at reduced pressure, diluted with water, and freeze-dried. Fractions 34–169 (2.4 g.) contained II.

A 500-mg. sample of the above material, still containing some I, was dissolved in the minimum amount of ethyl acetate (10 volumes), butanol (1 volume), saturated with water, and adjusted to pH 3 with hydrochloric acid. The solution was charged onto a powdered cellulose[16] column 2.7 cm. × 36.0 cm. impregnated with the same solvent system, which was also used for development. Fractions 2–25 (of 50) were combined and the solvent removed to give 78.6 mg. of amorphous yellow solid.

Crystallization was accomplished in a mixture of methanol–isopropyl alcohol–0.1 *N* hydrochloric acid. The crude, crystalline, hydrochloride could not be recrystallized satisfactorily, although it was nearly homogeneous on paper chromatograms. A 100-mg. sample of the hydrochloride was dissolved in 3 ml. of 0.01 *N* methanol–hydrochloric acid, and 17 mg. of sulfosalicylic acid added. The clear solution was stored at 5° overnight. After removal of 3 mg. of amorphous precipitate 2 ml. of water was added and the suspension warmed. On cooling 21 mg. of light yellow crystals of 2-acetyl-2-decarboxamido-7-chlorotetracycline sulfosalicylate monomethanolate separated. Second crops totaled 61 mg. The pure salt consisted of golden needles, m.p. > 200° dec.

Anal. Calcd. for $C_{23}H_{24}NO_8Cl \cdot C_7H_6O_6S \cdot CH_3OH$: C, 51.13; H, 4.71; N, 1.92; Cl, 4.84; acetyl (acid hydrolysis) 5.92. Found: C, 50.93; H, 5.22; N, 1.85; Cl, 4.93; acetyl (acid hydrolysis) 5.54.

Bioassay, 205 tetracycline units/mg. (310 units/mg. as free base.)

Ultraviolet absorption peaks were located at (in methanol 0.01 *N* in hydrochloric acid): 232 mμ (ε 28,740), 279 mμ (ε 15,890), 310s mμ, 337s mμ, 373 mμ (ε 14,190); (in methanol 0.01 *N* in sodium hydroxide): 237 mμ (ε 22,500), 278 mμ (ε 19,660), 387 mμ (ε 16,630). In dioxane solution the sulfosalicylate salt of III showed a sharp infrared absorption peak at 5.96 μ.

Acid Degradation of 2-Acetyl-2-decarboxamido-7-chlorotetracycline.—Acid degradation by either of the methods mentioned for I above gave what appeared on paper chromatograms (systems C and D) to be an anhydro derivative. Confirmation was obtained by the ultraviolet examination of a sample eluted from a paper strip with methanol 0.01 *N* in hydrochloric acid, which showed the peak at 437 mμ characteristic of anhydro 7-chlorotetracycline.[2]

Hydrogenation of 2-Acetyl-2-decarboxamido-7-chlorotetracycline. An 11-mg. sample of the sulfosalicylate salt was dissolved in 13 ml. of methanol and 10 mg. of triethylamine. The solution was added to a suspension of 10 mg. of prereduced 10% palladium-on-charcoal catalyst in 10 ml. of methanol. The course of the hydrogenation was followed by paper chromatography. After 42 hr. 2-acetyl-2-decarboxamido-7-chlorotetracycline had been replaced completely by 2-acetyl-2-decarboxamidotetracycline. Confirmation of the chromatographic identification was obtained by elution of a sample adequate for ultraviolet absorption and comparison with the spectrum of an authentic sample.

Acknowledgment.—The mutations and initial screening leading to these compounds were carried out by Edward Tynan of these laboratories. We wish to acknowledge also the technical assistance in this work of Mr. Robert Brown and Mr. Roger Bliss, and to thank our Physical Measurements Department, Bioassay Group, and Biochemical Pilot Plant for their contributions.

The Use of Arenesulfonhydrazides as Amino-Containing Bases in Papain- and Ficin-Catalyzed Reactions with *N*-Acylamino Acids

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Six arenesulfonhydrazides have been subjected to papain-catalyzed reactions at pH 4.0 and 40° with *N*-acylamino acids to form 1-acyl-2-arylsulfonylhydrazines. Each sulfonhydrazide underwent a reaction with hippuric acid, carbobenzoxyglycine, carbobenzoxy-L-alanine, and carbobenzoxy-DL-alanine. Resolution of carbobenzoxy-DL-alanine took place with each of the arenesulfonhydrazides to yield chiefly the L-product of reaction. The optimum pH for the reaction between benzenesulfonhydrazide and carbobenzoxyglycine was 3.8, under the conditions of concentrations employed. Ficin was substituted successfully for papain in catalyzing certain of these reactions. An attempted McFayden-Stevens synthesis was unsuccessful with 1-hippuryl-2-*p*-tolylsulfonylhydrazine as the reactant.

