suspension was transferred to another flask filled with nitrogen. To this there was added 2.6 g. (0.01 mole) of triphenylcarbinol dissolved in 50 ml. of ether. Very little heat was evolved during the addition while the reaction mixture became slightly deeper in color. After 48 hours of stirring at room temperature Color Test I[8] was still positive. Water was added to the mixture and it was filtered to separate 1.8 g. of cream-colored solid melting from 230–235°. This crude product was dissolved in hot benzene and filtered to remove a trace of gray specks. From the filtrate there was obtained 1.6 g. (48%[9]) of pure tetraphenylsilane (mixed m.p.) melting at 234–235°. The ethereal solution from the hydrolysis mixture was separated and dried over sodium sulfate. Evaporation of the solvent left 5.5 g. of a colorless solid residue melting from 145–151°. This was dissolved in hot petroleum ether (b.p. 60–70°) and allowed to cool. The first fraction of 0.2 g. of white solid melting at 225° was shown to be impure tetraphenylsilane after recrystallization. The petroleum ether solution was concentrated to give 2.7 g. of colorless crystals melting at 149–151°. It was suspected that this material might be a mixture of triphenylcarbinol and triphenylsilanol. A prepared mixture of about equal parts of triphenylcarbinol (m.p. 160–162°) and triphenylsilanol (m.p. 150–151°) melted at 151–153°. After some preliminary studies the reaction products were separated in the following manner.

The mixture of reaction products was refluxed in formic acid (Baker and Adamson, 98–100%) for 15 hours. Some colorless crystals were formed. The resulting mixture was diluted with 4 volumes of water and filtered. The solid residue (2.3 g.) was dissolved in petroleum ether (b.p. 60–70°) to give 0.5 g. of lustrous plates melting at 227–229°; a mixed melting point with hexaphenyldisiloxane showed no depression. This quantity corresponded to 10% of triphenylsilanol (presumably from the hydrolysis of excess triphenylsilylpotassium) originally present in the mixture of triphenylcarbinol and triphenylsilanol. The mother liquor after the removal of hexaphenyldisiloxane was evaporated to yield 1.6 g. of white crystals melting at 90–92°; a mixed melting point with an authentic sample of triphenylmethane was not depressed. The amount of triphenylmethane thus obtained was equivalent to 66% recovery of the triphenylcarbinol.

Reaction of Triphenylsilylpotassium with Benzohydrol.—The procedure used in this reaction was exactly the same as that described in the previous section except that 0.01 mole of benzohydrol was used instead of triphenylcarbinol. Following hydrolysis there was obtained, by filtration, 3.0 g. of crude tetraphenylsilane. This was purified as described above and 2.7 g. (80%[9]) of pure tetraphenylsilane (mixed m.p.) was isolated. From the ethereal solution 2.8 g. of white solid melting at 67–69° was obtained by recrystallization. Analyses of this product showed that it contained 3.0–3.5% of silicon. Melting point studies also pointed out that the presence of triphenylsilanol does not depress appreciably the melting point of benzohydrol. However, no attempt was made to separate this mixture.

Reaction of Triphenylsilylpotassium with Benzyl Alcohol.—Using exactly the same procedure described in the reaction with triphenylcarbinol except that 0.01 mole of benzyl alcohol was used instead of triphenylcarbinol, there was obtained 2.6 g. of crude and 2.3 g. (68%[9]) of pure tetraphenylsilane (mixed m.p.) melting at 234–235°. The ethereal solution was worked up as usual to give 1.0 g. (18%) of triphenylsilanol (mixed m.p.). Evaporation of the mother liquor resulted in an oil.

Reaction of Triphenylsilylpotassium with Triphenylsilanol.—The procedure used in this reaction was also the same as that for the triphenylcarbinol except that 0.01 mole of triphenylsilanol was used instead of triphenylcarbinol. Following hydrolysis there was obtained 3.2 g. of an insoluble solid melting at 232–235°. Recrystallization of this crude product gave 2.9 g. (86%[9]) of tetraphenylsilane (mixed m.p.) melting at 234–235°. The solid obtained from the ethereal solution was recrystallized twice from petroleum ether (b.p. 60–70°) to yield 3.2 g. of pure triphenylsilanol (mixed m.p.) melting at 150–151°. It was believed that part of the triphenylsilanol was the starting material and the rest came from the hydrolysis of triphenylsilylpotassium.

