16α,17α-Dimethyl-3-ethylenedioxy-5,9(11)-androstadiene-17β-ol, (Va) *and 16α-17β-dimethyl-3-ethylenedioxy-5,9(11)-androstadiene-17α-ol* (Vb). A solution of 8.8 g. of IVa, m.p. 172–175°, in 200 ml. of benzene was dried by azeotropic distillation. This was added to a solution of methylmagnesium iodide (prepared from 1.50 g. of magnesium, 24 ml. of methyl iodide, and 240 ml. of anhydrous ether) and allowed to stir overnight at 25°. Water was added and the product extracted with chloroform. The chloroform phase was dried over sodium sulfate and concentrated *in vacuo*. Chromatography on 300 g. of acid-washed alumina (Merck) and elution with ether–petroleum ether (1:9) yielded 1.02 g. (12%) of starting material. Elution with ether–petroleum ether (3:7 and 4:6) afforded 5.91 g. (75%) of 16α,17α-dimethyl-3-ethylenedioxy-5,9(11)-androstadiene-17β-ol, m.p. 170–191°. A sample for analysis was obtained from methanol, m.p. 194–198°; α_D^{27} −35° (*c* 1.3, benzene).

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.06; H, 9.35.

Further elution with ether–petroleum ether (5:5) afforded 260 mg. (3%) of 16α,17β-dimethyl-3-ethylenedioxy-5,9(11)-androstadiene-17α-ol, m.p. 152–156°. Recrystallization from methanol afforded the analytical sample, m.p. 155–157°; α_D^{23} −37° (*c* 1.0, benzene).

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.65; H, 9.94.

16α,17α-Dimethyl-4,9(11)-androstadiene-17β-ol-3-one (VI). A solution containing 0.4 g. of Va and 50 mg. of *p*-toluenesulfonic acid in 50 ml. of acetone was allowed to stand overnight. The solution was diluted with aqueous sodium bicarbonate and extracted with chloroform. The chloroform layer was dried over sodium sulfate and concentrated *in vacuo*. The crude product was adsorbed on 25 g. acid-washed alumina (Merck) from benzene. Elution with ether–petroleum ether (6:4, 7:3, and 8:2) afforded 0.25 g. crude VI. Several crystallizations from methanol–ether yielded a sample of 16α,17α-dimethyl-4,9(11)-androstadiene-17β-ol-3-one for analysis, m.p. 187–189°; α_D^{26} +34°; $\lambda_{max}^{CH_3OH}$ 240 mμ, ε 16,600.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 80.49; H, 9.86.

9α-Bromo-16α,17α-dimethyl-4-androstene-11β,17β-diol-3-one (VII). A suspension of 175 mg. of VI and 140 mg. of *N*-bromosuccinimide in 2.34 ml. of acetone was cooled in an ice bath and 0.56 ml. of 0.27*N* perchloric acid (0.46 g. 70% perchloric acid in 16.5 ml. water) was added. The suspension was stirred by means of a magnetic stirrer for 30 min., an additional 1 ml. of acetone was added and stirring continued for 40 min. Excess *N*-bromosuccinimide was destroyed by the addition of 0.24 ml. of allyl alcohol. The reaction mixture was diluted with 30 ml. of water and the product, 180 mg., was separated, and air dried. The crude 9α-bromo-16α,17α-dimethyl-4-androstene-11β,17β-diol-3-one, m.p. 183–188° dec., was used in the next step.

16α,17α-Dimethyl-9β,11β-oxido-4-androstene-17β-ol-3-one (VIII). A suspension of 90 mg. of bromohydrin above and 75 mg. of anhydrous potassium acetate in 2 ml. of anhydrous ethanol was refluxed under nitrogen for forty min. The suspension was cooled, diluted with ice water, and extracted with chloroform. The chloroform layer was dried over sodium sulfate and concentrated *in vacuo*. The crude product was adsorbed on 7.0 g. of acid-washed alumina (Merck) from benzene. Elution with ether–chloroform (9:1 to 5:5) afforded 32 mg. of 16α,17α-dimethyl-9β,11β-oxido-4-androstene-17β-ol-3-one. The analytical sample was crystallized from ether, m.p. 178–181°; α_D^{23} −67°; $\lambda_{max}^{CH_3OH}$ 244 mμ, ε 14,800.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 75.66; H, 8.99.

