Isolation and Characterization of Two New Tetracycline Antibiotics[1]

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Two new tetracycline antibiotics have been isolated from cultures of a mutant of *Streptomyces aureofaciens* Duggar. One of these has been characterized as 2-acetyl-2-decarboxamidotetracycline (I). The other is 2-acetyl-2-decarboxamido-7-chlorotetracycline (II). The two antibiotics are less active than the corresponding 2-carboxamido compounds against a number of bacteria.

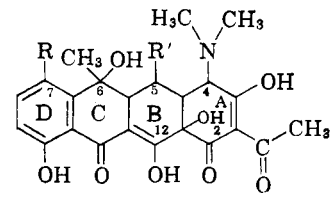
A mutant of *Streptomyces aureofaciens* has been found which produces 7-chlorotetracycline and two new antibiotics of the tetracycline type.

Pure 2-acetyl-2-decarboxamidotetracycline (I, $C_{23}H_{25}NO_8$) was obtained as the free base monohydrate in the form of yellow needles, m.p. 179–186°. This new antibiotic shows an ultraviolet absorption spectrum which is identical with that of tetracycline or of 5-oxytetracycline above 300 mμ, a region characteristic of the BCD-ring system.[2] The absorption peak in the 220–300-mμ region, however, occurs at a significantly higher wave length than that of tetracycline or 5-oxytetracycline, and matches that of 2-acetyl-2-decarboxamido-5-oxytetracycline.[3] This difference is a reflection of the difference between a 2-acetyl group and a 2-carboxamido group on the A-ring chromophore.

This ultraviolet absorption spectrum evidence for a 2-acetyl-2-decarboxamidotetracycline nucleus is confirmed by analytical data. These data show the expected single nitrogen atom, present as a dimethylamino group, and two C-methyl groups. Moreover, as in the case of 2-acetyl-2-decarboxamido-5-oxytetracycline,[3] the new compound contains an acetyl group readily and completely hydrolyzed by acid, but only partially hydrolyzed by alkali. The carbonyl band at 5.95 μ in the infrared absorption spectrum also is characteristic of a 2-acetyl group, and is not observed in spectra of the 2-carboxamidotetracyclines. It is evident, then, that our new substance must be a 2-acetyl-2-decarboxamidotetracycline type, differing from the known 2-acetyl-2-decarboxamido-5-oxytetracycline (III) only in its lack of one hydroxyl group.

Only two hydroxyl groups of the 5-oxytetracyclines, those at C-5 and at C-6, can be absent without affecting the ultraviolet-absorbing chromophore. Tetracycline itself as well as 7-chlorotetracycline dehydrate readily in acidic solutions to yield the C-5a:C-6 anhydro derivatives with a characteristic new chromophore.[2] Under similar conditions 5-oxytetracycline or 2-acetyl-2-decarboxamido-5-oxytetracycline undergo a more extensive transformation, which involves not only dehydration at C-5a:C-6, but also cleavage between positions C-12 and C-12a with lactone formation from C-12 to the C-5-hydroxyl group to form products which also are recognizable by their characteristic ultraviolet absorption patterns.[3,4]

Treatment of the new tetracycline antibiotic with hot, dilute mineral acid or with anhydrous hydrofluoric acid causes only dehydration at C-5a:C-6 to the anhydro derivative (IV). The ultraviolet absorption spectrum of this derivative resembles that of anhydrotetracycline and differs from the naphthalenediol-like spectra of the products obtained by acid treatment of the 5-oxytetracyclines. The new antibiotic, then, must have structure I.



		R	R′
I	2-Acetyl-2-decarboxamidotetracyline	H	H
II	2-Acetyl-2-decarboxamido-7-chlorotetracycline	Cl	H
III	2-Acetyl-2-decarboxamido-5-oxytetracycline	H	OH

The second, less polar antibiotic was separated from I by repeated chromatography on a cellulose column, using two solvent systems. It was obtained first as a crystalline hydrochloride. Purification was accomplished by means of the sulfosalicylate salt, $C_{23}H_{25}NO_8Cl \cdot C_7H_6SO_6$.

The ultraviolet absorption spectrum closely resembled that of 7-chlorotetracycline in the 300–400-mμ region. This resemblance together with the co-production of 7-chlorotetracycline in the same fermentation dictates assignment of the chlorine atom to position C-7. With the variations just noted the same structural arguments used for I apply to this second component of the mixture, indicating the structure of the latter to be II.

