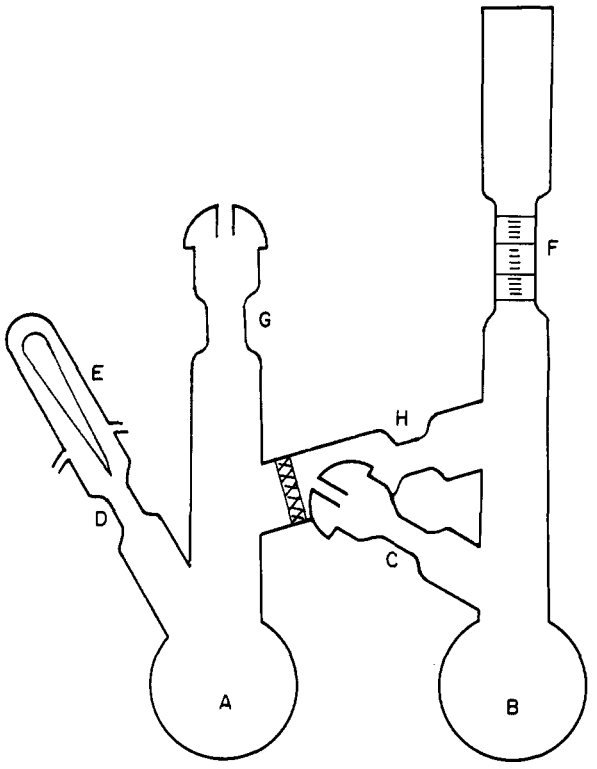

Fig. 3. Apparatus used for measuring the absorbency index of 2,6-di-t-butyl-4-butyoxyphenoxy and for studying the equilibrium

While still frozen, assembly *B* was rotated to break the enclosed ampule containing approximately one ml. of mercury. Constriction *C* was then sealed and the apparatus placed in a mechanical shaker. At the end of the shaking period (*ca.* 2 hr.) the blue solution was filtered through the medium grade frit. By cooling the bulb, benzene was distilled back into the reaction flask and used to rinse the bulb and frit. When rinsing was completed, the volume was determined from the calibrations at *D*, and the absorbency measured in a Beckman DU Spectrophotometer. The absorbency index at 625 mμ was found to be 400 ± 3; at 445 mμ, 11 ± 3. The result at 625 mμ agrees very well with a value of 410 ± 8 obtained by an entirely different technique.[7] At room temperature, the radical solutions proved to be very stable, losing about 1% of their absorbency on standing 24 hr.

B. Determination of the absorbency indices of 2,6-di-t-butyl-4-t-butyoxyphenoxy and of 2,6-di-t-butyl-4-methoxyphenoxy and the equilibrium constants. For these determinations, the apparatus shown in Fig. 3 was used. A five- to sixfold excess of the alkoxyphenol in low boiling petroleum ether (carefully purified) was added to bulb *B*, and the ether removed by suction applied to the ball joint at *C*. The constriction at *C* was then sealed off at the torch and 2,4,6-tri-*t*-butylphenoxy generated in flask *A* as described under part A. The phenoxy radical was filtered thru the frit into *B* and after several rinsings, the solution was frozen and the apparatus sealed off at *H*. The absorbency of the resultant red solution was then measured at 625 mμ and at 445 mμ. Since there was a large excess of alkoxyphenol, the equilibrium (Eq. 1) was displaced far to the right, and from the absorbency measurements and the volume it was possible to directly calculate approximate extinction coefficients for the alkoxyphenoxy radicals. These were applied to the equilibrium data (see below) to calculate approximate equilibrium constants. The constants were then used to correct the total absorbency for the small amounts of tri-*t*-butylphenoxy radical present in the system. As these corrections were on the order of only 3%, the method was considered highly satisfactory. At 625 mμ, 2,6-di-*t*-butyl-4-*t*-butoxyphenoxy had an absorbency index of 58.2 ± 2.7; at 445 mμ 105.9 ± 3.7; 2,6-di-*t*-butyl-4-methoxyphenoxy gave 31.2 ± 3 at 625 mμ and 89.5 ± 3.4 at 445 mμ.

