mg/kg iv (equivalent to 5 mg of INH/kg), successively for 3 days and collected urine during each 24 hr following the protocol used earlier.[3]

Aliquots of the urine collections were analyzed for INH,[9] for total hydrolyzable INH, and for total hydrolyzable isonicotinic acid derivatives.[10] Table I

TABLE I

URINARY EXCRETION OF RELATED COMPOUNDS BY DOGS RECEIVING N^1-SULFATO-N^2-ISONICOTINYLHYDRAZINE POTASSIUM SALT (9.6 mg/kg) INTRAVENOUSLY

Dog	No. of treatments	—Mean %[a] of the admind dose in 24-hr urines— INH[b]	Total hydrolyzable INH[c]	Total hydrolyzable isonicotinic acid[d]
A	3	0.0 (0.0–0.2)	97.1 (89.5–102)	97.3 (91.0–103)
B	3	0.0 (0.0–0.0)	94.3 (88.4–103)	96.0 (84.6–105)
C	2	0.0 (0.0–0.0)	104 (97.0–110)	100 (93.5–107)
D	3	0.0 (0.0–0.0)	93.2 (80.8–104)	95.6 (78.6–110)

[a] Mean of the indicated number of successive daily treatments. Values in parenthesis are the ranges observed. [b] Measured by vanillin procedure[9] before hydrolysis. [c] Measured by vanillin procedure[9] after hydrolysis in 1 *N* HCl, 24 hr at 45°. [d] Measured by isonicotinic acid procedure[10] after sealed-tube hydrolysis (5 *N* HCl, 3 hr, 120°).

presents the results obtained. It is apparent that the INH-sulfate conjugate was not split in the body since no INH could be detected in the urine. Qualitative paper-chromatographic examination of aliquots of urine[3] yielded only one INH derivative at an R_f (0.25) identical with that of the injected compound. Furthermore, the nearly quantitative recovery of the dose administered as either total hydrolyzable INH or total hydrolyzable isonicotinic acid derivatives indicates that the INH-sulfate conjugate was excreted unchanged in the urine. These results indicate that the INH-sulfate conjugate is chemically and metabolically stable like the noncaloric sweetening agent, sodium N-cyclohexylsulfamate.[11] Table II compares the antimycobacterial activity of the INH-sulfate conjugate with that of INH.[12] It is clear that this INH derivative possesses only a small fraction of the activity of INH.

TABLE II

ANTIMYCOBACTERIAL ACTIVITY OF N^1-SULFATO-N^2-ISONICOTINYLHYDRAZINE AGAINST *M. tuberculosis* VAR. *hominis* 5159 AND $H_{37}Rv$[a]

Drug	—Min inhib concn, μg/ml— 5159	$H_{37}Rv$
N^1-Sulfato-N^2-isonicotinylhydrazine potassium salt	12.8[b]	12.8[b]
INH	0.05	0.05

[a] Tests were carried out in Dubos liquid medium, incubation at 37° for 9 days. [b] Equivalent to 6.6 μg of INH/ml.

(9) J. R. Maher, J. M. Whitney, J. S. Chambers, and D. J. Stanonis, *Am. Rev. Tuberc. Pulmonary Diseases*, **76**, 852 (1957).

(10) J. H. Peters, *Am. Rev. Respirat. Diseases*, **82**, 153 (1960).

(11) (a) J. P. Miller, L. E. M. Crawford, R. C. Sonders, and E. V. Cardinal, *Biochem. Biophys. Res. Commun.*, **25**, 153 (1966); (b) J. D. Taylor, R. K. Richards, and J. C. Davin, *Proc. Soc. Exptl. Biol. Med.*, **78**, 530 (1951).

(12) We are indebted to Dr. R. C. Good for these determinations.

Experimental Section[13]

N^1-Sulfato-N^2-isonicotinylhydrazine Potassium Salt.—Redistilled chlorosulfonic acid (5.0 g, 43 mmoles) was added dropwise to dry pyridine (50 ml) maintained at 15° in a stoppered flask. Solid INH (5.9 g, 43 mmoles) was added in small portions with gentle agitation. After 1 hr the mixture was allowed to warm to room temperature. After 23 hr the orange-red solution was poured into 100 ml of 1 *N* KOH. Following extraction with three 100-ml portions of Et_2O, the aqueous phase was evaporated to one-half volume on a steam bath, without the product appearing after cooling. A pH check showed the solution to be weakly acid. Therefore it was alkalinized with additional KOH, yielding a copious precipitate. The mixture was reheated to dissolve the precipitate and allowed to stand overnight at 5°. The crude product (9.0 g, 86%) was crystallized from 80% MeOH. The yield was 4.6 g (41%). A small sample was dried to constant weight at 65° over P_2O_5; mp 170–180°. *Anal.* ($C_6H_6KN_3O_4S \cdot 0.5H_2O$) C, H, S; N: calcd, 15.9; found, 16.4.

