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.

DEPARTMENT OF RESEARCH AND DEVELOPMENT
THE AMERICAN TOBACCO CO.
RICHMOND, VA.

(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

RICHARD G. PETERSON[1] AND MAYNARD A. JOSLYN

Received March 12, 1959

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

(1) Present address, E. & J. Gallo Winery, Modesto, Calif.

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

color test.[3] That the impurity is formaldehyde was shown by the procedure below.

Although it is not surprising that formaldehyde can occur in reagent glacial acetic acid, apparently its occurrence is not generally known.[4] However, this small amount is extremely important in causing white onion juice to redden, and it is felt that it might be responsible for other chemical changes observed by other investigators where an odd specificity for acetic acid is noted.

EXPERIMENTAL

Five l. of glacial acetic acid (Baker and Adamson reagent grade, code 1019) were steam distilled in 750-ml. lots. The first 150 ml. of each distillate were collected, combined, and again steam distilled giving 500 ml. of strongly chromotropic acid–positive distillate containing 43% acetic acid. This was redistilled directly in an all-glass still using a 20-in. air condenser filled with Raschig rings as a fractionating column. The first 100 ml. of distillate, containing most of the chromotropic acid–positive substance, were mixed with an excess of dry calcium carbonate and allowed to stand overnight at room temperature. The mixture was then distilled, giving about 50 ml. of distillate containing the impurity in water solution free from acetic acid. The following tests were made:

A modified Schiff's reagent[5] was prepared containing 0.2 g. of rosaniline hydrochloride, 2 g. of anhydrous sodium bisulfite, and 2 ml. of concentrated HCl in 200 ml. of solution. Spot tested at room temperature, the color formed was stable for more than 6 hr.—a positive indication of formaldehyde in the presence of other aliphatic aldehydes.[6]

Alkaline resorcinol[7] reacted with the unknown yielding a yellow color which changed to red, giving an indication of formaldehyde.

The albumin-nitrite reagent[8] caused a color change from red to violet.

The unknown solution was compared polarographically with a standard formaldehyde solution. As in the procedure of Boyd and Bambach,[9] the reduction was carried out in 0.05*N* LiOH and both solutions exhibited similar waves at about −1.6 volts.

No other waves appeared in either sample. This, in conjunction with the chemical evidence above, demonstrated that the impurity in glacial acetic acid was formaldehyde.

A standard curve for quantitative formaldehyde determination by the chromotropic acid procedure was prepared. Using this method, one can easily, quickly, and accurately determine the formaldehyde content of acetic acid. Analysis of about 15 different lots of Baker and Adamson reagent glacial acetic acid showed a concentration range of 1 to 3 p.p.m. formaldehyde present.

DEPARTMENT OF FOOD TECHNOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIF.

(3) C. E. Bricker and W. A. Vail, *Anal. Chem.*, **22**, 720 (1950).

(4) *Reagent Chemicals, ACS Specifications*, W. J. Murphy, ed., ACS, Washington, D. C., 1955, pp. 24–26; also Baker and Adamson Regional Office, Personal Communication (1958).

(5) J. G. Reynolds and M. Irwin, *Chem. & Ind. (London)*, 419 (1948).

(6) F. R. Georgia and R. Morales, *Ind. Eng. Chem.*, **18**, 305 (1926).

(7) Lebbin, *Chem. Zentr.*, **I**, 90 (1906).

(8) E. Voisenet, *Bull. soc. chim. France*, **33**, 1198 (1905).

(9) M. J. Boyd and K. Bambach, *Ind. Eng. Chem., Anal. Ed.*, **15**, 314 (1943).

Ninhydrin Degradation of Hexosamines and Periodic Acid Oxidation of Hexoses[1]

P. J. STOFFYN[2]

Received March 13, 1959

Oxidative degradation with ninhydrin of hexosamines into the corresponding pentoses seems to proceed, for each pair of C_2 epimers, through an intermediate compound.[3] These rather unstable substances are detectable with ammoniacal silver reagent or aniline hydrogen phthalate on paper chromatograms of the reaction mixtures as long as the presence of unchanged hexosamine indicates that the degradation is not complete. Their R_f values are higher than those of the corresponding pentoses and depend on the nature of the original hexosamine. Consequently, it was suggested that these "faster moving compounds" are carbohydrate derivatives, intermediates in the degradation process, which are ultimately transformed into the pentoses. The final formation of pentoses shows clearly that deamination and oxidative cleavage of the bond between C_1 and C_2 are essential steps of the degradation of hexosamines with ninhydrin. On the assumption that hexosamines react in their more stable pyranose form, this degradation process could result in the formation of 4-*O*-formyl esters of the corresponding pentoses which are further hydrolyzed into formic acid and the free pentoses. The observed properties of the intermediates are in agreement with such a structure. Further evidence supporting this proposition is presented here.

Formyl esters of pentoses having been postulated, and in some cases characterized, as alkali-sensitive intermediates in the oxidation of hexoses with glycol splitting reagents,[4] we have compared the products of periodic acid oxidation of hexoses with the products of ninhydrin degradation of the corresponding hexosamines. Thus, glucose, mannose, and galactose were oxidized with periodic acid, 0.5 mole of oxidant being used per mole of sugar in order to stop the reaction in its early stages. On the other hand, glucosamine and galactosamine were reacted for a short time with ninhydrin in neutral aqueous alcoholic solution in order to retain the unstable intermediate compounds. The different reaction mixtures were then analyzed in

(1) This is publication No. 255 of the Robert W. Lovett Memorial Unit for the Study of Crippling Disease, Department of Medicine, Harvard Medical School. Address: Massachusetts General Hospital, Fruit Street, Boston 14, Mass. This work has been done partly during the tenure of a Research Fellowship of the American Heart Association.

(2) Research Fellow of the American Heart Association. Present address: Massachusetts General Hospital, Boston 14, Mass.

(3) P. J. Stoffyn and R. W. Jeanloz, *Arch. Biochem. Biophys.*, **52**, 373 (1954).