

**COMPOSITION STUDIES ON TOBACCO. XI**  
**High Molecular Weight, Cyclic Hydrocarbons From**  
**Flue-Cured Leaves**

**Introduction**

**D**URING preliminary work in a previous study (Stedman and Rusaniwskyj, 1959), hydrocarbon mixtures were isolated from Type 12 leaves which showed infrared spectra having more extensive chain branching and shorter unbroken methylene chains than those expected for the aliphatic n- and iso-paraffins (Carruthers and Johnstone, 1959; Johnstone and Plimmer, 1959) known to be in tobacco. Thus, the presence of highly branched hydrocarbons was indicated. The present report concerns the isolation from flue-cured leaves of branched cyclic hydrocarbon mixtures which superficially resemble the higher cycloparaffins of petroleum.

**Methods and Results**

*Isolation*

Cured, unaged, ground Type 12 leaf webs (39.6 kg) were extracted with Skellysolve B<sup>2</sup> as previously described (Dymicky and Stedman, 1959). The Skellysolve extract was reduced in volume, refrigerated, and filtered. The filtrate was then extracted with 90 per cent methanol, giving 1443 g of methanol-insoluble material (S3, Figure 1). S3 was chromatographed batchwise on Merck acid-washed alumina (activated at 150° C. for 15 hours) and the columns

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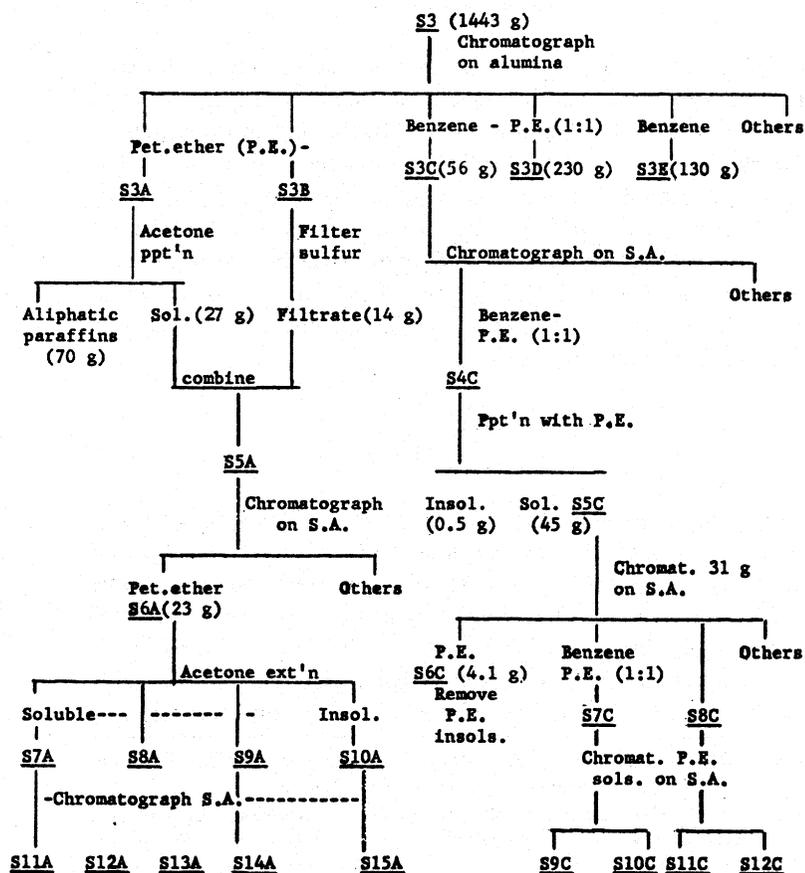
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\*\* Socony-Mobil Oil Company, Paulsboro, New Jersey.

1. Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture. Address all inquiries to this Division.

