g. also was isolated. Elemental analysis indicated it to be chloro-*t*-butylmesitylene.

Anal. Calcd. for $C_{13}H_{19}Cl$: C, 74.09; H, 9.09. Found: C, 73.98; H, 8.90.

Preparation of N-Benzenesulfonylalkylmesidines.—To a pyridine solution of the crude amine hydrochloride was added 1.2 molar equivalents of benzenesulfonyl chloride. The solution was heated at 60–70° for one hour, cooled, and poured into a mixture of ice and hydrochloric acid. The material which precipitated was recrystallized from 95% ethanol until a constant melting point was attained. A yield of 62% of N-benzenesulfonylisoduridine, m.p. 178–179°, was obtained.

Anal. Calcd. for $C_{16}H_{19}NO_2S$: C, 66.40; H, 6.62; N, 4.84. Found: C, 66.44; H, 6.74; N, 5.13.

N-Benzenesulfonyl-*t*-butylmesidine, m.p. 177–178°, was formed in 48% yield.

Anal. Calcd. for $C_{19}H_{25}NO_2S$: C, 68.84; H, 7.60; N, 4.23. Found: C, 68.65; H, 7.85; N, 4.50.

Preparation of N-Benzenesulfonyl-N-carboxymethylalkylmesidines.—To a warm, stirred ethanolic solution of the N-benzenesulfonylalkylmesidine and 2 molar equivalents of potassium ethoxide was added 1.2 molar equivalents of ethyl bromoacetate. Precipitation of potassium bromide occurred immediately. After heating for 40 minutes, the mixture was cooled and the precipitate collected. The solid was washed with hot ethanol, and the wash combined with the original filtrate. To this solution was added a large excess of 20% aqueous sodium hydroxide and the solution was heated until all the ethanol had distilled. Water was added periodically to maintain a nearly constant volume. The residual solution was heated an additional 3 hours, cooled, and made acid to congo red with cold concd. hydrochloric acid. The solid which precipitated was dissolved in aqueous sodium carbonate, the solution filtered, and the acid reprecipitated. Recrystallization was achieved from benzene. A yield of 71% of N-benzenesulfonyl-N-carboxymethylisoduridine was obtained.

Anal. Calcd. for $C_{18}H_{21}NO_4S$: C, 62.24; H, 6.10; N, 4.03. Found: C, 62.42; H, 5.81; N, 4.13.

N-Benzenesulfonyl-N-carboxymethyl-*t*-butylmesidine also formed in 71% yield.

Anal. Calcd. for $C_{21}H_{27}NO_4S$: C, 64.76; H, 6.98; N, 3.60. Found: C, 64.62; H, 7.11; N, 3.83.

Resolution of N-Benzenesulfonyl-N-carboxymethylisoduridine.—A solution of 4.20 g. of N-benzenesulfonyl-N-carboxymethylisoduridine, 3.54 g. of cinchonine and 325 ml. of ethyl acetate was prepared by warming the mixture and filtering the hot solution. The volume was reduced to 200 ml. by passing an air stream over the solution at room temperature. The solution was cooled in a refrigerator for several days, and 2.85 g. of colorless prisms was collected, m.p. 185–188°. After recrystallization from ethyl acetate the melting point was 186–188°, and the specific rotation unchanged; rotation: 0.0508 g. made up to 10 ml. at 30° with ethanol gave α_D +0.50°, *l* 1; $[\alpha]^{30}D$ +98.5°.

The optically active acid was regenerated from the pure salt in a manner previously described.[11] From 1.48 g. of the pulverized salt was obtained 0.75 g. (94%) of (+)-N-benzenesulfonyl-N-carboxymethylisoduridine, m.p. 184–186°; rotation: 0.0185 g. made up to 1.607 ml. with ethanol at 29° gave α_D +0.08°, *l* 1; $[\alpha]^{29}D$ + 6.9°.

Racemization of N-Benzenesulfonyl-N-carboxymethylisoduridine.—A dimethylformamide solution of 0.7670 g. of the (+)-acid was made up to 10 ml. and the racemization carried out at 118° (boiling point of *n*-butyl alcohol) in a manner described previously.[3] The following results were obtained: 0.0 hr., $\alpha^{30}D$ +0.22°; 1.5 hr., $\alpha^{30}D$ +0.19°; 2.5 hr., $\alpha^{30}D$ +0.17°; 4.0 hr., $\alpha^{30}D$ +0.15°; 5.0 hr., $\alpha^{30}D$ +0.14°; 6.0 hr., $\alpha^{30}D$ +0.13°.

