Constituents of the Cotton Bud. Sesquiterpene Hydrocarbons

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Sesquiterpene hydrocarbons obtained from the buds (squares) of Deltapine Smoothleaf cotton by steam distillation and column chromatography were identified by gas chromatographic techniques, infrared, proton resonance, and mass spectroscopy. Components identified and their percentages of the essential oil were: copaene (0.24), *l-trans*-α-bergamotene (0.33), *l*-caryophyllene (7.51), farnesene (0.03), α-humulene (2.54), *cis*-γ-bisabolene (2.90), *l*-δ-guaiene (1.02), and *l*-δ-cadinene (0.24).

THE terpene hydrocarbons present in the essential oil of Deltapine Smoothleaf variety cotton (*Gossypium hirsutum*) plant buds (squares) have been reported (*17*). This work deals with the sesquiterpene hydrocarbons present in the same source.

Characterization of the cotton plant volatiles is being undertaken as part of a study of the plant attractant(s) for the boll weevil (*Anthonomus grandis* Boheman). Accordingly, the entire terpene and sesquiterpene hydrocarbon fraction of the plant oil was assayed for weevil attractancy by Hardee's procedure (*8*). These results, though negative, will be published later in more detail.

Experimental

Apparatus. In addition to gas chromatographic equipment previously described (*17*), a Barber-Colman Model 5000 unit with a flame-ionization detector was used for quantitation. A catalytic hydrogenator constructed according to Beroza (*4*) was attached to an Aerograph HY-FI chromatograph inlet during a hydrogenation study, in which neutral palladium catalyst (National Instrument Laboratories, Inc., Rockville, Md.) and hydrogen-carrier gas were employed. Gas chromatographic operating conditions are given in Table I.

Beckman IR-5A and Perkin-Elmer 521 infrared spectrophotometers.

Varian Model A-60 Analytical NMR Spectrometer, all spectra run in Spectrograde CCl_4.

Kern Full-Circle polarimeter; 1-dm. cell and sodium emission lamp (SLA-5C; George W. Gates and Co.).

Isolation of Sesquiterpene Hydrocarbons. The hydrocarbon fraction obtained by chromatography of cotton square oil on Carbowax 20M coated silica gel columns (*17*) was gas chromatographed on Carbowax 4000 (Table I) and found to contain appreciable quantities of sesquiterpenes. In subsequent isolations for identification purposes, 250 ml. of *n*-pentane solvent was used rather than the 400 ml. necessary for quantitative recovery, since the larger volume occasionally eluted some low-molecular-weight, polar compounds. All materials found in the 400-ml. procedure were present in the 250-ml. procedure, although the more polar components having longer retention times on Carbowax were obtained in poorer yields than the less polar ones eluting earlier in both GLC and liquid chromatographic separations. The 400-ml. procedure was used in quantitation.

Table I. Gas-Chromatographic Operating Conditions

	Preporative Carbowax 4000	*Preporative Apiezon L*	*Analytical Carbowax 4000*	*Carbon Skeleton Carbowax 4000*
Detector	Therm. cond.	Therm. cond.	H_2 flame	H_2 flame
Column length, ft.	10	10	10	20
Column diam., in.	1/4	1/4	1/4	1/8
Column material	Al	Cu	Al	Cu
Per cent stationary phase, w./w.	28.5	20.0	28.5	28.5
Solid support	60- to 80-mesh Gas Chrom P[a]	60- to 80-mesh Gas Chrom P[a]	60- to 80-mesh Gas Chrom P[a]	60- to 80-mesh Gas Chrom P[a]
Carrier gas flow rate, ml./min.	He: 75	He: 75	N_2: 63	H_2: 18
Inlet pressure, p.s.i.g.	38	40	11	24
Outlet pressure, p.s.i.g.	0	0	0	0
Temperatures, °C.				
Column	175	200	175	175
Injector	220	253	268	200
Detector	260	268	212	175

[a] Hexamethyldisilazane-treated.

Analytical Gas Chromatography. A serious impediment to gas chromatographic studies of the sesquiterpenes is the unavailability of standards. All preliminary retention values were expressed relative to caryophyllene, the only readily available commercial standard. The caryophyllene ratio, R_c, was computed as

$$R_c = \frac{T_x - T_a}{T_c - T_a} \qquad (1)$$

where T_x, T_c, and T_a were retention times of the unknown, caryophyllene, and air, respectively.

