[CONTRIBUTION NO. 240 FROM THE RESEARCH LABORATORIES OF HOFFMANN-LA ROCHE INC.]

SYNTHETIC TUBERCULOSTATS. II. AMINO- AND HYDROXY-PYRIDINE CARBOXYLIC ACID DERIVATIVES

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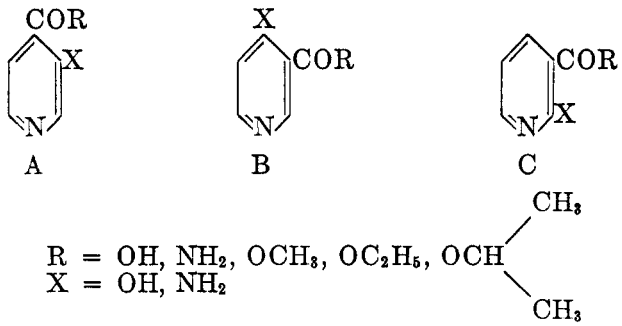
Received December 3, 1951

The discovery of the tuberculostatic activity of 3-aminoisonicotinic acid and its methyl ester (1) suggested the possibility that such activity might also be found in its position isomers and in other closely related compounds. It was decided, therefore, to synthesize the *o*-amino- and *o*-hydroxy-pyridine monocarboxylic acids, their amides, and some of their esters.

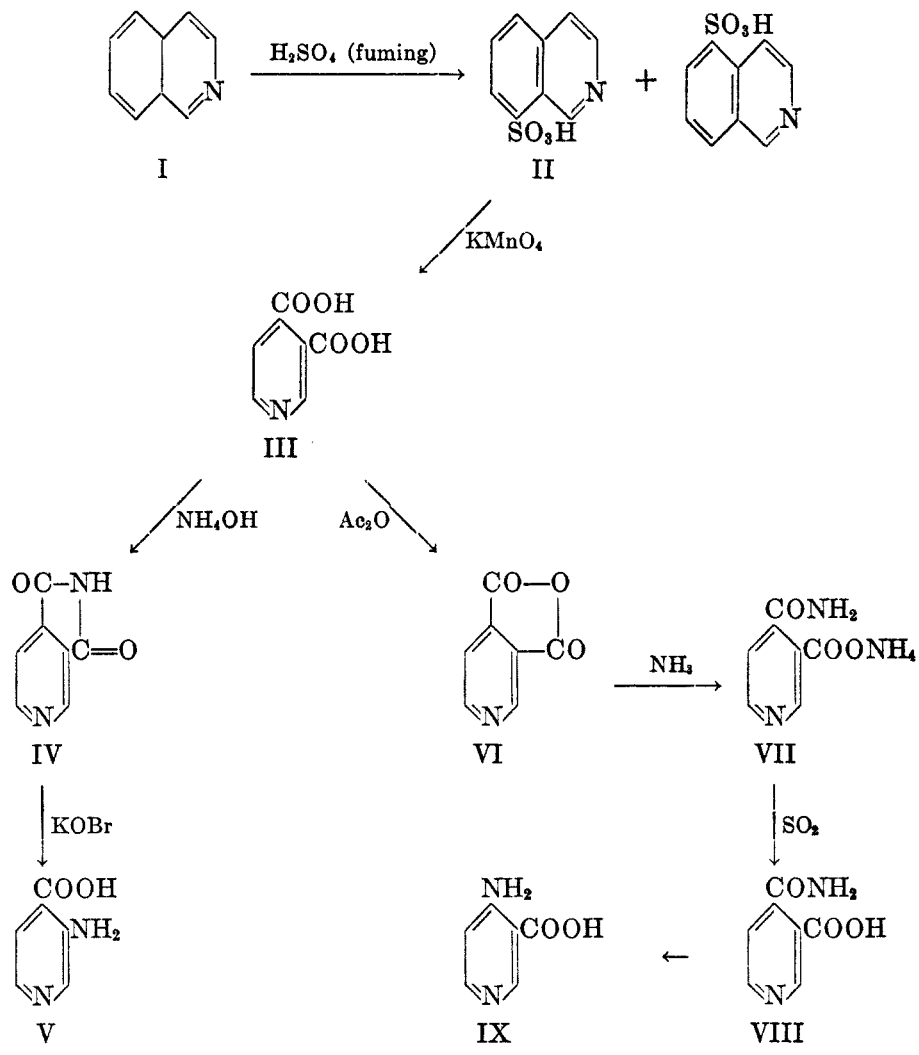


Three series, namely, Series A, B, and C have thus far been investigated—at least in part.

