

NBS

TECHNICAL NOTE

427

**Organic Chemistry: Air Pollution Studies;
Characterization of Chemical Structures;
Synthesis of Research Materials; Novel
Research Materials; Isotopic Methods for
Analysis of Carbohydrates; Occurrence,
Preparation, and Properties of Naturally
Occurring Monosaccharides (Including
6-Deoxy Sugars); Standard Reference
Materials (Organic)
July 1966 through June 1967**



**U.S. DEPARTMENT OF COMMERCE
National Bureau of Standards**

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ISSUED OCTOBER 1967

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Edited by Robert Schaffer

Organic Chemistry Section
Analytical Chemistry Division
Institute for Materials Research

NBS Technical Notes are designed to supplement the Bureau's regular publications program. They provide a means for making available scientific data that are of transient or limited interest. Technical Notes may be listed or referred to in the open literature.

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 a highly important output of our laboratories. 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 about our programs. A word is perhaps in order about the philosophy of these yearly progress reports. In any research program a large amount of information is obtained and techniques developed which never find their way into the literature. This includes the "negative results" which are so disappointing and unspectacular but which can often save others considerable work. Of importance also are the numerous small items which are often explored in a few days and which are not important enough to warrant publication--yet can be of great interest and use to specialists in a given area. Finally there are the experimental techniques and procedures, the designs and modifications of equipment, etc., which often require months to perfect and yet all too often must be covered in only a line or two of a journal article.

Thus our progress reports endeavor to present this information which we have struggled to obtain and which we feel might be of some help to others. Certain areas which it appears will not be treated fully in regular publications are considered in some detail here. Other results which are being written up for publication in the journal literature are covered in a much more abbreviated form.

At the National Bureau of Standards publications such as these fit logically into the category of a Technical Note. In 1967 we plan to issue these summaries for all of our sections. The following is the third annual report on progress of the Organic Chemistry Section.

W. Wayne Meinke, Chief
Analytical Chemistry Division

PREFACE

This is the third in a series of NBS Technical Notes which are prepared annually to summarize the technical activities of the Organic Chemistry Section of the Analytical Chemistry Division. The present report, which covers the period from July 1, 1966 to June 30, 1967, describes much work that is still in progress and also provides accounts of studies that were completed during the year and are, or are about to be, published.

For a great many years, and as it will continue to be, the chemistry of the carbohydrates has been a subject of major interest in the Section. The competences that have been developed in studying carbohydrate synthesis and reaction mechanisms, in developing methods of separation and purification, and in the characterization of molecular structure and conformation, are skills that are applied to work in other areas of organic chemistry, where the Section also serves in meeting the scientific needs of the Nation. Thus, the chemical properties of polycyclic, aromatic hydrocarbons, which occur as air-pollutants, are under intensive study. Also, the numerous metallo-organic compounds that are used as standards for the analysis of metals in oil are products of this Section's work.

Dr. H. S. Isbell, who served with great distinction as Chief of this Section, and Dr. Harriet L. Frush, who figured prominently in all of the Section's activities, are now serving as Consultants to the Analytical Chemistry Division, and therefore, a description of their on-going research work is not included in this Technical Note.

Robert Schaffer, Chief
Organic Chemistry Section

TABLE OF CONTENTS

	<u>PAGE</u>
1. AIR POLLUTION STUDIES.....	1
Detection of Impurities in, and Purification of, Commercial Compounds.....	1
Polycyclic, Aromatic Hydrocarbons.....	1
Related Compounds.....	11
Oxidation Products of Perylene.....	12
Determination of <u>ortho</u> -Quinones.....	16
Filtration of Aqueous Solutions of Polycyclic, Aromatic Hydrocarbons.....	17
Charge-transfer Complexes of Polycyclic, Aromatic Hydrocarbons.....	17
Photochemistry of Aromatic, Air Pollutants.....	17
Pyrene on Soil from Takoma Park, Maryland.....	18
Pyrene on Soil from Gaithersburg, Maryland.....	20
Perylene on Soil from Gaithersburg, Maryland.....	20
Fluorene on Soil from Gaithersburg, Maryland.....	24
1,4-Dihydroxyanthraquinone on Silica Gel.....	25
Anthracene on Carbon.....	25
Naphthacene in Solution.....	27
2. CHARACTERIZATION OF CHEMICAL STRUCTURES..	32
Infrared Absorption Spectra of 2-Oxo-1,3-bis(phenylhydrazono) Derivatives and Related Bis- and Tris-(phenylhydrazones).....	32

	<u>PAGE</u>
Phenylhydrazono—Phenylazo Tautomerism: Structures of 2-Oxo-1,3-bis(phenylhydrazono) Compounds and Related Compounds.....	33
Novel Aromatization of Inositols in Methyl Sulfoxide—Acetic Anhydride.....	34
3. SYNTHESIS OF RESEARCH MATERIALS.....	37
A Disaccharide of D-Lyxofuranose.....	37
Acetylation and Deacetylation.....	37
Chemical Properties of the New Product.....	38
Oxidation to the Crystalline Di-O-isopropylidenealdobionic Lactone.....	39
Reduction to the Crystalline (Di-O-isopropylidene-glycosyl)alditol.	39
Hydrolysis.....	39
Analysis by Nuclear Magnetic Resonance.....	40
4. NOVEL RESEARCH MATERIALS.....	42
1-Acylamido Derivatives of Aldoses (Furanoid, Pyranoid, and Acyclic).....	42
Reaction of Sulfonic Esters of Alditols with Methoxide.....	42
Infrared Spectroscopy of Carbohydrates....	51
5. ISOTOPIC METHODS FOR ANALYSIS OF CARBOHYDRATES.....	53

TABLE OF CONTENTS (CONT'D)

	<u>PAGE</u>
6. OCCURRENCE, PREPARATION, AND PROPERTIES OF NATURALLY OCCURRING MONOSACCHARIDES (INCLUDING 6-DEOXY SUGARS).....	54
7. STANDARD REFERENCE MATERIALS.....	56
Quebrachitol.....	56
<u>levo</u> -Inositol.....	58
1,2- <u>O</u> -Isopropylidene- β - <u>L</u> -idofuranose.....	60
1,2:5,6-Di- <u>O</u> -isopropylidene- β - <u>L</u> - idofuranose.....	60
1,2- <u>O</u> -Isopropylidene- β - <u>L</u> -idofuranose from the Diacetone Sugar.....	62
<u>L</u> -Idose from 1,2- <u>O</u> -Isopropylidene- β - <u>L</u> - idofuranose.....	63
2,3- <u>O</u> -Isopropylidene- β - <u>D</u> - <u>threo</u> - pentulofuranose.....	65
Polycyclic, Aromatic Hydrocarbons.....	66
8. PERSONNEL AND ACTIVITIES.....	67
Personnel Listing.....	67
Publications.....	67
Manuscripts in Process of Publication.....	68
NBS Reports.....	69
Lectures.....	70

TABLE OF CONTENTS (CONT'D)

	<u>PAGE</u>
9. ACKNOWLEDGEMENTS.....	71
10. REFERENCES.....	72

LIST OF FIGURES

<u>FIGURE NO.</u>	<u>PAGE</u>
1. (Top) High-resolution, grating, infrared spectrophotometer having an attached recorder for ordinate expansion. (Bottom) Preparation of thin-layer plates of silica gel G for chromatography.....	30
2. Infrared absorption spectra of (A) quebrachitol, (B) <u>levo</u> -inositol, and (C) 1,2-O-isopropylidene- β - <u>L</u> -idofuranose monohydrate.....	64

LIST OF TABLES

<u>TABLE NO.</u>	<u>PAGE</u>
1. Peaks (in nm) in the ultraviolet spectrum of authentic carbazole and of an impurity (carbazole) in zone-refined anthracene.....	4
2. Thin-layer chromatography of three samples (I, II, and III) of commercial anthracene.....	8
3. Ultraviolet and visible spectra of perylene derivatives.....	14
4. $\frac{R_f}{f}$ values in solvent A.....	22
5. $\frac{R_f}{f}$ values in solvent B.....	23

JULY 1966 TO JUNE 1967

Edited by R. Schaffer

ABSTRACT

This report describes work in progress in the Organic Chemistry Section of the Analytical Chemistry Division of the NBS Institute for Materials Research. The following research areas are discussed:

Air Pollution Studies. Information has been obtained on the detection of impurities in, and the purification of, commercial samples of certain polycyclic, aromatic hydrocarbons that are known to occur in polluted air. The oxidation of these hydrocarbons under conditions resembling those encountered in polluted areas has been studied, and some of the products have been identified.

Characterization of Chemical Structures. The infrared absorption spectra of a group of 2-oxo-1,3-bis(phenylhydrazono) derivatives have been studied, and compared with the spectra of four tris(phenylhydrazones) and six related mono- and bis-(phenylhydrazono) derivatives, in order to identify the important features of the bands arising from N=N, N-H (bending), and Ph-N groups. In addition, the phenylhydrazono-phenylazo tautomerism of 2-oxo-1,3-bis(phenylhydrazono) compounds has been elucidated. A novel aromatization of inositols in methyl sulfoxide-acetic anhydride has been discovered.

Synthesis of Research Materials: A Disaccharide of \underline{D} -Lyxofuranose. A compound formed in the high-vacuum distillation of 2,3- \underline{O} -isopropylidene- α - \underline{D} -lyxofuranose has been obtained crystalline by acetylation, crystallization of the diacetate, and saponification of the latter. From the results of oxidation, reduction, hydrolysis, and nuclear magnetic resonance studies, the compound is believed to be the di- \underline{O} -isopropylidene acetal of 5- \underline{O} - α - \underline{D} -lyxofuranosyl- α - \underline{D} -lyxofuranose. This is the first known example of a thermal condensation providing a reducing disaccharide in which both sugar moieties are furanoid.

Novel Research Materials. The reaction of sulfonic esters of alditols with methoxide has been examined, and a new dianhydrohexitol has been isolated. The literature on the infrared spectroscopy of carbohydrates has been critically evaluated and systematized.

Isotopic Methods for Analysis of Carbohydrates. The various methods of isotope-dilution analysis, the isotope effects that may accompany studies with labeled molecules, and the methods for determining isotopic distribution have been assessed and assembled.

Occurrence, Preparation, and Properties of Naturally Occurring Monosaccharides, Including 6-Deoxy Sugars. A survey has been made of all of the monosaccharides and 6-deoxy sugars that are known to occur naturally, and their preparation has been reviewed. In addition, their chemical and physical properties have been collated.

Standard Reference Materials. The preparation of highly pure samples of quebrachitol, levo-inositol, 1,2-O-isopropylidene- β -L-idofuranose, and 2,3-O-isopropylidene- β -D-threo-pentulofuranose has been achieved, and the compounds have been characterized by melting point, specific optical rotation, and infrared absorption spectrum. These compounds are now available to the public as standard reference materials.

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.

Key words:

Air pollution studies, oxidation of polycyclic aromatic hydrocarbons, characterization of chemical structures, phenylhydrazono-phenylazo tautomerism, disaccharide of D-lyxofuranose, infrared absorption methods for analysis of carbohydrates, naturally occurring monosaccharides and 6-deoxy sugars, standard reference materials, quebrachitol, levo-inositol, 1,2-O-isopropylidene- β -L-idofuranose, 2,3-O-isopropylidene- β -D-threo-pentulofuranose.

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

Polluted air contains many polycyclic, aromatic hydrocarbons and certain of their oxidation products. Information on the properties of these compounds has been obtained, the oxidation of the hydrocarbons under conditions resembling those encountered in polluted areas has been studied, and the products have been identified.

B. Detection of Impurities in, and Purification of, Commercial Compounds

A very pure sample of each hydrocarbon to be studied must be prepared and characterized before meaningful studies of its oxidation can be made. In addition, a sample must be prepared of each oxidation product that may be encountered in the oxidation studies, and this material must also be pure and adequately characterized.

1. Polycyclic, Aromatic Hydrocarbons

For most of the polycyclic hydrocarbons that are known to occur in polluted air, commercial samples have been purchased; for a number of these samples, the purity has been determined by chromatographic and spectrophotometric techniques and the impurities present have been identified.

a. Anthracene

(A. Cohen)

In Technical Note 405, we recorded the observation that, by use of petroleum ether saturated with 97% methanol (aqueous),

thin-layer chromatograms of commercial anthracene (refined by zone melting to 99.99% minimum purity) on Eastman Kodak Chromagram silica gel sheets (activated for 0.5 hr at 115 °C) showed no impurities in the anthracene. Similarly, we reported that, when a sample of this zone-refined anthracene was applied as a spot to silica gel G and developed with benzene, only anthracene was detected under ultraviolet light; however, when this plate was now sprayed with concentrated sulfuric acid (30% solution in absolute methanol) and heated on a hot plate, a bluish purple spot appeared that was neither anthracene nor anthraquinone. In this way, we demonstrated that the zone-refined anthracene contained an impurity.