The expression for the racemization rate of compounds exhibiting optical activity of the type studied is: $\ln \alpha_0/\alpha_t = 2kt$, where α_0 is the observed rotation at time 0 and α_t is the observed rotation at time *t*.[12] A plot of α *vs.* time on semilogarithmic paper afforded a straight line from whose slope was derived the rate constant $k = 4.7 \times 10^{-2}$ hr.$^{-1}$ and the half-life $t_{1/2}$ = 7.3 hr.

Resolution of N-Benzenesulfonyl-N-carboxymethyl-*t*-butylmesidine.—The cinchonidine salt of N-benzenesulfonyl-N-carboxymethyl-*t*-butylmesidine was prepared in a manner similar to that of the cinchonine salt of the isoduridine derivative, using ethyl acetate as resolving solvent. The less soluble salt was purified by recrystallization from ethyl acetate, m.p. 195–196°; rotation: 0.0611 g. made up to 10 ml. at 28° with ethanol gave α_D −0.22°, *l* 1; $[\alpha]^{28}D$ −36.0°. It was treated in the manner previously described to yield pure (+)-N-benzenesulfonyl-N-carboxymethyl-*t*-butylmesidine, m.p. 171.5–172.5°; rotation: 0.0064 g. made up to 1.607 ml. with ethanol at 26° gave α_D +0.17°, *l* 1; $[\alpha]^{25}D$ +42.5°.

Racemization of N-Benzenesulfonyl-N-carboxymethyl-*t*-butylmesidine.—A dimethylformamide solution of 0.1542 g. was made up to 10 ml. and the racemization carried out in the usual manner. The following results were obtained: 0.0 hr., $\alpha^{26}D$ +0.58°; 0.75 hr., $\alpha^{26}D$ +0.42°; 1.5 hr., $\alpha^{26}D$ +0.30°; 2.0 hr., $\alpha^{26}D$ +0.24°; 3.0 hr., $\alpha^{26}D$ +0.14°; 4.0 hr., $\alpha^{26}D$ +0.09°. The rate constant derived was $k = 2.32 \times 10^{-1}$ hr.$^{-1}$ and the half-life, $t_{1/2}$ = 1.49 hr.

(11) R. Adams and J. R. Gordon, THIS JOURNAL, **72**, 2456 (1950).

(12) D. F. Smith, *ibid.*, **49**, 43 (1927).

URBANA, ILLINOIS

[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

The Reaction of Hydroxylamine with Activated Acyl Groups. I. Formation of O-Acylhydroxylamine[1]

BY WILLIAM P. JENCKS[2]

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The reaction of *p*-nitrophenyl acetate or benzoate with dilute aqueous or alcoholic hydroxylamine at neutral *p*H gives as a major initial product an unstable compound which has been identified as the corresponding O-acylhydroxylamine. These compounds give no color with ferric chloride, are rapidly split by dilute base and react with concentrated hydroxylamine to give hydroxamic acid. Evidence for the formation of varying amounts of O-acylhydroxylamines with other acylating agents has been obtained.

Although the reactions of hydroxylamine with chlorosulfonic acid[3] and (on the basis of indirect evidence) certain phosphoric anhydrides[4] give O-substituted compounds, the products isolated from the reaction of hydroxylamine with acylating agents have been N-substituted hydroxylamines (hydroxamic acids)[5] with the apparent single exception of O-anthranoylhydroxylamine, which is formed from hydroxylamine and isatoic anhydride.[6] This re-

(1) For a preliminary report see W. P. Jencks, *Biochim. et Biophys. Acta*, **27**, 417 (1958).

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(3) F. Sommer, O. F. Schulz and M. Nassau, *Z. anorg. allgem. Chem.*, **147**, 142 (1925).

(4) B. Jandorf, THIS JOURNAL, **78**, 3686 (1956).

(5) H. L. Yale, *Chem. Revs.*, **33**, 209 (1943); F. Mathis, *Bull. soc. chim.*, **20**, D 9 (1953).

