TABLE IV

O-ALKYL BENZALDOXIMES (*syn*-V AND *anti*-IX) AND N-ALKYL-α-PHENYLNITRONES (VI)

| Alkyl | Mp or bp, °C (mm) | $n^{20}D$ | C, % | H, % | N, % |
|---|---|---|---|---|---|
| O-Methyl Va[a] | 95 (20) | 1.4578 | 70.93 | 6.73 | 10.28 found |
| IXa[b] | 86 (10) | 1.5469 | 70.93 | 6.68 | 10.20 found |
| N-Methyl VIa[c] | 82–83 | ... | 70.37 | 6.68 | 10.15 found |
| | | | 71.09 | 6.71 | 10.36 calcd |
| O-Ethyl Vb[d] | 98 (8) | 1.5369 | 72.35 | 7.53 | 9.39 found |
| IXb[e] | 98 (8) | 1.5351 | 72.33 | 7.52 | 9.60 found |
| N-Ethyl VIb | 116 (0.8) | 1.6065 | 72.70 | 7.60 | 9.22 found |
| | | | 72.46 | 7.43 | 9.39 calcd |
| O-Isopropyl Vc | 104 (8) | 1.5298 | 73.47 | 7.98 | 8.77 found |
| IXd[f] | 107 (10) | 1.5244 | 73.59 | 8.20 | 8.80 found |
| N-Isopropyl VIc | 164 (8) | 1.5868 | 73.54 | 8.17 | 8.82 found |
| | | | 73.59 | 8.03 | 8.58 calcd |
| O-Benzyl Vd[d,g] | 123 (0.5) | 1.5927 | 79.75 | 6.38 | 6.74 found |
| N-Benzyl VId[h] | 80–81 | ... | 79.29 | 6.23 | 6.42 found |
| | | | 79.59 | 6.20 | 6.63 calcd |

[a] J. Petraczek, *Chem. Ber.*, **16**, 826 (1883); J. Traube, *ibid.*, **53**, 1486 (1920). [b] Contains *ca.* 7% of the *syn* isomer. K. Auwers and B. Ottens, *Chem. Ber.*, **57**, 456 (1924). [c] H. Goldschmidt, *ibid.*, **23**, 2177 (1890); E. Beckmann, *Ann.*, **365**, 205 (1909). [d] E. Beckmann, *Chem. Ber.*, **22**, 1536 (1889). [e] Contains *ca.* 11% of the *syn* isomer. [f] Contains *ca.* 12% of the *syn* isomer. [g] P. Grammaticakis, *Compt. Rend.*, **224**, 1568 (1947). [h] E. Beckmann, *Chem. Ber.*, **22**, 435, 438 (1889).

ether (bp 30–60°) or fractional distillation under vacuum of the oils furnished the pure nitrones VIa–d in yields of about 50–80% (Table IV).

**Alkylation of *anti*-Benzaldoxime VIII in the Presence of Silver Oxide.**—To 5 g of *anti*-benzaldoxime[32] and 10 g of silver oxide, 40–45 ml of alkyl iodide was added with stirring. After the initial exothermic reaction subsided, the mixture was refluxed and stirred for an additional hour. The hot reaction mixture was filtered, the solid was washed with chloroform, and the combined filtrates were evaporated. The remaining oils were distilled under vacuum to give colorless liquids which contained predominantly the O-alkyl *anti*-benzaldoximes (IXa–c, Table IV).

**Product Analysis by Nmr.**—Reactions for product analysis (O/N ratio in Table I) were carried out on a 0.01 *M* basis (alkyl halide in 5–10% excess) in 20–25 ml of absolute ethanol. After completion of the alkylation, the solution was evaporated at room temperature[33] and the remaining product was freed of inorganic material as previously described. An aliquot of the solvent-free residue was dissolved in enough DMSO-$d_6$ to make *ca.* a 20% solution. The regions used for ratio determinations by nmr have been fivefold expanded ($H_B$ in Table II and $H_{B'}$ in Table III; in some cases, *e.g.*, methylation and benzylation, the $H_D$ or $H_{D'}$ protons can also be utilized).

