748. *Amido-oxy-peptides.* NO-*Peptides of Amino-oxyacetic Acid and of Canaline.*

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Peptide-like condensates of amino- and amino-oxy-acids containing the amido-oxy-linkage present in the antibiotic cycloserine, are described. Methods for the preparation of amido-oxy-peptides are developed.

Hydrogenolysis, hydrolysis, and alcoholysis of, and certain chemical and physical properties associated with the amido-oxy-peptide link are studied.

In a preliminary Note [1] we have reported the preparation of amido-oxy-peptides containing the amido-oxy-linkage $-NH\cdot CH(R)\cdot CO\cdot NH\cdot O\cdot CH(R)\cdot CO_2H$, between an amino- and an amino-oxy-acid, analogous to the amide link of the normal peptides. In this Paper, synthetic methods to obtain amido-oxy-peptides and related compounds are developed and some chemical and physical properties of these compounds are studied. The amido-oxy-peptides investigated here are condensation products of α-amino-acids with α-amino-oxyacetic acid and with the γ-amino-oxy-group of canaline (α-amino-γ-amino-oxybutyric acid). In addition, related peptide-like condensates of amino-oxy-acids are described.

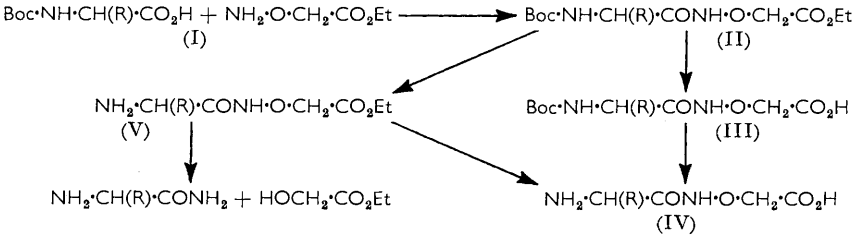
An internal amido-oxy-link formed by an α-amino-acid appears in cycloserine,[2] an *NO*-lactamic form of the lower homologue of canaline, and the amido-oxy-peptides reported here can be considered as linear analogues of cycloserine. The amido-oxy-peptides, as water soluble and easy hydrolysable acylic derivatives of amino-oxyacetic acid, may be of interest as possible competitive inhibitors of γ-aminobutyric acid–ketoglutaric acid transaminase.[3]

[1] Frankel, Knobler, and Zvilichovsky, *Tetrahedron Letters*, 1960, No. 18, 28.

[2] Kuehl, Wolf, Trenner, Peck, Howe, Hunnewel, Dowing, Newstead, Buhs, Putter, Ormond, Lyons, Chaiet, and Folkers, *J. Amer. Chem. Soc.*, 1955, **77**, 2344; Hidy, Hodge, Young, Harned, Brewer, Runge, Stanley, Pohland, Boaz, and Sullivan, *ibid.*, p. 2345.

[3] Wallach, *Biochem. Pharmacol.*, 1960, **5**, 323; Schumann, Paquette, Heinzelman, Wallach, DaVanzo, and Greig, *J. Medicin. Pharmaceut. Chem.*, 1962, **5**, 464.

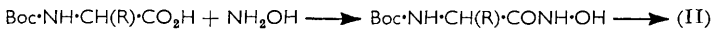
For coupling, the use of chlorides of *N*-protected amino-acids, the mixed anhydride method, and the *NN'*-dicyclohexylcarbodi-imide procedure [4] were tried, but in practice the dicyclohexylcarbodi-imide method was employed as the most effective. α-Benzyloxycarbonylamino-acids were used as acylating components with ethyl amino-oxyacetate (I) as the base. The *N*-protected amido-oxy-esters (II) were obtained in 60—70% yield. The esters (II) were converted to the *N*-protected amido-oxy-acids (III) in 80–90% yield by mild alkaline hydrolysis, to which the *NO*-bond proved stable.



Boc = Ph·CH_2·O·CO; (a) R = Me; (b) R = CH_2·Ph.

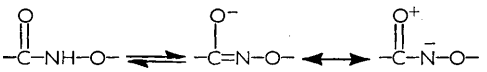
In addition, trifluoroacetyl-amino-acids were coupled with ethyl amino-oxyacetate by the dicyclohexylcarbodi-imide method, giving trifluoroacetylamido-oxy-esters. Treatment of these esters with sodium hydroxide led to the amido-oxy-acids (IV).

Another route to amido-oxy-peptides involved alkylation of an α-aminohydroxamic acid by condensing ethyl α-bromoacetate with an α-benzyloxycarbonylaminohydroxamic acid.



Benzyloxycarbonylamino-amido-oxy-esters (II) thus prepared were identical with the esters made from the amino-oxy-ester (I); they proved very hard to purify and to solidify.

Catalytic hydrogenation cleaves *O*-alkyl ethers of hydroxylamine to the alcohol and ammonia, and canaline was degraded by this method.[5] Catalytic hydrogenolysis of amido-oxy-peptides, both to remove the protecting benzyloxycarbonyl group and to reduce the amido-oxy-peptide to an amino-acid amide and glycolic acid, was carried out in a stepwise manner: it enabled debenzyloxycarbonylation to be carried out without cleavage of the amido-oxy-bond. Removal of the benzyloxycarbonyl group from the amido-oxy-acids (III) required a hydrogenation period of about 4 hours, and the deacylated amido-oxy-acids (IV) were sufficiently stable to resist further hydrogenation. With the esters (II), the benzyloxycarbonyl group was removed much faster (45 min.) and the resulting amido-oxy-esters (V) underwent scission after 4—6 hours to the amide and ethyl glycollate. The behaviour of the esters (II) towards hydrogenation resembled that of cycloserine. The more stable amido-oxy-acids (IV), contain the amido-oxy-group in an uncharged form and behave similarly to the acylamino-oxy-esters ethyl benzamido-oxyacetate (VI) and ethyl α-benzamido-γ-benzamido-oxybutyrate. Both the ester (VI) and ethyl α-benzamido-γ-benzamido-oxybutyrate are resistent to reduction under the same conditions; hydrogenolysis was achieved only after the addition of triethylamine. On the basis of the above findings, the behaviour of the acyl and peptidic derivatives of amido-oxy-compounds leads to the generalization that the ease of hydrogenolysis of the amido-oxy-linkage depends on the amount of enol ion present.



The amido-oxy-group of the ester (V) is charged, forming an internal salt with the adjacent α-amino-group, and is thus easily reducible. In the amido-oxy-acid (IV) both

[4] Sheehan and Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.
[5] Kitagawa and Monobe, *J. Biochem.* (*Japan*), 1933, **18**, 333.

carboxylic and amino-groups participate in internal salt formation, the amido-oxy-group remains uncharged, and the stability of the compound to hydrogenolysis is enhanced. The same effect may be brought about by elimination of the enolic charge of the amido-oxy-group of the ester (V) by addition of hydrochloric acid: the external salt formed is much more resistant to hydrogenation.

