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2. **Shichiro Kakimoto and Ken-ichi Yamamoto**: Studies on Antitubercular Compounds. X[1]. Condensation Products of Aldehydes and Acid Hydrazides of Pyridine Group.

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In the previous work,[2] 1-isonicotinoyl-2-isonicotinidenehydrazine proved to be more active *in vitro* than isonicotinic acid hydrazide, but not so *in vivo* in guinea pigs.[3] The mechanism of this difference between *in vivo* and *in vitro* are being studied in this institute, while other condensation products of aldehydes and acid hydrazides of pyridine group have to be studied in relation to chemical constitution and antitubercular activity, even only *in vitro*.

By the condensation of pyridine-α-, -β-, and -γ-aldehydes with α-, β- and γ-acid hydrazides of pyridine-carboxylic acids, nine condensation products were prepared. These compounds were all examined for antibacillary activity against the $H_{37}Rv$ strain of the human type tubercle bacilli on Kirchner's medium, containing 10% bovine serum.

From the tuberculostatic activities shown in Table I, it is clear that the compounds condensed with the effective acid hydrazides are also effective. The condensation products with isonicotinic acid hydrazide are most effective and the derivatives of nicotinic acid hydrazide are not, and that the activity of acid hydrazide *in vitro* is promoted by the condensation with pyridine aldehyde.

Table I.

	Condensation product		Min. inhibiting concn. γ/cc.	Crystal water	m. p. (dried subst.)
	Aldehyde	Acid hydrazide			
I	α	α	10	0	158°
II	〃	β	>100	2	102°(159°)
III	〃	γ	1	1	95°(168°)
IV	β	α	50	1	167°(167°)
V	〃	β	>100	2	211°(211°)
VI	〃	γ	1	0	238°
VII	γ	α	50	0	195°
VIII	〃	β	>100	4	80°(195°)
IX	〃	γ	1	2	231°(231°)
X	INAH (Control)		5		

The aldehyde was prepared by the oxidation of α- and γ-picoline with selenium dioxide. The 1-oxide of α-picoline was derived to the aldehyde.[4] β-Aldehyde was not obtained by these methods, but was prepared by the oxidation of nicotinic acid hydrazide with potassium periodate.[5] Condensation products were obtained by refluxing the aqueous or ethanolic solution of the two components for 1 to 0.5 hour. Crystal water and melting points are shown in Table I.

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Experimental

Pyridine-2-aldehyde—Pyridine-2-aldehyde diacetate, used for the preparation of the compounds (I), (II), and (III), was prepared through the 1-oxide of α-picoline and pyridine-2-methanol acetate[4] was obtained. α-Picoline was oxidized directly with SeO_2 at 130° to pyridine-2-aldehyde.[2]

1-Picolinoyl-2-picolinidenehydrazine (I)—A soultion of 1 g. of pyridine-2-aldehyde diacetate and 0.5 g. of picolinic acid hydrazide in 5 cc. of water was refluxed for 0.5 hr. After cool, the mixture was neutralized with Na_2CO_3, the separated crude crystalline precipitate was washed, dried, and recrystallized from water. Yield, 0.6 g. Fine needles, m.p. 158°. *Anal.* Calcd. for $C_{12}H_{10}ON_4$: C, 63.70; H, 4.50. Found: C, 63.48; H, 4.89.

1-Nicotinoyl-2-picolinidenehydrazine (II)—Prepared by the same method as above, from 1 g. of the aldehyde diacetate and 0.5 g. of nicotinic acid hydrazide. Yield, 0.5 g. Fine prisms, m.p. 102°, with 2 moles of crystal water. The dried substance melted at 159°. By admixture with (I), m.p. 158°, depression of about 20° was observed. *Anal.* Calcd. for $C_{12}H_{10}ON_4 \cdot 2H_2O$: C, 54.95; H, 5.38; H_2O, 13.73. Found: C, 54.59; H, 5.48, H_2O, 13.82.