2. Mention of a specific commercial product does not constitute endorsement by the United States Department of Agriculture. This solvent contained approximately 0.0008 per cent non-volatile residue (b p > 73° C.).

were developed by a flowing technique. Sixteen liters petroleum ether (on four columns) eluted a light brown, semisolid material (S3A); subsequently, 20 liters petroleum ether removed a viscous oil containing pale yellow needles (S3B). The solvent was changed to petroleum ether-benzene, 1:1, and two fractions were collected, the first with 16 liters (S3C) and the second with 32 liters (S3D). Subsequently, 63 liters benzene eluted S3E.



*S3A and S3B.* S3A was precipitated with acetone to give an insoluble portion containing mostly aliphatic paraffinic hydrocarbons, and a soluble portion. A small amount of petroleum ether was added to S3B and the yellow crystals (1.3 g) were filtered off. The filtrate was then pooled with the soluble portion from S3A, giving S5A. The yellow crystals were subjected to elemental analysis after recrystallization and showed 100 per cent sulfur.

Thirty-eight g of S5A were chromatographed on activated silicic acid (Mallinckrodt). One and two-thirds liters petroleum ether eluted 23 g of a pale yellow, viscous oil  $n_D^{24}$  1.4745, (S6A) and the other components on the column were eluted with solvents and mixtures thereof of increasing polarity. The infrared spectrum of S6A indicated the presence of hydrocarbon(s) with slight unsaturation at  $11.25\mu$  ( $R_1R_2C=CH_2$ ), extensive branching and unbroken methylene chains of moderate length. S6A was then extracted with acetone four times, yielding three extracts (S7A,  $n_D^{21}$  1.4710; S8A,  $n_D^{21}$  1.4723; S9A,  $n_D^{21}$  1.4766) and an insoluble residue (S10A,  $n_D^{21}$  1.4710), all four being similar in physical appearance.

These four fractions were each chromatographed on activated silicic acid. The columns containing S7A (5.9 g), S9A (2.5 g), S10A (8.2 g) and one-half (2.9 g) of S8A were run at 0° C. using the apparatus described by Clements (1958), and the other half of S8A was run at room temperature. In each case, the bulk of the sample was obtained in a number of fractions eluted with 0.4-1.0 liter petroleum ether and the nature of these eluates was quite similar, appearing as a semisolid white wax, a free flowing, clear, colorless oil or a mixture of the two. The columns were then rinsed with diethyl ether and the remaining material (10-30 per cent of total) was eluted; no effort was made to identify this material. In general, the patterns of elution of S8A at 0° C. and room temperature were not strikingly different.

Several samples of the above semisolid wax and clear oils were selected for further study: S11A (oil from S7a); S12A (oil from S8A chromatographed at room temperature); S13A (oil from S8A eluted from chromatography at 0° C.); S14A (oil from S9A); S15A (wax from S10A). Further work on these substances is described below.

*S3C and S3D.* S3C was chromatographed on activated silicic acid. Elution with 1:1 petroleum ether-benzene removed 45.5 g of a

thick, viscous, pale yellow oil (S4C). By precipitation with petroleum ether at  $-14^{\circ}$  C., approximately 0.5 g of a solid was removed from S4C; the solid melted at  $59^{\circ}$ - $63^{\circ}$  C. and showed the infrared spectral characteristics of a long chain, aliphatic ester. The filtrate (S5C) from this precipitation yielded a viscous oil the infrared spectrum of which exhibited a highly branched hydrocarbon structure having traces of unsaturation ( $R_1R_2C=CH_2$ ) and a carbonyl component.

An aliquot (31 g) of S5C was chromatographed on activated silicic acid. Petroleum ether (1.0 liter) eluted 4.1 g of a viscous, almost semisolid oil (S6C). Further amounts of this solvent eluted another 1.2 g of a similar oil from the column in two fractions. Benzene-petroleum ether, 1:1, then removed 11 g of a resin-like, slightly brown material (S7C) followed by 6.5 g of a darker resin (S8C).