9α-Fluoro-16α,17α-dimethyl-4-androstene-11β,17β-diol-3-one (IX). A solution of 200 mg. of VIII in 1.6 ml. of chloroform cooled to −40° was added to 10 ml. of a reagent consisting of 10 ml. of a 2:1 mixture by weight of hydrogen fluoride in tetrahydrofuran, 10.8 ml. of tetrahydrofuran, and 10 ml. of chloroform maintained at −80°. The tetrahydrofuran was reagent grade dried over potassium hydroxide and filtered. The reaction mixture was placed in a Dewar flask and maintained at −30° for 4 hr. After this time the reaction mixture was poured into a stirred mixture consisting of 5 g. of anhydrous potassium carbonate, 15 ml. of ice and water, and 25 ml. of chloroform. The chloroform layer was separated, and the aqueous phase was re-extracted with chloroform. The combined chloroform layer was dried over sodium sulfate and concentrated *in vacuo*. Chromatography on 20 g. acid-washed alumina (Merck) and elution with chloroform–ether (8:2) afforded 94 mg. of crude 9α-fluoro-16α,17α-dimethyl-4-androstene-11β,17β-diol-3-one. Crystallization from ethyl acetate afforded 71 mg., m.p. 256–259°; α_D^{23} +85°; $\lambda_{max}^{CH_3OH}$ 239 mμ, ε 17,800.

Anal. Calcd. for $C_{21}H_{31}O_3F$: C, 71.95; H, 8.91; F, 5.42. Found: C, 72.37; H, 8.89; F, 5.21.

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Synthesis of *N*-Substituted Derivatives of Carnosine and Homocarnosine

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The preparation of *N*-substituted derivatives of dipeptides in high yield cannot readily be achieved by direct attack on the free amino group of the dipeptide. The alternative method is to condense the carboxyl group of the *N*-substituted amino acid with the amino group of the second esterified amino acid such as in the *p*-nitrophenyl ester procedure which is used here. It has been previously applied, with considerable success, to the synthesis of large peptides.[1]

These compounds have been prepared for use as substrate analogs for enzyme specificity studies, including carnosine forming and splitting enzymes and the acylases. They will also serve as starting materials for the preparation of substituted lactam derivatives of the dipeptides which will be reported later.

TABLE I

Compound	Yield, %	M.P., Obs. (uncorr.)	M.P., Lit.	Ref.
Acetyl-β-alanine	85	78–80	78–81	[a]
Benzoyl-β-alanine[b]			133–134	[c]
Phthaloyl-β-alanine	90	152–153	152–153	[d]
Phthaloyl-γ-amino-butyric acid	95	117–119	115–117.5	[e]

[a] P. J. Fodor *et al.*, *J. Biol. Chem.*, **178**, 503 (1949). [b] Obtained from Mann Research Labs. Inc., N. Y. [c] C. C. Barker, *J. Chem. Soc.*, 317 (1954). [d] R. A. Turner, *J. Am. Chem. Soc.*, **75**, 2388 (1953). [e] G. Kushinsky *et al.*, *Biochem. Z.*, **327**, 314 (1955); *Chem. Abstr.*, **50**, 7164 (1956).

(1) M. Bodansky and V. duVigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).

TABLE II

p-Nitrophenyl Ester of	Yield, %	M.P. (uncorr.)	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
Acetyl-β-alanine	60	111–113	52.38	4.76	11.11	52.47	5.06	10.89
Benzoyl-β-alanine	90	163–164	61.14	4.49	8.92	61.21	4.70	9.63
Phthaloyl-β-alanine	50	210–212	60.00	3.56	8.24	60.23	3.71	8.42
Phthaloyl-γ-aminobutyric acid	85	131–132	61.02	3.95	7.91	61.29	4.24	7.79

TABLE III

Methyl Ester of	Yield, %	M.P., (uncorr.)	$[\alpha]_D^{28}$ (C = 1)	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
Acetyl-β-alanyl-*l*-histidine[a]	65	151–152	−3.9 (Water)	51.06	6.38	19.86	51.67	6.77	19.10
Benzoyl-β-alanyl-*l*-histidine	80	131–132	−4.3 (Water)	59.30	5.81	16.28	59.10	6.07	16.11
Phthaloyl-β-alanyl-[d] *l*-histidine[a]	69	192–193	−8.0 (DMF)						
Phthaloyl-γ-aminobutyryl-*l*-histidine	70	145–148	−8.5 (Chloroform)	59.38	5.21	14.61	58.69	5.50	15.03

[a] The *N*-substituted dipeptides, acetyl-β-alanyl-*l*-histidine and phthaloyl-β-alanyl-*l*-histidine, have been previously prepared.