(1) This work was reported to the Division of Medicinal Chemistry of the American Chemical Society at the 140th Meeting in Chicago, Ill., in September, 1961.

(2) C. R. Stephens, L. H. Conover, R. Pasternack, F. A. Hochstein, W. T. Moreland, P. P. Regna, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *J. Am. Chem. Soc.*, **76**, 3568 (1954).

(3) F. A. Hochstein, M. Schach von Wittenau, Fred W. Tanner, Jr., and K. Murai, *ibid.*, **82**, 5934 (1960).

(4) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. O. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *ibid.*, **75**, 5455 (1953).

In addition, catalytic hydrogenation of 2-acetyl-2-decarboxamido-7-chlorotetracycline under conditions which convert 7-chlorotetracycline to tetracycline[2] yielded 2-acetyl-2-decarboxamidotetracycline.

2-Acetyl-2-decarboxamidotetracycline is about 5–50% as active as tetracycline against many bacteria (Table I), while 2-acetyl-2-decarboxamido-7-chlorotetracycline is about 10–100% as active as tetracycline.

TABLE I

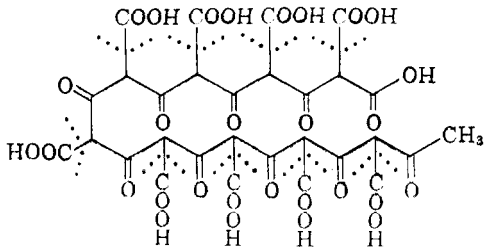
ANTIBACTERIAL ACTIVITY MINIMUM INHIBITORY CONCENTRATIONS (γ/ML.)

Bacterium	2-Acetyl-2-decarboxamido-tetracycline	Tetracycline
Corynebacterium diphtheriae	6.3	3.12
Malleomyces mallei	100	6
Bacterium ammoniagenes	6.3	0.8
Neisseria gonorrhoeae	3.12	.2
Hemophilus influenzae	1.56	.08
Listeria monocytogenes	12.5	.78

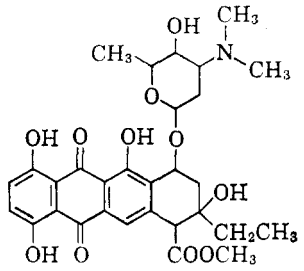
In a turbidity assay against *Klebsiella pneumoniae* 2-acetyl-2-decarboxamidotetracycline was about 10% as active as tetracycline, and of the same order of activity as 2-acetyl-2-decarboxamido-5-oxytetracycline.[3] By the same technique 2-acetyl-2-decarboxamido-7-chlorotetracycline was about 30% as active as tetracycline.

The fermentative formation of the 2-acetyl-2-decarboxamidotetracyclines might have been anticipated on biogenetic grounds. The tetracyclines are known to be derived predominantly from acetate.[5] Recent studies on the biogenesis of fatty acids[6] as well as other metabolites[7] indicate that the true biological intermediate is malonate rather than acetate. Thus while the nucleus of the 2-carboxamidotetracyclines can be envisaged as derived from malonate entirely or initiated by a half amide of malonate, the nucleus of the 2-acetyl-2-decarboxamidotetracyclines may be initiated by an acetate unit, followed by consecutive condensations of malonate units. The polyketomethylene chain (V), while not proposed as an actual intermediate, serves to illustrate the concept.[8]

Discovery of the 2-acetyl-2-decarboxamidotetracyclines seems to emphasize a biogenetic relationship between the tetracycline antibiotics and another class of streptomycete-produced compounds which resembles the tetracyclines in that its more highly colored chromophore also may be considered as a naphthacene derivative. This



V



VI

class includes the pyrromycin,[9] rhodomycin,[10] cinerubin,[11] aklavin,[12] and perhaps the quinocycline[13] complexes. Both glycosides and aglycones have been isolated. Certain of the aglycones of members of this class, *e.g.*, ε-pyrromycinone (rutilantinone), have been shown to be derived largely from acetate, but the ethyl side chain as well as the ring carbon atom to which it is attached has been shown to be derived from propionate.[14] It is likely, then, that in these cases propionate or methylmalonate rather than acetate or malonate is the chain initiator, and that further elaboration of the nucleus involves consecutive condensations (and decarboxylations) of malonate units.[15]

