compared with that of an authentic sample of *cis*-cyclopropane-d_2 prepared by the catalytic hydrogenation of cyclopropene.[18] The *cis* and *trans* isomers are easily differentiated by the fact that the *trans* isomer has a strong band at 12.77μ whereas the *cis* isomer has no band at this position. No cyclopropene was formed in the reaction giving the Grignard reagent.

***cis*-Cyclopropanedicarboxylic Acid.**—The Grignard reagent was prepared as described above using 11 g. of 1,2-dibromocyclopropane and 2.9 g. of magnesium in 40 ml. of ether. The solution was cooled in a Dry Ice–acetone-bath and dry carbon dioxide was added with stirring. After the reaction appeared complete, the reaction mixture was hydrolyzed with 25% sulfuric acid. The aqueous layer was saturated with sodium chloride, and extracted with ether. The combined ether solutions were evaporated giving a viscous oil which could not be induced to crystallize. The acid was converted to the ethyl ester by heating for 3 hours with 50 ml. of absolute ethanol and a few drops of concentrated hydrochloric acid. The solvent was removed by distillation and the residue was taken up in ether. The latter solution was extracted with 10% potassium carbonate solution, the solution was dried and the ether was removed by distillation. The residue (10% based on dibromocyclopropane) was found to have infrared and n.m.r. spectra identical with that of authentic diethyl *cis*-cyclopropane-1,2-dicarboxylate prepared by the esterification of the anhydride of *cis*-cyclopropane-1,2-dicarboxylic acid.

Argentic Complex.—Purified cyclopropene was passed into a 30% aqueous solution of silver nitrate. The cyclopropene was quantitatively absorbed, and after about a minute a black precipitate formed. In another experiment, cyclopropene was bubbled into ammoniacal silver nitrate solution. Again, complete absorption occurred, and a black precipitate was formed almost immediately. The black precipitates were found to be for the most part soluble in 30% perchloric acid solution, whereas silver (as formed in a Tollens test) is not. A yellow-white gum was formed in the reaction. This was found to be insoluble in all common solvents.

Reaction with Diphenyldiazomethane.—To a solution of diphenyldiazomethane prepared from 2.5 g. of benzophenone hydrazone in 50 ml. of methylene chloride was added 0.5 g. of finely divided copper powder. The solution was cooled in liquid nitrogen and approximately 1 g. of cyclopropene was distilled into the reaction flask. The solution was kept in a Dry Ice–acetone-cooled bath for 1.5 hours with occasional shaking, and it was then allowed to warm to room temperature. During this time the color lightened to yellow. The solution was decanted from the copper powder and evaporated to a small volume under reduced pressure. The remaining oil was taken up in hot 95% ethanol, the solution was filtered and then cooled giving 1 g. of material, m.p. 119–121°, in the form of yellow-tan needles.

Anal. Calcd. for $C_{16}H_{14}N_2$: C, 81.1; H, 6.4. Found: C, 81.7; H, 6.1.

Oxidation with basic permanganate gave benzophenone, identified as the 2,4-dinitrophenylhydrazone, m.p. 235–237°, mixed m.p. with an authentic sample, 235–237.5°.

Reaction with Ethyl Diazoacetate.—To a solution of 3.0 ml. of ethyl diazoacetate in 10 ml. of methylene chloride which was cooled in liquid nitrogen was added 1.5 ml. of cyclopropene. The reaction mixture was placed in a Dry Ice–acetone-bath and then allowed to warm to room temperature. At about −40°, an exothermic reaction occurred. Distillation gave 1.5 g. of material, b.p. 90–100° at 0.4 mm., and some higher boiling material.

Anal. Calcd. for $C_7H_{10}O_2N_2$: C, 54.5; H, 6.5. Found: C, 54.5; H, 6.4.

Pyrolysis of Cyclopropyldimethylamine Oxide.—To an ice-cooled solution of 25 ml. of 30% hydrogen peroxide in 50 ml. of water was added 8.5 g. of cyclopropyldimethylamine with stirring and cooling, at such a rate as to keep the temperature below 10°. The mixture was allowed to warm to room temperature, a slight exothermic reaction being noted. The mixture was stirred overnight. The excess hydrogen peroxide was then decomposed with a small amount of platinized asbestos and the solution was concentrated under reduced pressure to about 15 ml.

The solution was pyrolyzed at 320° using the apparatus employed for the pyrolysis of the quaternary ammonium hydroxide. The products of the reaction were swept with nitrogen through a trap containing 10% hydrochloric acid and then through a drying tower (calcium sulfate) and a liquid nitrogen cooled trap. The hydrochloric acid solution was made basic with potassium hydroxide, and the amine was isolated in the usual way. There was obtained 2.0 g. of material, b.p. 58°, whose infrared spectrum was found to be identical with cyclopropyldimethylamine. No cyclopropylmethylamine was found. The liquid nitrogen-cooled trap was found to contain no cyclopropene. The reaction was also carried out using platinized asbestos as a catalyst, as in the Hofmann degradation, but again no cyclopropene was formed.

[CONTRIBUTION FROM THE OAK RIDGE NATIONAL LABORATORY,[1] BIOLOGY DIVISION, OAK RIDGE, TENN.]

Characterizations and Some Chemical Reactions of Periodate-oxidized Nucleosides[2]

BY JOSEPH X. KHYM AND WALDO E. COHN

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Phenylhydrazine reacts with the periodate oxidation products of adenosine, guanosine, uridine and cytidine to form bisphenylhydrazones, which have been isolated and characterized. These substances react further with phenylhydrazine to form adenine, guanine, uracil, and cytosine and 2- and 3-carbon fragments. Sodium borohydride reduces both aldehyde groups of the oxidized nucleosides when the reaction is carried out under alkaline conditions, but selective reduction of the aldehyde group distal to the purine or pyrimidine ring occurs under slightly acidic conditions. Ion exchange and paper chromatographic methods have been developed for the examination of the dialdehydes and the corresponding reduction products obtained from them. Evidence is presented indicating that the acid hydrolysis of the completely reduced products, the tri-alcohols, is probably initiated at the glycosidic linkage. A new micro method for measuring and removing periodate and iodate essentially in one step has been developed.