**α-Phenyl-N-triphenylmethylnitrone (VIe).**—A solution was prepared from 1.21 g (0.01 mole) of *anti*-benzaldoxime[32] in 20 ml of absolute ethanol containing 0.23 g (0.01 mole) of sodium. To the stirred solution 2.78 g (0.01 mole) of triphenylmethyl chloride was added. A slightly exothermic reaction took place and a precipitate formed. The mixture was stirred for 10 min and cooled overnight. Filtration yielded 3 g of a crude white product with mp 132–134°. Recrystallization from 80% ethanol gave 2.7 g of pure product, mp 143–144° ($\lambda_{max}^{EtOH}$ 252 mμ (log ε 4.23); nmr showed two complex patterns centering around τ = 1.86 and 2.51 ppm).

*Anal.* Calcd for $C_{25}H_{21}NO$: C, 85.44; H, 6.02; N, 3.99. Found: C, 85.50; H, 5.79; N, 3.89.

**O-Triphenylmethyl Benzaldoxime (Ve). A. *syn*-Benzaldoxime** was treated the same way previously described for the formation of VIe. Filtration of the cooled reaction mixture yielded 3 g of crude white product with mp 114–117°. Recrystallization from 90% ethanol gave 2.5 g of pure product, mp 118° (lit.[11] mp 119.5–120.5° cor), $\lambda_{max}^{EtOH}$ 260 mμ (log ε 4.27); nmr showed a singlet at τ = 1.46 ppm due to CH=N and a complex pattern centering around τ = 2.62 ppm due to the $C_6H_5$ groups (hydrogen ratio 1/20, respectively).

*Anal.* Calcd for $C_{25}H_{21}NO$: C, 85.44; H, 6.02; N, 3.99. Found: C, 85.36; H, 5.80; N, 3.77.

**B. Thermal Isomerization of VIe to Ve.**—An evacuated, sealed glass tube containing 0.36 g of VIe was kept in a metal bath at 200° for 30 min. When cool, the oily product became a glassy solid. Recrystallization from ethanol–water yielded 0.34 g of Ve, mp 116–118°. A mixture melting point with Ve (obtained from procedure A) showed no depression, and the nmr and ultraviolet spectra were identical with those of Ve.

**Acknowledgment.**—I wish to acknowledge the assistance of Mr. Marvin J. Olsen with the nmr data, the advice of Dr. Robert J. Cushley in their interpretation, and the discussions with, and interest of, Dr. George Bosworth Brown.

(33) Because of the volatile nature of the O-alkyl benzaldoximes Va–c, particular care should be taken in preparation of the samples, especially during solvent removal.

# A General Synthesis of N-Hydroxyamino Acids[1a]

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A general synthesis for nitrones and N-substituted hydroxylamines has been applied to the synthesis of a series of N-hydroxyamino acids.

Several N-hydroxyamino acids have been identified in recent years as components of various antibiotics isolated from microbial fermentations. The N-hydroxyamino acids characterized from naturally occurring peptides are N-hydroxyglycine (from hadacidin),[2] N-hydroxyleucine (from pulcherrimin),[3] N-hydroxyisoleucine (from aspergillic acid),[4] N-hydroxytyrosine and -alanine (from mycelianamide),[5] as well as δ-N-hydroxyornithine (from ferrichromes[6] and albomycin)[7] and ε-N-hydroxylysine (from mysobactin).[8]

Since many of these N-hydroxy peptides have antibiotic and antitumor activities[2,9] or represent potent microbial growth factors,[10] several synthetic methods

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(5) A. J. Birch, R. A. Massy-Westropp, and R. W. Rickards, *J. Chem. Soc.*, 3717 (1956).

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(7) J. Turkova, O. Mikes, and F. Šorm, *Collection Czech. Chem. Commun.*, **27**, 591 (1962).