Because it is essential that we should have anthracene that is free from impurities (for use in studies on the photooxidation of anthracene), we decided to attempt the isolation and characterization of the impurity, so that methods for its removal from the zone-refined hydrocarbon could be devised.

(1) Carbazole as an Impurity

In a trial experiment, 2.6 mg of zone-refined anthracene was applied as 35 spots (74.4 µg per spot, in chloroform) to a thin-layer plate of activated silica gel G, and the plate was developed with benzene. The dried plate was protected with another glass plate, except for a narrow, exposed region that was sprayed with sulfuric acid in methanol in order to reveal the positions of the constituents. The approximate R_f values were: anthracene, 0.65; impurity, 0.51. The region containing the impurity was scraped off with a razor blade, and the scrapings were extracted under nitrogen with hot 2,2,4-trimethylpentane and then with benzene. The extracts were combined and evaporated to dryness, and the ultraviolet

spectrum of the material was recorded.

From the experience gained, the material was now isolated on a larger scale by use of the "dry column" technique. Zone-refined anthracene (0.1 g) was dissolved in chloroform and adsorbed on 1 g of silica gel. The dry mixture was placed on top of a dry column (i.d. 11.5 mm) of 50 g of silica gel (100-200 mesh), and developed for 2 hr with benzene. Elution under nitrogen pressure gave a series of fractions, and those containing mainly the impurity (as shown by thin-layer chromatography, as above) were combined and examined by ultraviolet spectroscopy. The spectrum showed peaks corresponding to those of the material previously isolated, together with peaks indicating the presence of a small proportion of anthracene. The material was therefore rechromatographed on a dry column (i.d. 6 mm) of 10 g of silica gel, with benzene as developer, and eluted under nitrogen pressure. Evaporation of the eluate gave 1.55 mg of material, and examination by ultraviolet spectroscopy showed that the anthracene had been removed. The ultraviolet spectrum was now identical with that of carbazole (in methanol and in 2,2,4-trimethylpentane); see table 1. Its infrared absorption spectrum was also identical with that of carbazole. On thin-layer chromatograms on silica gel G, it had the same R_f value as that of carbazole. The respective R_f values were: 0.49 (benzene), 0.72 (methanol), and 0.0 (2,2,4-trimethylpentane). After the sulfuric acid spray, the colors produced on heating were essentially the same for the material and for carbazole.

(ii) Other Impurities

A sample of the zone-refined anthracene was re-examined by thin-layer chromatography on silica gel G, with benzene as

Table 1. Peaks (in nm) in the ultraviolet spectrum of authentic carbazole and of an impurity (carbazole) in zone-refined anthracene

In methanol		In 2,2,4-trimethylpentane	
Impurity ^a	Carbazole [1]	Impurity ^a	Carbazole ^b
336	335.5	330	330
323	323	322(i)	322(i)
312(sh)	314	317	318
---	---	306	306
291	292	290	290
287(sh)	286.5	285	284
281(i)	---	279	279
---	---	274(i)	274(i)
---	---	272(i)	270(i)
256	256.5	254	254
243(sh)	242(sh)	245	245
233	233.5	231	232
228(sh)	228(sh)	---	---
212(sh)	212.5	209	209

^a Key: i = inflection; sh = shoulder.

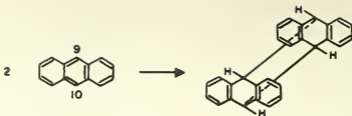
^b Commercial sample.

the developer; when the plate was sprayed with 20% sulfuric acid in methanol, and heated on a hot plate, two spots appeared, namely, that for anthracene and the purplish spot for carbazole. However, on examination under ultraviolet light, a very faint spot having the same faint-pink fluorescence and R_f value (0.27) as anthraquinone was noted. On prolonged heating, this spot became faintly visible under room light. Thus, commercially zone-refined anthracene contains, besides anthracene, a small proportion of carbazole and a trace of impurity that may be anthraquinone.

The commercial product was next examined at higher concentration, by thin-layer chromatography on silica gel G with benzene as developer, and a third impurity was discovered by examining the chromatogram under ultraviolet light (after spraying with methanolic sulfuric acid, and prolonged heating); this impurity moves slightly faster than anthracene and has R_f 0.81. The spot was barely visible under ultraviolet light, and the material responsible for this spot has not yet been identified. Visualization of the two impurities other than carbazole is not always reproducible.

(iii) 9,10,11,16-Tetrahydro-9,10[9',10']-anthracenoanthracene

Almost 100 years ago, Fritzsche [2] discovered that, if a cold, saturated solution of anthracene in benzene is kept in sunlight, dimerization occurs to give a bianthracene; this is sparingly soluble and crystallizes out. The compound has been called 9,9':10,10'-bianthracene; the systematic name is given in the heading.



Anthracene

9,9':10,10'-Bianthracene

Because of the possibility that exposed samples of anthracene might contain the dimer, or that it might be formed during photo-oxidation under ultraviolet light, a sample of the dimer was prepared as a reference compound. Anthracene (0.5 g) was dissolved in 100 ml of benzene, the flask and contents were heated to drive out most of the air, and the flask was stoppered. The solution was irradiated with ultraviolet light (366 nm) until it began to acquire a pale-yellow color (7 hr). During this time, the dimer continuously crystallized out. It was collected by filtration, washed with benzene, and dried; yield, 73%. The dimer is insoluble in most of the organic solvents tested, but we discovered that it can be recrystallized from boiling N,N-dimethylformamide (1 g of dimer per 150 ml of solvent); mp 254-259 °C (bath preheated to 240 °C, temperature rise of 6 °C per min); lit. mp 242-244 °C [3], 244-245 °C [4], 270-280 °C (rapid heating) [5]. A sample sealed in a melting-point tube under a pressure of 0.4 mm Hg sublimed at 251 °C (without melting). Its ultraviolet and infrared spectra were recorded.

(iv) Comparison of Commercial Anthracenes

In order to establish specifications of purity for anthracene as a possible NBS standard, three commercial samples of anthracene were examined: I, "high purity"; II, synthetic; and III, zone-refined. Three standard solutions were prepared, each containing 0.127 g of the respective anthracene per 25 ml of solution in benzene; and 2, 4, and 6 μ l of each solution were applied to thin-layer plates of silica gel G (250 μ m thickness) which had been air-dried overnight, activated for 1 hr at 115 $^{\circ}$ C, and stored over phosphorus pentoxide. The samples were applied in the dark (except for the use of a 50-watt, dark-ruby, photographic dark-room bulb) and dried with nitrogen.

The chromatograms were developed for 12 cm in three separate solvents, namely, benzene, cyclohexane, and methanol. The developed plates were examined under room light, and no spots could be seen. They were examined under ultraviolet light, and then sprayed with methanolic sulfuric acid, charred (for 10 min at 115 $^{\circ}$ C), and re-examined, with the results shown in table 2.

From table 2, it is seen that the synthetic anthracene (II) is the purest of the three samples: it contains no detectable proportion of carbazole. Moreover, the zone-refined anthracene (III) contains less carbazole than the "high purity" material (I). Each sample may contain anthraquinone and traces of impurities that show up near the origin in the chromatograms described in table 2.

Table 2. Thin-layer chromatography of three samples (I, II, and III) of commercial anthracene

Developing solvent	Conditions of examination ^a	R _f value of sample of anthracene			Comments ^b ; conclusion
		I	II	III	
Benzene	A	0.62			yellowish blue fl.; anthracene
			0.62	0.62	blue fl.; anthracene
		0.025	0.025	0.025	pale-blue fl.; unknown
B		0.008			yellow fl.; unknown
					green; anthracene
		0.44		0.44	blue (weaker for III); carbazole
C		0.25	0.25	0.25	faint pink fl.; not always reproducible; possibly anthraquinone
		0.025	0.025	0.025	pale-blue fl. (yellow under 254-nm light); unknown
		0.008			pale-yellow fl. (yellow under 254-nm light); unknown

Table 2. Continued

Developing solvent	Conditions of examination ^a	R _f value of sample of anthracene			Comments ^b ; conclusion
		I	II	III	
Cyclohexane	A	0.16	0.16	0.16	blue fl.; anthracene
		0.00			yellow fl.; unknown
	B	0.00	0.00	0.00	faint blue fl.; unknown grayish green; anthracene
Methanol		0.00	0.00	0.00	blue; carbazole
	A		0.02	0.02	faint gray; unknown
	C		0.02	0.02	violet fl. (yellow halo); anthracene
Methanol		0.00	0.02	0.02	yellow fl.; unknown
	A		0.7	0.7	blue fl. (bright under 254-nm light); unknown
	C	0.7			blue fl.; anthracene yellow fl.; changes to blue on prolonged exposure to u.v.; anthracene

Table 2. Continued

Developing solvent	Conditions of examination ^a	R_f value of sample of anthracene			Comments ^b ; conclusion
		I	II	III	
B		0.7	0.7	0.7	greenish gray; anthracene (possibly overlapped by carbazole of same R_f)
C		0.7	0.7	0.7	pink fl. (head) with purple fl. (tail); anthracene
		0.00	0.00	0.00	violet fl.; unknown

^aKey: A, under ultraviolet light; B, visible, after treatment with sulfuric acid; C, under ultraviolet light, after treatment with sulfuric acid.

^b fl. = fluorescence.

b. Fluorene

(A. J. Fatiadi)

The impurities in a sample of commercial fluorene ("white label" grade) were detected by thin-layer chromatography. A sample (about 2 μg) of the fluorene was spotted on a glass plate coated with silica gel G (250- μm layer, 5 x 20 cm) which was then placed in a chamber and developed with 1:1 (v/v) heptane—benzene; developing time, 60 min. The plate was then dried in a hood, sprayed with a 10% solution of concentrated sulfuric acid in methanol, and heated at 100 °C for 3 min. The following spots were observed: (1) colorless to very pale blue (blue fluorescence), R_f 0.86 \pm 0.01, fluorene; (2) deep blue-purple (not fluorescent), R_f 0.37 \pm 0.02, carbazole (approximate concentration 0.5 to 0.75%); (3) colorless (pale bluish green fluorescence), R_f 0.00 \pm 0.02, unknown.

The commercial fluorene was then purified by column chromatography on a column of Florisil (100-200 mesh). A solution of fluorene in benzene (reagent grade) was added to the column, and elution was conducted with 1:1 (v/v) heptane—benzene. The fractions exhibiting a blue fluorescence were collected, combined, and evaporated to dryness, and the crystalline product was recrystallized from absolute ethanol. The pure fluorene was colorless, had mp 116-117 °C (lit. mp [6], 116 °C), and showed only one spot (blue fluorescence) on a thin-layer chromatogram.

2. Related Compounds

a. Fluoren-9-one

(A. J. Fatiadi)

Commercial fluoren-9-one ("white label" grade) was examined by thin-layer chromatography, and found to be contaminated

with a small proportion of fluorene. The commercial fluoren-9-one was then purified by chromatography on a column of silica gel, with 1:1 (v/v) ethyl acetate—benzene as the eluant. The fractions having a yellow color were collected, combined, and evaporated to dryness, and the crystalline product was recrystallized from absolute ethanol. The pure compound was obtained as yellow crystals, mp 85-86 °C (lit. mp [7], 84-86 °C), and showed only one (yellow) spot on a thin-layer chromatogram on silica gel G with 1:1 (v/v) ethyl acetate—benzene.

b. Carbazole

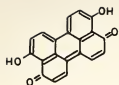
(A. J. Fatiadi)

Commercial carbazole ("white label" grade) was examined by thin-layer chromatography on silica gel G, with 1:1 (v/v) ethyl acetate—benzene as developer, and found (10% methanolic sulfuric acid spray) to contain a contaminant as shown by a blue spot faster than that of carbazole. The impurity has not yet been identified.

c. Oxidation Products of Perylene

(A. J. Fatiadi)

Pure reference compounds of perylene, particularly the various oxidation products, were needed in connection with a study of the products resulting from photochemical reactions of perylene adsorbed on various particulates. The preparation and purification of 3,10-perylenequinone have been described in Technical Note 405. We have now prepared 1,12-perylenequinone and 4,9-dihydroxy-3,10-perylenequinone. The 1,12-dione was obtained by a published procedure [8] involving cyclization of 2,2'-dihydroxy-1,1'-binaphthyl with aluminum chloride,



3,10-Perylene-
quinone



1,12-Perylene-
quinone

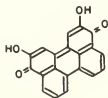


4,9-Dihydroxy-
3,10-perylene-
quinone

to give 1,12-perylenediol which was then oxidized with air and lead dioxide to give crude 1,12-perylenequinone. We have found that this procedure is not very efficient, as the desired dione is a labile compound which is difficult to purify. Preliminary values for peaks in its ultraviolet and visible spectra are given in table 3.