Introduction

Studies on the oxidation of sugar glycosides by periodate to yield dialdehydes have played an important role in the determination of carbohydrate configurations as first shown by Jackson and Hudson[3] and later extended by Smith and Van Cleve.[4] The reaction as applied to the nucleosides[5] distinguishes between deoxy- and ribonucleosides[6]

(1) Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

(2) Parts of this communication were presented to the American Chemical Society, Atlantic City, N. J., September, 1959, and at Cleveland, Ohio, April, 1960.

(3) E. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 944 (1937).

(4) F. Smith and J. W. Van Cleve, *ibid.*, **77**, 3091 (1955).

(5) (a) J. Baddiley in "The Nucleic Acids," Vol. 1, eds., E. Chargaff and J. N. Davidson, Academic Press, Inc., New York, N. Y., 1955, p. 147; (b) G. R. Barker, *Advances Carbohydrate Chem.*, **11**, 285 (1956).

(6) D. M. Brown and B. Lythgoe, *J. Chem. Soc.*, 1990 (1950).

and was used in the final confirmation of the structure of the latter.[7] The technique initially was used to determine whether synthetic 9-glycosylpurines are furanoses or pyranoses[8] and then was applied to both synthetic and natural nucleosides which, on oxidation with periodate, yield for a given pair the same dialdehyde.[7] However, the dialdehydes obtained from adenosine and cytidine and their picrate derivatives did not show characteristic melting point behavior, and they were identified on the basis of elementary composition and optical properties at different *p*H values. Degradative proof of the structure of these compounds was not obtained, and no information has been presented for the dialdehyde of guanosine, nor have analyses other than optical properties been given for the uridine compound.[7]

As pointed out by Bobbitt,[9] the dialdehydes obtained by periodate oxidation polymerize readily and are difficult to handle, so that these compounds are best studied by oxidation to carboxylic acids or reduction to the corresponding alcohols. This latter technique, common in the reduction of oligosaccharides, has been applied to periodate-oxidized nucleosides by Viscontini, *et al.*,[10] who used sodium borohydride to reduce the dialdehydes from N'-pentofuranoses. The resulting trialcohols were hydrolyzed by heating in hydrochloric acid. The presence of glycerol in the hydrolyzates was qualitatively established by paper chromatography, and the authors postulated that a 2-carbon alcohol derivative of the base fraction was the other component.

The experiments described here were designed to characterize more fully the dialdehydes of the nucleosides and their corresponding alcohol derivatives. A new micro method for measuring and removing periodate and iodate essentially in one step has been developed. Phenylhydrazine can be used either to isolate the bisphenylhydrazones of periodate-oxidized nucleosides or to degrade them completely to free base with the production of the phenylosazones of the 2- and 3-carbon fragments. A selective reduction of a single aldehyde group of the oxidized nucleosides occurs when the reduction with sodium borohydride is carried out in dilute acid. A mechanism different from that proposed by Viscontini, *et al.*,[10] for the hydrolysis of the acyclic alcohols produced from nucleosides is suggested.

Experimental

Periodate Oxidations. Methods.—Periodate–iodate mixtures are readily assayed in the ultraviolet region (222–235 mμ).[11] Since the molar extinction of iodate is 0.1 that of periodate, this method can be conveniently used where the product oxidized does not interfere and where it is not necessary to remove IO_3^- and IO_4^- for further treatment of the reaction mixture. A method for the simultaneous removal of IO_3^- and IO_4^- by anion exchange resins from reaction mixtures has been developed by Smith and Willeford,[12] who eluted both ions from the resin with NaOH and determined the periodate in the mixture by titration with standard I_2. Our method represents a combination of these procedures. A typical example is given.

The compound (15–200 μmoles) in a 5-ml. volume was treated with 2 ml. of 0.1 *M* $NaIO_4$. After 15 minutes, the solution was percolated through a 2-ml. column of Dowex-1-acetate. After a water wash, the iodate was removed from the column with 30–40 ml. of 0.1 *M* NH_4Cl and measured directly at 232 mμ. A molar extinction coefficient for IO_3^- of 900 was obtained by adding 200 μl. of 0.1 *M* $NaIO_4$ to 200 μl. of 1 *M* glycerol in a 50-ml. volume and measuring at 232 mμ. The IO_4^- was recovered, when desired, by reducing it to IO_3^- with 3 ml. of 1 *M* glycerol placed on the top of the resin bed; 1 ml. was allowed to flow through the column at once, the second after 10 minutes, and the third after an additional 10 minutes, followed by an amount of NH_4Cl solution equal to that used for the original IO_3^- elution. When acetate exchangers are used, a small blank of about 0.12 optical density unit (caused by acetate absorption at 232 mμ) is subtracted from the total absorption at 232 mμ before calculations are made.

Agreement within ±2% of the calculated amount of periodate that should be reduced was obtained when this method was applied to ethylene glycol, glycerol and the nucleosides. For the first two compounds, formaldehyde in the effluent was assayed by the chromotropic acid method. The effluents from oxidized nucleosides are suitable for direct treatment with phenylhydrazine or sodium borohydride solutions.

The chromotropic acid method for the determination of formaldehyde[13] was slightly modified as follows. The test solution (1 ml.), containing about 10 μg. of CH_2O, was mixed with 1 ml. of 1% aqueous chromotropic acid and then 5 ml. of concentrated H_2SO_4. After heating at 100° for 30 minutes, the samples were cooled, diluted to 10 ml., and read in a colorimeter with a green filter.

Preparation of the Diglycolic Aldehyde Derivatives of the Nucleosides.—Adenosine, uridine, cytidine or guanosine (40 mg. each) were dissolved in 2 ml. of 0.1 *M* $NaIO_4$ and, after 20 minutes, the mixtures excluding the guanosine solution were washed through 2-ml. columns of Dowex-1-acetate with 20 ml. of 0.02 *M* acetic acid. Because of its low solubility, the guanosine mixture was triturated often during the oxidation period; the volume was then increased to about 10 ml. with water, 0.5 ml. of 1 *M* acetic acid was added and, after 10 minutes of stirring, the mixture was poured on the exchange column and washed through with 10 ml. of 0.02 *M* acetic acid. If the solution was still cloudy, it was passed through a sintered glass funnel.

