NBS TECHNICAL NOTE

405

Organic Chemistry: Air Pollution Studies; Kinetic Behavior of Sugars in Solution Carbon-14- and Tritium-Labeled Carbohydrates; Characterization of Chemical Structures, Phenylhydrozono-Phenylazo Tautomerism; Synthesis of Research Materials, Cyclopentitols and Related Substances; Novel Research Materials; Standard Reference Materials (Organic) July 1965 through June 1966

Edited by Horace S. Isbell

U.S. DEPARTMENT OF COMMERCE
National Bureau of Standards

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TECHNICAL NOTE 405

ISSUED SEPTEMBER 30, 1966

Organic Chemistry: Air Pollution Studies; Kinetic Behavior of Sugars in Solution; Carbon-14- and Tritium-Labeled Carbohydrates; Characterization of Chemical Structures, Phenylhydrazono-Phenylazo Tautomerism; Synthesis of Research Materials, Cyclopentitols and Related Substances; Novel Research Materials; Standard Reference Materials (Organic) July 1965 through June 1966

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Organic Chemistry Section
Analytical Chemistry Division
Institute for Materials Research

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FOREWORD

The Analytical Chemistry Division was established as a separate Division at the National Bureau of Standards on September 1, 1963, and became part of the Institute for Materials Research in the February 1, 1964 reorganization. It consists at present of nine sections and about 100 technical personnel encompassing some 45 different analytical competences from activation analysis and atomic absorption to vacuum fusion and X-ray spectroscopy. These competences, and in turn the sections which they comprise, are charged with research at the forefront of analysis as well as awareness of the practical sample, be it standard reference material or service analysis. In addition it is their responsibility to inform others of their efforts.

Formal publication in scientific periodicals is highly important. In addition, however, it has been our experience that informal, annual summaries of progress describing efforts of the past year can be very valuable in disseminating information. At the National Bureau of Standards such publications fit logically into the category of a Technical Note. In 1966 we plan to issue these summaries for all of our sections. The following is the second annual report on progress of the Organic Chemistry Section.

W. Wayne Meinke, Chief
Analytical Chemistry Division

PREFACE

This is the second in a series of annual progress reports of the Organic Chemistry Section of the Analytical Chemistry Division. The report, in the form of an NBS Technical Note, covers the principal activities of the Section for the period July 1, 1965 to June 30, 1966.

We have been greatly pleased with the interest accorded our last Technical Note (No. 274). It appears to have filled a real need for providing the public with desired information on work in progress. In addition, we have found the report to be a convenient means for answering inquiries made to the Bureau concerning certain problems of current interest. The report provides an overall view of the work of the Section, and furnishes more information than can be given by letter.

In the present report, we have sought to convey not only information as to what we are doing but as to why we are doing it. In some instances, we have presented a historical resume of our work to give perspective regarding our present activities. We trust that this treatment will provide the scientific public with a better understanding of our overall research goals. At various points in the Note, reference is made to unpublished infrared and ultraviolet spectrograms. We shall be pleased to furnish copies of these on specific request.

In order to describe experimental procedures adequately, it has occasionally been necessary to identify commercial materials and equipment in this report. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

Horace S. Isbell, Chief
Organic Chemistry Section

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JULY 1965 TO JUNE 1966

Edited by Horace S. Isbell

ABSTRACT

This report describes work in progress in the Organic Chemistry Section of the Analytical Chemistry Division of the NBS Institute for Materials Research. It includes certain historical material, presented to give perspective to the overall program. The following research areas are discussed:

Air-Pollution Studies. Work in this field is directed toward the study of the oxidation of polycyclic aromatic hydrocarbons. Results are reported for the oxidation of naphthalene, anthracene, phenanthrene, pyrene, and perylene with a variety of oxidants. The results obtained with periodic acid as an oxidant are particularly noteworthy. Studies of photo-oxidation of the polycyclic hydrocarbons on silica gel, alumina, soil, and air-borne particulate matter have revealed, for each hydrocarbon, products that may arise in contaminated air under smog conditions.

Kinetic Behavior of Sugars in Solution. Pioneer work at NBS is reviewed with respect to recent interest in the relationship between conformation and chemical reactivity. In some instances, reactions take place either with a change in conformation or with a change in configuration. It has been shown that oxidation of beta anomers of aldopyranoses by bromine takes place in large measure directly, but oxidation of the alpha anomers takes place (a) by direct oxidation with change in conformation and (b) by way of anomerization. For an alpha (axial) anomer, the extent of the reaction that takes place by way of anomerization parallels the conformational stability of the anomer. Another important contribution in this area is an explanation, in terms of dipole moments, for the (previously observed) large influence of the hydroxyl group of C-3 on the conformation of the pentopyranose ring. The ring tends to assume that conformation in which this hydroxyl group is equatorially oriented, because the dipole—dipole interaction with the ring oxygen atom is then at the minimum. Other work reported on this project includes results from the study of mutarotation reactions in H_2O and D_2O, measurement of rates of enolization of sugars by hydrogen—tritium exchange, and a chromatographic method for ascertaining the distribution of carbon-14 in ^{14}C-labeled "α"-D-glucosaccharinic acid and in ^{14}C-labeled acetic acid.

Carbon-14- and Tritium-labeled Carbohydrates. Syntheses are reported for α-L-rhamnose-1-^{14}C, α-L-fucose-1-^{14}C, D-glucose-4-t, D-galactose-4-t, and D-glucitol-4-t. New and improved techniques are described for heavy-paper chromatography and for the non-destructive location of bands on developed chromatograms.

A unique application of a liquid scintillation counter was made in a study of the interaction of fluorescent hydrocarbons with oxygen. It was found that the scintillation properties of pyrene and 1,1'-bipyrene on exposure to a carbon-14 or a tritium source are extremely sensitive to the presence of oxygen. Mixtures of pyrene and 1,1'-bipyrene in toluene were found to be effective media for study of scintillation counting.

Characterization of Chemical Structure; Phenylhydrazono-phenylazo Tautomerism. The positions of the phenylhydrazono groups in xylo-4,5,6-trihydroxy-2-oxo-1,3-bis(phenylhydrazono)-cyclohexane were established by degradation. A series of new 4-oxo-1-phenyl-5-(phenylhydrazo)-3-pyridazine derivatives were prepared. A simple procedure was devised for preparing inososes from their phenylhydrazones by means of ion-exchange resins.

Synthesis of Research Materials: Cyclopentitols and Related Substances. Application of the nitromethane synthesis to a dialdehyde (previously prepared in this laboratory from 2,3-O-isopropylidene-D-lyxose) gave two nitrocyclopentitols which, on reduction, gave two unique aminocyclopentitol derivatives. The structures of these compounds were determined by chemical and physical methods.

Novel Research Materials. A highly efficient procedure was developed for introduction of nonterminal unsaturation into alditol derivatives. The infrared spectra of 60 carbohydrate acetamido derivatives were measured and critically evaluated, and no bands were found that could be correlated with the absence or presence of the pyranoid or furanoid ring.

Standard Reference Materials. Preparation of a series of standard reference carbohydrates was begun, and results obtained in the preparation of 1,2-O-isopropylidene-L-idose are reported.

Certain oil-soluble calcium and cadmium chelates were investigated for use as new metallo-organic standards. The solubilization technique previously developed is discussed.

Key words:
Air-pollution studies, oxidation of polycyclic aromatic hydrocarbons, sugars in solution, conformational analysis, carbon-14- and tritium-labeled carbohydrates, large-scale paper chromatography, phenylhydrazono-phenylazo tautomerism, cyclopentitols and related substances, unsaturated alditols, infrared spectra of acylamido derivatives, carbohydrate reference materials, metallo-organic standard reference materials.

1. AIR POLLUTION STUDIES

(R. S. Tipson, A. Cohen, and A. J. Fatiadi)

This work is supported, in part, by the Division of Air Pollution, Public Health Service, U. S. Department of Health, Education, and Welfare.

A. Introduction

Because of the presence of many polycyclic, aromatic hydrocarbons and certain of their oxidation products in polluted air, information was needed on such hydrocarbons. In addition, studies are being conducted on the oxidation of polycyclic, aromatic hydrocarbons found in polluted air. Reactions with a variety of oxidants are being examined, and the oxidation products (peroxides, aldehydic acids, quinones, etc.) are being identified.

B. Reference Compounds; Preparation, Purification, and Characterization

Before proceeding to study the oxidation of a hydrocarbon, a very pure sample of the hydrocarbon is required. Furthermore, a pure sample of each of the anticipated oxidation products is needed.

1. Techniques

In addition to the customary techniques of purification and characterization, the following have proved useful.

a. Adsorption Chromatography on a Dry Column [1].

The dry column is useful for preparative work, and its separative efficiency is reported to approach that of thin-layer plates. Use of pressure (nitrogen) with micro-columns facilitates development. Before use in dry columns, silica gel was treated with glacial acetic acid, washed with water

until neutral, and activated at 115 °C. In the course of the work, we found that silica gel G contains an impurity (or impurities), dissolved by methyl alcohol, that absorbs at 228 and 231 nm (nanometer), with a shoulder at 261 nm (in dichloromethane).

b. Visualization of Spots on Thin-layer Chromatograms. In visualization of the spots on thin-layer chromatograms, a spray of concentrated sulfuric acid (alone or in methyl alcohol), followed by heating, has been found useful; it reveals products not visible under ultraviolet light prior to spraying. Such spots are then further characterized by their appearance under ultraviolet light.

c. Characterization of Compounds. This was accomplished with the aid of visible, ultraviolet, infrared, and nuclear magnetic resonance spectrometry, and by gas—liquid chromatography (see figure 1).

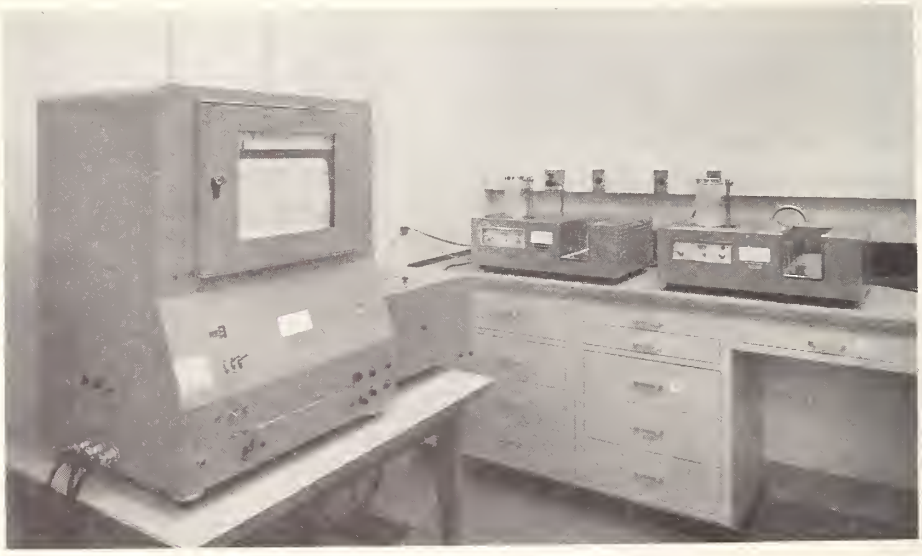


Figure 1. Portion of equipment used in measurement of ultraviolet, visible, and infrared spectra.

2. Polycyclic, Aromatic Hydrocarbons

Commercial sources for most of the polynuclear hydrocarbons that have been detected as air pollutants were located, and 22 of these were purchased. They are being examined for purity by ultraviolet, infrared, and chromatographic techniques.

a. Anthracene. Two commercial samples of anthracene were examined (1, reagent grade; 2, highly purified by zone melting). By use of petroleum ether saturated with 97% methyl alcohol (aqueous), and thin-layer chromatograms on commercial silica gel sheets (activated at 115 °C for 0.5 hr), sample 1 showed two impurities, but sample 2 showed no impurities. A sample of 2 was now applied, as a spot, to silica gel G and developed with benzene. Under ultraviolet light, only anthracene was detected. However, when the plate was sprayed with concentrated sulfuric acid and heated on a hot plate, a bluish-purple spot appeared that was not anthracene (which gives a grey spot); neither was it anthraquinone (which gives a yellow spot). This is under further study.

b. Naphthacene. This compound was found, by thin-layer chromatography on silica gel (with benzene as the solvent), to contain four impurities, and two of these reacted with iodine vapor. Recrystallization from (a) concentrated sulfuric acid and water, and (b) boiling xylene failed to remove these impurities. Attempted purification by vacuum sublimation eliminated two of the impurities, giving a product of mp 341 °C (in an open capillary tube, with darkening); lit. mp, 341 °C. This is under further study.

c. Pyrene. Commercial pyrene is slightly yellow; ordinarily it contains tetracene [2]. It has been found that the impurities can be removed easily by treatment, at room temperature, of an acetone solution of crude pyrene with solid

potassium permanganate (with stirring), followed by filtration using a small amount of decolorizing carbon. Concentration of the solution gave a white product which was washed successively with water saturated with $NaHSO_3$ and then with water, and air-dried in the dark. Crystallization of the material from glacial acetic acid gave a highly pure, colorless product (mp 152-154 °C), which showed one spot on thin-layer chromatograms.

d. Perylene. This compound was purified by chromatography on a column of silica gel (with glacial acetic acid as the solvent) giving product of mp 276-278 °C (lit. mp, 273-274 °C).

3. Oxidation Products of Polycyclic, Aromatic Hydrocarbons

a. Commercial Compounds. Oxidation products (needed for reference) that are commercially available were purchased and tested for purity. To aid in the identification of possible products, the infrared absorption spectra were recorded for commercial samples of: I, anthracene (1, reagent grade; 2, zone refined); II, anthraquinone (sublimed); III, 1,2-dihydroxyanthraquinone; IV, 1,4-dihydroxyanthraquinone; V, 1,5-dihydroxyanthraquinone; VI, 1,8-dihydroxyanthraquinone; VII, 2,6-dihydroxyanthraquinone; VIII, 1,2,3-trihydroxyanthraquinone; IX, 1,2,4-trihydroxyanthraquinone; X, 1,2,7-trihydroxyanthraquinone; XI, 1,2,10-anthratriol; XII, 1,4,9,10-anthratetrol; and XIII, anthrone.

To aid in subsequent identification, thin-layer chromatograms were run on commercial silica gel sheets (activated for 0.5 hr at 115 °C) for I, II, and IV, using benzene as the solvent. A mixture of these 3 compounds was readily resolved by use of this solvent.

Pure samples of certain hydroxy derivatives of anthraquinone were needed for use in identifying the products from the photo-oxidation of anthracene on particulate matter.

A sample of commercial 1,2-dihydroxyanthraquinone (III) was sublimed at 193 °C/0.27 mm; it then had mp 290-291 °C (lit. mp, 291-292 °C); after recrystallization from absolute ethyl alcohol, mp 288-291 °C, (4° per min); 290-292 °C (12° per min). Thin-layer chromatography of III on silica gel G with (1) petroleum ether saturated with 97% methyl alcohol gave a purple spot at the origin; (2) glacial acetic acid gave slight migration with tailings (all purple); and (3) 75:24:1 benzene—ethyl acetate—glacial acetic acid gave a yellow spot with a violet tail. When sprayed with magnesium acetate in methyl alcohol, the yellow spot became purple. When the acetic acid in the solvent mixture was replaced by formic acid, the separation was greater, but the materials had similar colors and overlapped. Thin-layer chromatography of III on kieselguhr G with benzene as the solvent gave a yellow front with a pale-violet tail; when sprayed with magnesium acetate in methyl alcohol, this became completely purple.

Partial separation of III was attempted on a dry column (6 mm i.d. x 22 in.) of silica gel, using 0.050 g of the material and a nitrogen pressure of less than 2 lb/in.^{2}; the developer was 75:24:1 benzene—ethyl acetate—formic acid. Pale-green and pale-violet impurities remained on the column. The material from the eluate was re-examined by thin-layer chromatography on kieselguhr G with benzene; the violet tail was no longer present. However, with silica gel G and 75:24:1 benzene—ethyl acetate—formic acid, it was found that complete separation had not yet been attained. This is under further study.

A sample of 1,8-dihydroxyanthraquinone (VI) was recrystallized from hot glacial acetic acid; mp 191-193 °C (lit. mp 193-197 °C; 196 °C). Thin-layer chromatography of

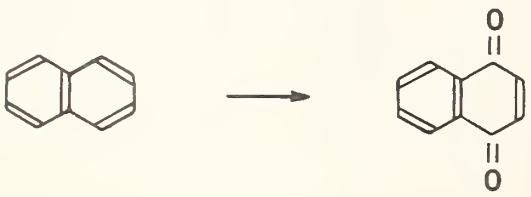
VI on silica gel G, with benzene as the solvent, showed one spot. However, chromatography on Whatman No. 1 paper, with 97% aqueous methyl alcohol and detection with methanolic magnesium acetate, gave a yellow and an orange spot which overlapped. This is under further study.

Work on the other compounds is in progress.

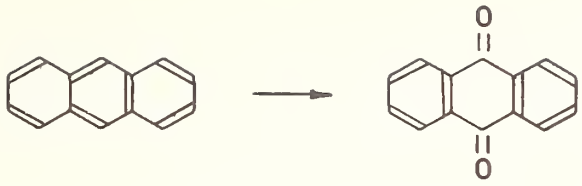
b. Miscellaneous Synthetic Compounds. Exploratory experiments were conducted on the oxidation of naphthalene, anthracene, phenanthrene, and pyrene with a variety of oxidants. The oxidation studies were directed to a study of (a) the reactions of polycyclic, aromatic hydrocarbons with oxidants heretofore not adequately investigated, and (b) reactions pertinent to the basic chemistry of the oxidation reactions, and the intermediates and oxidation products formed from polycyclic, aromatic hydrocarbons.

Significant results were obtained with periodic acid as the oxidant. This reagent has only once before been employed for oxidation of a compound (cholesterol) in this field. By oxidation of the hydrocarbons with periodic acid in a 1:4 ratio, the following gave the products in the yields cited in parentheses:

1. Naphthalene in glacial acetic acid gives 1,4-naphthoquinone (69 to 75%).



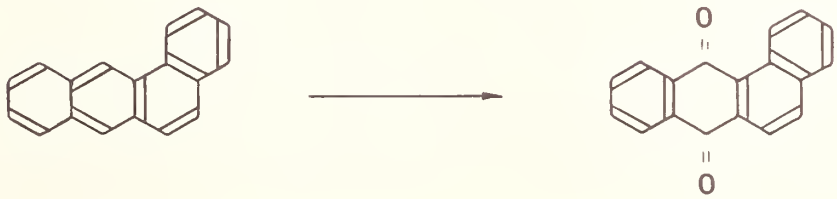
2. Anthracene in N,N-dimethylformamide gives anthraquinone (92 to 95%).



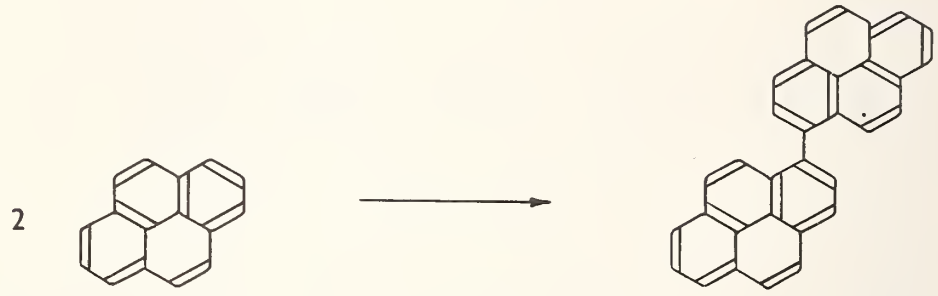
3. Naphthacene in N,N-dimethylformamide gives naphthacenequinone (80%).

4. Phenanthrene in p-dioxane gives phenanthrenequinone (45 to 55%).

5. Benz[a]anthracene in glacial acetic acid gives benz[a]anthracene-7,12-dione (20%).



c. Pyrene Derivatives. Pyrene in glacial acetic acid gave, with periodic acid, an unexpected condensation product (yield, nearly quantitative). The compound crystallizes in light-yellow plates that melt at 334 to 336 °C. The substance has a strong blue fluorescence; it has been identified as 1,1'-bipyrene, formed by hydrogen abstraction and dimerization.

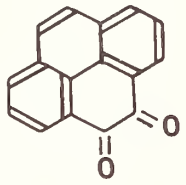


This compound had been synthesized earlier from 1-bromopyrene [3]. The prior synthesis was expensive because it required preparation of the 1-bromo derivative. The newly discovered preparation begins with readily available raw-materials and is very simple. Hence, the compound can now be readily made in any desired quantity. A manuscript describing the method is being prepared for publication.

The residue from the preparation of 1,1'-bipyrene gave a small yield of 1,6-pyrenedione, 1,8-pyrenedione, and an iodo derivative of unknown structure.

Oxidation of 1,1'-bipyrene with chromic acid in glacial acetic acid did not yield the anticipated quinones. Nitration gave a mixture, from which two crystalline nitro compounds have been separated. The fluorescence spectrum of 1,1'-bipyrene was found to be 35 times as strong as that of pyrene.

Pyrene with sodium periodate (catalyzed with ruthenium dioxide) in aqueous acetone is reported [4] to give an 11% yield of 4,5-pyrenedione in 16 days.



4,5—Pyrenedione

We have found that, if this oxidation is conducted in aqueous N,N-dimethylformamide containing 20 to 25 percent of glacial acetic acid, a similar yield (10%) of 4,5-pyrenedione is obtained after 36 to 48 hr. (If the oxidation is permitted to proceed for 72 hr, ring-opening occurs.) Sodium periodate may be replaced by sodium chlorate, which is cheaper.

Studies in progress have revealed that pyrene reacts readily with $Ce(SO_4)_2$, $K_2S_2O_8$, active manganese dioxide, oxygen on platinum, benzoyl peroxide, and m-chloroperoxybenzoic acid, whereas it appears inert to KBO_3, Bi_2O_3, and $HClO_4$.

The following pyrene derivatives were prepared by known methods, and their visible, ultraviolet, and infrared absorption spectra were recorded:

(1) 1,1'-bipyrene;

(2) 1-bromopyrene, 1-iodopyrene, 1-nitropyrene, and 1,6- and 1,8-dinitropyrene;

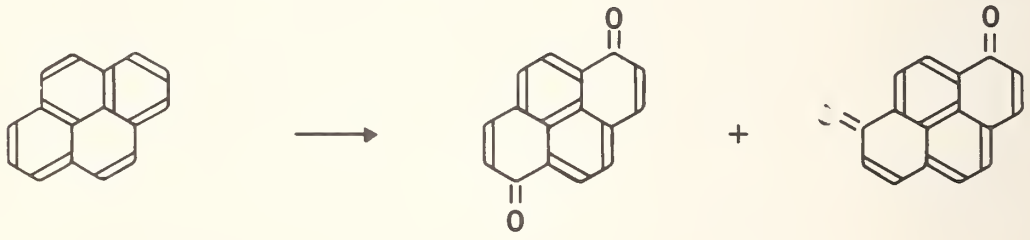
(3) 1,6-pyrenediol, and 1,6-pyrenediol diacetate; 1,8-pyrenediol, and 1,8-pyrenediol diacetate; and 4,5-pyrenediol;

(4) 1,6-pyrenedione, 1,8-pyrenedione, and 4,5-pyrenedione; and

(5) 1-pyrenol, 3-pyrenol, 4-pyrenol, and 1,3,6,8-pyrenetetrol.