4,9-Dihydroxy-3,10-perylenequinone was prepared by heating a solution of 3,4,9,10-tetranitroperylene in concentrated sulfuric acid at 140 °C for six hr [9]. The product was recrystallized from nitrobenzene to give lustrous, blue crystals, mp 483-485 °C (with sublimation and some decomposition). Preliminary values for peaks in its ultraviolet and visible spectra are given in table 3.

2,11-Dihydroxy-3,10-perylenequinone was prepared by a Friedel—Crafts condensation of 1,2-naphthoquinone, by a modification of a published procedure [10].



2,11-Dihydroxy-3,10-perylenequinone

Table 3. Ultraviolet and visible spectra of perylene derivatives

1,12-Perylenequinone		4,9-Dihydroxy-3,10-perylenequinone	
λ_{max} (nm)		λ_{max} (nm)	
in methanol		in methanol	in DMF ^a
223			
238		231(sh)	
---		267(sh)	
278(sh)		276	
292(sh)		280(sh)	
307(sh)		302(sh)	
319		---	
346(sh)		---	
---			400(sh)
---			424
458			450
			512(sh)
			522
			595

^a N,N-Dimethylformamide.

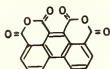
4-Oxo-4H-benz[de]anthracenedicarboxylic anhydride ("peryleneic anhydride") was prepared by oxidation of perylene



4-Oxo-4H-benz[de]anthracenedicarboxylic Anhydride

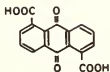
with chromic acid [11], by oxidation of 3,10-perylenequinone with manganese dioxide in concentrated sulfuric acid [11], or by oxidation of perylene with hydrogen peroxide—vanadium pentaoxide complex.

Phenanthrene-1,8,9,10-tetracarboxylic dianhydride was prepared by a modification of a published procedure [13].



Phenanthrene-1,8,9,10-tetracarboxylic Dianhydride

Anthraquinone-1,5-dicarboxylic acid was prepared by



Anthraquinone-1,5-dicarboxylic Acid

oxidation of perylene or of 3,10-perylenequinone with chromic acid [13].

Detailed procedures for preparation and purification of these compounds, together with their infrared spectra, R_f values, and other physical properties, are given in an article entitled "Preparation and Purification of Some Oxidation Products of Perylene," prepared for publication in J. Res. NBS.

D. Determination of ortho-Quinones

(A. Cohen)

We have shown that photo-oxidation of anthracene gives anthraquinone and hydroxylated derivatives thereof, but have not conclusively demonstrated that an ortho-quinone is not formed. The o-quinones and their homologs may be detected by sintering with guanidine carbonate, to give a blue-violet color [14], and preliminary work has now been done in applying this test in thin-layer chromatography. The following compounds were spotted on thin-layer plates of silica gel G: 1,4-naphthoquinone (1), 1,2-naphthoquinone (2), anthraquinone (3), acenaphthenequinone (4), 1,8-pyrenedione (5), 1,6-pyrenedione (6), p-benzoquinone (7), benz[a]anthracene-7,12-dione (8), phenanthrenequinone (9), and 1,4-dihydroxyanthraquinone (10). The plates were sprayed with 20% aqueous guanidine carbonate, and heated at 120 °C for 5 min. Compounds 2, 4, and 9, which are o-quinones, might have been expected to show some similarity, either under visible or ultraviolet light; however, this was not found. Under the conditions tried, compound 2 gave a reddish brown spot (visible) that was blue under u.v.; compound 4 gave a faint yellow spot (visible) that was bluish white under u.v.; and compound 9 gave an

orange spot (visible) that was not seen under u.v. The other compounds gave spots of the following colors: (visible) 1, reddish brown; 3, pink; 5, reddish brown; 6, brown; 7, brown with a green circumference; 8, yellow; and 10, violet; (ultraviolet) 1, blue-green; 3, pink; 5, negative; 6, blue; 7, blue-green; 8, negative; and 10, negative.

E. Filtration of Aqueous Solutions of Polycyclic, Aromatic Hydrocarbons

(M. N. Inscoe)

An article entitled "Losses Due to Adsorption During Filtration of Aqueous Solutions of Polycyclic, Aromatic Hydrocarbons," by M. N. Inscoe, was published in Nature, 211, 1083 (1966). This work was described in Technical Note 274, p. 89.

F. Charge-transfer Complexes of Polycyclic, Aromatic Hydrocarbons

(J. H. Gould)

An article entitled "Charge-transfer Complexes of Polycyclic, Aromatic Hydrocarbons with 2,4,7-Trinitrofluoren-9-one," by J. H. Gould, has been written.

G. Photochemistry of Aromatic, Air Pollutants

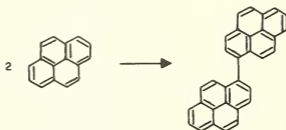
1. Introduction

In Technical Note 405, we described photochemical changes of several hydrocarbons adsorbed on various particulate matters when exposed to air and ultraviolet radiation. For example, we studied the products from pyrene adsorbed on silica gel, silicic acid, alumina, certain other metal oxides, and other particulates. This work has been continued and extended.

2. Pyrene on Soil from Takoma Park, Maryland

(A. J. Fatiadi)

Because pyrene is a constituent of polluted air, and because garden soil is a wide-spread, natural adsorbent, the work was extended to include these two materials. In Technical Note 405, a preliminary account was given of the reaction on this Maryland soil. It was found that irradiation for 240 hr at 32 °C gave a mixture of products that could be separated by thin-layer and column chromatography. The thin-layer chromatogram obtained showed 8 spots. Spot No. 1 was identified as unreacted pyrene. Spot No. 7 has now been identified as 1,1'-bipyrene, a compound formed from pyrene by hydrogen abstraction and dimerization. This material comprising spot No. 7 is slow-moving and has a strong blue fluorescence; its $\frac{R_f}{\text{—}}$ value is identical with that of authentic



1,1'-bipyrene. Extraction of the reaction mixture (from 2.5 g of pyrene) with toluene yielded about 60 mg of 1,1'-bipyrene which, on recrystallization from toluene—pyridine, had mp 334-336 °C. Six other compounds were formed, and four of these have been identified, namely, 1,6- and 1,8-pyrenediones, and 1,6- and 1,8-pyrenediols.

In order to evaluate the significance of the various elements present in this soil sample, particularly in the formation of 1,1'-bipyrene, the soil has been analyzed for metallic constituents by a general, qualitative, spectrochemical method (by Bourdon F. Scribner and Elizabeth K. Hubbard), with the following results: less than 0.001% of each, Ag, B, Be, Cu, Li, Pb; 0.001 to 0.01% of each, Ni, Rb, Sr, V, Zn; 0.01 to 0.1% of each, Ba, Ca, Cr, Mn; 0.1 to 1% of each, K, Na, Ti; 1 to 10%, Al, Fe, Mg; greater than 10%, Si.

When garden soil with adsorbed pyrene was stirred in the dark at 32 °C for 240 hr, the yield of 1,1'-bipyrene was lowered (from 4 to 6%) to 0.3%, and only traces of the two diones were formed; however, on treatment in the dark at 110 °C for only 8 hr, the yield of each dione was increased to 2.5%. When other particulates (silica gel, alumina, Florisil, etc.) were used instead of garden soil, 1,1'-bipyrene was not formed.

Experiments in which radical-capture agents were incorporated showed lower yields of 1,1'-bipyrene, indicating that the reaction caused by ultraviolet irradiation involves a free-radical mechanism. The yields of the diones were unchanged with these agents present, suggesting that the diones are formed by attack of adsorbed oxygen on photo-excited pyrene molecules.

The unusual reactions of pyrene adsorbed on garden soil may be representative of the reactions undergone by other polycyclic, aromatic hydrocarbons exposed to solar radiation, oxygen, and heat. The work has been written up as an article, entitled "Effects of Temperature and of Ultraviolet Radiation of Pyrene Adsorbed on Garden Soil," by A. J. Fatiadi, and submitted to Environmental Science and Technology for publi-

cation.

In the course of this work, 1,1'-bipyrene was prepared as a reference material; the procedure has been written up as an article entitled, "1,1'-Bipyrene," by A. J. Fatiadi, and accepted for publication in the Journal of Organic Chemistry.

3. Pyrene on Soil from Gaithersburg, Maryland

(A. J. Fatiadi)

A sample of soil from Gaithersburg, Maryland (some 25 miles from Takoma Park, Maryland) was pretreated as described in Technical Note 405. In order to evaluate the significance of the various elements present in this soil sample, the soil was analyzed for metallic constituents by a general, qualitative, spectrochemical method (by Bourdon F. Scribner and Elizabeth K. Hubbard), with the following results: less than 0.001% of each, Ag, B; 0.001 to 0.01% of each, Co, Cu, Ni, Pb, Zn; 0.01 to 0.1% of each, Cr, Sr, V; 0.1 to 1% of each, Ba, Ca, Mg, Mn, Na, Ti; 1 to 10% of each, none; greater than 10% of each, Al, Fe, Si. Pyrene was adsorbed onto a sample of the treated soil, and irradiated with ultraviolet light (366 nm) as before. From the reaction mixture, 1,1'-bipyrene was isolated and identified. Work on the other oxidation products obtained is in progress.

4. Perylene on Soil from Gaithersburg, Maryland

(A. J. Fatiadi)

A sample (9 g) of soil from Gaithersburg, Maryland, pretreated as previously described, was spread on a glass plate (20 x 20 cm) in a hood. A warm solution of 200 mg of perylene (reagent grade) in 15 ml of toluene was carefully pipetted onto the plate, starting in the middle of the plate. The mixture was then spread, while still wet with toluene, until

a homogeneous layer was obtained. After evaporation of the toluene, the plate was heated at 90 °C for 3 min to remove final traces of the solvent. The plate was then irradiated, in air, for 230 hr with an ultraviolet lamp (General Electric, high pressure, mercury vapor, 100 watts, spot bulb, equipped with a filter, 366 nm). The material was then scraped off the plate with a razor blade, a small portion of the material was extracted with 1:5:1 (v/v) N,N-dimethylformamide—toluene—acetic acid, and the extract was examined by thin-layer chromatography, which was conducted as follows.

A standard, glass plate (20 x 20 cm) was placed on a level surface and, along two opposite sides of the upper surface of the plate, three successive layers of paper tape were placed, to a thickness of 250 to 300 nm. Silica gel G (6 g) was mixed with 12 ml of water in a 25-ml, glass-stoppered bottle, and the resulting slurry was dumped onto the plate and spread over the entire plate by means of a glass rod, to give a homogeneous layer. The layer was then activated by heating at 110 °C for 3 hr. Each such plate could be separately spotted with seven different compounds (3 to 5 µg), the distance between spots being about 2.5 cm. It was found advisable that the unknown material should be used for the first and last spots as a check on the homogeneity of the layer; reference compounds could be applied as spots No. 2 to 6. Two different solvent mixtures were used for developing the chromatograms, namely, solvent A: 18:1:1 (v/v) benzene—N,N-dimethylformamide—acetic acid (having a resolution time of 100-120 min; see table 4), and solvent B: 2:1:1 (v/v) heptane—toluene—acetic acid (having a resolution time of 60-70 min; see table 5).

Table 4. R_f Values in solvent A.

Perylene quinone	3,10-Perylene- quinone	1,12-Perylene- quinone	Perylenic acid ^a	Reaction mixture from perylene on Gaith- ersburg soil	Comments ^b ; conclusions
0.86-0.91				0.87-0.91	strong blue fl.; yellow fl. in center if concentrated
				0.83-0.86	orange-red; unknown
0.67				0.68	weak pink fl.; impurity in the perylene
	0.64-0.69			0.63-0.68	yellow; 3,10-perylene- quinone
		0.68-0.70			cherry-red
			0.19-0.21		very strong, light-green fl.
				0.06	diffused, blue fl.; unknown
				0.00	bluish fl.; unknown

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

^b fl. = fluorescence.

