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SYNTHETIC TUBERCULOSTATS. V. ALKYLIDENE DERIVATIVES OF ISONICOTINYLHYDRAZINE

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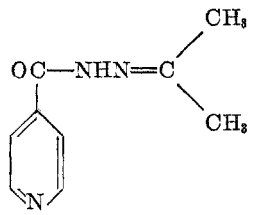
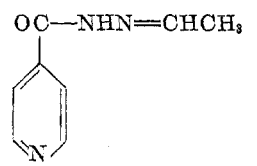
The search for tuberculoactive pyridine derivatives which led to the discovery of the remarkable *in vivo* activity of isonicotinyl hydrazine against *M. tuberculosis* (1–6) had shown that for most of the pyridine tuberculostats the structure-activity relationships were quite specific and that slight structural modifications resulted in marked diminution or total abolition of activity. With regard to the hydrazides, it was evident that certain changes, such as the shifting of the hydrazide grouping from the *gamma* to the *beta* position, were incompatible with activity (6).

To explore this field further, isonicotinyl hydrazine was reacted with acetone to give 1-isonicotinyl-2-isopropylidene hydrazine (I) which proved to be very active. It was decided therefore to systematically investigate alkylidene derivatives of isonicotinyl hydrazine with the two-fold view of discovering superior tuberculostats and determining, if possible, the structural limits of activity. Since the isopropylidene group is a branched chain substituent, the study was extended to the straight chain alkylidenes by preparing 1-isonicotinyl-2-ethylidene hydrazine (II) from isonicotinyl hydrazine and acetaldehyde. Not only did the ethylidene derivative show powerful tuberculostatic activity but, in fact, it was soon discovered that every alkylidene derivative of the 1-isonicotinyl-2-alkylidene hydrazine type—whether straight or branched chain—was actively tuberculostatic *in vivo*. Similarly, cycloalkylidene and arylalkylidene derivatives were also notable tuberculostats.



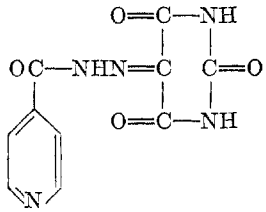
I



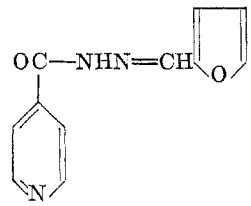
II

The effect of introducing heterocyclic nuclei into the hydrazide grouping was determined by condensing isonicotinyl hydrazine with alloxan to give 1-iso-

nicotinyl-2-(2,4,6-trioxohexahydro-5-pyrimidylidene)hydrazine (III) and with furfuraldehyde to give 1-isonicotinyl-2-(2-furfurylidene)hydrazine (IV). Both compounds were active.

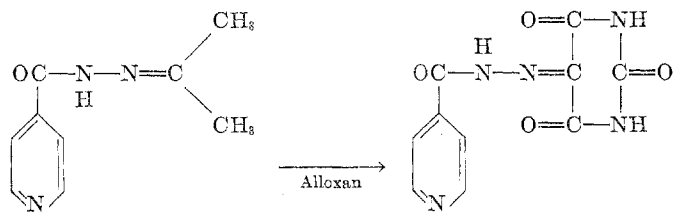


III



IV

Chemically, all of the compounds of this series with the possible exception of the alloxan derivative were characterized by the ease with which they could be hydrolyzed. Indeed, some of the compounds with large substituent groups appeared to be susceptible to scission even in the solid state since it was practically impossible to free them from the odor of the aldehydes or ketones used in their preparation. The apparent stability of the alloxan derivative (III) was probably due to its insolubility—a property which could be usefully employed to detect and remove fairly small quantities of isonicotinyl hydrazine in the presence of its water-soluble, N^2, substitution derivatives. Thus, isonicotinyl hydrazine could be effectively removed from a mixture with 1-isonicotinyl-2-isopropylhydrazine by treating a dilute aqueous solution of the mixture with alloxan. This use could not, however, be applied to mixtures of isonicotinylhydrazine with 1-isonicotinyl-2-alkylidene hydrazines since the more soluble alkylidene derivatives decomposed after a short while in the presence of an alloxan solution, to give the insoluble alloxan derivative (III). For example, a water solution of 1-isonicotinyl-2-isopropylidenehydrazine (I) on treatment with alloxan yielded compound (III).