Experimental

Melting points were determined on a Kofler hot stage. Bioassays were made using a *Klebsiella pneumoniae* turbidity technique against tetracycline as the standard. Useful paper chromatography systems were, for (I) and (II): A—nitromethane (20 volumes), toluene (10 volumes), butanol (5 volumes), pyridine (3 volumes) on paper wet with pH 3.5 McIlvaine's buffer and B—nitromethane (20

(5) J. F. Snell, A. J. Birch, and P. L. Thomson, *J. Am. Chem. Soc.*, **82**, 2402 (1960).

(6) Salih J. Wakil, Edward B. Titchener, and David M. Gibson, *Biochim. et Biophys. Acta*, **29**, 225 (1958); David M. Gibson, Edward B. Titchener, and Salih J. Wakil, *ibid.*, **30**, 376 (1958); Salih J. Wakil, *J. Am. Chem. Soc.*, **80**, 6465 (1958).

(7) R. Bentley and John G. Keil, *Proc. Chem. Soc.*, 111 (1961).

(8) Note added in proof: This concept has been confirmed by recently reported labeling studies with 5-oxytetracycline [Sten Gatenbeck, *Biochem. Biophys. Res. Commun.*, **6**, 422 (1961)].

(9) (a) Hans Brockmann and Burchard Franck, *Chem. Ber.*, **88**, 1792 (1955); (b) Hans Brockmann and Werner Lenk, *ibid.*, **92**, 1880, 1904 (1959); (c) Hans Brockmann and Hans Brockmann, Jr., *Naturwissenschaften*, **47**, 135 (1960); (d) H. Brockmann, H. Brockmann, Jr., J. J. Gordon, W. Keller-Schierlein, W. Lenk, W. D. Ollis, V. Prelog, and I. O. Sutherland, *Tetrahedron Letters*, **No. 16**, 17 (1959).

(10) (a) Hans Brockmann and Ilse Borchers, *Chem. Ber.*, **86**, 261 (1953); (b) Hans Brockmann and Peter Patt, *ibid.*, **88**, 1455 (1955); (c) Hans Brockmann and P. Boldt, *Naturwissenschaften*, **44**, 616 (1957); (d) Hans Brockmann and Enno Spohler, *ibid.*, **42**, 154 (1955) and other papers in this series.

(11) Leopold Ettlinger, Ernst Gäumann, Rolf Hütter, Walter Keller-Schierlein, Friederich Kradolfer, Lucien Neipp, Vlado Prelog, Pierre Reusser, and Hans Zähner, *Chem. Ber.*, **92**, 1867 (1959).

(12) F. Strelitz, H. Flon, U. Weiss, and I. N. Asheshov, *J. Bacteriol.*, **72**, 90 (1956).

(13) W. D. Celmer, K. Murai, K. V. Rao, F. W. Tanner, Jr., and W. S. Marsh, "Antibiotics Annual 1957–1958," Medical Encyclopedia, Inc., New York, p. 484.

(14) W. D. Ollis, I. O. Sutherland, R. C. Codner, J. J. Gordon, and G. A. Miller, *Proc. Chem. Soc.*, 347 (1960).

(15) This view was expressed to us privately by Professor W. D. Ollis during a profitable discussion of the role of malonate in the biosynthesis of microbial metabolites.

volumes), chloroform (10 volumes), pyridine (3 volumes) on paper wet with pH 3.5 McIlvaine's buffer. Useful paper chromatography systems for (IV) were: C—toluene (20 volumes), and pyridine (3 volumes) on paper saturated with pH 4.2 McIlvaine's buffer and D—benzene (3 parts) and chloroform (1 part) on paper saturated with pH 4.2 McIlvaine's buffer. (All solvent mixtures were saturated with the buffer solution.) Whatman No. 4 paper was used throughout for the paper chromatograms.