Before turning attention toward various other fundamental problems centered about papain-catalyzed syntheses of amides and amide-like compounds, it was important to round out a small, definitive, investigation involving the use of a few arenesulfonhydrazides as the amino-containing, basic reactants. Bergmann and Fraenkel-Conrat[5] demonstrated in 1937 that papain and also ficin could effectively catalyze anilide and phenylhydrazide formation from *N*-acylamino acids because of the water insolubility of these products, which displaced the equilibrium favorably. Subsequent investigations pertaining to the amino-containing

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(5) M. Bergmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937).

bases[6] established that substituted anilines, hydrazides, 3-aminoquinoline, 3-hydrazinoquinoline, and *m*-(1-hydroxyethyl)aniline with an asymmetric center in this amino-containing reactant could be utilized when papain was the catalyst. The purpose of these studies was multifold. One of the main objectives was to determine the ability of a variety of amino-containing substances to produce these amide types of compounds.

Selection of sulfonhydrazides was an obvious step in this particular objective. Each of these sulfonhydrazides was subjected to reactions with four *N*-acylamino acids in the formation of a 1-acyl-2-arylsulfonylhydrazine

$$\mathrm{ArSO_2NHNH_2 + HOCOCHRNHCOR'} \xrightarrow{\text{papain}} \mathrm{ArSO_2NHNHCOCHRNHCOR' + H_2O}$$

Ordinarily, the preparation of the sulfonhydrazides followed similar directions to those of Curtius and Lorenzen[7] for the synthesis of benzenesulfonhydrazide. A given arenesulfonyl chloride was dissolved in alcohol or another suitable solvent and was subsequently added to a slight excess of twice the molar quantity of 95% hydrazine in a small amount of the same solvent. This was accomplished by vigorous stirring with cooling, or at least avoidance of any appreciable rise in temperature.

$$\mathrm{ArSO_2Cl + 2NH_2NH_2} \xrightarrow[\text{cool}]{\text{appropriate solvent}} \mathrm{ArSO_2NHNH_2 + NH_2\overset{+}{N}H_3Cl^-}$$

Previous syntheses of 1-acyl-2-arylsulfonylhydrazines have followed two routes.[8,9] The first employed a carboxylic ester and hydrazine to form a hydrazide, followed by a reaction of the hydrazide with an arenesulfonyl chloride. In the second procedure the arenesulfonyl chloride was treated with hydrazine, with subsequent addition of an acyl chloride to the intermediate sulfonhydrazide

$$\mathrm{RCOOC_2H_5} \xrightarrow{\mathrm{NH_2NH_2}} \mathrm{RCONHNH_2} \xrightarrow{\mathrm{ClSO_2Ar}} \mathrm{RCONHNHSO_2Ar} \quad (1)$$

$$\mathrm{ArSO_2Cl} \xrightarrow{\mathrm{NH_2NH_2}} \mathrm{ArSO_2NHNH_2} \xrightarrow{\mathrm{ClCOR}} \mathrm{ArSO_2NHNHCOR} \quad (2)$$

Interest in these disubstituted hydrazines was chiefly centered about the McFayden-Stevens synthesis[8-10] of aldehydes.

$$\mathrm{RCONHNHSO_2Ar} \xrightarrow[\substack{\text{addition of finely divided}\\ \text{solid for surface contact}}]{\text{glycerol, 160°, base}} \mathrm{RCHO + N_2 + NaO_2SAr}$$

Aromatic and heterocyclic acyl radicals were found to be satisfactory in this synthesis but not the few aliphatic ones that were tried. However, the alicyclic radical of 1-benzenesulfonyl-2-cyclopropylcarbonylhydrazine did yield cyclopropanecarboxaldehyde, under conditions of pyrolysis.

The six arenesulfonhydrazides employed in the present papain-catalyzed syntheses were benzenesulfonhydrazide, *o*- and *p*-toluenesulfonhydrazides, *p*-fluorobenzenesulfonhydrazide, *p*-chlorobenzenesulfonhydrazide, and *p*-bromobenzenesulfonhydrazide. Other sulfonhydrazides were planned for inclusion in the study but various unpredictable circumstances were unfavorable for their usage, such as inability to synthesize some of them, prevention of reaction due to their water insolubility when an attempt was made to use certain of them as reactants, or various other deterrent factors. Among the ones that could not be used were methanesulfonhydrazide, ethanesulfonhydrazide, *p*-acetamidobenzenesulfonhydrazide, *o*-nitrobenzene-sulfonhydrazide, *p*-nitrobenzenesulfonhydrazide, *m*-benzenedisulfonhydrazide, and 2-thiophenesulfonhydrazide. The four *N*-acylamino acids selected were hippuric acid, carbobenzoxyglycine, carbobenzoxy-DL-alanine, and carbobenzoxy-L-alanine, because of the ease of usage that was found in previous work.

