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).

added. The mixture was heated at reflux for one hour, and the reaction mixture concentrated to dryness. The residue was taken up in 50 ml. of 1.2 *M* hydrochloric acid and heated on a water-bath at 40–50° for 15 minutes. The mixture was concentrated to dryness under reduced pressure in a water-bath at 50°. The residue was dispersed in 30 ml. of water, and the insoluble phthalylhydrazide removed by filtration. The aqueous solution, on dilution with 75 ml. of 95% ethyl alcohol, precipitated 2.9 g. of carnosine hydrochloride as an amorphous, hygroscopic solid. It was converted to carnosine by passing an aqueous solution of the hydrochloride through an ion exchange column containing Deacidite. The aqueous eluate was concentrated to dryness and the residue was recrystallized from aqueous alcohol to give 0.6 g. of carnosine.

Anal. Calcd. for $C_9H_{14}O_3N_4$: N, 24.7. Found: N, 24.5.

(**Method B**).—The procedure was a modification of that described by Shuman and Boissonnas.[4d] To 1.78 g. of phthalylcarnosine (0.005 mole), there was added 25 ml. of 95% ethyl alcohol, 0.5 g. of triethylamine and 1.55 g. of phenylhydrazine. The mixture was refluxed for three hours on a water-bath. At the completion of the heating period, the clear yellow solution was cooled, and acidified with 1 g. of glacial acetic acid, and the mixture poured into 80 ml. of methyl ethyl ketone. An amorphous precipitate was obtained which was dissolved in 5 ml. of water and reprecipitated by the addition of 75 ml. of 95% ethyl alcohol. The dried product weighed 0.63 g., and after recrystallization from aqueous ethyl alcohol, 0.41 g. of carnosine was obtained.

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Stereochemistry of 1,4-Addition. II. The Bromination of Butadiene

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A recent paper[1] reports the 1,4-adduct of bromine and butadiene, 1,4-dibromo-2-butene (m.p. 53°), to have the *cis* configuration. This claim rests on the observed Raman frequency of 1655 cm.$^{-1}$, associated with *cis*-ethylenic double bonds,[2] and is the basis for the assumption that butadiene enters into reaction with bromine in the "bent" or s-*cis* form.

It has been shown that a frontal transition state cannot be of any appreciable importance in the 1,4-addition of chlorine to butadiene[3]; the implied claim that this argument does not apply in the case of bromine addition would therefore be of considerable interest. The following observations may however be marshaled as convincing evidence in favor of the identity of 1,4-dibromo-2-butene (m.p. 53°), I, with *trans*-1,4-dibromo-2-butene. (a) The infrared spectrum of I exhibits a pronounced and characteristic[4] *trans* peak near 10.3 μ, absent in the saturated analog.[5]

(b) Lithium aluminum hydride reduction of I affords *trans*-2-butene, as evidenced by conversion to *meso*-2,3-dibromobutane.[6] The present author has repeated this experiment and obtained *meso*-2,3-dibromobutane, b.p. 46° (14 mm.), $n^{25}D$ 1.5088 (repd.[7] $n^{25}D$ 1.5091).

(c) The dipole moment of I, 1.63 *D*, is similar to that of *trans*-1,4-dibromo-2,3-dimethyl-2-butene, 1.72 *D*, but smaller than that of the *cis*-isomer 2.49 *D*.[8]

(d) A *cis*-1,4-dibromo-2-butene (II), prepared from authentic *cis*-2-butene-1,4-diol,[9] differs from I in a manner characteristic[4a] of the relative properties of *cis* and *trans* isomers. Thus, the melting point of I is higher than that of II, II is thermally unstable with respect to I, and I and II give different 1,2,3,4-tetrabromobutanes, m.p. 116 and 39°, respectively.

The evidence here adduced compels us to maintain that as yet no satisfactory experimental basis exists for the view of frontal attack in the 1,4-addition of halogen to butadienes.[10] Equally, the tetrabromides, m.p. 116 and 39°, must be assigned the *meso* and racemic configurations, respectively, the claim[1] to the contrary notwithstanding.

(1) Ya. M. Slobodin and S. A. Zaboev, *Zhur. Obshchei Khim.* (*J. Gen. Chem., U. S. S. R.*), **22**, 603 (1952).

(2) *E. g.*, N. Sheppard and D. M. Simpson, *Quart. Revs.*, **6**, 1 (1952).

(3) K. Mislow and H. M. Hellman, THIS JOURNAL, **73**, 244 (1951).

(4) *E.g.* (a) L. Crombie, *Quart. Revs.*, **6**, 101 (1952); (b) L. Crombie, *J. Chem. Soc.*, 2997 (1952); (c) F. Sondheimer, THIS JOURNAL, **74**, 4040 (1952); (d) K. Mislow, *ibid.*, **74**, 5155 (1952).

(5) A Baird Model B instrument with 0.1-mm. cells was employed. In this connection, the assistance afforded by correspondence with Dr. Ralph Nusbaum and his staff, Spectroscopy Section, Atomic Energy Project, U. C. L. A., Los Angeles, Calif., is gratefully acknowledged.

(6) L. W. Trevoy and W. G. Brown, THIS JOURNAL, **71**, 1675 (1949).

(7) R. T. Dillon, W. G. Young and H. J. Lucas, *ibid.*, **52**, 1953 (1930).

(8) O. J. Sweeting and J. R. Johnson, *ibid.*, **68**, 1057 (1946).

(9) A. Valette, *Ann. chim.*, [12] **3**, 644 (1948).

(10) Some recent developments pertaining to this concept as originally expressed (ref. 3): the 1,4-addition of sulfur dioxide to terminally substituted butadienes involves the s-*cis* form of butadiene (O. Grummitt and J. Splitter, THIS JOURNAL, **74**, 3924 (1952)); halonium ions can be incorporated in a stable symmetrical 5-membered ring as part of a diphenyl system (R. B. Sandin and A. S. Hay, *ibid.*, **74**, 274 (1952)); the argentation constants of monoargentated *cis*-1,2-diiodoethylene and *o*-diiodobenzene are indicative of the existence of symmetrical 5-membered onium rings (L. J. Andrews and R. M. Keefer, *ibid.*, **73**, 5733 (1951)).

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D-Glucuronolactone Isonicotinyl Hydrazone

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D-Glucuronolactone isonicotinyl hydrazone, a new compound with comparatively low toxicity and very high antitubercular activity *in vitro* as well as *in vivo*,[1] may be prepared easily by the following procedure.

D-Glucuronolactone (Eastman Kodak Co., 88 g.) was placed in a 3-l. round-bottomed flask and covered with 1.5 liters of methyl alcohol (acetone-free). The mixture was boiled gently on the steam-bath for 10 minutes when a clear solution was obtained. To the hot solution, isonicotinic acid hydrazide (Pfizer, 70 g.) was added all at once. The mixture was boiled vigorously for 10 minutes and the clear solution filtered without suction through a piece of lens paper into a 2-l. erlenmeyer flask. After standing for 24 hours at room temperature, the beautiful crystals (white rods and narrow plates) were filtered off with suction, washed with a small amount of methyl alcohol, and sucked completely to dryness. The product was dried in a vacuum desiccator for 3 days; yield 148 g. The product thus ob-

(1) Biological tests were performed by W. B. Sutton of the Lilly Research Laboratories, Indianapolis, Indiana, and the results later confirmed by Dr. E. G. Roberts of Stanford University School of Medicine. The new drug is now undergoing clinical trial. Results will be reported elsewhere.