of the tube corresponded to complete addition of the chlorine. The reaction product was a nearly colorless, dense, highly viscous liquid. Distillation gave about 90% of the theoretical quantity of product, b.p. 160–164°/0.4 mm., d_{20}^{20} 1.745. Calcd.: MrD^{20} 82.40. Found: 82.98.

Anal. Calcd. for $C_8H_5O_2Cl_9$: C, 21.25; H, 1.11; Cl, 70.5. Found: C, 21.15; H, 1.16; Cl, 70.6.

Because the reaction product was very viscous, some product was held up in parts of the distillation apparatus, lowering the yield. The crude undistilled reaction mixture was analyzed.

Anal. Found: C, 21.03; H, 1.02; Cl, 71.0.

Preparation of bis(1,2,3,4,5,6-hexachlorocyclohexyl) carbonate. In a thick-walled borosilicate glass tube were placed 10.7 g. (0.050*M*) of diphenyl carbonate, 31.6 g. (0.44*M*) of chlorine, and 18.0 ml. of carbon tetrachloride. The tube was sealed and irradiated by a black light fluorescent lamp for 17 hr. After the tube was vented, there was a 29.6 g. weight gain in the nonvolatile materials. The product was dissolved in methanol, precipitated by the addition of water, and rapidly filtered and dried. In the first crop, 15.0 g. of product was obtained, m.p. 84–90°.

Anal. Calcd. for $C_{13}H_{10}Cl_{12}O_3$: C, 24.4; H, 1.58; Cl, 66.5. Found: C, 25.3; H, 1.7; Cl, 63.4.

Subsequent materials which were precipitated were lower melting and gave greater deviations from the theoretical values in the elemental anaylsis.

Hydrolysis Experiments. The acid hydrolysis of I was effected by stirring a mixture of 15.0 g. (0.0332*M*) of I, 60 ml. of dioxane, 30 ml. of conc. HCl, and 60 ml. of water at reflux for one hour. The hydrolysis mixture was saturated with salt and the organic material separated by extraction with chloroform. After evaporation of the chloroform, distillation of the residue gave 8.3 g. of one fraction, b.p. 120–124°/39 mm. and 1.4 g. of 2,4,6-trichlorophenol, identified by elemental analysis and a mixed m.p. with an authentic sample. The first fraction was a binary mixture (apparently azeotropic) containing about an equimolar ratio of 2,4,6-trichlorophenol to trichloroacetic acid. The total amount of 2,4,6-trichlorophenol obtained was 5.9 g., or a 90% yield.

The hydrolysis of 0.08*M* of the addition-chlorinated phenyl trifluoroacetate in dioxane-water at 10–25° followed by chloroform extraction and distillation of the extract gave a 70% yield of the 2,4,6-trichlorophenol. The phenol was identified by a mixed melting point; benzoate, m.p. 74–75°.

PAINESVILLE, OHIO

[FROM THE DEPARTMENT OF BACTERIOLOGY, THE UNIVERSITY OF KANSAS]

Adduct Formation between Chloroacetone and *N'*-Alkyl Substituted Pyridine Bases and Its Biological Significance[1-3]

J. M. AKAGI* AND D. PARETSKY

Received April 29, 1959

The reaction involving adduct formation between DPN[4] and various carbonyl compounds have been extensively studied by Burton and Kaplan.[5,6] The mechanism for this reaction, proposed by these workers, involves a prior ionization of a proton from the carbon alpha to the carbonyl carbon, resulting in the formation of a negatively charged molecule. This is followed by an addition reaction with the positively-charged 4-carbon of the pyridinium moiety of DPN. The reaction can be followed by the appearance of a characteristic maximum absorption for that particular adduct. In this report will be presented results of adduct formation obtained between chloroacetone and *N'*-alkyl substituted pyridinium bases. When *N'*MeN is caused to react with chloroacetone in basic solutions, the formation of an adduct is apparent by the formation of a new maximum in the 360 mμ region of the absorption spectrum. Substituting the carbamoyl for a carboxaldehyde group resulted in a pyridinium compound (*N'*MePyAl), which in smaller quantities was capable of reacting with chloroacetone at a rate faster than *N'*MeN. When, instead of aldehyde, an acetyl group was attached to the pyridine ring, a compound was obtained which was intermediate between *N'*MeN and *N'*MePyAl in adduct-forming abilities. Table 1 summarizes these findings.