Square oil hydrocarbons gas chromatographed on Carbowax 4000 at 175° C. (Table I) gave a sesquiterpene chromatogram (Figure 1) with seven maxima

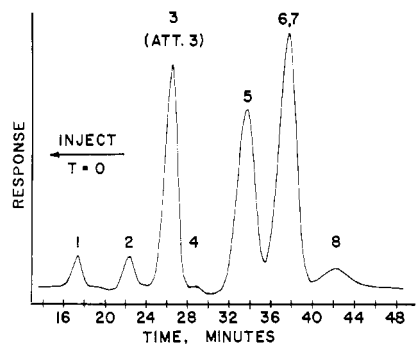


Figure 1. Analytical chromatogram of cotton square sesquiterpene hydrocarbons on Carbowax 4000

Maxima: 1. Copoene. 2. *trans*-α-Bergamotene. 3. Caryophyllene. 4. Farnesene. 5. α-Humulene. 6. *cis*-γ-Bisabolene. 7. δ-Guaiene. 8. δ-Cadinene

apparent. Components collected by bubbling into CCl_4 were rechromatographed on Apiezon L at 200° C. (Table I). Fraction 6 yielded two maxima, 6 and 7; the others gave only one.

Component 3 was readily identified as caryophyllene by comparing its infrared spectrum with that of an authentic GLC purified standard. Thereafter, R_c values of the unknowns were calculated based on component 3. Kováts indices (I_k) (*14*) were computed for all maxima on both phases. All retention data are given in Table II.

A linear-calibration curve was constructed by plotting R_c values for caryophyllene and limonene obtained on the Carbowax column as a function of the values calculated from the chromatogram reported by Hunter and Brogden (*10*). R_c values for other sesquiterpenes (*10, 15*) were obtained by interpolation or extrapolation. Admittedly, a curve based on two calibration points is subject to error, but caryophyllene and limonene were the only two mutual standards available to us, and the curve was used only to make preliminary identification of unknown compounds.

In a manner analogous to that employed with the terpenes (*17*), log log plots of ($R_c \times 10^3$) for Apiezon L and Carbowax 4000 for 16 sesquiterpenes reported by Lukeš and Komers (*15*) were constructed. The best three curves were drawn through the points corresponding to acyclic and monocylic, bicyclic, and tricyclic structures, respectively. Insufficient numbers of examples in certain skeletal classes and large deviations of others from the expected family curve limited the usefulness of this plot, although again some tentative information was obtained.

The sesquiterpenes were quantitated as described for the terpenes (*17*), using *n*-tetradecane as the internal standard.

Preparative Gas Chromatography. The hydrocarbon fraction, 15 ml., was chromatographed in 0.5-ml. portions on the preparative Carbowax 4000 column, and the components were trapped in *n*-pentane. Each fraction was rechromatographed on Carbowax, and the purified sesquiterpenes were condensed in a 10-cm. piece of 2-mm. i.d. Teflon tubing at the exhaust port. Maximum 6 was again trapped in *n*-pentane and rechromatographed on Apiezon L, from which components 6 and 7 were condensed in a Teflon tube. Each sesquiterpene also was collected into CCl_4 and ethanol for other determinations.

Infrared spectra were determined on CCl_4 solutions or on the neat sesquiterpenes between NaCl windows, using 5× ordinate scale expansion on the minor components. Proton-resonance spectra and optical rotations were determined on CCl_4 solutions of each component. The mass spectrum of component 1 was run on a sample of GLC purified liquid.

Table II. Retention Values and Quantitative Data for Sesquiterpenes in Cotton Square Oil

Peak No.	Identification	Carbowax 4000 R_c[a]	Carbowax 4000 I_k[a]	Apiezon L R_c	Apiezon L I_k	Cotton Oil, %
1	Copaene	0.65	1571	0.80	1438	0.24
2	*l-trans-α*-Bergamotene	0.84	1637	0.87	1459	0.33
3	*l*-Caryophyllene	1.00	1682	1.00	1487	7.51
4	Farnesene	1.09	1706	1.03	1496	0.03
5	*α*-Humulene	1.27	1747	1.17	1524	2.54
6	*cis-γ*-Bisabolene	1.42	1775	1.20	1532	2.90
7	*l-δ*-Guaiene	1.42	1776	1.32	1556	1.02
8	*l-δ*-Cadinene	1.59	1808	1.42	1572	0.24

[a] R_c = caryophyllene ratio; I_k = Kováts index.

