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.

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

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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

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parallel runs by paper chromatography. Under these conditions glucose or mannose were partially transformed into two compounds migrating respectively at the same rates as the two compounds derived from glucosamine, namely arabinose (R_f 0.19) and the corresponding faster moving intermediate (R_f 0.32). Similarly, galactose gave 2 compounds having R_f values identical to those of lyxose (R_f 0.25) and of the corresponding faster moving intermediate derived from galactosamine (R_f 0.42). When the chromatograms were sprayed with hydroxylamine–ferric chloride reagent,[5] all the faster moving substances gave identical purple spots. When sodium metaperiodate was used instead of periodic acid, only the intermediate compounds could be detected after 3 hours, the pentoses appearing much later in appreciable quantity. On the other hand, after a long time of reaction with periodic acid, the intermediates had completely vanished and the pentoses were the sole detectable reaction products. This is obviously attributable to a faster rate of hydrolysis of the intermediates at a lower *p*H.

Further comparison was made by submitting each of the above described reaction mixtures to a two-dimensional chromatographic procedure. The intermediate faster compounds were separated during the first run from unchanged hexosamine or hexose, and from the pentose already formed. Then, they were reacted with ammonia, either by allowing the dried paper to hang for a few hours in a closed vessel containing concentrated ammonia solution at the bottom, or by spraying with a small amount of a methanolic ammonia solution and drying at room temperature. After the second run, ammoniacal silver reagent revealed that the intermediates had been transformed in all four cases into the corresponding pentoses.

The similarity of chromatographic behavior, and the conversion into pentoses of the faster moving compounds observed in the course of ninhydrin degradation of hexosamines as well as of periodic acid oxidation of corresponding hexoses, have shown that these substances are identical intermediates in both degradation processes. The formation of pentoses by oxidation of hexoses with limited amounts of periodic acid proves that the splitting of the carbon chain occurs preferentially between C_1 and C_2.[6] By analogy with known cases of oxidation of hexoses and their derivatives with glycol splitting reagents, the alkali labile intermediates detected here are formyl esters of pentoses. This view was further supported by the identification of formic acid as the one carbon fragment appearing with the pentoses in the degradation of hexosamines with ninhydrin, and by the purple color developed when the faster moving compounds were treated with hydroxylamine–ferric chloride reagent. The location of the formyl group in position 4 on the pentose is assumed on the basis of the pyranose form of the starting material.

EXPERIMENTAL

Paper chromatographic evidence. Four % solutions of glucose, mannose, or galactose were mixed with equal volumes of a 2% solution of periodic acid—about 0.5 mole of oxidizing agent per mole of sugar—and the mixtures were left at room temperature for 3 hr. Glucosamine or galactosamine, also in 4% solutions, were mixed with equal volumes of an 8% solution of ninhydrin in a 50% mixture of ethanol and water, and the mixtures were heated at 100° for 20 min.

The different reaction mixtures were then analyzed in parallel runs by chromatography on Whatman No. 1 paper. The chromatograms, developed with a 4:1:1, 1-butyl alcohol, ethanol, water mixture containing 0.5% of acetic acid, were revealed after drying, with ammoniacal silver reagent.[3]

Identification of formic acid after ninhydrin degradation of hexosamines. A solution containing 107.5 mg. of glucosamine hydrochloride, or of galactosamine hydrochloride, in 20 ml. of a 2% aqueous solution of ninhydrin containing 4% of pyridine, was heated for 2 hr. at 100° in a sealed tube. After cooling, 6 ml. of a saturated solution of potassium hydrogen sulfate was added to the purple-black mixture, which was then frozen and lyophilized. The presence of formic acid in the lyophilizate was first shown by the intense purple color obtained with chromotropic acid after reduction of an aliquot to formaldehyde with magnesium and hydrochloric acid.[7] The remainder of the lyophilizate was neutralized with dilute sodium hydroxide, concentrated to a volume of 2 ml. and heated in a sealed tube at 100° for 1 hr. with 70 mg. of *p*-bromophenacyl bromide in 2 ml. of ethanol. After cooling, part of the alcohol was evaporated under a stream of nitrogen, and the crystalline precipitate was collected and recrystallized from dilute ethanol. The melting point, 137–140°, was unchanged in admixture with an authentic sample of the *p*-bromophenacyl formate.

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Anal.: Calcd. for $C_9H_7O_3Br$: C 44.47; H 2.90. Found: C 44.42; H 3.07%.