(6) A. W. Scott and B. L. Wood, *J. Org. Chem.*, **7**, 508 (1942).

TABLE I

PRINCIPAL INFRARED ABSORPTION BANDS OF ACYLHYDROXYLAMINES[d,e]

Substance	Wave length, microns										
	N–H (m)	Bonded O–H (m)	Bonded N–H (w)	C=O amide (s)	C=O ester (s)	(s)	C–N (?) (m)	(m)	C–O ester (s)	Benzoate (m)	N–O (?) (m)
Octanoylhydroxamic acid	2.95	3.13		6.01			7.20				10.25
Benzohydroxamic acid[a,b]	2.94	3.15		6.01			7.22				11.15
Dibenzohydroxamic acid[b]	3.03		3.15	5.86	5.68	6.89	7.25	7.60	8–8.5	9.26	11.14
O-Acetyl-N-benzoyl hydroxylamine[b]	3.02		3.13	5.88	5.60	6.89	7.32		7.9–8.5		11.14
O-Benzoyl-N-acetyl hydroxylamine	3.01		3.13	5.84	5.67	6.89	7.32	7.60	7.9–8.5	9.24	10.84
O-Benzoylhydroxylamine[c]	3.04		3.14		5.78	6.89		7.60	7.8–8.5	9.25	10.80
p-Nitrobenzaldoxime benzoate					5.70	6.89		7.62	7.9–8.5	9.29	10.33

[a] 1.6 mg./ml.; also weak O–H band at 2.75 μ. [b] Spectra for solid given by F. Mathis, *Compt. rend.*, **232**, 505 (1951). [c] Also 6.09 μ (w) and 6.44 μ (m). [d] Aromatic compounds also have bands at 3.3, 6.24, 6.32, 6.71–6.80 and 9.78–9.9 μ. [e] 1% solutions in $CHCl_3$.

port describes the identification and isolation of O-acylhydroxylamine derivatives as major initial products of the reaction of hydroxylamine with a number of acylating agents at neutral *p*H.

Results

Identification of O-Acylhydroxylamines.—Addition of hydroxylamine to a dilute alcoholic solution of *p*-nitrophenyl benzoate results in the immediate quantitative release of *p*-nitrophenol, but gives only a small fraction of the expected yield of hydroxamic acid, measured by the ferric chloride test.[7] The reason for this discrepancy became apparent upon the isolation and crystallization of a labile, low melting compound from the reaction mixture which gave the same analysis as benzohydroxamic acid but did not give the color reaction with ferric chloride characteristic of hydroxamic acids containing a free –NOH group.[5] In dilute solution this material is stable for several days at room temperature; however on reaction with 1.6 *M* hydroxylamine it is converted to benzohydroxamic acid and if the pure liquid is heated briefly it is converted to benzohydroxamic acid and dibenzohydroxamic acid. It is immediately destroyed by 0.01 *M* NaOH at room temperature and is not converted to hydroxamic acid by dilute acids and bases other than hydroxylamine. This compound was identified as O-benzoylhydroxylamine by reaction with acetic anhydride to give O-benzoyl-N-acetylhydroxylamine, by reaction with *p*-nitrobenzaldehyde to give *p*-nitrobenzaldoxime benzoate, and by its infrared spectrum (Table I). The carbonyl group of O-benzoylhydroxylamine absorbs at the same wave length, 5.78 μ, as the similar compound, perbenzoic acid (5.77 μ)[8] and at a slightly longer wave length than O-benzoylhydroxylamine derivatives containing electron-withdrawing substituents; the absorption is at too low a wave length to be assigned to an N-benzoyl group as in benzohydroxamic acid and its derivatives. In addition O-benzoylhydroxylamines show strong ester bands as well as two unidentified bands at 6.89 and 7.60 μ which are not present in simple hydroxamic acids. A band at 7.20–7.32 μ which is found in all N-acylhydroxylamines, possibly due to C–N stretching, is absent from O-benzoylhydroxylamine and from *p*-nitrobenzaldoxime benzoate. The O–H and N–H assignments are by analogy with perbenzoic acid, which shows a hydrogen bonded O–H band at 3.08 μ.[8] A definite amide II band could not be assigned in the hydroxamic acid spectra.

