The material (4.1 g.) was acetylated with 1.5 g. of sodium acetate and 33 ml. of acetic anhydride as described above to produce a sirup; yield 5.27 g. Crystallization from ethanol produced 290 mg. of crystalline material, m.p. 190–191° undepressed by authentic β-gentiobiose octaacetate. The X-ray powder diffraction pattern was identical with that of β-gentiobiose octaacetate. The mother liquors were evaporated to a sirup, dissolved in benzene and placed on a column (300 × 70 mm., diam.) of Magnesol–Celite[12] (5:1 by wt.). The column was developed with 3000 ml. of benzene–2-methyl-2-propanol (100:1 by vol.) and then extruded. The zones were located by streaking with indicator (1% potassium permanganate in 10% sodium hydroxide solution). Four zones 10–35 mm., 35–93 mm., 93–150 mm. and 150–175 mm. from the column top, were located and sectioned. Each zone was extracted with acetone, the solutions evaporated to sirups, and the material crystallized from ethanol.

The material from the top zone appeared to be a mixture of α-β-gentiobiose octaacetates and was not further investigated; yield 88 mg., m.p. 173°.

The material from the second zone from the column top exhibited an X-ray powder diffraction pattern identical to that of β-sophorose octaacetate; yield 123 mg., m.p. 186° undepressed on admixture with authentic β-sophorose octaacetate.

(12) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

The material from the third zone from the column top appeared to be impure β-isomaltose octaacetate; yield 324 mg. It was rechromatographed on a Magnesol–Celite (5:1 by wt.) column (180 × 30 mm., diam.) and developed with 1100 ml. of benzene–2-methyl-2-propanol (100:1 by vol.). The material isolated from a zone 90–130 mm. from the column top crystallized and some crystals of β-sophorose octaacetate were separated mechanically; yield 10 mg.; m.p. 186°, the remaining material crystallized as β-isomaltose octaacetate; yield 75 mg., m.p. 146°. The melting point of each was undepressed upon admixture with authentic samples and exhibited X-ray powder diffraction patterns identical with those of known β-sophorose octaacetate and β-isomaltose octaacetate, respectively.

The material from the fourth zone from the column top produced crystals which exhibited an X-ray powder diffraction pattern identical with that of known β-maltose octaacetate; yield 92 mg., m.p. 157–158° undepressed by a known sample.

Paper chromatography of fraction 4 indicated the presence of a disaccharide and some trisaccharides. The amorphous material (2.73 g.) was acetylated with 1.4 g. of sodium acetate and 27 ml. of acetic anhydride as described above. The material which crystallized from ethanol exhibited an X-ray diffraction pattern identical with that of β-cellobiose octaacetate; yield 218 mg., m.p. 192° undepressed upon admixture with known β-cellobiose octaacetate.

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[CONTRIBUTION FROM THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION]

Infrared Spectra and the Structure of Polyriboadenylic Acid

BY R. S. MORGAN[1] AND E. R. BLOUT[2]

RECEIVED MARCH 18, 1959

Infrared spectra have been obtained from films of polyriboadenylic acid (poly A) prepared from acid and alkaline solutions. Such films rapidly exchange their OH and NH hydrogens upon exposure to D_2O. The infrared measurements have demonstrated the existence of two forms of poly A in the solid state. One of these forms, that obtained from acid solutions, may be oriented and then shows marked infrared dichroism. From the dichroic measurements certain conclusions may be made concerning the spatial arrangement of the purine, ribose and phosphate groups. These conclusions are compared with the proposed models for the acid form of poly A.

Introduction

Polyriboadenylic acid (poly A) is the best known member of a class of enzymatically synthesized polymers, the polyribonucleotides, first prepared by Grunberg-Manago and Ochoa.[3] On the basis of fiber X-ray patterns, two models for the spatial structure of the polymer have been proposed.[4,5] Subsequent to the conception of these models, two new facts became known: first, that poly A possessed a titrable group with a pK near 6 (identified as the N or N_{10}-amino group of the adenine moieties)[6] and second, that accompanying the protonation of this group, the polymer underwent a structural change in solution.[7] In the solid state, such a change was first observed by the present infrared study. X-Ray diffraction studies on solutions[8] and films[9] then established that the fiber pattern earlier known was due to the protonated polymer, which we will refer to here as "acid" poly A.