In order to determine whether or not the group attached to the positive nitrogen atom influenced adduct formation, the carbon density around the ring nitrogen was increased by preparing the ethyl, isopropyl, and tertiary butyl derivatives of the pyridine bases. Rate studies comparing adduct formation between *N'*-methyl and the larger alkyl derivatives showed that with increasing carbon density around the ring nitrogen decreasing rates of addition reactions were obtained. This can be seen in Fig. 1 where the reactivity of *N'*MePyAl

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(1) Taken from a thesis submitted by J. M. Akagi in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

(2) This investigation was supported in part by the American Cancer Society, Institutional Grant 57 M 578-G, and by the University of Kansas General Research Fund.

(3) This report was presented at the Missouri Branch, Society of American Bacteriologists' Meeting in Manhattan, Kansas, in April 1958, and at the Midwest Regional Biochemistry Meeting in Lawrence, Kansas, October 1958.

(4) The following abbreviations will be employed in this paper: diphosphopyridine nucleotide (oxidized), DPN; *N'*-methylnicotinamide, *N'*-MeN; *N'*-ethylnicotinamide, *N'*-EtN; *N'*-isopropylnicotinamide, *N'*iPrN; *N'*-methyl-3-acetylpyridine, *N'*MeAP; *N'*-methyl-pyridine-3-carboxaldehyde, *N'*MePyAl; *N'*-tertiary butylpyridine-3-carboxaldehyde, *N'*-*tert*-BuPyAl.

(5) R. M. Burton and N. O. Kaplan, *J. Biol. Chem.*, **206**, 283 (1954).

(6) N. O. Kaplan, *Record of Chem. Prog.*, **16**, 177 (1955).

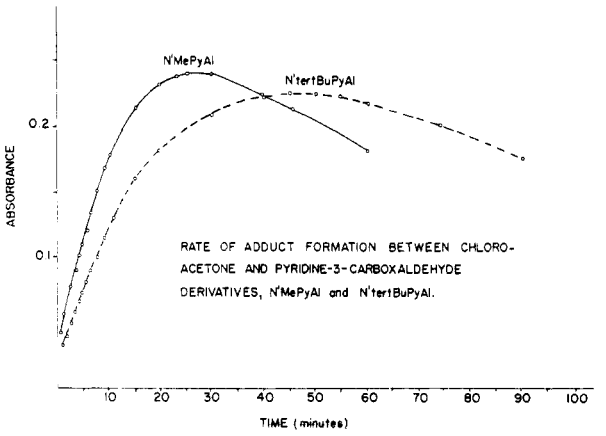


Fig. 1. Reaction rates were measured at the maximum absorption peaks of the respective chloroacetone adducts, *i.e.*, N¹MePyAl-chloroacetone = 340 mμ; N¹tertBuPyAl-chloroacetone = 390 mμ. Each reaction mixture consisted of 1.0 μmole pyridinium base; Tris buffer, 0.1 M, pH 10.2; reaction initiated by addition of 75 μmoles chloroacetone; the entire system made to a volume of 4.0 ml. with distilled water; temperature, 25°.