Fermentation of 2-Acetyl-2-decarboxamidotetracycline [2-Acetyl-4-dimethylamino-4a,5a,6,12a-tetrahydro-3,6,10,-12,12a-hexahydroxy-6-methyl-1,11-(4H,5H)-naphthacenedione]. (I).—A mutant strain, bearing our code number 2243, of *Streptomyces aureofaciens* was used. It produced predominantly I rather than 7-chlorotetracycline. The mutant can be grown on a medium similar to the one used for the production of 7-chlorotetracycline.[16] This medium included 75 g./l. of cornstarch, 25 g./l. of corn steep liquor, and 10 ml./l. of soybean oil. After a 2-day inoculum incubation, 1000-gal. tanks were run for 5 days at 26° with 12 cu. ft. of sterile air/hr., terminal pH 6.2.

Isolation of 2-Acetyl-2-decarboxamidotetracycline (I).—The isolation procedure involved strong mineral acid solutions not optimum for isolation of total antibiotics but useful since they decomposed selectively the contaminating 7-chlorotetracycline. A 9000-l. batch of broth was adjusted to pH 2.0 with hydrochloric acid. The acidified broth was filtered, and the filtrate extracted with one fourth its volume of butanol in a Podbielniak countercurrent extractor. The acid-butanol extract was concentrated at reduced pressure (30–40°) to 38 l.

The butanol concentrate was extracted eight times with 12 l. of 1 *N* hydrochloric acid; the combined hydrochloric acid extracts were treated with 100 g. of activated charcoal (Darco), and the pH raised to 7.0 with triethylamine. The first crop of crude cake which precipitated weighed 1548 g. (bioassay = 22 μ/mg.).

A 1000-g. sample of crude I was mixed with 8 l. of water, the pH adjusted to 1.0 with sulfuric acid, and filtered from 330 g. of insolubles. The filtrate was treated with 200 g. of activated charcoal (Darco), then adjusted to pH 2.5–3.0 with 20% sodium hydroxide. The 137 g. of black tar which separated was discarded. The filtrate was extracted seven times with a total of 18 l. of butanol. Concentration of the butanol extract to 4 l., filtration, and reconcentration to 100 ml. yielded 370 g. of crude antibiotic in two crops. This 370-g. sample was dissolved in 3 l. of methanol, adjusted to pH 1.8 with sulfuric acid, the solution treated with 250 g. of Darco activated charcoal, then with 25 g. of sodium ethylenediamine tetraacetate (Perma-Clear). The latter was added intermittently with enough sulfuric acid to maintain pH 2. Both the charcoal and the Perma-Clear were discarded. The filtrate was adjusted to pH 5.8 with 20% sodium hydroxide, the solution concentrated to 1 l., 1 l. of isopropyl alcohol, and 500 ml. of water were added, and the solvent mixture was concentrated at 10 mm. (less than 35°) until cloudy. The mixture was seeded at this point to induce crystallization, concentrated further, then cooled to 5° and filtered. In this way 166 g. of clean, crystalline I base monohydrate, m.p. 160–180°, was isolated. Further concentration of the filtrate yielded a second fraction (38 g.) with the same melting range. Reworking of side streams and charcoal yielded an additional 53 g. of similar material; overall yield, 257 g. (28 mg./l.).

Recrystallization was accomplished by dissolving 100 g. of first crop I in 500 ml. of methanol, adding 250 ml. of isopropyl alcohol and 100 ml. of water, and adjusting pH to 5.9. The solution was concentrated at 10 mm. (<35°) until cloudy, then seeded and concentrated further to give a first crop of 86 g. of recrystallized I monohydrate. After drying for 14 hr. at 60° (0.1 mm.) this sample, m.p. 179–186° dec., was analyzed.

(16) B. M. Duggar, U.S. Patent 2,482,055 (1949).

Anal. Calcd. for $C_{23}H_{23}NO_8 \cdot H_2O$: C, 59.86; H, 5.90; N, 3.04; C—CH_3 (2), 6.80; acetyl (1), 9.32. Found: C, 59.61; H, 6.30; N, 3.08; C—CH_3, 6.80; acetyl (acid hydrolysis) 8.86; (alkaline hydrolysis) 3.99.

Ultraviolet absorption peaks were located at (in methanol 0.01 *N* in hydrochloric acid): 220 mμ, 240s mμ, 277 mμ (ϵ 15,040), 316s mμ, 332s mμ, and 360 mμ (ϵ 15,040). (In methanol 0.01 *N* in sodium hydroxide): 236s mμ, 270 mμ (ϵ 19,230), 381 mμ (ϵ 18,802). Pertinent infrared absorption data are described in the discussion section. $[\alpha]^{25}D$ −125° (*c* 1, in methanol, 0.5 *N* in hydrochloric acid).

