov et al. (1963), Sadykov and Padkudina (1964), and Sadykov (1965) identified *n*-alkanes C_{24}, C_{26}, C_{28}, C_{30}, C_{32}, C_{35}, and C_{36} and *n*-primary alcohols C_{28}, C_{30}, and C_{32} from ground leaves, boll walls, and flowers of a *Gossypium* species, but gave no details on the methods of identification. Struck et al. (1968) extracted cotton squares and flowers (whole and ground) with petroleum ether and identified the high molecular weight *n*-alkanes C_{23}–C_{33} by mass spectrometry.

EXPERIMENTAL SECTION

Isolation and Fractionation. Terminal shoots were collected at intervals over a 1-year period from fruiting Bayou SM1 cotton plants grown in the greenhouse. The wax isolated from each collection was treated separately. The shoots were weighed and the wax extracted immediately by three 10-s dips in purified chloroform (Martin and Juniper, 1970). The chloroform extracts were combined and dried over anhydrous sodium sulfate and filtered and the solvent removed in vacuo at 20°C. The wax material was weighed, dissolved in solvent, and maintained at 0°C until fractionation.

The total wax was fractionated by high-speed liquid chromatography using a 30 cm × 4 mm i.d. 10-μ porous silica column. Fractions were eluted by programming a 0 to 100% solvent gradient of chloroform in hexane at 4 ml/min flow rate at 2000 psi. *n*-Alkanes were eluted with 100% hexane, *n*-primary alcohols at 10–20% chloroform in hexane, and three sterol–triterpenoid fractions were collected at 50–100% chloroform in hexane. The fractions were monitored by refractive index, a scanning uv–visible spectrophotometer interfaced with the liquid chromatograph, silica gel TLC, and GLC.

Analytical GLC–MS. Bayou SM1 wax isolated at different times was analyzed by two GLC/MS systems. The first analysis was obtained by introducing fractions into an AEI Scientific double-focusing MS-30 from a 275-cm column packed with 3% OV-1 on 60–80 mesh Gas-Chrom Q. The GLC unit was programmed from 175 to 320°C at 4°C/min. Mass spectra were obtained at 70 eV. The data were collected and processed by a computer.

The second analysis was obtained from a Hewlett-Packard 5930 quadrupole mass spectrometer at 70 eV using GLC conditions cited above. The two analyses provided corresponding data.

Identifications of the *n*-alkanes, *n*-primary alcohols, and six of the sterols and triterpenoids were made by direct comparisons with authentic standards and published spectra (Knights, 1967; Stenhagen et al., 1969, 1974; Mercer et al., 1974). The proposed identities of 24ξ-methyl-$\Delta^{5,22}$-cholestadien-3β-ol, 4,4,14α-trimethyl-$\Delta^{7,9,(11),24}$-cholestrien-3β-ol, and 24-methylcycloartanol (Table I) are based on spectral interpretation. The unidentified components (Table I) were classified as sterols and triterpenoids based on characteristic ionic species produced in the high mass range of their mass spectra (Friedland et al., 1959; Ryhage and Stenhagen, 1960; Knights, 1967; Budzikiewicz, 1972; Mercer et al., 1974).

The gas chromatographic profile obtained with a flame ionization detector was used to estimate the relative concentrations of wax components. Material balance observations were made by peak triangulation and normalization to 100%.

Table I. Sterols and Triterpenoids Isolated from the Surface Lipids of Bayou SM1 Cotton

M⁺	Identity: Mol formula	Identity: Name/or high mass ionic species[a]	% of total wax
386	$C_{27}H_{46}O$	Cholesterol	0.7
398	$C_{28}H_{46}O$	24-ξ-Methyl-$\Delta^{5,22}$-cholestadien-3β-ol	0.4
410	$C_{29}H_{46}O$	395, 392, 377, 297, 257	0.4
412	$C_{29}H_{48}O$	Stigmasterol	2.7
412	$C_{29}H_{48}O$	Fucosterol	4.5
412	$C_{29}H_{48}O$	24-Methylenelophenol	3.4
412	$C_{29}H_{48}O$	397, 394, 379, 231, 213	6.5
424	$C_{30}H_{48}O$	409, 406, 391, 299, 297	3.2
424	$C_{30}H_{48}O$	409, 406, 391, 243, 218	4.2
424	$C_{30}H_{48}O$	4,4,14α-Trimethyl-$\Delta^{7,9(11),24}$-cholestatrien-3β-ol	0.8
424	$C_{30}H_{48}O$	409, 406, 391, 302, 175	1.5
426	$C_{30}H_{50}O$	411, 408, 393, 285, 247	3.5
426	$C_{30}H_{50}O$	24-Ethylidenelophenol	3.6
426	$C_{30}H_{50}O$	411, 408, 393, 327, 299	2.7
428	$C_{30}H_{52}O$	413, 410, 395, 231, 213	2.1
430	$C_{30}H_{54}O$	415, 412, 397, 259, 227	1.3
430	$C_{30}H_{54}O$	415, 412, 397, 255, 229	1.3
440	$C_{31}H_{52}O$	24-Methylenecycloartanol	0.8
442	$C_{31}H_{54}O$	24-Methylcycloartanol	1.0