The present work was undertaken in the hope of adducing additional information concerning the spatial configurations of this polymer by means of infrared spectroscopy. This paper will present the spectra of oriented films of acid poly A obtained with polarized infrared radiation, the infrared spectra of the two forms (acid and alkaline) of the polymer in the solid state, and the spectra obtained from the corresponding deuterated polymers.

Experimental

Films for infrared study were prepared by evaporating solutions of the polynucleotide of known pH onto AgCl disks. The films then were examined in a Perkin–Elmer model 21 double-beam spectrophotometer with a sodium chloride prism. The films were either exposed to room air or sealed (by means of a second AgCl window and a spacer) over a reservoir containing D_2O.[10] For dichroic measurements on acid poly A, orientation was achieved by wetting an acid film with 40% ethanol and stroking it until dry with a glass spatula. The resultant film was quite negatively birefringent, ($\Delta\eta$ −0.04). Although very weak negative birefringence could be produced in alkaline films by similar means, these films did not show definitive infrared dichroism. Oriented films were examined with a fixed AgCl polarizer in the sample beam only, the film being rotated so that the orientation axis was either parallel or perpendicular

(1) Huntington Laboratory, Massachusetts General Hospital, Boston 14, Mass.

(2) Chemical Research Laboratory, Polaroid Corporation, Cambridge 39, Mass.

(3) M. Grunberg-Manago and S. Ochoa, THIS JOURNAL, **77**, 3165 (1955).

(4) J. D. Watson, "The Chemical Basis of Heredity," Johns Hopkins Press, Baltimore, Md., 1957, p. 552.

(5) R. S. Morgan and R. S. Bear, *Science*, **127**, 80 (1958).

(6) R. F. Beers and R. F. Steiner, *Nature*, **179**, 1077 (1957).

(7) J. Fresco and P. Doty, THIS JOURNAL, **79**, 3928 (1957).

(8) J. Fresco, *J. Mol. Biol.* in press.

(9) R. S. Morgan and R. Byrne, submitted to *J. Mol. Biol.*

(10) H. Lenormant, A. Baudras and E. R. Blout, THIS JOURNAL, **80**, 6191 (1958).

TABLE I

THE INFRARED ABSORPTION BANDS OF POLYADENYLIC ACID

Acid form						Alkaline form			
Undeuterated			Deuterated			Undeuterated		Deuterated	
Frequency[a]	Strength	Polarization	Frequency[a]	Strength	Polarization	Frequency[a]	Strength	Frequency[a]	Strength
3350	s	⊥	3400[b]	w	⊥	3350	s	3350	w
3200	s	⊥	3200	w	⊥	3200	s	3150	w
2950	w	⊥	2950	w	⊥	2950	m	2950	w
			2500	s	⊥			2500	s
								2400	s
1710	m	⊥							
1685	m	⊥	1665	m	⊥	1655	s		
1645	m	⊥?	1620	m	⊥	1640	s	1620	vs
1610	m	⊥				1605	m		
1575	w	⊥	1575	w	⊥	1580	m	1575	m
1475	m	⊥	1475	m	⊥	1475	m	1475	m
1410	m	⊥	1405	w	⊥	1420	m	1420	w
						1370	w	1370	w
1325	m	⊥	1335	w	⊥	1325	m	1335	w
			1290	w	⊥	1290	m	1295	w
1240	s	⊥	1235	vs	⊥	1240	s	1235	vs
1215	s	⊥				1210?	m		
			1160	w	⊥				
1125	m	∥	1125	m	∥	1070–1050	s	1070–1050	s
1080	s	∥	1080	s	∥				
1045	m	∥	1065	s	∥			1025	m
1030	m	⊥	1015	m	⊥				
985	w	⊥							
950	w	∥				950	m	950	w
905	m	∥	900	m	∥				
865	w	∥	865	m	∥	875–850	w	860	w
840	m	∥	840	m	∥				
795	w	⊥	795	w	⊥	810	m	815	w
						790	m	790	w
765	vw	⊥?							
710	w	⊥?	705	w		710	w	705	w

[a] In cm.$^{-1}$. Braces are used to signify a group of related absorption bands.

to the electric vector. The spectra are presented in Figs. 1 and 2; the absorption bands are listed in Table I.