The ampholytic character of the amido-oxy-esters (V) and acids (IV), is well illustrated in the infrared absorption spectra and was estimated by potentiometric titration [two pK_a values for the ester (V) and three pK_a values for the acid (IV)]. The dipolar character of the linear amido-oxy-esters reflects that of the cyclized analogue cycloserine.

Modifications of hydroxamic acid derivatives are described in the literature, having identical chemical analyses but showing different melting points and infrared spectra.[6] Hydrogenolysis of benzyloxycarbonylphenylalanylamino-oxyacetic acid (IIIb) gave a modification of phenylalanylamino-oxyacetic acid (IVb) previously obtained by alkaline hydrolysis of the ethyl ester (Vb). In addition to identical analytical values, both forms had markedly different melting points and showed differences in their infrared absorption spectra. The form obtained by hydrogenolysis of the acid (IIIb) was transformed into the second one by sodium hydroxide. Two forms of the ester (Vb) were also obtained. A transition temperature (108—110°) was found, and reconversion was carried out by recrystallization from ethanol. Variations in melting point were also found among benzyloxycarbonyl derivatives of amido-oxy-acids and their esters, as well as with ethyl benzamido-oxyacetate.

The amido-oxy-peptidic linkage is much more sensitive towards acid-catalysed solvolysis than the normal peptide link, as is to be expected for the weakly basic amino-oxy leaving group. Alcoholysis and hydrolysis of amido-oxy-esters (V) and of their benzyloxycarbonyl derivatives (II), in ethanol and in hydrochloric acid were investigated.

The results listed in the Table were obtained by following the formation of ethyl amino-oxyacetate at different hydrogen chloride concentrations; the red colour with alkaline picrate [7] is specific for NH_2–O-groups [8] and the optical density was determined at 525 mμ.

Alcoholysis of amido-oxy-compounds (in %).

Conditions: Time / Compound	N-HCl, 20°				N-HCl, reflux temp.				4N-HCl, 20°			0·1N-HCl, 20°
	3 min.	30 min.	60 min.	6 hr.	3 min.	30 min.	60 min.	3 hr.	2 hr.	3 hr.	6 hr.	10 hr.
IIa	15	70	80	95	50	95	—	—	—	—	—	20
IIb	10	50	70	90	50	95	—	—	—	—	—	15
Va	—	15	30	60	—	—	75	—	—	—	—	5
Vb	—	—	2	10	5	40	50	75	20	30	35	0
Ethyl benzamido-oxyacetate	—	20	40	70	—	—	—	—	—	—	—	6
Ethyl αγ-dibenzoyl-canalinate	—	—	3	15	10	70	80	100	25	40	50	0

The rate of ethanolysis of the benzyloxycarbonylamido-oxy-esters (II) in N-ethanolic hydrogen chloride at room temperature during 1 hour is considerably greater than that of analogous peptides.[9] The unprotected amido-oxy-ester (V) salts are relatively more resistant; nevertheless, they undergo acid catalysed ethanolysis much more readily than peptide ester salts. Alcoholysis of ethyl benzamido-oxyacetate, which does not form an ammonium salt like the amido-oxy-peptides, occurs more readily. It should be noted that the removal of the γ-benzoyl group from ethyl α-benzamido-γ-benzamido-oxybutyrate

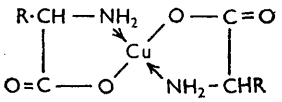
[6] Jones and Werner, *J. Amer. Chem. Soc.*, 1917, **39**, 413; Borek and Clarke, *J. Biol. Chem.*, 1938, **125**, 479; McHale, Green, and Mamalis, *J.*, 1960, 225.
[7] Knobler and Weiss, *Experientia*, 1958, **14**, 332.
[8] Kitagawa and Takani, *J. Agric. Chem. Soc., Japan*, 1935, **11**, 1077.
[9] Gawron and Draus, *J. Org. Chem.*, 1958, **23**, 1040.

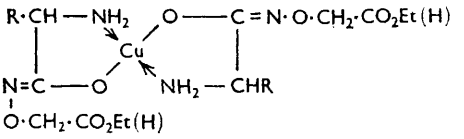
is more inhibited, due to the increased basicity of the leaving group, than the debenzoylation of ethyl benzamido-oxyacetate (α-NH_2O-, $pK_b = 10{\cdot}7$; γ-NH_2O-, $pK_b = 9{\cdot}8$).

Acid catalysed hydrolysis was somewhat less effective than ethanolysis, but in 4N-hydrochloric acid was rapid enough to cleave the amido-oxy-linkage of ethyl alanylamino-oxyacetate (Va) in 5 hours at room temperature. Hydrolysis of this ester was detectable after some hours in 0·25N-hydrochloric acid.

Acidolysis by hydrogen chloride in ether was slower than ethanolysis but could be observed at room temperature.

Free amido-oxy-acids and their esters (but not their acylated compounds) produce a violet colour with alkaline copper sulphate (biuret test). The copper complex seems to be structurally related to the deep blue co-ordination compounds of copper with α-amino-acids.





Ethyl amino-oxyacetate hydrochloride (I) was prepared by the condensation [8] of benzhydroxamic acid and ethyl bromoacetate in ethanolic sodium ethoxide, which gave ethyl benzamido-oxyacetate (VI), followed by debenzoylation in ethanolic hydrogen chloride. Benzyloxycarbonyl and trityl derivatives were prepared. Benzyloxycarbonylamino-oxyacetic acid (VII) was prepared by acylation of amino-oxyacetic acid in aqueous bicarbonate solution; esterification of the acid (VII) gave the ethyl ester (VIII) identical with the product of benzyloxycarbonylation of the ester (I) hydrochloride.

$$\underset{\text{(VI)}}{Ph{\cdot}CO{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CO_2Et} \longrightarrow (I) \longrightarrow \underset{\text{(VIII)}}{Boc{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CO_2Et} \longleftarrow \underset{\text{(VII)}}{Boc{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CO_2H}$$

A model amino-oxyamido-oxy-peptide ester derived from two amino-oxy-acids was also prepared. The acid (VII) was coupled with the ester (I) by the dicyclohexylcarbodiimide procedure to give benzyloxycarbonylamino-oxyacetylamino-oxyacetate (IX).

$$Boc{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CO_2Et \quad \text{(IX)}$$

The ester (IX) could not be debenzyloxycarbonylated by catalytic hydrogenation without reduction of the amino-oxy end group. The acid (VII) gave ammonia under the same conditions.