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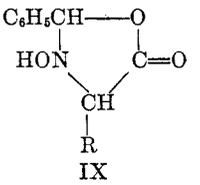
TABLE I

$C_6H_5CH$
‖
O←NCHCOOR′
|
R

| Nitrones[a] | R | R′ | Procedure | Yield, % | Mp, °C | Calcd, % C | Calcd, % H | Calcd, % N | Found, % C | Found, % H | Found, % N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IVa[b] | H | H | B | 91 | 178–179 | 60.33 | 5.06 | 7.82 | 59.98 | 5.50 | 7.59 |
| IVb | H | Et | A | 63 | 53–54 | 63.76 | 6.32 | 6.76 | 63.87 | 6.24 | 6.75 |
| V[c] | $CH_3$ | H | B | 64 | 148–149[c] | 62.17 | 5.74 | 7.25 | 62.04 | 5.80 | 7.50 |
| VI | $C_6H_5$ | Et | A | 88 | 113 | 72.06 | 6.05 | 4.94 | 71.94 | 6.09 | 4.88 |
| VIIa | $CH_2C_6H_5$ | Et | A | 70 | 122–123 | 72.71 | 6.44 | 4.71 | 72.59 | 6.44 | 4.59 |
| VIIb | $CH_2C_6H_5$ | H | A[d] | 19[d] | 170 | 71.36 | 5.61 | 5.20 | 71.40 | 5.82 | 5.13 |
| VIII | COOEt | Et | A | 54 | 114 | 60.20 | 6.14 | 5.02 | 60.38 | 6.08 | 5.13 |

[a] The nmr spectra of IV–VIII confirm the presence of the nitrone function. [The phenyl protons are split into two multiplets whose hydrogen ratio is 3:2 ($\tau$ 2.50 and 1.60); the signal of the CH=NO function appears at $\tau$ 2.00.[20]] Because of their free carboxyl group the derivatives Va and VIIb could have the alternative structure IX. [b] Reference 19a. [c] Reference 19b reports mp 167–170° (dependent upon rate of heating). [d] VIIb is a by-product during the formation of VIIa.



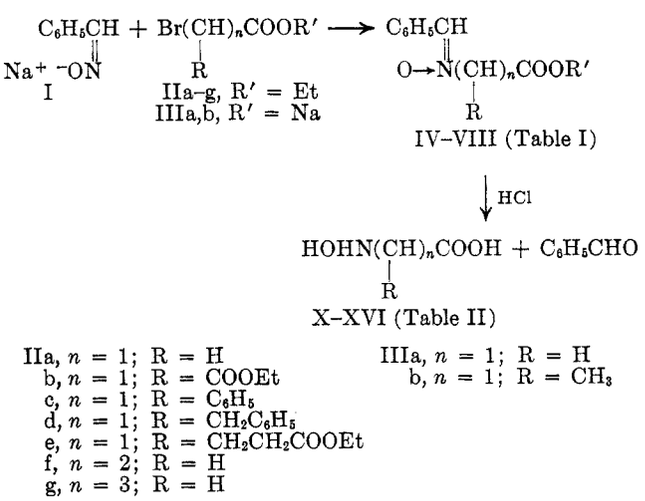
IX

have been designed for the synthesis of the constituent N-hydroxyamino acids. These methods include the reaction of nitrogen oxide with 1,3-diketo derivatives,[11] addition of HCN to aldoximes followed by hydrolysis,[11b,12] addition of hydroxylamine to $\alpha,\beta$-unsaturated carboxylic acids,[13] treatment of halogenocarboxylic acids with hydroxylamine,[14] and partial reduction of nitrocarboxylic acids.[15] In spite of the variety of routes reported, the N-hydroxyamino acids remained difficult to obtain[16] because of poor yields and limited applicability of each of the methods.[16–18] N-Hydroxyglycine was prepared in 1896 from the nitrone obtained from $\alpha$-chloroacetic acid and a "solid benzaldoxime."[19a] There has been one subsequent attempt to apply this reaction, but only the nitrone, and not the N-hydroxyamino acid, was characterized.[19b]

We present here a general synthesis of N-hydroxyamino acids, based upon the selective N-alkylation of sodium antibenzaldoximate described in the preceding paper.[20] The approach involves a general synthesis of nitrones, and subsequent hydrolysis to N-substituted hydroxylamines,[21] of which N-hydroxyamino acids are of special interest.