Table 5. R_f Values in solvent B.

Perylene quinone	3,10-Perylene- quinone	1,12-Perylene- quinone	Perylenic acid ^a	Reaction mixture from perylene on Gaith- ersburg soil	Comments ^b ; conclusions
0.82-0.83				0.81-0.83	strong blue fl. when not concentrated
	0.32-0.34			0.67-0.68	orange-red; unknown
		0.28-0.29		0.33-0.35	yellow; 3,10-perylene- quinone
			0.09-0.12		cherry-red; weak fl. weak yellow; strong light-green fl.
				0.4	blue fl.; unknown
				0.00	blue fl.; unknown

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

^b fl. = fluorescence.

The approximate \underline{R}_f values of the various unknown components of the reaction mixture were measured in the usual way; and average \underline{R}_f values were calculated from the results of three separate experiments and expressed as ranges. However, the \underline{R}_f values for the reference compounds are for pure samples especially prepared for this study. From Tables 4 and 5, it may be seen that 3,10-perylenequinone is a constituent of the photoreaction mixture; the other compounds formed are still under investigation.

5. Fluorene on Soil from Gaithersburg, Maryland

(A. J. Fatiadi)

A solution of purified fluorene (300 mg) in absolute ethanol (10 ml) was mixed with 6 g of purified garden soil; the resulting slurry was spread on a glass plate (20 x 20 cm), dried, and irradiated with ultraviolet light (366 nm) for 200 hr. The mixture was then extracted with acetone, and the extract was examined by thin-layer chromatography on silica gel G (250- μ m layer, 5 x 20 cm) on a glass plate, by use of two developers, namely, 18:1:1 (v/v) benzene—N,N-dimethylformamide—glacial acetic acid (solvent A), and 8:1:1 (v/v) heptane—glacial acetic acid—ethyl acetate (solvent B).

The following spots were observed. (1) (a) With solvent A (75 min): colorless; blue fluorescence; \underline{R}_f 0.95 ± 0.01 ; unreacted fluorene; (b) with solvent B (60 min): colorless; blue fluorescence; \underline{R}_f 0.73 ± 0.01 ; unreacted fluorene. (2) Yellow; pink fluorescence; \underline{R}_f 0.84 ± 0.03 (solvent A), 0.47 ± 0.02 (solvent B); fluoren-9-one. On spraying the chromatogram with 10% methanolic sulfuric acid and heating it for 3 min at 100 °C, the pink fluorescence of the spot changes to a deep-yellow fluorescence; this is characteristic of fluoren-9-one. (3) Colorless; light-blue fluorescence; \underline{R}_f

0.63 ± 0.02 (solvent A), 0.33 ± 0.03 (solvent B); unknown.

(4) Colorless; pinkish fluorescence; R_f 0.59 ± 0.02 (solvent A), 0.25 ± 0.02 (solvent B); unknown. (5) Orange-brown; faint pinkish fluorescence; R_f 0.55 ± 0.01 (solvent A); tentatively identified as 1,2-bis(2,2'-biphenylene)ethylene. (6) Colorless; greenish blue fluorescence; R_f 0.51 ± 0.02 (solvent A), 0.11 ± 0.01 (solvent B); unknown.

The material comprising spot 2 (yellow) was found to be identical, by ultraviolet and visible spectroscopy, with an authentic sample of pure fluoren-9-one.

6. 1,4-Dihydroxyanthraquinone on Silica Gel

(A. Cohen)

1,4-Dihydroxyanthraquinone was applied as spots to plates of activated silica gel G, and to Eastman Chromagram sheets (silica gel). These were irradiated with a high-intensity ultraviolet lamp (366 nm) for 4 hr. The quinone was then applied as the reference compound, and the plates and sheets were developed twice with benzene. Examination under ultraviolet light, and after spraying with methanolic sulfuric acid and charring, revealed no new spots. Consequently, the quinone does not undergo photo-oxidation on silica gel in 4 hr.

7. Anthracene on Carbon

(A. Cohen)

Charcoal (Norit, Darco) was successively extracted with acetone, methanol, and benzene in a Soxhlet extractor, each extraction being conducted for 24 hr. The resulting carbon was dried under high vacuum at room temperature.

A sample (5 g) of the extracted carbon was added to a solution of 0.5 g of zone-refined anthracene in chloroform in a flask, the mixture was concentrated to dryness, and the residue was dried under high vacuum. The flask was attached to a condenser, open to the air, and the mixture was irradiated for 24 hr with a high-intensity ultraviolet lamp (366 nm) while being stirred magnetically in a waterbath below 40 °C. The mixture was then extracted as described above, the combined extracts were evaporated to dryness, and the residue was dried at high vacuum, affording a quantitative recovery (0.5 g) of unchanged starting-material. Its ultraviolet and infrared spectra were identical with those of the starting material.

A sample of this material was examined by thin-layer chromatography on silica gel G, with benzene as the developer. Examination under high-intensity ultraviolet light revealed only a spot for anthracene. When the plate was sprayed with 20% sulfuric acid in methanol and heated on a hot plate, two spots appeared; one was the spot for anthracene and the other (purplish blue under room light) was that for the carbazole previously shown to be present (see p. 2) as an impurity in zone-refined anthracene. On examination under ultraviolet light, a very faint spot having the same fluorescence and R_f value as anthraquinone was noted; on prolonged heating, this spot became faintly visible under room light. However, as mentioned on p. 5, the zone-refined anthracene used as starting material also gave this very faint spot for anthraquinone. Consequently, anthracene does not undergo photo-oxidation on carbon under the conditions described, and the

carbazole present as an impurity appears to be unaffected also.

8. Naphthacene in Solution

(A. Cohen)

It has been stated [15] that, in contrast to anthracene, naphthacene does not dimerize under ultraviolet irradiation. On the other hand, formation of the naphthacene dimer in solution has been reported [16], but although its ultraviolet absorption spectrum was recorded, the compound was not isolated. As this dimer would be a useful reference compound in studies on the photo-oxidation of naphthacene, its preparation was attempted.

A solution of naphthacene in benzene was irradiated in a sealed vessel containing a small amount of air until the fluorescence disappeared (45 min). On processing the solution, a 50% yield of naphthacenequinone was isolated; after recrystallization from glacial acetic acid, it had mp 289-291 °C (lit. mp 281 °C [17], 294-5 °C [18], 294 °C [19]). However, when the reaction was repeated in an open flask, with vigorous stirring, over-oxidation occurred, and only a 5% yield of the quinone was isolated. When the reaction was repeated in a sealed flask with the exclusion of air, with irradiation for 12 hr, a 67% yield of unchanged naphthacene was recovered; the remaining 33% of material is being examined.

To obtain an authentic sample of naphthacenequinone for comparison, naphthacene was oxidized with fuming nitric acid [19], giving a 36% yield of crude product which, from its infrared spectrum, consisted of the quinone contaminated with

nitro derivatives. On recrystallization from glacial acetic acid, a 17% yield of the quinone was obtained; mp 287-289 °C.

As this photo-oxidation of naphthacene to naphthacenequinone in a yield of 50% might prove to be a useful procedure for preparation of the quinone, a study was made of the oxidation under a variety of conditions.

By changing the length of time for the oxidation, the following yields were obtained: 55% (90 min); 50% (45 min); and 64% (35 min).

The reaction was followed by thin-layer chromatography, to determine whether other reaction products are formed. A solution of 0.1 g of naphthacene in 100 ml of benzene was stirred in an open flask at room temperature for 90 min while being irradiated at 366 nm. Every 5 min, a sample (2 μ l) of the solution was applied, as a spot, to a sheet of silica gel (Eastman Chromagram, 8 x 8") having a fluorescent indicator. After 90 min, the sheet was chromatographed for 12 cm, with benzene as the developer. Naphthacene and naphthacenequinone were used as reference compounds, and the sheet was examined under ultraviolet light (366 nm and 254 nm).

After 5 min of reaction, three spots were evident under 366-nm light, at R_f 0.0, 0.03, and 0.57 (the last being unreacted naphthacene). Under 254-nm light, another spot, having R_f 0.34, was visible. After 15 min of reaction, naphthacenequinone, R_f 0.29, became evident, and at 30 min, practically all of the naphthacene had reacted. At 65 min, the spot having R_f 0.34 was no longer present. Finally, at 90 min, three spots remained: naphthacenequinone (0.29) and the spots at R_f 0.0 and 0.03.

To find out whether formation of the material having R_f 0.03 occurs at a lower temperature, the reaction was conducted at 0 °C, with toluene as the solvent. Within 5 min, the spot having R_f 0.03 was present. After 25 min, the spot having R_f 0.34 was barely evident, and no naphthacenequinone was detectable. The spot having R_f 0.0 was evident at 5 and 25 min. Thus, the spot having R_f 0.34 may represent a precursor of naphthacenequinone, and formation of the products having R_f 0.0 and 0.03 occurred at room temperature and at 0 °C.

To isolate the products for characterization, a suspension of 0.050 g of naphthacene in 50 ml of benzene was photo-oxidized for 105 min, and the solution was evaporated, giving 0.069 g of residue. This was applied to a plate (1 mm thick) of activated silica gel G, and developed twice with chloroform.

The material having R_f 0.03 was recognizable because it gave a blue fluorescence under 366-nm light; it was collected, and rechromatographed on a silica gel plate (250 μ m thick). The crystals isolated were almost colorless, but gradually became pale yellow; wt. 0.003 g (4% of the total product). The infrared spectrum showed bands for a hydroxyl group and for aromatic carbonyl. The ultraviolet absorption peaks were at 518(w), 482(w), 455(w), 410(sh, w), 362 and 355 (doublet), 315 and 301 (doublet), 274 and 264 (doublet), 255 (infl.), and 229(sh) nm. The product was twice recrystallized from chloroform, and examined under polarized light in a Kofler melting-point apparatus. A definite melting point was not observed. Some of the clumps of crystals began to disappear near 180 °C, and beautiful needles, highly polarized, began to form. By 240 °C, melting was complete.

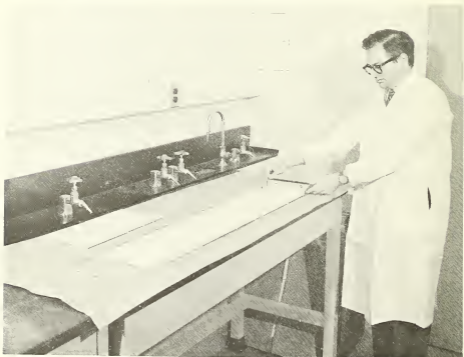
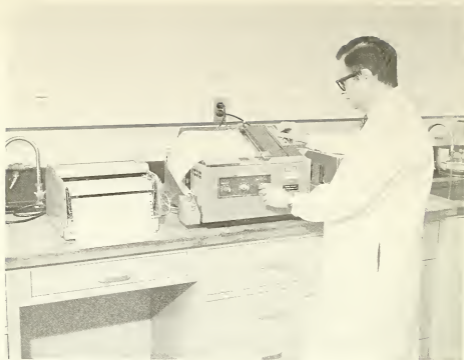


Figure 1. (Top) High-resolution, grating, infrared spectrophotometer having an attached recorder for ordinate expansion. (Bottom) Preparation of thin-layer plates of silica gel G for chromatography.

A brown sirup was isolated from the origin of the developed chromatogram; wt. 16.5 mg (24% of the total product). Also, naphthacenequinone was isolated; wt. 34 mg (57% yield, based on naphthacene taken). The two unidentified products having R_f 0.03 and 0.34 are being studied further.

2. CHARACTERIZATION OF CHEMICAL STRUCTURES

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

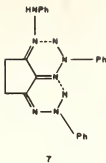
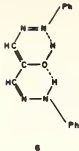
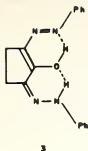
A. Infrared Absorption Spectra of 2-Oxo-1,3-bis(phenylhydrazono) Derivatives and Related Bis- and Tris-(phenylhydrazones)

(A. J. Fatiadi)

The main objective of the present study was to record the infrared absorption spectra of selected, characteristic members of a group of 2-oxo-1,3-bis(phenylhydrazono) derivatives. These compounds have attracted interest because of their unique structure, in which an electrophilic oxo group is in proximity to two phenylhydrazono groups. Interaction of these groups can give rise to tautomeric phenylhydrazono—phenylazo structures, and the expected N=N vibration frequencies (generally difficult to recognize) in the compound formed are of particular interest. Consideration of the infrared absorption spectra of solid phenylhydrazono—phenylazo derivatives was limited to the important features of the absorption bands arising from N=N, N-H (bending), and Ph-N groups.

