TABLE I

No.	R	Yield,[a] %	Crystn solvent	Mp, °C	Formula
		Amines:	$RNH(CH_2)_{10}NHR$		
2	9-Acridyl	52.7	C_6H_6–C_6H_{14}	139–145	$C_{36}H_{38}N_4$
3	2-Pyridyl	24.4	DMF–toluene	161–163	$C_{20}H_{30}N_4$[c,d]
4	3-Pyridyl	37.7	C_6H_6	139–142	$C_{20}H_{30}N_4$
5	4-Pyridyl	28.1	C_6H_6–C_7H_{16}	107–113	$C_{20}H_{30}N_4$
		Amides:	$RNHCO(CH_2)_8CONHR$		
6	2-Pyridyl	24.2	MEK	132–133	$C_{20}H_{36}N_4O_2$
7	3-Pyridyl	70.0	MEK	160–161	$C_{20}H_{36}N_4O_2$
8	4-Pyridyl	24.4	MEK	149–151	$C_{20}H_{36}N_4O_2$

[a] Yields are for analytically pure materials. [b] All compounds were analyzed for C, H, and N. Values are within ±0.4% of theoretical except where indicated. [c] Dihydrochloride. [d] C: calcd, 60.14; found, 59.72.

TABLE II

	Min inhib concn, μg/ml[a]					
No.	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*	*Trichomonas vaginalis*	*Trichophyton mentagrophytes*	*Candida albicans*
1	125	500	250	10	100	10
9	25	PI 100	25	100	N	6
2	N	N	N	100	10	100
6	N	N	N	N	PI 250	N
7	N	N	N	N	PI 500–100	N
8	100	500	500	500	PI 100	500

[a] PI = partial inhibition, N = no activity at 500 μg/ml.

filtered and worked up in the usual manner. The resulting compounds (free base or 2HCl salt) were purified by crystallization.

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N^1-Sulfato-N^2-isonicotinylhydrazine. A Potential Metabolite of Isoniazid[1]

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During studies on the metabolism of the antituberculous isoniazid (INH), in animals[3] and man,[4] we considered the possibility that one of the group of acid-labile urinary metabolites was an *N*-sulfate conjugate of INH. This was suggested by earlier *in vivo*[5,6] and *in vitro*[7] studies, using various laboratory animals, in which arylamines such as aniline and 1- and 2-naphthylamine were conjugated with sulfate to form highly acid-labile sulfamates. Since INH is acetylated by the same pigeon liver enzyme that acetylates arylamines,[8] a similar parallel activity seemed possible for sulfate conjugation. If highly acid labile, an INH-sulfate conjugate would contribute to the incompletely identified acid-labile fraction of urinary INH metabolites.

Therefore, we synthesized the INH analog of aryl sulfamates, N^1-sulfato-N^2-isonicotinylhydrazine, by procedures employed previously.[5] It was found to be stable to the mild acidic conditions known to hydrolyze the pyruvic and α-ketoglutaric acid hydrazones of INH[3] and the aryl sulfamates[5] (pH 1.0, room temperature). However, it could be split quantitatively to INH by heating at 45° in 1 *N* HCl for 24 hr, conditions developed for the hydrolysis of acetylisoniazid to INH without the decomposition of INH.[4a] When this hydrolysis procedure was applied to urine collected from dogs receiving INH in studies reported previously,[3] no increase of INH content could be detected. These results indicated that the sulfate conjugate was not a metabolite of INH in the dog. Therefore we tested the *in vivo* stability of the sulfate compound in subsequent experiments.

For preliminary tests on the toxicity of this compound, we administered single doses of 48.1 mg/kg iv (equivalent to 25 mg of INH/kg) to six 26-day-old Holtzman female rats. Similar groups of untreated rats and rats receiving 25 mg of INH/kg served as controls. No untoward reactions were noted in the rats receiving either INH or the INH-sulfate conjugate. The rats were weighed immediately before injection and daily for the next 9 days. Necropsies were performed after killing the animals with $CHCl_3$, with special attention being given to the liver, kidneys, and spleen, which were examined internally as well as externally. No gross changes from the untreated rats were noted in the treated groups. In addition, no toxic effects of the compounds were noted in the growth of the animals, as evidenced by nearly identical weight gains in the treated and untreated groups during the study period.

To determine the *in vivo* stability of the INH-sulfate conjugate, we injected four young adult female beagle dogs (weight range, 5.2–9.7 kg) with doses of 9.6

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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.

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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.

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

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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

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