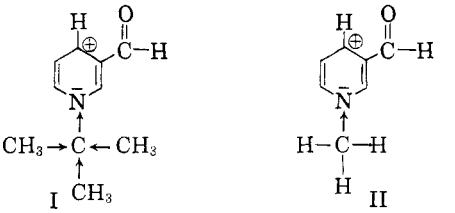
TABLE I

ADDUCT FORMATION BETWEEN CHLOROACETONE AND PYRIDINIUM BASES

Compound	Conc. μmoles per 4.0 ml.	Adduct observed formed with chloroacetone	Relative rate of reaction[a]
*N'*MeN	1.0	−	—
*N'*MeN	10.0	+	100
*N'*MePyAl	1.0	+	203
*N'*MePyAl	10.0	+	Too rapid to be measured
*N'*MeAP	1.0	−	—[b]
*N'*MeAP	10.0	+	—[b]

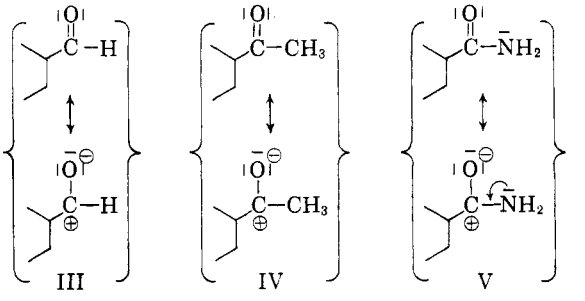
[a] Each system consisted of the pyridinium compounds which were caused to react with 75 μmoles of chloroacetone (with *N'*MeAP, 5 μmoles of chloroacetone were employed); Tris buffer, 0.1*M*, *p*H 10.2; made to a final volume of 4.0 ml. with distilled water. An arbitrary value of 100 was given to *N'*MeN expressed as the change in absorbancy per unit time (5 min.). The wave lengths at which the reactions were measured were: *N'*MeN = 365 mμ; *N'*MePyAl = 340 mμ; for *N'*MeAP there was an initial peak at 310 mμ followed by the slow formation of a new peak at 390 mμ after 24 hr. [b] The rate studies could not be made, since there was apparently rearrangement of the adduct to another product indicated by a shift in the absorption peak, as indicated above.

is compared with that of *N'-tert*-BuPyAl. The latter compound forms an adduct at a slower rate than the corresponding *N'*-methyl compound. This is in accordance with the fact that alkyl groups repel electrons through an inductive effect; a higher carbon density around the nitrogen would be expected to alleviate the positivity of this atom. Thus, the resonance structure in which the formal positive charge appears on the 4-carbon makes less of a contribution to the state of the pyridinium ion when the *N'*-substituent is *tert*-butyl (I) rather than methyl (II). Similar results were obtained



I II

with the nicotinamide series when *N'*-MeN was compared with *N'*EtN and *N'*iPrN. The *N'*EtN reactivity was very close to that of *N'*MeN, but there was a noticeable difference in rate between *N'*MeN and *N'*iPrN. Two conclusions may be drawn from the results obtained in these studies: 1. The side chain moiety of the pyridinium compound controls to a large extent the reactivity of the molecule. It appears that the more positive the carbonyl carbon of this side chain, the more reactive the compound as illustrated below:



III IV V

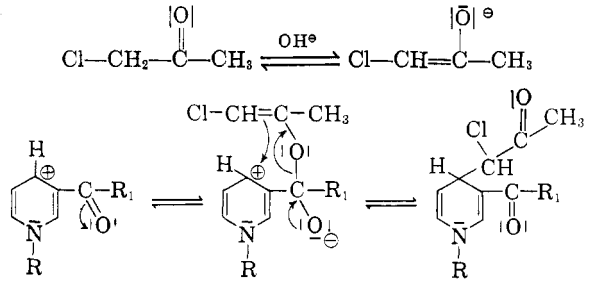
The carbonyl carbon atom of the carboxaldehyde, III, exhibits a greater electrophilic character than the carbonyl carbon atom of IV or V. Structure IV has a carbonyl carbon atom with a greater electrophilic character than V, since the amide group in V can help share the positive charge on the carbonyl carbon by donating its free pair of electrons of the nitrogen atom. The order of reactivity of the pyridinium compounds was found to be *N'*RPyAl>*N'*RAP>*N'*RN; *i.e.*, in the contribution of the side chain (—C(=O)—R¹) to pyridinium reactivity, R¹ = H>CH_3NH_2.