These stock solutions were then used for experiments with phenylhydrazine and sodium borohydride. The dialdehydes and some related derivatives to be described were examined on descending chromatograms (Whatman No. 1 paper). The solvent system was butanol saturated with water and the positions of the spots were detected with ultraviolet light; R_f values are recorded in Table I.

TABLE I

R_f VALUES RELATIVE TO URACIL[a] FOR DIALDEHYDES, ALCOHOLS AND MONOALDEHYDES RUN IN BUTANOL–WATER[b]

Derivatives of	Dialdehyde[c]	Monoaldehyde	Alcohol[d]
Adenosine	1.22	0.52	0.45
Cytidine	1.11	.49	.35
Guanosine	0.52	.18	.12
Uridine	1.57	.87	.58

[a] Uracil runs at the rate of about 0.6 cm./hr. [b] In isopropyl alcohol–H_2O–NH_3 all the compounds run at essentially the same rate as uracil. [c] The dialdehydes appear as characteristically elongated spots, 3–5 times as long as the reference spot of uracil. [d] Alcohol is used here for the completely reduced compound.

The Use of Phenylhydrazine to Form Intermediates and to Degrade Periodate Oxidized Nucleosides. Preparation of the Bisphenylhydrazones of Purine or Pyrimidine

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(8) B. Lythgoe and A. R. Todd, *ibid.*, 592 (1944).

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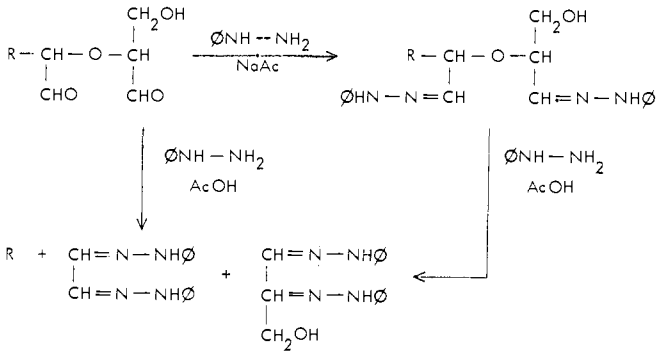


Fig. 1.—Reactions of the dialdehydes with phenylhydrazine.

Hydroxymethyl Diglycolic Aldehydes and the Phenylosazones of Reference Compounds.—Adenosine, cytidine, uridine or guanosine (100 mg. each) were oxidized as before except that the effluents, from the ion-exchange step, were collected in receivers containing 1 g. of sodium acetate. Phenylhydrazine (100 μl.) was added, and the solutions were stirred for about 1.5 hours at room temperature. The precipitates were filtered, washed with water, and dried *in vacuo* over P_2O_5. The yields of the pale yellow hydrazones ranged from 60–75%. They could not be recrystallized even at low temperatures without degrading to form some glyoxal phenylosazone. They do not melt sharply but decompose over a wide temperature range (*e.g.*, bisphenylhydrazone of adenine m.p. 101–108°, of cytosine m.p 90–96°, of uracil m.p. 87–94°, of guanine m.p. 116–122°, and at room temperature in the presence of air and light change in a few days to red-orange substances. The bisphenylhydrazones absorb characteristically in the 220 to 340 mμ region with well-defined maxima between 265 and 278 mμ.

Anal. Calcd. for bisphenylhydrazone of uracil-hydroxymethyldiglycolic aldehyde, $C_{21}H_{22}N_6O_4$ (422.44): C, 59.95; H, 5.3; N, 19.98. Found: C, 60.03; H, 5.5; N, 20.06; λ_{max} 278, ϵ_{max} 29,000 (in 95% ethanol). Calcd. for bisphenylhydrazone of adenine-hydroxymethyldiglycolic aldehyde monohydrate, $C_{22}H_{23}N_9O_2.H_2O$ (463.49): C, 57.01; H, 5.44; N, 27.20. Found: C, 56.91; H, 4.92; N, 27.75; λ_{max} 265, ϵ_{max} 30,000 (in 95% ethanol). Calcd. for bisphenylhydrazone of cytosine-hydroxymethyldiglycolic aldehyde monohydrate, $C_{21}H_{23}N_7O_3.H_2O$ (439.47): C, 57.39; H, 5.73; N, 22.31. Found: C, 57.86; H, 5.46; N, 21.50; λ_{max} 278 ϵ_{max} 33,000 (in 95% ethanol). Calcd. for bisphenylhydrazone of guanine-hydroxymethyldiglycolic aldehyde monohydrate, $C_{22}H_{23}N_9O_3.H_2O$ (479.49): C, 55.10; H, 5.26; N, 26.29. Found: C, 54.61; H, 5.61; N, 26.40; λ_{max} 275, ϵ_{max} 30,000 (in 95% ethanol).

Glyceraldehyde, glycolic aldehyde and dihydroxyacetone were purchased commercially[14] as were glyoxal (30% aqueous) and phenylhydrazine.[15] The last-mentioned compound was distilled under diminished pressure before use.

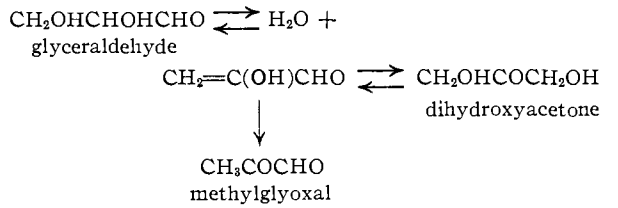
Glycerose phenylosazone was prepared from glycerol by Fischer's method[16] and from dihydroxyacetone by Engel's method.[17] The product was recrystallized several times from benzene and finally dried *in vacuo* over P_2O_5, m.p. 132–133°.