[a] Mercer et al., 1974; Table I, p 848.

Table II. *n*-Alkanes and *n*-Primary Alcohols Isolated from the Surface Lipids of Bayou SM1 Cotton

M⁺	Identity: Mol formula	Identity: Name	% of total wax
		n-Alkanes	
380	$C_{27}H_{56}$	*n*-Heptacosane	6.7
394	$C_{28}H_{58}$	*n*-Octacosane	2.6
408	$C_{29}H_{60}$	*n*-Nonacosane	28.7
422	$C_{30}H_{62}$	*n*-Triacontane	1.4
436	$C_{31}H_{64}$	*n*-Hentriacontane	7.7
450	$C_{32}H_{66}$	*n*-Dotriacontane	0.3
464	$C_{33}H_{68}$	*n*-Tritriacontane	1.4
478	$C_{34}H_{70}$	*n*-Tetratriacontane	0.1
492	$C_{35}H_{72}$	*n*-Pentatriacontane	0.3
506	$C_{36}H_{74}$	*n*-Hexatriacontane	0.1
520	$C_{37}H_{76}$	*n*-Heptatriacontane	0.1
534	$C_{38}H_{78}$	*n*-Octatriacontane	0.5
		n-Primary Alcohols	
382	$C_{26}H_{54}O$	*n*-Hexacosanol	0.5
396	$C_{27}H_{56}O$	*n*-Heptacosanol	0.6
410	$C_{28}H_{58}O$	*n*-Octacosanol	4.4

RESULTS

Bayou SM1 wax averages 0.68 mg/g (range 0.61–0.75 mg/g) of plant tissue. The wax is predominately *n*-alkanes (49.9%), with *n*-nonacosane ($C_{29}H_{60}$) as the major wax constituent (28.7%). *n*-Primary alcohols and sterols/triterpenoids account for 5.5 and 44.6%, respectively, of total surface wax.

The identities and percentages of the *n*-alkanes and *n*-primary alcohols isolated from the wax are listed in Table II. Table I lists the sterols and triterpenoids isolated from the wax with identities of the five characteristic high mass ionic species (Mercer et al., 1974) and percentages. Several wax samples (different collections) were studied and the relative concentrations of wax components were consistent.

DISCUSSION

Sterols (i.e., β-sitosterol and stigmasterol) and triterpenoids (i.e., ursolic, oleanolic, and amyrin derivatives) have been reported in some plant waxes, but they are

usually minor constituents (Martin and Juniper, 1970). The complex nature and large percentage (44.6) of sterols and triterpenoids found in Bayou SM1 wax have provided some interesting data to consider in cotton–pest interactions.

In consideration of cotton interactions with boll rot organisms, Bean (1973) has summarized reports which show that certain phytosterols (i.e., cholesterol, cholestanol) may cause an inhibition in growth and reproduction of certain microorganisms and be stimulatory to others, thus resulting in resistance or susceptibility of the plant to disease. This could possibly explain why cotton cuticular extracts were fungistatic to nine species of fungi frequently associated with boll rot, and were ineffective for *Penicillum spinulosum* (Wang and Pinckard, 1973).

The sterols found in Bayou SM1 wax can have an influence on cotton–insect interaction. Svoboda et al. (1975) reported that insects lack the capacity for de novo biosynthesis of the steroid nucleus; to date, all insects that have been examined critically have been shown to require an exogenous source of sterol to achieve normal growth, development, and reproduction. Cholesterol or some sterol that can be converted to cholesterol must be obtained from the host plant to meet this critical need. Sterols are, by definition, vitamins for insects (Heftmann, 1975). Therefore, insects can obtain needed sterols from Bayou SM1.