A γ-amido-oxy-peptidic derivative of canaline was prepared employing α-*N*-protected ethyl canalinate. Ethyl α-*N*-benzoylcanalinate was coupled with benzyloxycarbonylalanine to form ethyl α-*N*-benzoyl-γ-*NO*-benzyloxycarbonylalanylcanalinate (X).

$$Boc.NH{\cdot}CHMe{\cdot}CO{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH{\cdot}CO{\cdot}Ph){\cdot}CO_2Et \quad \text{(X)} \longrightarrow$$

$$Boc.NH{\cdot}CHMe{\cdot}CO{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH{\cdot}CO{\cdot}Ph)CO_2H \quad \text{(XI)} \longrightarrow$$

$$NH_2{\cdot}CHMe{\cdot}CO{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH{\cdot}CO{\cdot}Ph){\cdot}CO_2H \quad \text{(XII)}$$

The ester (X) was hydrolysed with dilute alkali to the acid (XI) and the latter was selectively hydrogenated to α-*N*-benzoyl-γ-*NO*-alanylcanaline (XII).

$$NH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH{\cdot}CO{\cdot}Ph){\cdot}CO_2Et \longrightarrow \underset{\text{(XIII)}}{H{\cdot}[NH{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH{\cdot}CO{\cdot}Ph){\cdot}CO]_3{\cdot}OEt}$$

Heating α-*N*-benzoylcanalinate in ethanol gave ethyl α-*N*-benzoyl-γ-*NO*-[-α-*N*-benzoylcanalyl-γ-*NO*-(α-*N*-benzoylcanalyl)]-canalinate (XIII).

Ethyl α-*N*-benzoyl-γ-*NO*-benzoylcanalinate (ethyl α-benzamido-γ-benzamido-oxybutyrate) was selectively γ-*NO*-debenzoylated in ethanolic hydrogen chloride when heated for 3 hr.

The diphenylmethylidene group provided an additional type of selective protection,

surviving heating under reflux in 20% ethanolic hydrogen chloride. Heating ethyl dibenzoylcanalinate in ethanolic hydrogen chloride in the presence of benzophenone results in a direct replacement of the protecting group to yield α-*N*-benzoyl-γ-*NO*-diphenylmethylidenecanalinate. Removal of the diphenylmethylidene group could only be carried out by refluxing in (1 : 1) ethanol–20% hydrochloric acid.

Reaction of methyl canalinate with 2 equivalents of benzyloxycarbonylphenylalanine (dicyclohexylcarbodi-imide method) gave a mixed tripeptide (XIV).

$$Boc{\cdot}NH{\cdot}CH(CH_2{\cdot}Ph){\cdot}CO{\cdot}NH{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH(CO_2Me){\cdot}NH{\cdot}CO{\cdot}CH(CH_2{\cdot}Ph){\cdot}NH{\cdot}Boc$$
(XIV)

Experimental

Unless stated otherwise, infrared spectra were obtained in Nujol.

Ethyl Benzamido-oxyacetate (VI).—To a solution of benzhydroxamic acid (6·85 g.) in ethanol (50 ml.), sodium ethoxide, prepared by dissolving sodium (1·15 g.) in ethanol (50 ml.), was added. On adding ethyl bromoacetate (8·35 g.), the precipitated sodium benzhydroxamate dissolved. The solution was refluxed for 5 hr. and filtered; the filtrate was evaporated *in vacuo* to small bulk, cooled, and the product was precipitated by the addition of water. Recrystallization from water or isopropyl alcohol gave *ethyl benzamido-oxyacetate* (VI) (7·8 g., 70%), m. p. 86°, $\nu_{max.}$ 3225s, 1725vs, 1640s, 1565w, 1500w, 1410m, 1290w, 1210vs, 1150s, 1080s, 1065s, 1020s, 970w, 890s, 735m, 710s, 700s cm.$^{-1}$ (Found: C, 59·2; H, 5·7; N, 6·3; OEt, 19·1. $C_{11}H_{13}NO_4$ requires C, 59·2; H, 5·9; N, 6·3; OEt, 20·1%).

In one experiment, the product, obtained by the same procedure, had m. p. 123° and showed $\nu_{max.}$ 3335s, 1725vs, 1665vs, 1590w, 1560w, 1540w, 1470s, 1300w, 1280m, 1250s, 1145m, 1125s, 1090vs, 1010w, 925w, 890w, 800w, 740w, 720vs, 690s cm.$^{-1}$.

Ethyl Amino-oxyacetate Hydrochloride (I).—The ester (VI) (4·46 g.) was refluxed in ethanolic hydrogen chloride (10%, 100 ml.) for 3 hr. The solution was concentrated *in vacuo*, cooled, and ether was added to turbidity. After keeping (0°) for 12 hr., the product was filtered off. Additional portions of ether were added to the filtrate when further crops of the product precipitated on cooling (0°). Recrystallization from ethanol gave *ethyl amino-oxyacetate hydrochloride* [1] (2·5 g., 80%), m. p. 116°; $\nu_{max.}$ 3450w, 2660—2500m, 1960m, 1750s, 1570s, 1500s, 1410m, 1240s, 1075—1065s, 1020s, 950m, 890s, 860m, 730—725vs cm.$^{-1}$ (Found: C, 30·6; H, 6·5; Cl, 21·8; N, 8·8; OEt, 29·9. $C_4H_{10}ClNO_3$ requires C, 30·9; H, 6·4; Cl, 22·8; N, 9·0; OEt, 29·0%).

Triphenylmethylation with triphenylmethyl chloride in methylene chloride in the presence of 1 equiv. of triethylamine gave *ethyl triphenylmethylamino-oxyacetate* (60%), m. p. 88—89°, from ethanol (Found: N, 4·2; OEt, 12·5. $C_{23}H_{23}NO_3$ requires N, 3·9; OEt, 12·4%).

Ethyl Benzyloxycarbonylamino-oxyacetate (VIII).—The ester (VI) (2·23 g.) was refluxed in ethanolic hydrogen chloride (10%, 50 ml.) for 3 hr. The solution was evaporated to dryness *in vacuo*, the residue was dissolved in 20% aqueous pyridine (30 ml.), and benzyloxycarbonyl chloride (2 g.) was added in portions. On cooling and acidification with dilute hydrochloric acid to Congo-Red, the oily *ethyl benzyloxycarbonyl derivative* (2 g., 80%) separated (Found: N, 5·4; OEt, 17·6. $C_{12}H_{15}NO_5$ requires N, 5·5; OEt, 17·8%).