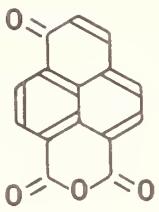
Arnold and Larson [5] reported that pyrene in boiling glacial acetic acid is oxidized with 30% H_2O_2 to give a mixture of 1,6- and 1,8-pyrenedione. This preparation was repeated, but close examination of the reaction product revealed that it was a complex mixture which could not be separated by chromatography on silica gel, deactivated Al_2O_3, or Florisil. The IR spectrum of the product showed peaks at 5.8 μm (indicative of the presence of opened-ring components) and 6.14 μm (conjugated, aromatic quinones).

An improved method for preparing the 1,6- and 1,8-pyrenediones was devised in which pyrene in glacial acetic acid is oxidized at 85 °C with 30% H_2O_2, catalyzed by V_2O_5. The oxidation proceeds vigorously, and temperature control is required for mild oxidation. The method gave a mixture of 1,6-pyrenedione and 1,8-pyrenedione in 60% yield. It was found that the formation of 1,8-pyrenedione is favored by low



Pyrene 1,6–Pyrenedione 1,8–Pyrenedione

temperatures. The reaction mixture, which was separated by column chromatography, also contained unreacted pyrene, "pyrenic anhydride," and hydroxylated pyrene derivatives.



1-Oxo-1H-phenalene-6,7-dicarboxylic anhydride ("Pyrenic anhydride")

A direct method for the separation of 1,6- from 1,8-pyrenedione has been developed and published [6]. The method consists of chromatography on a column of silica gel, using glacial acetic acid as the eluant. The 1,6-pyrenedione is then purified by chromatography on a column of activated alumina, with benzene as the solvent (see figure 2).

Figure 2. Column separation of oxidation products of pyrene.

Work was begun on the oxidation of pyrene with lead tetraacetate, a reagent not heretofore used with pyrene. The hydrocarbon, in glacial acetic acid, was treated for 92 hr at room temperature, with 2.4 moles of $Pb(OAc)_4$ per mole. The major product, identified as 1,6-diacetoxypyrene, was obtained in 40-50% yield.

The oxidation of pyrene with active MnO_2 was also studied. Active MnO_2 is known to oxidize labile primary alcohols to aldehydes, but heretofore no oxidation of hydrocarbons with MnO_2 had been reported. Reaction of pyrene with active MnO_2 in benzene gave 5% of 1,6-pyrenedione; reaction in glacial acetic acid gave 25% of 1,6-diacetoxypyrene and 7% of 1,6-pyrenedione.

In connection with our studies on the preparation and separation of pyrenediones, we have observed some unusual photochemical changes. When a solution of a pyrenedione in methyl alcohol is exposed to daylight, chemical changes occur which can be followed with the ultraviolet spectrophotometer. The changes in the absorption spectra in the visible and ultraviolet regions are summarized in table 1.

From table 1, it may be seen that the changes for 4,5-pyrenedione are greater than for 1,6-pyrenedione and 1,8-pyrenedione; there is complete disappearance of the conjugated quinoid structure, indicating extensive degradation and fragmentation of the molecule. For 1,6- and 1,8-pyrenedione, the appearance of strong bands at 243-248 nm possibly indicates change from the quinoid to a benzenoid structure (e.g., by formation of the corresponding dimethoxypyrenes). For the solution of 1,6-pyrenedione, strong bands appear at 352-355 nm, 379-383 nm, and 401-404 nm after only 3 to 4 days of exposure to daylight. For pyrenic anhydride, the rate of photo-induced

change was lower than for the pyrenediones; the appearance of strong bands at 230 and 328 nm possibly indicates change from the benzenoid to a conjugated quinonoid structure. The presence of new products was verified by evaporation of each of the solutions and thin-layer chromatography of the residue.

Table 1. Photochemically induced changes in the absorption spectra of pyrenediones in methanol.

Pyrenedione	Initial conc. (10^{-5} mole/liter)	Observed bands[a] (nm) 30 min after dissolution	90 days of exposure to daylight
1,6-	2.75	237, 265, 277, 400, 428, 448	239 (infl.), 242, 272, 282, 355, 383, 404, 447 (infl.)
1,8-	2.47	238, 282, 351, 366, 395, 458	243, 366, 425 (infl.)
4,5-	3.01	238, 254, 280, 291, 308, 322, 420	No bands; infl. at 239, 257, 304, 372, 415
"Pyrenic anhydride"	3.09	256, 367, 396 (infl.), 408 (infl.)	230, 251 (infl.), 328, 410 (infl.)

[a] infl. = inflection.

d. Perylene Derivatives. Reference compounds obtained by the chemical oxidation of perylene were needed for comparison with oxidation products resulting from its photo-oxidation.

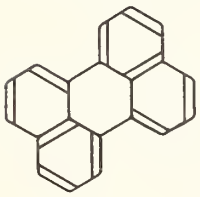
Oxidation of commercial perylene by published procedures [7,8] gave impure 3,10-perylenedione that could not be purified by many recrystallizations; thus, after successive recrystallization from pyridine—N,N-dimethylformamide,

N,N-dimethylformamide—o-dichlorobenzene, and nitrobenzene, the material still contained impurity showing bands at 5.65 and 5.80 μm in the infrared spectrum.

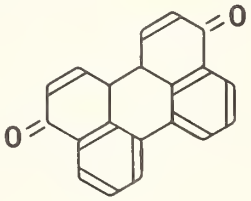
3,10-Perylenedione has now been obtained pure by adsorption chromatography using two different systems: (a) silica gel and glacial acetic acid, and (b) Darco adsorption carbon and nitrobenzene. The purified dione had mp 390-392 °C (decomp.); the bands in its ultraviolet and visible spectra were somewhat different from those recorded in the literature [8] (see table 2).

The bands observed for purified 4-oxo-4H-benz[de]-anthracene-7,8-dicarboxylic anhydride and 1,5-anthraquinone-dicarboxylic acid are listed in table 2, as these compounds are also formed in the oxidation.

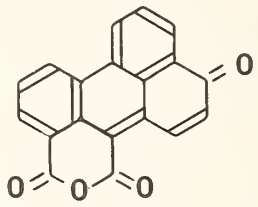
The preparation and purification of other perylene derivatives are in progress.



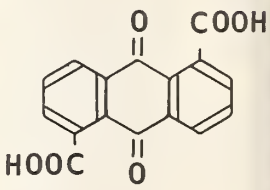
Perylene



3,10-Perylenedione



4-Oxo-4H-benz[de]-anthracene-7,8-dicarboxylic anhydride



1,5-Anthraquinone-dicarboxylic acid

e. Benz[a]anthracene Derivatives. A survey of the literature and of chemical catalogs revealed that none of the anticipated products of the oxidation of benz[a]anthracene are commercially available. Hence, we have started the preparation of such benz[a]anthracene derivatives for use as reference compounds.

Table 2. Ultraviolet and visible spectra[a] of some perylene derivatives.

3,10-Perylenedione			4-Oxo-4H-benz[de]anthracene-7,8-dicarboxylic anhydride			1,5-Anthraquinone-dicarboxylic acid
λmax (nm)			λmax (nm)			λmax (nm)
MeOH	Conc. H_2SO_4	Conc. H_2SO_4[8]	MeOH	Conc. H_2SO_4	Conc. H_2SO_4[8]	MeOH
					215	
				230sh	230	
238	241		238			
245sh	246sh			246.5		250
258			256.5	254sh		
266	265sh	271				
272sh				270sh		272sh
	285	285			278-283	
293sh			297sh	290	290	
307				307		
	314	315	311	312sh	311	
319			325			328
335	334sh			335		
	343sh	347		341		
350	352	354		352		
	362	362			363	
		375				
392sh			403			390
411				412sh		
415sh			422sh	432		
	488	489	488	485sh	470	
	548	543				
		569				
	580	584				

[a] sh = shoulder.

The first compound of interest, benz[a]anthracene-7,12-diol, was prepared as the diacetate, by the method of Badger and Lynn [9]. This entailed treating benz[a]anthracene-7,12-dione (previously prepared from benz[a]anthracene and $Na_2Cr_2O_7 \cdot 2H_2O$ in acetic acid) with zinc and boiling acetic anhydride for 1.5 hr, and hydrolyzing; a yield of more than 85% of crude product was obtained in two crops.

C. Photo-oxidation of Polycyclic, Aromatic Hydrocarbons on Solids

1. Introduction

In a preliminary communication [10] from this laboratory, it was shown that, for certain polycyclic, aromatic hydrocarbons, when a sample is adsorbed on a suitable solid (e.g., silica gel G) and then irradiated with ultraviolet light in the presence of air or oxygen, there may result a complex mixture of oxidation products. For example, in the mixture of products from pyrene, 1,6-pyrenedione and 1,8-pyrenedione were identified.

Presumably, some of the reactions of atmospheric pollutants involve oxidation of polycyclic hydrocarbons adsorbed on particulate matter (soot, dust, etc.) in the presence of oxygen (and other oxidants) and light. Consequently, the study has been extended, in order to permit us to identify other products and to determine the proportions of the products.

A simple and convenient procedure for studying this type of reaction has been devised. The procedure includes exposure of the hydrocarbon, adsorbed on a solid, to air and ultraviolet light, extraction of the products from the solid, and column or thin-layer chromatography of the extract.

2. Anthracene on Silica Gel

To permit us to evaluate and then select the techniques to be used in extending our study of the oxidation of polycyclic,

aromatic hydrocarbons on particulate matter, an investigation was started on the photo-oxidation of anthracene (as a model compound) on silica gel G.

A sample (10 g) of a 2.5% dispersion of zone-refined anthracene adsorbed on silica gel G was prepared by adding a chloroform solution of the hydrocarbon to the adsorbent and evaporating off the chloroform on a rotary evaporator. The resulting white powder was irradiated with ultraviolet light (366 nm) during 24 hr while being continuously stirred (egg-shaped, magnetic stirring-bar). The powder was then placed in a cellulose Soxhlet thimble (pre-extracted with boiling absolute methyl alcohol) and successively extracted with a variety of solvents; each extract was evaporated to dryness. For each fraction, the infrared spectrum was recorded, and the material was examined by thin-layer chromatography with benzene as the eluant. The results are given in table 3.

Thus, photo-oxidation of anthracene under the conditions described gives anthraquinone together with material which does not migrate when benzene is the eluant. This material gave a negative test with methanolic magnesium acetate, indicating the absence of phenolic derivatives of anthracene. No evidence for the formation of 1,4-dihydroxyanthraquinone was found.

The first two extracts (table 3) were chromatographed on a dry column [1] of silica gel, with benzene as the developer, and fractions were collected. Approximately 19.6 mg of "anthracene" was recovered from the photo-oxidation of 250 mg of anthracene, indicating that 92% had been oxidized in 24 hr. However, although the shape of the ultraviolet spectrum of this recovered "anthracene" was identical with that of pure anthracene (the molecular extinction coefficients were not

Table 3. Products from the photo-oxidation of anthracene (0.25 g) on silica gel.

Extract No.	Extractant	Material extracted Wt(g)	Color	TLC and IR show
1	cold hexane	0.130	pale yellow	only anthracene (I) + anthraquinone (II)
2	boiling hexane	0.085	light yellow	mainly II, plus some I[a]
3	boiling ethyl acetate	0.138	yellowish brown	mainly II, and a trace of I[b]
4	boiling methyl alcohol	0.033	yellowish brown	II, plus hydroxylated material[c]
5[d]	cold glacial acetic acid	0.027		inorganic acetate plus other material[e]

[a] And an unidentified IR band at 13.08 μm.
[b] And an unidentified IR band at 12.68 μm.
[c] Did not migrate in benzene; pale yellow in the visible, and greyish blue under ultraviolet light (253.7 nm).
[d] At this stage, the silica gel had a red tinge.
[e] On washing with water, this gave 0.0032 g of water-insoluble, reddish material which reacted with aqueous permanganate. It was insoluble in chloroform, but dissolved in hot, dilute, aqueous hydrochloric acid.

determined), thin-layer chromatography of it, with visualization under ultraviolet light, revealed the presence of a constituent which had a greenish-yellow fluorescence and which moved slightly faster than anthracene. Further visualization, by use of concentrated sulfuric acid aided by ultraviolet light, showed another constituent (possibly in trace amounts). Further fractionation, with 1:9 benzene—hexane, separated the faster-moving component (compound A) and a component (compound B) that moved slightly slower than anthracene.

Compound B had a blue fluorescence; its ultraviolet spectrum was very similar to that of anthracene, but the bands at the higher wavelengths had all shifted to higher wavelengths

by approximately 11 nm and three new bands appeared, at 273 (shoulder), 285, and 292 nm. A band at 255 nm (for anthracene) had shifted to 259 nm.

In the spectrum of compound A (as compared with that of anthracene), the band at 378 nm had shifted to 388 nm, and other bands had shifted to higher wavelengths. Also, the relative intensities of the bands at higher wavelengths were altered, so that the spectrum bears less resemblance to that of anthracene than does that of compound B. In addition, there was a great increase in the intensity of the bands at 287 and 291 nm. The bands in this region may be related to the "quinoid" bands [11]. In table 4, the bands are listed for anthracene and compounds A and B.

The infrared spectra of A and B do not match those of any anthracene oxidation products that we have yet recorded. Consequently, it will be necessary for us to record the ultraviolet and infrared spectra of many more hydroxylated anthracene and anthraquinone derivatives.

Table 4. Ultraviolet absorption bands[a] of anthracene and compounds A and B[b].

Anthracene (nm)	Compound A (nm)	Compound B (nm)
-	388	391
378	377 (sh)	-
-	368	370
358	348	352
341	337	334
325	327	321
311	ca. 317 (infl.)	ca. 309
292	291	292
-	287 (sh)	285
-	-	273 (sh)
254	255	259
248 (sh)	240 (sh)	252 (sh)
233 (sh)	235 (sh)	233

[a] In dichloromethane, using a Cary Model 14 spectrophotometer.
[b] sh = shoulder; infl. = inflection.

3. Pyrene on Silica Gel G

Pyrene adsorbed on silica gel G is colorless, but has a strong, pale-greenish fluorescence when examined in ultraviolet light. When the material is exposed in a thin layer to room light for some time, the pyrene mixture becomes tan and gradually darkens, losing all fluorescence. The rate at which the brown color develops depends in part on the presence of solvents and on the amount of exposure to ultraviolet light. When the material is exposed to ultraviolet light while moist with carbon tetrachloride, the tan color appears almost at once; but, when moist with ethyl alcohol, or after drying before being examined under ultraviolet light, development of a visible color-change requires several days. Although ultraviolet light accelerates the reaction, samples of the material kept in darkness show a gradual change in color, also.

The reaction appears to be a surface reaction, and its course is influenced by the adsorbent used. No visible change was observed with pyrene adsorbed on paper or on a plate coated with kieselguhr G, and no colored product has been obtained from pyrene on activated charcoal. On membrane filters prepared from cellulose esters (Millipore and Polypore filters) or from polyethylene (Mipor solvent-resistant filters), pyrene turns tan rather slowly. On chromatographic alumina, the reaction of pyrene and air gives a reddish color (instead of the yellowish-tan observed on silica gel G.)

By elution of the brown material formed from pyrene adsorbed on silica gel G, and chromatography of the eluate, five products were obtained in sufficient quantities to observe their UV spectra. Three of these are yellow, with yellow or red fluorescence on silica gel G, one is reddish, and one is

purple. (From pyrene on alumina, the proportions of the yellow products obtained are relatively small and the purple product seems to preponderate.) Work is progressing on (a) the separation of the reaction products from the large excess of unreacted pyrene and (b) the identification of these products.

Studies are also in progress on the role of the adsorbent, the effect of light, the effect of concentration, and other variables.

Two of the products described in table 5 have been identified as 1,6-pyrenedione and 1,8-pyrenedione by comparison with authentic samples prepared by methods reported in the literature [6]. Spectral studies suggest the presence of at least twelve other substances in the reaction mixture. Of these, five have been obtained in sufficiently pure form to be characterized by their spectra, but these have not yet been identified. In light of the reports of the production of dihydroxyanthraquinones by the photochemical oxidation of anthracene on alumina [12], it is possible that the polar, purple and gray materials obtained from pyrene may be hydroxylated quinones.

In an attempt to identify certain dark-purple compounds separated by thin-layer chromatography, preparations of 1,6-pyrenediol, 1,8-pyrenediol, and 1,4-pyrenedione were undertaken for use as reference compounds. The diols were separated in the form of the crystalline acetates. The 4,5-dione was separated by column chromatography.

Table 5. Some products of the oxidation of pyrene on silica gel.

Appearance on silica gel G		Approximate R_f values on silica gel G		Prominent absorption bands (nm, in 95% ethyl alcohol)				
Room light	Ultraviolet light	With EtOAc	With CCl_4					
Orange-red (1,8-pyrenedione)	Red	0.7	0	238	352		465	
Yellow (1,6-pyrenedione)	Dark red	0.8	0	239	402,429		450	
Yellow	Dark red	0.9	0.2	234	288	376	399	
Colorless	Blue-white	0.95	0.8	242	276	343	378	
Colorless	White	0.2	0	252,268,276			347	362
Tan	Dark, yellow when dilute	0-0.05	0	241	276	344		
Purple	Dark	0.1-0.15	0				450	555

For small-scale work, the proportion of hydrocarbon to particulate matter was 300 mg of pyrene adsorbed on 5 g of silica gel G; this was spread in a thickness of ca. 300 μm on glass plates (20 x 20 cm), giving an approximate distribution of 0.75 mg of pyrene per cm^2. The coated plates were irradiated directly with a UV lamp (>270 nm) by double exposure; namely, 10 to 12 hr on one side and then 8 to 10 hr on the other. By double exposure, the yield of oxidation products was raised by 25 to 35%.

For work on a larger scale, a solution of 3 g of purified pyrene in hexane was mixed with 50 g of a solid support (e.g., silicic acid), the hexane was evaporated off, and the solid was dried and powdered. The powder was stirred magnetically (egg-shaped stirrer) in a stoppered Pyrex flask under irradiation (>270 nm) for 100 to 120 hr (or 200-240 hr), and then extracted with warm glacial acetic acid. The extract was evaporated to dryness, weighed, and resolved by column chromatography with a suitable solvent. The resulting fractions were checked by thin-layer chromatography, identified, evaporated to dryness, and weighed.

By treatment of 3 g of pyrene on 50 g of silicic acid for 120 hr, 2.95 g of material was recovered. Thin-layer chromatography revealed at least 8 spots; three were fluorescent, two were purple, one was orange, one was yellow, and there was a dark-brown spot which did not migrate with the solvent. By column chromatography of this mixture (2.95 g) on silica gel with glacial acetic acid as the solvent, there was obtained 2.81 g of unreacted pyrene and ca.0.14 g (ca. 4.5%) of oxidation products. Further column chromatography of this mixture (0.14 g) gave 0.042 g of 1,6-pyrenedione

(1.4% oxidation), 0.036 g of 1,8-pyrenedione (1.2%), 0.008 g of 1,6-pyrenediol (0.3%), 0.012 g of 1,8-pyrenediol (0.4%), and a small residue that probably contained other hydroxylated pyrenes.

From the preliminary results on the photo-oxidation of pyrene on silicic acid and on silica gel, the following conclusions were drawn.

(1) The ratio of 1,6- to 1,8-pyrenedione is approximately 1:1.

(2) The proportions of products may depend on the nature of the solid support; for example, the proportion of 1,8-pyrenedione produced with silica gel differed from that obtained with silicic acid.

(3) More of the hydroxylated pyrene material was produced by 220-265 nm radiation than by the radiation >270 nm.

4. Pyrene on Other Solids

The oxidation of pyrene on alumina was found to yield relatively small proportions of the pyrenediones and considerably more of the darker-colored materials. A purple substance isolated from this reaction has an absorption band at a longer wavelength (580 nm) than was observed with any of the products obtained on silica gel. The isolation of other products of this reaction is continuing.

Preliminary studies have shown that pretreatment of the silica gel or alumina with acid or base has a considerable effect on the character of the reaction products. Pretreatment with a solution of potassium hydroxide appears to increase the proportion of darker-colored materials produced, whereas, after treatment with hydrochloric acid, a browner color is observed.

Pyrene shows considerable change in color on colloidal silica also, and some changes on zinc oxide or magnesium oxide. Other hydrocarbons which have been found to undergo color changes on silica gel or alumina include anthracene, phenanthrene, benz[a]anthracene, triphenylene, and perylene.

Investigations on the nature of these reactions are continuing. The formation of cation radicals, such as is observed on some silica—alumina catalysts [13] may be involved in the mechanism of the reaction. The products of oxidation on zinc oxide, asbestos, or acetylated cellulose (21%) were found to be quite different from those previously found for oxidation on silica gel or alumina. In particular, very little of the 1,6- and 1,8-pyrenediones (which are among the major products obtained on silica gel or alumina) were found. It was discovered that pre-treatment of alumina or silica gel with acid increases the proportion of diones obtained in the reaction, whereas pre-treatment with a base decreases the proportion of diones (especially the 1,6-dione), and increases the proportion of anionic purple and pink materials related to the diones.

5. Perylene on Silica Gel G

A sample (200 mg) of purified perylene (mp 278-280 °C) was dissolved in 15 ml of hot toluene (reagent grade) and mixed with 6 g of silica gel G. The slurry was spread on a glass plate (20 x 20 cm) which was then heated at 90 °C for 10 min. The layer was now illuminated by ultraviolet radiation (>270 nm) at 30 to 35 °C by the double-exposure procedure for 72 hours. By this time, the original bluish-yellow fluorescence had lost its intensity, and the white background had become tan in color. The mixture was extracted with

acetone, the extract was evaporated to dryness, and the residue was examined by thin-layer chromatography on silica gel G, using 18:1:1 benzene—*N*,*N*-dimethylformamide—glacial acetic acid. From the top to the bottom, at least five spots were distinguishable: (1) yellow (strong; light-blue fluorescence)——unreacted perylene; (2) orange-brown (weak)——unknown; (3) light brown (strong; light-green fluorescence); (4) yellow (medium strong; not fluorescent); and (5) dark brown (strong; not fluorescent)——unknown.

Spot 3 was tentatively identified as consisting of 4-oxo-4*H*-benz[*de*]anthracene-7,8-dicarboxylic acid or its anhydride. Spot 4 was positively identified as 3,10-perylenedione by comparison of R_F values and of ultraviolet and visible spectra with those of an authentic specimen.

6. Other Hydrocarbons on Silica Gel G or Alumina

Work was begun on the photochemical oxidation of fourteen other hydrocarbons on particulate matter. Phenanthrene, triphenylene, chrysene, and picene did not show rapid photochemical oxidation when adsorbed on either silica gel or aluminum oxide. These four hydrocarbons are structurally related and possess no highly active positions. Many of the other hydrocarbons were quite similar in behavior to pyrene. Benz[*a*]anthracene and benzo[*a*]pyrene reacted more rapidly than did pyrene.

A more quantitative study was made of the effects of experimental variables on the oxidation of pyrene adsorbed on alumina and silica gel and in addition, the behavior of pyrene, benz[*a*]anthracene, and similar hydrocarbons on a variety of other particulate materials was investigated.

Studies were continued on the identification and

quantitative determination of the products formed by treatment of benz[a]anthracene (adsorbed on silica gel and aluminum oxide) with light and oxygen.

7. Anthracene, and Pyrene, on Air-borne, Particulate Matter from Cincinnati, Ohio

A sample of air-borne, particulate matter, collected in Cincinnati, Ohio by the Taft Sanitary Engineering Center and freed of polycyclic aromatic hydrocarbons by exhaustive extraction with benzene and then with acetone—methanol, was presented to us by Elbert C. Tabor.