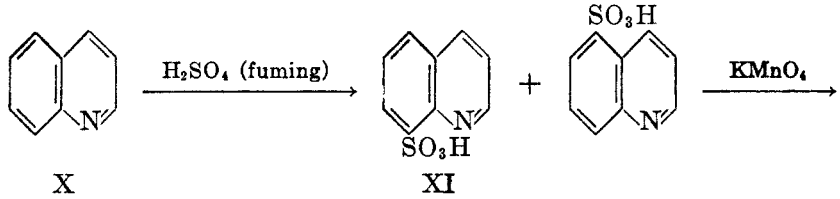
The starting point for the synthesis of the compounds of Series A and B was isoquinoline (I) which was sulfonated according to the method of Hoogewerff and Van Dorp (2). The mixed isoquinoline sulfonates (II) were oxidized with $KMnO_4$ to cinchomeronic acid (III). At this point the procedure was varied, depending upon the series desired. For Series A the cinchomeronic acid was converted to cinchomeronimide (IV) which was in turn degraded to 3-aminoisonicotinic acid (V) (3). For compounds of Series B cinchomeronic acid was converted to the anhydride (VI) (4) which gave the ammonium salt of the monoamide (VII) on treatment with ammonia (5). The free acid (VIII), obtained from the ammonium salt with sulfur dioxide, was subjected to a Hofmann reaction to give 4-aminonicotinic acid (IX) (5, 6).

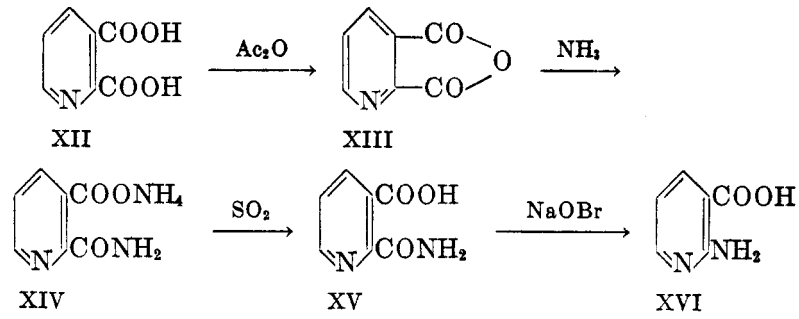
The compounds of Series C were obtained by sulfonating quinoline (X) and oxidizing the mixed quinoline sulfonates (XI) to quinolinic acid (XII) which was in turn converted to the anhydride (XIII). Treatment of the anhydride with ammonia gave the ammonium salt of quinolinic acid monoamide (XIV) which was changed to the free acid (XV) with sulfur dioxide. Compound XV on treatment with sodium hypobromite gave 2-aminonicotinic acid (XVI) (7).

This paper concerns, in the main, the compounds synthesized during the investigation of these series. Most of the compounds so prepared are listed in Table I.

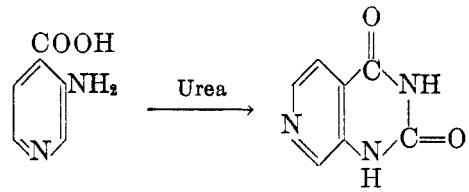


In addition to the simple derivatives of the three basic structures, bicyclic pyridopyrimidines were prepared by condensing urea or thiourea with the appropriate *o*-aminopyridine carboxylic acid. For example, 3-aminoisonicotinic acid (V) gave copazoline-2,4(1*H*,3*H*)-dione (XVII) when heated with urea. These bicyclic derivatives are listed in Table II.

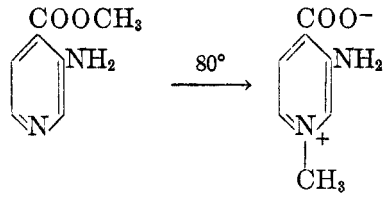




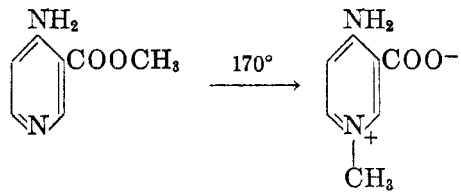
The effect of substituting a sulfur atom for the ethylenic linkage in nicotinamide was studied by preparing the thiazole analog of nicotinamide — 5-thiazolecarboxamide (8, 9).