However, phytosterols can also act in diverse capacities against insects. Heftmann (1975) has summarized reports of certain phytosterols that inhibit insect metamorphosis, others that are potent antimitotic agents, some that act as chemosterilants against certain insects, and others that act as repellents.

Elliott and Knights (1969) proposed that the relative amounts of sterols of the plant may be more important than the level of any one sterol in conferring resistance or susceptibility of a plant to certain insects and diseases. With the diversity of sterols and triterpenoids found in Bayou SM1 wax, these components could be important in the nonpreference type of resistance of this glabrous cotton to certain insects.

Chemical studies of several cotton strains, both glabrous and pubescent, indicate that cotton wax in general contains a large percentage of sterols and triterpenoids (unpublished data, this laboratory). GC profile comparisons were made of Bayou SM1 wax fractions and those of its near isogenic pubescent counterpart, Bayou. Profile differences were observed in the sterol and triterpenoid fractions, but GC/MS analyses of Bayou's wax are needed before any conclusions may be drawn.

Techniques are being developed for the bioassay of the wax fractions of Bayou SM1 and Bayou with several cotton insects which show nonpreference for glabrous cotton. Results will be reported when adequate techniques are obtained.

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LITERATURE CITED

Bean, G. A., *Adv. Lipid Res.* 1, 193 (1973).
Budzikiewicz, H., in "Biochemical Applications of Mass Spectrometry", Waller, B. R., Ed., Wiley-Interscience, New York, N.Y.,1972, p 251.
Chibnall, A. C., Piper, S. H., Pollard, A., Williams, E. F., *Biochem. J.* **28**, 2189 (1934).
Davis, D. D., Ellington, J. J., Brown, J. C., *J. Environ. Qual.* **2**, 530 (1973).
Elliott, C. G., Knights, B. A., *J. Sci. Food Agric.* **20**, 406 (1969).
Friedland, S. C., Lane, G. H., Longman, R. T., Train, D. E., O'Neal, M. J., *Anal. Chem.* **31**, 169 (1959).
Heftmann, E., *Phytochemistry* **14**, 891 (1975).
Knights, B. A., *J. Gas Chromatogr.* **5**, 273 (1967).
Lukefahr, M. J., Cowan, C. B., Jr., Bariola, L. A., Houghtaling, J. E., *J. Econ. Entomol.* **61**, 661 (1968).
Lukefahr, M. J., Cowan, C. B., Houghtaling, J. E., *J. Econ. Entomol.* **63**, 1101 (1970).
Lukefahr, M. J., Houghtaling, J. E., Graham, H. M., *J. Econ. Entomol.* **64**, 486 (1971).
Martin, J. T., Juniper, B. E., "The Cuticles of Plants", St. Martin Press, New York, N.Y., 1970, p 45.
Mercer, I. E., London, R. A., Kent, I. S. A., Taylor, A. J., *Phytochemistry* **13**, 845 (1974).
Power, F. B., Chesnut, U. K., *J. Am. Chem. Soc.* **47**, 1751 (1925).
Power, F. B., Chesnut, U. K., *J. Am. Chem. Soc.* **48**, 2721 (1926).
Ryhage, R., Stenhagen, E., *J. Lipid Res.* **1**, 361 (1960).
Sadykov, A. S., *J. Sci. Ind. Res. India* **24**, 77 (1965).
Sadykov, A. S., Isaev, K. I., Ismailov, A. I., *Uzb. Khim. Zh.* **7**, 53 (1963).
Sadykov, A. S., Padkudina, Z. P., *Nauchn. Tr. Tashk. Gos. Univ.* **263**, 88 (1964).
Stenhagen, E., Abrahamson, S., McLafferty, F. W., Ed., "Atlas of Mass Spectra Data", Interscience, New York, N.Y., 1969, p 1.
Stenhagen, E., Abrahamson, S., McLafferty, F. W., Ed., "Registry of Mass Spectra Data", Wiley-Interscience, New York, N.Y., 1974, p 1.
Struck, R. F., Frye, J. L., Shealy, Y. F., *J. Agric. Food Chem.* **16**, 1028 (1968).
Svoboda, J. A., Kaplanis, J. N., Robbins, W. E., Thompson, M. J., *Annu. Rev. Entomol.* **20**, 205 (1975).
Waldron, J. D., Gowers, D. S., Chibnall, A. C., Piper, S. H., *Biochem. J.* **78**, 435 (1961).
Wang, S., Pinckard, J. A., *Phytopathology* **63**, 315 (1973).

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