The more labile O-acetylhydroxylamine could not be obtained in the pure state because of its spontaneous conversion in concentrated solution to acetohydroxamic acid and diacetohydroxamic acid; in dilute solution it is stable for several days at room temperature and for months at −15°. On reaction with benzoyl chloride it gave O-acetyl-N-benzoylhydroxylamine.

O-Acylhydroxylamine Formation with Other Acylating Agents.—The reaction of a number of acylating agents with hydroxylamine proceeds in the same manner as that of *p*-nitrophenyl esters to give a rapid quantitative liberation of base from the acylating agent accompanied by the formation of only a fraction of the theoretical amount of hydroxamic acid; at relatively high concentrations of hydroxylamine this is followed by a slow formation of hydroxamic acid which shows (pseudo) first-order kinetics and which may be ascribed to the conversion of O-acylhydroxylamine which was formed in the initial rapid reaction to hydroxamic acid. The relative amounts of O- and N-acylation occurring in the initial rapid reaction could therefore be estimated by adding acylating agents to moderately concentrated hydroxylamine solutions and comparing the immediate formation of hydroxamic acid, from a (small) extrapolation to zero time, with that formed when the O-acylhydroxylamine had been converted completely to hydroxamic acid (Table II). The relative importance of O-acylation was found to vary widely with the nature of the acylating agent, ranging from 92% with diacetohydroxamic acid to an insignificant amount with benzoyl chloride, and, in general, to increase with increasing electron-donating character of the leaving group. No evidence for O-acyl hydroxylamine formation could be obtained with ethyl acetate, which reacted with hydroxylamine at a slower rate than did O-acetylhydroxylamine, or with acetyl chloride, which reacted with solvent more rapidly than with hydroxylamine.

Discussion

The occurrence of a significant amount of O-acylation will lead to incorrectly low results in the quantitative determination of activated acyl groups by reaction with hydroxylamine to give hydroxamic acid[7] if the further reaction of O-acylhydroxylamine to give hydroxamic acid is not complete or if the

(7) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).
(8) W. H. T. Davidson, *J. Chem. Soc.*, 2456 (1951).

TABLE II

INITIAL YIELD OF HYDROXAMIC ACID FROM THE REACTION OF HYDROXYLAMINE WITH VARIOUS COMPOUNDS AT 25° AND *p*H 6.8–7.0

Compound	(*M*)	NH_2OH[a] (*M*)	Other components	Hydroxamic acid formed in init. reacn., % of final value
Acetic anhydride	0.01	0.2	EDTA 10^{-4} *M*[b]	49
Acetylimidazole	.01	.2	EDTA 10^{-4} *M*	14[c]
p-Nitrophenyl acetate	.00055	.18	0.27 *M* NaCl	25[d]
Diacetohydroxamic acid	.001	.1	0.1 *M* phosphate buffer	8[e]
p-Nitrophenyl benzoate	.0005	1.6	36% ethanol	41[d]
2,4-Dinitrophenyl benzoate	.0023	1.8	40% EtOH, EDTA 10^{-4} *M*	63[d]
Benzoyl chloride	.023	1.8	40% EtOH, EDTA 10^{-4} *M*	96

[a] 85% as free base, 15% as $NH_2OH \cdot HCl$. [b] Ethylenediaminetetraacetic acid. [c] Spectrophotometric measurement at 245 mμ indicated that acetylimidazole disappearance was complete in less than 30 sec. [d] Nitrophenol liberation complete in less than 1 min. [e] *p*H 7.4. This value does not include one mole of hydroxamic acid which was liberated immediately.

labile O-acylhydroxylamine is destroyed before it is converted to hydroxamic acid. This provides an explanation for the low yield of hydroxamic acid and the accumulation of small amounts of a labile intermediate in the reaction of hydroxylamine with enzymatically activated fatty acids and for similar losses in the non-enzymatic reactions of dilute hydroxylamine with acetyl adenylate and S-acetylglutathione[9]; these losses were more marked if an apparent heavy metal-catalyzed decomposition of O-acetylhydroxylamine was not prevented by the addition of chelating agents. The accumulation of O-acylhydroxylamine would not be expected in the presence of the high concentrations of neutral hydroxylamine ordinarily used in preparative work or in base-catalyzed hydroxamic acid formation[10] since it would be converted rapidly to hydroxamic acid or hydrolyzed under these conditions.