A sample of anthracene adsorbed on this particulate matter was photo-oxidized for 24 hr under ultraviolet light (366 nm). The resulting mixture was successively extracted with benzene, acetone, and methyl alcohol. The product was examined by thin-layer chromatography on silica-gel sheets (Eastman Chromagram), with the following developers: hexane, benzene, chloroform, ethyl acetate, acetone, and glacial acetic acid. The spots were visualized under ultraviolet lamps at 366 and 353.7 nm. Most of the material was unchanged anthracene; a small proportion was anthraquinone; and some polar material (which may have come from the silica gel itself) remained at the origin.

Pyrene (0.5 g) adsorbed on this particulate matter (5 g) was photo-oxidized for 240 hr at 32 ±3 °C with ultraviolet light filtered through Pyrex glass ($\lambda > 270$ nm). Successive extraction of the reaction mixture with acetone and toluene yielded a trace of oxidation products. Examination of this on a thin-layer chromatogram (silica gel G and ethyl acetate) revealed a very weak, yellow spot of 1,6-pyrenedione (λ_{max}^{MeOH} 429 and 449 nm); no other components could be identified.

8. Pyrene on Maryland Soil

A sample of Maryland soil was freed of polycyclic aromatic hydrocarbons by successive, exhaustive extraction with water, acetone, toluene, and ethyl alcohol, and was dried at 40 °C to constant weight.

Pyrene (2.5 g) was adsorbed on 50 g of this Maryland soil, and irradiated for 240 hr at 34 ± 3 °C in the presence of air, as for pyrene on Cincinnati dust. Extraction of the reaction mixture with acetone and then with toluene gave a 1.2 to 1.8% yield of reaction products (the balance being unreacted pyrene). Thin-layer chromatography (silica gel, ethyl acetate) revealed at least 8 spots. On spraying the chromatogram with 10% sulfuric acid in methyl alcohol, and heating for 10 min at 90 °C, fluorescent spots No. 1 and 7 disappeared, indicating that these spots consisted of hydrocarbons. Spot No. 7 is slow moving, and has a strong blue fluorescence. Spot No. 1 is unreacted pyrene. The work is being continued.

9. Pyrene on a Silica Gel—Alumina Mixture

Alumina was pretreated by washing with acid and then with water; it was dried to constant weight. A 1:1 mixture of silica gel and this alumina was prepared, and pyrene was adsorbed on it. The mixture was irradiated, as in the previous experiment, at 32 ± 3 °C for 240 hr. On extraction, pyrene was revocered, together with a 3.5 to 3.8% yield of oxidation products. By thin-layer chromatography, the major components were identified as 1,6-pyrenedione and 1,8-pyrenedione. Trace amounts of diols were detected.

2. KINETIC BEHAVIOR OF SUGARS IN SOLUTION

(H. S. Isbell, H. L. Frush, and C. W. R. Wade)

A. Introduction

Differences in rates of reaction have been widely used by chemists for determination of structure and study of reaction mechanisms, but techniques based on reaction kinetics have not been generally recognized as an important and distinct area of analytical chemistry. In many instances, kinetic methods open up exciting new avenues of research, and reveal previously unknown phenomena.

In our last report, we pointed out that we are studying, by means of radiochemical and isotopic methods, the main processes involved in the transformations of sugars in alkaline solution. These include (a) conformational changes, (b) mutarotation reactions, (c) enolization reactions, (d) condensation and cleavage reactions, and (e) rearrangements of the saccharinic acid type. Knowledge of the manifold reactions that the sugars undergo in solution is essential for adequate understanding of biological chemistry, because all areas of biological chemistry touch in some manner on carbohydrate chemistry. For this reason, some of our earlier work on the composition of sugar solutions will be reviewed, and then some of our work in progress will be described briefly.

B. Conformational Analysis

1. Pioneer Work at NBS

As pointed out on page 46 in the Westheimer Report [14], in recent texts, and in review articles [15 to 17], consideration of the conformation, or shape, of molecules in solution has become extremely useful for understanding chemical reactions and for directing them to desired products. In view of the extremely important position that conformational analysis has

assumed, it is of interest to review in historical perspective our work on this subject.

Our laboratory was the first to attempt to correlate the effect of conformation on reaction rates and properties of organic compounds. In 1929, Haworth suggested that certain sugar derivatives have a strainless ring structure, and that several forms or "conformations" are possible. In 1930, the ring structure of the sugars was the subject of a bitter controversy. Hudson contended from comparisons of optical rotatory power, that certain sugars and glycosides which Haworth had shown to possess 1,5 rings do not have this structure, but have 1,3 and 1,4 ring structures. Studies of the oxidation of the sugars with bromine did not support Hudson's hypothesis, and Isbell sought to ascertain how the sugars that Hudson considered exceptional differ one from another. Thus, in 1930, Isbell [18] wrote, "The parallelism between the optical rotations of the α- and β-forms of 4-glucosido-mannose with the corresponding forms of mannose indicates that neither α- nor β-mannose has a 1,4 ring structure. We cannot definitely state at this time which form of mannose has a structure similar to the normal form of glucose. It is entirely possible that Hudson's classification of ring structures is in reality a classification of a new type of isomerism. It seems reasonable to presume that there is a difference in the structures of α- and β-mannose, the exact nature of which must remain for the future to determine."

In the next few years, numerous sugars and sugar derivatives were prepared and studied, to reveal unknown structural differences and to ascertain the effect of configuration on these differences. The following quotation from the Journal of Research NBS in 1937 [19] is noteworthy, because it was the

first attempt to correlate the effect of conformation on reaction rates and to classify sugar derivatives according to what we now call axial and equatorial substituents.

"CONFORMATION OF THE PYRANOSE RING

It was pointed out by Haworth that various geometric forms are possible for the pyranose ring. Thus if the carbon and oxygen atoms comprising the ring were coplanar the valence angles would be larger than 109°, required to give a strainless ring; if the ring is strainless the valence angles for the carbon atoms will be about 109° and the carbon and the oxygen atoms cannot lie in a single plane. As measurements of dipole moments for simple substances containing the pyranose ring reveal that the oxygen valence bonds form an angle considerably less than 109°, it seems probable that the pyranose ring is bent or puckered. Although ten or more strainless ring structures are possible they can be reduced to three types, as represented in fig. 4: (I) a trans structure in which four atoms lie in one plane, and the two remaining atoms lie on opposite sides of this plane; (II) a cis or boat-shaped structure in which 2 atoms lie on the same side of the plane formed by the four remaining atoms; (III) and (IV) structures in which the carbon atoms are coplanar and the ring-oxygen is not in the same plane. The monoplanar strained model is represented by (V).

The X-ray investigations of Cox and others indicate that the carbon atoms forming the ring in many sugars are coplanar, while the ring-oxygen lies outside the plane of the carbon atoms. Two structures of this character are possible for each hexose; in one of these the sixth carbon lies approximately in the plane of the carbon ring, as in (III); in the other it lies opposite the ring-oxygen, directed towards the first carbon as in (IV). At this time there is not a satisfactory chemical method for deciding which models correspond to the free sugars. Several sugar derivatives exist which for stereomeric reasons are limited to certain models. Thus trimethyl 1,4-anhydroglucopyranose requires the conformation of a boat-shaped ring in which carbons 1 and 4 lie in the trough. The model which will serve for this compound, however, will not serve for 2,3-4,6-diacetone α-methyl mannopyranoside because in order to form a 1,4-anhydro derivative the hydroxyl on carbon 4 (which is the same

for mannose and glucose) must be directed towards carbon 1 which is in opposition to carbon 6. It is thus apparent that the conformation of the pyranose ring is not the same for all sugar derivatives. It is possible that the various modifications exist in dynamic equilibrium. Consequently the particular modification which results after condensation with a second group is not necessarily the same modification as that of the original

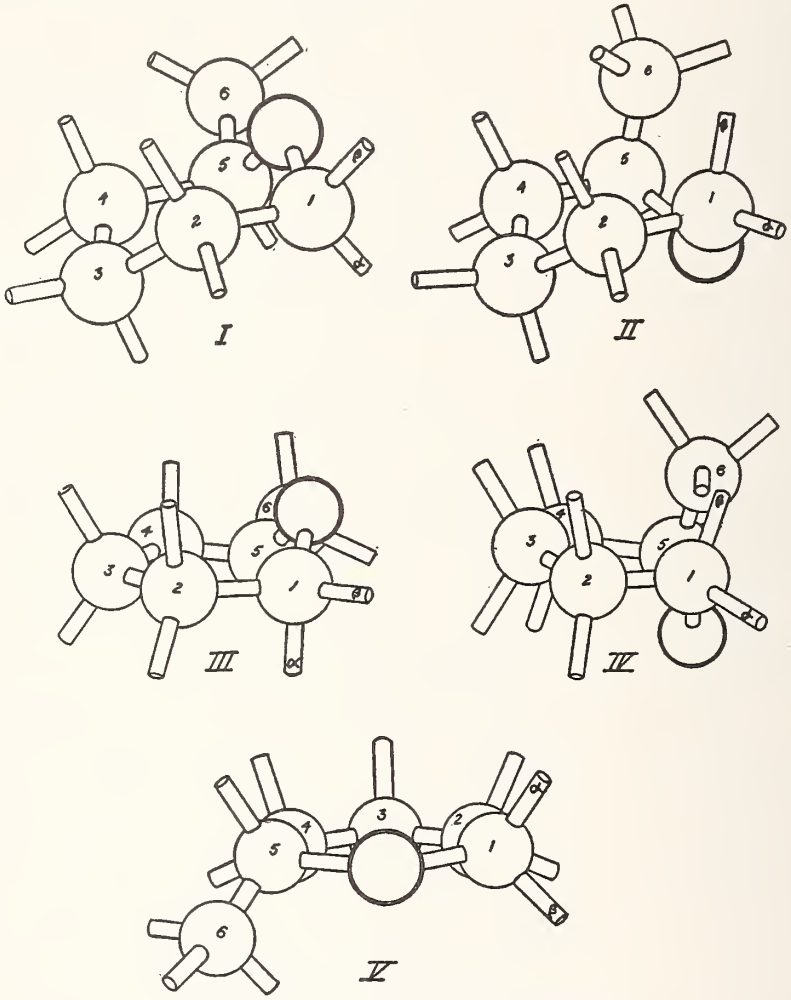


FIGURE 4.—*Models for the pyranose ring.*

sugar. It seems probable, however, that structurally related substances such as α-l-arabinose, α-d-galactose, α-d-fucose, α-l-fructose, α-d-α-mannoheptose, etc., have similar ring conformations. It can be observed from models (III) and (IV), illustrated in figure 4, that the ring-oxygen might be directed to either side of the plane of the carbon atoms. But since no more than two isomers of any one ring type are known, it seems probable that

each free sugar occurs in only one ring conformation. The separate sugars, however, may have different conformations.

If the sugars had the coplanar ring, the alpha and beta positions would be symmetrically located with respect to the plane of the carbon-oxygen ring, and there would be no fundamental difference between the alpha and beta sugars. But if the oxygen and carbon atoms forming the ring did not lie in one plane, the alpha and beta positions would not be symmetrically located with respect to the carbon-oxygen skeleton, and would be influenced to different degrees by the oxygen of the ring. Presumably this unequal influence would result in differences in the reactivity of the alpha and beta modifications. The positions of the hydrogen and hydroxyl groups of carbons 2,3, and 4 might also affect the reaction rates of the alpha and beta sugars, either directly or by causing alterations in the conformation of the pyranose ring to give various strainless ring isomers. Thus a comparison of the reaction rates of the alpha and beta pyranoses should provide information about the conformation of the pyranose ring."

In the same paper, it was pointed out that, "In every case the beta aldose is oxidized by bromine water more rapidly than the alpha, and the equilibrium solution contains two or more substances which differ in reaction rates," and that "The marked difference in the reactivity of the alpha and beta sugars supports the hypothesis that the pyranose ring is dissymmetric and contains a strainless rather than a monoplanar strained ring. Attention is called to 1,4-anhydroglucopyranose and to 2,3,4,6-diacetone α-methyl mannopyranoside which for stereomeric reasons cannot have like strainless ring structures. Thus the conformation of the ring may vary from sugar to sugar . . . Consequently, rotational differences may not correspond to the rotations of the separate asymmetric carbons but rather to the difference in the rotations of the molecules as a whole." As a result of these ideas, Isbell sought to classify the alpha and beta sugars according to what we now

call the axial and equatorial positions of the anomeric hydroxyl group. At this early date, the concept of conformation was just beginning to emerge, and there was no basis for actual assignment of a specific conformation.

Attempts were, however, made to correlate optical rotation, mutarotation, and bromine oxidation measurements for reducing sugars having like arrangements for the five carbon atoms comprising the pyranose ring. The beginning of conformational analysis was made by Isbell and Frush in 1940, in a paper [20] entitled, "Alpha and Beta Methyl Lyxosides, Mannosides, Gulosides, and Heptosides of Like Configuration." At this time, an unexpectedly large influence of the configuration at carbon atom 3 on the properties of the glycosides was noted, and the close resemblance of each pentose to the corresponding hexose which has the trans configuration for carbon atoms 3 and 5 was pointed out. After 26 years, we can now understand and rationalize this important observation (see page 37). Interest in conformation continued at NBS, and many sugars and sugar derivatives were prepared to study this subject in the period 1937 to 1947.

Papers by Hassell and Ottar [21] in 1947 and by Reeves [22] in 1949 established the conformations which are now accepted for the more important sugars. Modern interest in the relationship between conformation and chemical reactivity was awakened by Barton [23] in 1950, when he directed attention to differences in the chemical behavior of equatorial and axial substituents of cyclohexanes (see page 71 of [15]).

Even before that time, the Section had in progress a project supported by the Office of Naval Research entitled, "The Investigation of the Structure, Configuration, and Ring Conformations of the Sugars and Their Derivatives by Infrared

Absorption Measurements."

Work initiated under this project led to the publications cited in reference [24].

2. Current Work at NBS

The present intense interest in conformational analysis arises from recognition that consideration of conformation greatly enhances the abilities of chemists to direct chemical reactions to desired products. Knowledge of conformational free-energy differences has enabled chemists to predict the favored conformation, and new techniques, particularly NMR, have provided the experimental means for definitely establishing the particular conformation adopted by an organic compound.

a. Oxidation of Aldoses by Bromine. Conformation is a dynamic property; non-bonding interactions determine the stability or instability of conformers, but a change in conformation may occur during the reaction. In some instances, reactions may take place either with a change in conformation or with a change in configuration.

In development of this concept, Isbell [25] pointed out that the oxidation of beta anomers of aldoses by bromine takes place in large measure directly, but oxidation of the alpha anomers takes place (a) by direct oxidation with change in conformation and (b) by way of anomerization. Recently, it was found [26] that "In each instance, the extent of the reaction that takes place by way of anomerization parallels the conformational stability of the anomer. Aldoses which have an axial hydroxyl group at C1 in a highly stable conformation (e.g., α-D-glucopyranose-CA* and α-D-galactopyranose-CA), resist the change in conformation necessary for

*The system for indicating conformation is described in [27].

direct oxidation, and react largely through anomerization (change in configuration). Aldoses of lower conformational stability (e.g., α-D-lyxopyranose-CA, α-D-talopyranose-CA, and α-D-mannopyranose-CA) react chiefly by a change in conformation without a change in configuration.

The rate of the direct reaction depends on the difference in free energy between the reactants in the ground state and in a transition state in which the anomeric oxygen atom lies in the same plane as C1, C2, C5, and the ring oxygen atom. The free energy required in order to reach this transition state depends on the structure and the configuration. With molecules having an axial hydroxyl group at C1 in a highly stable conformation, much energy is required for so changing the conformation as to bring the hydroxyl group into an equatorial position. α-D-Glucopyranose and α-D-galactopyranose have high stability in that chair conformation having an axial hydroxyl group at C1; this fact accounts for the low rates of oxidation and the tendency of these sugars to react through a change in configuration rather than conformation."

It should be noted that the rate of oxidation does not depend on the free energy of the sugar, but rather on a free-energy difference. In fact, some of the most stable sugars are oxidized at the highest rate. Differences in the rates for the equatorial anomers may be ascribed to steric hindrance, but such hindrance may also be considered to be a factor in the energy of activation. In the oxidation of aldoses with bromine, the relative importance of the two reaction-paths depends on the reaction conditions; and hence, the relative rates, as such, are of little consequence. It is highly desirable, however, to understand the basic phenomena involved. Many reactions involve concurrent changes in conformation and

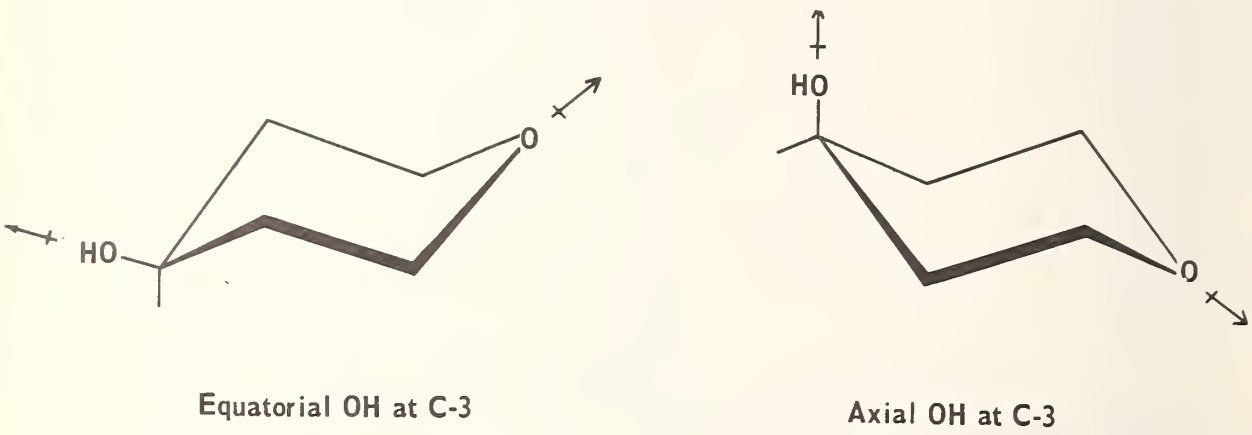
configuration, and development of methods for measuring the two types of change is highly important.

We have recently devised a new method for determining the two types of reaction in the oxidation of aldoses with bromine. The method is based on a difference in the isotope effect for anomerization of the aldose in H_2O and D_2O, and the isotope effect for the direct oxidation of the aldose. There are wide differences in the isotope effects, and the relative importance of the paths may be estimated from these. If, as claimed by Barker, Overend, and Rees [28], the rate-determining step in the oxidation of α-aldoses consists very largely of anomerization of the α-aldose to the β-aldose, the rates of oxidation of the α-aldoses in D_2O should parallel the rates of anomerization. Measurements in progress will ultimately answer this question.

b. Influence of the Configuration of Carbon Atom 3 on the Conformation of Aldopentopyranoses. Each pentose is configurationally related to two hexoses, which differ in the configuration of C-5. Isbell and Frush [20] pointed out that each pentopyranose closely resembles, in properties, the corresponding hexopyranose in which C-3 and C-5 have trans configurations. Thus, L-arabinose, D-xylose, D-lyxose, and L-ribose respectively resemble D-galactose, D-glucose, D-mannose, and D-talose more closely than they resemble L-altrose, L-idose, L-gulose, and L-allose. It was also noted that "carbon 3 lies opposite the oxygen of the ring and its attached groups appear to be in a particularly favorable position to influence the conformation of the ring" (page 133 of [20]). This influence may now be explained by conformational analysis.

In the hexopyranose series, when the configurations of C-5 and C-3 are opposite, both the CH_2OH group of C-5 and the OH

group of C-3 are equatorial in the stable, CA conformation. In the pentopyranose series, the large influence of the OH group of C-3 may be attributed to a dipole—dipole interaction with the ring oxygen atom. The molecule assumes that conformation in which this dipole—dipole repulsive force is at a minimum, i.e., in which the OH group of C-3 is equatorially oriented (see figure 3). The conformation then resembles the CA



Equatorial OH at C-3

Axial OH at C-3

Figure 3. Influence of OH group at C-3; direction of dipole moments.

conformation of the hexopyranose having opposite configurations for C-3 and C-5. This concept accounts for the previously noted influence of the configuration of C-3 on the conformation of the pentopyranoses. The explanation somewhat resembles that developed by Lemieux and Chü for the "anomeric effect" (see page 375 of [15] and page 738 of [17].

C. Mutarotation Reactions

Recent progress in NMR and gas chromatography of silyl ethers has resulted in a renaissance of interest in the mutarotation reaction and in the study of the composition of sugar solutions. In continuation of our study of anomerizations and pyranose-furanose interconversions, we have made numerous

new measurements of the mutarotations of D-fructose and D-glucose in D_2O and H_2O. The work, part of which was described in our last report, has been incorporated in a manuscript entitled: "Deuterium Isotope Effects in α-β-Pyranose and Pyranose-furanose Interconversions." An abstract of the paper is as follows:

"Rates of mutarotation, activation energies, catalytic coefficients, and isotope effects are reported for the mutarotations of α-D-xylose, α-D-glucose, and β-D-fructose in H_2O and in D_2O at 20° and at 3.9 °C. The isotope effects (k_H/k_D) for the mutarotation of β-D-fructose (pyranose-furanose interconversion) parallel in striking manner the isotope effects for the mutarotation of α-D-glucose (α-β pyranose anomerization). For both sugars, the isotope effect is highest in neutral, and lowest in strongly acid, solution; the parallelism of the values obtained for the isotope effects for the two reactions under a variety of conditions shows that the rate-determining steps in the two reactions are much alike. Presumably, in both instances, the overall mutarotation arises from concurrent reactions operating on different species of the sugar and showing substantially different isotope effects.

The following isotope effects were found for the mutarotation of α-D-glucose at 20 °C:

$$k_{H_3O^+}/k_{D_3O^+} = 1.39;\ k_{H_2O}/k_{D_2O} = 3.87;\ \text{and}\ k_{OH^-}/k_{OD^-} = 3.03,$$

where k_{OH^-} and k_{OD^-} represent the effects of base catalysts in H_2O and D_2O, respectively.

The following isotope effects were found for the mutarotation of β-D-fructose at 20 °C:

$$k_{H_3O^+}/k_{D_3O^+} = 1.39;\ k_{H_2O}/k_{D_2O} = 3.87;\ \text{and}\ k_{OH^-}/k_{OD^-} = 2.88.$$

Mechanisms are presented for the several concurrent acid- and base-catalyzed mutarotation reactions."

D. Enolization Reactions

1. Measurement of Rates of Primary Enolization

With reference to our study of the behavior of sugars in alkaline solutions, the method which we developed for measuring the primary enolization of sugars mentioned in our last report [29] has been applied to D-glucose under a variety of conditions, and to several other sugars under limited conditions. The method involves treating the sugar with a base catalyst in the presence of tritiated water, whereby the sugar molecule is converted into an enediol which, upon treatment with acid, reverts to an isomerized sugar mixture containing one tritium-labeled atom for each enediol structure formed. All labile tritium is removed by freeze-drying, and the sample is assayed for the amount of tritium fixed. The percent enolization is calculated by assuming that one tritium-labeled atom is fixed for each enediol molecule formed. The following example illustrates the technique: Eighteen mg (0.1 mmole) of D-glucose is placed in a 4-ml vial, fitted with an air-tight rubber cap through which hypodermic needles may be inserted. Two needles are inserted in the stopper, nitrogen (free from carbon dioxide) is passed through the vial for five min at a rate of 50 ml/min to displace the air. Next, 0.5 ml of 0.035 N NaOH (2.212 mCi of tritium/ml) is injected into the vial and, after removal of the needles, the sealed vial is placed in a test tube containing enough water to provide for heat transfer but not enough to reach the top of the vial. The tube is placed in the constant-temperature bath illustrated in figure 4. At the end of the reaction time, the vial is removed from the bath; two hypodermic needles are inserted, and carbon dioxide is passed into the vial for 5 min. The solution is left in an

Figure 4. Apparatus used in the determination of enolization by radiochemical techniques.

atmosphere of carbon dioxide for 30 min, after which 3 drops of glacial acetic acid are added.

