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.

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

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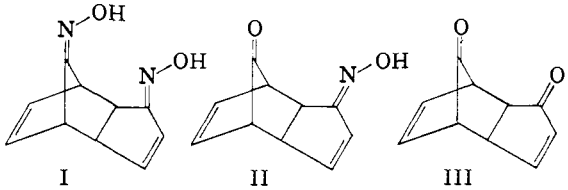
It has been found that levulinic acid is an excellent reagent for hydrolyzing oximes and 2,4-dinitrophenylhydrazones. Conjugated oximes hydrolyze enough more slowly than non-conjugated, that it has been possible to hydrolyze selectively a dioxime to a keto oxime.

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).

Keeney[4] had already shown that levulinic acid cleaved 2,4-dinitrophenylhydrazones of α,β-unsaturated ketones more slowly than it did those of unconjugated ketones, and our results tended to confirm this for oximes. We were led by the success of our initial experiments to examine a few typical oximes to see if the method was generally applicable. When the oxime of α-phenylcyclohexanone[5] was stirred overnight at room temperature with levulinic acid and hydrochloric acid the corresponding ketone could be isolated in greater than 90% yield by filtration of the product from the reaction mixture after dilution with water. We next tried a typical, unconjugated ketone, and found that dibenzyl ketone could be obtained in 98% yield from its oxime after three hours reaction at room temperature.[6] In this case the product did not crystallize from solution, but the levulinic acid was easily removed from the product by diluting with water, extracting with methylene chloride, and washing the extracts with water and bicarbonate. These two experiments indicated that the hydrolysis of normal, aliphatic cyclic and acyclic oximes proceeded readily and in nearly quantitative yield with levulinic acid.

It next became of interest to determine under what conditions α,β-unsaturated and aromatic ketones would be liberated from their oximes. Keeney[4] had already demonstrated a rate difference for conjugated and non-conjugated 2,4-dinitrophenylhydrazones under the conditions of his analytical method. It remained to be seen whether the rate difference would hold for oximes, and whether it would be large enough to permit selective hydrolysis on a preparative scale. Consequently we examined the hydrolysis of several conjugated ketoximes, and also of benzaldoxime. The results are listed in Table I.

TABLE I

YIELDS OF CARBONYL COMPOUNDS FROM HYDROLYSIS WITH LEVULINIC ACID

Compound	Yield, % (room temp.)	Yield, % (100°)
Acetophenoxime	28	85
Benzaldoxime	20	64
Benzophenoxime	13	72
Dibenzylketoxime	99	..
Dicyclopentadienoxime	94[a]	70[b]
Mesityl oxide oxime	19	75
2-Oximino-1-indanone	53	[c]
α-Phenylcyclohexanoxime	90	..
Δ^4-Cholesten-3-one 2,4-DNP	..	95[d]

[a] Hydrolysis of one oximino group. [b] Hydrolysis of two oximino groups. [c] Decomposes on heating. [d] Reaction mixture contained chloroform; see Experimental.

Finally it was decided to examine the hydrolysis of a 2,4-dinitrophenylhydrazone so that some information would be available about this reaction on a preparative scale, and so that some assessment of the relative merits of levulinic and pyruvic acids for this purpose could be made. This result is also included in Table I.

(5) We wish to thank Mr. B. Barnett, I.S.C., for conducting this experiment.

(6) We chose dibenzylketoxime rather than a simple ketone so that losses during the work-up would be minimized.

Discussion

It is clear from the results in the table that levulinic acid containing 10% of 1 *N* hydrochloric acid is an excellent reagent for removing oxime groups from carbonyl compounds. Non-conjugated oximes are hydrolyzed at room temperature, and the yields were 90% or greater in all three cases investigated. The α,β-unsaturated and aromatic ketoximes were hydrolyzed significantly slower, slow enough indeed to show that non-conjugated ketoximes could be selectively hydrolyzed in the presence of conjugated oximes. The hydrolysis of I to II demonstrates this selective hydrolysis extremely well, although this is somewhat of a special case in that the bridge ketoxime may be especially susceptible to hydrolysis. Nevertheless, the reaction conditions were vigorous enough to hydrolyze other non-labile oximes. These results show that it should be possible to use an oximino group as a protecting group for a conjugated ketone in a molecule containing both conjugated and unconjugated ketones. And of course the ease of hydrolysis, and the high yields obtained, make oximes attractive as derivatives which might be purified and then be hydrolyzed.