2. The group linked to the positive nitrogen atom is partly responsible for the shift of electrons from the ring to the nitrogen. This results in the formation of the positive center at the 4-carbon. It has been suggested that the side chain moiety of the pyridinium compound has an influence on the electrophilic nature of the 4-carbon.[7]

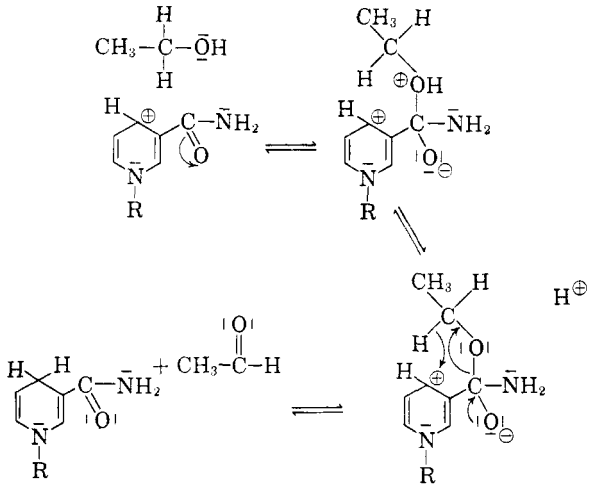
Although this effect is undoubtedly partially operative, it is the opinion of the authors that the main function of the side chain is to participate in addition reactions by coordinating with the nucleophilic carbonyl compound. The interaction of

(7) N. O. Kaplan and M. Ciotti, *J. Biol. Chem.*, **221**, 823 (1956).

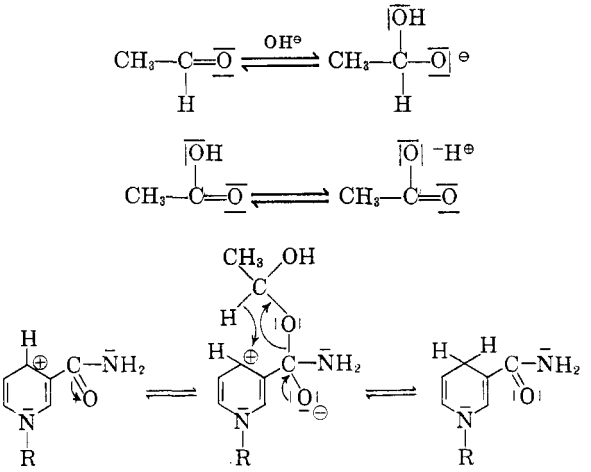
chloroacetone and the pyridinium bases employed in these studies may be explained as follows:



Extending this hypothesis to biological systems such as ethanol oxidation, a possible mechanism for the reduction of DPN would be as follows:



This is related to the Meerwein-Ponndorf-Verley reduction mechanism involving a hydride ion transfer as proposed by Woodward *et al.*[8] Mahler and Douglas[9] have considered a similar mechanism for DPN reduction involving zinc as a participant in the reaction. Wallenfels and Sund[10] also presented a mechanism for DPN reduction involving zinc; the metal is proposed to coordinate with enzyme, substrate, and the adenine moiety of DPN. In contrast are the views of van Eys and Kaplan[11] who suggested that zinc is linked to DPN through a pyrophosphate bond. In a subsequent report, van Eys *et al.*[12] stated that zinc is not an integral part in the actual catalysis of substrate oxidation. The action of aldehyde dehydrogenases may be explained by the mechanism which we propose in the present communication. Aldehyde dehydrogenases are pyridine nucleotide-linked enzymes which catalyze the oxidation of aldehydes to the respective acids. This can be visualized by an initial attack on the carbonyl group of the aldehyde by a hydroxyl ion in a manner similar to that found in the Cannizzaro reaction.[13] The following scheme demonstrates this reaction:



Aldehyde dehydrogenases and alcohol dehydrogenases may operate under the same mechanism as proposed in this communication.

EXPERIMENTAL

Melting points are uncorrected. Microanalyses were prepared by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

Rate studies were measured in the Beckman Model B spectrophotometer while the maximum absorption curves were determined in the Beckman DU spectrophotometer, employing cuvettes having a 1.0 cm. light path.