Results and Discussion

A. Acid *vs.* Alkaline State.—The spectra of these two forms are most obviously distinguished by their sharpness, that shown by the acid form having much sharper bands especially in the 1240 and 1080 cm.$^{-1}$ regions than that shown by the alkaline form. This fact further emphasizes that the acid state possesses the more ordered structure.

Of the absorption bands appearing in spectra of acid films and not present in those of alkaline films, we may assign that at 1685 cm.$^{-1}$ to the protonated adenine group, since a similar band appears in the spectrum of films of adenylic acid at *p*H 1 and 4.[11] The band at 1710 cm.$^{-1}$, however, has no counterpart in the monomer spectrum. In the deuterated state, both acid and alkaline, polymer bands in the 1650 cm.$^{-1}$ region match monomer bands quite closely. The acid polymer is further notable for a moderately strong absorption at 840 cm.$^{-1}$ not possessed by alkaline films. This band does not change on deuteration and is therefore probably not due to any NH or OH vibration. It shows marked parallel dichroism. Its assignment is unknown.

On being exposed to D_2O vapor, both acid and alkaline poly A films exchange within an hour most of their N- and O- bound hydrogens for deuteriums (compare ref. 12 on deuteration of DNA). There results a great diminution of absorption near 3300 cm.$^{-1}$ with a corresponding increase near 2500 cm.$^{-1}$, an isosbestic point for the reaction being near 2700 cm.$^{-1}$. When films of either sort are covered with liquid D_2O, the exchange occurs within a minute. This rapid exchange of labile hydrogens with D_2O indicates that such H-bonding as occurs in these structures is less strong than that observed in the helical configurations of certain polypeptides.[12]

B. Band Assignments.—Polynucleotides, like nucleic acids,[13,14] absorb infrared radiation strongly in four main regions whose frequencies center near 3300, 1650, 1240 and 1080 cm.$^{-1}$. In attempting to analyze the data from poly A films, difficulties were encountered. As far as we are aware no normal coördinate treatments of any groups which might be applicable to polynucleotides have been performed. Furthermore we have at present no certain means of identifying the effects of secondary structure or of intermolecular interactions upon either the frequency or intensity of infrared absorptions in these systems. Finally, existing theory does not enable one always to pass with assurance

(11) H. Lenormant and E. R. Blout, *Compt. rend. Acad. Sci. (Paris)*, **239** 1281 (1954).

(12) E. R. Blout and A. A. Ferguson, to be published.

(13) E. R. Blout and M. Fields, *J. Biol. Chem.*, **178**, 335 (1949).

(14) G. B. B. M. Sutherland and M. Tsuboi, *Proc. Roy. Soc. (London*, **A239**, 446 (1957).

