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Chemical Research

MRR-C, 1961, No. 9

To: Mr. Kenneth H. Hoover
Director of Research

September 20, 1961

Re: MONTHLY RESEARCH REPORT
Chemical Research
1961, No. 9

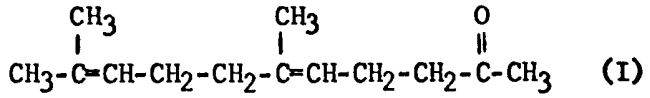
Period Covered:
August 25 to September 20

## A. ISOLATION AND CHARACTERIZATION OF COMPONENTS OF TOBACCO

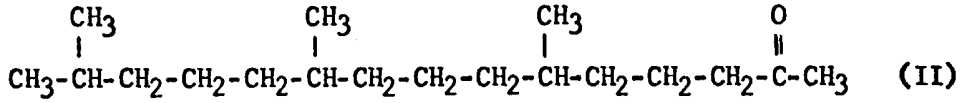
### I. Components of Turkish Tobacco

Separation of the flavorful, volatile portion (298 g.) of a hexane extract from one ton of Turkish tobacco sand into twelve crude chromatographic fractions was reported last month. During the coming months the several fractions with promising flavoring properties will be separated in attempts to isolate pure flavoring components.

This month Fraction C (18.5 g.), one of the early chromatographic fractions with promising flavoring properties, was separated into ketone (13.9 g.) and lactone (4.6 g.) fractions. Column chromatography of the ketone fraction gave 350 subfractions, several of which were essentially pure compounds. Geranyl acetone (I) and hexahydrofarnesyl acetone (II), also known as phytone, have been identified in the various fractions. An isomer (III) of geranyl acetone appears to be present, along with compounds



(I)



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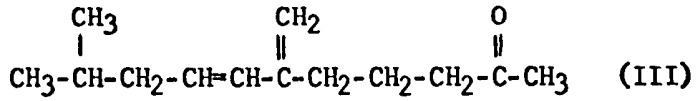


(III)

tentatively identified as pseudoionone and farnesyl acetone. All of these ketones have rather strong, pleasant odors which presumably contribute to the overall aroma of Turkish tobacco, but none of the odors are specifically characteristic or suggestive of the odor of Turkish tobacco or its smoke. Attempts are being made to identify other components of Fraction C.

## II. Components of Flue-cured Tobacco

This month further study has been made of ketone fractions separated from the previously described volatile and semivolatile flavoring materials obtained from flue-cured tobacco sand.

Earlier reports describe isolation of a flavorful unsaturated ketone, $C_{13}H_{18}O$, designated Compound K-1. Elucidation of the structure of Compound K-1 is in progress. This month a new ketone, designated Compound K-2, has been isolated. Preliminary physical data indicate that the new, flavorful ketone is similar in structure to Compound K-1. The ketone (III) isolated from Turkish tobacco sand, as described in the preceding section, was also isolated from flue-cured tobacco sand this month. Isolation of 2-acetylpyrrole from flue-cured tobacco sand was reported last month; a sample of this material will be synthesized for comparison with the isolate and for flavor evaluation.

At this writing, menthol has just been isolated from a volatile fraction from flue-cured tobacco sand. The amount isolated represents about 100 mg. per ton of the sand. The source of the extract and method of isolation strongly indicate that menthol is a natural component of flue-cured tobacco. A detailed report of this work will be given when present experiments have been completed.

## III. Components of Burley Tobacco

In a continuing study, extracts of burley tobacco and dust are being processed by various techniques in a systematic search for burley flavorants. In the course of work done this month one new compound, designated BT-10, of dubious flavoring quality, was isolated. The new compound appears to be an unsaturated hydroxyketone having a basic triterpene or steroid structure.

Characterization studies continue on various compounds in the classes designated M-II and BT. This work will be described when structures have been established.