1-Isonicotinoyl-2-picolinidenehydrazine (III)—From 2 g. of the aldehyde diacetate and 1.5 g. of isonicotinic acid hydrazide, 1.5 g. of (III) was obtained as fine needles, m.p. 95°, with 1 mole of crystal water. The dried substance melted at 168°. *Anal.* Calcd. for $C_{12}H_{10}ON_4$ (Anhyd. sub.): C, 63.70; H, 4.50. Found: C, 63.48; H, 4.86.

Pyridine-3-aldehyde—Prepared by the oxidation of nicotinic acid hydrazide with KIO_3 instead of Na salt,[4] and the chloroform solution of the aldehyde was used directly for the following condensation without separation.

1-Picolinoyl-2-nicotinidenehydrazine (IV)—To the chloroform solution of pyridine-3-aldehyde, prepared from 3 g. of nicotinic acid hydrazide as mentioned above, after distilling off the solvent, 15 cc. of EtOH and 1 g. of picolinic acid hydrazide were added. The mixture was refluxed for 1 hr. After evaporation of the solvent, the residue was recrystallized from water. Yield, 0.3 g. Fine needles, m.p. 167°, with 1 mole of crystal water. Dried substance also melted at 167°. By admixture with (III) (anhyd. sub., m.p. 168°) depression of about 30° was observed. *Anal.* Calcd. for $C_{12}H_{10}ON_4 \cdot H_2O$: H_2O, 7.35. Found: H_2O, 7.77. Calcd. for $C_{12}H_{10}ON_4$ (Anhyd. sub.): C, 63.70; H, 4.50. Found: C, 63.82; H, 4.83.

1-Nicotinoyl-2-nicotinidenehydrazine (V)—The aldehyde and nicotinic acid hydrazide were condensed to give (V). Yield, 0.7 g. Fine needles, m.p. 211°, with 2 moles of crystal water, and the dried substance also showed m.p. 211°. *Anal.* Calcd. for $C_{12}H_{10}ON_4 \cdot 2H_2O$: C, 54.95; H, 5.38; H_2O, 13.73. Found: C, 54.76; H, 5.68; H_2O, 13.96.

1-Isonicotinoyl-2-nicotinidenehydrazine (VI)—The aldehyde and isonicotinic hydrazide were condensed to give (VI). Yield, 0.4 g. Prisms, m.p. 238°. *Anal.* Calcd. for $C_{12}H_{10}ON_4$: C, 63.70; H, 4.50. Found: C, 63.82; H, 4.83.

Pyridine-4-aldehyde—Previously,[2] it was obtained by the oxidation of γ-picoline with SeO_2 and the reaction mixture, after separation of Se, directly used for the condensation with acid hydrazide. It was found better to extract the reaction mixture 3 times with ether, and the ethereal solution was dried, filtered, concentrated, and used for the condensation.

1-Picolinoyl-2-isonicotinidenehydrazine (VII)—The concentrated ethereal solution of the 4-aldehyde, prepared from 8 cc. of γ-picoline, was condensed with 1 g. of picolinic acid hydrazide in EtOH. Then the reaction mixture was dried up on a water bath, and the residue was recrystallized from water. Yield, 1 g. Fine needles, m.p. 195°. *Anal.* Calcd. for $C_{12}H_{10}ON_4$: C, 63.70; H, 4.50. Found: C, 63.73; H, 4.73.

1-Nicotinoyl-2-isonicotinidenehydrazine (VIII)—Preparated as above. Yield, 1.2 g. Fine needles, m.p. 80°, with 4 moles of crystal water. The dried substance melted at 195°. *Anal.* Calcd. for $C_{12}H_{10}ON_4 \cdot 4H_2O$: C, 48.31; H, 6.08; H_2O, 24.16. Found: C, 48.10; H, 6.31; H_2O, 23.98.

1-Isonicotinoyl-2-isonicotinidenehydrazine (IX)[2]—It has 2 moles of crystal water. The dried substance melted also at 231°. The dried substance was analyzed in previous paper.[2] *Anal.* Calcd. for $C_{12}H_{10}ON_4 \cdot 2H_2O$: H_2O, 13.73. Found: H_2O, 13.55.