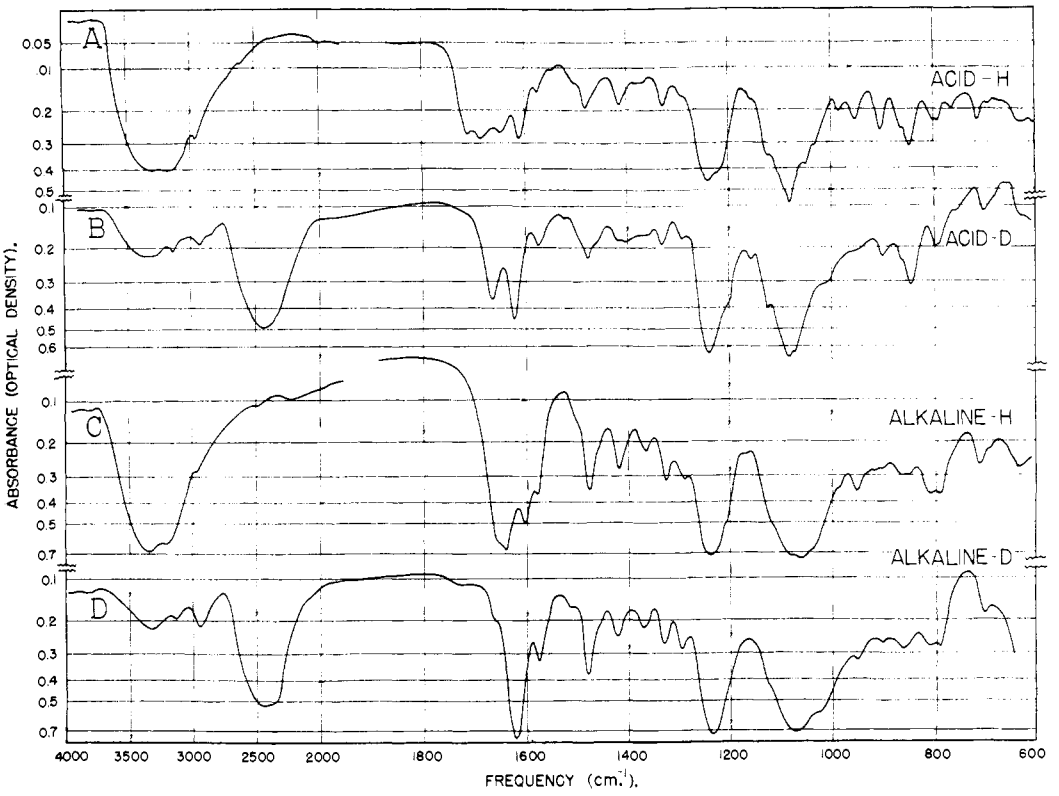


Fig. 1.—Spectra of cast films of polyriboadenylic acid: top two spectra, films cast from acid solutions; bottom two spectra, films cast from alkaline solutions. Of each pair the lower spectrum shows the deuterated state.

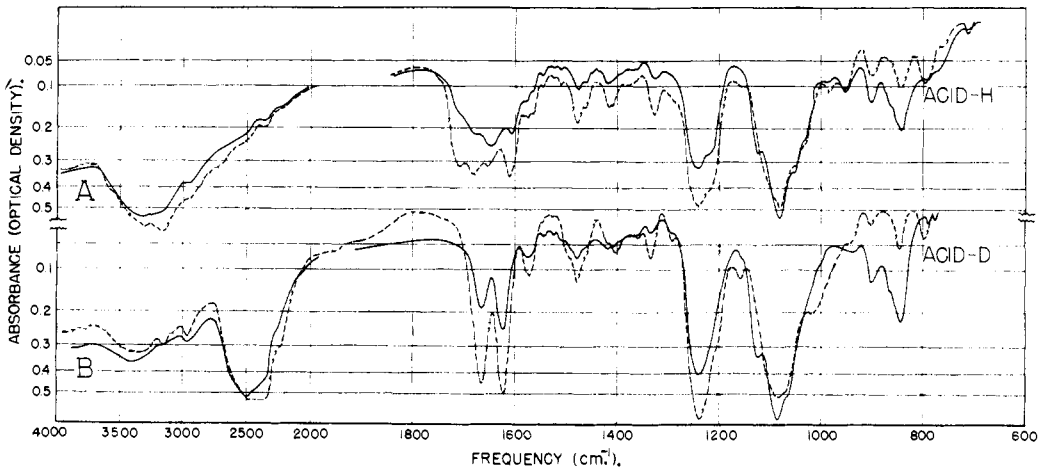


Fig. 2.—Spectra of oriented films of polyriboadenylic acid: top spectrum, film exposed to room air; bottom spectrum, film exposed to D_2O vapor; – – – –, electric moment of incident radiation perpendicular to orientation direction; ————, electric moment of incident radiation parallel to orientation direction.

from an observed dichroism to the exact spatial arrangement of the atoms involved—even assuming an accurate assignment of an absorption band. Rather than attempt to assign each observed band to a molecular vibration, we will review the component chemical groups in poly A in order to see what absorptions such groups should show.