For the equilibrium constant runs, appropriate known mixtures of the two phenols were added to bulb *B*, solutions of 2,4,6-tri-*t*-butylphenoxy then added from bulb *A*, and the apparatus sealed at *H* after freezing. From the absorbencies at 625 mμ and 445 mμ it was then possible to calculate the concentration of each species in the equilibrium mixture. During these reactions small decreases in radical concentration were noted; the total radical concentration can be calculated both from the spectral data and from the amount of bromocyclohexadienone used at the start. The former gave results averaging 4% less than the latter. This small loss was not sufficiently consistent to be some constant error in technique, and was most probably due to small amounts of the bromocyclohexadienone entering the frit and not reacting with the mercury. (Unlike the situation in part A, it is impossible in this case to return the materials to flask *A* and reshake to ensure complete reaction.)[8] The equilibrium constants did not vary in any regular way with the size of the loss, and it was therefore presumed that they did not introduce any major error.

All spectral measurements were made after the samples had equilibrated at the appropriate temperature in a constant temperature cabinet mounted on top of the spectrometer cell compartment. Little, if any, loss of radical occurred, during a run; it was found that the equilibrium mixtures averaged a loss of 2.4% per day in radical concentration on standing at room temperature.

Numerous runs were made at 25° to ensure that the technique worked and then four runs were carried over the full temperature range. These four runs are numbered on Fig. 1. Had the plot been confined to these runs, the van't Hoff isotherm would have been a bit lower but would have given essentially the same value for the enthalpy change.

Although solutions of 2,6-di-*t*-butyl-4-methoxyphenoxy gave good results on extinction coefficient measurements, attempts to measure its equilibrium constant on reaction with tri-*t*-butylphenol were generally frustrated due to the instability of the solutions. This was apparently due to some extraneous factor since two runs gave satisfactorily stable solutions. From them we estimate the equilibrium constant (written in the same direction as Eq. 1) to be on the order of 210 at 25° and ΔH to about −5 kcal/mole.

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(7) C. D. Cook and B. E. Norcross, *J. Am. Chem. Soc.*, 81, 1176 (1959).

(8) The bromocyclohexadienone has been shown to rapidly interact with the alkoxy phenols; probably a bromine atom is transferred followed by decomposition of the alkoxybromocyclohexadienone to yield 2,6-di-*t*-butylquinone. Direct attempts to brominate either of these two alkoxy phenols lead to such a result.

Identification of Neophytadiene in Burley Tobacco and in Cigarette Smoke[1]

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During the investigation of the hydrocarbons in aged burley tobacco, a pentane extract was

chromatographed on a column of silicic acid. Elution with hexane yielded a mixture containing principally saturated aliphatic hydrocarbons. After precipitation of the paraffins from chilled acetone, there remained an oily liquid whose ultraviolet spectrum[2]—maximum 225 mμ, log ε 4.23 (in isooctane)—indicated the presence of a monosubstituted acyclic conjugated diene. Evidence for a conjugated diene was substantiated by a 6.24μ band in the infrared spectrum.[3] The infrared spectrum also indicated the nature of the alkene groups constituting the conjugated system; the 10.07μ and 11.08μ bands [R—CH=CH_2] and the 11.22μ band [R—C(R)=CH_2]. The spectroscopic evidence pointed to the presence of a *beta* substituted butadiene, CH_2=C(R)—CH=CH_2. The infrared bands at 7.24μ and 7.30μ [CH_3—], 8.54μ [CH_3—$C(CH_3)H$—], 13.56μ [$(CH_2)_n$ with n = 2 or 3], and the shoulder at 8.65μ [R—C—C(C)—C—R], offered evidence of multiple methyl branching in the *beta* substituent of the butadiene system. The mass spectrum showed a parent peak at 278. The catalytic hydrogenation product, phytane (3,7,11,15-tetramethylhexadecane), gave a parent peak at 282, indicating the presence of only two double bonds in the original molecule. From the foregoing data and the elemental analysis, it was concluded that the unsaturated hydrocarbon isolated from aged burley tobacco was a phytadiene, 7,11,15-trimethyl-3-methylene -1-hexadecene.