Glyoxal and Methylglyoxal Bisphenylhydrazones.—The first mentioned compound was prepared from 30% aqueous glyoxal and the latter compound by the distillation of dihydroxyacetone from 1:5 H_2SO_4 and the subsequent reaction of the distillate with phenylhydrazine. The bisphenylhydrazine of glyoxal was recrystallized from benzene and dried *in vacuo* over P_2O_5, m.p. 170–171°, as was methylglyoxal bisphenylhydrazone, m.p. 144–146°.

Spectrophotometric Analysis of Osazones.—The osazones of glyoxal, methylglyoxal and glyceraldehyde, prepared as described, absorb characteristically in the range of 240–440 mμ and microgram quantities of these osazones can easily be detected.[18] The osazone fractions from oxidized nucleosides were taken up in benzene and adsorbed on neutral or acid alumina columns.[19] The columns (1 sq. cm. × 23 cm.) were washed with 50 ml. of benzene to remove dark brown impurities presumably arising from phenylhydrazine, glyceraldehyde or both. The osazone of glyoxal was eluted with about 40 ml. of 1% ethanol in benzene after 75 ml. of this reagent had been discarded. The benzene was removed and the residue taken up in 10 ml. of 95% ethanol for spectrophotometric observations.

The total osazone formed in a reaction mixture can be estimated directly in the 320–440 mμ range, the region of maximum absorption for the major peak of these osazones. The other products found in the reaction mixture, such as phenylhydrazine, N-acetylphenylhydrazine, pyrimidine or purine bases, and impurities removed by the alumina treatment, do not absorb significantly in this region. The values presented in the tables are those determined before the osazones were purified on alumina columns from which there is only about a 60% recovery of authentic compounds. The extinctions for the phenylosazone of glyoxal (ε 48,000), methyglyoxal (44,000) and glyceraldehyde (22,000) agree with those reported by Barry, *et al.*[18]

In the position where authentic glycerose phenylosazone is eluted from the column with 5% ethanol in benzene, only a trace of "osazone" was eluted and its curve was not characteristic of the glyceraldehyde compound. Although glyceraldehyde was found by Barry, *et al.*, 3-carbon fragments are difficult to isolate by this procedure, especially if only small amounts are present. As shown in the preceding section, glycerose phenylosazone is best prepared from dihydroxyacetone rather than glyceraldehyde. Our attempts to prepare the phenylosazone using glyceraldehyde and measure it spectrophotometrically resulted in unsatisfactory spectra, less than 6% yield, and high yields of the impurities just mentioned. Glyceraldehyde is very reactive in solution, and the following reactions occur in either acidic or basic solutions.[20,21]



If either glyceraldehyde or dihydroxyacetone is distilled in mineral acid, methylglyoxal appears in the distillate.[20,22] Glyceraldehyde was therefore identified by the distillation of periodate-oxidized nucleosides (the 2-carbon fragment, glyoxal, is destroyed by this procedure) from sulfuric acid solutions. The distillate was collected in a receiver containing phenylhydrazine, and after a short digestion period at 37° the phenylosazone of methylglyoxal was formed. It was purified on alumina columns, as described for the glyoxal compound.

Degradations with Phenylhydrazine.—It has been shown that the dialdehydes resulting from periodate oxidations of sucrose and polysaccharides, when treated with phenylhydrazine, give rise to intermediates that, if further treated with the same reagent in dilute acetic acid, degrade to 2- and 3-carbon fragments that can be identified as their osazones.[23] The bisphenylhydrazones of the nucleosides are degraded with excess phenylhydrazine in dilute acetic acid according to the scheme of Fig. 1 and the reaction apparently is complete in about 5 minutes at reflux temperature or in several hours in a water-bath at 37°. A typical example is as follows: 15 mg. (~35 μmoles) of the uridine hydrazone was dissolved in 1 ml. of ethanol, 1 ml. of H_2O

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(15) Eastman Organic Chemicals.

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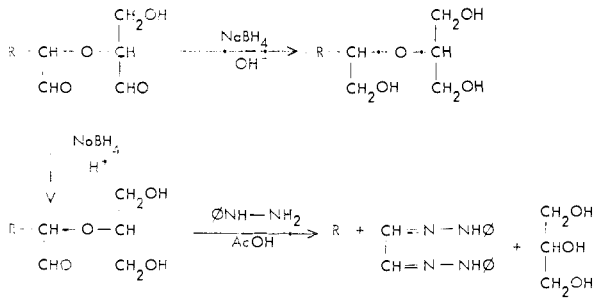

Fig. 2.—Reactions of the dialdehydes with sodium borohydride (ϕ for Ph or C_6H_5).

and 2 ml. of 4 *M* acetic acid. To this was added 25 μl. of phenylhydrazine. The mixture was refluxed for 5 minutes or set aside at 37° for 8–16 hours. The reaction mixture was diluted to 10 ml. with water and extracted with an equal volume of ether. (For the guanine derivative, the mixture was vacuum-distilled, taken up in ethanol, and centrifuged. The precipitated guanine was washed with alcohol, and the washings were combined for osazone analysis.) After the ether layer was washed with water, it was analyzed for osazone spectrophotometrically, using an aliquot dissolved in 95% ethanol. After removal of the ether, the osazone was taken up in benzene, purified on the alumina column, and its total spectrum compared to authentic material.

The purine and pyrimidine bases in the aqueous phases (guanine was dissolved in NH_4OH) were analyzed and identified by ion exchange[24] or paper chromatographic methods,[25] and by spectral shifts at different *p*H values.[26] Yellow-colored impurities in the aqueous phase were removed by making the solutions alkaline (~1–3 *M* NH_4OH), setting them aside for ~ 16 hours, and, after removing the NH_3, percolating them through small columns (~ 2 ml.) of Dowex-1-acetate to remove the precipitated impurities. The recovery of the products from some of these experiments is given in Table II.