In an attempt to synthesize 2-(3-aminoisonicotinamido)thiazole, methyl 3-aminoisonicotinate was heated with 2-aminothiazole at 140–150° for about 2 hours. A compound was obtained which possessed neither the anticipated properties of the thiazole derivative nor those of the original ester, even though its elemental analysis corresponded exactly to that of the latter. The new compound proved to be the betaine of methyl 3-aminoisonicotinate which could be obtained by merely heating the ester to its melting point.

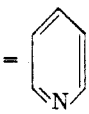


Similarly, when the melting point of methyl 4-aminonicotinate was being taken, it was observed that the compound first melted at 173–173.5° and then resolidified in the capillary tube to decompose finally at 293°. It was surmised, therefore, that the resolidification which took place was due to betaine forma-



tion. This was confirmed by heating a suspension of methyl 4-aminonicotinate in mineral oil to 170°, at which temperature the ester melted and then changed over to the solid betaine. On cooling the mineral oil filtrate, some unconverted ester precipitated out. This was an unexpected result which merited investigation. Accordingly, a small quantity of ester was dissolved in hot mineral oil and was heated to over 200°. On cooling, the unchanged ester was recovered. The experiment was repeated using propylene glycol (b.p. 189°) as a solvent, and here too, although the temperature of the solution was considerably higher than

TABLE I

PYRIDINE CARBOXYLIC ACID DERIVATIVES R = 

SUBSTITUENT					SALT	M.P., °C. (CORR.)	REF.
Nucleus	1	2	3	4			
R			$COOH$	NH_2	–	*ca.* 330 (dec.)	(6)
R		NH_2	$COOH$		–	229	(7)
R			CH_3COO	$COOH$	–	226 (dec.)	E
R		NH_2	$COOC_2H_5$		–	94–96	E
R			$COOCH_3$	NH_2	–	173–173.5	(5)
R	CH_3^+		NH_2	$-COO^-$	–	269–270	E
R			$COOC_2H_5$	NH_2	–	109–111	E
R	CH_3^+		$-COO^-$	NH_2	–	293 (dec.)	E
R		OH	$COOH$		–	258–260	(7)
R			NH_2	$COOCH(CH_3)_2$	HCl	185.5	E
R			NH_2	$COOCH(CH_3)_2$	–	78–79	E
R			$CONH_2$	NH_2	–	229.5–230.5 (dec.)	E
R		NH_2	$COOCH_3$		–	83–84	(11)
R			NH_2	$COOC_2H_5$	–	64–66	E
R		NH_2	$CONH_2$		–	191.5–193.5	(11)
R			CH_3CONH	$COOCH_3$	–	93.5–94.5	E
R			OH	$COOCH_3$	–	78.5–80.5	E

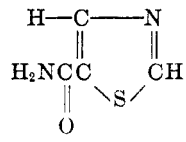
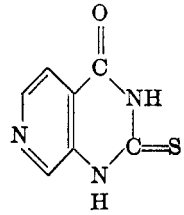
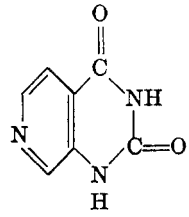
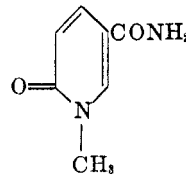
E = See Experimental

the rearrangement temperature, no betaine was formed. It was concluded, therefore, that rearrangement from methyl 4-aminonicotinate to the betaine does not take place in solution—whether the solvent be polar or non-polar—even at temperatures far in excess of the normal rearrangement temperature. The same

observation was subsequently made regarding the rearrangement of methyl 3-aminoisonicotinate to the betaine as described earlier in this report. When methyl 3-aminoisonicotinate was boiled in xylene at 137–140° (about 60° beyond the rearrangement temperature), no change was observed.

