[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE SCHOOL OF PHARMACY, UNIVERSITY OF MARYLAND]

PEPTIDES *VIA* AMIDES OF α-BENZYLOXIMINO ACIDS[1a]

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The convenient preparation of oximino acids in good yields and their ready hydrogenation to the corresponding amino acids (1, 2, 3) has led to the investigation of the possible use of these compounds as intermediates in the synthesis of peptides. Schemin and Herbst (4) were able to reduce the oximes of several oximino acid amides to the corresponding dipeptides. However, their route to the intermediates leaves much to be desired.

Waters and Hartung (5) found that the chlorides of oximino acids themselves are not directly accessible. The α-alkoximino acids, however, lend themselves admirably for the preparation of acid chlorides, from which amides may be synthesized. Amides of α-amino acids suggest the possibility for a new route to the synthesis of peptides, thus:

$$\underset{\underset{\text{NOR''}}{\|}}{\text{RCCOCl}} + \text{H}_2\text{NCHR'COOH} \rightarrow \underset{\underset{\text{NOR''}}{\|}}{\text{RCCONHCHR'COOH}} \xrightarrow{\text{H}_2} \underset{\underset{\text{NH}_2}{|}}{\text{RCCONHCHR'COOH}}$$

I II

The O-alkyl ethers of oximes have received some study and, in particular, Adkins and Reeve (6) have shown that ethyl O-ethyloximinoacetoacetate was reduced by Raney nickel catalyst to the desired threonine. Hydrogenolysis of benzyl ethers proceeds at room temperature and lower pressures (7). Therefore, O-benzyl ethers of oximino acids (I) should be easily cleaved by hydrogenolytic procedures, with subsequent further reduction of the resulting oximino group to the amino group (II).

The benzylation of oximino acids has been reported by Waters and Hartung (5). In the course of the present work, it was observed that yields are improved if benzylation is conducted in an alcoholic medium rather than in aqueous acetone. Four α-oximino acids were benzylated in this way and their properties are given in Table I. They are low-melting, white crystals when obtained from ethanol and water.

Waters observed some difficulty in the preparation of the acid chlorides of the α-alkoximino acids using thionyl chloride. Even though redistilled purified thionyl chloride was refluxed with the acids, the reaction often did not go to completion. It is now found that with thionyl chloride and phosphorus pentachloride the acid chlorides are more consistently obtained in good yields than with either reagent alone. More than theoretical quantities of phosphorus pentachloride give a yellowish discoloration to the products.

[1a] Paper No. 9 on amino acids; for No. 8 see Barry, Mattocks, and Hartung, *J. Am. Chem. Soc.*, **70**, 693 (1948). [b] Present address: Medical College of Virginia, Richmond 19, Virginia. [c] Present address: University of North Carolina, Chapel Hill, North Carolina.

The benzyloximino acid chlorides, when pure, are colorless, rather high-boiling liquids. The acid chlorides of lower molecular weight possess peculiar, pungent, straw-like odors. They boil under reduced pressures without appreciable decomposition, but slowly turn dark on storage. Residues of the acid chlorides turned to red solids when stored for several months in glass-stoppered bottles. When the acid chlorides are poured into water they do not generate heat and react rather slowly. No attempt was made to analyse them, but they were characterized through their anilides.

The α-benzyloximino acid anilides were not prepared by the two-phase method, used with the amino acids, because of the availability of aniline to serve as a base