Former experiments commonly incorporated benzoyl-DL-alanine and benzoyl-L-alanine in the series of *N*-acylamino acids utilized. The fact that benzoyl-DL-alanine was usually resolved easily in such a way that papain selected the L-isomer from the racemic mixture, whereas commercially labelled benzoyl-L-alanine by itself consistently did not undergo a reaction, led to the disclosure that the commercial material was actually the D-isomer.[11] Since this discovery was made long after the present investigation was under way, this provocative situation was avoided entirely in the present research. It was only significant to prove that papain would resolve a racemic *N*-acylamino acid, not necessarily benzoyl-DL-alanine, in reactions with arenesulfonhydrazides.

Another objective of this research was to substitute ficin for papain as the enzymatic catalyst in a few reactions, in order to compare the two enzymes. The ficin used here had its origin in South America. A further objective was to test 1-hippuryl-2-*p*-tolylsulfonylhydrazine by pyrolysis in the McFayden-Stevens synthesis, as a route to hippuraldehyde. Hippuraldehyde is an aldehyde whose properties are known,[12] as are the properties

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of its phenylhydrazone. If this should be successful, a general synthesis of *N*-acylamino aldehydes would be provided.

Experimental

Preparation of Activated Papain.—Dried papaya latex, imported from Ceylon, was purchased from the Wallerstein Laboratories, New York N. Y., in a vacuum packed metal container and was used in this study. Papin, was extracted in the usual way[6,13] with cold water, activated with hydrogen sulfide, precipitated with methanol, and dried over phosphorus pentoxide. It was stored at 5° in a stoppered vial, enclosed in a brown bottle fitted with a screw cap.

Synthesis of Arenesulfonhydrazides.—Benzenesulfonhydrazide, *p*-fluorobenzenesulfonhydrazide, *p*-chlorobenzenesulfonhydrazide, and *p*-bromobenzenesufonhydrazide were prepared by very similar procedures. Nearly the same directions were followed as those given by Curtius and Lorenzen[7] for the preparation of benzenesulfonhydrazide. In place of the hydrazine hydrate of their method, 95% hydrazine was employed. Twenty five grams of the arenesulfonyl chloride was dissolved in about 15 or 20 ml. of absolute ethanol. This solution was added at a moderate rate, over a period of 0.5–1 hr., with stirring and avoidance of a rise in temperature, to very slightly more than twice the molar quantity of 95% hydrazine in about 10 ml. of absolute ethanol. At the end of the reaction period a large amount of solid was formed. The mixture was cooled in an ice bath and filtered rapidly by cold suction filtration. The solid on the filter paper was removed and then extracted with two 50-ml. portions of ice-cold water, which dissolved the hydrazine hydrochloride. The remaining solid was then dissolved in a minimum amount of boiling water. The resultant solution was filtered hot, but not boiling, to remove a small amount of solid that formed from an oil, if the temperature was lowered somewhat below the boiling point of water. A small amount of decolorizing carbon was used in treatment of the hot filtrate, which was then filtered hot three times to remove all traces of carbon and then placed in the refrigerator overnight. Large, colorless, crystals of the arenesulfonhydrazide formed. They were removed by filtration and air-dried. An additional amount of crystals could be obtained from working up the ice-cold alcoholic filtrate at the step where the hydrazine hydrochloride and sulfonhydrazide were removed. This filtrate was treated with ice-cold water, in an ice bath, with the formation of additional insoluble sulfonhydrazide, which was removed by filtration. The yields of sulfonhydrazides varied from about 35 to 65%, depending on the variations of experimental procedures and the particular sulfonhydrazide being prepared. They could be purified by dissolving in hot ethanol, treating with decolorizing carbon, filtering three times by suction filtration, and then allowing the solution to evaporate to dryness near the vent of a hood.

An alternative method of synthesis was employed for *p*-toluenesulfonhydrazide, as well as some of the *p*-halobenzenesulfonhydrazides. Modifications of the procedure of Freudenberg and Blummel[14] were used. The arenesulfonyl chloride was dissolved in the minimum amount of benzene and was subsequently added, slowly and with stirring, to slightly more than twice the molar quantity of 95% hydrazine. The rate of addition was such as to avoid any appreciable rise in temperature. Hydrazine hydrochloride was removed by filtration. Extraction of the filtrate with hot water, with subsequent cooling of the aqueous extract in the refrigerator overnight, yielded solid *p*-toluenesulfonhydrazide.

o-Toluenesulfonhydrazide was obtained from 85% *o*-toluenesulfonyl chloride, Eastman grade. A solution of 85% *o*-toluenesulfonyl chloride dissolved in dioxane was added dropwise to slightly more than twice the molar quantity of 95% hydrazine at 15–20°, with constant stirring. Solid material was removed by filtration and the filtrate was treated with carbon, then filtered again. Removal of the dioxane by slow evaporation yielded a clear, yellow, very viscous oil of impure *o*-toluenesulfonhydrazide, which was used directly in papain-catalyzed syntheses without further purification. Properties of arenesulfonhydrazides are listed in Table I.