TABLE II

BICYCLIC DERIVATIVES AND RELATED COMPOUNDS

STRUCTURE	NAME	M.P., °C. (CORR.)	REF.
	5-Thiazolecarboxamide	201–203	(9)
	2-Thiocopazoline-2,4(1*H*, 3*H*)-dione	>360	E
	Copazoline- 2,4(1*H*, 3*H*)-dione	>320	(3)
	1,6-Dihydro-1-methyl-6-oxonicotinamide	209–211	(10)

E = See Experimental.

The experimental details are given only for those compounds that have not been reported in the literature. All of the compounds listed in Tables I and II are without significant *in vivo* activity.

Acknowledgment. The author acknowledges his indebtedness to Dr. R. J. Schnitzer, Dr. E. Grunberg, and the staff of the Chemotherapy Department for evaluating the compounds herein reported. The author also wishes to thank Dr. A. Steyermark and his staff for the microanalyses.

EXPERIMENTAL

All melting points are corrected.

3-Acetoxyisonicotinic acid. A mixture of 3.7 g. of 3-hydroxyisonicotinic acid was acetylated with sodium acetate and acetic anhydride. The acetoxy compound was recrystallized from a mixture of acetic anhydride and benzene. Fine, white crystals, dec. 226°. It hydrolyzes readily in water.

Anal. Calc'd for $C_8H_7NO_4$: C, 53.0; H, 3.9.

Found: C, 53.5; H. 4.0.

Ethyl 2-aminonicotinate. 2-Aminonicotinic acid (38 g.), 250 cc. of dry ethanol, 130 cc. of dry benzene, and 30 cc. of conc'd sulfuric acid were refluxed for 24 hours using a water-trap to facilitate water removal. When the esterification was complete, the excess benzene and ethanol were removed, the residual oil was dissolved in ice-water, and the resulting solution was made alkaline with sodium carbonate. The alkaline mixture was extracted with chloroform to give 42 g. of the ethyl ester. Large, white spires from Skellysolve "B"; m.p. 94–96°. Soluble in the common organic solvents, hot water, and hot Skellysolve "B".

Anal. Calc'd for $C_8H_{10}N_2O_2$: C, 57.8; H, 6.0.

Found: C, 58.0; H, 5.8.

Betaine of methyl 3-aminoisonicotinate. Methyl 3-aminoisonicotinate (10 g.) was heated to about 80° in an oil-bath. The ester melted at first, and the melt then solidified with evidences of some decomposition. The solid betaine was recrystallized from a mixture of water-ethanol-acetone as golden yellow crystals, dec. 269–270°. It was very soluble in water; insoluble in organic solvents.

Anal. Calc'd for $C_7H_8N_2O_2$: C, 55.3; H, 5.3; N, 18.4.

Found: C, 55.0; H, 5.3; N, 18.3.

Ethyl 4-aminonicotinate. 4-Aminonicotinic acid (46 g.), 300 cc. of ethanol, 157 cc. of dry benzene, and 36 cc. of conc'd sulfuric acid were refluxed for 48 hours under a water trap. The reaction mixture was worked up as described above to give 29 g. of the ester. Long, colorless needles from ligroin; m.p. 109–111°.

Anal. Calc'd for $C_8H_{10}N_2O_2$: C, 57.8; H, 6.0.

Found: C, 57.7; H, 6.0.

Betaine of methyl 4-aminonicotinate. A suspension of 17 g. of methyl 4-aminonicotinate in mineral oil was heated carefully in an oil-bath until the internal temperature was about 170°, at which point the ester began to melt and then—with considerable evolution of heat—changed over to the solid betaine. If heating was not carefully controlled, extensive decomposition took place at this stage even though the betaine, once formed, does not decompose until 293°. The mixture was filtered hot, and the insoluble betaine was washed with ligroin and recrystallized from a mixture of water-2-propanol-acetone to yield 12 g. of glistening white crystals, dec. 293° (with previous darkening). Very soluble in water and insoluble in organic solvents.

Anal. Calc'd for $C_7H_8N_2O_2$: C, 55.3; H, 5.3.

Found: C, 55.6; H, 5.4.

Isopropyl 3-aminoisonicotinate hydrochloride. 3-Aminoisonicotinic acid (15 g.) was esterified in the customary way using 100 cc. of 2-propanol, 10 cc. of conc'd sulfuric acid, and 200 cc. of dry benzene—the latter to facilitate removal of water. The free base of the ester was converted to the hydrochloride which was recrystallized from a mixture of isopropanol-acetone-ether as yellow needles, dec. 185.5°.