TABLE IV

Compound	Yield, %	M.P., Obs. (uncorr.)	M.P., Lit.	Ref.	$[\alpha]_D^{29}$ Obs. (C = 1)	$[\alpha]_D^{23}$, Lit. (C = 1)	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
Acetyl-β-alanyl-*l*-histidine (Acetylcarnosine)			209–210	[a]								
Benzoyl-β-alanyl-*l*-histidine (Benzoyl-carnosine)	73	214–216			+10.0° (H_2O)		58.18	5.46	16.97	58.00	6.00	16.44
Phthaloyl-β-alanyl-*l*-histidine (Pthaloyl-carnosine)	80	221–224	221–224	[b]	+21.5° (H_2O)	+21.6 (H_2O)						
Phthaloyl-γ-amino-butyryl-*l*-histidine (Phthaloyl-homocarnosine)	80	211–213			+18.0° (H_2O)		58.38	4.87	15.14	58.21	4.69	15.06

[a] A. Lukton and A. Sisti, *J. Org. Chem.*, 26, 617 (1961). [b] See footnote *d*, Table I.

In this procedure the *N*-substituted derivatives of β-alanine or γ-aminobutyric acid were condensed with histidine methyl ester using *N,N'*-dicyclohexylcarbodiimide[2] as the condensing agent. The resulting *N*-substituted dipeptide methyl esters were then converted to the free acids by mild hydrolysis.

The *N*-substituted β-alanine and γ-aminobutyric acid derivatives were prepared. Their melting points agreed with those previously reported. The values are given in Table I. In Table II, melting points and elemental analyses are given for the *p*-nitrophenyl esters of the *N*-substituted amino acids. Table III shows the melting points, specific rotation values, and elemental analyses for the *N*-substituted dipeptide methyl esters. Table IV lists the individual dipeptides and their properties.

(2) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, 77, 1067 (1955).

EXPERIMENTAL

p-Nitrophenyl esters of N-substituted amino acids. To a 0.3*M* solution of the *N*-substituted amino acid in ethyl acetate, *p*-nitrophenol was added in 20% excess of the calculated amount. *N,N*-dicyclohexylcarbodiimide was then added in 10% excess at 0°. After 0.5 to 5 hr., the mixture was allowed to come to room temperature. The *N,N*-dicyclohexylurea was filtered off and washed with ethyl acetate. The combined filtrate and washings were evaporated to dryness. The residue was dissolved in hot absolute ethanol and the solution cooled. In the preparation of *p*-nitrophenyl esters of phthaloyl-β-alanine and phthaloyl-γ-aminobutyric acid dioxane was the solvent used.

Methyl esters of N-substituted dipeptides. A chloroform solution of the *p*-nitrophenyl ester was added in equimolar quantity to a previously prepared chloroform solution of *l*-histidine methyl ester. The histidine solution was prepared by the method of Davis and Smith.[3] The yellow solution that resulted was allowed to stand at room temperature

(3) N. C. Davis and E. L. Smith, *Biochem. Prep.*, 4, 38, (1955).

for two days. After removal of the solvent, ether was added to the residue and the suspension stirred at room temperature. The precipitate which formed was filtered and washed with ether. The impure dipeptide esters were recrystallized from normal propanol.

N-Substituted dipeptides. Three grams of the dipeptide methyl ester was added to a mixture of 50 ml. of acetone and 50 ml. of water. To this mixture was added approximately 8 ml. of 1*N* sodium hydroxide, and the solution was stirred for 1 hr. The reaction mixture was then neutralized with 6*N* hydrochloric acid and evaporated to dryness. The residue was extracted with several 5-ml. portions of hot absolute ethanol. The sodium chloride was filtered off and the filtrate evaporated to dryness *in vacuo*. The impure dipeptide was crystallized from propanol. In the case of benzoyl-*β*-alanyl-*l*-histidine, isopropanol was used as the solvent for crystallization.