TABLE I

p-HALOBENZENESULFONHYDRAZIDES

Halogen substituent	*p*-Fluoro	*p*-Chloro	*p*-Bromo
Per cent N calcd.	14.75	13.56	11.16
Per cent N found	14.81	13.77	11.34
Melting point	90–92	112–114	120–122
Per cent yield	55	40	50

Preparation of Acetone Derivatives of *p*-Halobenzenesulfonhydrazides.—A sample of 0.2 g. of the *p*-halobenzenesulfonhydrazide was dissolved in 2 ml. of acetone and the solution was filtered into a test tube. This solution was placed in a water bath and maintained at 50° for one hr. The solution was then filtered and evaporated to dryness on a watch glass. Since each of the solid products showed a rather wide range in melting point, each was dissolved in a few milliliters of acetone. Decolorizing carbon was added, the mixture was warmed a few minutes and then filtered three times, with the use of a new filter paper and carefully cleaned apparatus for each filtration. After the final filtration, each solution was poured onto a large watch glass and evaporated to dryness by allowing the solution to stand near the vent of a hood for several days. Melting points of these derivatives are recorded in Table II.

TABLE II

N^1-ISOPROPYLIDENE-*p*-HALOBENZENESULFONHYDRAZIDES

Halogen substituent	*p*-Fluoro	*p*-Chloro	*p*-Bromo
Per cent N calcd.	12.17	11.36	9.62
Per cent N found	12.55	11.70	9.90
Melting point	137–139	129.5–130.5	155–157

Dependence of Yield on pH for the Papain-Catalyzed Synthesis of 1-(*N*-Carbobenzoxyglycyl)-2-phenylsulfonylhydrazine from Benzenesulfonhydrazide and Carbobenzoxyglycine.—To each of ten glass-stoppered, conical flasks were added the following substances: 0.0100 mole of benzenesulfonhydrazide; 0.0100 mole of carbobenzoxyglycine; 0.5000 g. of L-cysteine hydrochloride monohydrate; and a different buffer solution to each flask. The buffers were at these pH values: 3.00; 3.50; 3.60; 3.75; 3.87; 4.00; 4.25; 4.50; 5.00; and 5.50. About 110 ml. of a given buffer was added to the solid mixture. The mixture was heated to bring about rapid solution and then filtered after first cooling to room temperature. Then 0.2500 g. of papain was dissolved in another 10 ml. of the given buffer solution and added to the main volume of the solution. Sufficient more buffer was added to bring the solution to a total volume of 125 ml. The pH was readjusted, if necessary, to the intended pH with the aid of a pH meter by adding either hydrochloric acid or sodium hydroxide. The solutions were then incubated at 40° and precipitates were collected by filtration at the end of 24, 48, and 72 hr. Readjustment of the pH to its original value was made after each filtration. The precipitates were dried and weighed. Results are given in Fig. 1.

Papain-Catalyzed Syntheses of 1-Acyl-2-arylsulfonylhydrazines from Arenesulfonhydrazides and *N*-Acylamino Acids.—These reactions were all carried out at pH 4.0 in 125 ml. total solution, buffered with sodium lactate and lactic acid. To each flask was added 0.0100 mole of the arenesulfon-

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[13] W. Grassmann, *Biochem. Z.*, **279**, 131 (1935).

[14] K. Freudenberg and H. Blummel, *Ann.*, **440**, 51 (1924).

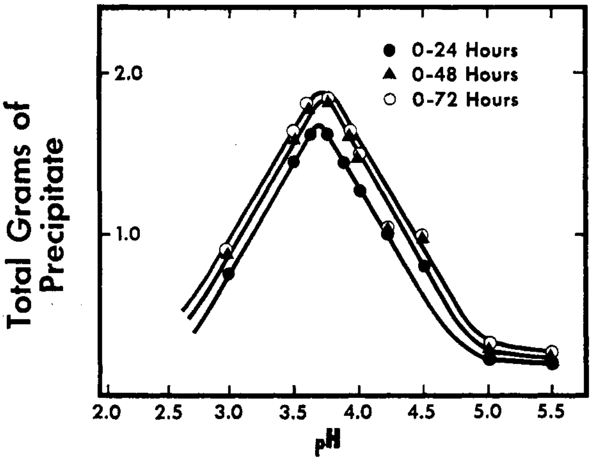

Fig. 1.—Dependence of yield on pH for the papain-catalyzed synthesis of 1-(N-carbobenzoxyglycyl)-2-phenylsulfonylhydrazine from benzensulfonhydrazide and carbobenzoxyglycine (0.0100 mole of carbobenzoxyglycine; 0.0100 mole of benzenesulfonhydrazide; 0.500 g. of L-cysteine hydrochloride monohydrate; 0.250 g. of activated Wallerstein papain; sufficient buffer to give a total of 125 ml. of solution at pH adjusted exactly to desired value; solution incubated at 40°.