Carbon Skeleton Chromatography. Ten-microliter samples of GLC-purified sesquiterpenes in ethanol were injected into a carbon-skeleton unit (*4*) packed with neutral palladium catalyst held at 200° C. (Table I). This low temperature and large sample volume decreased the hydrogenation efficiency of the catalyst so that intermediate hydrogenation states of the sesquiterpenes as well as the parent compound could be detected in the resulting chromatogram. The number of double bonds and, consequently, the structure class—i.e., acyclic, monocyclic, etc.—thus were established.

Results and Discussion

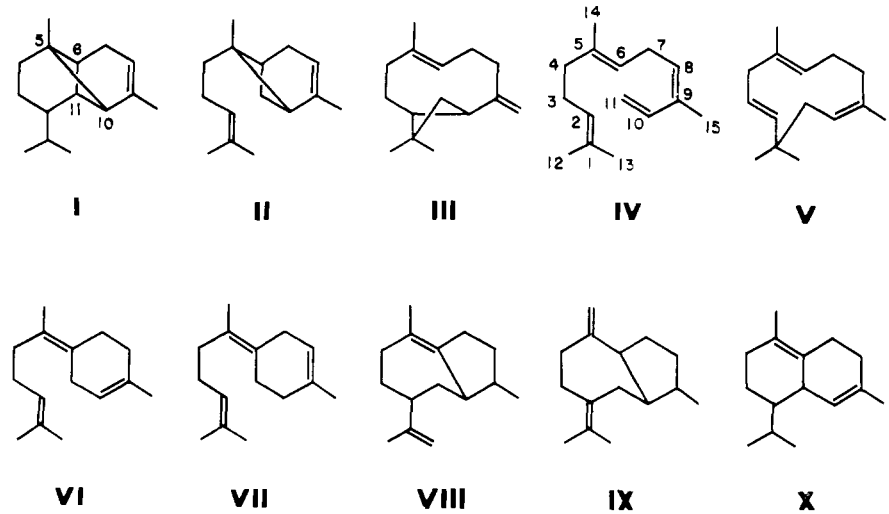
The sesquiterpene hydrocarbons comprise 14.81% of the essential oil of the cotton bud. The percentages of each of the components and GLC retention values on two columns are given in Table II.

Copaene. Component 1 was identified as copaene (I) by its infrared, mass, and PMR spectra. The infrared spectrum agreed at 29 maxima in wavenumber and relative intensity with spectra of authentic copaene from three different sources (*9, 16*), and the mass spectra of the two were identical (*9*). The PMR spectrum agreed with that given by Kapaida *et al.* (*12*) with the exception of an impurity maximum at δ = 1.26 p.p.m. (singlet) in our spectrum. The infrared spectrum of 1 does not agree with that given for copaene by Plíva and others (*20*), which appears to be in error in view of the agreement between copaene spectra of other investigators.

Optical rotation of this material collected from 15 ml. of hydrocarbons averaged +0.01°.

***trans*-α-Bergamotene.** Component 2 was identified as *trans*-α-bergamotene (II) by comparison of its infrared spectrum with a standard (*20*). Thirty-seven maxima agreed in wavenumber and relative intensity with the standard. The PMR and infrared spectra also agreed with those of Kováts (*13*), who pointed out that the PMR spectrum is consistent with a trans configuration of the side chain with respect to the ring unsaturation. Optical rotation of this component collected from 15 ml. of hydrocarbons was −0.15°.