TABLE II

MOLE PER CENT. OF PRODUCTS RECOVERED BY THE USE OF PHENYLHYDRAZINE

Initial nucleoside	Degradations with phenylhydrazine of Bisphenylhydrazones Base	Glyoxal	Hydroxymethyldiglycolic aldehydes Base	Glyoxal	Distillation experiments methylglyoxal
Adenosine	90 (85)[a]	86 (88)	92 (95)	82 (85)	67
Cytidine	91 (82)	90 (100)	92 (92)	71 (72)	48
Guanosine	100 (93)	94 (100)	84 (93)	77 (93)	55
Uridine	99 (95)	71 (75)	90 (63)	68 (55)	61
Dihydroxyacetone					68
Glyceraldehyde					71
Glyoxal					<3

[a] Values in parentheses represent results of experiments carried out at 37°; others were refluxed.

Degradations, as indicated in the equation of Fig. 1 (R = purine or pyrimidine, also in equations that follow), were also carried out directly on the original dialdehyde solutions. Aliquots of these stock solutions (2 ml.) were treated with excess phenylhydrazine (20 μl.) in 25% ethanol solutions 1–2 *M* in acetic acid as described before. These results are also shown in Table II. In experiments with the dialdehyde of uridine, the solution was varied from 3 *M* acetic acid to buffered acetate solutions at *p*H 5.7, and the reflux time varied from 5–20 minutes. Approximately a 70% yield of glyoxal phenylosazone was obtained in each case.

Determination of the 3-Carbon Fragment as Methylglyoxal.—Aliquots (5 ml.) of the dialdehydes were placed in a small one-piece distillation apparatus and 2 ml. of concentrated H_2SO_4 plus 5 ml. of H_2O were added to the samples. Distillation at about 185° yielded about 6 ml. of distillate that was collected in a large test-tube containing 2 ml. of ethanol plus 2 ml. of 2*M* acetic acid and 25 μl. of phenylhydrazine. The outlet tube was kept submerged in the phenylhydrazine solution during the distillation. After digesting at 37° for at least 4 hours, osazone formation was measured as before. These results are shown in Table II.

Reductions of the Dialdehydes. Reduction of both Aldehyde Groups.—To 40 mg. of each nucleoside, after oxidation with periodate and passage through the acetate exchanger, was added 80 mg. of solid $NaBH_4$. The dilute alkaline solutions were set aside for about 16 hours. Borate was removed from the uracil compound by adding 3 ml. of Dowex-50-H^+, stirring for a few minutes, filtering through a sintered glass funnel, and vacuum-distilling to dryness twice in the presence of methyl alcohol.[27] For the adenine and cytosine compounds, 3 ml. of 1 *M* acetic acid was added, and the solutions were passed through 3-ml. columns of Dowex-50-H^+. After a water wash, the trialcohols were eluted with 30 ml. of 5 *N* NH_4OH, which was then removed by vacuum distillation, and the compounds were stored in a known volume of water. The guanine compound was vacuum-distilled to a 5-ml. volume after addition of 3 ml. of 1 *M* acetic acid. Over 90% of the trialcohols was recovered. They contained less than 3 mole % free glycerol; this was determined by passing an appropriate aliquot through about 2-ml. columns of 50–200 mesh cocoanut charcoal,[28] which adsorbs the trialcohols but not the glycerol. Glycerol was determined by periodate uptake and formaldehyde production.

Selective Reduction of a Single Aldehyde Group.—A high preference (90–95% yield) for the reduction of only the aldehyde group distal to the purine or pyrimidine base, in accordance with Fig. 2, is found if the reduction is carried out in a way similar to that used by Frush and Isbell[29] to reduce aldonic lactones in a slightly acid medium. The following procedure was used to prepare the monoaldehydes of all four periodate-oxidized nucleosides.

To 40 mg. of a nucleoside, after oxidation with periodate and removal of IO_3^- and IO_4^-, was added 5 ml. of saturated H_3BO_3 plus 2.5 ml. of 1 *M* acetic acid. Over a period of 40–60 minutes 80 mg. of $NaBH_4$ dissolved in 10 ml. of water was added drop by drop to the dialdehyde solutions, which were contained in narrow tubes (for more efficient reduction) over a magnetic stirrer. After the reactions, the solutions were vacuum-distilled in the presence of methyl alcohol to remove the boric acid. Each monoaldehyde and its corresponding fully reduced derivative are readily separated by paper chromatography (see Table I) and, except for the cytosine compounds, by chromatography on strong-base ion exchangers with NH_4OH–NH_4Cl buffers. In contrast to the completely reduced compounds, the monoaldehydes react readily with phenylhydrazine to give free base, glyoxal and glycerol. They are readily converted to their corresponding full alcohol derivatives by treating neutral or slightly alkaline solutions of them with solid $NaBH_4$. The products obtained are identical to those produced by the direct treatment of the dialdehydes with alkaline $NaBH_4$.

Reaction of the Monoaldehydes with Phenylhydrazine.—Thirty to forty μmoles of monoaldehyde in 1 ml. were treated with a 5- to 7-fold excess of phenylhydrazine after the initial solution had been adjusted to about 1 *M* acetic acid and 25% with respect to ethanol. After refluxing about 5 minutes, the glyoxal phenylosazone was separated and measured as before. The 3-carbon fragment appears as glycerol in the aqueous phase with the free base and was separated from it by the adsorption, in the case of uracil, from ammonical solution on small (2-ml.) Dowex-1-acetate columns or, in the case of cytosine or adenine, by adsorption on small Dowex-50-H^+ columns. The bases were eluted with 0.1 *M* acetic acid and 5 *N* ammonium hydroxide, respectively, the solvents vacuum-distilled, and purine or pyrimidine identified by ion-exchange or paper chromatography.[24,25]

(24) W. E. Cohn in "The Nucleic Acids," *op. cit.*, p. 211.

(25) G. R. Wyatt in "The Nucleic Acids," *op. cit.*, p. 243.

(26) G. H. Beaven, E. R. Holiday and E. A. Johnson in "The Nucleic Acids," Ref. 5, p. 493.

(27) L. P. Zill, J. X. Khym and G. M. Chenlae, THIS JOURNAL, **75**, 1339 (1953).