TABLE I

α-BENZYLOXIMINO ACIDS, ACID CHLORIDES, AND ANILIDES

$$RC(=NOCH_2C_6H_5)COR'$$

R	R′	YIELD, %	M.P., °C.[a]	B.P., °C./MM.	N, %[d]	
					Calc'd	Found
$C_6H_5CH_2$—	OH	66	78–79[b]			
CH_3—	OH	57	73–75		7.26	7.07
CH_3CH_2—	OH	45	86		6.79	6.92
$(CH_3)_2CHCH_2$—	OH	79	79–80		5.95	6.23
$C_6H_5CH_2$—	Cl	84		164/.7		
CH_3—	Cl	45		95–100/.75		
CH_3CH_2—	Cl	80		105–107/.3		
$(CH_3)_2CHCH_2$—	Cl	67		117–118/.85		
$C_6H_5CH_2$—	NHC_6H_5	81	72–73[c]			
CH_3—	NHC_6H_5	84	70–71		10.44	10.75
CH_3CH_2—	NHC_6H_5	84	71		9.93	10.33
$(CH_3)_2CHCH_2$—	NHC_6H_5	77	57		9.03	9.15

[a] Melting points on calibrated block. [b] Waters (5) reported 79–80°. [c] Waters (5) reported 73.5–74°. [d] Micro-analyses by Oakwold Laboratories, Alexandria, Virginia.

and of the ease of separation of aniline hydrochloride from the anilide produced. All the anilides are white solids melting below 100°. They are sometimes difficult to crystallize, cooling and stirring being necessary to induce crystallization.

Coupling amino acids with the α-benzyloximino acid chlorides was accomplished in a two-phase system of ether and aqueous alkali, similar to the procedure of McKie (8). Such a method saves valuable intermediates and allows easy isolation of the reaction product. Furthermore, acid sensitive substances such as tryptophane may be acylated.

The amides so obtained are white solids, usually melting around 100°. Often some difficulty is encountered in inducing crystallization; the products frequently separate as oils. The amides of lower molecular weight are slightly soluble in water, but the higher amides may be crystallized, as a rule, almost quantitatively from ethanol and water. The variation in the melting points of the amides

suggests the possible use of the acid chlorides as reagents for identification of amino acids. Constants for the anilides and amides of the amino acids are found in Tables I and II.

Reduction of the α-benzyloximino acid amides is expected to provide the desired dipeptides. In the course of this work, the reduction of β-phenyl-α-benzyloximinopropionylalanine was studied in an attempt to prepare phenylalanylalanine. Conventional procedures using palladium catalysts with the amide in 95% ethanol and added hydrochloric acid yield the diketopiperazine in small amounts.

TABLE II

α-BENZYLOXIMINO AMIDES RCCONHCHR'COOH
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NOCH$_2$C$_6$H$_5$

R	R'	YIELD, %	M.P., °C.[a]	N, %[b]	
				Calc'd	Found
$C_6H_5CH_2$—	H—	90–98	96.5–97	8.59	8.70
$C_6H_5CH_2$—	CH_3—	91	112	8.23	8.46
$C_6H_5CH_2$—	L-(+)$(CH_3)_2CHCH_2$—	77	86–87	7.33	7.10
$C_6H_5CH_2$—	$C_6H_5CH_2$—	85	140–141	6.73	6.97
$C_6H_5CH_2$—	L-(+)$HOOCCH_2CH_2$—	58	110	7.03	6.90
CH_3—	H—	60	127	11.20	11.39
CH_3—	CH_3—	54	118	10.14	10.43
$(CH_3)_2CHCH_2$—	H—	45	58–59	9.56	8.25
$(CH_3)_2CHCH_2$—	CH_3—	94	70	9.15	8.81
$(CH_3)_2CHCH_2$—	$C_6H_5CH_2$—	73	116–116.5	7.33	7.33
$(CH_3)_2CHCH_2$—	$(CH_3)_2CHCH_2$—	92	45–46	8.09	8.25
$(CH_3)_2CHCH_2$—	$C_8H_6NCH_2$—	86	90	9.97	10.43
$(CH_3)_2CHCH_2$—	L-(+)$HOOCCH_2CH_2$—	74	101	7.69	7.41
CH_3CH_2—	H—	90	106	10.60	10.64
CH_3CH_2—	CH_3—	84	94	10.06	10.31
CH_3CH_2—	$(CH_3)_2CHCH_2$—	77	87	8.75	8.39
CH_3CH_2—	$C_6H_5CH_2$—	82	89	7.91	8.17
CH_3CH_2—	L-(+)$HOOCCH_2CH_2$—	52	92–93	8.33	7.89

[a] Melting points on calibrated block. [b] Micro-analyses by Oakwold Laboratories, Alexandria, Va.