hydrazide, 0.5000 g. of L-cysteine hydrochloride monohydrate, and 0.0100 mole of the N-acylamino acid if it was the L-antipode or nonracemic, or 0.0200 mole if its was racemic. Nearly 115 ml. of buffer was added, the mixture was heated nearly to boiling to bring about rapid solution, and the solution was cooled to 40° and filtered. A few milliliters of the filtered solution was used to dissolve 0.5000 g. of activated Wallerstein papain by grinding in a mortar and this was poured back into the reaction flask and mixed thoroughly. The mortar and pestle were rinsed back into the reaction flask by using several milliliters of the resultant solution. Enough more buffer was added to bring the total volume to 125 ml. The pH was adjusted exactly to 4.0 and incubation was carried out at 40°. At the end of 24 hr. and 48 hr., precipitates were collected, dried, and weighed.

Purification of the products was usually accomplished by treating with hot water to remove water-soluble impurities. Then the remaining precipitate was dissolved in the minimum amount of hot pyridine, decolorizing carbon was added, the mixture was warmed for a few minutes with stirring and then filtered three times, with the use of new filter paper and clean apparatus for each filtration. The filtrate was poured into ice-cold water containing chipped ice. After the ice had melted, the white or cream-colored solid was removed by filtration, washed two or three times with hot water, and allowed to dry in an oven at 40°. Portions of these precipitates were used for nitrogen analyses, which were all run at the Schwarzkopf Microanalytical Laboratory of Woodside, New York. Products of carbobenzoxy-L-alanine and carbobenzoxy-DL-alanine were checked for specific rotations in pyridine at 25°. About 2–3% solutions were utilized in these determinations.

In a few instances ethanol was substituted for pyridine in recrystallization. The crude precipitate from the papain-catalyzed reaction was washed free of water-soluble impurities by means of hot water. The remaining precipitate was dissolved in hot ethanol and filtered. Decolorizing carbon was added to the filtrate, which was stirred vigorously and kept hot. The solution was filtered, hot, three times, with a change of filter paper and apparatus for each filtration. The resultant filtrate was allowed to evaporate to dryness overnight in the hood. The white or cream-colored solid consisted of pure product. A summary of details is given in Table III.

An Attempted McFayden-Stevens Synthesis of Hippuraldehyde.—1-Hippuryl-2-p-tolylsulfonylhydrazine (0.65 g.) was dissolved in 31 ml. of hot ethylene glycol. The mixture was heated to 160–165° and glass wool was added for a high degree of solid surface contact. Then 0.217 g. of anhydrous sodium carbonate was added slowly, with vigorous stirring, over a period of 4 min. The solution was cooled in an ice bath, then diluted with 30 ml. of water and filtered. There was no precipitate formed.

A phenylhydrazine reagent was made up by mixing 1 g. of phenylhydrazine hydrochloride with 1.5 g. of crystalline sodium acetate in 10 ml. of water. One third of the pyrolysis product above, after the dilution with water, consisting of 20 ml. of the resultant diluted solution, was added to the 10 ml. of phenylhydrazine reagent. The mixture was allowed to cool overnight. Subsequent work-up of the material showed no evidence of hippuraldehyde phenylhydrazone.

The attempted pyrolysis was repeated under identical conditions of concentration but the temperature was raised to 180–185°. A mixture of glass wool and Celite filter aid (John Manville Diatomaceous Earth L-655A) was used as the solid for surface contact. The mixture was stirred vigorously at this temperature for 4 min. during the addition of anhydrous sodium carbonate, then cooled in an ice bath and filtered through a coarse-grade sintered-glass filter with suction filtration.

One third of the filtered solution was tested as previously described for phenylhydrazone formation. Hippuraldehyde was not present. Another one-third portion of the filtered solution was tested for 2,4-dinitrophenylhydrazone formation, also with negative results.