*Ethyl Benzyloxycarbonyl-*DL*-alanylamino-oxyacetate* (IIa).—*N*-Benzyloxycarbonyl-DL-alanine (2·23 g.) was dissolved in methylene chloride (30 ml.) and *NN'*-dicyclohexylcarbodi-imide (2·06 g.) in methylene chloride (10 ml.) was added with cooling (ice-bath). Ethyl amino-oxyacetate hydrochloride (1·55 g.) and triethylamine (1·01 g.) in methylene chloride (20 ml.) were introduced, and the mixture was stirred for 2 hr. (ice-bath), then for 10 hr. at room temperature. *NN'*-Dicyclohexylurea was filtered off and the solution was washed with water (30 ml.), sodium hydrogen carbonate (3%, 30 ml.), and water. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to 20 ml. Some impurities were filtered off and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in ethyl acetate (2 ml.) and kept at −10° for some hours. *Ethyl benzyloxycarbonylalanylamino-oxyacetate* (IIa) (2·4 g., 75%) was filtered off and crystallized from a small amount of ethyl acetate or from ethanol, m. p. 85—87°; $\nu_{max.}$ 3220s, 1740s, 1670s, 1650vs, 1515s, 1410w, 1310w, 1250vs, 1075s, 1055m, 1030w, 950w, 900w, 780w, 745m, 695s cm.$^{-1}$ (Found: C, 55·4; H, 6·4; N, 8·7; OEt, 13·9. $C_{15}H_{20}N_2O_6$ requires C, 55·5; H, 6·2; N, 8·6; OEt, 13·9%).

*Benzyloxycarbonyl-*DL*-alanylamino-oxyacetic Acid* (IIIa).—The ester (IIa) (1·62 g.) was shaken with N-sodium hydroxide (20 ml.) for 6 hr. The solution was filtered, cooled, and carefully acidified with 2N-hydrochloric acid to Congo-Red. The product was immediately extracted with ethyl acetate (3 × 20 ml.). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. *Benzyloxycarbonylalanylamino-oxyacetic acid* (IIIa) (1·2 g., 80%), crystallized from ethyl acetate (2 ml.), had m. p. 102—104°; $\nu_{max.}$ 3335s, 3125s, 2565w, 1725vs, 1615vs, 1515vs, 1265s, 1235vs, 1110w, 1100w, 1065vs, 1020s, 950m, 910m, 880w, 840w, 785m, 735m, 700s cm.$^{-1}$ (Found: C, 52·3; H, 5·4; N, 9·6. $C_{13}H_{16}N_2O_6$ requires C, 52·7; H, 5·4; N, 9·5%).

DL-*Alanylamino-oxyacetic Acid* (IVa).—The acid (IIIa) (0·8 g.) was dissolved in ethanol (10 ml.), 10% palladized charcoal (0·12 g.) was added and the mixture was hydrogenated at room temperature at 3 atm. for 4 hr. The precipitated product was filtered off and separated from the catalyst by extraction with water (5 ml.) followed by reprecipitation with ethanol. The *alanylamino-oxyacetic acid* (IVa) (0·25 g., 57%), crystallized from water–ethanol, m. p. 196—197° (decomp.); $\nu_{max.}$ 3330w, 3125s, 2085w, 1665s, 1620vs, 1580vs, 1540m, 1420s, 1325s, 1275m, 1220w, 1165m, 1125w, 1050s, 1020s, 1000m, 950s, 945s, 885m, 730—725vs cm.$^{-1}$ (Found: C, 36·7; H, 6·2; N, 17·3. $C_5H_{10}N_2O_4$ requires C, 37·0; H, 6·2; N, 17·3%). It gave a weak red coloration with ninhydrin and a violet colour with alkaline cupric sulphate (biuret reagent), specific for free amido-oxy-peptides.

Ethyl DL-*Alanylamino-oxyacetate* (Va).—The ester (IIa) (3·2 g.) was dissolved in ethanol (20 ml.), 10% palladized charcoal (0·2 g.) was added and the mixture was hydrogenated at room temperature at 3 atm. for 40 min. After separation from the catalyst by dissolution in boiling ethanol, *ethyl alanylamino-oxyacetate* (Va) (1·3 g., 70%) had m. p. 156—157°; $\nu_{max.}$ 3225s, 2600s, 2500s, 2130m, 1740vs, 1615vs, 1565vs, 1345s, 1290s, 1210vs, 1135s, 1100vs, 1090vs, 1020m, 1000w, 900w, 860w, 830s, 725m, 690w cm.$^{-1}$ (Found: C, 44·4; H, 7·5; N, 14·6; OEt, 23·8. $C_7H_{14}N_2O_4$ requires C, 44·2; H, 7·4; N, 14·7; OEt, 23·7%). It gave a pale red colour with ninhydrin and a violet colour with the biuret reagent.

*Ethyl Benzyloxycarbonyl-*DL*-phenylalanylamino-oxyacetate* (IIb).—This was made from *N*-benzyloxycarbonyl-DL-phenylalanine (2·99 g.) as described above for the ester (IIa). Crystallization from ethanol gave the *ester* (IIb) (2·6 g., 65%), m. p. 107—109°; $\nu_{max.}$ 3330s, 3225s, 1755s, 1695s, 1665s, 1515s, 1490m, 1400w, 1280vs, 1250m, 1210s, 1150m, 1100w, 1060s, 1035m, 1000w, 950w, 915m, 910m, 870w, 850w, 775w, 750m, 740s, 700s cm.$^{-1}$ (Found: C, 63·1; H, 6·0; N, 7·0; OEt, 11·2. $C_{21}H_{24}N_2O_6$ requires C, 63·0; H, 6·0; N, 7·0; OEt, 11·2%). On recrystallization from ethyl acetate, and sometimes from ethanol, the product melted (on rapid heating) at about 80°. After the melted product had been kept at 100° for 15 min. it solidified and remelted at 109°. The infrared spectrum of the product, m. p. 80°, was the same except for the absence of a maximum at 870 cm.$^{-1}$ and a weaker band at 750 cm.$^{-1}$.

*Benzyloxycarbonyl-*DL*-phenylalanylamino-oxyacetic Acid* (IIIb).—This was made from the ester (IIb) (1·5 g.) as described for the acid (IIIa). The *acid* (IIIb) (1·16 g., 80%), after several crystallizations from ethyl acetate, had m. p. 162—163°. Recrystallization from ethanol gave a form, m. p. 165—166°. Both forms gave the same analytical results but some differences could be noticed in the infrared spectra between 1400—700 cm.$^{-1}$: the form, m. p. 165—166°, had additional absorption maxima at 870 and 1650 cm.$^{-1}$ (Found: C, 60·3; H, 5·3; N, 7·5. $C_{19}H_{20}N_2O_6$ requires C, 61·3; H, 5·4; N, 7·5%).