The compound was easily separated from INH by descending paper chromatography[3] (R_f of INH, 0.51; of product, 0.25). On paper chromatograms, the product could be detected by uv absorption or with color reagents specific for unsubstituted pyridine N.[3] Tests with reagents for unsubstituted hydrazine N were negative. Hydrazine content was found to be 104% of theory by a procedure for total hydrazine.[14] Total hydrolyzable isonicotinic acid content[10] was 101% of theory. Tests of the recovery of the INH-sulfate conjugate from urine by the procedure for INH[9] yielded less than 0.5% of the theoretical INH content.

(13) Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Microanalyses were performed by Clark Microanalytic Laboratory, Urbana, Ill. Where analyses are indicated by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(14) J. H. Peters, *Am. Rev. Respirat. Diseases*, **81**, 485 (1960).

Preparation and Antibacterial Activity of α-(5-Tetrazolyl)benzylpenicillin[1]

J. M. ESSERY

Research Division, Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York 13201

Received March 17, 1969

A recent publication from these laboratories[2] discussed some examples of the replacement of the carboxyl group by the comparably acidic 5-tetrazolyl group[3,4] in biologically active compounds. Such a substitution has now been made in an antibiotic without loss of antibacterial potency. The Beecham group has disclosed[5] that the semisynthetic penicillin, disodium α-carboxybenzylpenicillin (carbenicillin), exhibited activity against certain *Pseudomonas* and *Proteus* species high enough to warrant clinical investigation.[6] A similar order of *in vitro* activity was shown by the analogous compound dipotassium α-(5-tetrazolyl)benzylpenicillin (IV) which was prepared according to the reaction sequence depicted in Scheme I.

Chemistry.—Treatment of 5-benzyltetrazole (I) with 2 equiv of *n*-butyllithium followed by carbonation afforded α-(5-tetrazolyl)phenylacetic acid (II). This acid, an analog of phenylmalonic acid, was readily

(1) This compound, and related ones, have been described by J. M. Essery, U. S. Patent 3,427,302 (1968).

(2) P. F. Juby, T. W. Hudyma, and M. Brown, *J. Med. Chem.*, **11**, 111 (1968).

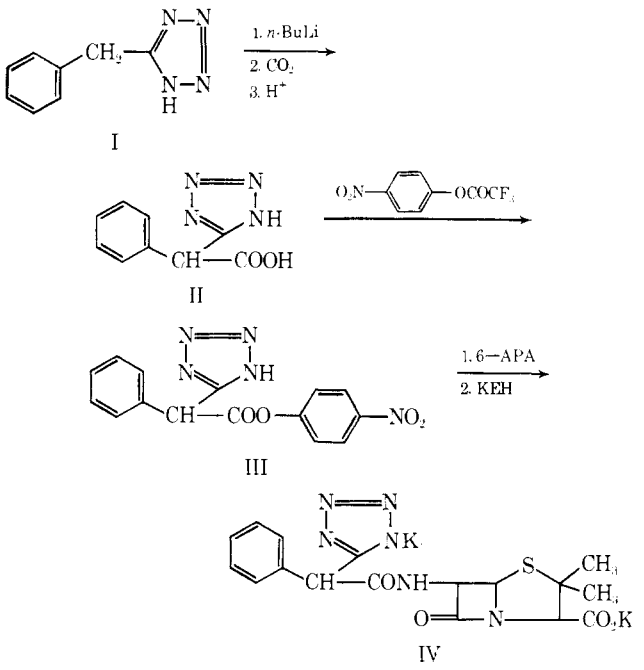
(3) F. R. Benson, *Chem. Rev.*, **41**, 1 (1947).

(4) R. M. Herbst, "Essays in Biochemistry," John Wiley and Sons, Inc., New York, N. Y., 1956, p 141.

(5) P. Acred, D. M. Brown, E. T. Knudsen, G. N. Rolinson, and R. Sutherland, *Nature*, **215**, 25 (1967).

(6) W. Brumfitt, A. Percival, and D. A. Leigh, *Lancet*, **1**, 1289 (1967).