The reaction of hydroxylamine with potassium cyanate gives rise to two isomers of hydroxyurea, one of which is labile, low melting, easily reduced, weakly basic, ether soluble and rapidly destroyed by 0.1 *M* KOH[11a]; from these properties and by analogy with the reactions described here it seems quite likely that this unstable isomer is also a product of O attack by hydroxylamine and has the formula $H_2NC(=O)NH_2$, rather than previously suggested formulas of the $H_2NC(OH)=NOH$ type.[11b]

Experimental

Materials.—A stock solution of 4 *N* hydroxylamine hydrochloride was neutralized with an equal volume of 3.5 *N* NaOH just before use. Identical results were obtained with recrystallized hydroxylamine. Benzohydroxamic acid, m.p. 128°,[12] dibenzohydroxamic acid, m.p. 152–154°, *p*-nitrophenyl benzoate, m.p. 145°, 2,4-dinitrophenyl benzoate m.p. 132–133°, *p*-nitrobenzaldoxime benzoate, m.p. 192–193°, and O-benzoyl N-acetyl hydroxylamine, m.p. 98–99°, were prepared by the action of benzoyl chloride on the appropriate base in neutral or alkaline aqueous solution.[13] *p*-Nitrophenyl acetate,[14] m.p. 78–79°, was recrystallized from hexane and stored over $CaSO_4$. Aqueous solutions were made up to 10^{-3} *M* concentration by prolonged stirring at room temperature and were stable for several days at 3°; *p*-nitrophenyl benzoate and 2,4-dinitrophenyl benzoate were made up to 10^{-3} *M* in 71% ethanol. Acetylimidazole[15] was stored over $CaSO_4$ at −15° and dissolved in water immediately before use.

Measurements.—Hydroxamic acids and their derivatives were determined as the $FeCl_3$ complex by a modification of the procedure of Lipmann and Tuttle,[7] using the appropriate hydroxamic acids as standards. Free hydroxamic acids were determined directly by adding 4 ml. of 10% $FeCl_3 \cdot 6H_2O$ in 0.7 *N* HCl to 1 ml. of sample and then adding 1 ml. of a freshly prepared mixture of equal parts of 4 *N* $NH_2OH \cdot HCl$ and 3.5 *N* NaOH. The absorbance was measured at 540 mμ with a Coleman Jr. spectrophotometer and was found to increase linearly with hydroxamic acid concentration within the usable range of the instrument. O-Acylhydroxylamine derivatives were incubated at room temperature for 10 minutes (O-acetyl, 1.0-ml. sample) or 30 minutes (O-benzoyl, 0.1-ml. sample followed by 0.9 ml. of water at the end of incubation) with 1 ml. of neutralized hydroxylamine solution. After incubation 4 ml. of $FeCl_3$ solution was added. Under these conditions O-acylhydroxylamines give one mole and diacylhydroxylamines give two moles of hydroxamic acid. Diacylhydroxylamines were further identified by incubation for 10 minutes (O-acetyl, 1.0-ml. sample) or 30 minutes (O-benzoyl, 0.1-ml. sample followed by addition of 0.9 ml. of water after incubation) with 0.5 ml. of 3.5 *N* NaOH, followed by the addition of 4 ml. of $FeCl_3$ reagent and then 0.5 ml. of 4 *N* $NH_2OH \cdot HCl$; under these conditions O-acylhydroxylamines give no color and diacylhydroxylamines are hydrolyzed to give one mole of hydroxamic acid.

Liberation of *p*-nitrophenol and 2,4-dinitrophenol was followed by measurement with the Coleman Jr. spectrophotometer of the absorbance at 400 mμ due to the nitrophenolate anion in the reaction mixture or a diluted aliquot thereof. Each reading was corrected for deviations from Beer's law by reference to a standard curve constructed by measurement of serial dilutions of the appropriate compound in phosphate buffer, *p*H 6.8. Ultraviolet and visible spectra were measured with a Cary model 11M or Beckman model DU spectrophotometer and infrared spectra were measured with a Perkin–Elmer spectrophotometer in NaCl cells. *p*H measurements were made with a Beckman model G *p*H meter.