(28) Fisher Scientific Co.

(29) H. L. Frush and H. S. Isbell, THIS JOURNAL, **78**, 2844 (1956).

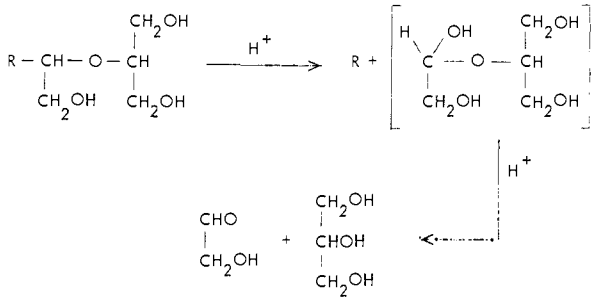

Fig. 3.—Mechanism of hydrolysis for the alcohol derivatives.

Glycerol (from the guanine compound, it was found in the ethanol washes from precipitated guanine and was recovered by solvent extraction with water–ether after removing the ethanol) was freed of phenylhydrazine impurities by passing it through small columns of cocoanut charcoal. The glycerol in the effluent from the charcoal columns was vacuum-distilled to a small volume and chromatographed in butanol–water; its presence was demonstrated by spraying with ammonical silver nitrate.[30] It was quantitatively analyzed by periodate uptake with release of formaldehyde.

The equivalence of free purine or pyrimidine base and of glyoxal and glycerol that is formed when the monoaldehydes react with phenylhydrazine is demonstrated in Table III.

TABLE III

MOLE PER CENT. PRODUCTS RECOVERED FROM THE MONOALDEHYDES AFTER REACTION WITH PHENYLHYDRAZINE

Monoaldehyde	Base	Glyoxal	Glycerol[a]
Adenosine	93	86	85
Cytidine	87	73	71
Guanosine	92	75	70
Uridine	97	89	80

[a] Glycerol recoveries ranged from 70–100%; the average value from a number of determinations was about 80%.

Hydrolysis of the Trialcohol Derivatives.—Hydrolysis of the full alcohol derivatives, which can be considered as heterocyclic N-analogs of diethylene glycol, yields free base, glycolic aldehyde and glycerol (Fig. 3). These products were determined in a manner similar to the procedures already described. Glycolic aldehyde was detected through its osazone, forming the same osazone as does glyoxal, and by the colorimetric diphenylamine method.[31] Since glycolic aldehyde is partially destroyed, depending on the length of heating and concentration of acid used for the hydrolysis experiments, the course of the reaction is best followed by detection of free base and of glycerol.

The stability of these alcohol derivatives to mineral acids is markedly different among the four compounds studied. The alcohol from cytidine is only slightly degraded after 45 minutes in 6 *N* HCl at 100° while the uridine derivative is about 50% hydrolyzed in 3 *N* acid at 100° in 50 minutes. The alcohols derived from adenosine and guanosine partially hydrolyze in 0.5*N* HCl at 100° when heated for short periods and are completely hydrolyzed when heated under the same conditions for 5 minutes.

The following is a typical example of the separation of products after hydrolysis. Twenty to forty μmoles of trialcohol was heated in mineral acid and, when cooled, the diphenylamine test was applied to a diluted sample. Another sample was taken for chromatographic analysis. The rest was put through Dowex-1-acetate to remove chloride or sulfate. The effluent was vacuum-distilled to about 1 ml., alcohol, acetic acid and phenylhydrazine were added as before, and the sample was refluxed. After separation of the osazone, the base fraction and phenylhydrazine were separated from glycerol by passing the mixture through charcoal columns. Glycerol was analyzed as described before. Some of these results are presented in Table IV.

TABLE IV

MOLE PER CENT. OF PRODUCTS RECOVERED AFTER HYDROLYSIS OF TRIALCOHOL DERIVATIVES

Alcohol derived from	HCl at 100° Time, min.	Concn., M	Base fraction[a]	Glycolic aldehyde[b]	Glycerol
Adenosine	0.5	0.5	55(45)[c]	19	44
Cytidine	45	6	11(89)	..	9
Guanosine	3	0.5	27(73)	11	26
Uridine	50	3	55(45)	8	51
Glycolic aldehyde	0.5	0.5		38	

[a] Bases and alcohols were separated by ion-exchange chromatography. [b] Determined by osazone formation. [c] Values in parentheses represent recoveries of unreacted alcohols.

Stability of Dialdehydes in Alkali.—Before applying the phenylhydrazine method to degrade the dialdehydes, their stability in dilute alkali was tested in a manner similar to that used by Whitfeld[32] for the determination of nucleotide sequence in polyribonucleotides. Solutions of oxidized cytidine, uridine and adenosine in 0.01 *M* NaOH, 0.1 *M* borax and 0.1 *M* glycine buffered at *p*H 10.5 were allowed to incubate at 37°. Periodically, samples were placed on paper and run in butanol–water together with control solutions of cytosine, uracil and adenine. At time zero, the characteristically elongated spots of the dialdehydes (see Table I) were noted for all three oxidized nucleosides applied to the paper from NaOH and the borax solutions but the dialdehydes in glycine solutions all remained at the origin. At the end of 3 days, definite base formation (<20%) was noted in the glycine solutions but not in the others, and the majority of material from these solutions had a tendency to remain at the origin.

Results and Discussion

The composition of the bisphenylhydrazones of the purine and pyrimidine hydroxymethyldiglycolic aldehydes definitely shows that a phenylhydrazine molecule is condensed on each aldehyde group of the dialdehydes. Apparently this is not a general property of dialdehyde compounds. Barry, *et al.*[23] discuss several dialdehyde compounds where condensation is complete when one phenylhydrazine molecule has condensed for each dialdehyde group.

The spectra of the bisphenylhydrazones may be used to indicate the purity of the compounds with respect to degradation and the formation of osazones. If care is not taken to avoid acidic conditions during their formation, or if attempts are made to recrystallize the bisphenylhydrazones, the presence of the phenylosazone of glyoxal is demonstrated readily by an increase in absorption in the 340–440 mμ region.