Pyridinium compounds. The compounds employed during this investigation, *N'*MeN,[14] *N'*EtN,[15] *N'*PrN, *N'*MePyAl[16] and *N'*tertBuPyAl iodides were prepared by the methods of Karrer *et al.*[14]

N'-Isopropylnicotinamide iodide. Yellow crystalline product, m.p. 185–186°. *Anal.* Calcd. for $C_9H_{13}N_2IO$: C, 36.96; H, 4.45; N, 9.58; I, 43.49; O, 5.48. Found: C, 37.45; H, 4.54; N, 9.69; I, 43.7; O, 4.62.

N'-Methyl-3-acetylpyridine iodide. Yellow shiny powder, m.p. 160–163°. *Anal.* Calcd. for $C_8H_{10}NIO$: C, 36.5; H, 3.8; N, 5.33; I, 48.3; O, 6.08. Found: C, 36.94; H, 3.9; N, 5.43; I, 48.22; O, 5.51.

N'-tert-Butylpyridine-3-carboxaldehyde iodide. Yellow flaky powder, m.p. 192°. *Anal.* Calcd. for $C_{10}H_{14}NIO$: C, 41.2; H, 4.81; N, 4.81; I, 43.7; O, 5.5. Found: C, 41.0; H, 4.92; N, 4.81; I, 43.5; O, 5.77.

Adduct formation. The procedures employed during this investigation consisted of reacting the cited concentrations of pyridinium salts with chloroacetone in the presence of Tris buffer, 0.1*M*, *p*H 10.2, made to a final volume of 4.0

(8) R. B. Woodward, N. L. Wendler, and F. J. Brutschy, *J. Amer. Chem. Soc.*, 67, 1425 (1945).

(9) H. R. Mahler and J. J. Douglas, *J. Amer. Chem. Soc.*, 79, 1159 (1957).

(10) K. Wallenfels and H. Sund, *Biochem. Z.*, 329, 59 (1957).

(11) J. van Eys and N. O. Kaplan, *Biochim. Biophys. Acta*, 23, 574 (1957).

(12) J. van Eys, A. San Pietro, and N. O. Kaplan, *Science*, 127, 1443 (1958).

(13) E. R. Alexander, *J. Amer. Chem. Soc.*, 69, 289 (1947).

(14) P. Karrer, G. Schwarzenbach, F. Benz, and U. Solmssen, *Helv. Chim. Acta* 19, 811 (1936).

(15) P. Karrer and F. J. Stare, *Helv. Chim. Acta*, 20, 418 (1937).

(16) L. Pannizzon, *Helv. Chim. Acta*, 24, 24E (1941).

ml. with distilled water. All reactions were conducted at room temperature (23–25°).

Acknowledgment. The authors wish to acknowledge their indebtedness to Dr. W. E. McEwen, Department of Chemistry, for his helpful suggestions and constructive criticisms during the course of this work.

Addendum. During the preparation of this report an abstract by B. Kadis[17] appeared which proposed a similar Meerwein-Ponndorf-Verley reduction mechanism for DPN reduction.

LAWRENCE, KAN.

(17) B. Kadis, Abstracts, 135th Meeting of the American Chemical Society, Boston, Mass., April 1959, page 24–0.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Ethyl 1-Thio-α-D-galactofuranoside

M. L. WOLFROM, Z. YOSIZAWA,[1] AND B. O. JULIANO[1]

Received April 30, 1959

Partial demercaptalation of D-galactose diethyl dithioacetal (mercaptal) (I) leads to the synthesis of ethyl 1-thio-α-D-galactofuranoside (II) characterized by periodate oxidation and by its crystalline tetraacetate IV.