DL-*Phenylalanylamino-oxyacetic Acid* (IVb).(*a*) The acid (IIIb) (1 g.), was hydrogenated in the same way as the acid (IIIa) giving *phenylalanylamino-oxyacetic acid* (IVb) (0·18 g., 28%), m. p. 174—175° (decomp.); $\nu_{max.}$ 3350w, 3200w, 2175w, 1665vs, 1610s, 1480s, 1405m, 1305m, 1210w, 1160w, 1095s, 1070m, 1030m, 935w, 915w, —750s, 720s, 700s cm.$^{-1}$ (Found: C, 55·2; H, 6·4; N, 11·8. $C_{11}H_{14}N_2O_4$ requires C, 55·4; H, 5·9; N, 11·8%). (*b*) The ester (Vb) (0·53 g.) in 2N-sodium hydroxide (3 ml.) was left overnight at room temperature. The solution was acidified with 2N-ethanolic hydrogen chloride (3 ml.), and, after keeping for several hr. at 0°, furnished a form of the acid (IVb) (0·35 g., 74%), m. p. 186—188° (decomp.); $\nu_{max.}$ 3350w, 3125m, 2080w, 1720s, 1620vs, 1540—1490vs, 1405m, 1300m, 1210w, 1140w, 1115w, —1050s, 1015m, 975m, 955m, 935w, 915w, 850m, 750s, 715w, 700s cm.$^{-1}$ (Found: C, 55·2; H, 5·95; N, 11·5%).

When the acid (IVb) (0·1 g.), in 2N-sodium hydroxide (0·5 ml.) was kept overnight at room temperature and the solution was acidified with 2N-ethanolic hydrogen chloride (0·5 ml.) and kept overnight at 0°, the form, m. p. 186—188° (decomp.), identified by the infrared spectrum, was precipitated.

Thin-layer chromatography (kieselgel, 0·25 mm.) gave the following R_F values for the acid (IVb) and the form, m. p. 186—188° (decomp.): in 80% phenol–water (ninhydrin), 0·28 and 0·33; in butanol–water–acetic acid (4 : 1 : 1), 0·43 and 0·47, respectively.

Ethyl DL-*Phenylalanylamino-oxyacetate* (Vb).—The ester (IIb) (3 g.), treated in the same way as the ester (IIa) yielded the *ester* (Vb) (0·45 g., 23%), m. p. 108° (Found: C, 58·5; H, 6·85; N, 10·0. $C_{13}H_{18}N_2O_4$ requires C, 58·6; H, 6·8; N, 10·5%). $\nu_{max.}$ 3500m, 2150w, 1730s, 1650w, 1640—1595m, 1540w, 1300s, 1230s, 1110s, 1035s, 840s, 750m, 740m, 730m, 700s, 675m cm.$^{-1}$. The ester gave a pale red colour with ninhydrin and a violet colour with the biuret reagent. It was transformed by heating to a form, m. p. 122° (Found: C, 58·1; H, 6·8; N, 10·0%). $\nu_{max.}$ 3080m, 2100w, 1730s, 1650m, 1640m, 1590m, 1565s, 1540m, 1300s, 1230s, 1110s, 1035s, 840s, 750w, 740m, 700s cm.$^{-1}$, and generated by recrystallization from ethanol.

R_F values in thin-layer chromatography (kieselgel, 0·25 mm.) for the ester (Vb) and the form, m. p. 122° in 80% phenol–water (ninhydrin), 0·93 and 0·99; in butanol–water–acetic acid (4 : 1 : 1), 0·62 for both.

*Ethyl Trifluoroacetyl-*DL-*alanylamino-oxyacetate.*—*N*-Trifluoroacetyl-DL-alanine (1·85 g.) in methylene chloride–ether (3 : 1; 20 ml.) was treated with *NN'*-dicyclohexylcarbodi-imide (2·06 g.) in methylene chloride (30 ml.) followed by ethyl amino-oxyacetate, (1·55 g.) and triethylamine (1·01 g.). The mixture was stirred for 12 hr. at room temperature, dicyclohexylurea was filtered off and the filtrate was washed with water (50 ml.). The recovered residue was shaken with ether giving *ethyl trifluoroacetylalanylamino-oxyacetate* (1·15 g., 40%), m. p. 110°; $\nu_{max.}$ (KBr disc) 3280vs, 3125s, 1755vs, 1725vs, 1665vs, 1565s, 1495m, 1410m, 1265m, 1250—1150vs, 1075vs, 1020m, 925m, 950m, 900w, 880m, 860w, 740s, 725s cm.$^{-1}$ (Found: N, 9·9; OEt, 14·7. $C_9H_{13}F_3N_2O_5$ requires N, 9·8; OEt, 15·7%).

The derivative (0·5 g.) was treated with 4N-sodium hydroxide (1 ml.) for 2 hr. at room temperature, the solution was acidified to pH 6 with N-ethanolic hydrogen chloride (4 ml.), excess of ethanol was added (10 ml.) and precipitated sodium chloride was filtered off. After 48 hr. at room temperature, crystals of alanylamino-oxyacetic acid (IVa) m. p. 185°, containing some sodium chloride, were precipitated and identified by colour reactions and the infrared spectrum.

Ethyl Trifluoroacetylglycylamino-oxyacetate.—Trifluoroacetylglycine (1·71 g.) was treated as for the alanine derivative. *Ethyl trifluoroacetylglycylamino-oxyacetate* (1 g., 37%) (crystallized from ether) had m. p. 125° (Found: N, 10·2; OEt, 15·9. $C_8H_{11}F_3N_2O_5$ requires N, 10·3; OEt, 16·5%).

Upon treatment with 4N-sodium hydroxide *glycylamino-oxyacetic acid,*[10] m. p. 150°, was obtained.

N-DL-*Alanylhydroxylamine.*—To hydroxylamine hydrochloride (7 g.) in methanol (50 ml.) was added potassium hydroxide (5·6 g.) in methanol (50 ml.) and, after heating to 50°, precipitated potassium chloride was filtered off. The filtrate at 0° was added to freshly distilled alanine ethyl ester (13·1 g.) in methanol (50 ml.). After keeping at room temperature for 2 days, *N*-alanylhydroxylamine precipitated, (7 g., 70%), m. p. 164°.[11] After some months the m. p. rose to 175° (Found: C, 34·7; H, 7·6; N, 26·8. Calc. for $C_3H_8N_2O_2$: C, 34·6; H, 7·7; N, 26·9%).

With excess potassium hydroxide, pure *N*-alanylhydroxylamine, m. p. 172°, was obtained instead of the expected potassium salt.