Caryophyllene. Component 3 was identified as caryophyllene (III), its infrared spectrum being superimposable over that of gas chromatographically purified commercial caryophyllene and identical to that reported by Nigam and Levi (*18*). The proton resonance spectrum had maxima at δ (p.p.m.) = 0.95 (singlet, 6 protons); 1.54 (unsymmetrical doublet, 8 protons); 2.03



(multiplet, broad, 7 protons); 4.80 (quadruplet, J = 6.0, 2.0 c.p.s., 2 protons); and 5.20 (multiplet, broad, 1 proton). Carbon-skeleton chromatography clearly indicated the two double bonds present in this compound. Optical rotation of the quantity collected from 21 ml. of hydrocarbons measured −0.30°.

Farnesene. Component 4 was tentatively identified as farnesene (IV) on the basis of its gas chromatographic behavior on the two columns employed (*15*). Incomplete separation of this trace component from caryophyllene, the major sesquiterpene present, precluded trapping and positive spectral identification. Inasmuch as α-humulene and caryophyllene were present and are well known relatives of farnesene, its appearance might be expected.

α-Humulene. Component 5 was shown to be α-humulene (V) by comparing its infrared and proton resonance spectra with those given by Benešová, Herout, and Šorm (*3*) and Dev (*6*). Twenty-seven maxima in the infrared spectrum agreed in wavenumber and relative intensity with those of α-humulene, although the presence of a small amount of β-humulene could not be rigorously excluded on the basis of the infrared spectrum alone. The proton resonance spectrum was identical to that reported by Dev (*6*). The vinyl proton count (3.3) agreed more nearly with the expected value of 4 for α-humulene than with 5 as required for β-humulene, indicating that component 5 is essentially pure α-humulene.

***cis*-γ-Bisabolene.** Three double bonds (carbon-skeleton chromatography) in component 6 and the position of the component on the log-log retention plot indicated a monocyclic structure.

Relative PMR signal areas for 6 (Figure 2) are: δ (p.p.m.) = 1.54; 1.62 (12 protons total, 3:9 ratio); 1.95 (6 protons); 2.23 (2 protons); 2.65 (2 protons); 5.02 (1 proton); and 5.26 (1 proton). The authors concluded, on the basis of the similarity of the PMR spectrum to that of terpinolene (Figure 2), that 6 incorporated the terpinolene structure in its skeleton. Of particular interest were the olefinic signal at δ 5.26 p.p.m., the broad 2-proton peak at 2.65 p.p.m. due to the doubly allylic ring methylene, the 2.23-p.p.m., 2-proton doublet, the 1.95-split signal, and the 3 vinyl methyls signal at 1.62 p.p.m., all of which appeared in the terpinolene spectrum at nearly the same positions. The attachment site and possible configuration of the additional isoprene unit then were deduced from consideration of the PMR and infrared data on 6 and terpinolene.

The unsplit methyl symmetric bending infrared absorption at 1377 cm.$^{-1}$, plus the presence of only one additional vinyl proton (δ = 5.02 p.p.m.) strongly indicated a terminal isopropylidene structure in the added isoprene unit. These two new isopropylidene methyls should have increased the 1.59-p.p.m. signal of terpinolene from 9 to 15 protons, but instead increased it only 3 protons. Additionally, the 1.95-p.p.m. signal characteristic of methylenes alpha to isolated unsaturations (*6*) increased from 2 to 6 protons.

These facts indicate either structure VI or VII for component 6, for either of which a consistent assignment of all PMR signals (Figure 2A) may be made. The ring vinyl proton signal (δ = 5.26 p.p.m.) in 6 was shifted 0.03 p.p.m. upfield from the corresponding signal (δ = 5.29 p.p.m.) in terpinolene, although the vinyl methyl signal (1.62 p.p.m.) was shifted only 0.007 p.p.m. upfield in an average of four spectra of each material. Systematic errors, concentration and temperature shifts, and different volume susceptibilities of bisabolene and terpinolene could have given rise to this admittedly small shift difference between the vinyl proton and essentially unshifted vinyl methyl signals in the