# B. ISOLATION AND CHARACTERIZATION OF COMPONENTS OF TOBACCO SMOKE

## I. Components of Turkish Tobacco Smoke

In January 1960, the smoke condensate from 20,560 Turkish cigarettes was collected and partitioned between equal volumes of hexane and 9:1 methanol:water. Since that time, repeated chromatography of the various partition fractions has permitted identification of the following compounds or groups of compounds: saturated hydrocarbon fraction (RDR, 1960, No. 21); phytadiene fraction and neophytadiene (RDR, 1960, No. 21, cf. RDR, 1959, No. 11); solanesenes (RDR, 1960, No. 21, cf. RDR, 1960, No. 3); solanesyl acetate (RDR, 1960, No. 21, cf. RDR, 1958, No. 22); solanesyl ester fraction

(RDR, 1960, No. 21, cf. RDR, 1959, No. 2); phytosteryl ester fraction (RDR, 1960, No. 21, cf. RDR, 1959, No. 2); α-tocopherol (RDR, 1960, No. 21, cf. RDR, 1959, No. 23); phytosterol fraction (RDR, 1960, No. 21); solanesol (RDR, 1960, No. 21, cf. RDR, 1958, No. 22); sclareolide (RDR, 1960, No. 8); α- and β-levantenolide (RDR, 1961, No. 21); phenol, o- and p-cresol (RDR, 1961, No. 10); hydroquinone, 1-naphthol, eugenol, and isoeugenol (RDR in preparation); 6-acetyl-2,3,4-tris-d-β-methylvaleryl-β-D-glucopyranoside (RDR, 1961, No. 42); long-chained fatty acid fraction (RDR, 1961, No. 15); lactones.

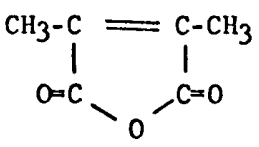
The more polar fractions from the above-mentioned partition have yielded small amounts of short-chained fatty acids, phenols, and lactones with interesting flavoring possibilities. To obtain these compounds in greater amount for characterization, the smoke condensate from 47,200 Turkish blend cigarettes has been collected. Fractionation of this smoke condensate is in progress and this month resulted in isolation of a crystalline phenol, yet to be identified.

As reported last month fractionation of a subfraction from one of the more polar partition fractions from the smoke condensate from the 20,560 Turkish cigarettes yielded 12α-hydroxy-13-epimanoyl oxide, m.p. 138-139° C., $[\alpha]_D^{25}$ + 43.3° (chloroform). During the present research period this same subfraction yielded two additional crystalline alcohols.

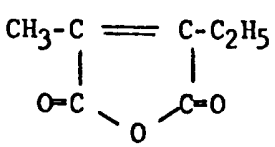
One of these, molecular weight 304, m.p. 109-110° C., has an infrared absorption spectrum identical with that of Compound X, of unknown structure, isolated from Turkish tobacco.

## II. Components of Burley Tobacco Smoke

In previous work three anhydride fractions were isolated from the strong acid fraction of burley tobacco smoke. Two of the anhydride fractions which had tobacco-type odors have been characterized. The first anhydride, a crystalline solid, was identified as 2,3-dimethylmaleic anhydride (IV). The second anhydride fraction with a tobacco-type odor was an oil, molecular weight 140. The data suggested that this material was 2-methyl-3-ethyl-maleic anhydride (V). The third anhydride fraction has not been characterized.