O-Benzoylhydroxylamine.—Fifteen ml. of an aqueous mixture of equal parts of 4 *N* $NH_2OH \cdot HCl$ and 3.5 *N* NaOH were added all at once with shaking to 20 mmoles of *p*-nitrophenyl benzoate in 500 ml. of 95% ethanol at 65° and the mixture was allowed to stand for 20 minutes at room temperature. Assay of aliquots taken after 15 minutes showed that *p*-nitrophenol liberation was complete and that 15% of the theoretical amount of hydroxamic acid had been formed. The mixture was evaporated to 95 ml. under reduced pressure, 95 ml. of water was added and the mixture extracted twice with 100 ml. of ether. The ether solution was dried with sodium sulfate for 15 minutes and evaporated

(9) W. P. Jencks and F. Lipmann, *J. Biol. Chem.*, **225**, 207 (1957), and unpublished experiments.

(10) S. Hestrin, *ibid.*, **180**, 249 (1949).

(11) (a) H. Kofod, *Acta Chem. Scand.*, **11**, 5 (1957); **9**, 455 (1955); **8**, 485 (1954); **8**, 494 (1954); **7**, 274 (1953); **7**, 938 (1953). (b) The O-carbamylhydroxylamine structure for this isomer has also recently been suggested by O. Exner, *Coll. Czechoslov. Chem. Communs.*, **22**, 335 (1957), and considered by Kofod, *Acta Chem. Scand.*, **11**, 1276 (1957), on the basis of infrared absorption data.

(12) Melting points are uncorrected.

(13) A. I. Vogel, "Practical Organic Chemistry," Longmans Green & Co., London, 1956, p. 263.

(14) F. D. Chattaway, *J. Chem. Soc.*, **134**, 2495 (1931).

(15) J. H. Boyer, THIS JOURNAL, **74**, 6274 (1952).

under reduced pressure to a yellow oil which at −15° gave 4.9 g. of yellow mixed crystals that were stable at this temperature for at least a week; 1.5 g. was dissolved in 100 ml. of cold $CHCl_3$ (or ether) and extracted rapidly with 100 and 25 ml. of cold 0.2 *M* Na_2CO_3. The combined aqueous layers were washed with 50 ml. of $CHCl_3$ which was washed with fresh Na_2CO_3 solution. The combined $CHCl_3$ solutions were evaporated under reduced pressure to an oil which on crystallization from ether–petroleum ether at −78° gave 363 mg. of O-benzoylhydroxylamine as white plates recrystallized to m.p. 8°. A sample was prepared for analysis by drying over P_2O_5 for 2 days at −10°, brought to room temperature and the liquid analyzed immediately; ultraviolet spectrum: λ_{max} 230 mμ, ε 10,900, with shoulders ending at 274 mμ, ε 1,010, and 282 mμ, ε 760 in 95% ethanol.

Anal.[16] Calcd. for $C_7H_7O_2N$: C, 61.3; H, 5.2; N, 10.2. Found: C, 61.0; H, 5.3; N, 10.2.

O-Benzoyl hydroxylamine (157 mg.) in 5 ml. of methanol was treated with 0.15 ml. of acetic anhydride and 1.5 ml. of 1 *M* aqueous sodium acetate at room temperature and evaporated at reduced pressure to 0.5 ml. to give 136 mg. of product which on recrystallization from ether gave O-benzoyl-N-acetylhydroxylamine, m.p. 98–99°, no depression on mixed m.p. and identical infrared spectrum with an authentic sample.

p-Nitrobenzaldehyde (45 mg) and 1.5 ml. of acetic acid added to 42 mg. of O-benzoylhydroxylamine gave an immediate crystalline precipitate. After standing 5 minutes at room temperature the mixture was heated 10 minutes on the steam-bath, cooled, and 59 mg. of crystals was collected, m.p. 192–192.5°, no depression mixed m.p. and identical infrared spectrum with *p*-nitrobenzaldoxime benzoate. The same reactants in alcohol without acetic acid gave a crystalline product, m.p. 120°, which resolidified and then melted at 172–174° and displayed infrared carbonyl bands in $CHCl_3$ solution characteristic of the starting materials, presumably an addition or molecular complex.