Fractions S6C, S7C, and S8C were found to contain small amounts of a tacky material insoluble in hot petroleum ether which was filtered off. The filtrate from S6C was evaporated to a pale yellow, semisolid resin which was used in the analytical work described below. The filtrates from S7C and S8C were evaporated and aliquots of each of the residues chromatographed on silicic acid. From 6.2 g of S7C were obtained several fractions, of which two were selected for further study: S9C (2.5 g) eluted by 10 per cent benzene in petroleum ether and S10C (2.2 g) removed by 75 per cent ethyl ether in benzene. Similarly, S8C (4.3 g) yielded a number of fractions including S11C (1.3 g eluted by 10 per cent benzene in petroleum ether) and S12C (0.7 g removed by 10-50 per cent ethyl ether in benzene). S6C and S9-12C were studied in detail as described below.

*S3D and S3E.* After removal from S3D and S3E of small amounts of materials insoluble at  $-14^{\circ}$  C., the filtrates were repeatedly chromatographed on silicic acid. Several fractions were obtained which were similar to those isolated from the above fractions and discussed in detail below.

#### *Analytical Data*

*Vapor phase chromatography.* Several of the fractions were screened by this procedure to determine purity. A number of experimental conditions were employed. S11A, S12A, and S13A were each found to have eight or more components, and several components

having similar retention times were found in each fraction. S14A showed one distinct peak of low retention time, but indications of slowly eluted high boiling components were observed. The findings with S15A were difficult to evaluate and purity could not be claimed. S6C gave one major peak and possibly two minor constituents at 240° C.

*Microanalyses.* The appearances, melting ranges, elemental analyses, average molecular weights (Rast), empirical formulas and refractive indices of the fractions are shown in table 1. It should be emphasized that all fractions were mixtures and that the data represent mean values. S15A was the only fraction giving a total of 100 per cent carbon and hydrogen, although the amounts of oxygen present in S11A, S12A, and S6C were too small to account for one atom of oxygen per molecule based on the molecular weight by the Rast procedure. The carbon:hydrogen ratios of all fractions suggested the presence of possible cyclization, unsaturation or aromaticity; the fractions in the "C" series showed a much higher degree of these characteristics than the "A" series.

TABLE 1.  
PROPERTIES OF ISOLATED FRACTIONS

Fract.	Appearance	Melt. Range (° C.)	Microanalys. (%)*			Mol. Wt.	Empirical Formula***	$n_D^{20}$
			C	H	C + H			
S11A	Colorless oil	25-30	84.63	13.66	98.29	530	C <sub>37</sub> H <sub>72</sub> O <sub>0.56</sub>	1.4715
S12A	Colorless oil	25-30	83.80	13.23	97.03	515	C <sub>36</sub> H <sub>68</sub> O <sub>0.96</sub>	1.4740
S13A	Colorless oil	20-25	83.81	13.38	97.19	—	—	1.4764
S14A	Colorless oil	20-25	84.94	13.50	98.44	—	—	1.4787
S15A	Opaque wax	35-40	86.18	13.94	100.12	515	C <sub>37</sub> H <sub>72</sub>	1.4814
S6C**	Yellow resin	—	86.51	11.84	98.35	560	C <sub>40</sub> H <sub>66</sub> O <sub>0.58</sub>	1.5307
S9C	Yellow resin	—	83.80	11.02	94.82	525	C <sub>37</sub> H <sub>57</sub> O <sub>1.7</sub>	1.5320
S10C	Brown resin	—	77.78	10.36	88.14	760	C <sub>49</sub> H <sub>78</sub> O <sub>5.6</sub>	—
S11C	Yellow resin	—	77.33	10.97	88.30	730	C <sub>47</sub> H <sub>79</sub> O <sub>5.3</sub>	1.5310
S12C	Brown resin	—	74.55	10.33	84.88	900	C <sub>56</sub> H <sub>92</sub> O <sub>8.6</sub>	—

\* All fractions were negative for N, S and halogen.

\*\* Showed  $[\alpha]_D^{28}$ -9.8 (c, 2.12 in chloroform). Optical rotations on other samples were not determined.

\*\*\* Assuming the difference between (C + H) values and 100 per cent is oxygen. As an alternate procedure, the empirical formulas may be corrected for this difference in the case of hydrocarbons having trace amounts of oxygen or other contaminants (Zechmeister and Sandoval, 1946). Such corrections give formulas of C<sub>38</sub>H<sub>74</sub>, C<sub>37</sub>H<sub>70</sub>, and C<sub>41</sub>H<sub>68</sub> for S11A, S12A, and S6C, respectively.