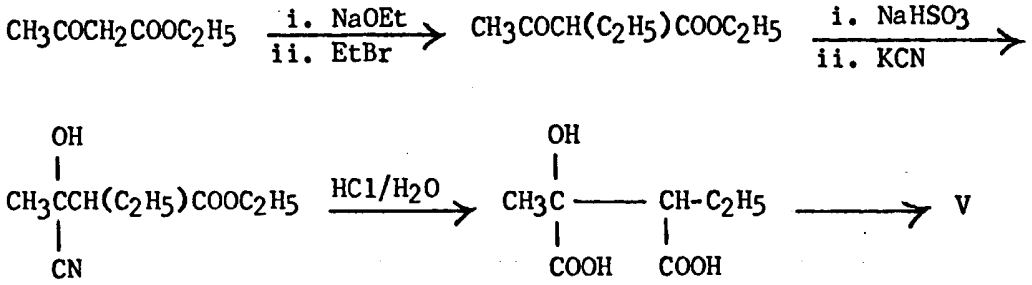
IV



V

During the present research period, 2,3-dimethylmaleic anhydride (IV) was studied as an additive for burley tobacco. The smoke from uncased K10X burley cigarettes containing this anhydride was much milder than the smoke from control cigarettes. The effect on burley smoke was much greater than the effect on flue-cured smoke which had been observed previously.

Synthesis of 2-methyl-3-ethylmaleic anhydride (V) has been accomplished, as outlined below. The infrared absorption spectra of the synthetic product and the anhydride fraction from the smoke condensate are identical, confirming the assigned structure. Future work will include synthesis of a larger quantity of V for a study of its contribution to the flavor of cigarette smoke.




## C. SYNTHESIS OF FLAVORING COMPONENTS OF TOBACCO; AND OTHER SYNTHESIS STUDIES

### I. Production of Sclareolide

Current work on operations and processes involved in production of sclareolide is described below. It will be recalled that sclareol, obtained from clary sage, is the only practical starting material known for production of sclareolide.

#### a. Experimental Growth of Clary Sage in Forsyth County (Crouse Farm)

##### 1. PARALLEL 1/20 ACRE PLANTINGS OF ENGLISH AND FRENCH CLARY SAGE (NEMATODE FREE)

Histories of these plantings, established in fumigated soil in late August 1960, have been given. The previous report presented general observations and comparative yield data for the 1961 harvest period. An additional experiment and further general observations are described below.

In parallel experiments, two 60-plant samples of flowering parts from French-type clary sage were harvested during the period

of optimum sclareol production. The control sample was extracted immediately by the standard procedure, and the extract was weighed and then assayed for sclareol. The companion sample was immersed in hexane for eight days, and was then extracted by the standard procedure. The extract was weighed and assayed for sclareol. Converting the data to a per acre basis, the control sample represented yields of 103 and 70.6 lbs. of concrete and sclareol, respectively, per acre, and the corresponding "immersed" sample represented yields of 104 and 67.8 lbs. of concrete and sclareol, respectively, per acre. This experiment indicates that harvested clary sage flowers may be stored under hexane for at least eight days without significant decrease in amount of concrete or sclareol recoverable. This result agrees with all previous observations.

At the end of the 1961 harvest season, following the usual practice, both plantings were mowed to within 4-5 inches of the ground and were cultivated. The plants have now put forth new vegetative growth, making a preliminary estimate of plant losses possible. It is estimated that 50 percent of the English plants and 90 percent of the French plants have failed to survive. These unexpectedly high losses, particularly for year-old plants at this time of year, may be due to several factors. High soil fertility, resulting from a very high rate of fertilization, would appear to be the primary cause of plant loss in view of the fact that losses were only 20 and 30 percent for English and French plants, respectively, in the 1/8 acre plantings (described below) which were lightly fertilized. Further, a report in the literature indicates that high soil fertility results in shortened life span of clary sage as compared to the life span in poor soil. The effect of soil fertility on the life span of clary sage should be the subject of a future investigation. Adverse weather conditions and excessive infestation of the plantings, particularly the French planting, with weeds may be other factors responsible for the abnormally high plant loss incurred this year.

Both plantings have been reseeded as required to fill voids, but germination has been very poor to date because of excessively dry weather.

### 2. PARALLEL 1/8 ACRE PLANTINGS OF ENGLISH AND FRENCH CLARY SAGE (NEMATODE INFESTED)

These plantings were established with no prior soil fumigation in late July 1960, as described previously. Results of experiments with fertilizers and herbicides together with general observations for the 1961 flowering season were presented last month.