Anal. Calc'd for $C_9H_{12}N_2O_2 \cdot HCl$: C, 49.9; H, 6.0.

Found: C, 49.6; H, 5.6.

The free base, *isopropyl 3-aminoisonicotinate*, was obtained by the addition of sodium bicarbonate to an aqueous solution of the hydrochloride. Long, white spires from Skellysolve "B"; m.p. 78–79°.

Anal. Calc'd for $C_9H_{12}N_2O$: C, 60.0; H, 6.7.

Found: C, 60.2; H, 6.6.

4-Aminonicotinamide. A mixture of 25 g. of methyl 4-aminonicotinate, 150 cc. of methanol, and 40 cc. of liquid ammonia was heated at 80–90° at 1000 lbs. nitrogen pressure for about 50 hours. The solid residue, remaining after removal of the methanol, was extracted with hot chloroform to remove unreacted ester. The chloroform-insoluble amide was recrystallized from water. White needles; m.p. 229.5–230.5° (with previous softening).

Anal. Calc'd for $C_6H_7N_3O$: N, 30.7. Found: N, 30.8.

Ethyl 3-aminoisonicotinate. A mixture of 44.6 g. of 3-aminoisonicotinic acid, 200 cc. of ethanol, 200 cc. of dry benzene, and 36 cc. of conc'd sulfuric acid was reacted in the usual way to give 37 g. of the ethyl ester. Long, pale yellow needles from ligroin (Skellysolve "B"); m.p. 64–66°.

Anal. Calc'd for $C_8H_{10}N_2O_2$: C, 57.8; H, 6.0.
Found: C, 57.9; H. 6.1.

Methyl 3-acetamidoisonicotinate. A mixture of 10 g. of methyl 3-aminonicotinate and 15 cc. of acetic anhydride was heated on a steam-bath for about 1 hour. To the reaction mixture was then added 10 cc. of water, and the resulting acetic acid was removed under a vacuum to leave a dry residue of the acetylated product. White needles from Skellysolve "B"; m.p. 93.5–94.5°.

Anal. Calc'd for $C_9H_{10}N_2O_2$: C, 55.7; H, 5.2.
Found: C, 55.7; H, 5.4.

Methyl 3-hydroxyisonicotinate. To 30 g. of methyl 3-aminonicotinate dissolved in 1200 cc. of 0.5 *N* sulfuric acid was added 14 g. of sodium nitrite. The mixture was then heated on a steam-bath until evolution of nitrogen ceased. The dark colored reaction mixture was concentrated under a vacuum to about 400 cc., and the concentrate was saturated with sodium acetate to yield a crystalline precipitate. The mixture (including the precipitate) was extracted several times with ether. The ether extracts were combined and dried, and the ether was finally removed, leaving methyl 3-hydroxyisonicotinate as the residue. Bright yellow needles from Skellysolve "B"; m.p. 78.5–80.5° with resolidification at about 190° (probably due to betaine formation). The product gives a red color with ferric chloride.

Anal. Calc'd for $C_7H_7N_3O$: N, 9.2. Found: N, 9.4.

2-Thiocopazoline-2,4(1H,3H)-dione. A suspension of 15 g. of 3-aminoisonicotinic acid and 12 g. of thiourea in mineral oil was heated and stirred at 170–180° for 5 hours. The product was filtered off and washed successively with ligroin, benzene, and acetone; yield, 21 g. For further purification the copazolinedione was dissolved in hot, dilute potassium hydroxide, filtered with Norit, and reprecipitated with glacial acetic acid. The resulting tan powder sublimed without melting at a temperature in excess of 360°; soluble only in strong alkali.

Anal. Calc'd for $C_7H_5N_3OS$: C, 46.9; H, 2.8.
Found: C, 47.3; H. 2.7.

CONCLUSION

On the basis of the work done in this study so far, it would appear that a high degree of specificity exists between structure and tuberculostatic activity, since any positional or structural deviation from 3-aminonicotinic acid and its methyl ester (1) resulted in loss of activity.

SUMMARY

Various *o*-amino- and *o*-hydroxy-nicotinic and isonicotinic acid derivatives were synthesized and studied for tuberculostatic activity. All the compounds were inactive.

NUTLEY 10, N. J.

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