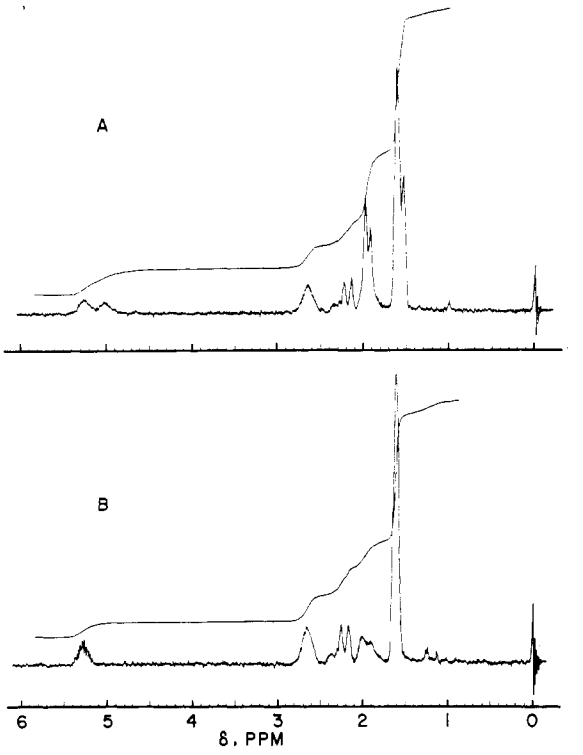


Figure 2. Proton resonance spectra of *cis*-γ-bisabolene (*A*) and terpinolene (*B*)

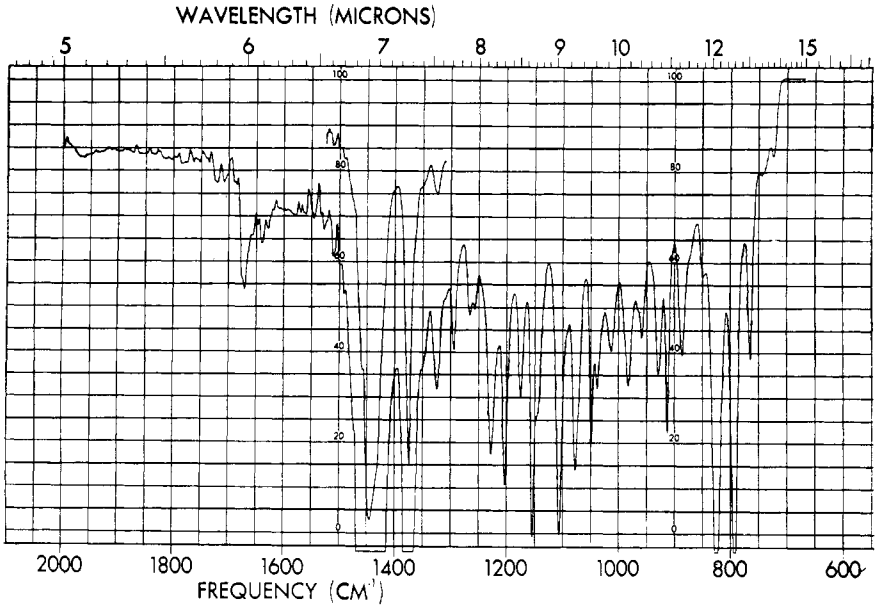


Figure 3. Infrared spectrum of *cis*-γ-bisabolene

two spectra. We feel, however, that the shift may be real, caused by the closer average approach and resultant shielding by the side chain to the "near side" (cis-) ring vinyl proton in (VI) than to the more distant trans-ring vinyl proton in isomer VII. Despite the uncertainty as to the geometric configuration of the side chain with respect to the ring unsaturation, the identification of component 6 as γ-bisabolene rests on a more certain basis.

Ruzicka and van Veen (*21*) suggested VI as the structure of γ-bisabolene nearly 40 years ago, pointing out its relationship to terpinolene. Bisabolene is ordinarily isolated from essential oils as the trihydrochloride (*7*) from which regenerated bisabolene may be recovered. According to Ruzicka (*21*) this regenerated product is principally the gamma isomer, although some isomers containing a terminal methylene could occur as contaminants (*19*). Regeneration from the trihydrochloride with the usual reagents (*7*) would not be expected to be stereospecific with respect to the configuration of the side chain and ring unsaturation, and should produce a mixture of VI and VII in addition to possible small amounts of other bisabolene isomers.