I III

The compounds prepared in this study are listed in Tables I, II, and III. All of them were markedly tuberculostatic in mouse tuberculosis.

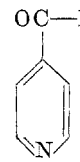
All of the melting points in Tables I, II, and III are corrected.

The preparations of those compounds which have not appeared previously in the literature are described in the Experimental.

Acknowledgment. The authors are indebted to Drs. R. J. Schnitzer and E. Grunberg and the staff of the chemotherapy laboratory for testing the compounds

TABLE I

STRAIGHT-CHAIN ALKYLIDENE DERIVATIVES

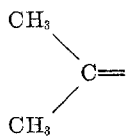
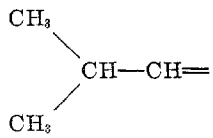
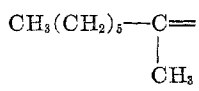
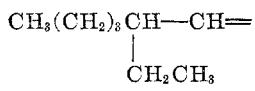
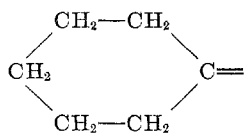


R	M.P., °C.	REF.
$CH_3CH=$	175.5–176	E[a]
$CH_3CH_2CH=$	142.5–143.5	E
$CH_3CH_2CH_2CH=$	113–114	E
$CH_3CH_2CH_2CH_2CH_2CH=$	92.5–93.5	E
$CH_3(CH_2)_5CH=$	101–102	E

[a] E = See Experimental.

TABLE II

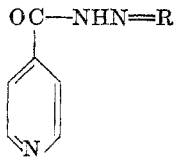
BRANCHED-CHAIN ALKYLIDENE DERIVATIVES

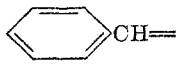
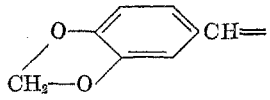
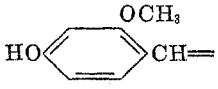
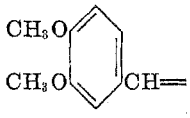
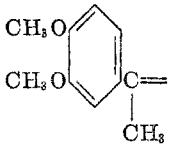
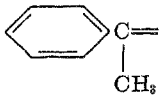
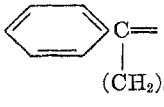
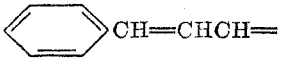
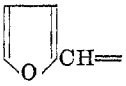
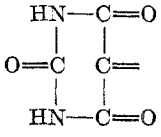
R	M.P. °C	REF.
	161–161.5	E[a]
	136.5–138	E
	75–77	E
	89.5–91.5	E
	167.5–169.5	E

[a] E = Experimental.

TABLE III

ARYLALKYLIDENE DERIVATIVES



R	M.P., °C	REF.
	199–200	(7)
	171.5–173.5	E[a]
	236–237	E
	229.5–230.5	(7)
	189–190	E
	189.5–190.5	E
	173–173.5	E
	125.5–126.5	E
	201–202	E
	217–218	E
	243.5 (dec.)	E

[a] E = See Experimental.

and to Dr. A. Steyermark and his staff for the microanalyses. Thanks are also due to Dr. W. Wenner for his helpful suggestions.

EXPERIMENTAL

All the melting points are corrected.

1. *1-Isonicotinyl-2-ethylidenehydrazine.* A mixture of 30 g. of isonicotinylhydrazine, 40 cc. of acetaldehyde, and 300 cc. of propanol-2 was heated on a steam-bath to solution. On cooling the reaction mixture, white crystals of practically pure 1-isonicotinyl-2-ethylidenehydrazine precipitated out. The product could be recrystallized from propanol-2 or chloroform. Yield 33 g.; m.p. 175.5–176°.