The reaction of ethyl bromocarboxylates (IIa–g) with the *anti*-benzaldoxime anion (I) (Scheme I) proceeds smoothly in ethanol at room temperature (procedure A) to the corresponding N-substituted $\alpha$-phenylnitrones (IVb, VI–VIII). Most of those can be isolated easily and in good yields (Table I).

SCHEME I




IIa, $n$ = 1; R = H
b, $n$ = 1; R = COOEt
c, $n$ = 1; R = $C_6H_5$
d, $n$ = 1; R = $CH_2C_6H_5$
e, $n$ = 1; R = $CH_2CH_2COOEt$
f, $n$ = 2; R = H
g, $n$ = 3; R = H

IIIa, $n$ = 1; R = H
b, $n$ = 1; R = $CH_3$

The nitrones (Table I) prepared from IIe–g were not isolated but were hydrolyzed directly (procedure D) to the corresponding N-hydroxyamino acids (XIV–XVI).

In contrast to the esters, the sodium salts of the bromocarboxylic acids (IIIa,b) were less reactive and require elevated temperatures for complete reaction (procedure B). Nitrone formation is considerably reduced with aqueous solvents[19] and sodium $\alpha$-chlorocarboxylates (*e.g.*, sodium chloroacetate).[19a] The sodium salts of the bromocarboxylic acids corresponding to the esters IIb–g rapidly undergo the expected solvolytic displacement of the bromide,[22] owing to the

(11) (a) W. Traube, *Chem. Ber.*, **27**, 1507 (1894); W. Traube, *ibid.*, **28**, 1785, 2297 (1895); (b) L. Neelakantan and W. Hartung, *J. Org. Chem.*, **23**, 964 (1958).

(12) L. W. Kissinger and H. E. Ungnade, *ibid.*, **25**, 1471 (1960); E. F. J. Duynstee and J. P. Hennekens, *Rec. Trav. Chim.*, **84**, 1442 (1965).

(13) (a) T. Posner, *Ann.*, **389**, 120 (1912); (b) N. Grossowicz and Y. Lichtenstein, *Nature*, **191**, 412 (1961); (c) T. T. Emery, *Biochemistry*, **2**, 1041 (1963).

(14) (a) A. H. Cook and C. A. Slater, *J. Chem. Soc.*, 4130 (1956); (b) G. C. Lancini, A. Diena, and E. Lazzari, *Tetrahedron Letters*, 1769 (1966).

(15) (a) J. B. Neilands and P. Azari, *Acta Chem. Scand.*, **17**, 190 (1963); (b) S. Rogers and J. B. Neilands, *Biochemistry*, **2**, 6 (1963).

(16) A. Chimiak, *Wiadomosci Chem.*, **19**, 803 (1965); *Chem. Abstr.*, **64**, 4881*a* (1966).

(17) The addition of HCN to oximes and the subsequent hydrolysis to $\alpha$-N-hydroxyamino acids[11b,12] is the best of the available procedures. Unfortunately, this method seems to be limited to aliphatic oximes.[18]

(18) E. Buehler, unpublished results.

(19) (a) A. Hantzsch and W. Wild, *Ann.*, **289**, 285 (1896); (b) C. D. Hurd and J. M. Longfellow, *J. Am. Chem. Soc.*, **73**, 2395 (1951).

(20) E. Buehler, *J. Org. Chem.*, **31**, 261 (1966).

(21) H. Lindemann and K. T. Tschang, *Chem. Ber.*, **60**, 1726 (1927); O. Exner, *Collection Czech. Chem. Comm.*, **16**, 258 (1951).

(22) A. Streitwieser, Jr., Solvolytic Displacement Reactions, Series in Advanced Chemistry, McGraw-Hill Book Co., Inc., New York, N. Y., 1962.