Drying at 2×10^{-5} mm. with liquid nitrogen cooled traps for 24 hr. at 80° yielded anhydrous I, m.p. 179–181° dec.

Anal. Calcd. for $C_{23}H_{25}NO_8$: C, 62.29; H, 5.68; N, 3.16. Found: C, 61.86; H, 5.78; N, 3.12.

Acid Degradation of 2-Acetyl-2-decarboxamidotetracycline to 2-Acetyl-4-dimethylamino-4a,12a-dihydro-3,10,11,12a-tetrahydroxy-6-methyl-1,12-(4H,5H)-naphthacenedione. (IV).—A 2-g. sample of I monohydrate was dissolved in 5% hydrochloric acid, heated at 50° for 30 min. and cooled. The dark oil which separated was dissolved in butanol. Removal of the solvent at reduced pressure left a gum, which was triturated with isopropyl alcohol, filtered, and washed with ethyl ether.

Alternatively, 2 g. of I monohydrate was dissolved in 20 ml. of anhydrous hydrogen fluoride, and the solvent allowed to evaporate in a good hood, leaving a dark solid.

Purification was achieved by cellulose column chromatography with a solvent system composed of a water-saturated mixture of 1 volume of chloroform to 3 volumes of benzene. A 120-g. sample of I dissolved in 20 ml. of this solvent system was charged into a Pyrex column 3.8 cm. × 13 cm., packed with powdered cellulose[17] impregnated with the same solvent mixture. Development and removal of solvent yielded 104 mg. of crystalline product. Recrystallization from boiling isopropyl alcohol, and drying at 54° (0.1 mm.) for 18 hr. gave paper chromatographically homogeneous apricot-colored needles, m.p. 204–205° dec.

Anal. Calcd. for $C_{23}H_{23}NO_7$: C, 64.92; H, 5.45; N, 3.29; acetyl (1) 10.1. Found: C, 64.79; H, 5.55; N, 3.38; acetyl, 12.0 (acid hydrolysis).

Ultraviolet absorption peaks were located at (in methanol 0.01 *N* in hydrochloric acid): 223 mμ (ϵ 31,450), 273.5 mμ (ϵ 52,650), inflection at 310 mμ (ϵ 6590), inflection at 323 mμ (ϵ 2591), 427.5 mμ (ϵ 9140). (In methanol 0.01 *N* in sodium hydroxide): 227 mμ (ϵ 25,500), 271.5 mμ (ϵ 43,620), 322.5s mμ (ϵ 6540), 334s mμ (ϵ 6130), and 433 mμ (ϵ 12,740).

Alkaline Degradation of I. Isolation of Dimethylamine Hydrochloride.—The alkaline degradation procedure resembled that used in the characterization of 2-acetyl-2-decarboxamido-5-oxytetracycline.[3] A 300-mg. sample of the base monohydrate yielded 20 mg. of dimethylamine hydrochloride, m.p. 169–171°, identity confirmed by infrared absorption spectrum. No ammonia was evolved during the alkaline degradation.

Isolation of 2-Acetyl-2-decarboxamido-7-chlorotetracycline (III).—The crude crystals of I contained a considerable amount of a less polar tetracycline (paper chromatography systems A and B) which inhibited *Bacillus subtilis* or *Klebsiella pneumoniae* on bioautographs. Complete separation was difficult. A large Pyrex column (9.8 cm. × 105 cm.) was packed with 2 kg. of powdered cellulose[17] saturated with a solvent system consisting of (by volume) ethyl acetate (40 parts), nitromethane (25 parts), chloroform (7 parts) with water enough to saturate the mixture of organic solvents. A 10-g. sample of the crude I was dissolved in 50 ml. of the packing and developing solvent system plus 5 ml. of methanol and 0.1 ml. of 12 *N* hydrochloric acid. A total of 911 fractions (75–90 ml. each) were collected. Each fraction was partially concentrated

(17) Whatman ashless cellulose powder (W. and R. Balston, Ltd., standard grade).

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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(3) Fresno County Heart Association Undergraduate Research Fellow.

(4) National Science Foundation Undergraduate Research Fellow, University of Redlands.

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