The observation that the bisphenylhydrazones have essentially equal extinctions at maximum absorption in contrast to the nucleosides themselves indicates a general property of the former that might be useful in determination of molecular weights of the less common nucleosides such as those recently found in nucleic acid digests.[33]

The degradation of the dialdehydes by phenylhydrazine (Fig. 1) proceeds equally well through their intermediates (*i.e.*, the bisphenylhydrazones)

(30) L. Hough, *Nature*, **165**, 400 (1950).

(31) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **180**, 1297 (1949).

(32) P. R. Whitfeld, *Biochem. J.*, **58**, 390 (1954).

(33) J. W. Littlefield and D. B. Dunn, *ibid.*, **70**, 642 (1958); J. D. Smith and D. B. Dunn, *ibid.*, **72**, 294 (1959); F. F. Davis and F. W. Allen, *J. Biol. Chem.*, **227**, 907 (1957); W. E. Cohn, *Biochim. et Biophys. Acta*, **32**, 569 (1959); W. E. Cohn, *J. Biol. Chem.*, **235**, 1488 (1960).

or with the dialdehydes themselves as starting material. The ease of the reaction at 37° was unexpected. Visual inspection of the amount of glyoxalosazone precipitated in reaction mixtures from the dialdehydes of adenosine, cytidine and guanosine indicated that the reactions were complete in less than 30 minutes at 37° and that only the compound from uridine required a longer digestion period and even then gave results consistently lower than the other compounds in regard to osazone and base formation. The high yield of base, even at the low temperature, shows that the action of phenylhydrazine on these dialdehyde compounds is to labilize the glycoside linkage, ordinarily resistant to dilute acetic acid at 100° for all the common nucleosides and especially the pyrimidines, which are stable at elevated temperatures even in the presence of mineral acids. Lack of identification of glyceraldehyde as the 3-carbon fragment in these phenylhydrazine mixtures is attributed to the many side reactions of this compound that lead only to a high yield of impurities whenever glyceraldehyde is indicated in a reaction mixture.

The determination of the 3-carbon fragment by the distillation of the dialdehydes to give methylglyoxal worked exceedingly well. It is fortunate that the 2-carbon fragment, glyoxal, is destroyed in this procedure (Table II) since the difference in the spectra of the two osazones of the compounds in question is slight. Also the separation of these two osazones on the alumina columns was incomplete so that any mixtures of the two osazones present in the phenylhydrazine digests would be difficult to analyze by the techniques presented.

The action of phenylhydrazine on the compounds classified as the monoaldehydes to give the products shown in Fig. 2 leaves little doubt as to the structure of these compounds. Glycerol could arise from carbon atoms C_3', C_4', and C_5' of the initial furanose structure only if C_3' were contained as part of a primary alcohol group. If the proximal group was considered as the group reduced in these monoaldehyde compounds, the nature of the products could not be explained at all. The completely reduced compounds are stable to phenylhydrazine so that the formation of quantitative yields of free base could only arise by an attack on the glycosidic linkage that becomes susceptible when the single aldehyde group is proximal to the purine or pyrimidine ring and not distal. Furthermore, when the monoaldehydes are distilled from sulfuric acid solution, osazone formation is not detected.

The reduction of a single aldehyde group with such high specificity is not readily explained. Ultraviolet spectra and polarographic studies indicate the absence of free aldehyde in certain periodate-oxidized glycosides.[34,35] Hurd, *et al.*,[35] suggest that substances such as methoxyhydroxymethyl-diglycolic aldehyde (from methyl α-D-glucopyranoside), in addition to hydrated structures, could cyclize to form "pyranose" structures of the form given in Fig. 4.

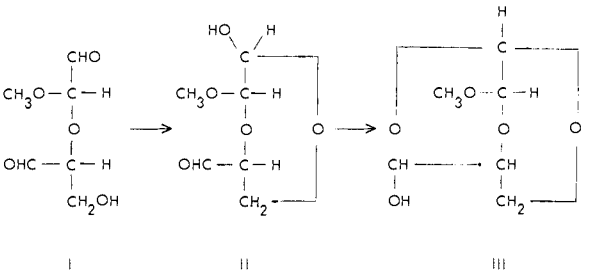

Fig. 4.

Abdel-Akher, *et al.*,[36] showed that both aldehyde groups of the diglycolic aldehyde derivative shown are reduced by hydrogenation with Raney nickel under pressure at 100° or with $NaBH_4$ at 25°, but only the aldehyde group nearest to the primary alcohol group is reduced if the hydrogenation under pressure is carried out at room temperature in the presence of palladium-charcoal catalyst. The hydrated form of structure II seems to fit most of the evidence that has been gathered from these dialdehyde compounds and has been used to explain the selective reduction.[35]

If the methoxy group of these structures were replaced by a purine or pyrimidine ring, the second cyclization might be prohibited thus leaving the distal aldehyde group exposed. According to Pigman,[37] the quantity of free reducible material in sugars increases rapidly as the *p*H is increased from 7.0 to more alkaline conditions. If the ring structure of these aldehyde compounds is favored in dilute acid, the relation between *p*H and selective reduction is readily explained, particularly since the remaining aldehyde group of these compounds is easily reduced when the reaction with $NaBH_4$ is carried out under alkaline conditions.

The four alcohol derivatives hydrolyze in mineral acid in accordance with the equation of Fig. 3. The data in Table IV and from ion exchange chromatography show the equivalence of free base, glycolic aldehyde and glycerol that result even if the alcohol compounds are only partially hydrolyzed. An excess of glycerol over free base produced was never found, even in the case of the cytosine compound, which was found to have the most resistant glycosidic linkage. This indicates that reaction is initiated at the nitrogen-carbon bond of the alcohols and that the ether link of these derivatives remains stable until the former bond is broken. It is proposed that the first step in the reaction is the formation of free base and the intermediate indicated by the brackets in Fig. 3. This intermediate is a hemiacetal, which would readily hydrolyze in acid to give glycolic aldehyde and glycerol in the manner of the methoxy-hydroxymethyldiethylene glycol compounds described by Smith and Van Cleve.[4] This mechanism is opposed to that of Viscontini, *et al.*,[10] who suggest that the ether link of the alcohol compounds is

(34) J. W. Rowen, F. H. Forzlati and R. E. Reeves, THIS JOURNAL, **73**, 4484 (1951).