Adenine.—From previous work[11-13,15,16] strong absorption from the purine ring in the 1650 cm.$^{-1}$ region is expected. We would point out that, of the bands seen in spectra of poly A films in this region, only that at 1575 cm.$^{-1}$ is unaltered with deuteration. Hence the others must involve motions of the amino hydrogens as well as the ring atoms.

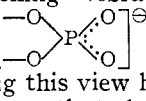
3′,5′-Diesterified D-Ribose.—Besides affording a methylene group (C_5', which is probably here absorbing at 1475 cm.$^{-1}$), the ribose parts of polyribonucleotides present the groups COC, C_2'-OH,

(15) E. R. Blout and M. Fields, THIS JOURNAL, **72**, 479 (1950).
(16) C. H. Willits, J. C. Decius, K. L. Dille and B. E. Christensen, *ibid.*, **77**, 2569 (1955).

and C′–O–P which might be expected to have characteristic absorption frequencies. Of these the first is the simplest to assign; cyclic ethers in general have very strong absorptions in the range 1070–1140 cm.$^{-1}$ [17] and tetrahydrofuran in particular has a strong band at 1076 cm.$^{-1}$, ascribed to an anti-symmetric vibration of the entire five-membered ring.[18] Ribose and ribose-containing compounds absorb strongly here.[11] Hence some part of the 1080 cm.$^{-1}$ complex of absorptions of poly A, and very likely the strong 1080 cm.$^{-1}$ band itself, is probably due to such an in-plane vibration of the furanose ring.

The C–O–P ester links also may be expected to absorb in the 1080 cm.$^{-1}$ region, but to separate such absorption from others occurring here would not seem possible.

Associated secondary alcoholic groups have been shown[19] to have a weak, broad absorption near 1400 cm.$^{-1}$. Poly A films show absorption around 1420 (alkaline) and 1410 cm.$^{-1}$ (acid) and since these bands both change slightly with deuteration these data are consistent with such an assignment. A positive identification of these polymer bands, however, would require more extensive comparisons.

Ionic Phosphate.—Previous workers[12,20] have given reasons for assigning the strong bands near 1240 cm.$^{-1}$ (seen in all polynucleotides) to the asymmetric stretching vibration of the ionic phosphate group  , and recently other evidence confirming this view has been produced.[21] These studies suggest that the related symmetric vibration will absorb near 1050 cm.$^{-1}$; if so, this adds a third motion producing absorption in the 1080 cm.$^{-1}$ complex of poly A.

NH, OH.—Poly A has an amino group on C_6 of the adenine ring and a hydroxyl on C_2' of the ribose both of which will absorb due to hydrogenic stretching vibrations in the 3300 cm.$^{-1}$ region. Such bands are seen; they are strong and broad and they disappear on deuteration.[22] That no peak of absorption in this region has a frequency greater than 3500 cm.$^{-1}$ indicates that, in these films, both these groups are hydrogen bonded although at this time it is impossible to say whether inter- or intramolecularly or both.

Finally, it may well be helpful to regard polynucleotides as a linked system of dialkyl phosphates, with the furanose and nitrogenous rings as side chains. A normal coördinate analysis of dimethyl phosphate, including if possible transition moments, would be most useful in this respect, and should be feasible with current techniques.

(17) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen, London, 1954.

(18) G. M. Barrow and S. Searles, THIS JOURNAL, **75**, 1175 (1953).

(19) A. V. Stuart and G. B. B. M. Sutherland, *J. Chem. Phys.*, **24**, 559 (1956).

(20) M. Tsuboi, THIS JOURNAL, **79**, 1351 (1957).

(21) J. W. Maarsen and M. C. Smit, *Rec. trav. chim. Pay-Bas*, **76**, 724 (1957).