We next examined the conditions which would be necessary for the hydrolysis of α,β-unsaturated oximes, and found that the best yields were obtained after heating on a steam-bath for three hours. The yields of pure carbonyl compound averaged about 75%, and some of the lower yields may have been due to losses in the work-up of these compounds, for they were more volatile than the unconjugated ones. It is clear that here too, levulinic acid is successful as a deoximating agent.

Finally we examined the hydrolysis of an α-oximino ketone, for these are common intermediates in the preparation of α-diketones. We chose 2-oximino-1-indanone because the hydrolysis of this oxime had recently been reported by Cava[7] using formaldehyde and strong hydrochloric acid. The starting material and product are both sensitive to heat and acid, and we were only able successfully to hydrolyze the oxime at room temperature. Our yield, 53%, was about the same as that reported by Cava, but he was able to recover most of the unreacted oximino-ketone whereas we were not able to do so. It appears then that levulinic acid may not be especially advantageous for the hydrolysis of this particular kind of oximes.

It seems probable that levulinic acid will be equally successful for hydrolyzing other derivatives of carbonyl compounds. One hydrolysis which has been much investigated is the cleavage of 2,4-dinitrophenylhydrazones of steroidal α,β-unsaturated ketones.[8] These derivatives often arise during the introduction of the α,β-double bond.[8a] Djerassi examined in some detail the problem of hydrolyzing these 2,4-dinitrophenylhydrazones using pyruvic acid and found that although the conditions were rather critical, 60–70% yields of ketone could be obtained. We therefore thought it pertinent to at-

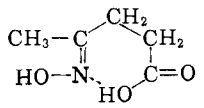
(7) M. P. Cava, R. L. Little and D. R. Napier, THIS JOURNAL, **80**, 2260 (1958).

(8) (a) V. R. Mattox and E. C. Kendal, *ibid.*, **70**, 883 (1948); (b) C. Djerassi, *ibid.*, **71**, 1003 (1949); (c) E. B. Hershberg, *J. Org. Chem.* **13**, 542 (1948).

tempt to hydrolyze a steroidal 2,4-dinitrophenylhydrazone using levulinic acid so that some comparison could be made. We therefore prepared the 2,4-DNP of Δ^4-cholestenone and treated it with levulinic acid, hydrochloric acid and enough chloroform to make the mixture homogeneous. After heating under reflux on the steam-bath for 3 hours, the reaction mixture was worked up in the usual way and the cholestenone was isolated by chromatography in 95% yield. We do not know that these conditions are the mildest that could be used, but it is obvious that levulinic acid can successfully be used for 2,4-dinitrophenylhydrazone hydrolyses also.

Levulinic acid has advantages over pyruvic acid over and above the higher yield obtained in this one case. It is cheaper, more easily purified, and more stable. The ketones obtained are not contaminated by any decomposition products from the keto acid, and the excess levulinic acid can, to a large extent, be recovered if necessary.

A word may be in order about why levulinic acid is so good for accomplishing these hydrolyses. We have no information which necessitates the postulation of anything but a simple equilibrium. However, it may be that the equilibrium is shifted in the case of levulinic acid in favor of the levulinic acid oxime by internal hydrogen bonding as in IV. This may make the reaction substantially irreversible and reduce complications. It is not impossible to



IV

imagine more complicated roles for the levulinic acid, but this would be idle speculation. We wish only to bring to general notice that levulinic acid can serve as an excellent reagent for these hydrolyses.

Experimental

1,8-Dioximino-4,7-methano-3a,4,7,7a-Tetrahydroindene (I) was prepared by the method of reference 2b.