As a consequence, the infrared spectrum of component 6 (Figure 3) would not be expected to agree perfectly with that of regenerated bisabolene (*7, 20*). Nevertheless, there are many points of agreement, although several minor maxima are altered or missing. Figure 3 is believed to be the spectrum of *cis*-γ-bisabolene (VI), nearly pure (95+%) because of the isolation technique. Especially notable is the nearly complete absence of a methylenic δ(CH) fundamental in the 865- to 895-cm.$^{-1}$ region, indicating almost no terminal methylenic contamination.

Component 6 was optically inactive as expected.

δ-Guaiene. Thirty maxima in the infrared spectrum of component 7 matched in wavenumber and relative intensity with their counterparts in the δ-guaiene (VIII) spectrum given by Plíva and coworkers (*20*). Optical rotation of this compound collected from 15 ml. of hydrocarbons was −0.23°.

Proton resonance maxima occurred at δ (p.p.m.) = 0.86 (doublet, J = 6.0 c.p.s., 3 protons); 1.11 (doublet, J = 11.0 c.p.s., 2 protons); 1.61 (wide, asymmetric, 9 protons); 2.03 (broad multiplet, 8 protons); 4.57 (singlet, 1.5 protons), and 5.11 (broad, diffuse multiplet, 0.5 proton).

The two-proton signal at 4.57 and 5.11 p.p.m. and the 892-, 1645-, and 1784-cm.$^{-1}$ infrared absorptions indicate a terminal methylene group, aliphatic or exocyclic to a 6-membered or larger ring. The unsplit 1382-cm.$^{-1}$ absorption indicates either an isopropylidene or isopropenyl structure. The second double bond observed in the carbon skeleton hydrogenation must be tetrasubstituted in view of the absence of any vinyl proton signal, and ring strain considerations make a 9, 10 placement of such a bond seem unlikely. These data indicate that the other methyl must be either a vinyl type or a terminal methylene, implying isopropylene (VIII) or isopropylidene (IX) structures, respectively, for the three-carbon side chain. This evidence supports that of Bates and Slagel (*2*), who recently showed that pyrolysis of bulnesyl acetate gave α- and β-bulnesene. They reported that α-bulnesene has structure VIII and is identical to δ-guaiene.

δ-Cadinene. Component 8 was shown to be δ-cadinene (X), since 33 maxima agreed in wavenumber and relative intensity with their counterparts in the standard spectrum (*20*). Proton resonance signals occurred at δ (p.p.m.) = 0.86 (quadruplet, J = 7.0, 10.0 c.p.s., 6 protons); 1.23 (singlet, 3 protons); 1.61 (multiplet, 7 protons); 1.94 (multiplet, wide 4 protons); 2.52 (multiplet, broad, 3 protons); and *ca.* 5.0 (multiplet, broad and diffuse, peak at 5.3, 1 proton).

Optical rotation of this component collected from 2 ml. of hydrocarbons was −0.18°.

General. The similarity of the PMR spectrum of bergamotene (II) to that of copaene (I) and particularly α-pinene is striking. Coupled with the GLC behavior, this strong resemblance led us to search for sesquiterpenes incorporating α-pinene in their skeletons as candidates for component 1. Recently both copaene (*16*) and α-ylangene (*11*) have been shown to fit this criterion. Also, copaene (I) and bergamotene (II) are related in the same manner as γ-bisabolene (VI) and δ-cadinene (X), specifically in the ring closure between positions 2 and 11.

The numbering scheme is based on IV, inasmuch as most sesquiterpenes with few exceptions can be considered as derivatives of farnesene. This system is believed to be superior to that suggested by Barton, de Mayo, and Shafiq (*1*) or Cekan, Herout, and Šorm (*5*) since it correlates a great number of sesquiterpene skeletons and may have some biosynthetic implications.

The structural similarities between the sesquiterpene hydrocarbons identified in this study are emphasized in formulas I–VI, VIII, and X. Since α-pinene, myrcene, and *trans*-β-ocimene are the three major terpenes of cotton (*17*), it is interesting that I and II contain an α-pinene nucleus, while structures III, V, VI, and X are cyclic derivatives of α- or β-farnesene (IV), which are themselves derivatives of myrcene and ocimene, respectively. The isopropenyl structure in δ-guaiene (VIII) may have originated by way of a carbonium-ion intermediate as suggested by Bates and Slagel (*2*).