The acidified solution is quantitatively transferred with 15 ml of water to a 125-ml, long-necked, round-bottomed flask, and freeze-dried. After three additional freeze-dryings, by use of 15 ml of water each time, the residue in the flask is dissolved in exactly 5 ml of water, and three 0.1-ml aliquots are removed and assayed for tritium.

All assays were carried out in a liquid scintillation spectrometer, by use of a p-dioxane scintillation solution and the technique described in our last report (page 30 of [29]).

The ratio of the counts per second per mmole of sample to the counts per second per milliequivalent of the tritiated water used (as reagent and solvent) times 100 gives the percent

enolization. Values for the pseudo first-order rate-constant were calculated from the relationship

$$k = 1/t \log A/(A-X)$$

where A is 100 and X is the percent enolization at time t (hr).

Some results for D-glucose are given in table 6. As expected, the rate of enolization increases with the temperature and with the concentration of the base. As the reactions proceed, the solutions turn slightly yellow, and the overall rate-constant changes, presumably from a variety of reactions. The initial reaction-rate is characteristic of the primary enolization. The subsequent changes give rise to pseudo-equilibrium mixtures which are being studied by isotope-dilution techniques.

2. Pseudo-Equilibrium States

Work was continued on the quantitative determination of the products of rearrangement of pentoses, hexoses, and heptoses under conditions approaching equilibrium. Considerable progress was made on the preparation of carbon-14 labeled D-altrose, D-allose, D-talose, D-gulose, and certain heptoses. These materials are needed for study of the rearrangements by means of the radiochemical methods which we have developed.

E. Reversible Condensation and Cleavage Reactions

Although our tracer methods provide an exciting new method for study of the condensation and cleavage reactions, it was not possible to pursue this subject during the past year, but we hope that we shall be able to return to it shortly.

F. Saccharinic Acid Rearrangements

To test the mechanism proposed in the last report (page 55 of [29]) for rationalization of the distribution of carbon-14 in the "α"-D-glucosaccharinic acid prepared by Sowden and co-workers [30] from D-mannose, we rearranged samples of

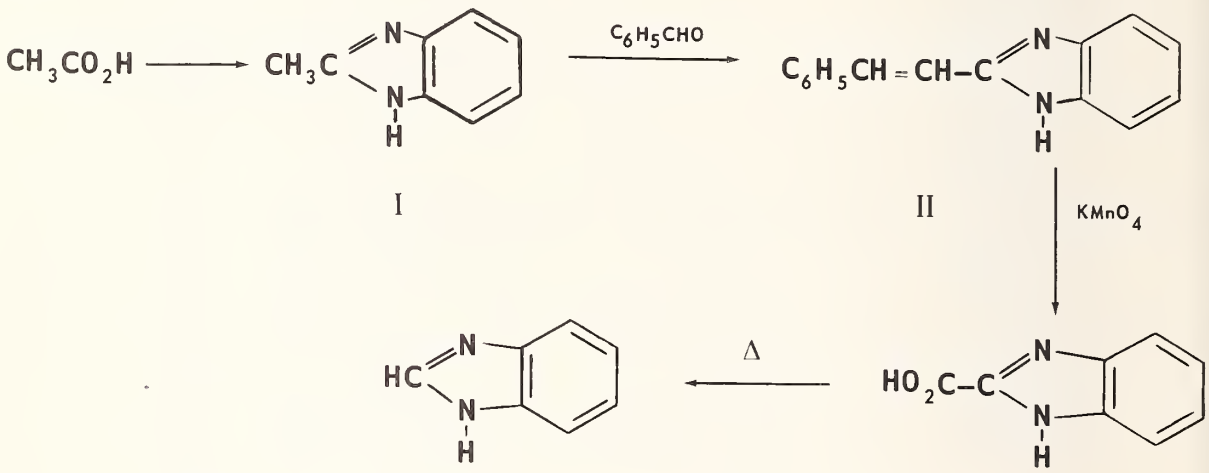
Table 6. .Enolization of D-glucose.

D-Glucose	NaOH	Ratio	Temp	(Atoms of T/mmole of sugar) x 10^{-2}						Rate constant[a] x 10^{3}					
mmole/ml	mmole/ml	NaOH/sugar	°C	1 hr	2 hr	3 hr	4 hr	5 hr	7 hr	1 hr	2 hr	3 hr	4 hr	5 hr	7 hr
0.1	0.175	1.75	35	2.24	9.52					9.8	21.7				
0.18	0.175	1.0	35	2.96	7.80	14.10				13.0	17.6	22.0			
0.36	0.175	0.5	35	2.10	5.68	7.96	12.42	15.54		9.2	12.7	12.0	14.4	14.7	
0.2	0.035	0.175	35	0.50	2.24		3.24		5.70	2.2	4.9		3.6		3.7
0.4	0.035	0.0875	35	0.22	0.74	1.04	1.94	2.30	3.46	1.0	1.6	1.5	2.1	2.0	2.2
0.18	0.175	1.0	15		0.74	1.14	1.04	1.32	1.82		1.6	1.7	1.1	1.2	1.1
0.18	0.175	1.0	20	0.4		0.94	1.32	1.64	2.14	1.7		1.4	1.4	1.4	1.3
0.18	0.175	1.0	26.4	1.00	1.96	2.68	3.74	5.36	10.38	4.4	4.3	3.9	4.1	4.8	6.8
0.19	0.175	1.0	30	1.58	3.50	6.20	8.94	12.26	19.4	6.9	6.7	9.3	10.2	11.4	13.4
0.18	0.175	1.0	35	2.96	7.80	14.10				13.0	17.7	22.0			

[a] $k = \frac{1}{t} \log \frac{A}{A-X}$, where A is 100, and X is the percent of the sugar enolized in t hours.

D-mannose-1-^{14}C and D-mannose-3-^{14}C, and separated the labeled saccharinic acid. Prior to degradation of our extremely scarce labeled material, it was necessary to carry out the procedure with non-labeled compounds to provide carriers and to perfect our techniques. All of the necessary intermediates have now been prepared, but the final analysis has not been attempted.

To ascertain the distribution of carbon-14 in the saccharinic acid, it is oxidized with periodate, and the resulting acetic acid and formic acid are assayed for carbon-14. In order to determine the position of the carbon-14 in the acetic acid, it is degraded by the series of reactions given below:



Intermediates I through IV should show the same radioactivity if the carbon atom of the carboxyl group contains carbon-14, but should show a change if carbon-14 is distributed between the CH_3 and COOH groups. However, if the carbon-14 is in the CH_3 group only, all of the radioactivity should be eliminated in passing to compound IV. It is imperative, therefore, that the purity of each intermediate be known before assays for carbon-14 are made.

Using the technique of thin-layer chromatography, we found that nanogram quantities of each intermediate can be rapidly analyzed for purity. One to five nanograms of the benzimidazole derivative I, dissolved in methyl alcohol, is spotted on silica gel, and the chromatograph is developed with 2-butanone. After the plates have been dried, the substances are detected with ultraviolet light, with iodine, or with sulfuric acid. The spots are sharply separated, and R_f values can be used to identify the impurities.

Because of the very small quantities of intermediates needed, the speed of the method, and the sensitivity of the reagents, the TLC technique will be invaluable in processing products, not only from saccharinic rearrangements, but for radiochemical assay of acetic acid in general.

3. CARBON-14- AND TRITIUM-LABELED CARBOHYDRATES

(H. L. Frush, H. S. Isbell, and N. B. Holt)

A. Introduction

Under our program on carbon-14- and tritium-labeled carbohydrates, services to the scientific public have been continued by supplying research workers with labeled compounds that were needed. Because of the competence of the staff in the field of isotopically labeled carbohydrates, requests are frequently received for additional kinds of labeled materials that have become important for biological research. However, developmental work of this nature has been limited in the past year, because of curtailment of staff through resignation and retirement. Nevertheless, improvements have been made in the synthesis of two radioactive sugars, L-fucose-1-^{14}C and L-rhamnose-1-^{14}C. The syntheses are described in this report, together with an improved synthesis yielding D-glucose-4-t, D-galactose-4-t, and D-glucitol-4-t. The last-named synthesis was carried out earlier by Dr. L. T. Sniegoski, as part of a study of isotope effects in bacterial oxidations.

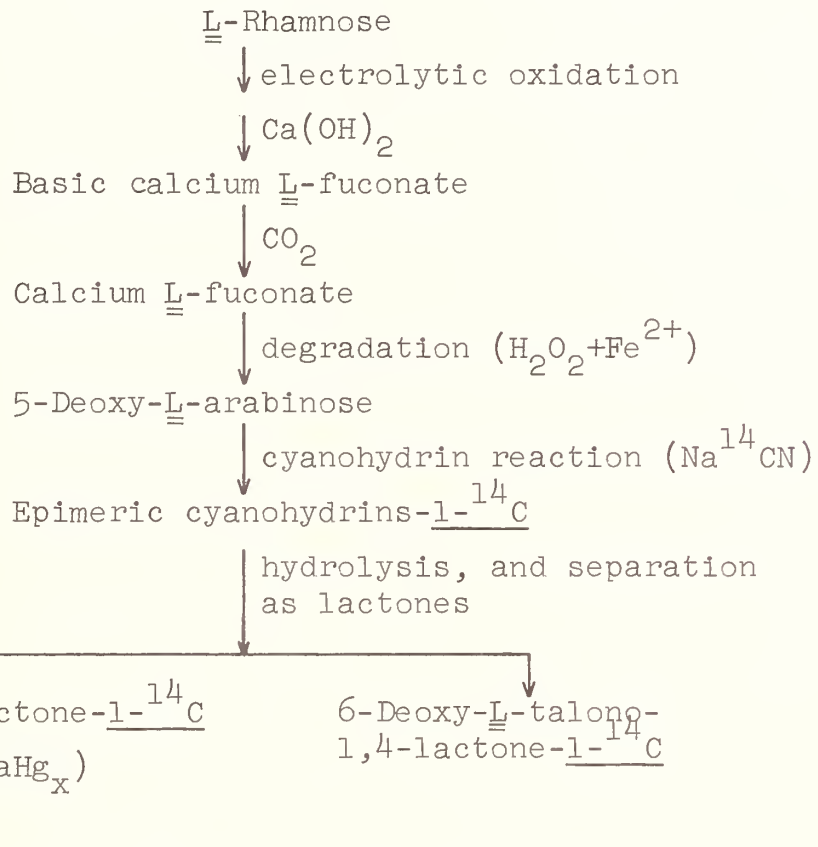
Work has been continued on analytical and purification techniques. Notable progress has been made in large-scale paper chromatography, and in the utilization of carbon-14 and tritium in double-label techniques. Preliminary studies of oxygen quenching in liquid-scintillation counting emphasize the need for further work in this area.

B. Synthesis of α-L-Rhamnose-1-^{14}C (6-Deoxy-α-L-Mannose-1-^{14}C)

1. Discussion of Method

L-Rhamnose occurs free in a few plants, and as a constituent of many glycosides, certain gums, and various

bacterial polysaccharides. It is readily prepared from "lemon flavin," a commercially available dyestuff obtained from black-oak bark (p. 460 of [31]). We have now synthesized α-L-rhamnose-1-^{14}C and have supplied the pure, radioactive sugar to numerous workers engaged in biological research. The method developed for the preparation is outlined below.



The L-rhamnose was oxidized electrolytically in the presence of calcium carbonate, by the method of Isbell and Frush [32], and the resulting calcium L-rhamnonate was purified through the intermediate separation of basic calcium L-rhamnonate [33]. The calcium salt was degraded to 5-deoxy-L-arabinose [34,35], and this was allowed to react with sodium cyanide-^{14}C. After hydrolysis of the cyanohydrins, the epimeric acids were separated as their lactones. L-Rhamnono-

1,4-lactone-1-^{14}C crystallizes readily, whereas the epimeric lactone does not. By reduction of L-rhamnono-1,4-lactone-1-^{14}C with sodium amalgam, and use of the nonradioactive sugar as the carrier, α-L-rhamnose-1-^{14}C hydrate was synthesized in an overall yield of 20.0 %.

2. Experimental Details

a. Calcium L-Rhamnonate. A mixture consisting of 22.8 g of α-L-rhamnose monohydrate (0.125 mole), 4 g of calcium bromide, and 6.5 g of calcium carbonate in 500 ml of water was placed in a 2-liter, 3-necked flask fitted with two graphite electrodes (1 cm diameter) and an efficient stirrer. The flask was cooled in ice water, and a direct current of 0.2 ampere was passed through the stirred solution. After 34 hr (6.8 ampere-hr) a test for reducing sugar with Benedict solution [36] was faint, and the electrolysis was stopped. The electrolyzed solution was filtered, and the calcium L-rhamnonate was precipitated as the basic calcium salt by the addition of 20 g of calcium hydroxide. The precipitate was collected on a Büchner funnel and washed with lime-water until free from bromide ion. It was then suspended in water, and carbon dioxide was bubbled through the (vigorously stirred) suspension until the supernatant liquid was almost neutral (colorless to phenolphthalein). The calcium carbonate was removed by filtration, and the filtrate was concentrated under reduced pressure to a sirup from which amorphous calcium L-rhamnonate was precipitated by the addition of ethyl alcohol. The crude product weighed 18 g.

b. 5-Deoxy-L-arabinose. A solution of 5 g (12.5 mmoles) of calcium L-rhamnonate and 0.245 g of barium acetate in 20 ml of water was mixed with a solution of 0.25 g of ferrous

sulfate heptahydrate in 20 ml of water. The mixture was diluted to a volume of 75 ml, placed in a water bath at 50 °C, and treated with 3 ml of 30% hydrogen peroxide. When the solution turned dark brown (about one hr), a second quantity (3 ml) of 30% hydrogen peroxide was added. After an additional hr, the mixture was diluted with 25 ml of methyl alcohol and 50 ml of ethyl alcohol, and filtered. The filtrate was concentrated under reduced pressure to about 25 ml. Titration of an aliquot with iodine in alkaline solution [37] showed the presence of 12.4 mmoles of reducing sugar (49.3% of the theoretical yield).

c. L-Rhamnono-1,4-lactone-1-^{14}C. A solution containing 2.0 mmoles of sodium cyanide-^{14}C (30 mCi) and 10 mmoles of sodium hydroxide in 55 ml was frozen in a 200-ml, round-bottomed flask. To the flask was added a solution of 1.13 g (12 mmoles) of ammonium carbonate and 2.1 mmoles of 5-deoxy-L-arabinose in 17 ml of water. The mixture was kept in a refrigerator for one day, and at room temperature for four days. Sodium carbonate (1.06 g, 10 mmoles) was added, and the mixture was heated at 100 °C, with the addition of water from time to time, until the evolution of ammonia ceased. The solution was passed through a column containing 75 ml of a cation-exchange resin, and the effluent was concentrated under reduced pressure to a sirup. The sirup was dissolved in methanol, and the solution was again concentrated; the process was repeated a third time. Finally, the residue was dissolved in a few drops of glacial acetic acid, and the solution was heated at 70 °C for one hour, and then concentrated in a stream of dry air. Heating and concentrating were repeated, and L-rhamnono-1,4-lactone-1-^{14}C crystallized

on nucleation. The crystals were separated, and a second crop was obtained from the mother liquor after dilution with 200 mg of nonradioactive L-rhamnono-1,4-lactone. The two crops were combined, and re-crystallized from methyl Cellosolve by the addition of ether; yield, 110 mg, 6.737 mCi. By treating the mother liquor with additional nonradioactive L-rhamnono-1,4-lactone, several crops were separated; these were combined and recrystallized; yield, 216 mg, 1.674 mCi, and 428 mg, 2.405 mCi. The total radiochemical yield (10.8 mCi) was 36.0 percent. The mother liquor containing 18.0 mCi (chiefly in the form of L-isorhamnono-1,4-lactone-1-^{14}C), was held for further work.

d. α-L-Rhamnose-1-^{14}C Monohydrate. The various crops of L-rhamno-1,4-lactone-1-^{14}C were reduced in quantities of about 100 mg per reduction tube, as previously described [38], by use of 15 ml of water in each tube, and, per mmole of lactone, 9.2 g of 5% sodium amalgam in the form of pellets [39] and 6.4 g of sodium hydrogen oxalate. Half of the amalgam and oxalate were added, and, after these had reacted, the remainder was added. Finally, the reduction products in the tubes were combined, and the residual acids were neutralized with sodium hydroxide. Five volumes of methyl alcohol were added, and the mercury and precipitated salts were separated on a filter, washed with methyl alcohol, and discarded. The filtrate was concentrated under reduced pressure to about 10 ml and diluted with five volumes of methyl alcohol. The salts that precipitated were removed by filtration, and the filtrate was concentrated, under reduced pressure, to remove the alcohol. The sirup was dissolved in water, and the solution was passed through a column containing 25 ml of a 1:1 mixture of cation- and anion-exchange resins. The effluent (which was shown to be salt free by

testing with a commercial conductivity meter) was concentrated to a sirup, and brought to crystallization by the addition of isopropyl alcohol.

By use of nonradioactive α-L-rhamnose as the carrier, three additional crops of crystals were removed from the mother liquor. The four crops, when combined and recrystallized, yielded 617 mg of α-L-rhamnose-1-^{14}C having 5.124 mCi of activity. Further use of carrier, and recrystallization of the product, yielded a crop of 453 mg, having 874 μCi of activity. The radiochemical yield (6.0 mCi) of chromatographically pure α-L-rhamnose-1-^{14}C monohydrate was 20.0% of the sodium cyanide-^{14}C employed in the synthesis.

C. Synthesis of α-L-Fucose-1-^{14}C (6-Deoxy-α-L-Galactose-1-^{14}C)

Polysaccharides containing L-fucose are found in the cell walls of marine algae, in a few gums, and in certain substances of animal origin, such as red blood-cells, blood serum, gastric mucin, ovarian cyst fluid, and many other materials of biological importance. Because of the wide-spread occurrence of L-fucose, the ^{14}C-labeled sugar is a valuable research tool. We have carried out the synthesis of α-L-fucose-1-^{14}C, by a method similar to that described for α-L-rhamnose-1-^{14}C monohydrate. The overall radiochemical yield was 30.2%. A manuscript describing this synthesis in detail has been prepared for publication in the NBS Journal of Research.

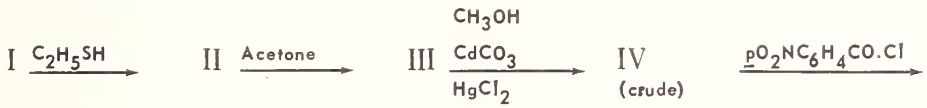
D. Synthesis of D-Glucose-4-t, D-Galactose-4-t, and D-Glucitol-4-t (H. S. Isbell and L. T. Sniegoski)

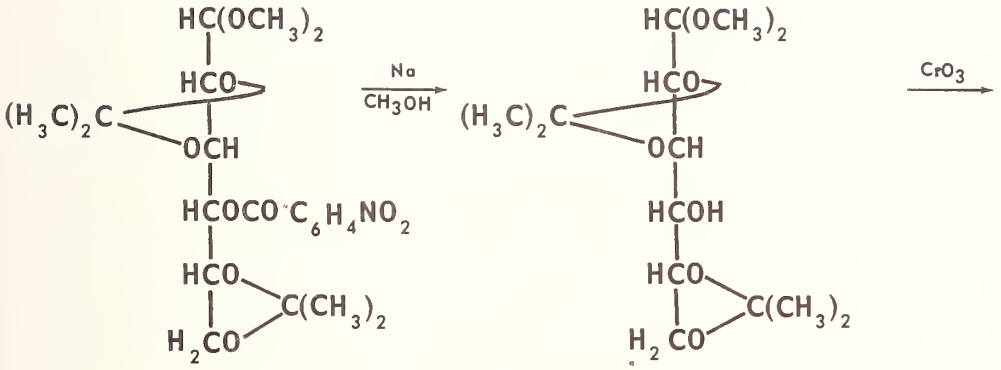
D-Glucitol-4-t was needed for the determination of isotope effects in certain bacterial oxidations, and both D-glucose-4-t and D-galactose-4-t have numerous applications in biochemical and biological research. All three compounds result from the synthesis depicted below. Briefly, this synthesis involves the

preparation, from D-glucose, of a compound having only one free hydroxyl group (at C-4). This >CHOH is oxidized to >C=O, and the latter is reduced with a tritiated reagent to >CHOH in both configurations (i.e., the D-gluco and D-galacto). Because the labeled D-galactose compound is formed only by the tritiated reagent, whereas the labeled D-glucose compound is diluted with that which was unoxidized at C-4, the resulting D-galactose has greater specific activity than the D-glucose.

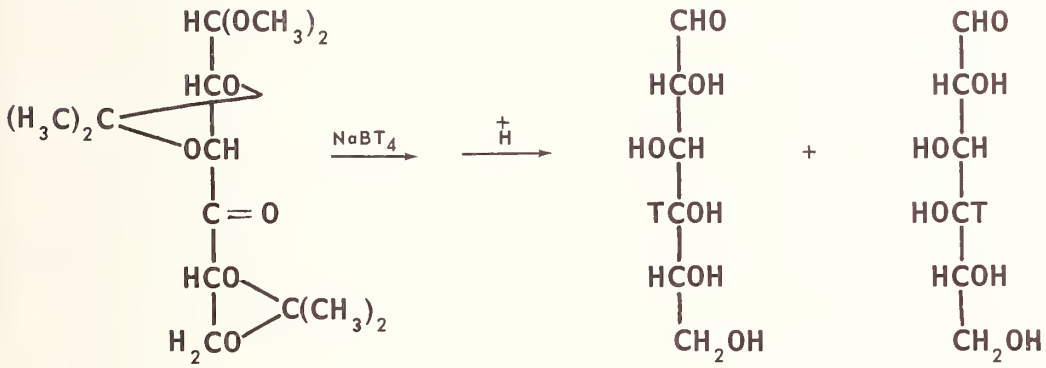
The synthesis employed is a simplification of that of Kohn and Kohn [40], which, in turn, derives from the work of Wolfrom et al. [41,42] on the aldehydo derivatives of the sugars. In our work, a radiochemical yield (85%) of D-glucose-4-t and D-galactose-4-t (considerably better than that of Kohn and Kohn) was obtained by the use of sodium borohydride-t in methyl alcohol (instead of lithium borohydride-t in ether) as the reducing agent, and by the use of ion-exchange resins for purifying the labeled compounds. D-Glucitol-4-t was obtained in nearly quantitative yield by the reduction of D-glucose-4-t with sodium borohydride.

The synthesis of the labeled compounds from D-glucose (I) involved the preparation, in sequence, of D-glucose diethyl dithioacetal (II), 2,3:5,6-di-O-isopropylidene-D-glucose diethyl dithioacetal (III), 2,3:5,6-di-O-isopropylidene-D-glucose dimethyl acetal (IV), purification of IV through crystalline 2,3:5,6-di-O-isopropylidene-4-O-(p-nitrobenzoyl)-D-glucose dimethyl acetal (V), saponification of V to IV, oxidation of IV to 2,3:5,6-di-O-isopropylidene-D-xylo-hexos-4-ulose dimethyl acetal (VI), reduction of VI with sodium borohydride-t, hydrolysis to a mixture of D-glucose-4-t (VII) and D-galactose-4-t (VIII), chromatographic separation of VII and VIII, and reduction of VII to D-glucitol-4-t.