Ficin-Catalyzed Syntheses of 1-Acyl-2-phenylsulfonylhydrazines from Benzenesulfonhydrazide and N-Acylamino Acids.—All of these reactions were conducted at pH 4.0 buffered with sodium lactate and lactic acid, in a total volume of solution amounting to 125 ml. Each flask contained 0.0100 mole of benzenesulfonhydrazide, 0.500 g. of L-cysteine hydrochloride monohydrate and 0.0100 mole of the N-acylamino acid if it was non-asymmetric or the L-antipode, or 0.0200 mole if it was racemic. About 115 ml. of buffer was added to the flask, the mixture was heated to permit rapid solution, and then the solution was cooled below 40° and filtered by suction filtration. Rapid weighing of 0.750 g. of ficin was necessary because it was very hygroscopic toward the moist atmosphere. This ficin was triturated with about 25 ml. of the solution and filtered on a couple of suction filters to bring about more rapid filtration. The solution was returned to the proper flask. Sufficient additional stock buffer solution was now added to bring the total volume to 125 ml. A pH meter was used in adjusting the pH exactly to 4.0, after which incubation was carried out at 40°. Precipitates were collected at the end of 24 hr; they were dried and weighed. Purification of the products followed the same procedure as with papain-catalyzed products. Melting points and specific rotations in pyridine at 25° were established. Essential details are provided in Table IV.

In other related work it was found that Celite filter aid could be used as a wet cake on a suction filter in speeding up filtration of a solution of ficin in just the buffer. Care must be used in such a process because some of the ficin is retained on and throughout the cake, even though washed with more buffer. Also, other solutions components should be absent because portions are held up on the cake. It is best to use aliquots of filtered ficin in buffer, in making up a series of ficin solutions of equal ficin concentration under these conditions.

Discussion of Results

The optimum pH for the reaction between benzenesulfonhydrazide and carbobenzoxyglycine, under papain catalysis at the given concentration

TABLE III. PAPAIN-CATALYZED REACTIONS BETWEEN ARENESULFONHYDRAZIDES AND *N*-ACYLAMINO ACIDS

Arenesulfonhydrazide reactant	*N*-Acylamino acid reactant			
Benzenesulfonhydrazide	Hippuric acid (HA)	Carbobenzoxyglycine (CBG)	Carbobenzoxy-DL-alanine (C-DL-A)	Carbobenzoxy-L-alanine (C-L-A)
Name of product[a]	1-Hippuryl-2-phenylsulfonylhydrazine	1-(*N*-Carbobenzoxyglycyl)-2-phenylsulfonylhydrazine	1-(*N*-Carbobenzoxyalanyl)-2-phenylsulfonylhydrazine	1-(*N*-Carbobenzoxy-L-alanyl)-2-phenylsulfonylhydrazine
$[\alpha]^{25}\mathrm{D}$ 2–3% in pyridine			−59.12°	−77.51°
Wt. of product, g.				
0–24 hr.	0.27	0.56	0.11	0.43
24–48 hr.	0.12	0.25	0.17	0.22
Melting point	219–220	190–191	159–160	160–161
Per cent N calcd.	12.61	12.10	11.14	11.14
Per cent N found	12.62	12.09	11.28	11.26
o-Toluenesulfonyhdrazide	(HA)	(CBG)	(C-DL-A)	(C-L-A)
Name of product	1-Hippuryl-2-*o*-tolylsulfonylhydrazine	1-(*N*-Carbobenzoxyglycyl)-2-*o*-tolylsulfonylhydrazine	1-(*N*-Carbobenzoxyalanyl)-2-*o*-tolylsulfonylhydrazine	1-(*N*-Carbobenzoxy-L-alanyl)-2-*o*-tolylsulfonylhydrazine
$[\alpha]^{25}\mathrm{D}$ 2–3% in pyridine			−55.30°	−55.34°
Wt. of product, g.				
0–24 hr.	0.17	0.67	0.48	1.58
24–48 hr.	0.04	0.52	0.05	0.10
Melting point	194–196	146–147	191–192	192–193
Per cent N calcd.	12.10	11.13	10.47	10.47
Per cent N found	12.19	11.01	(Mixed melting point with L-isomer was undepressed)	10.47
p-Toluenesulfonhydrazide	(HA)	(CBG)	(C-DL-A)	(C-L-A)
Name of product	1-Hippuryl-2-*p*-tolylsulfonylhydrazine	1-(*N*-Carbobenzoxyglycyl)-2-*p*-tolylsulfonylhydrazine	1-(*N*-Carbobenzoxyalanyl)-2-*p*-tolylsulfonylhydrazine	1-(*N*-Carbobenzoxy-L-alanyl)-2-*p*-tolylsulfonylhydrazine
$[\alpha]^{25}\mathrm{D}$ 2–3% in pyridine			−69.30°	−69.40°
Wt. of product, g.				
0–24 hr.	0.15	0.37	0.29	0.34
24–48 hr.	0.02	0.06	0.03	0.05
Melting point	228–229	181–182	191–192	191–193
Per cent N calcd.	12.10	11.14	10.47	10.47
Per cent N found	11.97	11.10	(Mixed melting point with L-isomer was undepressed)	10.60
p-Fluorobenzenesulfonhydrazide	(HA)	(CBG)	(C-DL-A)	(C-L-A)
Name of product	1-Hippuryl-2-*p*-fluorophenylsulfonylhydrazine	1-(*N*-Carbobenzoxyglycyl)-2-*p*-fluorophenylsulfonylhydrazine	1-(*N*-Carbobenzoxyal-L-anyl)-2-*p*-fluorophenylsulfonylhydrazine	1-(*N*-Carbobenzoxy-L-alanyl)-2-*p*-fluorophenylsulfonylhydrazine
$[\alpha]^{25}\mathrm{D}$ 2–3% in pyridine			−72.93°	−74.76°
Wt. of product, g.				
0–24 hr.	1.09	1.42	1.59	2.41
24–48 hr.	0.29	0.20	0.21	0.21
Melting point	247–249	204–206	210–211.5	210–211.5
Per cent N calcd.	11.96	11.02	10.63	10.63
Per cent N found	12.27	11.00	(Mixed melting point with L-isomer was undepressed)	10.90
p-Chlorobenzenesulfonhydrazide	(HA)	(CBG)	(C-DL-A)	(C-L-A)
Name of product	1-Hippuryl-2-*p*-chlorophenylsulfonylhydrazine	1-(*N*-Carbobenzoxyglycyl)-2-*p*-chlorophenylsulfonylhydrazine	1-(*N*-Carbobenzoxyalanyl)-2-*p*-chlorophenylsulfonylhydrazine	1-(*N*-Carbobenzoxy-L-alanyl)-2-*p*-chlorophenylsulfonylhydrazine
$[\alpha]^{25}\mathrm{D}$ 2–3% in pyridine			−75.85°	−79.21°
Wt. of product, g.				
0–24 hr.	1.26	1.05	1.10	1.65
24–48 hr.	0.08	0.06	0.06	0.05
Melting point	250–252	203–205	234–236	235–238
Per cent N calcd.	11.43	10.56	10.20	10.20
Per cent N found	11.46	10.72	(Mixed melting point with L-isomer was undepressed)	9.98