N-(*Benzyloxycarbonyl-*DL-*alanyl*)*hydroxylamine.*—Hydroxylamine hydrochloride (1·4 g.) in methanol (25 ml.) was added to potassium hydroxide (1·78 g.) in methanol (25 ml.) and the precipitated potassium chloride was filtered off. The filtrate was added to *N*-benzyloxycarbonyl-DL-alanine (4·46 g.) in methylene chloride (40 ml.), followed by *NN'*-dicyclohexylcarbodi-imide (4·12 g.) in methylene chloride (10 ml.). The reaction mixture was stirred at room temperature for 6 hr., precipitated dicyclohexylurea was filtered off and the organic layer was extracted with water (3 × 100 ml.). The extract was evaporated to dryness *in vacuo* at 40°. The residue was redissolved in water, cooled, and acidified with dilute hydrochloric acid. The precipitate was extracted with ethyl acetate (50 ml.) and the organic layer dried (Na_2SO_4) and evaporated *in vacuo* to dryness. N-(*benzyloxycarbonylalanyl*)*hydroxylamine* (2 g., 42%), from ethyl acetate, had m. p. 124°, $\nu_{max.}$ 3335s, 3225s, 1685vs, 1640vs, 1515vs, 1310s, 1250vs, 1165w, 1125w, 1090w, 1065s, 1045s, 1025s, 950m, 760m, 725m, 700s cm.$^{-1}$ (Found: C, 55·2; H, 5·9; N, 12·1. $C_{11}H_{14}N_2O_4$ requires C, 55·4; H, 5·9; N, 11·8%).

[10] Knobler, Bittner, and Frankel, following Paper.
[11] Cunningham, Newbold, Spring, and Stark, *J.*, 1949, 2091.

N-(*Benzyloxycarbonyl-*DL*-phenylalanyl*)*hydroxylamine*.—Benzyloxycarbonyl-DL-phenylalanine (6 g.) was treated as the alanine derivative in the above preparation. The *hydroxylamine* (40%) crystallized from ethanol–aqueous sodium chloride (3%); it had m. p. 130°; $\nu_{max.}$ 3335s, 3125m, 1965s, 1650s, 1515s, 1310s, 1250s, 1145w, 1080w, 1055w, 1020m, 995w, 930w, 770w, 740s, 695s cm.$^{-1}$ (Found: C, 64·7; H, 5·9; N, 8·5. $C_{17}H_{18}N_2O_4$ requires C, 64·95; H, 5·8; N, 8·9%).

Condensation of Benzyloxycarbonylaminohydroxamic Acids with Ethyl Bromoacetate.—The hydroxamic acid (2 m.moles) in ethanol (10 ml.) was treated with sodium (0·046 g.) in ethanol (4 ml.) followed by ethyl bromoacetate (0·23 ml.). The mixture was refluxed for 1 hr. and left overnight at room temperature. On addition of water, an oil separated. With both alanine and phenylalanine derivatives the products could not be crystallized. The infrared spectra were consistent with *ethyl benzyloxycarbonylalanyl-* (IIa) (Found: N, 8·5; OEt, 14·0. $C_{15}H_{20}N_2O_6$ requires N, 8·6; OEt, 13·9%) and *ethyl benzyloxycarbonylphenylalanylamino-oxyacetate* (IIb) (Found: N, 7·2; OEt, 10·7. $C_{21}H_{24}N_2O_6$ requires N, 7·0; OEt, 11·25%).

Both products gave a positive Jaffe's test after hydrolysis.

Hydrogenolysis of Amido-oxy-peptides.—The ester (Vb) (0·53 g.) in ethanol (25 ml.) in the presence of 10% palladized charcoal (0·12 g.) was hydrogenated for 6 hr. at room temperature and 3 atm. The catalyst was filtered off and the solution was concentrated *in vacuo* to small bulk. On cooling at −10°, phenylalanine amide separated (0·3 g., 90%), m. p. 139°, and gave a violet ninhydrin test (Found: N, 16·8; N (Van Slyke), 8·7. Calc. for $C_9H_{12}N_2O$: N, 17·1; N (Van Slyke), 8·55%).

Phenylalanine amide was also obtained by treating the ester (IIb) in the same manner. The ester (VI) and ethyl γ-benzamido-oxy-α-benzamidobutyrate did not undergo catalytic hydrogenation under the above conditions. In the presence of 1 equiv. of hydrogen chloride the ester (Vb) was resistant to hydrogenolysis under similar conditions, while the acid (IVb) was stable even in the absence of hydrogen chloride.

The alanyl derivatives behaved similarly.

Hydrogenolysis of Amido-oxy-compounds in the Presence of Triethylamine.—Ethyl benzamido-oxyacetate (1·12 g.) in ethanol (8 ml.) and triethylamine (1·5 g.) was hydrogenated for 6 hr. at room temperature (3 atm.) in the presence of 10% palladized charcoal (0·12 g.). The catalyst was removed by filtration, the solution was concentrated *in vacuo*, and water was added. On cooling, benzamide (0·5 g.; m. p. and mixed m. p. 129°) crystallized and was identified by the infrared spectrum.

Benzyloxycarbonylamino-oxyacetic Acid (VII).—Amino-oxyacetic acid hemihydrochloride (10·9 g.) was neutralized by N-sodium hydroxide (150 ml.). Sodium hydrogen carbonate (20 g.) was added, followed during 1 hr. (stirring) by benzyloxycarbonyl chloride (13·5 g.). After keeping overnight at room temperature, the solution was washed with ether and acidified to Congo-Red with hydrochloric acid. On shaking, a solid (20 g.; 90%) separated. *Benzyloxycarbonylamino-oxyacetic acid*, had m. p. 63° (from chloroform); $\nu_{max.}$ 3440s, 3280s, 1725vs, 1505m, 1250—1235s, 1135s, 1035m, 985m, 750vs, 705s cm.$^{-1}$ (Found: C, 52·4; H, 4·7; N, 6·3. $C_{10}H_{11}NO_5$ requires C, 53·3; H, 4·9; N, 6·2%).

The acid was esterified by refluxing in ethanol in the presence of a catalytic amount of toluene-*p*-sulphonic acid. The ester obtained was identical with the ester (VIII).

Ethyl Benzyloxycarbonylamino-oxyacetylamino-oxyacetate (IX).—The acid (VII) (2·25 g.) and ethyl amino-oxyacetate (1·55 g.) were condensed under the conditions used for the preparation of the ester (IIa) and gave *ethyl benzyloxycarbonylamino-oxyacetyl-amino-oxyacetate* (IX) (1·8 g., 58%) (Found: N, 8·7; OEt, 14·1. $C_{14}H_{18}N_2O_7$ requires N, 8·6; OEt, 13·8%).

Benzyloxycarbonylamino-oxyacetylamino-oxyacetic Acid.—The ester (IX) (1·63 g.) was shaken with N-sodium hydroxide (15 ml.) for 4 hr. After filtration, the solution was cooled in ice and acidified with N-hydrochloric acid. The precipitated *acid* (0·7 g., 45%) had m. p. 108° (Found: C, 48·0; H, 5·0; N, 9·2. $C_{12}H_{14}N_2O_7$ requires C, 48·3; H, 4·7; N, 9·4%).