V IV



VI VII VIII

Compound II was prepared from 119 g of anhydrous I, 101 ml of concentrated hydrochloric acid, and 100 g of ethanethiol according to the procedure in [41]. After one recrystallization, the yield was 102 g (54%), mp 127.5 °C (lit. value 127 °C).

Compound III was prepared in crude condition from 101 g of II, one liter of acetone, and 200 g of anhydrous cupric sulfate. The yield of sirupy III was 120.8 g. A total of 40 g of III was converted into IV by treatment with mercuric chloride and cadmium carbonate by the method of Wolfrom et al. [42] as modified by Kohn and Kohn [40] in the use of absolute methyl alcohol instead of ethyl alcohol, and a lower temperature (65-70 °C instead of 70-80 °C). The sirupy IV (20.2 g) was purified by conversion into V. The yield of re-crystallized product was 8.27 g (27.6% based on IV), mp 104.5-106 °C (lit. value, 106-107 °C).

A 4-g sample (8.8 mmoles) of V was saponified with sodium methoxide in methyl alcohol to sirupy IV (2.89 g), which was oxidized with 2.89 g (28.9 mmoles) of chromium trioxide in dry pyridine. The sirupy product (2.22 g) containing VI reduced Somogyi solution, and showed a carbonyl band in its infrared spectrum.

From Kohn and Kohn's data [40], we estimated that about a quarter of the 2.22 g of sirupy product, or 555 mg (1.8 mmoles), was the 4-ketose, compound VI. Without isolation of this, the sirup was reduced with 19 mg (0.52 mmole, 1.81 mCi) of sodium borohydride-t in anhydrous methyl alcohol at 0 °C. Half of the sodium borohydride-t was added four hr after the first half, in order to minimize any isotope effects which might occur during reduction. After 24 hr, 20.4 mg of ordinary sodium

borohydride was added to ensure complete reduction. After an additional 24 hr, no reducing sugar was present.

The solution was concentrated several times, with addition of methyl alcohol; cation-exchange resin was added to decompose excess borohydride and remove sodium ions, and concentration and addition of methyl alcohol were repeated several times. After removal of the resin by filtration, and concentration of the solution to a sirup, the product was hydrolyzed by adding 25 ml of 0.3 N hydrochloric acid and heating at 98-100 °C for 90 min. A test chromatogram using water-saturated tert-pentyl alcohol showed the presence of only D-glucose and D-galactose. The D-glucose-4-t and D-galactose-4-t spots, cut out and assayed with a liquid scintillation counter, gave 248 and 332 cps, respectively. Hence, the D-galactose configuration is favored in the reduction.

The solution was passed through a column of mixed (1:1) cation- and anion-exchange resins and concentrated, affording 2.05 g of sirupy D-glucose-4-t (VII) and D-galactose-4-t (VIII). Radioanalysis of the product showed 1.54 mCi (85.1% overall radiochemical yield). A crop of 52 mg of D-galactose-4-t (3.06 Ci/mg, 159 μCi) was obtained by recrystallization from water—methyl alcohol-isopropyl alcohol. The remaining material, containing D-glucose-4-t and D-galactose-4-t, was separated by large-scale paper chromatography on Whatman seed-test paper, with a wick of Whatman No. 3 MM paper (see page 61). A triple development was made, with 1-butanol—pyridine—water (45:25:40) as the solvent. The D-glucose-4-t and D-galactose-4-t were eluted from the paper, and each solution was deionized by passage through a column containing 30 ml of mixed (1:1) cation- and anion-exchange resins; yield, 551 mg of D-glucose-4-t

(0.72 μCi/mg, 397 μCi), 200 mg of D-galactose-4-t (3.24 μCi/mg, 648 μCi), and 143 μCi of a mixture of the two from an intermediate strip.

A sample of D-glucose-4-t (140 mg, 0.78 mmole) was reduced to D-glucitol-4-t with a seven-fold excess (50 mg) of sodium borohydride in aqueous solution [43]. After removal of cations with a cation-exchange resin and of borate by repeated concentration of the sirup in the presence of methyl alcohol, 143 mg of D-glucitol-4-t was obtained. Chromatography in 2-butanone—acetic acid—saturated aqueous boric acid solution (9:1:1) [44] and scanning of the chromatogram for radioactivity showed that the D-glucitol-4-t contained no D-glucose.

E. Liquid Scintillation Counting; Study of Oxygen Quenching

(H. S. Isbell, A. Cohen)

It is known that oxygen has a reversible quenching effect, not only on the fluorescence of polycyclic, aromatic hydrocarbons, but also on the scintillation from mixtures commonly used for radiochemical assays. These facts suggested the possibility of studying the interaction of polycyclic, aromatic hydrocarbons with oxygen by means of a liquid scintillation counter. It seemed probable that fluorescent hydrocarbons would act as scintillators in the counter, and that the reactions with oxygen might be more or less characteristic of the hydrocarbons.

Knowledge of the quenching effects of oxygen for various scintillators is desirable, because variations in the oxygen content of the solutions used in analyses cause substantial errors; the more sensitive the scintillator to oxygen-quenching, the higher the error.

In development of these ideas, a study of the scintillation properties of pyrene and 1,1'-bipyrene was made. The convenient method for preparation of 1,1'-bipyrene developed by

Mr. A. J. Fatiadi (see page 8) makes this heretofore scarce compound available for research and wide scientific use. The substance is highly fluorescent; this property offers many possible applications, as, for instance, a water-insoluble, invisible, ultraviolet marker, for activating electronic circuits. It seemed possible that it might have applications in scintillation counting of radioactivity, because the fluorescence is at long wavelengths.

For study of the scintillation properties of the hydrocarbons and the effect of oxygen on the scintillation, we prepared two hydrocarbon-soluble radioactive standards, hexa-O-acetyl-D-mannitol-1-t and hexa-O-acetyl-D-mannitol-1-^{14}C. These provide sources of beta radiation of widely different energy levels.

A variety of measurements was conducted, but only a few will be described here, as a more comprehensive study is being made. In experiments 1 to 6 of table 7, an accurately weighed quantity of the tritium source (2 to 4 mg) was placed in each of 6 vials containing a Teflon-coated stirring bar, followed by 10 ml of the solution to be tested for scintillation (see table 8). The vials were sealed with caps containing two holes and a rubber liner. The solutions were cooled in an ice bath, and a stream of nitrogen gas (1.5 to 2.5 liters) was passed in, through hypodermic needles inserted in the holes of each cap. Then, the solutions were counted in the liquid scintillation counter shown in figure 5. The samples were further successively treated (by the method described) with air and oxygen; after each treatment, the samples were counted.

Experiments 1 and 2 were conducted with conventional scintillation solutions used for assay of carbon-14 and tritium. In the conventional toluene and p-dioxane solutions, tritium was counted in air with efficiencies of 39 and 30%, respectively. The count in the p-dioxane solution is less sensitive to oxygen

Table 7. Effect of oxygen on counting efficiency for a tritium source.

Solution[a] employed	Flushed with	Counts/sec/mg of tritium source	Relative count	Counting efficiency %
1	N_2	506.56	1	45.4
	Air	433.55	0.86	38.9
	O_2	246.29	0.49	22.1
2	N_2	367.84	1	33.0
	Air	334.40	0.91	30.0
	O_2	225.02	0.61	20.2
3	N_2	35.38	1	0.32
	Air	2.53	0.07	0.02
	O_2	0.17	0.005	0.002
4	N_2	165.40	1	4.8
	Air	40.61	0.25	0.4
	O_2	4.37	0.03	0.04
5	N_2	22.10	1	0.20
	Air	6.41	0.29	0.06
	O_2	0.91	0.04	0.008
6	N_2	186.95	1	16.8
	Air	49.36	0.26	0.4
	O_2	3.42	0.02	0.03

[a]For composition, see table 8.

Table 8. Composition of scintillation solution employed[a].

Soln.	Toluene ml	p-Dioxane ml	Heptane ml	PPO g	Me_2POPOP g	Naphthalene g	Pyrene g	1,1'-Bipyrene g
1	1000			5	0.3			
2		1000		7	0.3	100		
3	500		500			100		
4	500		500				63	
5	500		500					0.18
6	500		500				32	0.09

[a]Per liter of solution.

Figure 5. Liquid scintillation counter used for determining carbon-14 and tritium.

than that in the toluene solution, but both are greatly affected by the presence of oxygen. Experiments 4,5, and 6 were conducted with pyrene and with extremely small quantities of 1,1'-bipyrene as scintillators. (The amount of the latter was limited by its insolubility.) Both hydrocarbons proved to be effective scintillators; a mixture of pyrene and 1,1'-bipyrene is more effective than either alone. The sensitivity of the counting efficiency of pyrene and 1,1'-bipyrene to the presence of oxygen is higher than that of the conventional scintillators used in experiments 1 and 2. The results are of an exploratory nature, and the investigation is being continued.

F. Large-scale Paper Chromatography

(H. L. Frush)

1. Introduction

Despite the multiplicity of available chromatographic techniques, paper chromatography continues to be one of the most versatile techniques for the separation, identification, purification, and analysis of carbohydrates. With respect to range in capacity, it has advantages over either thin-layer or column chromatography. In combination with radiochemical techniques, it affords sensitive quantitative analyses by non-quantitative isolation methods.

In the syntheses of position-labeled, radioactive compounds carried out in our laboratories, we have used paper chromatography not only for determining purity and for following the course of reactions, but also for accomplishing numerous large-scale separations and purifications in the actual syntheses [45].

Most large-scale preparative methods for paper chromatography depend on an increase in bulk achieved by the use of many layers of thin paper, pressed or rolled together. Some of these methods require the tedious loading of each sheet, and others suffer from a lack of uniformity of movement throughout the bulk.

In 1957, Brownell and co-workers [46] described a large-scale, heavy-paper technique for preparative chromatography in which the paper (equipped with a supporting stirrup and a wick of thinner paper) is used, after preliminary purification, in essentially the manner customary for single sheets. The authors stated that the dense paper (Whatman seed-test paper, 1.5 mm thick) is capable of separating 0.5 to 3.0 g of material per large sheet (18 x 22 in.), depending on the spread in R_f values. They also described a method for nondestructively locating the separated

bands by staining a "print" obtained by pressing a sheet of thin paper against the wet chromatogram.

However, despite the unusually high capacity of the heavy paper, and the inherent advantages of paper over column chromatography, the techniques described by Brownell et al. have not been widely adopted, presumably because of the inconvenience of preparation and the fact that movement of bands through the paper is not so uniform as that in other papers.

Despite these disadvantages, heavy-paper chromatography has found extensive use in our laboratories for separating both radioactive and nonradioactive mixtures. Certain simplified techniques, described below, have facilitated application of the paper to problems of separation. The techniques are equally applicable to Whatman No. 17 paper, and, on this paper, afford separations of 0.2 to 0.6 g per large sheet. The No. 17, like the seed-test, is a "fast" paper whose properties are improved by use of a thinner wick, which serves to retard the absorption of solvent.

2. Procedures

a. Preparation of the Heavy Paper. Figure 6 shows a simplified method for applying the wick and supporting stirrup. A strip of Whatman No. 3 MM paper (7 x 18 in.) is loosely machine-stitched* along its short sides, about 2 in. from the edge, and then folded as shown. The 18-in. edge of the seed-test paper is softened by momentarily dipping it in water, and blotting the excess. The dampened edge is inserted into the folded strip of thin paper, and the two are twice machine-stitched together. To facilitate dripping of the solvent, the lower edge is conveniently serrated by dampening it and tearing off small

*This stitching prevents tearing of the stirrup when the chromatogram is wet.

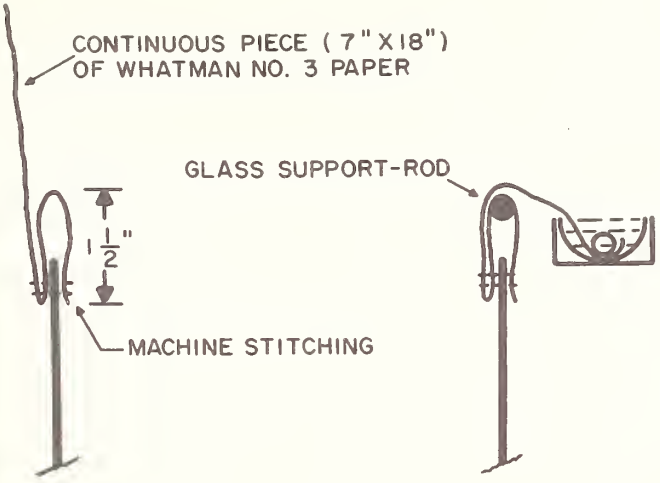


Figure 6. Simplified preparation of stirrup and wick for heavy-paper chromatogram.

pieces to form an irregular edge.

The papers are chromatographically washed with 0.01 N hydrochloric acid and then with water (two days in each solvent). The sheets are air-dried, and strips (1 in. or more in width) are trimmed from each long edge; stirrups and wicks are trimmed to match. A second wick (useful when the paper is washed, or is developed in a slow-moving solvent) need not be stitched, but can be added as shown in figure 7.

Aqueous or alcoholic solutions of mixtures to be chromatographed (as much as 2 ml) are applied as a narrow band, 2 cm below the stitching, by means of a pipet having a 1-ml bulb and a short tip of capillary tubing. The band is loaded from both sides of the paper, which is then air-dried for several hr. The No. 17 paper may be used without purification or trimming, but with the same stirrup and wick.

b. Nondestructive Location of Bands on the Developed Chromatogram. Figure 8 illustrates a convenient method for locating bands of material on the chromatogram after development. The wet sheet is laid on a glass plate in a well-ventilated hood,

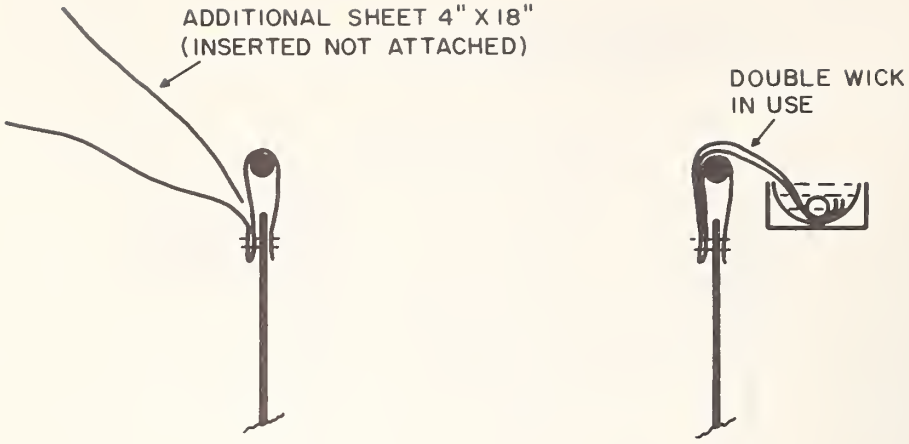


Figure 7. Addition of auxiliary wick to heavy-paper chromatogram.

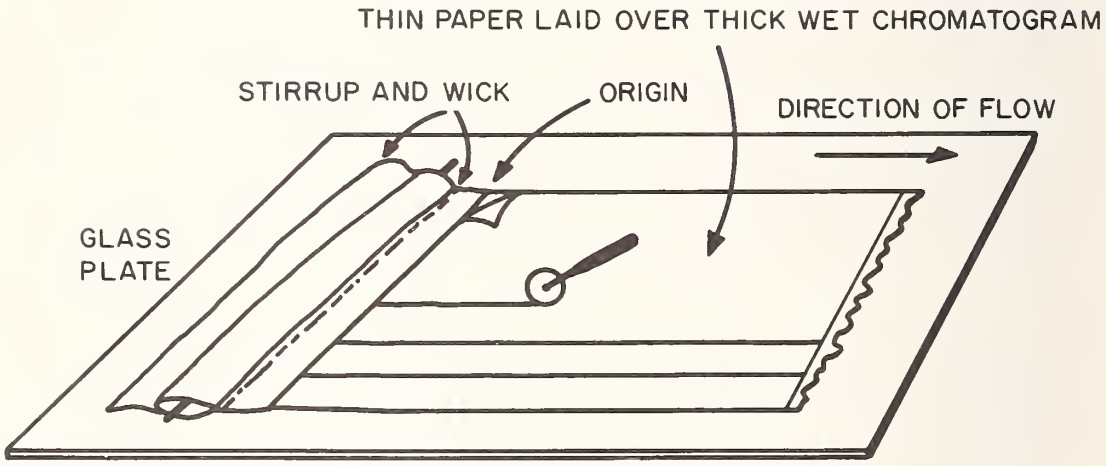


Figure 8. Method for non-destructively locating bands on developed, heavy-paper chromatogram.

and over-laid with a sheet of thin paper (such as Whatman No. 1) carefully positioned at the origin. A small plastic wheel* is drawn down the paper in a series of lines, in which good absorption from the wet chromatogram is caused by the pressure of the wheel. The tracing thus prepared is sprayed with a

*This is a pastry wheel available in stores selling kitchenware.

suitable color reagent. Because the color is intense in those portions of the lines that cross the bands, the limits of the bands are more clearly discerned than in a "print" [46] of the entire chromatogram. If it is desired only to ascertain the progress of a development, a single line, drawn by the wheel on a strip of paper placed lengthwise down the middle of the wet chromatogram, will suffice. When the separation of bands has become satisfactory, and a tracing has clearly defined them, the chromatogram is air-dried. The outlines of the bands sketched on the tracing are readily transferred to the chromatogram by means of a stylus.

In the chromatography of ^{14}C-labeled compounds, a radioautograph most accurately locates the separated bands. By outlining the bands on the film with series of punctures, positioning the film on the chromatogram, and dusting it with carbon, the outlines are transferred to the chromatogram, and the intact film may be saved for reference.

For tritium-labeled compounds, the tracing technique previously described has a further advantage. Tritium does not produce a satisfactory radioautograph from a chromatogram because of the weakness of its radiation. If insufficient compound is present to enable the bands to be located by staining the tracing, the technique illustrated in figure 9 is useful. Individual lines on the tracing are scanned in a paper-chromatogram scanner having a 1:1 correspondence of the paper and chart (figure 10). This technique has been used in the chromatography of high-activity, tritium-labeled compounds on the lower-capacity papers such as Whatman No. 3 MM.

c. Removal and Treatment of Bands of the Developed Chromatogram. The bands, located by one of the methods described, are conveniently removed by means of tinners' shears.

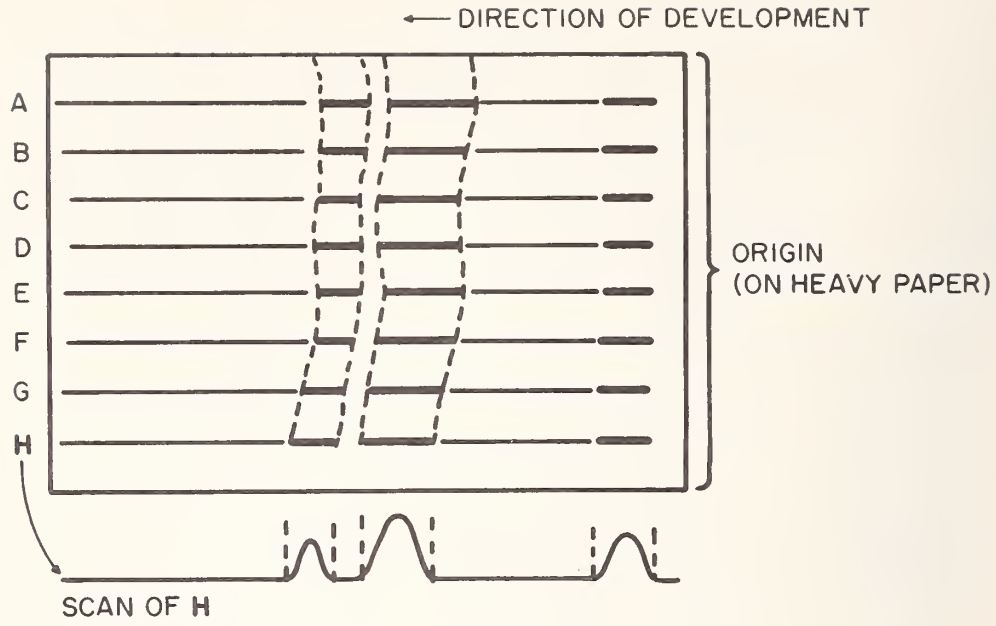


Figure 9. Use of tracing and chromatogram scanning to locate bands of high-activity, tritiated compounds.

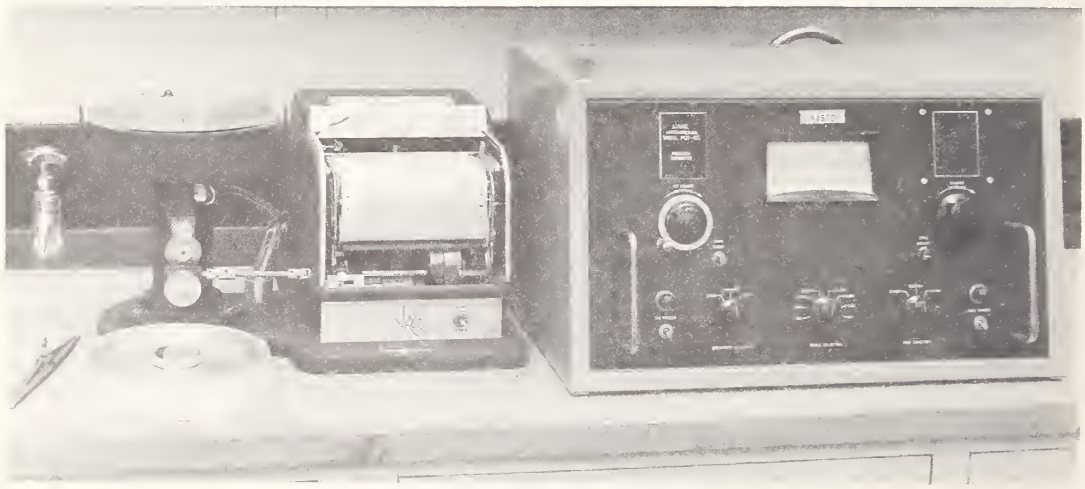


Figure 10. Paper chromatogram scanner used for assay of carbon-14- and tritium-labeled compounds.