The infrared absorption spectra of six 2-oxo-1,3-bis(phenylhydrazones) (two of them shown: 3 and 6), four tris(phenylhydrazones) (one of them shown: 7), and six related mono- and bis-(phenylhydrazono) derivatives were studied. For comparison, partial spectra of ten selected aromatic azo compounds were also studied.

The important features of the absorption bands arising from the N=N (1579 to 1558 cm^{-1} and 1447 to 1408 cm^{-1}), N-H (bending) (1557 to 1515 cm^{-1}), and Ph-N (1163 to 1123 cm^{-1}) groups were studied.



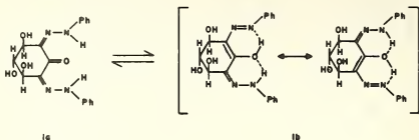
B. Phenylhydrazono—Phenylazo Tautomerism: Structures of 2-Oxo-1,3-bis(phenylhydrazono) Compounds and Related Compounds

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

The structures of xylo-4,5,6-trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane (1) [20], 2-oxo-1,3-bis(phenylhydrazono)cyclohexane (2) [21], 2-oxo-1,3-bis(phenylhydrazono)cyclopentane (3) [22], 3-oxo-2,4-bis(phenylhydrazono)cyclohexanecarboxylic acid (4) [23], 2-oxo-1,3-bis(phenylhydrazono)indane (5) [24], and 2-oxo-1,3-bis(phenylhydrazono)propane (6) [25] were studied by comparison of their nuclear magnetic resonance, ultraviolet, visible, and infrared spectra with the spectra of reference compounds of known structures.

The same results showed that the compounds exist in two forms. The red forms have an enolic (chelated) phenylhydrazono—phenylazo structure. A similarity of the compounds to the diphenylformazans was noted, and was substantiated by a comparison of absorption spectra. Compound 2 crystallized from aqueous ethyl alcohol in the form of a yellow hydrate

shown to have a phenylhydrazono structure. On dehydration, the yellow hydrate yields the red, enolic form. The structures of the 1,2,3-tris(phenylhydrazono) derivatives of cyclohexane, cyclopentane (7), and cyclohexanecarboxylic acid were also studied.



Tautomerism of 2-Oxo-1,3-bis(phenylhydrazones)

C. Novel Aromatization of Inositols in Methyl Sulfoxide—Acetic Anhydride

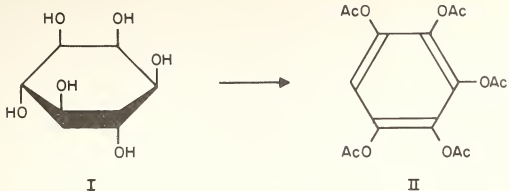
(A. J. Fatladi)

Methyl sulfoxide in acetic anhydride has proved to be an efficient reagent for the oxidation of an isolated or sterically hindered secondary alcoholic group to a ketone group in steroids [26] and partially substituted carbohydrates [27].

A unique, one-step aromatization of unsubstituted inositols (cyclohexanehexols) caused by treatment with methyl sulfoxide—acetic anhydride has been discovered.

myo-Inositol (I) (2 g) was stirred with a mixture of dry methyl sulfoxide (30 ml), acetic anhydride (10 ml), and dry pyridine (5 ml) at 60-65 °C for 45 min. Treatment of the red-brown reaction mixture with ice-water deposited 1.9-2.1 g (54-60%) of pentaacetoxybenzene (II), mp 153-158 °C. Recrystallization from 4:1 (v/v) ethanol—acetic acid raised the melting point to 166-168 °C. The product was identical

(infrared spectrum, mixture mp, and thin-layer chromatography) with an authentic sample [20, 28].



Paper and thin-layer chromatography of the reaction mixture [19:1 (v/v) acetone—water and 4:1 (v/v) ethyl acetate—benzene, respectively] revealed that II was the preponderant product. The conversion of I into II (an exothermic reaction) was also conducted at room temperature by stirring the reactants for 24 hr; the yield of II was not appreciably different.

Treatment of I with methyl sulfoxide—acetic anhydride in the absence of pyridine yielded II, but the yield was significantly lower.

Compound II has also been obtained in good yield from epi-inositol (III), dextro-inositol (IV), levo-inositol (V), muco-inositol (VI), and scyllo-inositol (VII). Apparently, methyl sulfoxide—acetic anhydride is not a stereospecific oxidizing agent, in contrast to catalytic [29] or bacterial [29] oxidants, as the inositols containing one, two, or three axial hydroxyl groups (compounds I, III, IV, V, and VI) are converted into II as readily as is VII, which has no axial hydroxyl groups and is known to be inert both to catalytic and

and to bacterial oxidation [29].

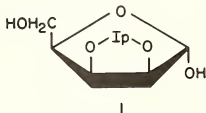
Compound II was not formed from either myo-inosose-2 or DL-epi-inosose-2, the known oxidation products obtained from myo-inositol [30]. However, II was produced when the starting material was DL-xylo-4,5,6-trihydroxycyclohexenediolic acid, the enolic form of a diketoinositol that is obtained by oxidation of myo-inositol with nitric acid [28]. Thus, the conversion of inositols into pentaacetoxybenzene probably proceeds through a form of the diketoinositol as an intermediate.

3. SYNTHESIS OF RESEARCH MATERIALS

A. A Disaccharide of D-Lyxofuranose

(R. Schaffer and A. Cohen)

Levene and Tipson [31] found that, in the high-vacuum distillation of monoacetone-D-lyxofuranose, a new substance of higher boiling point is formed. This was confirmed by Schaffer during a study in which the crystalline D-lyxose derivative was proved to be 2,3-O-isopropylidene- α -D-lyxofuranose (1) [32]. In order to learn the nature of the change that had occurred, the structure of the higher-boiling material was investigated.



1. Acetylation and Deacetylation

Efforts to induce the glassy, high-boiling material to crystallize were unsuccessful; however, a crystalline acetate (2) was obtained after treatment with acetic anhydride in pyridine. The elemental composition of 2 corresponded to the introduction of about one acetyl group per monoacetone-D-lyxose molecule. This ratio was confirmed by saponification of the acetylated product with sodium hydroxide in ethanol-water and titration of the excess alkali (to the phenolphthalein end-point).

The crystalline acetate (2) was next deacetylated catalytically with methanolic barium methoxide, and in this way, the higher boiling, new compound was obtained as a crystalline product (3). The i.r. spectrum of 3 showed an absorption band for hydroxyl, but none for a carbonyl group.

2. Chemical Properties of the New Product

A strong reducing test (Benedict's solution) was obtained when compound 3 was first hydrolyzed in warm mineral acid and then tested for reducing sugar. Without the prehydrolysis, compound 3 did not react to the test except that, after prolonged treatment, there was a slight suggestion of a possible reaction.

Compound 3 was found to be oxidized on treatment with sodium hypiodite according to the method described by Schaffer and Isbell [33], and the consumption of iodine corresponded to the oxidation of one aldehyde group for every two monoacetone-pentose residues.

Although the essentially negative Benedict test would suggest the absence of a reducing end-group in 3, whereas the apparently stoichiometric oxidation by sodium hypiodite would suggest its presence, and the two observations are therefore seemingly contradictory, such behavior has previously been observed with aldoses that are unsubstituted at O-1 but that have an isopropylidene substituent at O-2 and O-3; for example, compound 1 behaves in precisely this way. Conclusive evidence for the presence of the "reducing" end-group in 3 was obtained by (a) oxidizing 3, and isolating a product corresponding to the acid derivative of 3, and (b) reducing 3, and isolating the derived alditol. The elemental analyses of these two compounds showed them to have been derived from

a reducing disaccharide.

3. Oxidation to the Crystalline Di-O-isopropylidene-aldobionic Lactone (4)

For this preparation, a sample of the crystalline acetate, compound 2, was treated with barium methoxide, and the deacetylated derivative (obtained as a sirup) was oxidized in aqueous solution with bromine in the presence of barium carbonate. The di-O-isopropylidenealdobionic lactone (4) was obtained in crystalline form. Its infrared spectrum showed a band at 1776 cm^{-1} , which is typical of a γ -lactone [34].

4. Reduction to the Crystalline (Di-O-isopropylidene-glycosyl)alditol (5)

This reaction was performed in two ways. Lithium aluminum hydride was employed both to reduce and deacetylate compound 2. Secondly, sodium borohydride was employed with the deacetylated product. In the latter case, removal of the boric acid did not complicate the isolation of the crystalline, reduced compound. The same product (5) was obtained by both procedures.

The evidence thus obtained supports the formulation of 3 as a disaccharide whose "reducing" sugar residue has a furanose ring. Assuming a minimum of structural change in the condensation of two molecules of compound 1 to give compound 3, the isopropylidene groups would be expected to remain at O-2 and O-3 of each sugar residue. The glycosidic linkage would therefore connect C-1 of one residue to C-5 of the other.

5. Hydrolysis

The optical rotation of a dilute solution of compound 3 in aqueous oxalic acid at room temperature was found to increase significantly at first and then, during about two weeks,

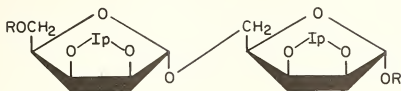
to decrease gradually to about the levorotatory value expected for a solution containing only D-lyxose. Paper chromatography of the final hydrolysis mixture revealed D-lyxose as the only sugar in the hydrolyzate. The initial change in rotation is believed to be due to a selective dioxolane ring hydrolysis, rather than to rupture of the glycosidic linkage, but this subject is still under study.

6. Analysis by Nuclear Magnetic Resonance

The nmr spectrum of the diacetate of 1, 1,5-di-O-acetyl-2,3-O-isopropylidene- α -D-lyxofuranose [32], chosen as a model compound, was measured in deuteriochloroform with a Varian A-60 spectrometer. The proton attached to the acetylated anomeric carbon atom appears as a sharp singlet at τ 3.93, the position of the absorption peak farthest down field in the spectrum. The absence of coupling of this proton to that at C-2 results from the trans relationship of these two protons on the furanose ring. (The C-2, C-3, and C-4 protons, which are cis, cis to each other, appear as a complex of multiplets at about τ 5.5 to 5.9.) The nmr spectrum of compound 2, measured similarly, shows two singlets as the most downfield peaks, each having an integrated intensity corresponding to one proton. One of these singlets is at τ 3.90, and it can be attributed to the proton attached to the anomeric carbon atom; thus, in analogy with the acetate of compound 1, the "reducing" end of the acetylated disaccharide is a 1-O-acetyl- α -D-lyxofuranose residue. The second singlet is at τ 4.97, and can be attributed to the proton at C-1 of the glycosidically attached D-lyxose residue, which, it is reasonable to assume, has a furanoside ring. As such, C-1 and

C-2 only would fail to show coupling if the configuration at C-1 is α -D.

We have therefore tentatively assigned the following structure, where R = H, to compound 3.



2, R = COCH₃

3, R = H

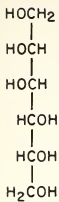
(R. S. Tipson and A. Cohen)

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

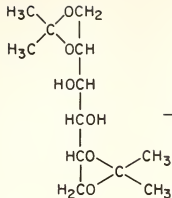
Work has been completed on the infrared spectra of some aldofuranoid, aldopyranoid, and acyclic 1-acylamido derivatives of sugars. The results were incorporated in a paper which has been published [35].