SCHEME I



decarboxylated as shown by attempted recrystallization from hot EtOAc which yielded 5-benzyltetrazole. Consequently, modification of the carboxyl function to provide an intermediate suitable for condensation with 6-aminopenicillanic acid (6-APA) was difficult. Thus, the acid chloride and mixed anhydride procedures for amide formation were found to be inapplicable, and the carbodiimide method of Sheehan and Hess[7] failed to provide a good sample of the penicillin (IV). It was found, however, that the activated ester III was formed when the acid II was treated with *p*-nitrophenyl trifluoroacetate in pyridine at room temperature. Condensation with 6-APA then proceeded smoothly to provide the penicillin IV which was isolated as the dipotassium salt. Resolution of the activated ester III and isolation of isomers of the penicillin IV were not attempted since nmr spectroscopy showed that the proton on the carbon atom bearing the tetrazole group was readily exchanged in the presence of deuterium oxide.

Biological Activity.—The new penicillin was strikingly similar to carbenicillin in its range and order of *in vitro* antibacterial activity. Thus, although less active than both benzylpenicillin and ampicillin against such gram-positive bacteria as *Staphylococcus aureus* and *Streptococcus pyogenes*, and susceptible to destruction by staphylococcal penicillinase, it was found to have a relatively high degree of activity against many species of gram-negative bacteria. The most noteworthy feature of carbenicillin is its activity against *Pseudomonas aeruginosa* and some *Proteus* strains,[5] such activity having been observed infrequently in semisynthetic penicillins. When tested against *Pseudomonas* and *Proteus* organisms resistant to the broad-spectrum penicillin, ampicillin, the new penicillin showed a level of activity comparable with that of carbenicillin. The minimum inhibitory concentrations (MIC) for IV, carbenicillin, and ampicillin as measured by twofold serial dilution in a medium comprised of antibiotic assay broth and 1.5% agar using the multiple-inoculator apparatus of Steers, *et al.*,[8] designed for replicate spotseeding of agar surfaces in Petri dishes are shown in Table I.

TABLE I

Organism		MIC, μg/ml IV	Carbenicillin	Ampicillin
Pseudomonas aeruginosa	A-9843	63	63	>500
	A-9923	125	63	>500
	A-9930	125	125	250
	A-15, 150	125	63	>500
	A-15, 194	>500	500	>500
Proteus mirabilis	A-9554	2	1	63
Proteus morganii	A-15, 153	2	2	250
Proteus vulgaris	A-9699	32	16	>500
	A-9526	8	32	125

The new penicillin showed a CD_{50} of >500 mg/kg, as compared with 66 mg/kg for carbenicillin, when administered intramuscularly to mice which had been infected with *Pseudomonas aeruginosa* A-9843. The ineffectiveness of IV *in vivo* may be attributed to its poor absorption properties since it was found that the peak blood level in noninfected mice when 0.11 mmole/kg of the compound was given intramuscularly was <2 μg/ml as compared with a peak value of 11 μg/ml for carbenicillin for the same dose level. When administered by the intraperitoneal route, the peak blood levels in mice were 6.4 and 8.7 μg/ml, respectively, for IV and carbenicillin following a dose of 0.056 mmole/kg.

In summary, it has been shown that the unusual *in vitro* antibacterial activity of a semisynthetic penicillin containing a carboxyl group in the side chain was unaltered when the carboxyl function was replaced by the 5-tetrazolyl group. This correlation did not, however, extend to the *in vivo* activity.

Experimental Section[9,10]

DL-α-(5-Tetrazolyl)phenylacetic Acid (II).—To a cold (−60°) solution of 0.4 mole of *n*-BuLi in hexane (240 ml) under N_2 was added with stirring a solution of 32.0 g (0.2 mole) of 5-benzyltetrazole in 400 ml of THF. The resulting bright yellow slurry was stirred 15 min. CO_2 was passed through the slurry until all of the color had disappeared. The mixture was cautiously added to 600 ml of H_2O containing NH_4Cl and the two-phase system was cooled in an ice bath while 42% H_3PO_4 was added with stirring to pH 3.0. The layers were separated and the aqueous phase was extracted (EtOAc, two 200-ml portions). After the combined extracts had been washed (cold H_2O) and dried ($MgSO_4$), the solvent was removed and the residue was recrystallized by dissolving it in THF at room temperature, diluting with Et_2O, and cooling to 0° to provide 31.7 g (72%) of the monohydrate, mp 106–107° dec. *Anal.* ($C_9H_8N_4O_2 \cdot H_2O$) C, H, N, H_2O.

p-Nitrophenyl DL-α-(5-Tetrazolyl)phenylacetate (III).—To a stirred solution of 2.22 g (0.01 mole) of DL-α-(5-tetrazolyl)-phenylacetic acid monohydrate in 20 ml of dry pyridine was added 4.70 g (0.02 mole) of *p*-nitrophenyl trifluoroacetate. The clear yellow solution was stirred 3 hr at room temperature. Most of the solvent was removed under reduced pressure and the residue was added to 20 ml of ice–water and 20 ml of $CHCl_3$

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

(8) E. Steers, E. L. Foltz, and B. S. Graves, *Antibiot. Chemotherapy*, **9**, 309 (1959).