TABLE III (*Continued*)

Arenesulfonhydrazide reactant	N-Acylamino acid reactant			
p-Bromobenzenesulfonhydrazide	(HA)	(CBG)	(C-DL-A)	(C-L-A)
Name of product	1-p-Bromophenylsulfonyl-2-hippurylhydrazine	1-p-Bromophenylsulfonyl-2(N-carbobenzoxyglycyl)-hydrazine	1-p-Bromophenylsulfonyl-2-(N-carbobenzoxyalanyl)-hydrazine	1-p-Bromophenylsulfonyl-2-(N-carbobenzoxy-L-alanyl)hydrazine
$[\alpha]^{25}D$ 2–3% in pyridine			−60.73°	−77.73°
Melting point	237–238	221–222	240–242	242–244
Per cent N calcd.	10.19	9.50	9.21	9.21
Per cent N found	10.35	9.64	Mixed melting point with L-isomer was undepressed	9.46
Wt. of product, g.				
0–24 hr.	0.85	1.06	1.58	2.18
24–48 hr.	0.06	0.03	0.05	0.11

[a] The class name 1-acyl-2-arylsulfonylhydrazine is used in naming these products. Specific products are then named with the 1-prefix placed before the radical name which contains the lower alphabet first letter, regardless of whether it is an acyl or arylsulfonyl radical.

TABLE IV

FICIN-CATALYZED REACTIONS BETWEEN BENZENESULFONHYDRAZIDE AND N-ACYLAMINO ACIDS

Arenesulfonhydrazide reactant	N-Acylamino acid reactant			
Benzenesulfonhydrazide	(HA)	(CBG)	(C-DL-A)	(C-L-A)
Name of product	See Table III			
Wt. of product, g.				
0–24 hr.	0.27	1.23	0.43	0.75
24–48 hr.	0.14	0.37	0.29	0.25
Melting point	219–220	190–191	162–164	160–162
$[\alpha]^{25}D$ 2–3% in pyridine			−59°	−77°
Analyses	Mixed melting points with products from papain-catalysis showed no depression; mixed melting point of ficin-catalyzed products from C-DL-A and C-L-A was 160–163°. Nitrogen analyses were unnecessary.			

of solution components, turned out to be about 3.8. This is shown in Fig. 1. Consequently all of the other reactions were carried out near this value at pH 4.0. Carbobenzoxy-DL-alanine was easily resolved by papain when a reaction took place with any of the six arenesulfonhydrazides used successfully in this investigation. The specific rotations of the 1-arylsulfonyl-2-(N-carbobenzoxy-L-alanyl)-hydrazines in pyridine at 25° were in the neighborhood of about 60 to 80°, with the exception of 1-(N-carbobenzoxy-L-alanyl)-2-o-tolylsulfonylhydrazine, which was about 55°. The extent of resolution of carbobenzoxy-DL-alanine can be seen by comparison of the rotations of the products with those of the products of carbobenzoxy-L-alanine.