Oximes.—The oximes were prepared by refluxing the ketones with a slight excess of hydroxylamine hydrochloride in a mixture of pyridine and absolute ethanol.[9] After two hours the solution was cooled, concentrated, and diluted with water. The organic layer was removed and the oxime recovered by crystallization or distillation.

Acetophenoxime, m.p. 56–58° (lit.[10] m.p. 60°); **benzaldoxime**, b.p. 102° (6 mm.), m.p. 34–35° (lit.[11] m.p. 35°); **dibenzylketoxime**, m.p. 120–121° (lit.[12] m.p. 120–121°); **mesityl oxide oxime**, b.p. 70–72° (5 mm.) (lit.[11] b.p. 84° (11 mm.)); **benzophenoxime**, m.p. 141–142° (lit.[13] 141–142°).

2-Oximino-1-indanone.—This keto-oxime was prepared in 76% yield from 1-indanone and ethyl nitrite according to the procedure of Cava[7]; m.p. 205–210° dec.

Δ^4-Cholesten-3-one.—This ketone was prepared by the oxidation of cholesterol according to reference 14. The 2,4-dinitrophenylhydrazone was prepared in the usual manner, m.p. 228.5–230° (lit.[8b] m.p. 232°).

α-Phenylcyclohexanone.—This ketone, prepared by oxidation of the commercially available α-phenylcyclohexanol with sodium dichromate and sulfur acid, had m.p. 55–56° (lit.[15] m.p. 50–52°). The oxime was prepared in the usual way and purified by recrystallization from alcohol; m.p. 168–170° (lit.[15] m.p. 168–170°).

1-Oximino-8-Keto-4,7-methano-3a,4,7,7a-Tetrahydroindene (II).—This keto-oxime was prepared by hydrolysis of the dioxime. After recrystallization from methylene chloride, it had a m.p. of 158–159°. The infrared absorption spectrum of II exhibited strong absorption at 5.6 μ.

Anal. Calcd. for $C_{10}H_9O_2N$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.35; H, 5.33; N, 7.73.

1,8-Diketo-4,7-methano-3a,4,7,7a-tetrahydroindene (III).—This diketone was prepared by hydrolysis of either the dioxime I or the keto-oxime II. After recrystallization from hexane, it had a m.p. of 101–101.5°. This compound exhibited characteristic absorption bands in the infrared at 5.6 and 5.85 μ.

Anal. Calcd. for $C_{10}H_8O_2$: C, 74.99; H, 5.03. Found: C, 74.96; H, 5.23.

Levulinic Acid.—A portion of the levulinic acid was generously supplied by the Quaker Oats Co., of Chicago. This was redistilled at 152–154° (20 mm.). The remainder of the acid was purchased from Eastman (technical) and redistilled as above.

General Hydrolysis Procedure. a. Unconjugated Oximes.—One gram of the oxime was mixed with 30 parts of a solution of 9 volumes of levulinic acid and one volume of 1.0 *N* hydrochloric acid. This mixture was placed in an erlenmeyer flask and stirred at room temperature for three hours. If the oxime was not immediately soluble, it gradually dissolved over the course of the three hours. The solution was then diluted with water, extracted with methylene chloride, and the extracts washed free of levulinic acid with bicarbonate solution. The methylene chloride was removed and the ketone recovered by chromatography or recrystallization.

b. Conjugated Oximes.—The procedure was the same except that the flask was stirred on the steam-bath for three hours. It was worked up in the same manner and the products isolated again by chromatography.

c. Δ^4-Cholesten-3-one 2,4-DNP.—One gram of the hydrazone was dissolved in 100 ml. of the usual levulinic acid–hydrochloric acid mixture and 100 ml. of chloroform. The mixture was heated under reflux for three hours on the steam-bath and worked up as described above. Chromatography gave 0.65 g. (95%) of cholestenone, m.p. 77–78°, mixture melting point with an authentic sample, 77–78°.

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