Schneider and co-workers[2,3] synthesized alkyl 1-thio-α-D-glucosides by treating an aqueous solution of D-glucose dialkyl dithioacetal (mercaptal) at room temperature with one mole of mercuric chloride and neutralizing the acid formed with alkali. With more mercuric chloride, complete demercaptalation occurred to produce the free sugar in aqueous solution or the alkyl glycoside in alcohol solution. However, attempts to prepare an ethyl 1-thio-D-galactoside (II) by treatment of D-galactose diethyl dithioacetal (I) with one mole of mercuric chloride, under neutral conditions, failed.[3,4] There was obtained instead, in ethanolic solution, equimolar amounts of ethyl β-D-galactofuranoside and starting material (I), which Green and Pacsu[4] ascribed to the reactivity of the thioglycoside (II) to solvolysis promoted by mercuric chloride. Green and Pacsu[4] concluded, on the basis of rotation values and ease of acid hydrolysis, that the glycosides formed from the dithioacetals were furanosides. Utilizing periodate oxidation data, Wolfrom and co-workers[5] verified this ring assignment for ethyl 1-thio-α-D-glucofuranoside derived from the dithioacetal.

We report herein the synthesis of sirupy ethyl 1-thio-α-D-galactofuranoside (II) and its crystalline tetraacetate (IV), using essentially the method of Green and Pacsu[4] but supplemented with chromatographic techniques not at the time available to these workers. A reappraisal of the feasibility of partial demercaptalation of I to II stemmed from the need of the analogous 2-acetamido-2-deoxy-1-thio-α-D-galactofuranoside as an intermediate in the synthesis of 2-amino-2-deoxy-L-arabinose from 2-amino-2-deoxy-D-galactose.[6] D-Galactose diethyl dithioacetal (I) was treated with an aqueous solution of mercuric chloride in the presence of mercuric oxide, to produce nearly equimolar amounts of ethyl 1-thio-α-D-galactofuranoside (II) and D-galactose (III). The latter substance (III) was removed by its exhaustive fractional precipitation from alcoholic solution. The mother liquor was acetylated and further purified by silicate column elution chromatography to give crystalline IV, recrystallized from diethyl ether–petroleum ether, m.p. 50.5–51.5°, $[\alpha]_D$ +118° (chloroform) and +127° (ethanol). This substance showed weak infrared absorption at 648 and 682 cm.$^{-1}$ Sheppard[7] cites 600–700 cm.$^{-1}$ as the region for C—S bond absorption.

The ring structures of II and IV were assigned on the basis of sodium metaperiodate oxidation (Table I) of sirupy II, $[\alpha]_D$ +124° (water), obtained from pure IV by deacetylation. The oxidation conditions employed were essentially those of Wolfrom and Yosizawa.[6] It has been shown[5,6] that the rapid liberation of one mole of formaldehyde by periodate ion is characteristic of 1-thiohexofuranosides and it is further known that the presence of the thioethoxyl group results in some overoxidation of an obscure nature.[8] Although a number of 1-thio-β-D-glycopyranosides have been reported,[8,9] to our knowledge II is the first 1-thio-D-galactoside to be recorded.

(1) National Science Foundation Research Associate (Z. Y.) and Predoctoral Fellow (B. O. J.), 1957–1958, under Grant NSF-G4494 to The Ohio State University.

(2) W. Schneider and Johanna Sepp, *Ber.*, **49**, 2054 (1916).

(3) W. Schneider, Johanna Sepp, and Ottilie Stiehler, *Ber.*, **51**, 220 (1918).

(4) J. W. Green and E. Pacsu, *J. Am. Chem. Soc.*, **59**, 1205 (1937).

(5) M. L. Wolfrom, S. W. Waisbrot, D. I. Weisblat, and A. Thompson, *J. Am. Chem. Soc.*, **66**, 2063 (1944).

(6) M. L. Wolfrom and Z. Yosizawa, *J. Am. Chem. Soc.*, **81**, 3474, 3477 (1959).

(7) N. Sheppard, *Trans. Faraday Soc.*, **46**, 429 (1950).

(8) L. Hough and M. I. Taha, *J. Chem. Soc.*, 3994 (1957).

(9) E. Fischer and K. Delbrück, *Ber.*, **42**, 1476 (1909); C. B. Purves, *J. Am. Chem. Soc.*, **51**, 3619, 3631 (1929).