(9) Melting points were determined in a Mel-Temp apparatus and are uncorrected. Ir spectra (KBr disks) were measured with a Beckman IR9 spectrophotometer and nmr spectra with a Varian Associates A-60 spectrometer. Spectra were consistent with structure in each case.

(10) Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical values.

with stirring. The aqueous phase was extracted ($CHCl_3$, three 10-ml portions) and the combined $CHCl_3$ phases were thoroughly washed (H_2O) to remove *p*-nitrophenol, dried ($MgSO_4$), and evaporated. The residual gum was triturated first with PhMe, then with Et_2O, to give a solid which was recrystallized from EtOH to give 1.5 g (45%) of colorless needles, mp 153.5–155°. *Anal.* ($C_{15}H_{11}N_5O_4 \cdot 0.5H_2O$) C, H, N.

Dipotassium 6-[DL-α-(5-Tetrazolyl)phenylacetamido]penicillanate (IV).—To a cold (0–5°) solution of 1.62 g (7.5 mmoles) of 6-aminopenicillanic acid and 1.515 g (15 mmoles) of Et_3N in 25 ml of dry CH_2Cl_2 was added with stirring 2.439 g (7.5 mmoles) of *p*-nitrophenyl DL-α-(5-tetrazolyl)phenylacetate. The mixture was stirred for 2 hr at 0–5° and for 17 hr at room temperature and was then added to 25 ml of ice–water with vigorous stirring. The aqueous phase was extracted ($CHCl_3$, two 20-ml portions) and acidified to pH 4.5 and reextracted (EtOAc, three 20-ml portions) to remove *p*-nitrophenol. The pH was then lowered to 2.0 with 42% H_3PO_4 and the penicillin was extracted into EtOAc (three 20-ml portions). After the extracts had been washed (H_2O) and dried ($MgSO_4$), 15 mmoles of a 50% solution of potassium 2-ethylhexanoate (KEH) in *n*-BuOH was added and the solvent was evaporated to half-volume under reduced pressure at 35°. The product precipitated on the walls of the flask from which it was recovered by trituration with dry Et_2O, filtration, and drying over P_2O_5 *in vacuo*. This yielded 2.5 g of hygroscopic material for which a satisfactory analysis could not be obtained; the purity of the penicillin was estimated from ir and nmr spectra to be 90%.

Acknowledgment.—The microbiological data were kindly supplied by Dr. K. E. Price and Dr. M. Misiek; microanalyses were provided by Mr. R. M. Downing and Mrs. C. Kalinowski.

Tetracyclic Phenothiazines. II.[1a] Antibacterial Evaluation of 3-Keto-3H-pyrido[3,2,1-*kl*]phenothiazine-2-carboxylate[1b,c]

DER-SHING HUANG, TIMOTHY J. YALE, AND ARNOLD R. MARTIN

College of Pharmacy, Washington State University, Pullman, Washington 99163

Received January 10, 1969

Extension of our efforts to prepare tetracyclic phenothiazine derivatives of potential medicinal interest led us to synthesize 3-keto-3H-pyrido[3,2,1-*kl*]phenothiazine-2-carboxylate (I). A structural relationship can be seen between I and the quinolone antibacterial agents, nalidixic acid[2] and oxolinic acid,[3] which are particularly effective against gram-negative organisms. The synthesis of nalidixic acid analogs is of particular interest as a result of the discovery that nalidixic acid selectively inhibits DNA synthesis in growing bacteria.[4]

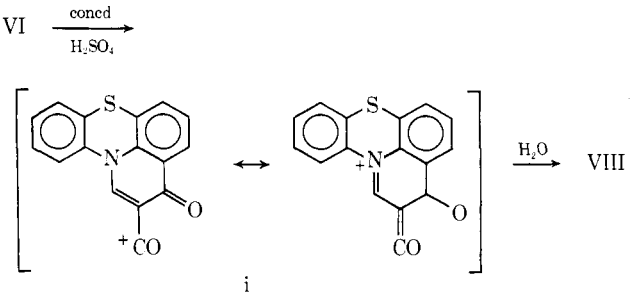
The reactions employed for the preparation of I (isolated as the salt, VIII) are outlined in Scheme I. The tetracyclic ketone II (prepared by procedures previously reported[5]) was converted *via* the glyoxalate III to corresponding β-keto ester IV. Bromination of IV gave the pyridophenothiazinium salt V,[6] which was not isolated but was converted directly to its conjugate base, the unsaturated β-keto ester VI. It was later discovered that VI could be obtained in greater over-all yield by ketone cleavage of the unsaturated glyoxalate VII using NaOEt. Compound VII was obtained from III by bromination followed by neutralization with triethylamine.