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Reactions of Nitric Oxide. Synthesis of Salts of *p*-*N*-Nitrosohydroxylamino-*N'*-nitroso Substituted Anilines

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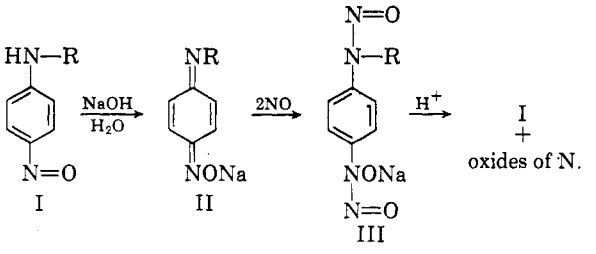
In a previous paper[2] we described the addition of nitric oxide to salts of quinonedioximes. We have extended this study to include the salts of quinonemonoxime imines (nitroso anilines).

In basic solution *p*-nitrosoanilines, I, tautomerize to quinone monoxime imines, II. This is analogous to the nitrosophenol–quinone monoxime system. The extent of formation of II is dependent on the solvent system and the concentration of the base. Under normal conditions I appears to have considerable stability as indicated by its green nitroso color and the large amount of base necessary to obtain a good conversion to III without impurities.

When a solution of I was dissolved in an excess of aqueous sodium hydroxide and treated with nitric oxide at 60 p.s.i., two moles of gas were absorbed to give the corresponding *p*-*N*-nitrosohydroxylamino-*N*-nitroso-*N'*-substituted anilines III. Using a methanol–methoxide system, a four molar absorption of nitric oxide was observed and an unstable diazonium compound was isolated and identified by infrared spectrum. The formation of this diazo compound is due to addition of nitric oxide to the nitroso group.[3,4]

Acidic decomposition of III (R = C_6H_5) resulted in the liberation of oxides of nitrogen, and 54% of I was isolated. Using precipitation techniques analogous to those of cupferron, the silver salt of III (R = C_6H_5) was prepared and converted to a methyl derivative. Because of reasons described previously[2] the exact structure of this compound is unknown.



EXPERIMENTAL

Apparatus. This reaction may be carried out in any type of stirred stainless steel pressure vessel or a Paar low pressure hydrogenator modified with a stainless steel tank and gauge. All tubing and valves are stainless steel. The nitric oxide was 99+ % pure (Olin Mathieson), and was passed through a stainless steel Kuentzel bomb (1 ft. long, 2 in. i.d.) packed with sodium hydroxide. Care must be taken that gas inlet and exit ports do not become clogged.

General Procedure. The *p*-nitroso-*N*-substituted aniline was dissolved in sodium hydroxide solution, filtered, placed in a reaction bottle, and cooled. The reaction bottle was placed in the Paar apparatus and the oxygen was removed by evacuation and flushing with oxygen-free nitrogen. The bottle was finally evacuated. Nitric oxide was admitted and the shaker started. When the absorption of gas was completed, the nitric oxide was removed by flushing with nitrogen and evacuation with a water aspirator. There is considerable foaming and care must be taken not to clog the exit ports. The product was isolated by filtration, washed, and dried. Compounds so prepared are listed in Table I.

Decomposition of III (R = C_6H_5). To 10 ml. of a stirred solution of concd. hydrochloric acid, 1.0 g. of III was added slowly. There was considerable foaming, and this was allowed to subside before additional III was added. One hour after all of the III was added, the solution was diluted with water and made alkaline with sodium hydroxide. Carbon dioxide was passed into this solution until precipitation was complete, and 0.70 g. of black solid, m.p. 110–113°, was isolated. The solid was redissolved in 20 ml. of 10% sodium hydroxide, filtered, and re-treated with carbon dioxide. The brown solid was collected by filtration, washed, and dried to give 0.38 g. (54%) of I (m.p. 141.5–143.5°). Infrared spectrum and mixed melting point were identical to that of an authentic sample.

Methyl ether of III (R = C_6H_5). To a filtered solution of 1.4 g. of III in 100 ml. of 50% aqueous methanol was added 0.90 g. of silver nitrate in 50 ml. of distilled water. The red precipitate was collected on a filter, washed, and dried to give 2.0 g. of the silver salt. To a suspension of this salt in 50 ml. of methanol was added 2 ml. of methyl iodide, and the mixture was stirred for 0.5 hour. The precipitate was collected on a filter and washed with methanol. Water was added to the

(1) Present address: American Viscose Corp., Marcus Hook, Pa.

(2) M. Danzig, R. Martel, and S. R. Riccitiello, *J. Org. Chem.*, **26**, 3327 (1961).

(3) Bamberger, *Ber.*, **30**, 508 (1897).

(4) Nesmezanov and Iaffe, *J. Gen. Chem. U.S.S.R.*, **11**, 392 (1941).