Anal. Calc'd for $C_8H_9N_3O$: C, 59.0; H, 5.5.
Found: C, 59.4; H, 5.9.

2. *1-Isonicotinyl-2-propylidenehydrazine.* To a mixture of 20 cc. of freshly distilled propionaldehyde and 100 cc. of methanol was added 20 g. of isonicotinylhydrazine. Heat was evolved spontaneously, and the isonicotinylhydrazine went into solution. The excess propionaldehyde and methanol were removed under a vacuum, and the white crystalline residue was recrystallized from ethyl acetate to yield 22.5 g. of pure colorless crystals of 1-isonicotinyl-2-propylidenehydrazine; m.p. 142.5–143.5°.

Anal. Calc'd for $C_9H_{11}N_3O$: C, 61.0; H, 6.2.
Found: C, 60.9; H, 5.9.

3. *1-Isonicotinyl-2-isopropylidenehydrazine.* A mixture of 40 g. of isonicotinylhydrazine and 600 cc. of acetone was refluxed until solution was complete. On cooling the reaction mixture, 49 g. of pure 1-isonicotinyl-2-isopropylidenehydrazine precipitated out. Fine white needles; m.p. 161–161.5°.

Anal. Calc'd for $C_9H_{11}N_3O$: C, 61.0; H, 6.2.
Found: C, 60.8; H, 6.1.

4. *1-Isonicotinyl-2-butylidenehydrazine.* A mixture of 40 g. of isonicotinylhydrazine, 40 cc. of freshly distilled butyraldehyde, and 300 cc. of methanol was reacted as described in Experiment 2 above to yield 48 g. of 1-isonicotinyl-2-butylidenehydrazine. White granular crystals from toluene; m.p. 113–114°.

Anal. Calc'd for $C_{10}H_{13}N_3O$: C, 62.9; H, 6.8.
Found: C, 63.2; H, 7.1.

5. *1-Isonicotinyl-2-isobutylidenehydrazine.* A mixture of 40 g. of isonicotinylhydrazine, 40 cc. of freshly distilled isobutyraldehyde, and 150 cc. of methanol was reacted as described in Experiment 2 above to yield 48 g. of 1-isonicotinyl-2-isobutylidenehydrazine. Colorless rhomboids from benzene; m.p. 136.5–138°.

Anal. Calc'd for $C_{10}H_{13}N_3O$: C, 62.8; H, 6.8.
Found: C, 62.7; H, 7.2.

6. *1-Isonicotinyl-2-hexylidenehydrazine.* A mixture of 40 g. of isonicotinylhydrazine, 40 cc. of freshly distilled hexaldehyde, and 200 cc. of methanol was reacted as described in Experiment 2 above to yield 57 g. of 1-isonicotinyl-2-hexylidenehydrazine. White needles from ethyl acetate or benzene; m.p. 92.5–93.5°.

Anal. Calc'd for $C_{12}H_{17}N_3O$: C, 65.8; H, 7.8.
Found: C, 65.9; H, 8.1.

7. *1-Isonicotinyl-2-heptylidenehydrazine.* Isonicotinylhydrazine (20 g.), 17 g. of freshly distilled heptaldehyde, and 150 cc. of water were mixed to form a two-phase oil-water system. On shaking, the oily phase was replaced by a white precipitate of 1-isonicotinyl-2-heptylidenehydrazine. Yield 31 g.; white needles from xylene; m.p. 101–102°.

Anal. Calc'd for $C_{13}H_{19}N_3O$: C, 67.0; H, 8.2.
Found: C, 66.7; H, 8.0.

8. *1-Isonicotinyl-2-(1-methylheptylidene)hydrazine.* A mixture of 40 g. of isonicotinylhydrazine, 50 cc. of methyl hexyl ketone, and 300 cc. of methanol was refluxed for 4 hours. The methanol and excess of ketone were then removed under a vacuum, and 200 cc. of ligroin

(b.p. 60–70°) was added to the residue. The mixture was heated with stirring to solution and finally cooled to give 60 g. of 1-isonicotinyl-2-(1-methylheptylidene)hydrazine. White needles from ligroin (b.p. 60–70°); m.p. 75–77°.