*Oxidation and reduction.* Results of tests with concentrated sulfuric acid, potassium permanganate in acetone and bromine in carbon tetrachloride are detailed in table 2. S6C and S15A were insoluble and relatively unreactive with concentrated sulfuric acid; fraction S11C gave a reaction typical of organic compounds attacked by this reagent. All tested fractions decolorized bromine (at least in part) by a substitution reaction. Microhydrogenation (in dioxane using palladium on carbon as catalyst) showed less than one double bond per molecule based on the Rast values.

*Infrared spectra.* The spectra of all samples showed the presence of a rather highly branched hydrocarbon skeleton containing no or slight unsaturation and amounts of oxygen-containing groups varying from a trace (S15A) to a considerable quantity (S12C). Branching was indicated by strong absorption at  $7.23\mu$  ( $\text{CH}_3$  —) relative to  $6.82\mu$  ( $-\text{CH}_2-$  and  $\text{CH}_3-$ ) combined with weak  $13.7\mu$

TABLE 2.  
PROPERTIES OF ISOLATED FRACTIONS

Fract.	Conc. $\text{H}_2\text{SO}_4$		$\text{KMnO}_4$ **	$\text{Br}_2$ in $\text{CCl}_4$ ***	Microhydro- generation****
	Color*	Solub.			
S11A	R-O	Insol.	O	+	0.50
S12A	—	—	—	—	0.24
S13A	R-O	Sl. sol.	±	++	—
S15A	S1Y	Insol.	O	+	0.025
S6C	S1Y	Insol.	O	+++	0.40
S9C	R-B	Insol.	±	—	0.42
S10C	—	—	—	—	0.23
S11C	B1	Sol.	—	—	0.32
S12C	—	—	—	—	0.56

\* R-O = red-orange, S1Y = slightly yellow, R-B = red-brown, B1 = black.

\*\* ± = Slight decolorization in 15 minutes, O = no change in 15 hours.

\*\*\* Speed of decolorization: + = slowest, +++ = fastest. All fractions evolved HBr (litmus test).

\*\*\*\* Number of double bonds per molecule.

(unbroken — CH<sub>2</sub> — chains) bands in all samples.<sup>3</sup> Aromatic nuclei were absent. Figure 2 shows the spectrum of S15A (in carbon disulfide), which was representative of those fractions having no or small amounts of oxygenated components; figure 3 shows the bands at 7.23 and 6.82 $\mu$  (in carbon tetrachloride) for S15A. Table 3 gives the ratios of absorptivities at 6.82 and 7.32 $\mu$  to illustrate the degree of chain branching in all fractions.

The oxygen-containing groups were characterized by broad bands in the 5.7-6.0 $\mu$  region, including some possible  $\alpha$ ,  $\beta$ -unsaturated

TABLE 3.  
CHAIN BRANCHING IN VARIOUS FRACTIONS BY INFRARED SPECTRAL ANALYSIS

Fraction	Ratio absorp. 7.23 $\mu$ *	
	absorb. 6.82 $\mu$	
S11A	0.609	
S12A	0.679	
S13A	0.719	
S14A	0.715	
S15A	0.678	
S6C	0.894	
S9C	0.908	
S10C	1.06	
S11C	0.946	
S12C	1.12	

\* All spectra determined in carbon tetrachloride. Absorp. = absorptivity.