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16α-Methoxy-5-pregnen-3β-ol-20-one

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Recently Mattox[1] reported the isolation of 16α-methoxy-5-pregnen-3β-ol-20-one acetate obtained by the action of methanolic hydrogen chloride on 5,16-pregnadien-3β-ol-20-one followed by acetylation of the product. Fukushima and Gallagher[2] isolated the same substance after saponifying pregnadienolone acetate in methanol and acetylating the crude product. Since we had previously isolated the unacetylated 16α-methoxy-5-pregnen-3β-ol-20-one by use of the Freudenberg[3] conditions, we are giving our results on this work.

During our studies on the acid hydrolysis of cortisone acetate, we isolated a product for which one possible structure was a 16,21-dimethoxypregnetrione.[1,4] In order to investigate whether the addition of methanol to the Δ^{16}-20-keto system would proceed by acid catalysis, we treated pregnadienolone in methanol with acetyl chloride and obtained a product having an E-value of 72 at 239 mμ, corresponding to the presence of about 25% of the original α,β-unsaturated ketone. The major product, 16α-methoxy-5-pregnen-3β-ol-20-one was separated by chromatography in about 35% yield, and was found to be identical with that obtained upon treatment of pregnadienolone acetate with methanolic alkali.

Treatment of the methoxypregnenolone with boiling acetic anhydride did not split out methanol, but only gave the known 3-acetate.[2] Cleavage was successful, however, when acetic anhydride catalyzed with hydrochloric acid was used, and it was possible to isolate 5,16-pregnadien-3β-ol-20-one acetate from the mixture.

Experimental[5]

16α-Methoxy-5-pregnen-3β-ol-20-one from 5,16-Pregnadien-3β-ol-20-one.—5,16-Pregnadien-3β-ol-20-one (2.0 g.) was dissolved in 160 ml. of abs. methanol and cautiously treated with 4 ml. of acetyl chloride (equiv. to 1% HCl). The mixture was allowed to stand 18 hours at room temperature. A solution of 5 g. of sodium acetate in 500 ml. of water was added and the precipitate which formed was collected by suction and washed with water. It weighed 2.1 g., m.p. 125–128°, E 72 (239 mμ in 95% ethanol).

One gram of the material was dissolved in benzene and chromatographed on 25 g. of Florisil as follows (100 ml. fractions):

Fraction	Eluant	Wt., mg.	M.p., °C.	$E^{1\%}_{1\text{ cm}}$	Product
1–4	Hexane	Neg.			
5–14	3:1 Hexane–C_6H_6	235	180–195	200	Pregnadienolone
15–22	1:1 Hexane–C_6H_6	50	150–180	150	Mixture
23–37	Benzene	360	144–154	22	16-Methoxy-pregnenolone
38–48	1% MeOH in C_6H_6	320	Oil		

Upon crystallization from methanol, fractions 23–37 gave 16α-methoxy-5-pregnen-3β-ol-20-one, m.p. 151–153°, $[\alpha]^{25}D$ −23.9° (EtOH). *Anal.* Calcd. for $C_{22}H_{34}O_3$: C, 76.26; H, 9.89. Found: C, 76.51; H, 10.00. The ratio to re-

(1) V. R. Mattox, THIS JOURNAL, **74**, 4340 (1952).
(2) D. K. Fukushima and T. F. Gallagher, *ibid.*, **73**, 196 (1951).
(3) K. Freudenberg and W. Jakob, *Ber.*, **74**, 1001 (1941).
(4) D. Gould and E. B. Hershberg, in press.
(5) All melting points are corrected. $E^{1\%}_{1\text{ cm}} = (l/cd) \log I_0/I$; ϵ = M.W. × E/10. Analyses and optical data obtained by the Microanalytical Department of these laboratories.

covered starting material taking account of the purity of fractions 5–14 was about 2:1.