When a mixed palladium-platinum catalyst was used under similar conditions, significant amounts of diketopiperazine were isolated. Similar results were obtained with several other amides. Considerable alcohol-soluble material was found in the course of isolation of the reduction products. Consequently, the possibility of ester formation with subsequent cyclization to the diketopiperazine may be suspected, a well known mechanism for the formation of peptide anhydrides (9). Preliminary studies indicate that water added to the reduction mixture suppresses diketopiperazine formation and increases the yield of dipeptides. Further work is being carried out in an effort to complete this reduction to the dipeptide more successfully.

EXPERIMENTAL[2]

Reagents. Many of the chemicals used in this work were purchased from various sources and were used without further purification. Some of the malonic esters, glycine, DL-alanine, L-(−)-leucine, DL-leucine, and L-(−)-tryptophane were purchased from the Eastman Kodak Company. Thionyl chloride and benzyl chloride were redistilled. Ethyl benzylmalonate was prepared in 66–67% yields in a modification of Marvel's preparation (10) by using three moles of ethyl malonate to two moles of benzyl chloride instead of equal molar quantities.

The oximino acids were prepared by the alkaline nitrosation of the substituted malonic esters as reported by Barry (2), with some modifications. The following preparation is exemplary of the type.

β-Phenyl-α-oximinopropionic acid. Sodium (11.5 g., 0.5 mole) was added to 1 liter of absolute alcohol contained in a 2-liter, 3-necked, round-bottomed flask equipped with a condenser, drying tube, stirrer, and dropping-funnel. After solution of the sodium 125 g. (0.5 mole) of ethyl benzylmalonate was added to the hot mixture, which was then cooled to 0° in an ice-salt bath. Then 103 g. (1 mole) of butyl nitrite was added slowly beneath the surface of the mixture over a period of about one hour while keeping the temperature below 5°. The cooling bath was removed, the mixture was allowed to reach room temperature slowly (one hour), and then was heated to reflux. Suction was applied to remove the butanol and ethanol until a volume of about 200 ml. remained. Then 600 ml. of ice and water was added, the product was acidified with hydrochloric acid, and extracted with ether. The ether extract was extracted with 10% sodium hydroxide, which was then heated on the steam-bath for about one hour. After adding ice, the mixture was acidified with concentrated hydrochloric acid and the precipitate which formed was dried *in vacuo* over phosphorus pentoxide. Yield, 95% of light-tan solid melting at 160°. Barry reported 169° for the recrystallized product. Materials of the purity obtained here were adequate for further benzylation.

Benzylation. The preparation of β-phenyl-α-benzyloximinopropionic acid is illustrative of the most satisfactory method found.

In a 1-liter apparatus of the previous type was placed 500 ml. of commercial absolute alcohol and 11.5 g. (0.5 mole) of freshly cut sodium. After the sodium had reacted, 45 g. (0.25 mole) of finely divided β-phenyl-α-oximinopropionic acid was added. Then to the hot solution, 64 g. (0.5 mole) of benzyl chloride was added all at once and the mixture was refluxed for two hours, or until it became neutral. Then 100 ml. of 20% potassium hydroxide in 95% ethanol was added and about 400 ml. of alcohol was distilled out of the mixture. Water and enough hydrochloric acid to make the solution acid were added and the mixture was extracted with ether. The ether along with a good portion of the benzyl alcohol was removed *in vacuo*. The dry residue in the flask was then dissolved in ethanol and water and allowed to crystallize. Usually a quantitative yield of brown solid was obtained. When this material was recrystallized from ethanol-water and decolorized with Nuchar, the yield of solid was 43.5 g. (66%), melting at 78–79°. Waters (5) reported 79–80°.