B. Reaction of Sulfonic Esters of Alditols with Methoxide

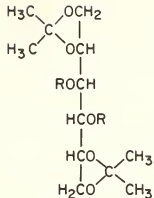
In 1947, Wiggins [36] prepared 1,4:3,6-dianhydro-D-iditol (6) from D-mannitol (1) by the following series of reactions. Compound 1 was condensed with acetone to give the 1,2:5,6-di-O-isopropylidene acetal (2) of 1, and 2 was p-toluenesulfonated to afford the 3,4-di-p-toluenesulfonate (3b) of 2. Compound 3b was then treated with 70% acetic acid at 70 °C during 3 hr, giving an unstated yield of an uncharacterized sirup which, it was supposed, was 3,4-di-O-p-tolylsulfonyl-D-mannitol (4b). This crude sirup was then acetylated with boiling acetic anhydride and sodium acetate, to give a 65% yield of a sirup having $[\alpha]_D -0.8^\circ$ (chloroform) which, it was supposed, was the 1,2,5,6-tetraacetate (5b) of 4b. Finally, by the action of sodium methoxide on the crude, sirupy 5b, Wiggins [36] obtained crude 1,4:3,6-dianhydro-D-iditol (6), by Walden inversion at C-3 and C-4, as a sirup in a gross yield of 54% (calculated on 5b). However, study of the experimental part of the article by Wiggins reveals that, for three reasons, the actual content of compound 6 in this sirup must have been less than 50% (which means that the actual yield of 6 was less than 27%). (a) On benzylation of the sirupy dianhydride (6), the yield of the 2,5-dibenzoate of 6 was only 48.5%



1

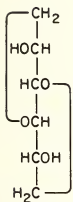


2

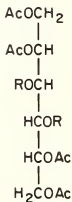


3a, R = Ms

3b, R = Ts

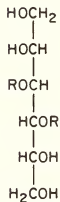


6



5a, R = Ms

5b, R = Ts



4a, R = Ms

4b, R = Ts

of the theoretical, instead of the almost 100% to be expected. (b) On methanesulfonation of the sirupy dianhydride (6), the yield of the 2,5-dimethanesulfonate of 6 was only 40.3% of the theoretical. (c) The sirupy D-dianhydride (6) had $[\alpha]_D -16.7^\circ$ (water); and, although it crystallized, Wiggins could not recrystallize it. In contrast, the L enantiomorph is readily crystallized and recrystallized, and has mp 64°C and $[\alpha]_D +20.8^\circ$ (water); hemihydrate, mp $43-45^\circ\text{C}$ and $[\alpha]_D +18.4^\circ$ (water). Consequently, it seems reasonable to conclude, from this evidence, that Wiggins' "dianhydro-D-iditol" must have been grossly impure.

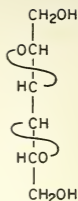
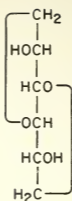
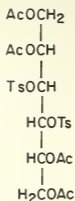
From these considerations, we decided to re-investigate some of the chemistry involved in the work of Wiggins [36]. In the present study, the preparation of each compound in the sequence 1 to 5b has been improved, and compounds 4b and 5b (that were obtained as sirups by Wiggins) have been crystallized, and characterized by melting point, specific optical rotation, and infrared absorption spectrum. In addition, compounds 4a and 5a have been synthesized from 3a (already known), and have been crystallized, and characterized by melting point, specific optical rotation, and infrared absorption spectrum.

A polarimetric study of the formation of 4b from 3b revealed that the sample of 4b obtained (as a sirup) by Wiggins must have been impure. Our study of the hydrolysis of 3b with 4:1 (v/v) glacial acetic acid—water at 65°C showed that, after the elapse of 45 min, hydrolysis of the isopropylidene acetal groups was complete; thereafter, slow hydrolysis

of the sulfonic ester groups occurred. In contrast, Wiggins had conducted the hydrolysis with 70% acetic acid at a higher temperature (70 °C) for 4 times as long (3 hr). Consequently, (1) his sirupy 4b must have contained some free p-toluenesulfonic acid and some D-mannitol (and/or anhydro-alditols); (2) his sirupy 5b, obtained by acetylating his impure 4b, must have contained some hexa-O-acetyl-D-mannitol and/or acetates of anhydro-alditols; and (3) his sirupy 6 might well have contained anhydro-alditols differing from 1,4:3,6-dianhydro-D-iditol (6).

We therefore decided to ascertain whether pure, crystalline 6 could be prepared in high yield by treating pure 5b with sodium methoxide under the conditions described by Wiggins [36]. A priori, there would appear to be no reason why any or all of four other dianhydro-alditols (7 to 10) should not be produced in this reaction, in addition to or instead of compound 6. However, certain considerations suggest that compound 7 would be the most likely product. The symmetrical molecule 5b would not be expected to give rise to the unsymmetrical molecules 9 or 10, and the four-membered ring in compounds 8 or 9 is not normally encountered.

Compounds 7, 9, and 10 have an epoxide ring, a three-membered ring that is susceptible to ring-opening by methoxide ion. Consequently if any, or all, of these three compounds is formed, prolonged contact with the methoxide reagent might be expected to cause such ring-opening. Scission, by methoxide ion, of ethylene oxide rings involving two secondary carbon atoms proceeds in two ways, because the methoxide anion (H_3CO^-) can approach either of the two secondary

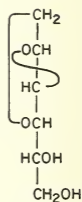
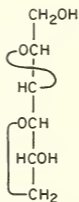
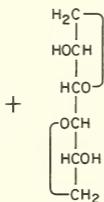


5b

6

2, 3: 4, 5-
DIANHYDRO-
D-IDITOL

7



1, 3: 4, 6-
DIANHYDRO-
D-IDITOL

8

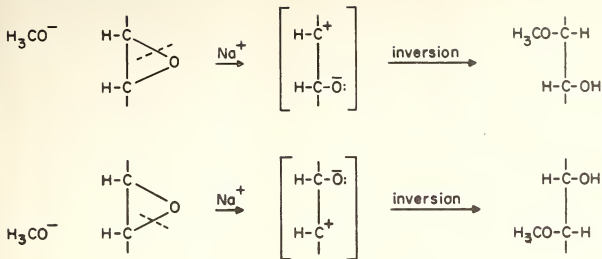
2, 3: 4, 6-
DIANHYDRO-
D-IDITOL

9

1, 4: 2, 3-
DIANHYDRO-
D-IDITOL

10

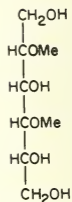
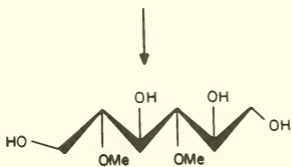
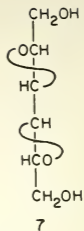
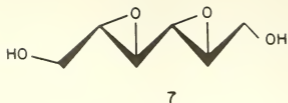
carbon atoms involved:



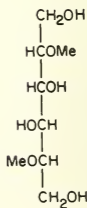
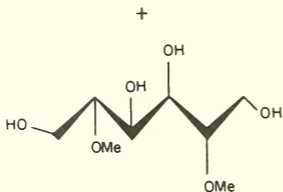
In both instances, the methoxyl group in the product is on the opposite side (of the carbon atom) to where the ethylene oxide ring had been. Consequently, in the zig-zag, planar conformation, the two new groups (hydroxyl and methoxyl) replacing the epoxide ring are in the trans relationship.

Although two newly substituted carbon atoms appear per epoxide ring, the relative proportions formed depend on the total structure of the anhydro compound. Thus, for example, the reaction of compound 7 with methoxide ion would be expected to give rise to a mixture of three di-O-methylhexitols. Similarly, should the epoxy-ring derivatives 9 and 10 be formed, each could react with methoxide ion to give a mixture of di-O-methylhexitols.

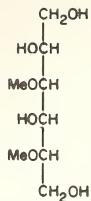
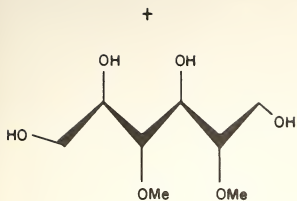
Treatment of pure, crystalline 5b in chloroform (1.33 ml per g) with an excess of sodium methoxide in methanol at 0 °C, followed by 4 hr at room temperature, with isolation as described by Wiggins [36], except that carbon dioxide was used



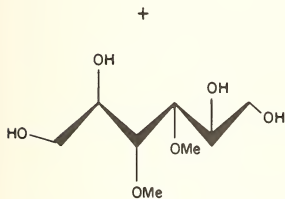
2, 4-Di-O-methyl-D-allitol



2, 5-Di-O-methyl-L-mannitol



2,4-Di-O-methyl-D-allitol



3,4-Di-O-methyl-D-mannitol

instead of N sulfuric acid to neutralize the base, gave a practically quantitative yield of product (calculated as dianhydrohexitol). The product consisted of colorless crystals and a colorless sirup; the crystals had an elementary analysis corresponding to that calculated for a dianhydrohexitol hemihydrate, but the melting point and $[\alpha]_D$ (in water)

were both much higher than those reported by Wiggins for the enantiomorph of compound 6, and the infrared spectrum differed from that of authentic 1,4:3,6-dianhydro-L-iditol (kindly supplied by Dr. H. G. Fletcher, Jr., of the National Institutes of Health, Bethesda, Maryland 20014). Thin-layer chromatograms on silica gel G [pretreated with water (2 ml per g), air-dried overnight, and activated at 110 °C for 1 hr], with ethyl acetate as the developer, showed the presence of three compounds, the fastest of which had the same R_f value as authentic 1,4:3,6-dianhydro-L-iditol.

When pure, crystalline 5a in chloroform was treated with methanolic sodium methoxide at 0 °C, and the mixture was kept in the refrigerator for 2 days and then at room temperature for four days, a practically quantitative yield (calculated as dianhydrohexitol) of sirup resulted. Thin-layer chromatography, as above, showed spots for six compounds; the fastest had the same R_f value as 1,4:3,6-dianhydro-L-iditol, but was much weaker than in the 4-hr experiment.

(A. Cohen) Because the reaction of 5a or 5b to give a dianhydrohexitol involves deacetylation, it seemed possible that the acetylation step might be unnecessary. This was verified; treatment of pure, crystalline 4a with methanolic sodium methoxide only (at 25 °C for 24 hr) gave a mixture similar to that from the tetraacetate. It was then decided to employ methanolic barium methoxide (without chloroform) instead of sodium methoxide, and to determine the effect of conducting the reaction at room temperature for 2 hr (instead of 4 hr). Under these conditions, compound 5b gave a 65% yield of a crystalline compound (X) having an elementary

analysis agreeing with that calculated for a dianhydro-hexitol. Its melting point and specific rotation were much higher than those for compound 6. Similar treatment of 5a or 4a gave a 53% and 68% yield, respectively, of X. The infrared spectrum of X differed from those of 1,4:3,6-dianhydro-L-l-iditol and 1,4:3,6-dianhydro-D-m-mannitol (prepared by refluxing a suspension of D-m-mannitol and p-toluenesulfonic acid in xylene for 75 min). Repetition of the preparation from 4a on a larger scale (0.02 mole) gave compound X in a yield of 90.8%. Compound X was unaffected by sodium metaperiodate in water during 24 hr at room temperature. Consequently, compound X does not have the structure of compound 10, the only one of the possible products that has two hydroxyl groups vicinal. Hence, the structure of compound X is that of 7, 8, or 9. On acetylation of compound X with acetic anhydride-pyridine at room temperature during 2.66 hr, a crystalline compound was isolated having an elementary composition agreeing with that calculated for a di-O-acetyl-dianhydrohexitol; yield 97%. It was characterized by melting point, specific optical rotation, and infrared absorption spectrum. A crystalline dibenzoate was also prepared and characterized. The work is being continued. (R. S. Tipson and A. Cohen)

C. Infrared Spectroscopy of Carbohydrates

An article has been written on the infrared spectroscopy of carbohydrates, in which all of the important work in this field has been collected and systematized. This was needed because previous articles in the literature were either out of date [37] or not sufficiently comprehensive [38], and certain statements that had been made were subject

to misinterpretation. The present article discusses principles and instrumentation, sampling techniques, comparison of samples, interpretation of spectra, functional groups of carbohydrates and their derivatives, correlations for the fingerprint region and beyond, and conformational studies. In addition, examples are discussed of the use of infrared spectra for qualitative and quantitative purposes and in the determination of structure. Special techniques are briefly described, including use of plane-polarized radiation, the technique of attenuated total reflection, and Raman spectra.

(R. S. Tipson)

5. ISOTOPIC METHODS FOR ANALYSIS OF CARBOHYDRATES

(R. Schaffer)

The incorporation of an isotope into a carbohydrate structure associates the isotope's distinctive properties with the carbohydrate. As a consequence, the highly sensitive detection and measurement techniques for isotopes become applicable for analysis of the compound and for following the course of the isotopic portion of its structure through complex reactions.