TABLE II

$HONH(CH)_nCOOH$ (R)

| N-Hydroxyamino acids | n | R | Procedure | Time, min | Yield, % | Mp, °C[a] | Calcd, % C | Calcd, % H | Calcd, % N | Found, % C | Found, % H | Found, % N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| X[b] | 1 | H | C | 4 | 74 | 139 | 26.38 | 5.53 | 15.38 | 26.60 | 5.56 | 15.48 |
| XI[c] | 1 | $CH_3$ | C | 5 | 59 | 146–147[c] | 34.29 | 6.71 | 13.34 | 34.53 | 6.73 | 13.18 |
| XII | 1 | $C_6H_5$ | C | 15 | 45 | 133 | 57.48 | 5.43 | 8.38 | 57.46 | 5.40 | 8.26 |
| XIII[d] | 1 | $CH_2C_6H_5$ | C | 15 | 51 | 156–157 | 59.69 | 6.12 | 7.73 | 59.89 | 6.25 | 7.75 |
| XIV | 1 | $CH_2CH_2COOH$ | D | 25[e] | 38[f] | 138 | 36.81 | 5.56 | 8.59 | 36.53 | 5.45 | 8.30 |
| XV | 2 | H | D | 20 | 62[f] | 108–109 | 34.29 | 6.71 | 13.34 | 34.46 | 6.76 | 13.11 |
| XVI | 3 | H | D | 10 | 53[f] | 114–115 | 40.33 | 7.62 | 11.76 | 40.26 | 7.64 | 11.71 |

[a] All derivatives melt under decomposition. [b] References 2 and 11a. [c] For N-Hydroxyalanine (XI) a decomposition point of 194–195° is reported.[11b] We have repeated this procedure and obtained a product identical with XI with mp 145–147°. [d] Reference 11b. [e] A decrease in the time of hydrolysis to 15 min for the formation of XIV provided a monoethyl N-hydroxyglutamate (XIVa) in a yield of 35%, mp 140–141°. *Anal.* Calcd for $C_7H_{13}NO_5$: C, 43.98; H, 6.85; N, 7.33. Found: C, 44.38; H, 6.90; N, 7.19. [f] Over-all yields for the N-alkylation and hydrolysis.

neighboring carboxylate, and fail to yield the corresponding nitrones.[23]

The use of the nitrone as an intermediate offers considerable advantages for the synthesis of N-hydroxyamino acids (Table II) particularly in comparison with the direct nucleophilic displacement of α-halogenocarboxylic acids with hydroxylamine.[14,19a] Since hydroxylamine can act both as a nucleophilic and as an oxidizing agent[24] the proportion of N-hydroxyamino acid formation is decreased sharply and the α-oximino derivatives are obtained instead. With the hydroxylamine incorporated into an oxime, this undesirable side reaction is completely eliminated. The N-hydroxyamino acids (X–XVI) are easily obtained by acid cleavage of the nitrones (procedure C or D), followed by neutralization to the approximate isoelectric point. The switterion character[11b] is confirmed by the presence of a carboxylic absorption in the infrared spectra ($OCO^-$ 1550–1600 $cm^{-1}$). The immediate reduction of Fehling's solution at room temperature provides a convenient test for detection of N-hydroxyamino acids in the reaction mixtures.

Catalytic reductions of X–XVI yielded the corresponding amino acids.

## Experimental Section

Melting points are uncorrected. The nuclear magnetic resonance (nmr) spectra were obtained on a Varian Model A-60 spectrometer in DMSO-$d_6$ (internal standard tetramethylsilane). The infrared spectra were carried out on a Perkin-Elmer Infracord spectrophotometer. The analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

**Preparation of Nitrones. Procedure A.**—A solution was prepared from 0.46 g (0.02 mole) of sodium, 2.42 g (0.02 mole) of *anti*-benzaldoxime,[20,25,26] and 40 ml of absolute ethanol. To the magnetically stirred solution 0.022 mole of the ethyl bromocarboxylate was added in one portion and the stirring was continued until a wet pH paper showed pH 7. The reaction mixture was filtered and the remaining solid was extracted twice with $CHCl_3$.[27] The combined filtrates were evaporated and the residue was taken up in ether and cooled overnight. The crystals obtained were essentially pure. For analysis (Table I) the samples were recrystallized from petroleum ether (bp 30–60°).