The other benzylated oximino acids shown in Table I were prepared similarly and recrystallized from ethanol and water. This method was used in preference to Waters' original aqueous-acetone system. The latter method always yielded significant amounts of unreacted oxime which interfered with the isolation of the product. In addition, separation of the benzyl ether from the excess benzyl alcohol encountered in this preparation is not easy. Modifications of reaction temperatures, amount of alkali and benzyl chloride, and reflux times in the acetone-water system did not improve yields over Waters' adopted procedure. Benzylation with benzylphenyldimethylammonium chloride (11) was unsuccessful.

Acid chlorides. The acid chlorides were most economically prepared using a molar equivalent of phosphorus pentachloride with added thionyl chloride. The following preparation of β-phenyl-α-benzyloximinopropionyl chloride was general for these acid chlorides.

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[2] Experimental work carried out at the Naval Research Laboratory, Washington 25, D. C.

To 26.9 g. (0.1 mole) of β-phenyl-α-benzyloximinopropionic acid in 200 ml. of benzene was added 20.8 g. (0.1 mole) of phosphorus pentachloride, in several portions with agitation, and finally a few ml. of thionyl chloride. The mixture was then refluxed for one-half hour. On distillation, 24.1 g. (84%) of product boiling at 164°/0.7 mm. was obtained. The boiling points of the acid chlorides are given in Table I.

Anilides. The preparation of β-phenyl-α-benzyloximinopropionanilide, which follows, is general for this group.

To 1.9 g. (0.02 mole) of aniline and 20 ml. of dry benzene in a small beaker was added, with continuous stirring, 2.9 g. (0.01 mole) of β-phenyl-α-benzyloximinopropionyl chloride. During the addition, a light yellow precipitate formed and the contents of the beaker became warm. The mixture was allowed to stand several hours, the aniline hydrochloride was filtered off, and the benzene evaporated from the filtrate on the steam-bath. The yellow oil remaining was taken up in ethanol and water and allowed to cool. An oil separated which after further cooling crystallized. The crystals were dried *in vacuo* over phosphorus pentoxide. The anilide melted at 72–73°; yield, 2.8 g. (81%).

The properties of the various anilides are given in Table I.

Amides. The preparation of β-phenyl-α-benzyloximinopropionylglycine which follows is rather general for this group.

Glycine, 0.75 g. (0.01 mole), was dissolved in 4 ml. of 10% sodium hydroxide (0.01 mole) and 6 ml. of water, and the solution was overlayered with 10 ml. of ether. Then, while stirring, 2.9 g. (0.01 mole) of β-phenyl-α-benzyloximinopropionyl chloride in 10 ml. of absolute ether was added alternately with 4 ml. of 10% sodium hydroxide (0.01 mole) in 6 ml. of water. A little heat was developed in the reaction flask. The mixture was then allowed to stand in the refrigerator overnight. The lower alkaline layer was neutralized with a small amount of concentrated hydrochloric acid. The oil which separated crystallized on cooling. After drying *in vacuo* over phosphorus pentoxide, 2.95 g. (90%) of white solid was obtained. After recrystallization from ethanol and water, the product melted at 96.5–97°. In larger runs, the yields ranged up to 98%.

For all the glutamic acid derivatives, an extra equivalent of alkali was used. The glutamic acid derivatives were in general very much more soluble in water than those of the monocarboxylic acids.

The α-benzyloximinoisocaproamides were recrystallized from water or very dilute ethanol. In general, this series was very difficult to crystallize, and repeated stirring and cooling were necessary to induce crystal formation.