***In vitro* Tests against Tubercle Bacilli**—Kirchner's medium containing 10% of bovine serum was used as a test medium. In the present study, use was made of cultures of a virulent human tubercle bacilli, strain $H_{37}Rv$, grown for 3 weeks on Ogawa's egg medium. The test inoculum employed consisted of 0.1 cc. of the aqueous suspension containing 3 mg./cc. of the culture. The compounds under investigation, sterilized by heat in propylene glycol solution, were tested in final concetrations of 100, 50, 10, 1, and 0.5 per cc. and the reading of the results was made visually for growth after 2 weeks of incubation at 37°. The minimum complete inhibitory concentrations thus obtained are presented in Table I. INAH was used as the control.

Summary

Nine condensation products of isomeric three aldehydes and three acid hydrazides of pyridine group were prepared and their tuberculostatic activity against virulent human tubercle bacillus, strain $H_{37}Rv$, was examined on Kirchner's medium containing 10% of bovine serum. The most active compounds were the condensation products of isonicotinic acid hydrazide with aldehydes and stronger than isonicotinic acid hydrazide *in vitro*.

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3. **Tsutomu Sugasawa** : Aconitum-Alkaloide. XI.[1]
Über die Shimoburobase. I.

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Die Aconitum-Knollen aus Shimoburo bzw. Sakuma der Prov. Awomori (Spezies: Ochiai Nr. 13)[2] enthalten ein Alkaloid, welches über sein im Wasser schwerlösliches Chlorhydrat (Zers. Pkt. 278~279°) leicht trennbar ist. Die freie Base stellt Prismen vom Schmp. 198~202° und von der Drehung $[\alpha]_D^{24}$: −141.1° (Methanol) dar und bildet noch ausserdem ein, prismatisches Bromhydrat vom Zers. Pkt. 275°, nadelförmiges Pikrat vom Zers. Pkt. 225~229° und ein prismatisches Jodmethylat vom Zers. Pkt. 258~260°. Die Analysenzahlen der freien Base sowie der Salze stimmen mit der Formel $C_{22}H_{31}O_3N$ oder $C_{21}H_{29}O_3N$ überein, die keine Methoxylgruppe enthält. Der Stickstoff in ihm ist tertiär und die N-Methylbestimmung gibt den Wert, der einer Methyl- oder Äthylgruppe entspricht. Aus seinem UV-Spektrum (λ_{max}^{EtOH} =290 mμ bei log ε =2.50) wurde eine Carbonylgruppe vermutet, was durch die Bildung vom Monosemicarbazon (Prismen vom Zers. Pkt. 245~246°) bestätigt wurde. Das Alkaloid wurde vorläufig als die Shimoburobase I (I) genannt und liess sich einer weiteren Untersuchung unterziehen.[3]

(I) zeigt eine deutliche Farbreaktion mit Tetranitromethan, aber keine Färbung mit Eisenchlorid und widersteht der Oxydation mit Perjodsäure in der Kälte. In seinem IR-Spektrum sind Banden bei 1709, 3546, 3448 und 1664 cm^{-1} vorhanden, was ausser der Carbonylgruppe auf zwei Hydroxylgruppen und eine C=C Doppelbindung hinweist.

(I) gibt beim Acetylieren mit Essigsäureanhydrid und Pyridin ein Diacetet (II) (Nadeln vom Schmp. 180°), in dessen IR-Spektrum findet man die beiden OH-Banden bei 3546 und 3448 cm^{-1} nicht mehr. (II) geht beim Verseifen mit methanolischer Kali-Lösung in (I) mit fast quantitativer Ausbeute über.

Bei der Reduktion von (I) nach Wolff-Kishner wurde eine nadelförmige Desoxybase (III) vom Schmp. 149~150° mit guter Ausbeute erhalten. Die letztere bildet ein nadelförmiges Chlorhyrat vom Zers. Pkt. 250~251° und von der Drehung $[\alpha]_D^{9}$: −25.8° (Wasser). In ihrem IR-Spektrum wurde das Verschwinden des CO-Bandes bei 1709 cm^{-1} bestätigt.

Hiermit wurde es festgestellt, dass die 3 Sauerstoffe in (I) aus 2 Hydroxylgruppen und einer Carbonylgruppe bestehen.

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