As this work was being completed, Rowland[4] described the isolation and identification of the same phytadiene from flue-cured tobacco and assigned the name neophytadiene. Within a very short time, Onishi, *et al.*,[5] reported the identification of the same diene in aged Japanese burley and flue-cured tobaccos, naming the compound γ-phytadiene. Thus the phytadiene was isolated from cured tobaccos, and its structure determined, as a result of three, almost simultaneous, independent investigations. The name neophytadiene is used in this paper because of its prior assignment by Rowland.[4]

In addition to its identification in aged burley tobacco, neophytadiene was identified in the mainstream smoke of domestic cigarettes.

EXPERIMENTAL

Neophytadiene from aged burley tobacco. A 150-g. sample of aged burley tobacco, U. S. type 31, 1953 crop, 7% moisture, was extracted with pentane in a Soxhlet apparatus for 8 hours. During the course of the extraction the solvent was changed every hour. The final accumulated volume of pentane was 6 l. The extract was concentrated to a small volume by evaporation at reduced pressure. The concentrated extract was chromatographed on 200 g. of silicic acid in a 600 × 40 mm. column. The silicic acid (Mallinckrodt AR, 100 mesh) had been previously dried at 100° for 16 hours. The hydrocarbons were eluted from the column with 1250 ml. of hexane (b.p. 65–67°) at a flow rate of 5 ml. per minute. The eluate was concentrated to a volume of 5 ml. Warm acetone (20 ml.) was added, the solution was cooled in an acetone–Dry Ice bath, and the precipitated paraffins were removed by cold filtration. The paraffins were dissolved again in warm acetone and precipitated as above. The filtrates were combined and dried over anhydrous sodium sulfate. Vacuum distillation of the solvent mixture left a residue of neophytadiene. A small quantity of residual paraffins was removed by precipitation from cold acetone. A yield of 139 mg. of neophytadiene was obtained in this manner corresponding to 0.10% of the dry weight of the tobacco. Neophytadiene is a colorless oil, soluble in organic solvents and devoid of a characteristic odor. The product is levorotatory $[\alpha]_D^{25}$ −1.66° b.p. 171° at 7 mm., n_D^{25} 1.4632.

Anal. Calcd. for $C_{20}H_{38}$: C, 86.25; H, 13.75. Found: C, 86.33; H, 13.59.

Neophytadiene in cigarette smoke. The smoking technique was that described by Bradford, Harlan and Hanmer,[6] collecting the smoke of domestic cigarettes in 0.1 *N* sulfuric acid. The hexane-soluble portion of the chloroform-soluble resins from the smoke collection media was chromatographed on a column of silicic acid. Elution of the column with hexane, followed by concentration of the eluate and removal of the paraffins by precipitation with acetone, yielded an oil whose ultraviolet and infrared spectra showed the presence of neophytadiene. Based on the absorbance at 225 mμ, the amount of neophytadiene corresponded to *ca.* 0.2 mg. per cigarette.

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(1) From paper presented at the 11th Tobacco Chemists' Research Conference, Conn. Agr. Exp. Sta., New Haven, Conn., Oct. 10–11, 1957.

(2) Recorded on a Cary model 14M recording spectrophotometer.

(3) Recorded on a Perkin-Elmer model 21A infrared spectrophotometer, equipped with a sodium chloride prism.

(4) R. L. Rowland, *J. Am. Chem. Soc.*, **79**, 5007 (1957).

(5) I. Onishi, M. Nagasawa, H. Tomita, and T. Fukuzumi, *Bull. Agr. Chem. Soc. Japan*, **22**, 57 (1958).

(6) J. A. Bradford, W. R. Harlan, and H. R. Hanmer, *Ind. Eng. Chem.*, **28**, 836 (1936).

Occurrence of Formaldehyde in Glacial Acetic Acid

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During recent investigations on the chemistry of the formation of a water-soluble, nitrogenous red pigment in Southport White Onion Tissue,[2] we discovered that pigment formation was accentuated in rate and extent by an impurity in the glacial acetic acid initially added to the onion puree. Addition of acetic acid prepared from sodium acetate and sulfuric acid did not produce pigmentation as rapidly or in concentrations approaching those produced by addition of an equivalent amount of reagent grade acetic acid. The presence of the impurity was followed by the chromotropic acid

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(2) M. A. Joslyn and R. G. Peterson, *J. Agr. Food Chem.*, 6, 754 (1958).