(22) Although some NH and OH absorptions are seen in the curves reproduced in Fig. 1 (curves B and D), further exchange of the films with fresh D_2O removes all absorption in the 3200–3400 cm.$^{-1}$ region. However, long exposures to a saturated D_2O solution sometimes results in solution of the film.

C. Infrared Dichroism.—Since the 1650 cm.$^{-1}$ bands are due to in-plane purine ring vibrations, their transition moment lies in this plane and the observed dichroism must be simply related to the spatial orientation of this plane. In acid poly A, these bands have perpendicular dichroism similar to that shown by DNA[12] but of less magnitude. Hence the purine rings of acid poly A are predominantly perpendicular to the orientation axis, in agreement with the deduction from the X-ray diffraction pattern[4,5] and from the negative birefringence of flow.

The 1240 cm.$^{-1}$ bands of acid poly A show perpendicular dichroism only, in contrast to those of DNA. If, as suggested by Sutherland and Tsuboi,[12] we may take the transition moment of these bands as lying along the O. . .O line in the ionic phosphate group, the dichroism here seen implies that this line in acid poly A is nearly perpendicular to the orientation axis.

Sutherland and Tsuboi relate the band at 1050 cm.$^{-1}$ seen in the spectra from DNA films to the symmetric PO_2^- stretching. With acid poly A a strong 1050 cm.$^{-1}$ band having parallel dichroism is found together with a moderately intense band near 1020 cm.$^{-1}$ showing perpendicular dichroism. In the absence of studies with isotopic oxygen which might allow firm assignments of these bands, it seems unwarranted to draw conclusions concerning the spatial orientation of the bisector of the OPO angle.

Finally we may expect that the transition moment of the furanose ring vibration near 1080 cm.$^{-1}$ will lie in the (near) plane of this ring. To understand the relation of the dichroism of this region shown by DNA films to the DNA structure as most lately given by Langridge, *et al.*,[23] we must assume that this moment lies not only in the ring but also along a direction within the ring approximating a line from C_1' to C_3'. If such be the case (and we may only pose the hypothesis here) this line in acid poly A must be more nearly parallel to the fiber axis, assuming that this moment is not too greatly altered by the hydroxyl group on C_2'. More definitive work on this point would be of considerable value in elucidating polynucleotide structures, as it would allow one to decide (within limits perhaps) the orientation of the sugar with respect to the base around the glycosidic bond, an important parameter of any polynucleotide structure.

D. Comparison of Proposed Models.—Table II gives the angles between several important lines in the models proposed for acid poly A and the orientation (helical) axis. We are indebted to Dr. Alexander Rich for showing us the coördinates of his group's model in advance of publication. Both models predict strong perpendicular dichroism for the bands associated with the purine rings and with the antisymmetric phosphate vibrations in agreement with that observed. Certain other infrared absorption bands cannot be definitely assigned to molecular groupings as yet and thus correlated with the proposed models. In particular

(23) R. Langridge, W. F. Seeds, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins and L. D. Hamilton, *J. Biochem. Biophys. Cyt.*, **3**, 767 (1957).

TABLE II

Line	Region of i.r. absorption in cm.$^{-1}$	Crick, Davies, Rich Watson model[24] Angle[a]	Crick, Davies, Rich Watson model[24] Expected infrared dichroism	Morgan, Bear model[5] Angle[a]	Morgan, Bear model[5] Expected infrared dichroism
Purine ring	1575 −1725	77°[b]	⊥, strong	90°	⊥, strong
O—O	1200 −1250	70°	⊥, strong	87°	⊥, strong
OPO angle bisector	1000 −1150	47°	⊥, weak	70°	⊥, strong
C_1'–C_3'	1000 −1150	29°	∥, weak	36°	None

[a] Angle between group indicated and helix axis. [b] Angle between normal to purine ring and helix.

acid poly A shows a highly dichroic group of absorption bands between 800 and 900 cm.$^{-1}$ which when assigned will yield structural information. On the basis of the data and interpretation presented here, however, there is no clear preference for either model.