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The C—H Stretching Bands of Aliphatic Amines

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The C—H stretching bands of saturated aliphatic hydrocarbons generally observed between 3.38 μ and 3.51 μ.[1] When the alkyl group is attached, to oxygen, bands are also found at 3.54–3.55 μ for compounds containing the methoxyl group[2] and at 3.55 μ and 3.68 μ for aldehydes.[3]

Recently, Hill and Meakins[4] and Braunholtz *et al.*[5] have shown that the infrared spectra of compounds containing the NMe or NMe_2 group includes absorption bands of medium to strong intensity between 3.55 μ and 3.62 μ. Absorption is near 3.55–3.57 μ when the group is attached directly to an aromatic system. When the NMe group in in an aliphatic or nonaromatic heterocyclic system the band occurs in the 3.56–3.60 μ range, while the NMe_2 group so attached has two specific bands, one at 3.54–3.56 μ and the other at 3.60–3.62 μ.

We would like to point out that not only methyl and dimethylamines but also a high percentage of other tertiary aliphatic amines and many secondary aliphatic amines have absorption bands in this range when examined as the free bases. For example, an intense band centered around 3.6 μ was observed in the spectra of a series of 2-alkyl derivatives of merimine,[6] 2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine (Fig. 1). This band was absent in merimine derivatives substituted in the 2-position by hydrogen, acetyl, benzoyl, carbethoxy, carbamoyl, or dimethylsulfamoyl.

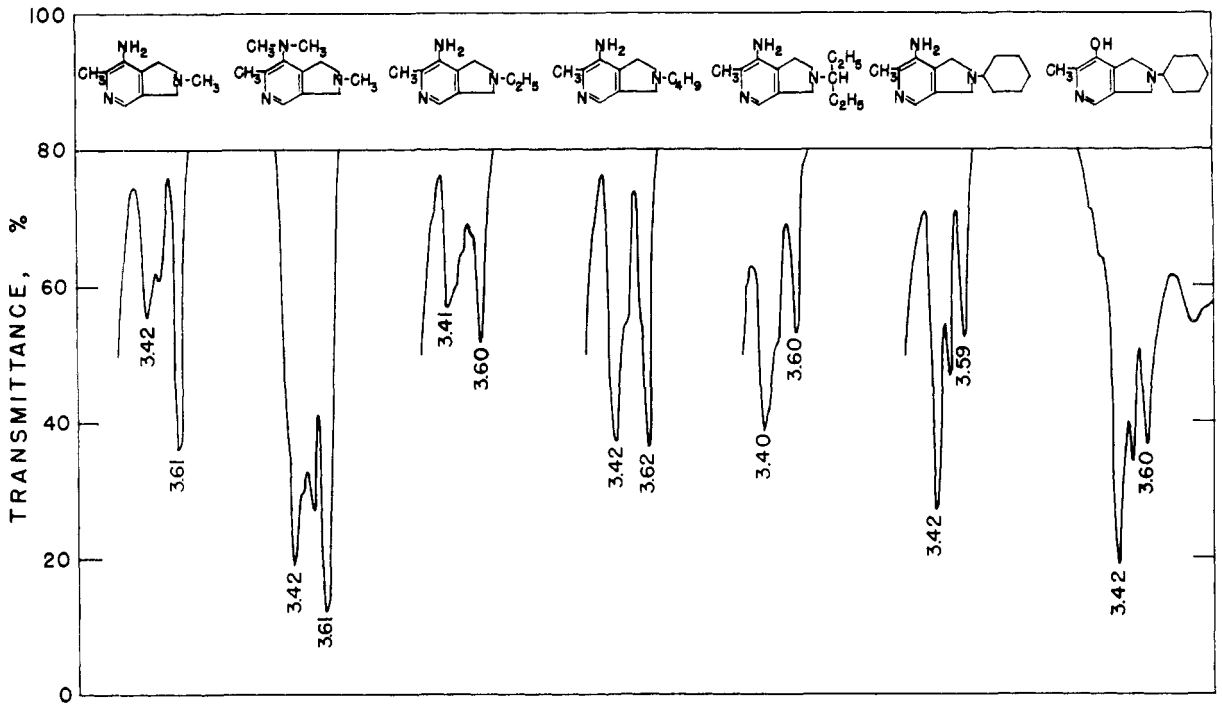

Fig. 1. The C—H stretching bands of 7-substituted-2-alkyl-6-methylmerimines. Spectra were recorded on the Perkin-Elmer Model 21 spectrophotometer. The second sample was a smear. All others were potassium bromide pellets

These observations prompted us to examine the infrared spectra of over one hundred other amines.

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