At the end of the 1961 flowering season both plantings were mowed to within 4-5 inches of the ground and were cultivated.

During the current report period new vegetative growth has appeared and it is now estimated that 20 and 30 percent, respectively, of the English and French plants have failed to survive. The losses appear to be concentrated in areas of severe nematode infestation. The combination of nematode damage and extremely dry weather may be responsible for the losses observed.

Both plantings have now been cultivated and fertilized. Numerous volunteer seedlings have appeared in both plantings, making reseeding to fill voids unnecessary. Sections of the English planting have been treated with various herbicides. These sections will be observed for evidence of plant damage and weed control. Final evaluation of the treatments may not be possible until Spring of 1962.

### 3. USE OF PRE-EMERGENCE HERBICIDES IN ESTABLISHING CLARY SAGE PLANTINGS

Small scale experiments continue for study of use of pre-emergence herbicides in establishing clary sage plantings. Additional small plots have been seeded with clary sage and sprayed with various levels of various herbicides. Herbicides tested to date have either inhibited germination of clary sage seed or have proved toxic to emerging clary sage seedlings. Additional experiments are in progress.

## b. Recovery of Sclareol from Clary Sage Concrete

### 1. PILOT PLANT OPERATION

Pilot plant operations for recovery of sclareol from clary sage concrete have been suspended throughout the current report period due to installation of equipment for study of production of sclareolide. It now appears that the installation work will continue for several more weeks. Pilot plant operations will resume when installation of equipment has been completed.

As a result of runs to date a total of 658 lbs. of 92 percent sclareol is on hand. This quantity of material is sufficient for at least five full-scale runs for preparation of sclareolide. The sclareol on hand will be ground and blended to provide a single, homogeneous batch of starting material for initial pilot plant runs in study of production of sclareolide.

### 2. LABORATORY STUDY OF A NEW PROCESS FOR RECOVERY OF SCLAREOL FROM CLARY SAGE CONCRETE

The so-called "detergent process" for recovery of sclareol from clary sage concrete has been described in detail in previous

reports. By using various modifications of this process, yields of sclareol have ranged from 70-90 percent of theory with product purities ranging from 85-100 percent. In experiments to date high yields result in low purity of product, and pure sclareol is obtained in relatively low yield. Various process variables are being studied in attempts to obtain high yields of high purity sclareol.

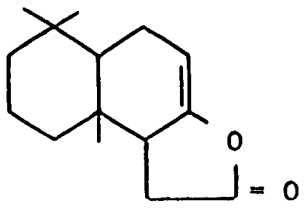
c. Chemical Conversion of Sclareol to Sclareolide

A process for production of sclareolide from sclareol has been developed in the laboratory and awaits further study on pilot plant scale. Most of the pilot plant equipment has now been received and installed. A special reactor, the only major piece of equipment still on order, is scheduled to be shipped at the time of this writing. On this basis all equipment should be installed and ready for operation by mid-October.

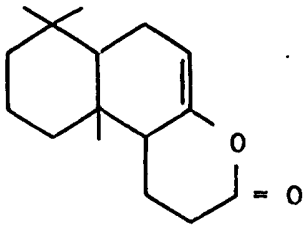
Quotations on various chemical raw materials for the pilot plant study have been obtained, and sufficient materials for initial runs will now be ordered. Plans are being made for operation of the pilot plant, and laboratory study of various aspects of the process continues.

II. Study of the Chemistry of Sclareol and Its Derivatives

Sclareol and several of its derivatives are on hand as a result of work on production of sclareolide. Study is being made of the chemistry of these compounds in the hope that useful information will result. In the course of an earlier study of the dehydration of sclareol, two enol lactones (VI and VII) with desirable flavoring properties were prepared. For patent purposes it became desirable to develop practical syntheses of the two compounds. A synthesis of (VI) was described last month.