Ethyl α-N-*Benzoyl*-ω-N-(*benzyloxycarbonyl-*DL*-alanyl*)-DL-*canalinate* (X).—To *N*-benzyloxycarbonyl-DL-alanine (2·23 g.) in methylene chloride (30 ml.) was added *NN'*-dicyclohexylcarbodiimide (2 g.) in methylene chloride (10 ml.) followed by ethyl α-benzamido-DL-canalinate hydrochloride [1,12] (3·03 g.) and triethylamine (1·42 ml.). The mixture was stirred for 2 hr. at 0°, then for 12 hr. at room temperature. The *NN'*-dicyclohexylurea was filtered off, the solution washed with water (30 ml.), sodium hydrogen carbonate (3%, 30 ml.), and water (30 ml.). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* (to 20 ml.), some impurities were filtered

[12] Frankel, Knobler, and Zvilichovsky, *J.*, 1963, 3127.

off, and the filtrate was evaporated *in vacuo* to dryness. The oily residue was dissolved in ethyl acetate and filtered, and light petroleum was added. The precipitated *ethyl* γ-NO-*benzyloxycarbonylalanyl*-α-N-*benzoylcanalinate* (X) (3 g., 63%), crystallized from ethyl acetate–light petroleum, had m. p. 126—127°; $\nu_{max.}$ 3250m, 1695vs, 1640vs, 1525s, 1505s, 1510w, 1335w, 1280m, 1250vs, 1220s, 1165w, 1110w, 1065m, 1020m, 895w, 840w, 765m, 700m cm.$^{-1}$ (Found: C, 61·4; H, 6·7; N, 9·0; OEt, 9·4. $C_{24}H_{29}N_3O_7$ requires C, 61·1; H, 6·3; N, 8·9; OEt, 9·6%).

α-N-*Benzoyl*-ω-N-(*benzyloxycarbonyl*-DL-*alanyl*)-DL-*canaline* (XI).—The ester (X) (3 g.) was shaken with N-sodium hydroxide (25 ml.) for 4 hr. After filtration, the solution was cooled and acidified to Congo-Red with N-hydrochloric acid. The precipitated *acid* (XI) (2·5 g., 88%) had m. p. 105°; $\nu_{max.}$ 3450w, 3225s, 1725s, 1680vs, 1640vs, 1515s, 1370w, 1310w, 1280w, 1235s, 1165w, 1125w, 1075s, 1055w, 1030w, 990w, 740m, 720m, 695s cm.$^{-1}$ (Found: C, 59·9; H, 5·9; N, 9·85. $C_{22}H_{25}N_3O_7$ requires C, 59·6; H, 5·7; N, 9·5%).

α-N-*Benzoyl*-ω-N-DL-*alanyl*-DL-*canaline* (XII).—The acid (XI) (1·1 g.) in ethanol (10 ml.) was hydrogenated for 4 hr. at room temperature (3 atm.) with 10% palladized charcoal (0·12 g.). The hydrogenation product was filtered off, together with the catalyst, and extracted with water. The solution was concentrated *in vacuo* and on addition of ethanol α-N-*benzoyl*-ω-N-*alanylcanaline* (XII) (0·3 g., 40%) was precipitated. It had m. p. 175—180° (decomp.); $\nu_{max.}$ 3125w, 1665s, 1615s, 1565s, 1515s, 1055s, 950w, 720s, 695s cm.$^{-1}$ (Found: C, 54·3; H, 6·0; N, 13·4. $C_{14}H_{19}N_3O_5$ requires C, 54·5; H, 6·2; N, 13·6%), and gave a pale red colour with ninhydrin and a violet colour with the biuret reagent.

Ethyl α-N-*Benzoyl*-ω-N-[α-N-*benzoyl*-DL-*canalyl*-ω-N-(α-N-*benzoyl*-DL-*canalyl*)]-DL-*canalinate* [1] (XIII).—Potassium acetate (0·98 g.) in ethanol (50 ml.) was added to ethyl α-*N*-benzoylcanalinate hydrochloride (3 g.) in ethanol (50 ml.). The mixture was heated to 70° and cooled, potassium chloride was filtered off, and the filtrate was refluxed for 3 hr. After cooling and filtration the solution was concentrated *in vacuo*. The oily residue was washed with water and crystallized from aqueous ethanol. The *tripeptide* (XIII) (2 g., 85%) had m. p. 95—100°; $\nu_{max.}$ 3225s, 1725m, 1640s, 1515s, 1075m, 1040m, 1020w, 855m, 805w, 715s, 690s cm.$^{-1}$ (Found: C, 60·0; H, 6·0; N, 11·9; OEt, 6·6. $C_{35}H_{42}N_6O_{10}$ requires C, 59·5; H, 5·9; N, 11·9; OEt, 6·4%).

Methyl αω-NN-*Di*(*benzyloxycarbonyl*-DL-*phenylalanyl*)-DL-*canalinate*.—Triethylamine (2·02 g.) was added to benzyloxycarbonyl-DL-phenylalanine (5·98 g.) in methylene chloride (30 ml.) at 0°, followed by methyl DL-canalinate dihydrochloride (2·2 g.) and *NN'*-dicyclohexylcarbodiimide (4·12 g.) in methylene chloride (30 ml.). The mixture was stirred for 2 hr. with cooling, then for 4 hr. at room temperature, and then kept at room temperature for 48 hr. Precipitated dicyclohexylurea was filtered off and the solution was washed with water (60 ml.), sodium hydrogen carbonate solution (3%, 60 ml.) and water (60 ml.). The organic layer was dried (Na_2SO_4), concentrated *in vacuo* to 10 ml., some insoluble matter was filtered off and the filtrate was evaporated to dryness *in vacuo*. The residue was redissolved in ethyl acetate and left for 6 hr. at −10°. The precipitate (4 g., 56%) still gave a positive Jaffe's test. After recrystallization from isopropyl alcohol (30% loss), Jaffe's test was negative; the *tripeptide ester* (XIV) melted at 102—104°; $\nu_{max.}$ 3335s, 3225m, 1725m, 1665s, 1640vs, 1515vs, 1280s, 1235s, 1030s, 1000m, 915w, 850w, 770w, 740s, 700s cm.$^{-1}$ (Found: C, 65·95; H, 5·9; N, 7·7; OMe, 4·0. $C_{39}H_{42}N_4O_9$ requires C, 65·9; H, 5·9; N, 7·8; OMe, 4·4%).