Ficin readily resolved carbobenzozy-DL-alanine when benzenesulfonhydrazide was the basic reactant. This ficin had its origin in South America. It is obtained from the viscous milky latex extracted from the trunk of the Higueron tree, in a similar manner to isolation of rubber latex. Such fig trees grow wild in tropical climates and bear the formal name *Ficus glabrata*. The latex is commonly called leche de Higueron from the Spanish word leche, meaning milk. This type of fig tree is considerably different from agriculturally productive California fig trees. Crude, dried, ficin was procured locally,[15] as well as from the Instituto de Investigaciones Technologicas of Bogotá, Colombia.[16] Leche de Higueron was obtained from the field, in a plastic bag, and was air-mailed from the American Embassy in Caracas, Venezuela. The latex can be processed to the ordinary crude, dried, enzyme by the method of Robbins.[17]

It is known that ficin is an enzyme similar to papain, whose activity depends on a mercapto group on a cysteine residue of the polypeptide chain located at the active site. The mechanism for its activity is not known, but because of its similarity to papain it might be explained, in part, by these equations[6,18]

It is not necessary to use hydrogen sulfide or sodium cyanide to activate crude ficin, as is usually done with papain. The dried, crude ficin will remain active for a very long time if protected from moisture and atmospheric oxygen and if kept refrigerated. However, it is very hygroscopic and is readily deactivated in contact with atmospheric oxygen when moist.

The McFayden-Stevens synthesis was attempted

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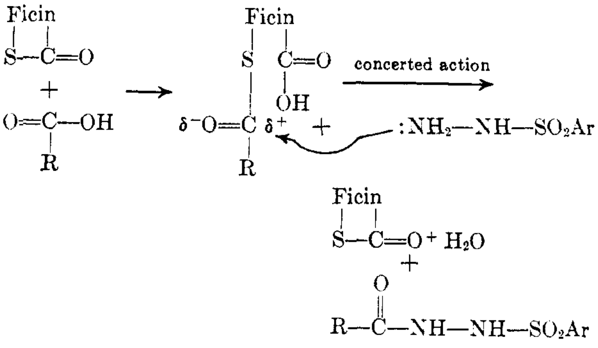
[15] The California Corp. for Biochemical Research. Los Angeles, California, dispenses the dried enzyme, which has a South American origin.

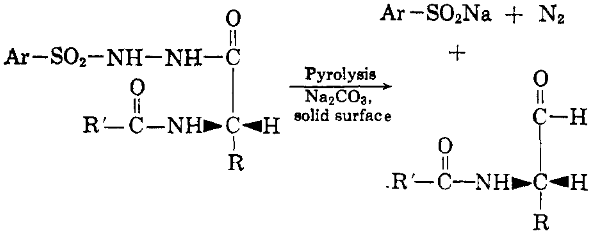
[16] Dr. Oliverio Phillips M., Director of this institution, generously supplied the dried enzyme.

[17] B. H. Robbins, *J. Biol. Chem.*, **87**, 251 (1930).

[18] E. L. Smith, *ibid.*, **223**, 1392 (1958).



by using 1-hippuryl-2-*p*-tolylsulfonylhydrazine under the improved conditions of Newman and Caflish[9] for such an aldehyde synthesis. Pyrolytic conditions were employed with a finely divided, insoluble, solid for surface contact. If the experiment had worked, it would have provided a general method for the synthesis of *N*-acylamino aldehydes. A route to L-*N*-acylamino aldehydes might have been established. Even if the reaction had worked, it might not have been possible to isolate separate antipodes. The basic conditions and high temperature might have caused racemization, although the short time of a few minutes for the reaction might have prevented this.



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6,16-Dimethylated Steroids. I. Studies on the Synthesis of 6,16α-Dimethylprogesterones[1a]

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Intermediates leading to 6,16α-dimethylprogesterones have been prepared by three different routes. Two of these involve conjugate addition of a methyl group to a Δ^{16}-20-ketone followed *in situ* by a forced attack on a $5\alpha,6\alpha$-epoxide or 6-ketone. Also described is a method for the stereospecific synthesis of $5\alpha,6\alpha$-epoxides in high yield. The most potent compound of the series, Δ^{6}-dehydro-6,16α-dimethylprogesterone (*8a*), has an oral progestational activity 1.5 times greater than ethinyltestosterone.

The enhancement of progestational activity by substitution of a methyl group at the 6α-position has been known for some time; later it was shown that further enhancement could be achieved by dehydrogenation to the corresponding Δ^{6}-dehydro derivative.[2] Recent reports[3] have indicated that a great increase in anti-inflammatory activity occurs on substitution of corticoids with methyl groups at both the 6α- and 16-positions. We have now found that this enhancement of activity is also observed in

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