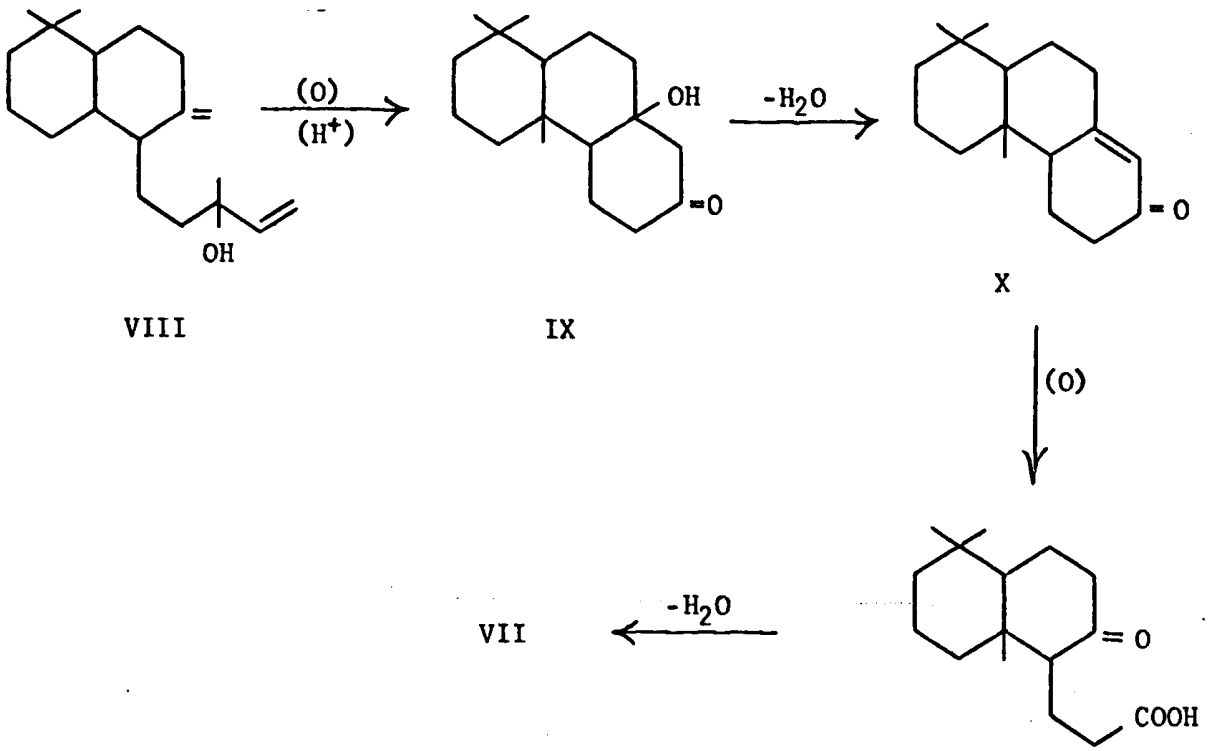



VI




VII

Synthesis of the other enol-lactone (VII) from manool (VIII) is in progress by the following scheme:



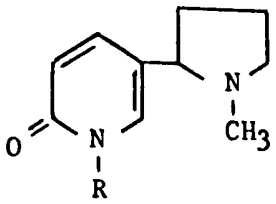

Preparation of intermediate ketones IX and X has now been achieved; completion of the synthesis will be attempted during the coming month.

III. Synthesis of Derivatives of Nicotine

Synthesis of potentially useful, salable derivatives of nicotine continues. Compounds synthesized to date are: two fluoronicotines; two chloronicotines; two aminonicotines; β-(N-6-oxo-1-nicotine)-propionitrile; thirteen 6-alkoxy-1-nicotines; and $Cu^{++}$ and $Hg^{++}$ complexes of 6-hydroxy-1-nicotine. The higher alkoxynicotines and the complexes of 6-hydroxy-1-nicotine have been submitted to the Biochemical Division for evaluation as germicides and fungicides.