O-Acetylhydroxylamine.—Two and one-half ml. of an aqueous mixture of equal parts of 4 *N* $NH_2OH \cdot HCl$ and 3.5 *N* NaOH was added rapidly with shaking to 5 mmoles of *p*-nitrophenyl acetate in 100 (or 50) ml. of absolute ethanol at 0°. The solution immediately turned bright yellow due to the liberation of *p*-nitrophenolate ion and then became almost colorless as the *p*H dropped and the anion was converted to *p*-nitrophenol. Assay indicated the presence in the reaction mixture of 0.8 mmole of acetohydroxamic acid, 3.7 mmoles of a compound which would react with hydroxylamine to form hydroxamic acid, and no diacetohydroxamic acid. The ethanol and O-acetylhydroxylamine were removed together by distillation under reduced pressure (30–40°, 15 mm.) and collected in an ice–alcohol trap. The distillate contained 2.6 mmoles of O-acetylhydroxylamine, no hydroxamic acid and no diacetohydroxamic acid and gave no yellow color with base. Ultraviolet spectroscopy showed only end absorption in the accessible region except for a small peak due to a trace of contaminating *p*-nitrophenol. Attempts to concentrate and purify this material resulted only in the formation of acetohydroxamic acid and diacetohydroxamic acid. Partial purification could also be obtained by extraction of the relatively lipid soluble product from the original reaction mixture with chloroform or ether. This product was converted to O-acetyl-N-benzoylhydroxylamine by addition of 5 mmoles of benzoyl chloride to the alcohol solution and allowing the mixture to stand for 15 minutes at room temperature. Assay indicated the presence of 2.4 mmoles of diacylhydroxamic acid (calculated with O-acetyl-N-benzoylhydroxylamine as a standard). Evaporation and crystallization gave 189 mg. of needles which were recrystallized from ether to constant m.p. 128–129°, no depression on mixed m.p. and identical infrared spectrum with O-acetyl-N-benzoylhydroxylamine from sodium benzohydroxamate and acetic anhydride.

Anal. Calcd. for $C_9H_9O_3N$: C, 60.3; H, 5.1; N, 7.8. Found: C, 60.4; H, 5.2; N, 7.6.

Treatment of an aliquot of the reaction mixture after benzoyl chloride addition with 0.1 *N* NaOH for 10 minutes at room temperature and subsequent descending chromatography of an aliquot on Whatman #1 paper developed with 4:1:5 amyl alcohol–acetic acid–water[17] indicated the presence of only benzohydroxamic acid on spraying with $FeCl_3$ solution. A similar experiment, but with the benzoylation carried out in 0.1 *M* sodium acetate buffer, gave predominantly benzohydroxamic acid, R_f 0.72, and a trace of acetohydroxamic acid, R_f 0.37 (possibly a result of the formation of some mixed anhydride of benzoic and acetic acids and acetylation to form diacetohydroxamic acid).

The ultraviolet and visible spectra of solutions of hydroxylamine and *p*-nitrophenyl acetate which had been allowed to react in phosphate buffer at *p*H 6.8 were identical to that of the same concentration of *p*-nitrophenol at the same *p*H, indicating that it is indeed free nitrophenol that is formed. When the reaction was allowed to proceed to completion in the absence of buffer, 0.43 equivalent of NaOH was required to restore the original *p*H of 6.65, indicating that no dissociated acid or base except *p*-nitrophenol (*pK* 7.0) was formed.

Decomposition.—Crystalline samples of O-benzoylhydroxylamine were allowed to come to room temperature, weighed, allowed to stand at 30° for 2 hours or heated at 100° for 10 minutes, dissolved in alcohol, and aliquots analyzed for hydroxamic acid directly and after incubation with NH_2OH or NaOH. At room temperature 45% conversion to hydroxamic acid and 11% to diacylhydroxamic acid was found after 2 hours. At 100° 88% hydroxamic acid and 3% diacylhydroxamic acid were found after 10 minutes. Only traces of hydroxamic acid and diacylhydroxamic acid were found after decomposition in dilute solution. O-Acetyl- and O-benzoylhydroxylamine were completely destroyed in 5 minutes at room temperature in 0.01 *M* NaOH. Dilute solutions of O-acylhydroxylamines were more stable in the presence of ethylenediaminetetraacetic acid, suggesting that decomposition was catalyzed by heavy metals, but this was not examined critically.

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(16) Analyses were carried out by S. Nagy and associates, Massachusetts Institute of Technology, Cambridge.

(17) A. R. Thompson, *Aust. J. Sci. Res.*, **B4**, 180 (1951).