(Seed-test paper is difficult to cut with scissors and tends to crumble when cut with a sharp knife.) For elution, the stirrup and wick are attached to one end of the excised band by means of several stainless-steel staples, vertically placed. The auxiliary wick is added as shown in figure 7, the lower

end of the band is trimmed to a point, and the strip is eluted, usually with 50% methyl alcohol. Subsequent treatment of the eluate may include the use of a small amount of a decolorizing carbon, and, in some instances, of ion-exchange resins.

d. Capacity of the Heavy Papers. Figures 11 and 12 illustrate the capacity of the heavy papers. Obviously, the ease of separation (and, therefore, the capacity) depends on

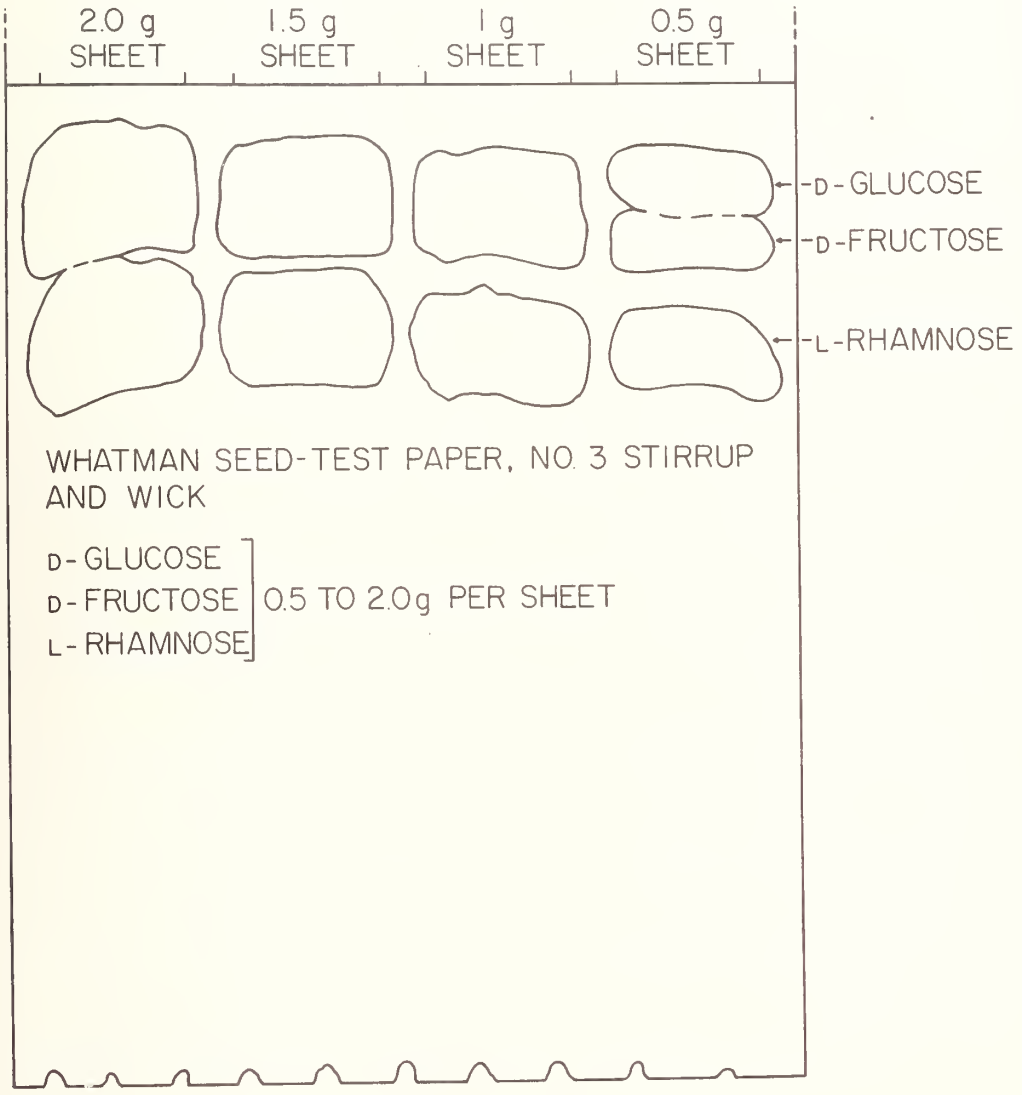


Figure 11. Illustration of capacity of heavy (seed-test) paper; dependence on relative R_f values.

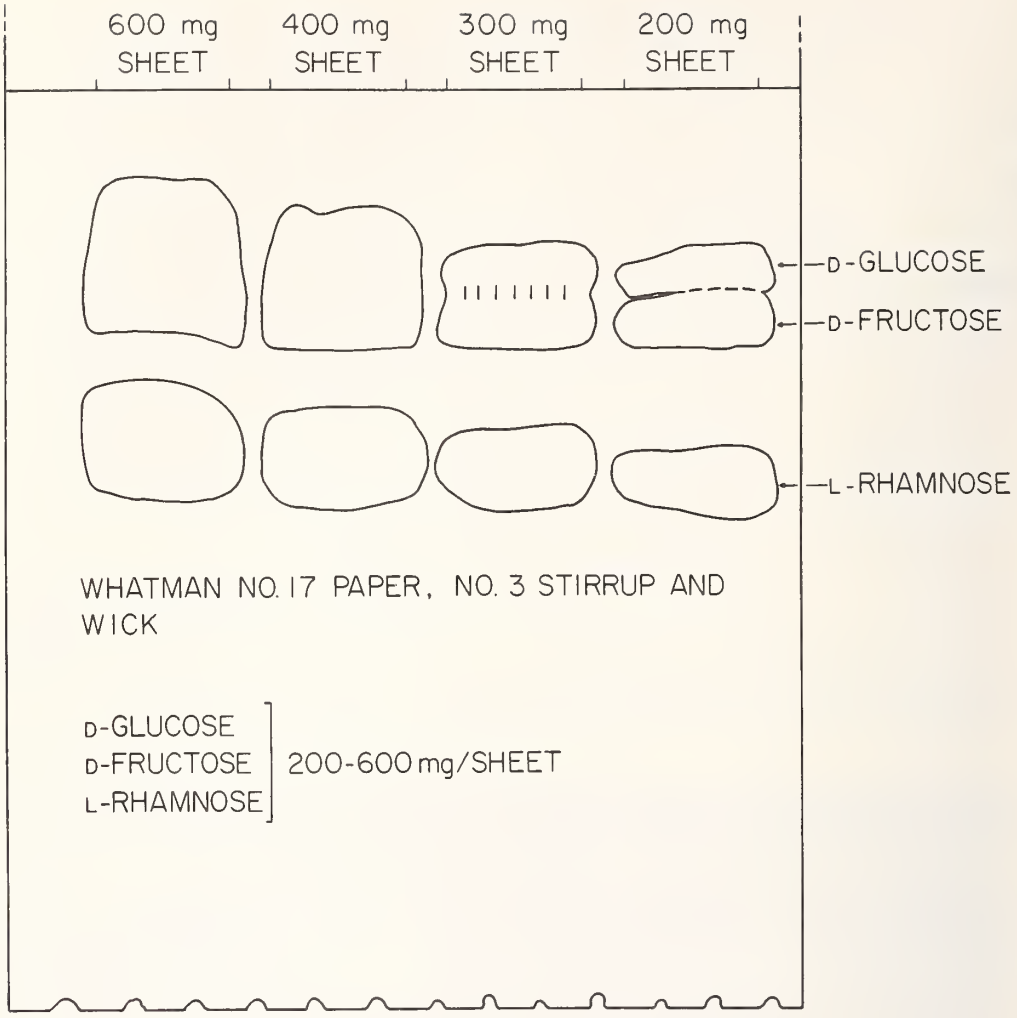


Figure 12. Illustration of capacity of fast, medium-weight paper, prepared with thinner wick.

the difference in R_f values. At most indicated levels of loading, L-rhamnose was separated from D-glucose and D-fructose. Further development, or a second development, would separate the last two from each other at the one-gram level on the seed-test paper, and at the 400-mg level on the No. 17 paper. The capacity of the heavy papers can be increased somewhat (if the decreased length is unimportant) by attaching the stirrup and wick to the long (22 in.) edge. The chromatographic properties of the seed-test paper are the same in the two directions. The R_f values for the seed-test paper are about 0.7 of those for Whatman paper No. 1.

4. CHARACTERIZATION OF CHEMICAL STRUCTURE; PHENYLHYDRAZONO—PHENYLAZO TAUTOMERISM

(H. S. Isbell and A. J. Fatiadi)

A. xylo-4,5,6-Trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane and 4-Oxo-1-phenyl-5-phenylazo-3-pyridazine Derivatives

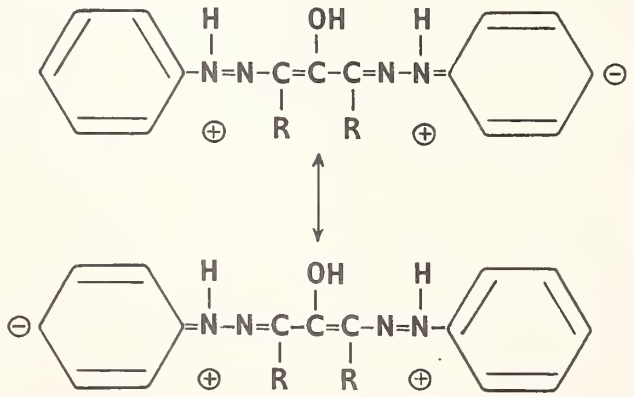
The positions of the phenylhydrazono groups in xylo-4,5,6-trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane were established by degradation of the compound with sodium metaperiodate, and identification of the reaction product. The dialdehyde initally formed by periodate oxidation (but not isolated) cyclized to 4-oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxaldehyde, which yielded a crystalline methyl hemiacetal, an oxime, a semicarbazone, and, by oxidation, the corresponding monocarboxylic acid and sodium salt. Upon treatment with phenylhydrazine at 50 °C, the carboxaldehyde gave a red, crystalline phenylhydrazone which, with phenylhydrazine at 130 °C, afforded a product that appears to be 4-oxo-1-phenyl-5-(phenylhydrazo)-3-pyridazinecarboxaldehyde phenylhydrazone.

The reactions provide a route to a series of new pyridazine derivatives which may prove valuable for the synthesis of unusual compounds for biological and medical research. This work has been incorporated in a paper which has been accepted for publication in Carbohydrate Research.

B. Structures of 2-Oxo-1,3-bis(phenylhydrazono) Compounds

A manuscript entitled "Phenylhydrazono—phenylazo Tautomerism, Part II. Structures of 2-Oxo-1,3-bis(phenylhydrazono) Compounds" was prepared, and will be submitted for publication in the near future. The paper presents measurements of NMR, infrared, ultraviolet, and visible spectra for twelve phenylhydrazono compounds, prepared to show that 2-oxo-1,3-bis(phenylhydrazono) derivatives have a novel phenylhydrazono—phenylazo structure and that they exist in tautomeric forms.

In an invited paper presented at an A.C.S. symposium on "New Reactions in Carbohydrate Chemistry," the structures of carboxylic acids, enediolic acids, diphenylformazans, and the enolic tautomers of 2-oxo-1,3-bis(phenylhydrazono) compounds were compared. From the comparisons of the structures, and consideration of the properties of the compounds, the concept was advanced that the enediolic acids are analogs of carboxylic acids in that the =O and -OH groups are attached to carbon atoms joined through a -C=C(OH)- group. Similarly, it was proposed that the enolic tautomers of the 2-oxo-1,3-bis(phenylhydrazono) compounds are analogs of the diphenylformazans in that the phenylazo and phenylhydrazono groups are also attached to carbon atoms joined through a -C=C(OH)- group. Certain novel reactions of the 2-oxo-1,3-bis(phenylhydrazono) compounds and the diphenylformazans were discussed. A reaction with strong acids, which we discovered and interpreted, is noteworthy. Thus, a deep-blue color is obtained when a 2-oxo-1,3-bis(phenylhydrazono) compound or a diphenylformazan is dissolved in a strong acid, such as phosphoric acid. Phenylosazones and other 1,2-bis(phenylhydrazono) compounds examined do not give this intense blue color. The color presumably arises from a cation of the following type:



The reaction is being investigated further.

C. Inosose Derivatives

Work was continued on the reactions and properties of inositol derivatives, especially with reference to aromatization by cleavage of acyloxy groups beta to enolic hydroxyl groups [47].

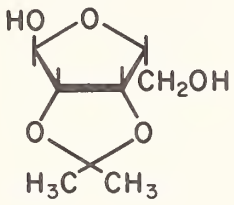
Procedures were developed and published [48] for preparing inososes from their phenylhydrazones and for separating an inosose phenylhydrazone from an inosose phenylosazone by means of ion-exchange resins. In the latter process, the phenylhydrazone—phenylosazone mixture is treated with a cation-exchange resin. The phenylhydrazone is hydrolyzed, and the inosose is obtained in the aqueous extract. The insoluble phenylosazone is separated with the resin, and extracted from it with N,N-dimethylformamide.

5. SYNTHESIS OF RESEARCH MATERIALS: CYCLOPENTITOLS AND RELATED SUBSTANCES

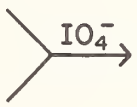
(R. Schaffer)

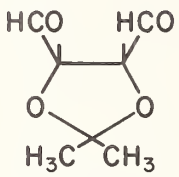
A. Introduction

In a study in which a long-known O-isopropylidene-D-lyxose (I) was finally characterized [49], one reaction afforded a new dialdehyde (II) that was found to crystallize as III by incorporating a water molecule in its structure. (The two hemiacetal functional groups in a single tetrahydrofuran ring make this an unusual ring structure for a carbohydrate.) A more economical source of III is the isopropylidene derivative (IV) of naturally occurring D-ribose, which unexpectedly undergoes oxidation by periodate at a much higher rate than I.

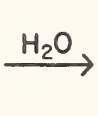


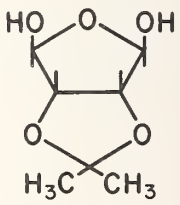
I



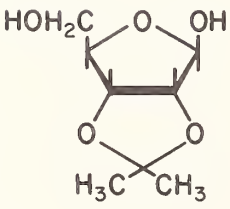


II





III



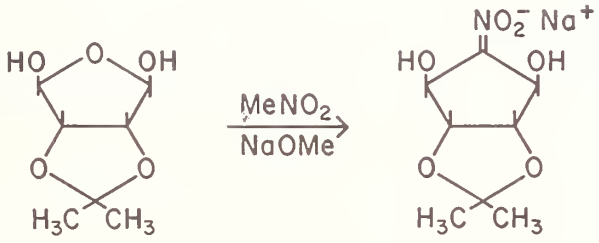
IV

The availability of compound III offered the prospect of synthesizing pentasubstituted cyclopentanes, 5-carbon-atom ring analogs of the hexasubstituted cyclohexanes, important

biochemicals that serve as model compounds of fundamental importance for studying the stereochemistry, conformations, and reaction mechanisms of substances having 6-carbon-atom rings. The pentasubstituted cyclopentanes hold promise of equal usefulness for this widespread ring-type.

B. Nitromethane Synthesis

Conversion of the novel dialdose (II) into cyclopentanes was achieved by treating it in alkaline solution with nitromethane. The reaction may be formulated as involving nitromethane (as the aci-nitromethane anion) adding to one of the aldehyde groups of II to form an open-chain, 5-carbon-atom intermediate having the unreacted aldehyde group at one end and an aci-nitro functional group at the other, followed by addition of the latter terminal group to the former to yield the sodium aci-nitrocyclopentane salt (V) that precipitates from the reaction mixture.



III V

The reaction products possible from this synthesis are the one with the meso structure depicted as V, another meso salt (like structure V, except that the two hydroxyl groups are up and the hydrogen atoms are down), and a DL pair (like V, except that only one hydroxyl group and one hydrogen atom are up). However, only compound V was isolated.

1. Structure of the Sodium Salt

The elementary composition ($C_8H_{12}O_6NNa$) according to chemical analysis of the isolated salt, the presence in its

infrared spectrum of peaks that indicate the dioxolane ring, the nitro group, and hydroxyl groups, and the absence of peaks for a carbonyl group are consistent with structure V. Its configuration of asymmetric carbon atoms was established by use of a proton magnetic resonance spectrometer (figure 13), a

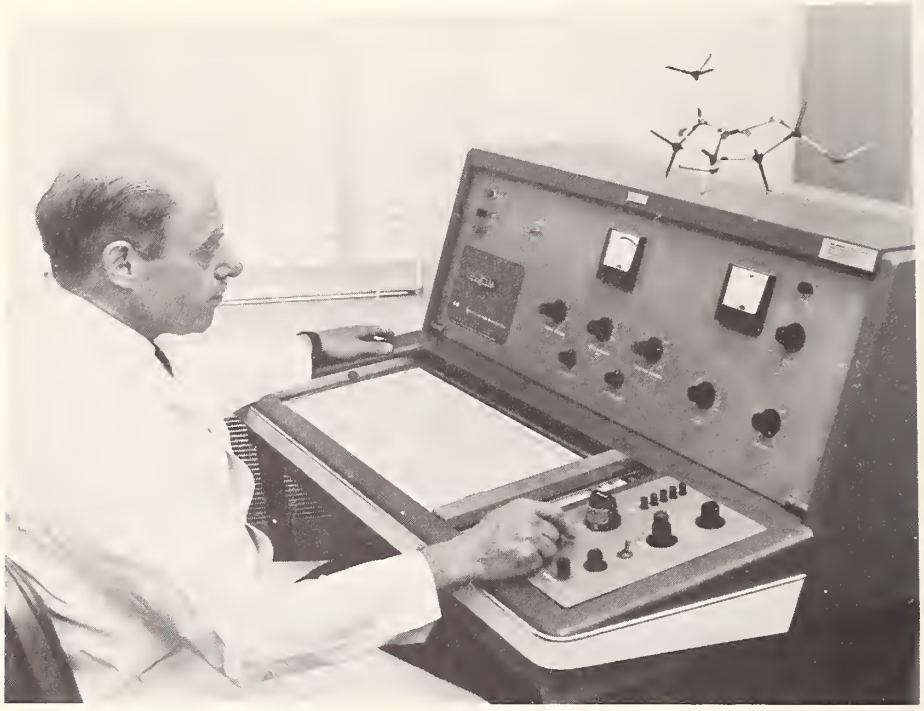


Figure 13. NMR spectrometer used for determining geometric arrangement of protons in cyclopentane derivatives.

powerful instrument capable of exhibiting many fine details of molecular structure. The magnetic resonance spectrum of the salt in deuterium oxide solution (figure 14) shows a pair of well-spaced singlets, each corresponding in intensity to two protons. By correlation with published information, the downfield 2-proton singlet is assigned to the protons on the carbon atoms to which the hydroxyl groups are bonded. Similarly, the upfield singlet is assigned to the two protons on the carbon atoms to which the dioxolane ring is bridged. Analysis shows

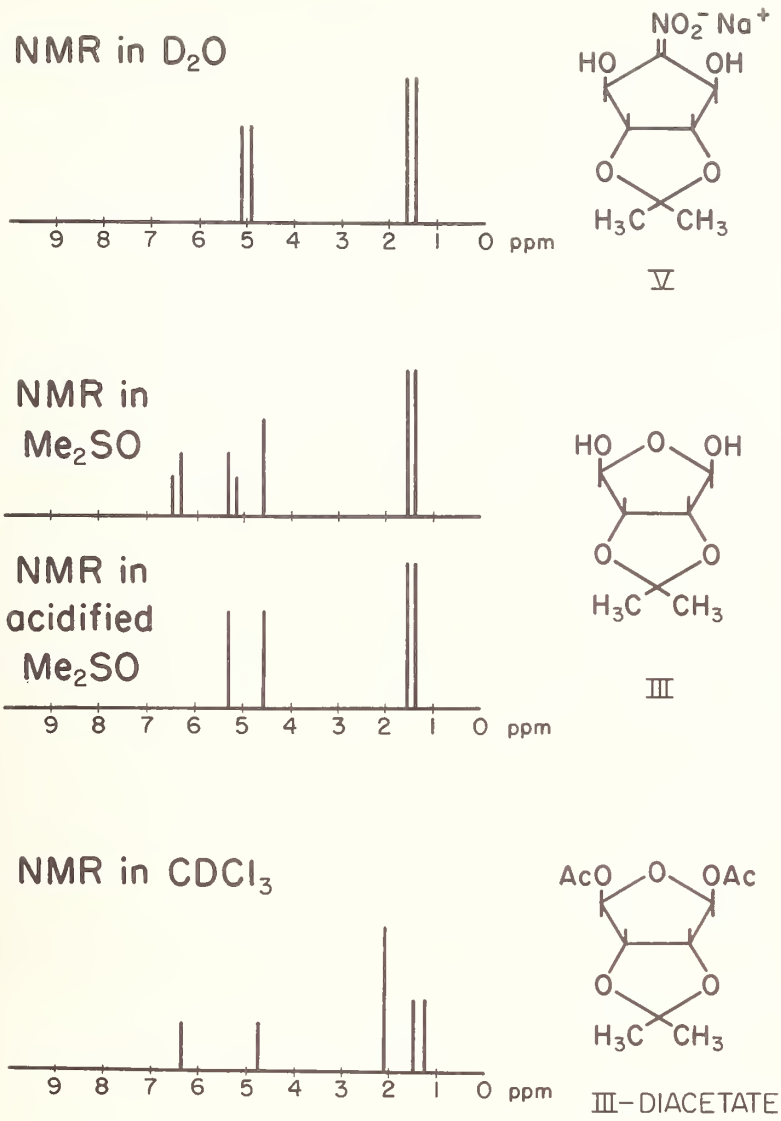
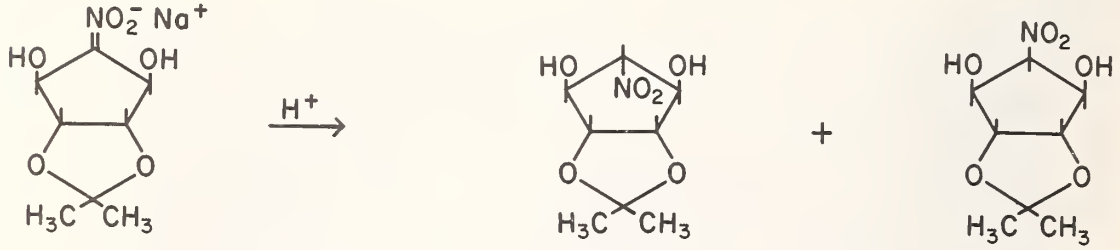


Figure 14. NMR spectra of V, III, and III-diacetate.

that this simple pattern can only be associated with the symmetrical trans-cis-trans geometrical arrangement of protons, which is the galacto configuration shown in structure V. This simple pattern was observed also in the NMR spectra of III and its diacetate. Conformational analysis would have predicted the galacto configuration for each of these structures; NMR provided the proof.

2. Conversion into Nitrocyclopentane Derivatives

The aci-nitro compound tautomerizes to the non-ionized nitro form when the sodium is replaced by hydrogen. This transformation generates VI and VII, whose structures differ only in their configurations at the new asymmetric center.



V VI VII

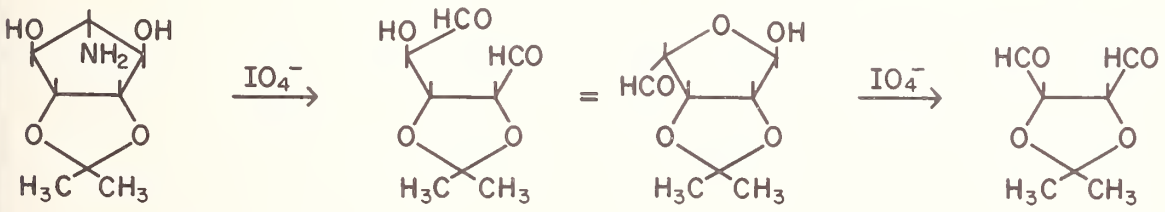
The nitrocyclopentane derivatives VI and VII crystallize as monohydrates from aqueous solution. The isolated crystals readily lose the water of hydration on drying in vacuo, but the dried products do not show characteristic melting points. Consequently, infrared spectra were employed to characterize the fractions of hydrated crystalline material isolated. The spectra were found to be of two distinctive patterns that were presumed to signify the separation of the individual nitro compounds. However, the reactions of samples of the "product type" that was obtained in greater abundance resulted in pairs of isomeric derivatives, and it was found that this product type was, in fact, a mixture resulting from co-crystallization of the two hydrated isomers. Separation into pure VI and VII was effected by fractional recrystallization of the anhydrous materials from chloroform and then from ether plus pentane.