Anal. Calc'd for $C_{14}H_{21}N_3O$: C, 68.0; H, 8.5.
Found: C, 68.3; H, 8.1.

9. *1-Isonicotinyl-2-(2-ethylhexylidene)hydrazine.* A mixture of 20 g. of isonicotinylhydrazine, 18 g. of 2-ethylhexaldehyde, and 150 cc. of water was reacted as described in Experiment 7 above to yield 30 g. of 1-isonicotinyl-2-(2-ethylhexylidene)hydrazine. White crystals from a xylene-ligroin mixture; m.p. 89.5–91.5°.

Anal. Calc'd for $C_{15}H_{21}N_3O$: C, 68.0; H, 8.5.
Found: C, 68.3; H, 8.3.

10. *1-Isonicotinyl-2-cyclohexylidenehydrazine.* A mixture of 20 g. of isonicotinylhydrazine, 15 g. (16 cc.) of cyclohexanone, and 250 cc. of water was reacted as in Experiment 7 to give 1-isonicotinyl-2-cyclohexylidenehydrazine in practically quantitative yield. Fine white needles from ethanol; m.p. 167.5–169.5°.

Anal. Calc'd for $C_{12}H_{15}N_3O$: C, 66.5; H, 6.9.
Found: C, 66.1; H, 7.2.

11. *1-Isonicotinyl-2-(p-methoxybenzylidene)hydrazine.* A mixture of 13.7 g. of isonicotinylhydrazine and a slight excess of anisaldehyde was heated on a steam-bath. Partial solution took place, followed by solidification of the reaction mixture to produce a practically quantitative yield of 1-isonicotinyl-2-(*p*-methoxybenzylidene)hydrazine. Lustrous white flakes from xylene; m.p. 171.5–173.5°.

Anal. Calc'd for $C_{14}H_{13}N_3O_2$: C, 65.9; H, 5.1.
Found: C, 66.1; H, 5.2.

12. *1-Isonicotinyl-2-piperonylidenehydrazine.* A mixture of 27.4 g. of isonicotinylhydrazine, 30 g. of piperonal, and 200 cc. of water was warmed to about 50° and shaken until the solid 1-isonicotinyl-2-piperonylidenehydrazine began to precipitate. The mixture was permitted to stand until it became almost solid. The precipitate was then filtered off and was suspended in hot propanol-2. On cooling the suspension, 42 g. of pure product was obtained. Colorless prisms or fine needles; m.p. 236–237°.

Anal. Calc'd for $C_{14}H_{11}N_3O_3$: C, 62.5; H, 4.1.
Found: C, 62.3; H, 4.2.

13. *1-Isonicotinyl-2-veratrylidenehydrazine.* A mixture of 42 g. of isonicotinylhydrazine, 51 g. of veratraldehyde, and 600 cc. of propanol-2 was reacted as in Experiment 1 to yield 78 g. of 1-isonicotinyl-2-veratrylidenehydrazine. Thick colorless prisms from propanol-2; m.p. 189–190°.

Anal. Calc'd for $C_{15}H_{15}N_3O_3$: C, 63.1; H, 5.3.
Found: C, 62.9; H, 5.3.

14. *1-Isonicotinyl-2-(α-methylveratrylidene)hydrazine.* A mixture of 28 g. of isonicotinylhydrazine, 37 g. of 3,4-dimethoxyacetophenone, and 100 cc. of propanol-2 was reacted as in Experiment 1 to yield about 40 g. of 1-isonicotinyl-2-(α-methylveratrylidene)hydrazine. Fine white needles from isopropanol-2; m.p. 189.5–190.5°.

Anal. Calc'd for $C_{16}H_{17}N_3O_3$: C, 64.2; H, 5.7.
Found: C, 64.1; H, 5.3.