The authors do not mean to imply that the sesquiterpene hydrocarbons in a biological system such as this are produced directly from the terpene hydrocarbons or by interconversions of sesquiterpene hydrocarbons themselves, though the possibility of this biosynthetic route should not be arbitrarily discarded. We do believe that the enzymatic and chemical systems prevailing may produce structurally similar intermediates at the mono-, sesqui-, and higher terpene stages that result in recognizable family types at each stage. If this hypothesis is valid, a thorough study of the more easily identified terpenes in a plant system would be of value as a guide in the study of the sesqui-, di-, and triterpenoids of the same system.

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BIODEGRADABILITY

Biodegradability of Ureaformaldehydes and Related Compounds

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A method based upon the principles of the BOD test was used to study the degradation of ureaforms. By making the nitrogen content the limiting factor in the growth media, a relationship between turbidity and nitrogen content allowed for measurement of the degree of degradation of ureaforms. The results correlated with previously reported nitrification studies. An additional parameter, the biodegradability index, was suggested to define more clearly the agronomic utility of ureaforms and processed plastic scrap.

UREAFORMS were biodegraded to the extent of 50 to 55% as measured by a Warburg respirometer technique (*7*). Separation of the undegraded residue and reincubation under the same test conditions showed no growth. While the residue was not degraded under the test conditions, nitrification and turf studies indicated that higher molecular weight fractions degraded to some extent (*3–5*). Evidently, the degradation of the higher molecular weight components of the ureaform was so slow that the growth of the organisms could not be supported.

Since the Warburg respirometer procedure used in the earlier study (*7*) apparently did not achieve the objective of measuring the total potentially available nitrogen in ureaforms, modification of the biodegradability methodology was required. The procedure used in this study did not alter the basic principles of the biological oxygen demand (BOD) test (*1*). All nutrients except the nitrogen source were provided for organism growth. Nitrogen available from the material to be studied was the limiting factor for growth. As the organisms degraded the nitrogen source and utilized it for growth, their numbers increased. As the microorganism population increased, cell protoplasm caused the media to become turbid. By developing a relationship between nitrogen content and the intensity of the turbidity, measurement of nitrogen utilization was possible.

The modified biodegradability procedure described herein offers a simple and rapid method of evaluating ureaforms and similar materials which can be correlated to the more commonly used nitrification studies. This method is useful, since the activity index determination is of limited value as an indication of quality for slow release nitrogen sources such as button scrap.

Method

Apparatus. The shaking apparatus was a Gyrorotary water bath shaker capable of maintaining a temperature of 25° ± 0.5° C., New Brunswick Scientific Co., Model GR 76, equipped with cooling coils to maintain temperature, or its equivalent.

Bausch & Lomb Spectronic 20 colorimeter with 1/2-inch cells, or its equivalent.

Reagents. A. Phosphate buffer solution, 8.5 grams of KH_2PO_4, 21.7 grams of K_2HPO_4, and 33.4 grams of $Na_2HPO_4 \cdot 7H_2O$ dissolved in 1 liter of distilled water (*1*).

B. Magnesium sulfate solution, 22.5 grams of $MgSO_4 \cdot 7H_2O$ dissolved in 1 liter of distilled water (*1*).

C. Calcium chloride solution, 27.5 grams of anhydrous calcium chloride dissolved in 1 liter of distilled water (*1*).

D. Ferric chloride solution, 0.25 gram of $FeCl_3 \cdot 7H_2O$ dissolved in 1 liter of distilled water (*1*).

E. Seed organisms, obtained from domestic sewage, collected, and stored for 24 hours in a refrigerator. Twenty-five milliliters of the supernatant is used as the inoculum.

F. Dilution Water. Add 10 ml. of solution A, 1 ml. each of solutions B, C, and D, 1 gram of glucose, and 25 ml. of seed organisms to a 1-liter volumetric flask and bring to volume with distilled water.

G. Adapted Seed Cultures. Add 50 mg. of the nitrogenous material to be studied to a 250-ml. Erlenmeyer flask. Add 100 ml. of the seeded dilution water; stopper with a cotton plug, and place on the shaking apparatus for 1 week. Turbidity in the media is indicative of the growth of organisms capable of