This month reaction of 6-hydroxy-1-nicotine with sodium hydroxide in alcoholic solution has been shown to result in formation of a solid sodio

derivative of the hydroxynicotine. This sodio derivative will now be used for preparation of a series of 1-alkyl-5-(N-methyl-2-pyrrolidyl)-2-pyridine derivatives (XI).



where R = $C_8$-$C_{18}$ alkyl group

XI

These compounds will probably act as detergents and may exhibit germicidal or fungicidal activity.

## D. STUDIES INVOLVING USE OF RADIOISOTOPES

### I. The Fate of Disodium Isopropylmalonate During Smoking

This study has been suspended, temporarily, to permit more effort on investigation of the degradation of sclareol during drying of clary sage, as described below.

### II. Study of the Degradation of Sclareol During Drying of Clary Sage Flowering Parts

This study was begun in the previous report period in the hope that knowledge of the processes involved in degradation of sclareol during drying of clary sage flowering parts might lead to development of methods for stabilizing sclareol in plant tissue. This would permit drying and storage of harvested flowering parts for later extraction.

The successful incorporation of sclareol-$C^{14}$ into excised clary sage flowering spikes was reported last month. Evidence was also obtained of extensive biochemical degradation of sclareol in the living plant. This suggests that the loss of sclareol during air-drying is a continuation of such a biochemical degradation for a time after harvesting. The loss during heat-drying, however, appears to be chemical rather than biochemical, e.g., dehydration, oxidation, or polymerization.

During the current report period two separate drying experiments were carried out. In a preliminary run, sclareol-$C^{14}$ was incorporated into several flowering spikes of clary sage by immersing the excised stalk in an

aqueous solution of the labeled terpene. The plant material was then divided into three equal piles. One pile was air-dried for 10 days, another was heat-dried at 150° C. for 30 minutes, and the third pile, extracted immediately, served as a control. Each pile was extracted with hexane, and the sclareol was isolated and assayed for radioactivity. The activity of the sclareol from the air-dried sample was reduced almost to zero while that of the heat-dried material was reduced by 81 percent from that of the control. In addition, a considerable portion of the radioactivity from all three samples was found in fractions other than sclareol, indicating substantial degradation of the labeled terpene. In the case of the heat-dried material there was a sizeable loss of activity on concentration of the hexane extract, presumably due to evaporation of a volatile degradation product.

It is known that sclareol is readily dehydrated at elevated temperatures under mildly acidic conditions. If such a degradation occurs on an acidic surface of the plant, the products (i.e., the sclarenes) might be sufficiently volatile to be lost during concentration of the hexane extract. The dehydration reaction might, however, be eliminated by neutralization of the plant surface, i.e., by ammonia, prior to heat-drying.

In a second experiment the incorporation was carried out as described above and the flowering spikes were divided into four piles. The first pile was heat-dried as described previously, the second was treated with ammonia and then heat-dried, the third was air-dried for five days and the fourth, extracted at once, served as the control. The results of this experiment are given in Table I.

TABLE I

RECOVERY OF SCLAREOL FROM DRIED SAMPLES OF CLARY SAGE FLOWERING SPIKES

| Treatment | Pre-Extraction Conditions | % Recovery of Sclareol* | |
|---|---|---|---|
| | | Weight | Radioactivity |
| Heat-dried | 150°/30 mins. | 34 | 21 |
| Ammonia/heat-treated | 2 hrs. in $NH_3$ atm., then 150°/30 mins. | 84 | 33 |
| Air-dried | 5 days | 87 | 88 |
| Control | Extracted Immediately | 100 | 100 |

* Based on control = 100%.