15. *1-Isonicotinyl-2-(α-methylbenzylidene)hydrazine.* A mixture of 40 g. of isonicotinylhydrazine, 40 cc. of acetophenone, and 200 cc. of methanol was refluxed for 2 hours. On cooling, the practically pure 1-isonicotinyl-2-(α-methylbenzylidene)hydrazine precipitated in good yield. White needles from methanol; m.p. 173–173.5°.

Anal. Calc'd for $C_{14}H_{13}N_3O$: C, 70.3; H, 5.4.
Found: C, 69.9; H, 5.2.

16. *1-Isonicotinyl-2-(α-heptylbenzylidene)hydrazine.* A mixture of 26 g. of phenyl heptyl ketone, 17.5 g. of isonicotinylhydrazine, and 100 cc. of methanol was refluxed for about 2 hours. The bulk of the methanol was then removed under a vacuum to yield 45 g. of 1-isonicotinyl-2-(α-heptylbenzylidene)hydrazine. Lustrous white needles from propanol-2-ligroin (60–72°) mixture; m.p. 125.5–126.6°.

Anal. Calc'd for $C_{20}H_{25}N_3O$: C, 74.3; H, 7.8.
Found: C, 74.6; H, 8.1.

17. *1-Isonicotinyl-2-cinnamylidenehydrazine.* A mixture of 27.4 g. of isonicotinylhydrazine, 26.4 g. of cinnamaldehyde, and 500 cc. of propanol-2 was reacted as in Experiment 1 to yield 46 g. of pure 1-isonicotinyl-2-cinnamylidenehydrazine. Small yellow crystals; m.p. 201–202°.

Anal. Calc'd for $C_{15}H_{13}N_3O$: C, 71.7; H, 5.2.
Found: C, 71.6; H, 4.9.

18. *1-Isonicotinyl-2-furfurylidenehydrazine.* A mixture of 40 g. of isonicotinylhydrazine, 30 cc. of freshly distilled furfuraldehyde, and 200 cc. of methanol was reacted as in Experiment 2 above. On cooling the reaction mixture, 61 g. of practically pure 1-isonicotinyl-2-furfurylidenehydrazine precipitated. Fine white needles from dilute propanol-2; m.p. 217–218°.

Anal. Calc'd for $C_{11}H_9N_3O_2$: C, 61.5; H, 4.2.
Found: C, 61.2; H, 4.0.

19. *1-Isonicotinyl-2-(2,4,6-trioxo-hexahydro-5-pyrimidylidene) hydrazine monohydrate.* To 27.4 g. of isonicotinylhydrazine in 375 cc. of water was added 32 g. of alloxan in 160 cc. of water. A quantitative yield of practically pure pyrimidylidene derivative was obtained. Fine white crystals; m.p. 243.5° (dec.). The product was insoluble in all solvents except strong acids and alkalis.

Anal. Calc'd for $C_{10}H_7N_5O_4 \cdot H_2O$: C, 43.0; H, 3.2.
Found: C, 43.2; H, 3.6.

Conclusion. On the basis of the work covered in this report, it is clearly apparent that a whole new field of endeavor has been opened up and that literally hundreds of active tuberculostats can be synthesized by the simple expedient of substituting alkylidene, cycloalkylidene, or arylalkylidene groupings for the two terminal hydrogen atoms on the hydrazine moiety of isonicotinylhydrazine. So far, no exception has been found to this generalization despite the variety of structures employed in this study. Not only can large numbers of tuberculostats be synthesized in this field, but the *in vivo* potency of several of them is of a higher order than that of streptomycin itself.

SUMMARY

A series of twenty-one alkylidene and arylalkylidene derivatives of isonicotinylhydrazine has been prepared and studied for tuberculostatic activity. All of them have been active and the *in vivo* activities of several of them are greater than that of any known synthetic tuberculostat other than that of isonicotinylhydrazine itself.

NUTLEY 10, N. J.

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