3. The Aminocyclopentane Derivatives and Their Structures

a. Reactions with Sodium Metaperiodate. The amino compounds VIII and IX obtained by catalytic hydrogenation of VI and VII, respectively, were particularly useful for determining

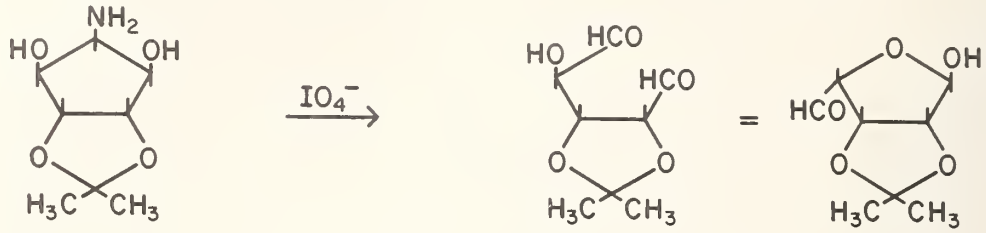
configurations at the asymmetric carbon atom attached to the nitro group. The amino derivatives were treated with sodium metaperiodate, which cleaves bonds between vicinal carbon atoms attached to hydroxyl and amino groups (and hydroxyl and aldehyde groups). Rates of this reaction are dependent on the cis (fast) or trans (slow) relationship of the hydroxyl and amino groups on the 5-membered ring. Open-chain α-hydroxy aldehydes are oxidized at intermediate rates. Although amino compounds VIII and IX could react with two mole-equivalents of sodium metaperiodate each, at completion the products of these reactions would be identical; but, on allowing the oxidation of each amino compound to occur with only one mole-equivalent of oxidant, the effects of their different structures on the course of the reaction (and, hence, their individual structures) are revealed by the nature of the reaction products that each gives.

Thus, one of the amino compounds, on equimolecular oxidation by periodate, gave only the 4-carbon-atom oxidation product (II), a result which implies that each oxidized molecule was oxidized a second time and that half of the original amount of the amino compound was not oxidized at all. Consequently, this amino compound is VIII, because both oxidizable groupings on its cyclopentane ring must be trans



VIII X II

for the open-chain 5-carbon-atom oxidation product (X), as it is formed, to undergo oxidation of its α-hydroxy aldehyde grouping more readily than either hydroxylamino grouping on the parent cyclopentane ring. The other amino compound, under

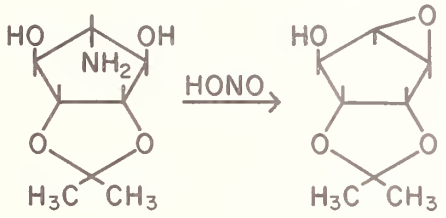


IX X

the same conditions, gave only the 5-carbon-atom open-chain product (X), and compound X has the arabino configuration. Hence, the cyclopentane ring in the present compound has only highly reactive cis hydroxyl and amino groupings. The parent is therefore IX. Cleavage of the cyclopentane ring yields the same open-chain 5-carbon-atom product (X) as would be obtained from VIII, but X accumulates here, since its cis-substituted cyclopentane ring precursor (IX) is a much more reactive reductant of periodate.

b. Reactions with Nitrous Acid. The configurations of the compounds assigned structures VIII and IX are supported by their behavior on treatment with nitrous acid. This reagent converts an amino into a diazo group, and the latter is eliminated from its attached carbon atom (nitrogen is evolved) with particular effectiveness if the diazo group and a vicinal trans hydroxyl group are substituents on a 5-membered ring. The result is the formation of a product having an epoxide ring. Compound VIII gave such a reaction with rapidity, thereby affording epoxide XI in high yield. On the other hand, with compounds where the amino group and the vicinal hydroxyl group

are cis to each other, reaction is sluggish and a number of products usually result. It was found that compound IX, which is assigned only cis configurations, showed little evidence of reaction with nitrous acid and no crystalline product could be isolated.



VIII XI

6. NOVEL RESEARCH MATERIALS

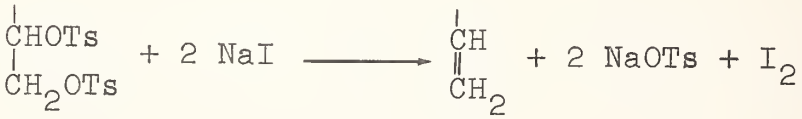
(R. S. Tipson and A. Cohen)

Advances in organic chemistry lead to new products for which improved methods of synthesis are needed; in addition, suitable methods of purification must be devised, and appropriate characterization must be developed.

A. Unsaturated Alditols

During the past few years, alditols and related compounds containing unsaturation have assumed considerable importance. Although, by the use of elimination reactions in aprotic, dipolar solvents, a method had been developed for the production of terminal unsaturation, a simple way of introducing nonterminal unsaturation was not available. We have now developed such a method, and a description has been published [50].

Unsaturation is readily introduced [51,52] at the two terminal carbon atoms of an alditol (or an acyclic polyhydroxyalkyl appendage of a cyclic sugar) by the action of sodium iodide (in a suitable solvent) on a derivative in which a primary and a secondary sulfonyloxy group are contiguous.

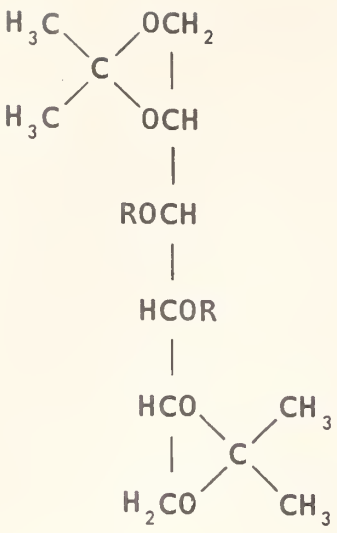


For sugar derivatives that are acyclic, there is only one example that has been recorded of a similar reaction involving two contiguous, secondary sulfonyloxy groups; it was found that methyl 4-deoxy-2,3-di-O-(methylsulfonyl)-DL-erythronate and -threonate react with sodium iodide [53] to

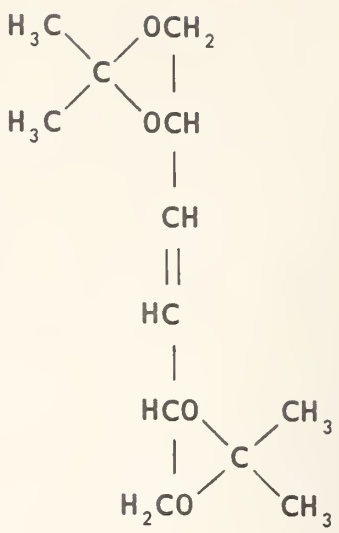
give the corresponding 2,3-unsaturated esters, but it is known that the carboxylic ester group has an activating effect [52].

"Unsuccessful attempts were made to prepare an unsaturated compound" [54] from the 3,4-dimethanesulfonate (IIa) and 3,4-di-*p*-toluenesulfonate (IIb) of 1,2:5,6-di-*O*-isopropylidene-D-mannitol (I). Consequently, we decided to re-investigate this reaction; for convenience, we avoided the use of an autoclave or of sealed tubes. We found that IIa is unaffected on treatment with a 10% solution of sodium iodide in acetone during eleven days at room temperature or eight hr at the boiling point. However, reaction occurred when various high-boiling solvents (for example, 2,5-hexanedione) were used at the respective boiling point; free iodine and the sodium methanesulfonate-iodide salt [53,55] were formed. Unfortunately, iodination took place, and this made isolation of III difficult. Hence, we made a search for (*a*) a solvent which was capable of dissolving sufficient sodium iodide and II(a or b) to be practical and which had a boiling point sufficiently high for the reaction to occur at a reasonable speed, and (*b*) a substance which, when present throughout the reaction, would combine with the iodine as fast as it was formed (thereby precluding iodination and promoting the reaction) and which would prevent the development of acidity.

We found that *N*,*N*-dimethylformamide provides a boiling point high enough for a reasonable rate of reaction and that zinc dust affords a rapid means for removing iodine. Treatment of IIa or IIb with an excess of sodium iodide (20% solution in dry *N*,*N*-dimethylformamide), in the presence of an excess of zinc dust, with efficient stirring and with vigorous boiling under reflux (drying tube) during 5 hr, followed by cooling,



I (R = H)
IIa (R = Ms)
IIb (R = Ts)



III

IIa + 2.5 NaI $\longrightarrow$ III + 2 NaOMs · 0.25 NaI + I_2

IIb + 2 NaI $\longrightarrow$ III + 2 NaOTs + I_2

filtering, extracting (heptane), evaporating the extract, retreating the residue with heptane, and evaporating, gave crystalline 1,2:5,6-di-O-isopropylidene-trans-3-hexene-D-threo-1,2,5,6-tetrol (III) (64%). This was sublimed at 45°/0.02 mm, and had mp 80-82 °C from aqueous ethanol; $[\alpha]_D^{24}$ + 57.5° (c 1.02, chloroform); $J_{3,4}$ 15.5 c.p.s.; it readily decolorized aqueous permanganate solution. These properties agree with those recorded [56,57] for compound III prepared by other methods. Compound III showed ν_{max} 1307 and 971 cm^{-1} (trans olefinic; not shown [58] by I) and 1156, 1134, and 1053 cm^{-1} (1,3-dioxolane rings; I shows [58] bands at 1160, 1126, and 1044 cm^{-1}).

When compound III was hydrolyzed with 80% acetic acid (5 hr at 25 °C), and the solution was evaporated to dryness, trans-3-hexene-D-threo-1,2,5,6-tetrol (IV) was obtained mp 64-65 °C (from ethanol—ethyl acetate); $[\alpha]_D^{25}$ - 13.8° (c 2.00, water); $J_{3,4}$ 15.5 c.p.s.; ν_{max} 1653, 1325, and 976 cm^{-1}. Anet [57] gave mp 114 °C; $[\alpha]_D$ - 2° (water); $J_{3,4}$ 15.5 c.p.s. For IV, Dr. Anet now finds mp 64-65 °C, $[\alpha]_D^{25}$ - 14.6° (c 0.5, water), and IR spectrum identical with ours (personal communication, Sept. 24th, 1965). Moreover, Dr. Haines has now prepared compound IV, and finds mp 60-62 °C, $[\alpha]_D^{24}$ - 15.3° (c 4.3, water), $J_{3,4}$ 15.9 c.p.s., and IR spectrum identical with ours (personal communication, Sept. 28th, 1965). Condensation of compound IV with acetone, in the presence of anhydrous cupric sulfate, regenerated III.

The above procedure for introducing nonterminal unsaturation should be applicable to a variety of alditol derivatives, and also to sulfonic esters of suitable derivatives of certain mono-, oligo-, and poly-saccharides, and cyclitols. Moreover, it should provide an improvement in the introducing of terminal unsaturation into alditol and other sugar derivatives by use of the appropriate sulfonic esters.

Upon conclusion of this phase of the project, work on it was suspended until that on the topic described in the next section could be completed.

B. 1-Acylamido Derivatives of Aldoses (Furanoid, Pyranoid, and Acyclic)

This work was conducted, in part, with compounds provided by Professor Venancio Deulofeu and Dr. Alberto S. Cerezo, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Perú 222, Buenos Aires, Argentina, and, in part, with compounds prepared at NBS on a project sponsored by the Office of Naval Research and described in NBS Report 1358 "Infrared Spectra of Sugar Acetates and Related Compounds" by H. S. Isbell, F. A. Smith, C. Creitz, J. D. Moyer, and H. L. Frush, December 1951.

The main object of the present project was to record the infrared absorption spectra of a variety of 1-acylamido derivatives of aldoses (having furanoid, pyranoid, or acyclic structures), in order to find whether previously reported correlations between the structure and specific bands in the infrared spectra of certain derivatives of sugars are also applicable to this group of compounds. In essence, the study consisted of answering two questions: (a) Do 1-acylamido compounds having certain structural features show, in their infrared spectra, the bands previously described as characteristic of such features, and (b) do 1-acylamido compounds that lack these structural features fail to show these bands in their infrared spectra? No infrared band can be regarded as unique for any feature, of course; and interpretation must be substantiated by other evidence.

For a paper now in process of publication, the spectra have been recorded for two glycofuranosylacetamides and their perbenzoates; eleven glycopyranosylacylamides and eight esters thereof; and thirteen 1,1-bis(acylamido)-1-deoxyalditols and eight esters thereof. In addition, a discussion is given of the spectra previously published [59] for five glycopyranosylacetamides and six acetates thereof, and for a 1,1-bis(acetamido)-1-deoxyalditol and its tetraacetate; the serial number given each of these compounds is that originally assigned [59], with the letter A suffixed thereto. Also, the spectra previously published [60] for three derivatives of D-ribosylamine have received consideration; the serial numbers for them are those originally given [60], with the letter B added.

Table 9 gives a list of the compounds studied, and an index to the spectrograms; the serial number of a compound is the same as the number of its spectrogram. The spectra were measured in the region of 4000 to 667 cm^{-1} for compounds 1 to 44, and arranged according to their code numbers [61].

In a series of articles [62,63,64], Barker and co-workers sought to identify, in the range of 960 to 730 cm^{-1}, infrared

bands characteristic of several aldopyranoses and their derivatives. They also noted [65] absorption bands for aldo- and keto-furanose derivatives. Verstraeten [66] has made a study of infrared bands characteristic of 2-ketoses, and Nanasi and co-workers [67] have made some correlations for glycosylamine derivatives.

To be of general usefulness in structural analysis, the correlations made by Barker and co-workers should be applicable to the furanoid and pyranoid forms, respectively, of sugar derivatives of all kinds, provided that allowance is made for bands attributable to such other features as benzene rings or acetate ester groups present in the molecule. Similarly, the other correlations should be applicable for the features mentioned.

We have made a careful study of the infrared spectra of the 60 compounds listed in table 9. We found that the useful correlations between structure and infrared absorption bands made by Barker and co-workers for certain carbohydrates, and by Nanasi and co-workers for some N-arylglycosylamines, cannot be extended to the 1-acylamido compounds we have studied. This conclusion confirms our previous reports that, for cyclic acetals of sugars, "distinctive absorption bands are not apparent for pyranose or furanose rings" [58], and that, for 1-acetamido derivatives of sugars, "no bands were noted which could be correlated with the absence or presence of the pyranoid ring" [68]. However, we found that certain of Verstraeten's correlations may have some diagnostic value.

This work has been brought to completion, and an article describing the results is being written.

Table 9. Compounds measured and index to spectrograms.

Compound	mp (°C)	$[\alpha]_D$, degrees	Reference	Spectrogram
A. N-GLYCOFURANOSYLACETAMIDES				
1. Unsubstituted				
N-Acetyl-				
α-D-xylosylamine	147-148	+100.0(H_2O)	1	1
α-D-glucosylamine	195-197	+87.6(H_2O)	2	2
2. Esters				
N-Acetyl-				
2,3,5-tri-O-benzoyl-α-D-xylosylamine	139-141	+31.8($CHCl_3$)	1	3
2,3,5,6-tetra-O-benzoyl-α-D-glucosylamine	153-155	-20.1($CHCl_3$)	3	4
B. N-GLYCOPYRANOSYLACYLAMIDES				
1. Unsubstituted				
N-Acetyl-				
β-D-xylosylamine	213-214	-0.7(H_2O)	4,5	(1A)
β-D-glucosylamine	230-260(dec.)	-22.8(H_2O)	4,5	(2A)
β-D-lyxosylamine	166-167	-47.0(H_2O)	6	5
β-D-mannosylamine, monohydrate	203-204	-47.4(H_2O)	4,5	(3A)
6-deoxy-β-L-mannosylamine	210-211	+65.7(H_2O)	6	6
α-L-arabinosylamine	222-224	+69.1(H_2O)	5,7	(4A)
α-D-galactosylamine	179-180	+194.9(H_2O)	6,8	7

β-D-galactosylamine	233 236-238	+9.8(H_2O) +10.2(H_2O)	5,8	(5A),8
α-D-ribosylamine	198-200	+17.8(H_2O)	9	(5B)
β-D-ribosylamine	195-197	-23.4(H_2O)	9	(6B)
N-Propionyl-β-D-glucosylamine	193-194	-23.2(H_2O)	3	9
N-Benzoyl-				
β-D-lyxosylamine	234-235	-22.0(H_2O)	6	10
α-D-arabinosylamine	209-211	-53.2(H_2O)	- [a]	11
β-D-glucosylamine	231-233	-12.2(H_2O)	3	12
β-D-mannosylamine	251-252	+5.8(py)	10	13
6-deoxy-β-L-mannosylamine	235-237	+18.9(H_2O)	11	14
β-D-galactosylamine	188-190	+17.3(H_2O)	6	15
2. Esters				
N-Acetyl-				
2,3,4-tri-O-acetyl-β-D-xylosylamine	172-173	+28.5($CHCl_3$)	4,5,12	(6A)
2,3,4,6-tetra-O-acetyl-β-D-glucosylamine	163-164	+17.4($CHCl_3$)	4,5,12	(7A)
2,3,4,6-tetra-O-acetyl-β-D-mannosylamine	188-189	-16.5($CHCl_3$)	4,5,12	(8A)
2,3,4-tri-O-acetyl-6-deoxy-β-L-mannosylamine	135-137	+6.2($CHCl_3$)	6	16
2,3,4-tri-O-acetyl-α-L-arabinosylamine	177-178	+89.6($CHCl_3$)	5,7,12	(9A)
2,3,4-tri-O-benzoyl-α-D-arabinosylamine	121-123	-270.6($CHCl_3$)	- [a]	17
2,3,4,6-tetra-O-acetyl-α-D-galactosylamine	172-173	+117.4($CHCl_3$)	5,8,12	(10A)
β anomer	173	+34.7($CHCl_3$)	5,8,12	(11A)
2,3,4-tri-O-acetyl-β-D-ribosylamine	128-130	+35.3($CHCl_3$)	9	(4B)

Table 9. (Cont'd.)

Compound	mp (°C)	$[\alpha]_D$, degrees	Reference	Spectrogram
N-Propionyl-2,3,4,6-tetra-O-propionyl-β-D-glucosylamine	103-104	+19.3($CHCl_3$)	3	18
2,3,4,6-tetra-O-benzoyl-β-D-glucosylamine	151-152	+68.7($CHCl_3$)	3	19
N-Benzoyl-2,3,4-tri-O-acetyl-α-D-arabinosylamine	162-163	-40.1($CHCl_3$)	- [a]	20
2,3,4-tri-O-benzoyl-β-D-xylosylamine	173-175	+1.9($CHCl_3$)	6	21
2,3,4-tri-O-benzoyl-α-D-arabinosylamine	214	-174.2($CHCl_3$)	- [a]	22
2,3,4,6-tetra-O-benzoyl-β-D-glucosylamine,	113-115	+23.0($CHCl_3$)	3	23
2,3,4,6-tetra-O-benzoyl-β-D-glucosylamine,	184	+22.9($CHCl_3$)	3	23a
C. 1,1-BIS(ACYLAMIDO)-1-DEOXYALDITOLS				
1. Unsubstituted				
1,1-Bis(acetamido)-1-deoxy-D-erythritol	210-211	+8.7(H_2O)	13	24
1-deoxy-L-arabinitol	189-191	+9.8(H_2O)	5,14	(15A)
1-deoxy-D-glucitol	165-167	+4.0(H_2O)	3	25
1,6-dideoxy-L-mannitol	238-240	+22.7(H_2O)	11	26
1-deoxy-D-galactitol	194-196	+8.4(H_2O)	15	27
1-Deoxy-1,1-bis(propionamido)-D-glucitol	154-156	+6.6(H_2O)	3	28
1,1-Bis(benzamido)-1-deoxy-L-erythritol	217-219	+13.5(py)	16	29
1-deoxy-L-arabinitol	194-196	-5.3(py)	16	30

1-deoxy-D-ribitol	190-192	-8.1(py)	16	31
1-deoxy-D-glucitol	199-201	+1.3(py)	10	32
1,2-dideoxy-D-arabino-hexitol	208-210	+12.0(py)	-[b]	33
1-deoxy-D-mannitol	222-224	+2.9(py)	10,17	34
1,6-dideoxy-L-mannitol	220-222	+14.1(py)	11	35
1-deoxy-D-galactitol	207-208	-6.8(py)	15	36
2. Esters				
1,1-Bis(acetamido)-1-deoxy-2,3,4,5-tetra-O-acetyl-L-arabinitol	218-219	-72.5($CHCl_3$)	5,12	(16A)
2,3,4,5,6-penta-O-acetyl-D-glucitol	188-190	+22.3($CHCl_3$)	3	37
1,1-Bis(benzamido)-1-deoxy-6-O-benzoyl-D-glucitol	208-209	+5.2(py)	18	38
2,3,4-tri-O-acetyl-5-O-benzoyl-D-lyxitol	184-186	+35.1($CHCl_3$)	19	39
2,3,4-tri-O-acetyl-5-O-benzoyl-D-arabinitol	192-193	+68.1($CHCl_3$)	19	40
2,3,4,5-tetra-O-benzoyl-D-arabinitol	134-136	+62.0($CHCl_3$)	16	41
2,3,4-tri-O-acetyl-L-erythritol	181-183	+9.2($CHCl_3$)	16	42
2,3,4,5-tetra-O-acetyl-D-ribitol	175-176	-10.2($CHCl_3$)	16	43
2,3,4,5,6-penta-O-acetyl-D-glucitol	189-191 (needles) 199-200 (plates)	-39.4($CHCl_3$)	20	44

[a] Prepared by A. S. Cerezo.

[b] Sample of this compound kindly provided by I. Mastronardi and J. O. Deferrari.

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7. STANDARD REFERENCE MATERIALS

(H. S. Isbell, H. L. Frush, and R. Schaffer)

A. New Program on Standard Reference Carbohydrates

1. Rare Hexoses

The development of highly sensitive, modern methods of instrumental analysis, and the heightened interest in carbohydrates because of their immense economic value and their many roles in biological processes, have resulted in a real and growing need for a large group of unavailable carbohydrates of high purity as standard reference materials. These are in demand for the identification of unknown compounds, for quantitative measurements by instrumental techniques, for studying and testing biological systems and processes, and as nucleating crystals for the preparation of sugars in other laboratories.

For many years, the Organic Chemistry Section has maintained a position of leadership in the development of methods for the synthesis of rare sugars and sugar derivatives. In a program covering a number of years, the Section has developed methods for synthesis of sugars position-labeled with carbon-14 and tritium, and supplied these radioactive sugars on a fee basis. The use of this service is world-wide. As the result of basic research and numerous publications, the scientific public looks to our Section for both information and materials in the carbohydrate field.

In a new project now underway, it is planned to prepare a series of standard reference carbohydrates, and to supply these on a fee basis. Certain commercially unavailable hexoses (D-allose, D-altrose, L-galactose, D-gulose, L-idose, D-sorbose, D-tagatose, and D-talose) will be prepared, in the highest purity feasible, in quantities of 10 to 100 g (depending on the individual characteristic of the sugar). Because the preparation of these materials is arduous and time-consuming, samples will be issued to the public in amounts of only 10 to 100 mg.

2. 1,2-O-Isopropylidene-L-Idose (R. Schaffer)

At present, there is no evidence for the natural occurrence of idose, but both mirror-image forms of synthetic idose have been prepared although neither has been obtained as a crystalline material [69]. This sirupy aldohexose is unstable. On storage, it is reported to transform spontaneously into the ketohexose sorbose [70], thus undergoing a reaction ordinarily catalyzed by alkali. Furthermore, in aqueous acid, the sugar tends to form an equilibrium mixture with its 1,6-anhydride, a product that results from loss of a molecule of water between the hydroxyl groups at C-1 and C-6 of the sugar [71]. Fortunately, derivatives of idose do not suffer such instability. Consequently, a derivative from which the free aldohexose can be readily liberated is of particular value for preserving the sugar and providing idose of optimum purity. Crystalline 1,2-O-isopropylidene-L-idose [72], which can be synthesized from D-glucose by a series of reactions, serves this purpose.