Ethyl α-N-*Benzoyl*-ω-N-(*trifluoroacetyl*-DL-*alanyl*)-DL-*canalinate*.—To *N*-trifluoroacetyl-DL-alanine (1·85 g.) in methylene chloride–ether (3 : 1, 20 ml.) was added *NN'*-dicyclohexylcarbodiimide (2·06 g.) in methylene chloride (30 ml.), ethyl α-*N*-benzoylcanalinate hydrochloride (3·03 g.), and triethylamine (1·01 g.). The mixture was stirred for 12 hr. at room temperature, dicyclohexylurea was filtered off, and the solution was washed with water (50 ml.). The organic layer was dried (Na_2SO_4) and evaporated to dryness *in vacuo*. On shaking the residue with ether, the *trifluoroacetylamido-oxy-ester* (3 g., 70%) was precipitated; it had m. p. 130°; $\nu_{max.}$ (KBr disc) 3335vs, 3030—2940w, 1740s, 1695vs, 1640vs, 1515vs, 1480w, 1450w, 1370—1265w, 1205s, 1175vs, 1150vs, 1055m, 1030m, 960w, 860w, 720s, 695s cm.$^{-1}$ (Found: N, 9·7; OEt, 10·5. $C_{18}H_{22}N_3O_6F_3$ requires N, 9·7; OEt, 10·4%).

With 4N-sodium hydroxide the ester gave the acid (XII) but separation from sodium chloride was very difficult.

Ethyl α-N-*Benzoyl*-ω-N-*diphenylmethylidene*-DL-*canalinate*.—Ethyl αω-*NN*-dibenzoylcanalinate [12] (1·85 g.) and benzophenone (0·91 g.) were refluxed during 8 hr. in 0·66N-ethanolic hydrogen chloride (6 ml.). The solution was kept overnight (−10°) and the product then filtered off. *Ethyl* α-N-*Benzoyl*-ω-N-*diphenylmethylidene*-DL-*canalinate* (1·95 g., 90%), crystallized from

ethanol; it had m. p. 101° (Found: C, 72·2; H, 6·1; N, 6·4; OEt, 10·3. $C_{26}H_{26}N_2O_4$ requires C, 72·5; H, 6·1; N, 6·5; OEt, 10·5%).

The diphenylmethylidene ester was also obtained from γ-amido-oxy-peptides of ethyl α-*N*-benzyoylcanalinate by the same procedure.

The diphenylmethylidene ester resisted hydrolysis by refluxing in 20% ethanolic hydrogen chloride during 5 hr. The protecting group was removed by refluxing in ethanol–20% hydrochloric acid (1 : 1) for 1 hr. and yielded ethyl α-*N*-benzoylcanalinate hydrochloride.[1,11]

Determination of the Rates of Alcoholysis of Amido-oxy-compounds.—The rates of alcoholysis of the amido-oxy-peptides, their benzyloxycarbonyl derivatives, and of *NO*-acyl derivatives of ethyl amino-oxyacetate and ethyl canalinate were measured by photometric determination of the ester liberated. The acid-catalysed ethanolysis was followed in N-, 2N-, and 4N-ethanolic hydrogen chloride, at different temperatures, and at concentrations of 2×10^{-5} mole per ml. The colorimetric method [7] based on Jaffe's reaction [8] was adapted for the measurements, by using the formation of a picrate complex after stopping the reaction by neutralization. The amido-oxy-compounds were stable during several hr. to alkaline cleavage under the conditions given, and no formation of coloured complex could be observed without previous treatment with ethanolic hydrogen chloride; interference due to alkaline hydrolysis is thus excluded. Typical results are listed in the Table.

The amido-oxy-compound (0·002 mole) was dissolved in ethanol (50 ml.), 2N-, 4N-, or 8N-ethanolic hydrogen chloride (50 ml.) was introduced rapidly and the solution was shaken in a closed bottle for 1 min. Samples (1 ml.) were withdrawn at timed intervals and the ethyl amino-oxyacetate was estimated as follows. N-, 2N-, or 4N-sodium hydroxide (1 ml.) was added followed by the rapid successive addition of ethanol (1 ml.), N-sodium hydroxide (1 ml.) and saturated picric acid solution (1 ml.). The solution was kept at room temperature for 40 min. (sufficient for the colour intensification desired). The optical density was compared with that of a control determination on a Fisher photoelectric colorimeter, used with a 525 mμ filter (3-ml. cylindrical cells).

Calibrations were obtained with colour standards prepared from known amounts of ethyl amino-oxyacetate, the ester of the acyl component, the amido-oxy-compound and aqueous ethanol.

The presence of sodium chloride in large excess has a slight effect on the optical densities, as found by comparison of the calibration curves with those of equivalent amounts of the components without sodium chloride. Only 1—3% differences could be detected, except in the case of the alcoholysis of ethyl αω-*NN*-dibenzoylcanalinate (5—10%).

Rates of Hydrolysis.—Rates of hydrolysis of amido-oxy-esters were determined by an analogous photometric estimation of the amino-oxy component liberated in 4N-hydrochloric acid at a concentration of 4×10^{-5} mole per ml., at room temperature. They were: for the ester (Vb); 6% (after 2 hr.), 20% (5 hr.): for the ester (Va); 20% (1 hr.), 40% (2 hr.), 60% (5 hr.).

No hydrolysis could be detected with more dilute hydrochloric acid in the case of the amido-oxy-ester (Vb), whereas with the ester (Va) the following results were obtained after storage overnight in hydrochloric acid: N-, 45%; 0·5N-, 20%; 0·25N-, 5%.

Acidolysis.—The ester (IIb) (200 mg.) in 1·5N-ethereal hydrogen chloride was kept overnight at room temperature and the ether was removed *in vacuo*. The residue was dissolved in ethanol (25 ml.); aliquot parts were estimated as above, and indicated 25% of acidolysis.

Determination of pK_a Values of Amido-oxy- and Amino-oxy-compounds.—A "Radiometer-Copenhagen" titrator with glass and calomel electrodes was used. To 0·06 m.mole of the compound in water 3 ml., 2M-sodium chloride 0·5 ml., and water 6·5 ml. were added. Titration was carried out either with 0·2N-sodium hydroxide or with 0·2N-hydrochloric acid. The pK_a values were taken from the mid-points of the pH neutralization curves:

Compound	pK_1 (COOH)	pK_2 (HO·C=N)	pK_3 (NH_3^+)
Ethyl DL-alanylamino-oxyacetate		6·3	9·35
DL-Alanylamino-oxyacetic acid	2·8 *	7·1	9·5
Ethyl DL-phenylalanylamino-oxyacetate		6·1	8·7
DL-Phenylalanylamino-oxyacetic acid	2·9 *	6·8	9·55
Ethyl amino-oxyacetate			3·3
Ethyl α-*N*-benzoyl-DL-canalinate			4·2

* ±5% accuracy.

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