The same product was obtained[6] when 50 g. of 5,16-pregnadien-3β-ol-20-one acetate was saponified by refluxing for 6 hours in 1200 ml. of methanol with 20 g. of potassium hydroxide in 50 ml. of water. The water-precipitated product was dissolved in chloroform, the solution was washed neutral with water, dried and evaporated. The residue was recrystallized twice from ethyl acetate to give 16α-methoxy-5-pregnen-3β-ol-20-one, m.p. 151–152°, $[\alpha]^{24}D$ −24.4° (EtOH), no absorption between 220 and 300 mμ. *Anal.* Calcd. for $C_{22}H_{34}O_3$: C, 76.26; H, 9.89. Found: C, 76.40; H, 9.53.

5,16-Pregnadien-3β-ol-20-one Acetate from 16α-Methoxy-5-pregnen-3β-ol-20-one.—When 16-methoxypregnenolone was refluxed one hour with acetic anhydride, the product was not that of dehydration, but was the known 16α-methoxy-5-pregnen-3β-ol-20-one acetate, m.p. 154–156°, $[\alpha]^{23}D$ −27.1° (EtOH).[2]

A sample of 5 g. of 16-methoxypregnenolone was dissolved in 20 ml. of acetic anhydride and treated with 2 ml. of concd. hydrochloric acid. The mixture was refluxed 20 minutes and chilled. There was obtained upon cooling 0.8 g. of crystalline 5,16-pregnadien-3β-ol-20-one acetate, m.p. 169–172°, m.p. mixed with authentic material, 171–174°, ε 9100 (234 mμ in *i*-octane).

From the mother liquor, there was obtained a low melting product which gave on crystallization from isopropyl alcohol 16α-methoxy-5-pregnen-3β-ol-20-one acetate, m.p. 153–155°.

(6) We are indebted to T. Clayton and J. R. Confroy for permission to report these experimental data.

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A New Synthesis of Carnosine[1]

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The use of N-phthalylamino acids as coupling agents in the syntheses of peptides has been studied by several groups of investigators.[4] The peptides prepared by this method include di- and tripeptides of the simple amino acids, as well as several peptides containing cysteine, glutamic acid and aspartic acid. There has been no reported use of this procedure for the preparation of peptides containing histidine.[5]

In a continuation of a previous investigation on the metabolism of β-alanine,[6] it was considered desirable to evaluate methods for the synthesis of carnosine labeled with N^{15} in the β-alanine portion of the molecule. An examination of the methods[7] available for the preparation of this interesting peptide indicated that the procedures were either too involved or that the yields were too low.

The phthalyl method for the preparation of carnosine was investigated since N-phthalyl-β-alanine could be prepared in good yields from phthalimide and methyl acrylate. N-Phthalyl-β-alanyl chloride was prepared by the method of Sheehan and Frank,[4b] but the direct coupling of the acid chloride with L-histidine in aqueous dioxane in the presence of magnesium oxide was unsuccessful. It was found that the N-phthalyl-β-alanyl chloride underwent a very rapid hydrolysis in aqueous solution. N-Phthalylcarnosine methyl ester was obtained by the direct condensation of either the acid chloride or the azide with L-histidine methyl ester in chloroform. However, difficulties were encountered in converting the methyl ester to phthalylcarnosine by both acid and mild alkaline saponification. The latter procedure yielded the phthalamic acid derivative of carnosine.

N-Phthalylcarnosine was obtained by treating the azide with the sodium salt of L-histidine in 50% dioxane. Removal of the phthalyl group was accomplished by the method of Sheehan and Frank[4b] which resulted in the isolation of carnosine hydrochloride as an amorphous solid. Treatment of N-phthalylcarnosine with phenylhydrazine by the method of Shuman and Boissonnas[4d] gave carnosine directly in moderate yields.