**Procedure B.**—A solution was prepared from 0.46 g (0.02 mole) of sodium, 1.21 g (0.01 mole) of *anti*-benzaldoxime, and 40 ml of absolute ethanol. To the clear solution was added in one portion 0.011 mole of the α-bromocarboxylic acid. The reaction mixture was stirred at *ca.* 70° until a wet paper showed pH 7. The solvent was evaporated and the remaining solid was taken up in little water, cooled, and acidified with 10–15 ml of 1 *N* hydrochloric acid. The white crystals were collected. For analyses the samples were recrystallized from ethanol and water (Table I).

**Hydrolysis to N-Hydroxyamino Acids. Procedure C.**—A solution of 1.5 g of the nitrone in 15–20 ml of concentrated hydrochloric acid was refluxed and stirred at 100–105° for various lengths of time (Table II). The reaction mixture was evaporated to dryness and the remaining solid was dissolved in a little water.[28] The pH of the solution was adjusted to pH 5.5–6.0 by dropwise addition of concentrated aqueous ammonia solution and then cooled. The white crystals were collected, washed with cold ethanol, and recrystallized from ethanol and water (Table II).

**Procedure D.**—Sodium *anti*-benzaldoxime (I) and the ethyl bromocarboxylates (IIe–g) were condensed as described in procedure A. The reaction mixture was evaporated and the residue was refluxed and stirred in 35–40 ml of concentrated hydrochloric acid at 100° for various lengths of time (Table II). After evaporation of the aqueous hydrochloric acid, the residue was taken up in a little water and the pH of the solution was adjusted to 5.5–6.0.[29] If after cooling of the neutralized solution a precipitate formed, further work-up was carried out according to procedure C (*e.g.*, XIV and XIVa) (see footnote *e* in Table II).

With XV and XVI the solution was evaporated to complete dryness and the remaining solid was extracted with boiling absolute ethanol until Fehling's test with the undissolved material was negative. The combined ethanolic filtrates were evaporated to a volume of about 40 ml and cooled. The white crystals were recrystallized from ethanol and water.

**Catalytic Reduction of N-Hydroxyamino Acids.**—A suspension of 200 mg of the N-hydroxyamino acid and 50 mg of $PtO_2$ in 50 ml of water was shaken in hydrogen at room temperature and 1 atm of pressure. After the hydrogen uptake subsided (2–4 hr), the catalyst was collected[30] and the filtrate was concentrated until a white solid formed. The crystals were collected, recrystallized, and compared by mixture melting points and/or infrared spectrum with authentic DL-amino acids.

**Acknowledgment.**—The authors wish to express their appreciation to Mr. Alex Sele for his capable assistance.

(23) The disodium bromomalonate corresponding to IIb decarboxylates and nitrone IVa was isolated.

(24) Here, $NH_2OH$ is behaving as the amide of HOOH.

(25) A. I. Vogel, "Practical Organic Chemistry," 3rd ed, Longmans, London, p 719.

(26) A. Sele and E. Buehler, submitted for publication.

(27) In the reaction between I and IId the remaining solid was dissolved in water and acidified with hydrochloric acid, which provided VIIb in 21% yield. Recrystallization was from ethanol and water.

(28) At that point the $C_6H_5CHO$ which remains undissolved can be either extracted with ether (*e.g.*, in the isolation of X) or brought into solution by addition of a little ethanol.

(29) For the isolation of N-hydroxyglutamic acid adjustment of the pH to 3 is sufficient.

(30) Water-insoluble amino acids remained with the catalyst and were extracted by boiling ethanol–water (1:1).