Several acid chlorides were allowed to react with L-(−)-tyrosine, but low analyses indicated that the pure desired monoacylated product was not obtained. In the preparation of the tryptophane derivative, care was taken to avoid excess acid chloride. This derivative had a tendency to fluff and gel which may account for the slightly high analysis shown in Table II. The amides from α-benzyloximinobutyryl chloride and the amino acids were characteristic in that they rarely separated from the crystallizing medium as oils.

Hydrogenation. The reductions of the acid amides, for the most part, were carried out at room temperature and 20 atmospheres pressure in a glass-lined vessel of such capacity that 0.01 mole of hydrogen gave approximately a 10–12 pound pressure drop, depending on the slight variations in amount of solvent used in the hydrogenations. The palladium catalyst was prepared by the method of Hartung (12) and the platinum catalyst was that of Adams (13).

β-Phenyl-α-benzyloximinopropionylalanine (0.15 mole) was dissolved in 100 ml. of 95% ethanol and 5 ml. of concentrated hydrochloric acid. After addition of 5 g. of palladium catalyst, another 0.5 g. of palladium chloride was added and the mixture was shaken at room temperature under 20 atmospheres of hydrogen. A definite odor of toluene was noted when the bomb was opened after hydrogen uptake was completed. The catalyst was filtered off, the filtrate was neutralized with sodium hydroxide, and then evaporated on the steam-bath. The residue was taken up in water, and alcohol added. A very small quantity of material melting at 268–270° precipitated. The diketopiperazine melts at 267–268° (14).

Anal. Calc'd for $C_{12}H_{14}N_2O_4$: C, 66.03; H, 6.47; N, 12.84.
Found: C, 65.78; H, 6.53; N, 12.86.

Use of an unfortified catalyst was unsuccessful and heating did not help in any way. Use of acetic acid, in place of hydrochloric acid, with an unfortified catalyst was also of no avail.

Since the fortified catalyst caused hydrogen to be taken up, a 40% palladium catalyst was used and the theoretical amount of hydrogen was absorbed. After removing the catalyst, the filtrate was dried *in vacuo* and ammonium hydroxide was added until neutrality was reached. After concentration, alcohol was added. Some of the precipitate which formed dissolved in water. The remainder was insoluble in alkali and acid, indicating anhydride formation.

When 0.015 mole of amide was reduced with 5 g. of 10% palladium catalyst and 0.15 g. of added platinum oxide in 95% ethanol and 5 ml. of concentrated hydrochloric acid, the theoretical amount of hydrogen was used. After neutralization with ammonium hydroxide, evaporation and washing with ethanol, a product was obtained which melted at 240°. The dipeptide melts at 241° (15). However, analyses showed impurities, probably due to diketopiperazine. The difficulties encountered here show how unreliable melting point information may be with products of this type.

In most of the reductions considerable alcohol-soluble material formed, indicating some product other than the diketopiperazine or the dipeptide.

When the amide was reduced in ethanol with added water and hydrochloric acid, considerable product, soluble in both acid and alkali was isolated, indicating more dipeptide formation than previously found. Addition of water, then, may aid in dipeptide formation at the expense of some other compound, possibly the ethyl ester of the amides.

The reductions did show that the benzyl group is readily removed by catalytic hydrogenation. However, the subsequent course of events and the influence of solvent require further study.

SUMMARY

1. An improved method for the preparation of certain α-benzyloximino acids is described.

2. The preparation of the acid chlorides of these acids has been carried out in good yield.

3. These acid chlorides may be condensed with a variety of amino acids, usually in good yield. The variation in melting point of the amides suggests possible use as amino acid derivatives for identification purposes.

4. A study of the reduction of these amides has shown that the benzyl group is readily removed by platinum-palladium catalysts at room temperatures and low pressures. The reaction products isolated indicate the oximes are reduced to the free amines in ethanol and aqueous ethanol with added hydrochloric acid. The isolation of diketopiperazines from these reduction mixtures suggests either the possible formation of ethyl esters or that an exceptionally easy method for anhydridization has been uncovered.

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