Experimental

Phthalyl-β-alanyl Chloride.—Phthalyl-β-alanine was converted to the acid chloride by the procedure described by Sheehan and Frank[4b] for the preparation of phthalylglycyl chloride. The compound was obtained in 85–90% yields, m.p. 102–103°.

Phthalyl-β-alanyl Azide.—A solution of 2.40 g. (0.01 mole) of phthalyl-β-alanyl chloride was dissolved in 25 ml. of cold acetone, and the solution mixed with 0.07 g. of sodium azide dissolved in 2 ml. of water. The reaction mixture was agitated for five minutes while the flask was immersed in an ice-bath. A white oil separated initially which gradually solidified after about ten minutes. The solid material was obtained by filtration, and dried *in vacuo* over phosphorus pentoxide. The crude material weighed 2.1 g., and decomposed explosively at 89–90°.

Phthalylcarnosine Methyl Ester.—A solution of histidine methyl ester in 25 ml. of chloroform was prepared from 2.42 g. (0.01 mole) of histidine methyl ester dihydrochloride by the method of Fischer and Cone.[8] To the chloroform solution was added 2.4 g. (0.01 mole) of phthalyl-β-alanyl azide, and the solution was allowed to stand overnight at room temperature. A white crystalline solid weighing 2.2 g. was obtained. The product was recrystallized from water, m.p. 193–194°.

Anal. Calcd. for $C_{18}H_{18}O_6N_4$: N, 15.1. Found: N, 14.9.

Phthalylcarnosine.—Histidine hydrochloride monohydrate, 1.05 g. (0.005 mole), was dissolved in 9.9 ml. of 1.02 *N* sodium hydroxide. The phthalyl-β-alanyl azide, 1.2 g. (0.005 mole), was dissolved in 25 ml. of dioxane, and the resulting solution poured into the aqueous histidine solution. The mixture was allowed to stand in the refrigerator overnight. The reaction mixture was neutralized with 4.7 ml. of 0.994 *N* sulfuric acid. The resulting mixture was filtered from a small amount of insoluble material, and concentrated to dryness at 50° under reduced pressure. The residue was extracted with three 25-ml. portions of boiling methanol, and the combined filtrates were placed in the refrigerator. A white crystalline solid was obtained weighing 0.7 g., m.p. 225–230°, dec. Recrystallization from methanol containing a trace of water brought about no change in the decomposition point.

Anal. Calcd. for $C_{17}H_{16}O_5N_4$: N, 15.68; neut. equiv., 357. Found: N, 15.55; neut. equiv., 357.

Carnosine (Method A).—The procedure used for the removal of the phthalyl group is identical with that described by Sheehan and Frank.[4b] Phthalylcarnosine, 3.56 g. (0.01 mole) was dispersed in 20 ml. of 95% ethyl alcohol, and 25 ml. of 1 *M* hydrazine hydrate in 95% ethyl alcohol was

(1) Presented before the Division of Biological Chemistry, A. C. S. Meeting, Atlantic City, N. J., 1952.

(2) American Cancer Society Research Fellow.

(3) Alrose Chemical Co., Providence, R. I.

(4) (a) F. E. King and D. A. Kidd, *J. Chem. Soc.*, 3315 (1949); (b) J. C. Sheehan and V. S. Frank, THIS JOURNAL, **71**, 1856 (1949); (c) W. Grassmann and F. Shulte-Uebbing, *Ber.*, **83**, 244 (1950); (d) I. Shuman and R. A. Boissonnas, *Nature*, **169**, 154 (1952).

(5) A private communication from Dr. R. A. Turner has disclosed the synthesis of carnosine from phthalylcarnosine.

(6) J. Graf and H. Hoberman, *J. Biol. Chem.*, **186**, 369 (1950).

(7) (a) R. H. Sifferd and V. du Vigneaud, *ibid.*, **108**, 753 (1935); (b) G. Barger and F. Tutin, *Biochem. J.*, **12**, 408 (1918); (c) L. Baumann and J. Ingvaldsen, *J. Biol. Chem.*, **35**, 263 (1918).

(8) E. Fischer and L. H. Cone, *Ann.*, **363**, 107 (1908).