Conclusions.—Infrared spectroscopy has demonstrated the existence of two forms of polyriboadenylic acid in the solid state, the forms depending on the *p*H of the solution from which the solid films were cast. One of these forms, that from solutions whose *p*H is below 6, may be oriented and then shows marked infrared dichroism. Both forms rapidly exchange their active hydrogens for deuteriums with D_2O vapor. Although at present a complete translation of dichroic measurements into structural conclusions is not possible, the observations here reported permit certain conclusions about the spatial relations of the purine, ribose and phosphate groups of this polymer in its acid state. The comparison of these conclusions with the two models proposed for acid poly A is presented and does not allow an unequivocal choice of one over the other. More definite conclusions must await future work on simpler systems, some directions for which are indicated.

Acknowledgment.—For gifts of poly A we are indebted to Dr. Roland F. Beers, Jr., of the Children's Hospital School, Baltimore, Md., and to Drs. Jacques Fresco and Paul Doty of the Department of Chemistry, Harvard University. This work was supported in part by U. S. Public Health Grants No. CY-3335 and A-2558.

(24) A. Rich, personal communication.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE UNIVERSITY]

Levulinic Acid as a Reagent for the Hydrolysis of Oximes and 2,4-Dinitrophenylhydrazones

BY C. H. DEPUY AND B. W. PONDER

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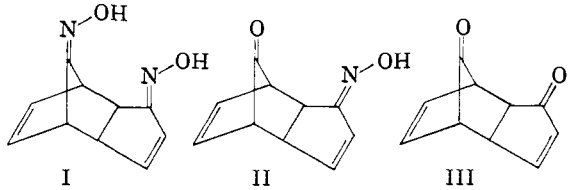
It has been found that levulinic acid is an excellent reagent for hydrolyzing oximes and 2,4-dinitrophenylhydrazones. Conjugated oximes hydrolyze enough more slowly than non-conjugated, that it has been possible to hydrolyze selectively a dioxime to a keto oxime.

Introduction

In connection with another problem,[1] we were interested in hydrolyzing the dimer of cyclopentadienone oxime[2] (I) to the corresponding diketone III. Compounds I, III and the intermediate II are all rather unstable, sensitive to strong acid, and III



I II III

decomposes near its melting point (97–98°). Attempted hydrolysis by acid with or without the presence of formaldehyde[3] led at low temperatures to the isolation of either unreacted starting material or to an intractable mixture of I, II and III. At higher temperatures, extensive decomposition occurred, and only traces III could be isolated. In many runs, under a variety of conditions, we could not obtain satisfactory yields of the dione.

At the time that this attempted hydrolysis was underway, Keeney[4] described a semi-micro colorimetric procedure for the estimation of 2,4-dinitrophenylhydrazones which involved the use of levulinic acid as an acceptor molecule in a transderivatization reaction. We were consequently encouraged to try levulinic acid for the preparative-scale deoximation of I. We found that when compound I was stirred at room temperature with levulinic acid to which 10 volume per cent of 1 *N* hydrochloric acid had been added, the dioxime gradually, over a period of about three hours, went into solution and from the resultant solution the keto-oxime II could be isolated in nearly quantitative yield. Under identical conditions the product from the formaldehyde-hydrochloric acid method was an oily mixture containing a mixture of I, II and even III, plus decomposition products. We then tried to hydrolyze the keto-oxime II to the diketone III and found that the same levulinic acid–hydrochloric acid mixture gave a 70% yield of III after three hours heating on the steam-bath. The stepwise procedure seemed to give somewhat better over-all yields of III than did the direct hydrolysis of I to III, although even on direct hydrolysis the yields were acceptable.

(1) C. H. DePuy and E. F. Zaweski, THIS JOURNAL, **81**, in press (1959).

(2) (a) J. Thiele, *Ber.*, **33**, 669 (1900); (b) W. von E. Doering and C. H. DePuy, THIS JOURNAL, **75**, 5995 (1953).

(3) J. A. Barltrop, A. J. Johnson and G. D. Meakins, *J. Chem. Soc.*, 181 (1951).

(4) M. Keeney, *Anal. Chem.*, **29**, 1489 (1957).