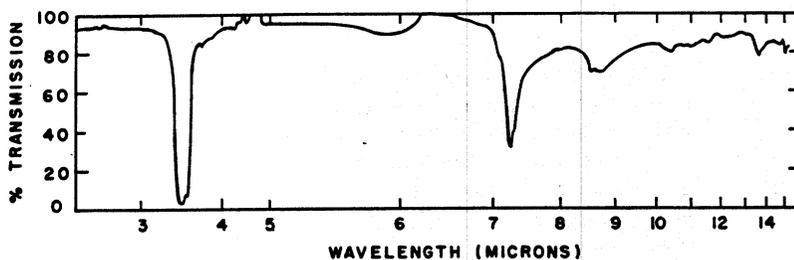


FIGURE 2  
INFRARED SPECTRUM OF S15A. THREE PER CENT SOLUTION IN  
CARBON DISULFIDE

3. For various reasons, the 7.23 $\mu$  band may frequently be stronger than the 6.82 $\mu$  band as in certain of the above fractions. Aliphatic n-paraffins of tobacco have  $\frac{\text{absorb. } 7.23\mu}{\text{absorb. } 6.82\mu}$  values of about 0.218.

carbonyl in at least two fractions (S6C and S9C). In the more highly oxidized fractions, small amounts of hydroxyl absorption were noted. A tendency was observed for slow, progressive auto-oxidation on prolonged storage, even under nitrogen, of fractions in the "C" series as judged by intensification of color and increased absorptivity of the oxygen-containing groups of the infrared spectrum; this tendency was not as marked in the "A" series of fractions and might have been a reflection of degree of chain branching or cyclization in the two series. A similar pattern is noted in the petroleum naphthenes.

*Mass spectra.* Four of the above fractions (S11A, S15A, S6C, and S6D) were submitted to mass spectrometric analysis. In addition, two other fractions (123-2 and 169-21) obtained by extensive chromatography on silicic acid of S3D and S3E were also run. The essential features of the mass spectra are given in table 4.

TABLE 4.  
MASS SPECTRA OF MIXTURES CONTAINING CYCLIC HYDROCARBONS  
FROM TYPE 12 LEAVES

Fraction	Significant Masses in Fragment and Parent Peak Regions ( $m/e$ )*		Components
S11A	278,**	527, 541, 555	Naphthene-like. Mono-, di- and tricyclic constituents present.
S15A	278, 317, 331, 345 359, 527, 541, 555, 609, 623, 637, 830		Similar to S11A.
S6C	325, 393, 461, 597, 612		Naphthene-like. Tetracyclics predominate. Moderate amount of pentacyclics. Small quantities of tricyclics. Possibly some (<5 percent) iso-paraffins.
S9C	323, 391, 608, 610, 612		Naphthene-like. Tetracyclics predominate. Some tri- and pentacyclics possibly present.
169-21	323, 391, 608, 610, 612		Almost identical with S9C.
123-2	142, 156, 170, 184, 393, 421		About 50 per cent iso-paraffins ( $C_{29}$ and $C_{31}$ ) and n-paraffins ( $C_{10}$ to $C_{13}$ ). Remainder are mono- and polycyclic compounds.

\* Mass spectrometer operated at 350° C./0.01 mm. Hg.; variation of  $\pm 2 m/e$  in high molecular weight peak ranges (>600).

\*\* Parent mass of neophytadiene (Gladding *et al.*, 1959).

In general, the spectra showed the presence in all fractions of hydrocarbons having cyclic structures superficially similar to the naphthenes found in petroleum. Specifically, however, the patterns of the spectra showed the components of the mixtures from tobacco to be quite unlike the cycloparaffins of petroleum. The dissimilarity was such that quantitative spectral evaluation for specific structural details by comparison with known petroleum naphthene characteristics was difficult in most instances. Despite extensive fractionation, the samples appeared to be complex mixtures.

In table 4, the major fragment and parent masses ( $m/e$ ) are indicated for each fraction. Each of the samples showed a number of high molecular weight fragments. Due to the variations in  $m/e$  values in the higher ranges, it was uncertain whether the peaks at  $>600$  were fragments or parent masses. Comparison of these peaks with the Rast molecular weights indicated that compounds having relatively high molecular weights predominate in the fractions. The major components in fractions having less chain branching and higher C:H ratios (S11A, S15A) were one-, two- and three-ring structures. Those samples showing lower C:H ratios consisted mainly of tetracyclic constituents in combination with some three- and five-ring compounds. Only one sample (123-2) gave a spectrum which, when evaluated according to a scheme devised for quantitative analysis of petroleum hydrocarbons, yielded rational values. This sample showed about fifty per cent iso-paraffins (approximately  $C_{31-33}$ ) and n-paraffins ( $C_{10-15}$ ), the remainder being ring compounds with monocyclics predominating.