The isotopically substituted carbohydrate is almost always employed in admixture with some proportion of that carbohydrate in "nonisotopic" (nonlabeled) form. In most experiments, the overall similarity in properties of the isotopic forms of a carbohydrate is so close that variations in the ratio of isotopic to nonisotopic molecules (called "isotope effects"), consequent to the physical and chemical processes in which the labeled carbohydrate is employed, are not detected, because, if variations occur at all, they fall within the experimental error of measurement. Constancy in the ratio of the isotopic species forms the basis for most quantitative uses of labeled compounds. However, in such applications, the absence of significant isotope effects cannot be assumed.

"Isotopic Methods," a manuscript dealing with the various methods of isotope-dilution analysis, the isotope effects that may accompany studies with labeled molecules, and the methods for determining isotopic distribution, has been submitted for publication in "The Carbohydrates," W. Pigman and D. Horton, editors, Academic Press, N. Y.

6. OCCURRENCE, PREPARATION, AND PROPERTIES OF NATURALLY
OCCURRING MONOSACCHARIDES (INCLUDING 6-DEOXY SUGARS)

(Robert Schaffer)

The sugars that are found in naturally occurring materials are of particular importance because of interest in their biological function and in their present or potential industrial application. To the chemist, these sugars are of value in providing, along with the alditols and uronic acids, starting materials for the preparation of both the numerous monosaccharides known only by synthesis and of those natural monosaccharides which (because of inadequate sources or difficulties attending their isolation) are more readily obtained by transformation of other abundant carbohydrates.

Prior to the advent of modern methods of separation and identification, the only sugars known to occur in botanic materials as free monosaccharides were two aldoses (D-glucose and L-arabinose) and four ketoses (D-fructose, I-sorbose, D-mannoheptulose, and sedoheptulose). Now, however, chromatographic techniques applied to extracts of plants have revealed traces of three free aldoses, already known to occur commonly in glycosidic combinations, namely D-xylose, D-galactose, and D-mannose, and twelve other free sugars (hexoses to nonuloses), nine of which had not previously been recognized as naturally occurring. As regards the occurrence of free sugars in animals, D-glucose is a normal constituent of blood, lymph, and other body fluids. The blood of the newborn has been found to contain D-fructose; this ketose is also found in semen. Trace amounts of several C_5 and C_6 sugars in normal human urine have been detected by use of paper chromatography. Increased levels of two sugars, L-"xylulose" and D-glucose, are excreted in cases of essential

pentosuria and diabetes, respectively. Thus, a surprising number of monosaccharides have been recognized as naturally occurring only in recent years.

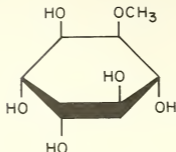
A chapter surveying the occurrence, preparation, and properties of these sugars has been submitted for publication in "The Carbohydrates," W. Pigman and D. Horton, editors, Academic Press, N. Y.

7. STANDARD REFERENCE MATERIALS

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

A. Quebrachitol

(A. J. Fatiadi)



Quebrachitol

Quebrachitol (1-O-methyl-levo-inositol), a naturally occurring inositol [29], was obtained as a crude, dark-brown material from the Plantation Division, U. S. Rubber Co., Rockefeller Center, New York, N. Y. The material was purified as follows: 1.5 kg of dry, crude quebrachitol was suspended in 2.6 liters of water in a 4-liter beaker, and the suspension was stirred at 90 °C for 1 hr, and kept at room temperature for 18 hr. The precipitate was filtered off, washed with about 700 ml of hot water, and dried at 50 °C for 48 hr, to yield a charcoal-like solid (300 g). Preliminary analysis of this material (by infrared and ultraviolet spectra) indicated the presence of phenolic and amino-phenolic components, and it was therefore discarded.

The dark-brown filtrate was heated at 90 °C, stirred, and treated with portions of decolorizing carbon to obtain a clear, pale-yellow solution; 500 g of carbon and 2.5 to 3 hr of stirring were required. The warm suspension was filtered on a layer of micro-cellulose, and the filter cake was thoroughly washed with warm water (total volume, about 4 liters).

If the filtrate plus washings showed the presence of an appreciable amount of sulfate ions (aqueous barium chloride test), it was either neutralized with saturated barium hydroxide solution (phenolphthalein) or stirred with solid barium carbonate. The suspension was then filtered, and the filtrate was passed through a column of Amberlite IR-120 (H⁺) ion-exchange resin, which was then thoroughly washed with water. (Note: water-soluble proteins and higher sugars can be removed by treatment of the solution with 25% aqueous lead acetate, followed by filtration, and removal of lead ions with hydrogen sulfide.)

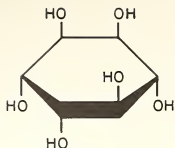
The clear solution was concentrated, in portions, at 60 °C until crystallization started; the concentrates were combined in a 2-liter beaker, kept in a refrigerator for 12 to 18 hr, and filtered, to give 490 to 500 g of fairly pure quebrachitol (crop 1). On concentration and cooling of the filtrate, second (250 g) and third (125 g) crops of crude quebrachitol were obtained. The partially purified quebrachitol was recrystallized from hot water (550 ml), with treatment with carbon (50 g); filtration, concentration, and cooling yielded colorless crystals which, after being washed with 1:1 (v/v) ethanol-water, and dried at 50 °C for 48 hr, amounted to 279 g; mp 189-190 °C.

Highly pure quebrachitol was obtained as follows. Recrystallized material (270 g) was dissolved in boiling water (350 ml) and the solution was treated with decolorizing carbon. The suspension was filtered, and the filtrate was concentrated at 60 °C, in portions, until the start of crystallization; each concentrate was heated to dissolve the crystals, transferred to a beaker (1 liter), and kept at 60-65 °C (on a water bath) until all of the concentrates had been combined. To the warm solution (50-60 °C) was added an equal volume of 95% ethanol, and the solution was kept at room temperature until crystallization started, stirred, and kept at 0 °C for 2 hr. The snow-white, lustrous crystals were then filtered off, washed successively with ice-cold 1:1 (v/v) water—ethanol and 95% ethanol, and dried in an oven at 50 °C for 48 hr and then in a vacuum desiccator for 24 hr. The yield of quebrachitol was 250 g; mp 191-193 °C, $[\alpha]_D^{21} -80.5^\circ$ (c 2.37, water); lit. [29] mp 190-191 °C, $[\alpha]_D -80^\circ$ (water). The product showed no impurities by paper chromatography in the following solvents, tert-butyl alcohol saturated with water, and 18:1 (v/v) water—acetone; when the chromatogram was sprayed with 5% aqueous silver nitrate containing 10% of ammonia, and then heated at 100 °C for 2 min, only one spot (dark) was detected. The infrared spectrum of pure quebrachitol is shown in figure 2 (see p. 64).

B. levo-Inositol

(A. J. Fatiadi)

levo-Inositol was obtained by demethylation of quebrachitol with hydriodic acid, followed by recrystallization. From experiments on the demethylation, it was found that the



levo-Inositol

best yield of levo-inositol was obtained when demethylation was conducted with 25-g portions of quebrachitol; larger or smaller samples gave lower yields of the cyclitol.

A suspension of 25 g of purified quebrachitol (mp 190-191 °C) in 50 ml of 47% hydriodic acid was refluxed for 30 min to give a clear solution. The warm solution was then added dropwise to 400 ml of hot, absolute ethanol with vigorous stirring (10 min). levo-Inositol crystallized as the mixture was stirred and allowed to cool to room temperature; the suspension was then refrigerated. The product was filtered off, washed with 95% ethanol, and air-dried; yield of crude levo-inositol, 20.6 to 23.8 g (89 to 99%).

The crude product was purified as follows. A solution of 195 g of crude levo-inositol (from several demethylations) in 250 ml of hot water was treated with decolorizing carbon (10 g), and the suspension was filtered through a layer of microcrystalline cellulose which was then washed with hot water. The filtrate and washings were combined (350 ml), stirred, and

heated to 85-90 °C, and then 50 ml of glacial acetic acid was added, followed by warm ethanol to permanent turbidity. The stirred mixture was allowed to cool to room temperature, and much of the product crystallized. Finally, the mixture was cooled (ice-bath), and filtered, and the crystals were washed successively with 85% ethanol and absolute ethanol. Drying in air (48 hr) and then in a vacuum desiccator over sodium hydroxide provided 150 g of levo-inositol, mp 243-245 °C (heating rate, 1°/min), $[\alpha]_D^{21} -64.5^\circ$ (c 2, water); lit mp 242-243 °C [39], 247 °C [40], $[\alpha]_D -65^\circ$ (water) [41]. Paper chromatography (as described for quebrachitol) showed that the product was free from impurities; spraying the chromatogram with an ammoniacal silver nitrate reagent gave one spot (dark). Further recrystallization did not significantly alter its physical properties. The infrared spectrum of pure levo-inositol is shown in figure 2 (see p. 64).

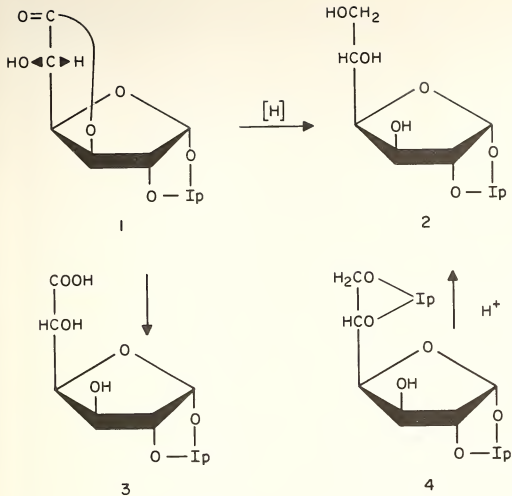
C. 1,2-O-Isopropylidene-β-L-idofuranose

(R. Schaffer)

The preparation of 1,2-O-isopropylidene-β-L-idofuranose (2) was discussed in Technical Note 405. Some interesting details of the work are presented here, together with an optimal procedure for obtaining free L-idose from the monoacetone sugar.

1. 1,2:5,6-Di-O-isopropylidene-β-L-idofuranose

The mother liquor from a crystallization of 1,2-O-isopropylidene-β-L-idofuranose (2), which had been prepared from 1,2-O-isopropylidene-β-L-idofuranurono-6,3-lactone (1) by treatment with lithium aluminum hydride, was found to contain uncrystallized, acetonated sugar and also 1,2-O-isopropylidene-β-L-iduronic acid (3)— the latter was present because of hydrolysis of unreduced uronolactone (1). The mother liquor, as a



dried sirup, was therefore treated for a new lactonization by dissolving the sirup in acetone, diluting the solution with toluene, and heating gently to boiling, as described previously [42], to remove the acetone from the solvent and the water liberated during lactonization. After concentrating to remove the toluene, and adding ether, the solution was treated with lithium aluminum hydride, the mixture was worked up, and the 1,2-O-isopropylidene-L-idofuranose (2) was crystallized; however, the resulting mother liquor, as a sirup, now spontaneously gave a crystalline product having a higher melting point and optical rotation than those of 1,2-O-isopropylidene-L-idofuranose (2). (For the properties of 1,2-O-isopropyl-

idene-L-idofuranose, see p. 63.) The new product showed mp 151-153 °C, $[\alpha]_D^{20} -36.4^\circ$ (c 1, water), and $[\alpha]_D^{20} -33^\circ$ (c 1, acetone), values that closely resemble numerically, with opposite rotational sign, the corresponding properties described by Iwadare [43] for 1,2:5,6-di-O-isopropylidene-D-idofuranose (4), mp 151-152.5 °C and $[\alpha]_D^{25} +36^\circ$ (c 2, acetone); the values are not entirely consistent with those which Baggett and Jeanloz [44] later reported for the L compound: mp 153-154 °C, $[\alpha]_D^{22} -22^\circ$ (c 0.6, water), and $[\alpha]_D^{23} -25^\circ$ (c 0.55, acetone). Hydrolysis, as described below, gave the 1,2-monoacetone sugar (2), thus establishing its correspondence to Iwadare's compound. Introduction of the second isopropylidene residue on the monoacetone-L-idose most probably occurred during the lactonization, where acetonation could have been catalyzed by the acidity provided from the free carboxylic acid of the acetonated uronic acid when the mixture of monoacetone sugar and monoacetone uronic acid was heated in the acetone-toluene solution.