The discrepancy between the recoveries based on weight and on radioactivity, especially for the ammonia-treated sample, indicates a preferential degradation of the sclareol-$C^{14}$ over the unlabeled material originally present in the flowering spike. Since the labeled and unlabeled compounds should be chemically indistinguishable, this discrepancy must reflect a biological difference, i.e., a difference in the physical location of the labeled sclareol and its unlabeled counterpart in the plant. Such a phenomenon might easily result from the incorporation method used. Thus a sizeable portion of the radioactive sclareol might remain in the vascular system of the plant or at some other internal location, while most of the unlabeled material might reside on the external surface. The exposed surfaces of the flowering spikes would be readily neutralized by the ammonia, while the internal portions would be less accessible and, therefore, less completely neutralized. The unlabeled sclareol would then be protected from degradation while the labeled compound in the interior would still be subject to acid degradation.

If the above hypothesis is correct, a more satisfactory incorporation method would be necessary. Another experiment, in which a solution of labeled sclareol is applied directly to the surface of the flowering spike, is being contemplated. These conditions would also permit a higher level of labeling and would reduce the complications introduced by biochemical degradation of the sclareol-$C^{14}$.

## III. Development of an Isotope Dilution Analysis for Sclareol

Sclareol-$C^{14}$, obtained from clary sage grown under an atmosphere of $C^{14}O_2$, is being used in development of an isotope dilution assay for sclareol in clary sage concrete and related materials. As described previously, the method of assay involves addition of a known weight and activity of sclareol-$C^{14}$ to the sample, recovery of sclareol from the sample by column chromatography, and recrystallization of the recovered sclareol to constant melting point and specific activity. The concentration of sclareol in the sample material can then be calculated.

Last month ten analyses of the same clary sage concrete gave an average sclareol content of 61.64 ± 0.29 percent, with an average deviation of ± 0.47 percent. This month duplicate samples of two different concretes were analyzed; the results for each concrete indicated a precision of ± 0.4 percent. Thus good precision is indicated. The accuracy of the method has now been studied by enriching a sample of concrete (containing 61.64 ± 0.29 percent sclareol) with known amounts of pure sclareol and analyzing the resulting mixtures. Using the original sclareol content of the concrete as a basis for calculation, the amounts of sclareol added could be determined with 100.2 ± 1.3 percent accuracy. As a separate check on the accuracy of the method, two determinations were made on highly purified sclareol; values of 100.0 ± 0.5 percent sclareol were obtained.

A sample of "ARWAX" prepared by Stafford Allen and Sons was analyzed and found to contain 96.7 percent sclareol.

Additional study of the isotope dilution assay will be made during the coming month. Samples analyzed by the standard infrared method will be re-analyzed by the isotope dilution method to permit comparison of results from the two methods.

## E. DEVELOPMENT AND USE OF A FLAVORING QUALITY INDEX FOR TOBACCO

Work continues on development of a flavoring quality index for tobacco. Burley tobacco is being used in establishing the experimental procedure. As described in RDR, 1961, No. 29, analysis of the volatile oils from a tobacco sample by gas chromatography gives a "profile" (gas chromatogram), the peak areas of which reflect the flavoring qualities of the tobacco. A flavoring quality number, N, may be calculated from the areas of certain peaks using the following equation:

$$N = \frac{\text{Area of Aromatic Peak}}{\text{Area of Floral Peak}} + \frac{\text{Area of Aromatic Peak}}{\text{Area of Green Peak}}$$

In preliminary experiments the N values obtained from various samples have been directly related to Company grades assigned to those samples.

It now seems desirable to evaluate a large number of burley tobacco samples to test the validity of the experimental procedure and calculated flavoring quality number, N. This month evaluation of a number of aged and unaged (refrigerated) samples from the 1954 through 1958 burley crops was begun. Results will be presented when all samples have been evaluated.

An earlier report described evaluation of flue-cured tobacco treated with MH-30. It will be recalled that small but distinct differences were apparent between the profiles from untreated tobacco and tobacco treated with MH-30. A more detailed experiment along this line is now planned. Samples of flue-cured tobacco grown with and without MH-30 treatment have been obtained from the current crop, and will be evaluated in the near future.

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