#### *Discussion*

Of the fractions studied completely, all were mixtures of compounds having high molecular weight, saturated, cyclic hydrocarbon structures therein. Some unsaturated linkages were found in each fraction, but these were comparatively few. At most, the low level of unsaturation might have caused an error in mass spectral evaluation of one ring, but, in all probability, was too slight to be of significance in this regard. The observed large fragment or parent masses and high average molecular weights (Rast) might indicate that molecules having repeating units of polycyclic hydrocarbons linked by methylene groups or aliphatic chains exist therein. Undoubtedly, substituents consisting of methyl groups or branched alkyl

chains are also present. Although the mass spectra of the more highly oxygenated fractions, S10C, S11C, and S12C, were not examined, these mixtures appeared to be basically related to the other samples, existing perhaps, as more complex structures of greater susceptibility to oxidation; in fact, S6C through S12C could be visualized as a relatively progressive series in this respect.

As far as we are aware, cyclic, saturated hydrocarbons of this type have not been previously reported in plants, and no mixture of known, fully characterized plant constituents could fulfill the physical properties outlined above.

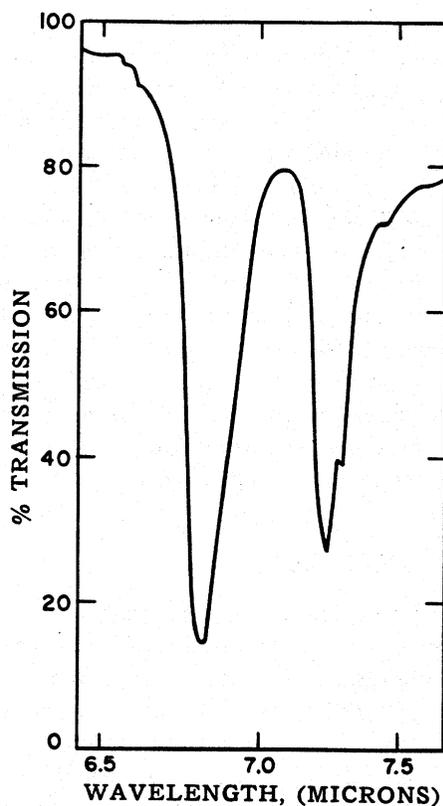


FIGURE 3

INFRARED ABSORPTION OF S15A IN THE 6.5-7.5 MICRONS REGION SHOWING DEGREE OF CHAIN BRANCHING. THREE PER CENT SOLUTION IN CARBON TETRACHLORIDE

Although the compounds are significantly unlike the petroleum naphthenes, the possibility that they are artifacts<sup>4</sup> has been extensively considered. The source of the compounds was not the non-volatile residues from the (petroleum-derived) extracting and chromatographic solvents for two reasons: repetition of the fractionation (on a smaller scale) using highly purified solvents essentially free of non-volatile residues resulted in the isolation of mixtures similar to the above; and the total amounts of non-volatile residues (4.9 g) in all solvents used in the above fractionation were significantly less than the estimated quantities of the mixtures isolated thus far from tobacco (greater than 40 g<sup>5</sup>). Perhaps part or all of the non-volatile residues carried over into the fractions; the n-paraffins from C<sub>10</sub> to C<sub>13</sub> in fraction 123-2 may be of such origin. Iso-paraffins in the C<sub>27-33</sub> range (cf. m/e, 393 and 421, fraction 420-123-2) have been previously reported in tobacco. Perhaps the elemental sulfur found above was derived from the extracting solvent rather than fungicidal residues on the leaves (Weybrew, 1959).

Polymerization of tobacco olefins on chromatographic columns<sup>6</sup> represents another possible extraneous source. Fink *et al.* (1950) and Gallaway and Murray (1958) mentioned the effect of low temperature in reducing polymerization of olefins on chromatographic columns but gave no details; other workers have commented on

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4. Another possible artifact is the deposition of residues on leaves during curing as a result of improper operation of the burners employed to provide heat. Since the isolated substances give mass spectra not identical with petroleum naphthenes, this possibility appears to be eliminated.