(35) C. D. Hurd, P. J. Baker, Jr., R. P. Holysz and W. H. Saunder, Jr., *J. Org. Chem.*, **18**, 186 (1953).

(36) M. Abdel-Akher, J. E. Cadotte, R. Montgomery, F. Smith, J. W. Van Cleve and B. A. Lewis, *Nature*, **171**, 474 (1953).

(37) W. Pigman in "The Carbohydrates," Academic Press, Inc., New York, N. Y., 1957, p. 55.

attacked during acid hydrolysis to yield glycerol and a 2-carbon alcohol that remains attached to the base fraction.

The observation that the periodate-oxidized nucleosides show entirely different chromatographic properties after treatment with glycine buffer in contrast to treatment with solutions of sodium hydroxide or borax seem to suggest the formation of a complex between the dialdehyde compounds and glycine. This concept is now being explored with particular attention directed to the alkaline reactions of periodate-oxidized nucleoside 5′-phosphates.

In investigations related to the determination of base sequence in ribopolynucleotides, two groups[32,38] have reported that oxidized nucleoside 5′-phosphates readily degrade in the presence of glycine buffer at *p*H 10.5 to produce inorganic phosphate. Preliminary investigations in this Laboratory indicate that periodate-oxidized nucleoside 5′-phosphates form addition compounds (α-hydroxy amine derivatives),[39,40] not only with glycine but also with ammonia and primary amines, and that phosphate is not eliminated during the formation of these complexes. It appears that these compounds slowly release inorganic phosphate in the *p*H range of 8 to 11 and rapidly when the compounds are acidified.

(38) D. M. Brown, M. Fried and A. R. Todd, *Chemistry & Industry*, 352 (1953); *J. Chem. Soc.*, 2206 (1955).
(39) M. M. Sprung, *Chem. Revs.*, **26**, 297 (1940).
(40) D. French and J. T. Edsall, *Advances in Protein Chem.*, **2**, 277 (1945).

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Macro Rings. XXII. 6-Hydroxy-7-keto-*trans*-cyclodecene and Derivatives[1]

BY DONALD J. CRAM AND LYLE K. GASTON[2]

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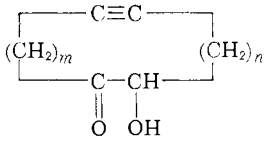
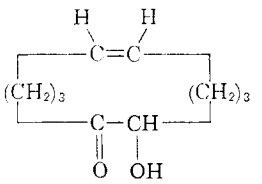
When submitted to the acyloin reaction, dimethyl *trans*-5-decenedioate gave about a 50% yield of 6-hydroxy-7-keto-*trans*-cyclodecene, which was converted to a number of derivatives. Transannular effects in both reactions and spectral properties were observed. Attempts to ring close dimethyl 5-undecynedioate failed.

Previous studies demonstrated that although a twelve-membered carbocyclic ring containing a carbon–carbon triple bond (Ia) could be closed with the acyloin reaction, the corresponding transformation failed when applied to the preparation of the ten-membered homolog (Ib).[3] However, an acyloin reaction gave a yield of cyclic olefin II substantially better than was obtained in the preparation of sebacoin, the saturated analog.[3] The purpose of this investigation was to explore



Ia, $m = n = 4$
b, $m = n = 3$
c, $m = 4, n = 3$



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further the steric limitations of the acyloin reaction, and to make available systems amenable to a study of transannular effects.

Syntheses and Reactions.—The *trans*-olefinic acyloin V was prepared from *trans*-olefin IV which in turn was obtained from the known acetylenic diacid III.[3] The yield in this acyloin reaction (about 50%) compares with the 80% yield of II and 60% yield of sebacoin obtained under similar conditions. Thus the ends of *trans*-olefin IV can be brought together without creation of great strain. The identity of V was demonstrated through its infrared spectrum, and by its conversion to sebacoin by catalytic hydrogenation. Acetylation of V gave acetate VII, whereas oxidation of V with cupric acetate gave diketone VI.[4]

An attempt to reduce acyloin acetate VII to the *thioketal* of 6-keto-*trans*-cyclodecene with trimethylenedithiol[5] resulted in a more deep-seated reduction, which led to the thioketal of cyclodecanone (VIII). The reduction of an isolated carbon–carbon double bond by a mercaptan is unprecedented, and it therefore seems probable that some transannular mechanism is operative which involves both the acyloin and olefinic linkages.

Acetylenic ester IX was prepared by the indicated sequence, but gave only polymer and starting material on repeated attempts at ring closure by the acyloin reaction. Clearly, the smallest ring containing a carbon–carbon triple bond that can be closed by the acyloin reaction is twelve-membered.

Spectra.—Table I gives the positions in the infrared spectra of the carbonyl band of a number of alicyclic ketones. Within experimental error, in these medium rings the corresponding ketones, acyloins and α-diketones all have the same carbonyl stretching frequency. As the alicyclic ketones get larger, the carbonyl band decreases in frequency until a minimum is reached in the ten-membered ring.[6] This effect seems to be associated with an increase in the C–C–C bond angle of the carbonyl group which is largest with the ten-membered

(1) This work was sponsored by the Office of Ordnance Research, U. S. Army.
(2) Dow Predoctoral Fellow, 1956–1957.
(3) D. J. Cram and N. L. Allinger, THIS JOURNAL, **78**, 2518 (1956).
(4) Bismuth oxide [W. Rigby, *J. Chem. Soc.*, 793 (1951)] oxidation failed to give diketone.
(5) D. J. Cram and M. Cordon, THIS JOURNAL, **77**, 1810 (1955).
(6) T. Burer and H. H. Gunthard, *Helv. Chim. Acta*, **39**, 365 (1956).