The transformation of D-glucose into 1,2-O-isopropylidene-L-idose involves the following steps: D-glucose (I) → 1,2:5,6-di-O-isopropylidene-D-glucose (II) → 1,2-O-isopropylidene-D-glucose (III) → 1,2-O-isopropylidene-L-xylo-pentodialdose (IV) → "dimer" (V) → 5-cyano-1,2-O-isopropylidene-D-gluco-pentose (VI) + 5-cyano-1,2-O-isopropylidene-L-ido-pentose (VII) → barium 1,2-O-isopropylidene-D-glucuronate (VIII) + barium 1,2-O-isopropylidene-L-iduronate (IX) → calcium 1,2-O-isopropylidene-L-iduronate (X) → 1,2-O-isopropylidene-L-idurono-6,3-lactone (XI) → 1,2-O-isopropylidene-L-idose (XII).

Details of the conversion of D-glucose into its acetone diacetal (II) and of the latter into monoacetone-D-glucose (III) have been presented in many places [73] and are therefore not discussed here. Periodate oxidation of III to the monoacetone-xylo-dialdose (IV), with crystallization of the "dimeric" product (V), was reported by Schaffer and Isbell [74].

a. Dimeric 1,2-O-Isopropylidene-D-xylo-pentodialdose Hemihydrate (V). A stirred solution of 50 g of sodium metaperiodate in 400 ml of water in a 1-liter flask, surrounded by an ice bath, was treated during 30 min with 50 g of 1,2-O-isopropylidene-D-glucofuranose, added in small portions. After the solution had been stirred an additional 20 min, the excess periodate was decomposed by the addition of ethylene glycol. The solution was concentrated by freeze-drying, and the residue was extracted with four 100-ml portions of chloroform. The extracts were combined, clarified by filtration through carbon, and concentrated under reduced pressure to a heavy sirup. By dissolution of the sirup in 100 ml of water and reconcentration under vacuum, the trace of chloroform was removed. The concentrate was dissolved in 50 ml of water, filtered, and stored in the refrigerator for several weeks. The crystals that formed were separated, washed with water, and then recrystallized from water to give 29.9 g of a molecular dimer of 1,2-O-isopropylidene-D-xylo-pentodialdofuranose which crystallizes with 0.5 molecule of water of hydration. Additional hydrate crystallized after lyophilization of the mother liquors, dissolution of the residue in an equal weight of water, and further storage at low temperature. Alternatively, this concentrate can be fractionally distilled at high vacuum, as described by Iwadare [75] in his original preparation of sirupy IV. The distilled sirup is not as pure as the crystalline product, but is nevertheless adequate as a starting material for the cyanohydrin synthesis. Extraction procedures provide a less pure sirupy product [76].

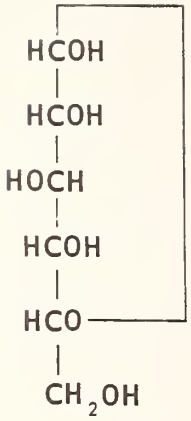
Conditions for adding cyanide to the free aldehyde group of IV were carefully studied [77] when the reaction was employed in this laboratory with radioactive cyanide for the synthesis of D-glucose-6-^{14}C. Since the cyanide addition reaction adds an asymmetric center to the L-xylo reactant, one product (VI) has the D-gluco and the other (VII) the L-ido-configuration.

The relative proportions of the two products (VI and VII) are affected by the buffer present in the reaction medium. We have found that, with an acetic acid-sodium acetate buffer, the reaction gives a maximum of the D-gluco epimer (VI) and that less acidic buffers lead to a diminished yield of VI but an increased yield of the L-ido epimer (VII). However, distinctly alkaline buffers were found to catalyze the aldol reaction of the dialdose IV [78], which resulted in lower yields of both VI and VII. The optimum yield of VII was obtained by use of a bisulfite-buffered reaction. Although our analytical details and an optimum-yield synthesis of the L-ido-epimer were published [79], a recently published procedure for preparing the L-ido product utilized a bicarbonate-buffered cyanohydrin reaction. In our procedure, the cyanohydrin addition products VI and VII are saponified by dilute alkali, and by use of cation-exchange resin are then converted into the salts VIII and IX. Advantage is taken of the marked difference in the solubilities of these barium salts to separate the epimers. Compound VIII crystallizes on concentrating an aqueous solution of VIII and IX, and replacement of the remaining water by methyl alcohol completes the separation, as compound IX remains in solution. The L-ido epimer is then purified by converting IX into the calcium salt X, which crystallizes readily. Other workers [76,80] have transformed the salt mixture obtained on saponification of VI and VII into their corresponding lactones, and separated the latter products by column chromatography. However, the salt-crystallization process we use is no less effective for the separation, and has the advantages of being much more rapid than the chromatographic method and readily utilizable for separating any quantity of material.

b. Calcium 1,2-O-Isopropylidene-L-idofuranuronate Dihydrate (X). An aqueous solution of 12.54 g of 1,2-O-isopropylidene-L-xylo-pentodialdofuranose (IV), 12.65 g of sodium pyrosulfite, and 3.4 g of sodium cyanide was prepared at 0° and allowed to warm to room temperature. After three days,

the solution was heated at 90° for one hr, and then treated with 7 g of sodium carbonate, and refluxed for three hr. The solution was cooled in an ice-bath, and passed through a column containing 350 ml of cation-exchange resin at ice temperature. The effluent, including washings, was delivered into a flask containing 41.6 g of barium hydroxide octahydrate, immersed in an ice-bath. After removal of excess barium hydroxide by carbonation and filtration, the filtrate was concentrated under vacuum to a sirup that yielded 8.5 g of crystalline barium 1,2-O-isopropylidene-D-glucofuranuronate monohydrate; addition of methyl alcohol gave a further 0.5 g. The mother liquor was passed through a column containing 75 ml of cation-exchange resin at ice temperature. The effluent, including washings, was delivered into a flask containing 5 g of calcium carbonate. The mixture was filtered, and the filtrate concentrated under vacuum. Calcium 1,2-O-isopropylidene-L-idofuranuronate dihydrate separated. The product, 4.7 g, was recrystallized from water with the addition of methyl alcohol.

c. 1,2-O-Isopropylidene-L-idurono-6,3-lactone (XI). Lactone XI is prepared from X by use of a cation-exchange resin to replace the calcium by hydrogen. The acid thus obtained loses water on refluxing in toluene solution. An almost quantitative conversion into XI is effected. An ice-cold, aqueous solution containing 20 g of X was passed through ice-cold cation-exchange resin, and the effluent and washings were concentrated under reduced pressure. After several additions of ethyl alcohol and reconcentrations, the concentrate was dissolved in acetone, toluene was added, and the solution was heated gently for 2 hr to allow the acetone to escape and the toluene to reflux. Filtration and concentration gave large crystals of XI.



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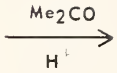

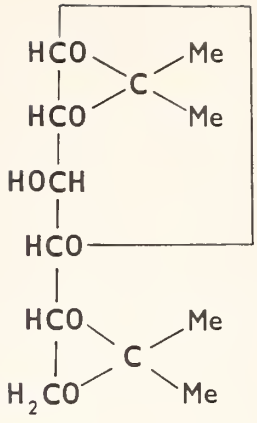

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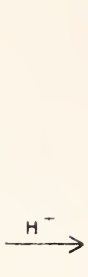

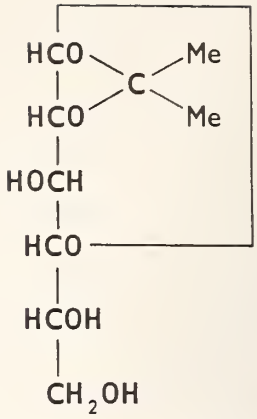

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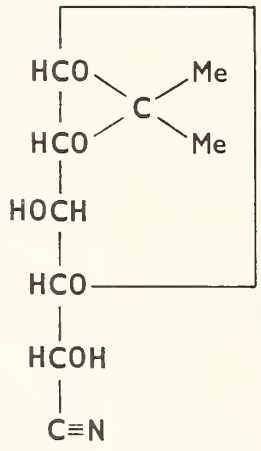

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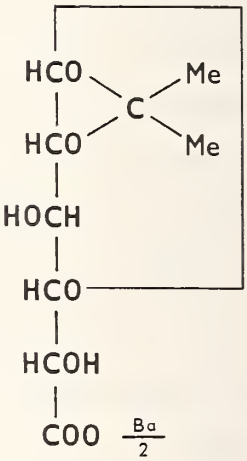
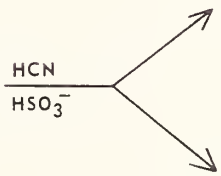


VIII

V
(or IV)



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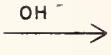

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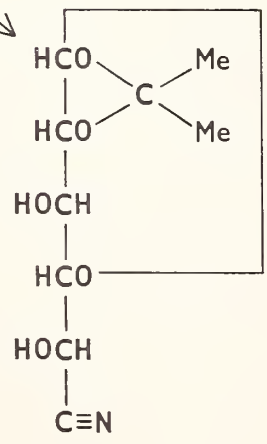
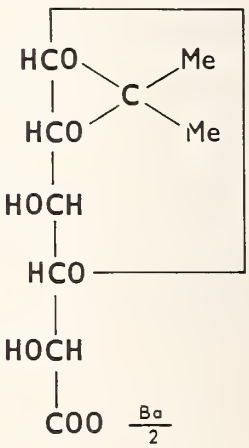
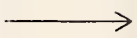
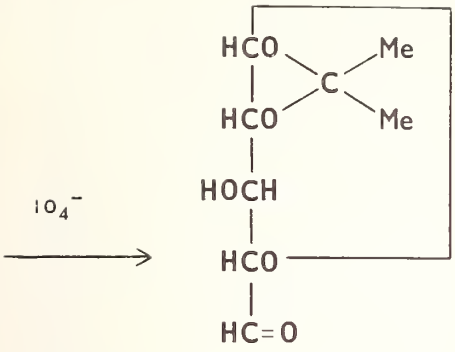


VII

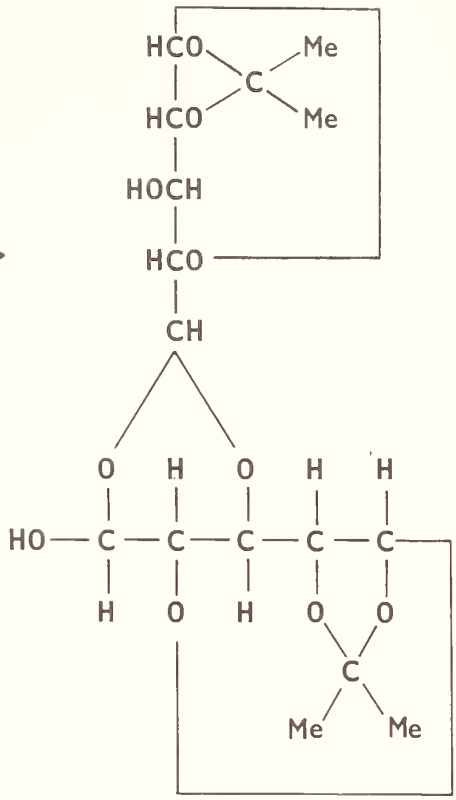


IX

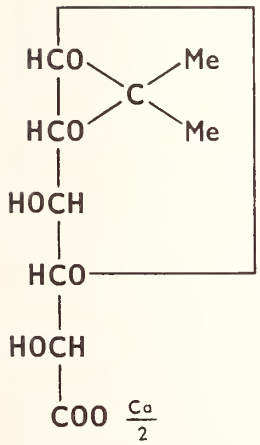



IO_4^-
HCO
Me
C
HCO
Me
HOCH
HCO
HC=O

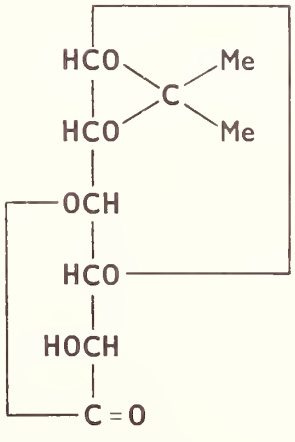
IV


HCO
Me
C
HCO
Me
HOCH
HCO
CH
O
H
O
H
H
HO
C
C
C
C
C
H
O
H
O
O
C
Me
Me

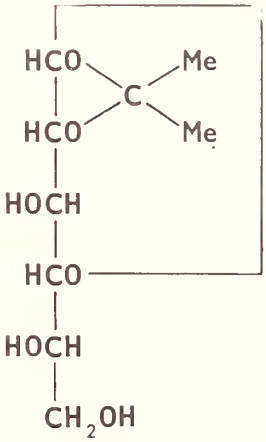
V


HCO
Me
C
HCO
Me
HOCH
HCO
HOCH
COO $\frac{Ca}{2}$

X


HCO
Me
C
HCO
Me
OCH
HCO
HOCH
C=O

XI


HCO
Me
C
HCO
Me
HOCH
HCO
HOCH
CH_2OH

XII

d. 1,2-O-Isopropylidene-L-idose (XII). Lithium aluminum hydride effectively reduces the lactone to XII. A stirred solution of 10 g of XI in anhydrous ether was treated slowly with a clear solution of lithium aluminum hydride in ether, and then refluxed for 15 min. Addition of 100 ml of ethyl alcohol (slow at first) led to the dissolution of precipitated solids, and, after a solution of 10 ml of concentrated acetic acid in 150 ml of water had been added, the ether was evaporated under a jet of nitrogen, and the aqueous solution was treated with cation-exchange resin at 0°. Concentration of the effluent, and reconcentrations with ethyl alcohol, gave a sirup that dissolved readily in ethyl acetate. 1,2-O-Isopropylidene-L-idose crystallized on concentration of the ethyl acetate solution. The product was recrystallized from ethyl alcohol plus pentane.

B. Metallo-organic Standards

1. Introduction (H. S. Isbell)

At the time of the initiation of the metallo-organic standards program at NBS, metal naphthenates were used for spectrographic measurements of metals in lubricating oils. Because of variations in the composition of naphthenic acid and lack of oil-solubility of some metal naphthenates, we sought other metallo-organic oil-soluble compounds as standard reference materials.

In development of this project, we prepared numerous salts of fatty acids; some of these had certain desirable properties, but certain salts formed gels with petroleum products. These gels are similar to Napalm (a coprecipitated aluminum soap from napthenic acid and the fatty acids of coconut oil, used for incendiary munitions). Presumably, gel formation arises from cross-linked metal complexes. To avoid formation of gels, we devised the technique of adding a highly fat-soluble beta-diketone to the mixture containing the standard. Presumably, the beta-diketone unites with some of the co-ordination centers of the metal, and thereby prevents cross-linking and formation of a gel. Use of this solubilizing technique permits considerable latitude in the compounds used in preparing solutions of the metals in lubricating oils. At the time we developed the solubilizing technique, we had already prepared supplies of some of the standards we now issue. With depletion of the stock of materials, we are now attempting to obtain improved products. Exploratory work has revealed that many of the metals of interest to this project form crystalline derivatives with 1-phenyl-1,3-butanedione. With the solubilizing technique, some of these compounds are suitable for use as standards. Procedures for

preparation of the chromium and iron chelates of 1-phenyl-1,3-butanedione were described in our last Technical Note (No. 274), and the preparation of the cadmium and calcium chelates is described here.

1. Cadmium and Calcium Standards (C. W. R. Wade)

Cadmium 2-ethylhexanoate and the cadmium derivative of 1-phenyl-1,3-butanedione were prepared, and studied for use as metallo-organic standards. Both compounds dissolve in the solubilizing mixture previously developed by us, and give stable solutions of cadmium in petroleum oils. Cadmium 2-ethylhexanoate is, however, liquid at room temperature and is therefore not suitable for use as a metallo-organic standard. The cadmium chelate of 1-phenyl-1,3-butanedione, however, was found to have the desired properties, and was selected for use as the standard material. Procedures for the preparation of both compounds are given below, as well as for the corresponding calcium compounds.

a. Cadmium 2-Ethylhexanoate. A solution of 7.98 g (0.03 mole) of cadmium acetate dihydrate in 50 ml of water was heated to 70 °C and stirred, while a hot, aqueous, 20% solution of sodium 2-ethylhexanoate (prepared from 4.32 g, 0.03 mole, of 2-ethylhexanoic acid) was added. Cadmium 2-ethylhexanoate separated as a viscous, amber sirup which was washed with two 5-ml portions of hot water and dissolved in 15 ml of ethyl alcohol. The solution was filtered and evaporated. Crystallization of the sample was effected by dissolving the viscous mass in 5 ml of pentane and refrigerating the solution. After about 24 hr, long thin needles separated, but on being warmed to room temperature, the crystals melted.

The compound dissolves readily in benzene, xylene, chloroform, methyl alcohol, toluene, heptane, or carbon tetrachloride; but, because of its low melting point, it is not suitable for use as a standard sample.

b. Cadmium Bis(1-phenyl-1,3-butanedione) Chelate. Cadmium acetate dihydrate (2.66 g, 0.01 mole) was dissolved in 5 ml of water and mixed with 5 ml of concentrated ammonium hydroxide (sp gr 0.90). The solution was heated to 80 °C and vigorously stirred, while 3.2 g (0.02 mole) of 1-phenyl-1,3-butanedione (benzoylacetone) in 5 ml of hot aqueous ethyl alcohol (50%) was added slowly.

The cadmium bis(1-phenyl-1,3-butanedione) chelate was precipitated immediately. After the mixture had been stirred for a further 30 min, the material was washed on the funnel with two 5-ml portions of hot water. The crude sample, after recrystallization from 30 ml of ethyl alcohol, weighed 3.9 g (89.6%) and melted at 210-211 °C with decomposition.

The solubility of the compound in oil, using 6-methyl-2,4-heptanedione and 2-ethylhexanoic acid as solubilizing agents, is equivalent to, or better than, that of cadmium cyclohexane-butyrate used as NBS Standard Sample 1053. Additional tests showed that the compound may be readily recrystallized from N,N-dimethylformamide, or from ethyl alcohol.

The preparation was repeated with 4 kg of cadmium acetate and 4.8 kg of 1-phenyl-1,3-butanedione. The crude crystalline product weighed 6.1 kg. After recrystallization from N,N-dimethyl-formamide, the compound will be used for a new cadmium organo-metallic standard.

c. Calcium 2-Ethylhexanoate - NBS Standard Sample 1074A. 2-Ethylhexanoic acid (1.8 kg, 12.5 moles) was neutralized with 20% aqueous sodium hydroxide, using phenolphthalein as the

indicator (faint pink). The resulting solution was heated on a water bath to about 90 °C and vigorously stirred, while a hot, aqueous solution of 763 g (6.88 moles) of calcium chloride in 2 l of water was slowly added. The calcium salt began to separate immediately as a sticky product which, after continued heating and stirring, became crystalline. The solid product was collected by filtration and washed three times with 500-ml portions of hot water. The crystalline material (1980 g) was recrystallized from 3 l of ethyl alcohol (water being added to incipient turbidity), and washed on the funnel with cold acetone—ethyl alcohol (1:4). The recrystallization was repeated, and the white compound was ground (agate mortar and pestle), sieved (60-mesh), and dried in a rotary vacuum drier (95 °C/5mm), with continuous mixing.

After the dried material had been resieved, the product was again thoroughly mixed, and submitted to Sections 310.05 and 310.02 for analyses.

Anal. Calcd. for $C_{16}H_{30}CaO_4$: Ca, 12.3. Found: Ca, 12.5.

Spectrochemical analysis: Silicon and strontium were each found to be less than 0.01 percent, and all other metallic impurities were each found to be less than 0.001 percent.

Results of the drying tests suggested that this standard should be dried in a desiccator over phosphorus pentaoxide for at least 48 hr.

d. Calcium Bis(1-phenyl-1,3-butanedione) Chelate. Calcium hydroxide (7.4 g, 0.1 mole) was mixed with 100 ml of water and heated to boiling, and the mixture vigorously stirred while 32.4 g of 1-phenyl-1,3-butanedione in 100 ml of ethyl alcohol was added. Reaction occurred immediately, with rapid dissolution of the calcium hydroxide and precipitation of the

calcium bis(1-phenyl-1,3-butanedione) chelate. The off-white precipitate was thoroughly washed with two 5-ml portions of hot water and air-dried; weight, 36.8 g (100%).

The dry, crude product was dissolved in 150 ml of hot ethyl alcohol, and the solution was kept at room temperature overnight. The resulting crystals were collected on a filter and recrystallized; weight 32 g, mp above 260 °C. The compound dissolves readily in the solubilizing mixture previously developed for use in spectrographic analyses, and will give a stable solution of 500 ppm of calcium in oil.

The use of this compound as a replacement for the present, oil-soluble, calcium standard is contemplated.

8. PERSONNEL AND ACTIVITIES

A. Personnel Listing

H. S. Isbell, Section Chief
R. S. Tipson
H. L. Frush
R. Schaffer
C. W. R. Wade
N. B. Holt - Retired Nov. 1965
A. J. Fatiadi
A. Cohen

B. Publications

Oxidation of Polycyclic, Aromatic Hydrocarbons.
R. S. Tipson, NBS Monograph 87 (Sept. 1965).

Synthesis of D-Glucose-3-^{14}C and Related Compounds.
H. L. Frush, L. T. Sniegoski, N. B. Holt, and H. S. Isbell, J. Res. NBS 69A, 535 (1965).

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D. NBS Reports

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NBS 8985 - Quarterly Report AIR POLLUTION PROGRAM - 7/1/65-9/30/65. R. Klein, R. E. Rebbert, R. Stair, R. S. Tipson.

NBS 9031 - Quarterly Report AIR POLLUTION PROGRAM - 10/1/65-12/31/65. R. Klein, R. E. Rebbert, R. Stair, R. S. Tipson.

NBS 9092 - Quarterly Report AIR POLLUTION PROGRAM - 1/1/66-3/31/66. R. Klein, R. E. Rebbert, R. Stair, R. S. Tipson.

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E. Lectures

9/15/65 - Reactions of 2-Oxo-1,3-bis(phenylhydrazones) and Diphenylformazans. 150th A.C.S. Meeting, Atlantic City, N. J. Symposium on New Reactions in Carbohydrate Chemistry. H. S. Isbell.

1/17/66 - The Use of Paper Chromatography in the Separation, Purification, and Analysis of Labeled Carbohydrates. A.C.S. Winter Meeting, Phoenix, Arizona. H. L. Frush

1/19/66 - 2,3-O-Isopropylidene-erythro-tetrodialdose and its Conversion into Nitro- (and Amino-)cyclopentanetetrols. A.C.S. Winter Meeting, Phoenix, Arizone. R. Schaffer

9. ACKNOWLEDGMENT

The Organic Chemistry Section wishes to thank Dr. J. K. Taylor and staff of the Analysis and Purification Section for assistance and use of equipment during the past year. We also wish to express our appreciation to Mrs. R. S. Maddock, Administrative Assistant for the Division of Analytical Chemistry in Charge of Publications, who assisted in preparing the report for publication.

The editor deeply appreciates the cooperation and help of all members of the staff of the Organic Chemistry Section in writing the report. He is particularly indebted to Dr. H. L. Frush who critically reviewed all parts of the report and made many improvements. Special thanks are expressed to Mrs. Y. S. Sladen, Section secretary, whose tireless efforts made expeditious publication possible and whose cooperation and patience made a difficult task pleasant.

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