5. These values are based on the weights of isolated fractions which appeared similar by inspection and other physical characteristics (index of refraction, infrared spectrum, and chromatographic behavior) to those studied more intensively. Although work on the separation of S3D and S3E has not been completed, a considerable amount of resin-like material similar to S6C and S9C (as well as smaller amounts of solids similar to S15A) has been obtained from these fractions, making the above estimate quite conservative.

6. Mention should be made of the striking difference between the chromatographic patterns of tobacco extracts on acid-washed alumina and on silicic acid. The latter does not isomerize neophytadiene and appears to be a stronger adsorbent than Merck acid-washed alumina. Petroleum ether eluates from silicic acid columns contain only aliphatic paraffins and neophytadiene, the latter being obtained as an acetone-soluble, colorless, light oil. Similar eluates from acid-washed alumina columns contain aliphatic paraffins, isomerized neophytadiene, some of the cyclic paraffins, unidentified unsaturated (11.25  $\mu$ ) hydrocarbons and an unidentified, dark brown oil. The acetone-solubles from such eluates are dark brown, thick oils. Repeated chromatography of the latter on silicic acid serves to separate the unsaturates from the saturated material to some degree, although traces of unsaturation usually persist.

isomerization of olefins under such conditions, including some negative findings (Saier *et al.*, 1954; Johnston *et al.*, 1948). To check this possibility, squalene (Kosak and Swinehart, 1958; Van Duuren and Schmitt, 1958) was chromatographed on acid-washed alumina and silicic acid. Although some isomerization was observed on the alumina column (but not on silicic acid), no compounds resembling the above cyclic hydrocarbons were isolated. In similar trials with neophytadiene (Rowland, 1957; Onishi *et al.*, 1958; Gladding *et al.*, 1959), only isomerization on acid-washed alumina was observed: the infrared spectral characteristics of the eluates suggested that phytylene C (Rowland *et al.*, 1957) was the principal isomer present.

Although the isolated hydrocarbons are subject to auto-oxidation, it is felt that the more highly oxidized fractions, e.g., S10C, exist as such *in situ*. Prolonged manipulation and exposure to air of fractions having low oxygen contents, e.g., S6C, has not resulted in their altering physical appearance or infrared spectral characteristics to a degree found in the more highly oxidized samples.

At least three practical implications of the present findings are evident. First, the extreme tackiness of such fractions as S6C suggests that they may contribute to the gummy or oily characteristics of the leaf, one of the desirable quality features. Secondly, the cyclic hydrocarbons may be quite easily dehydrogenated during pyrolysis and be involved in the formation of aromatic polynuclear hydrocarbons known to be present in smoke. Thirdly, the presence of such compounds in plants may have a bearing on current theories of petroleum origin which require the postulation of mineral-catalyzed polymerization of fatty acids or olefins to explain the presence of naphthenes therein (Brooks, 1950, 1951).

The failure to separate a single compound from the above mixtures is hardly surprising since prolonged, consistent effort by many workers to fractionate the higher naphthenes in petroleum has met with only limited success.

### Summary

The isolation of mixtures containing saturated, cyclic hydrocarbons from flue-cured tobacco leaves is described. The structural features of the compounds were established from microanalyses (carbon and hydrogen, catalytic hydrogenation and molecular weight); reactions to concentrated sulfuric acid and oxidizing agents;

and infrared and mass spectral characteristics. Most of the hydrocarbon mixtures exist in various degrees of oxidation and show mass spectra having fragment and parent masses in the 278-830 range; the major components of the mixtures superficially resemble the higher naphthenes of petroleum to some extent, although outstanding differences exist. The possible roles of these compounds in contributing to leaf gumminess, to the pyrolytic generation of aromatic polynuclear hydrocarbons during smoking, and to the current theory of the origin of naphthenes in petroleum were discussed.

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