2. 1,2-O-Isopropylidene- β -L-idofuranose from the Diacetone Sugar

Iwadare [43] had prepared the enantiomorph of this compound from his diacetonated D-idose by hydrolysis in 70% acetic acid for 2 hr at 50 °C, and the monoacetone compound he obtained showed mp 81-82 °C and $[\alpha]_D^{25} +35^\circ$ (c 1.3, water). Since we found that monoacetone-L-idose is readily hydrolyzed to the free sugar when heated at 70 °C in 70% acetic acid, gentler conditions for conversion of diacetone-idose (4) into monoacetone-idose (2) were investigated. At 22 °C, the optical rotation of our diacetone-L-idose in 70% acetic acid was found to undergo a change that essentially ceased after 3 hr. Freeze-drying of the hydrolyzate then gave an almost

quantitative yield of 1,2-O-isopropylidene-L-idose (2).

When recrystallized from chloroform without care to exclude moisture, the product isolated melts at about 85 °C. This behavior is best observed by immersing the compound in a capillary tube in a bath preheated to 85 °C or above, whereupon immediate melting takes place. Immersion at a lower temperature, or the more normal method of gradually heating the melting-point bath for determining a melting point, usually results in some sintering between 80 and 88 °C, and melting at 111-114 °C. On remelting the solidified melt, only the higher melting point is observed. The low-melting modification is a hydrated form of 1,2-O-isopropylidene-L-idose, and this can be seen from the strong absorption at 6.0 μm in its IR spectrum (see figure 2, C). Meyer and Reichstein [45] first reported the melting point of anhydrous 1,2-O-isopropylidene-L-idofuranose to be 112-114 °C and its $[\alpha]_D$ as -29° (c 1.4, water); recrystallization of our product from acetone provides well-formed prisms having mp 114-115 °C and $[\alpha]_D^{20}$ -27.8° (c 2, water). Its infrared spectrum is devoid of absorption at 6.0 μm.

3. L-Idose from 1,2-O-Isopropylidene- β -L-idofuranose

Hydrolysis of 1,2-O-isopropylidene-L-idose is readily effected in 70% acetic acid by warming at 70 °C for 2.5 hr, in which time the rapid change in optical rotation is nearly complete. Paper chromatography in 8:2:3 (v/v) butyl alcohol—acetic acid—water then no longer shows the presence of the starting material, and, in addition to the sugar, there is evidence of only a trace amount of a compound that moves faster than the sugar and that is presumed to be 1,6-anhydro-L-idopyranose.

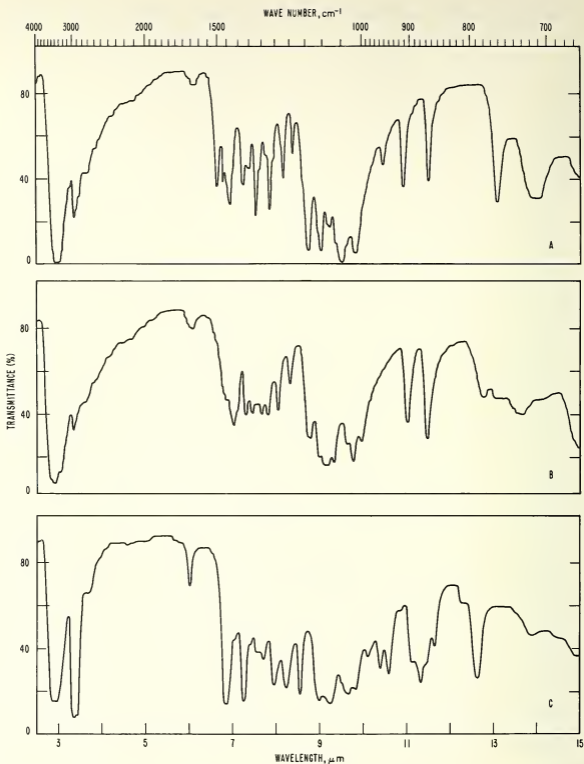


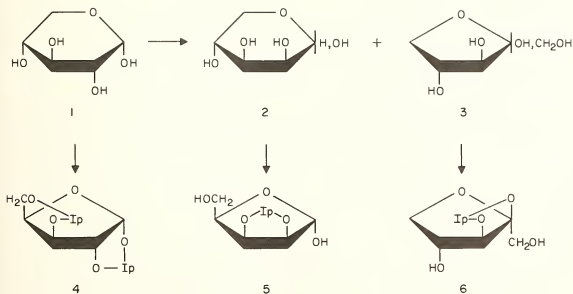
Figure 2. Infrared absorption spectra of (A) quebrachitol, (B) *levo*-inositol, and (C) 1,2-O-isopropylidene-β-D-idofuranose monohydrate.

D. 2,3-O-Isopropylidene-β-D-threo-pentulofuranose

(R. S. Tipson)

D-threo-Pentulose is a rare sugar of considerable interest to biochemists, as it is probably an intermediate in the photosynthetic cycle [46]. It was synthesized in 1933 by the pyridine-catalyzed epimerization of D-xylose (1) [47]. The preparative value of the reaction was improved by Levene and Tipson [31], who acetonated the mixture of D-xylose (1), D-lyxose (2), and D-threo-pentulose (3) that is formed, and separated the resulting mixture of 1,2:3,5-di-O-isopropylidene-α-D-xylofuranose (4), 2,3-O-isopropylidene-α-D-lyxofuranose (5), and 2,3-O-isopropylidene-β-D-threo-pentulofuranose (6) by fractional distillation followed by crystallization.

In order to make D-threo-pentulose available as a standard reference material, this work has now been repeated, and some improvements in procedures have been introduced. The concentration of the solution of D-xylose in pyridine has



been altered, affording a higher yield of the products and permitting the epimerization of a larger amount of D-xylose per preparation. Use of a different solvent, instead of 95% ethanol, for removal of unchanged D-xylose enhanced the proportion of the latter that could be removed from the reaction mixture prior to acetonation. Careful refractionation of the fractionally distilled "monoacetone" fraction permitted the isolation of crystalline 2,3-O-isopropylidene- α -D-lyxofuranose (5), whereas in the earlier work [31] this fraction had not been obtained crystalline, even on nucleation with authentic 5. Moreover, sufficient of an unknown di-O-isopropylidene-pentose byproduct previously encountered has now been accumulated to permit its characterization, and determination of its identity and structure. Highly pure 2,3-O-isopropylidene- β -D-threo-pentulofuranose (6), characterized by its melting point, specific optical rotation, and infrared absorption spectrum, is now available as a standard reference material.

E. Polycyclic, Aromatic Hydrocarbons

(A. Cohen and A. J. Fatiadi)

As a result of the work described in Section 1 (Air Pollution Studies), the presence of impurities in commercial samples of polycyclic, aromatic hydrocarbons has been recognized. Methods have been devised for removing these impurities, and it is intended to prepare highly pure samples of anthracene, naphthacene, pyrene, perylene, and other such hydrocarbons as standard reference materials.

8. PERSONNEL AND ACTIVITIES

A. Personnel Listing

R. Schaffer, Section Chief

R. S. Tipson

A. J. Fatiadi

A. Cohen

H. L. Frush, transferred

H. S. Isbell, transferred

B. Publications

Losses Due to Adsorption During Filtration of Aqueous Solutions of Polycyclic, Aromatic Hydrocarbons.

M. N. Inscoe, *Nature* 211, 1083 (1966).

NBS Technical Note 405. Organic Chemistry Section - 7/1/65 - 6/30/66.

Edited by H. S. Isbell (September, 1966).

Large-scale, Preparative Paper Chromatography.

H. L. Frush, *J. Res. NBS* 71A, 49 (1967).

Infrared Absorption Spectra of Some Aldofuranoid, Aldopyranoid, and Acyclic 1-Acylamido Derivatives of Sugars.

R. S. Tipson, A. S. Cerezo, V. Deulofeu, and A. Cohen, *J. Res. NBS* 71A, 53 (1967).

Synthesis of α -L-Fucose-1-¹⁴C (6-Deoxy- α -L-galactose 1-¹⁴C).

H. S. Isbell, H. L. Frush, and N. B. Holt, *J. Res. NBS* 71A, 133 (1967).

Novel Aromatization of Inositols in Dimethyl Sulfoxide—Acetic Anhydride.

A. J. Fatiadi, *Chem. Commun.* 1967, 441.

Deuterium Isotope Effects in α - β -Pyranose and in Pyranose-Furanose Interconversions.

H. S. Isbell and C. W. R. Wade, J. Res. NBS 71A, 137 (1967).

C. Manuscripts in Process of Publication

Phenylhydrazono-Phenylazo Tautomerism. Part II. Structures of 2-Oxo-1,3-bis(phenylhydrazono) Compounds and Related Compounds.

A. J. Fatiadi and H. S. Isbell. (Manuscript completed for Carbohydrate Research)

Charge-transfer Complexes of Polycyclic, Aromatic Hydrocarbons with 2,4,7-Trinitrofluoren-9-one.

J. H. Gould. (Manuscript completed for J. Res. NBS)

Effects of Temperature and of Ultraviolet Radiation on Pyrene Adsorbed on Garden Soil.

A. J. Fatiadi. (Manuscript completed for Environmental Science and Technology)

A Novel, Facile Preparation of 1,1'-Bipyrene.

A. J. Fatiadi. (Manuscript accepted for publication in Journal of Organic Chemistry)

Infrared Spectroscopy of Carbohydrates.

R. S. Tipson. (Manuscript completed for NBS Monograph)

Isotopic Methods in Carbohydrate Chemistry.

R. Schaffer. (Manuscript completed for "The Carbohydrates," edited by W. Pigman and D. Horton)

Occurrence, Properties, and Preparation of Naturally Occurring Monosaccharides, Including 6-Deoxy Sugars.

R. Schaffer. (Manuscript completed for "The Carbohydrates," edited by W. Pigman and D. Horton)

Infrared Spectroscopy of Carbohydrates.

R. S. Tipson and P. S. Parker. (Manuscript completed for "The Carbohydrates," edited by W. Pigman and D. Horton)

Periodic Acid, a Novel Oxidant of Polycyclic, Aromatic Hydrocarbons.

A. J. Fatiadi. (Manuscript completed)

Preparation and Purification of Some Oxidation Products of Perylene.

A. J. Fatiadi. (Manuscript completed for J. Res. NBS)

Infrared Absorption Spectra of 2-Oxo-1,3-bis(phenylhydrazono) Derivatives and Related Bis- and Tris-(phenylhydrazones).

A. J. Fatiadi. (Manuscript accepted for publication in J. Res. NBS)

D. NBS Reports

(Quarterly Reports on Air Pollution Program prepared jointly with certain Sections of the Division of Metrology and the Division of Physical Chemistry)

NBS 9441 - Quarterly Report AIR POLLUTION PROGRAM - 7/1/66 - 9/30/66. R. Klein, R. Stair, R. S. Tipson.

NBS 9445 - Quarterly Report AIR POLLUTION PROGRAM - 10/1/66 - 12/31/66. A. Cohen, A. J. Fatiadi, R. Klein, M. D. Scheer, R. Stair, R. S. Tipson.

NBS 9518 - Quarterly Report AIR POLLUTION PROGRAM - 1/1/67 - 3/31/67. A. Cohen, A. J. Fatiadi, R. Klein, M. D. Scheer, W. E. Schneider, R. S. Tipson.

NBS 9558 - Quarterly Report AIR POLLUTION PROGRAM -
4/1/67 - 6/30/67. A. Cohen, A. J. Fatiadi,
R. Klein, M. D. Scheer, W. E. Schneider,
R. S. Tipson.

E. Lectures

10/18/66 - Sugars in Acidic and Basic Media.
Biochemistry Department and Basic Science
Graduate School, New York Medical College,
New York, New York. R. Schaffer.

10/18/66 - Sugars in Solution. Biochemistry
Department and Basic Science Graduate School,
New York Medical College, New York, New York.
H. S. Isbell.

9. ACKNOWLEDGMENT

The Organic Chemistry Section wishes to thank Dr. J. K. Taylor and his staff in the Microchemical Analysis Section for providing numerous elementary analyses and for assistance with instrumental techniques. We also wish to thank 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 is most grateful to his associates in the Organic Chemistry Section for their earnest help in preparing this report. The editor's task was greatly eased by the expert effort of Dr. R. S. Tipson, who reviewed all of the report, making many improvements for which the editor is deeply grateful. Special thanks are expressed to Mrs. R. L. Keck, Section Secretary, whose effort made expeditious publication possible and whose enthusiasm made a difficult task pleasant.

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