The unsaturated β-keto ester VI proved to be surprisingly resistant to alkaline hydrolysis; treatment of VI with ethanolic KOH at reflux temperatures for 18 hr failed to yield I. Unchanged starting material was also obtained when 5% HCl or 72% H_2SO_4 were used as catalysts. Finally, the procedure employed for the hydrolysis of mesitoic ester[7] using concentrated H_2SO_4 was successfully carried out to give VIII, the conjugate acid of I.[8] Attempts to obtain I by ketone cleavage of VII with KOH at 25° gave an uncharacterizable residue. At 0° the glyoxylic acid IX was obtained.

Microbiological Testing.—Compound VIII was tested against a variety of microorganisms *in vitro* employing the method of turbidity measurement of growth in nutrient broth.[9] The results are shown in Table I. Inconclusive results were obtained when VIII was tested against *Streptococcus pyogenes*, *Shigella dysenteriae*, and *Neisseria gonorrhea* due to difficulty in forming uniform suspensions. Use of the paper disk–agar diffusion method[10] failed to show antibacterial activity of VIII, apparently as a result of poor diffusion of the compound in solid agar.

Experimental Section

Melting points were determined on a calibrated Fisher-Johns melting point apparatus. The microanalyses were performed by the Galbraith Laboratories, Knoxville, Tenn. The ir and uv spectra were obtained with a Beckman IR-8 spectrophotometer and Cary 15 spectrophotometer, respectively. Where analyses



The resistance of VI to hydrolysis in aqueous acid may be due to its conversion to the conjugate acid. The failure to obtain alkaline hydrolysis seems rather unusual, since esters of nalidixic acid and oxolinic acid and their analogs are readily hydrolyzed under the same conditions.

(1) (a) For paper I in the series, see A. R. Martin, G. G. Briggs, and T. J. Yale, *J. Pharm. Sci.*, **57**, 166 (1968). (b) This investigation was supported in part by the National Institute of Mental Health, National Institutes of Health, U. S. Public Health Service, Grant No. MH 12425. (c) Abstracted in part from the M.S. thesis of D. S. Huang, Washington State University, 1968.

(2) (a) G. Y. Lesher, E. J. Froelich, M. D. Gruett, J. H. Bailey, and R. P. Brundage, *J. Med. Pharm. Chem.*, **5**, 1063 (1962); (b) E. W. McChesney, E. J. Froelich, G. Y. Lesher, A. V. R. Crain, and D. Rosi, *Toxicol. Appl. Pharmacol.*, **6**, 292 (1964); (c) K. Shimizu, T. Harada, M. Hatakeyawa, O. Kumii, T. Zindachi, E. Yamada, and K. Shimada, *Chemotherapy* (Tokyo), **12**, 384 (1964).

(3) (a) D. Kaminsky and R. I. Meltzer, U. S. Patent 3,172,811 (March 9, 1965); (b) S. M. Ringel, F. J. Turner, D. Kaminsky, and B. S. Schwartz, Presented at the 67th Annual Meeting of the American Society for Microbiology, New York, N. Y., April 30–May 4, 1967.

(4) W. H. Dietz, T. M. Cook, and W. A. Goss, *J. Bacteriol.*, **91**, 768 (1966).

(5) M. Harfenist and E. Magnien, *J. Org. Chem.*, **28**, 1834 (1963).

(6) This result is analogous to the bromine-induced dehydrogenations of II and of its sulfoxide previously reported.[1a]

(7) H. P. Treffers and L. P. Hammett, *J. Am. Chem. Soc.*, **69**, 1232 (1947).

(8) The susceptibility of VI to hydrolysis in concentrated H_2SO_4 can be explained on the basis of its conversion to the resonance-stabilized acylium ion i, which is converted to VIII when the reaction mixture is poured into water.

(9) (a) Society of American Bacteriologists, "Manual of Microbiological Methods," McGraw-Hill Book Co., N. Y., 1957, p 173; (b) transmission readings were measured at 500 mμ on a Beckman DB spectrophotometer.

(10) R. N. Goodman in "Antibiotics, Their Chemistry and Non-medical Uses," H. S. Goldberg, Ed., Van Nostrand Co., New York, N. Y., 1959, pp 322–448.