

Base: White needles (petr. ether), m.p. 64~64.5°. *Anal.* Calcd. for  $C_{13}H_{21}ON$ : C, 80.86; H, 7.92; N, 5.24. Found: C, 80.7; H, 7.65; N, 5.15.

Oxalate-C was 8-(*p*-methoxybenzyl)-2-methyl-1,2,3,4,5,6,7,8-octahydroisoquinoline oxalate (V). It formed white and very hygroscopic semicrystalline mass (3.5 g.) from acetone.

Base: Colorless liquid of b.p.<sub>3</sub> 164~165°, which decolorized 2%  $KMnO_4$  solution in acetone.

Picrolonate: Yellow granules (EtOH+dioxane), m.p. 219~222°. *Anal.* Calcd. for  $C_{28}H_{33}O_6N_5$ : C, 62.79; H, 6.21; N, 13.08. Found: C, 62.65; H, 6.3; N, 13.35.

**3-Hydroxy-N-methyl-7-aza-des-N-morphinan (VIII)**—A solution of octahydroisoquinoline oxalate (V) (6.7 g.) in 48% HBr (70 cc.), containing ca. 20 mg. of hydroquinone as an inhibitor of polymerisation, was refluxed for 20 hrs. After removal of HBr *in vacuo*, the residue was dissolved in water, filtered with charcoal, basified with  $NH_4OH$ , and the separated voluminous white precipitate was extracted with ether. After being dried, solvent was concentrated to a volume of about 10 cc., cooled in an ice-box over night, and the crystalline solid (230 mg.) was collected. After evaporating the liquor, the residue was dissolved in benzene and chromatographed over  $Al_2O_3$ . Elution with acetone gave an additional 120 mg. of crude product. The combined product was recrystallized from AcOEt to colorless granules (300 mg.), m.p. 202~203°. It gave a yellow green color with  $FeCl_3$ . *Anal.* Calcd. for  $C_{17}H_{23}ON$ : C, 79.32; H, 9.01; N, 5.44. Found: C, 79.25; H, 9.2; N, 5.7.

### Summary

3-Hydroxy-N-methyl-7-aza-des-N-morphinan was synthesized. Analgesic action of this compound, contrary to expectations, was not strong.

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## 84. Morizo Ishidate and Terumi Nakajima: Structure of the Condensation Product of Isonicotinylhydrazine and Sodium Glucuronate.

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Passedonet, *et al.*<sup>1)</sup> first reported the reaction product of isonicotinylhydrazine (INAH) and glucuronolactone and gave it the structure of isonicotinylhydrazone of glucuronolactone (I) analogous to that of various aldoses.<sup>2)</sup>

When this product is treated with an equivalent amount of sodium hydroxide, a sodium salt (IIa) of the compound separates out. The latter compound is also obtained by heating an aqueous ethanolic solution of one mole each of sodium glucuronate and

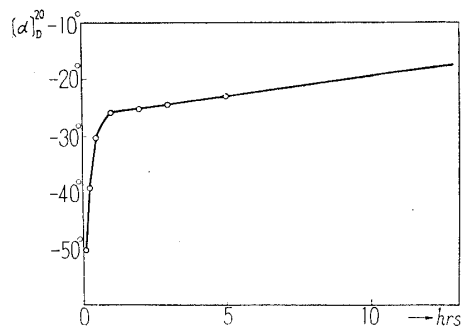


Fig. 1. Mutarotation of Sodium Isonicotinylhydrazone-(glucopyranosid)uronate (c=1.0, in water)

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1) H. Passedonet, P. Fusey, M. Roussos: *Rev. Tuberc. (Paris)*, **17**, 784(1953).

2) H. Zinner, W. Bock: *Chem. Ber.*, **89**, 1124(1956).

isonicotinylhydrazine.

By acidification of the sodium salt (IIa) with hydrochloric acid with cooling, the corresponding free acid (IIb) is obtained as fine needles of m.p. 178°. The same acid can also be obtained by the condensation of equimolar amounts of glucuronic acid with isonicotinylhydrazine in an aqueous medium.

The change in optical rotation of the solution of (IIa) is illustrated in Fig. 1. In this state, mutarotation after one hour comes to a definite value and the subsequent slight

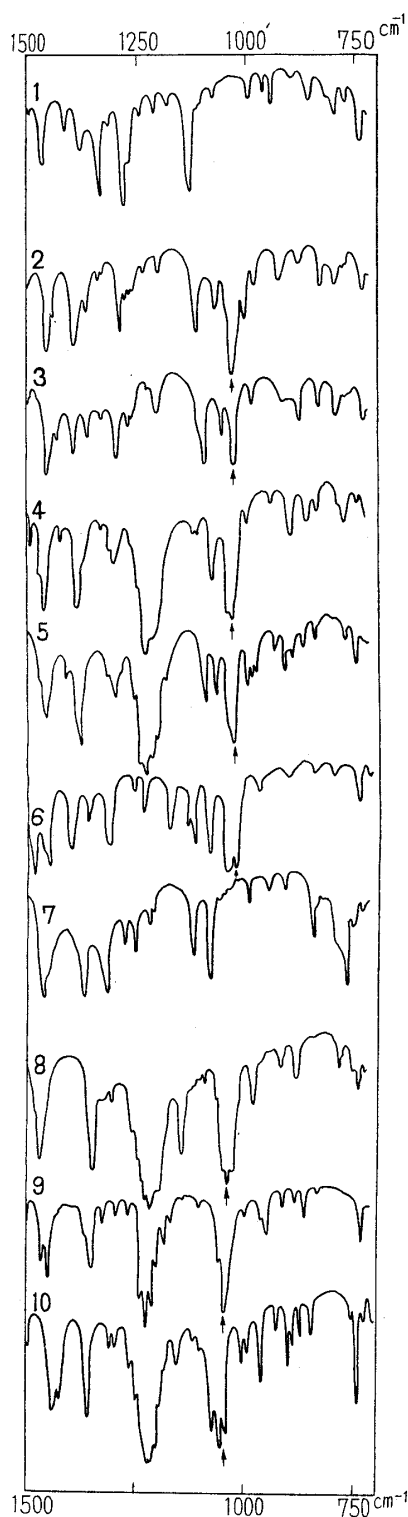


Fig. 2.

Infrared Absorption Spectra of *D*-Glucuronic Acid Derivatives of Isonicotinylhydrazine and Related Compounds (in Nujol mull, Hilger H-800)

1. *D*-Glucuronolactone isonicotinylhydrazone (I)
2. Sodium isonicotinylhydrazino-  
*D*-N-(glucopyranosid)uronate (IIa)
3. Isonicotinylhydrazino-  
*D*-N-(glucopyranosid)uronic acid (IIb)
4. *N,N'*-diacetyl-*N'*-isonicotinylhydrazino-  
2,3,4-tri-O-acetyl-  
*D*-N-(glucopyranosid)-uronic acid (III)
5. Methyl isonicotinylhydrazino-2,3,4-tri-O-  
acetyl-*N*-(glucopyranosid)uronate (VI)
6. Sodium glucuronate
7. Sodium glucuronate diethylmercaptal
8. Methyl 2,3,4-tri-O-acetyl-  
*D*-glucopyranuronate (V)
9. Anilino-2,3,4-tri-O-acetyl-  
(glucopyranosid)-uronic acid anilide
10. Methyl Anilino-2,3,4-tri-O-acetyl-  
(glucopyranosid)uronate

change might be attributed to gradual hydrolysis into its two components.

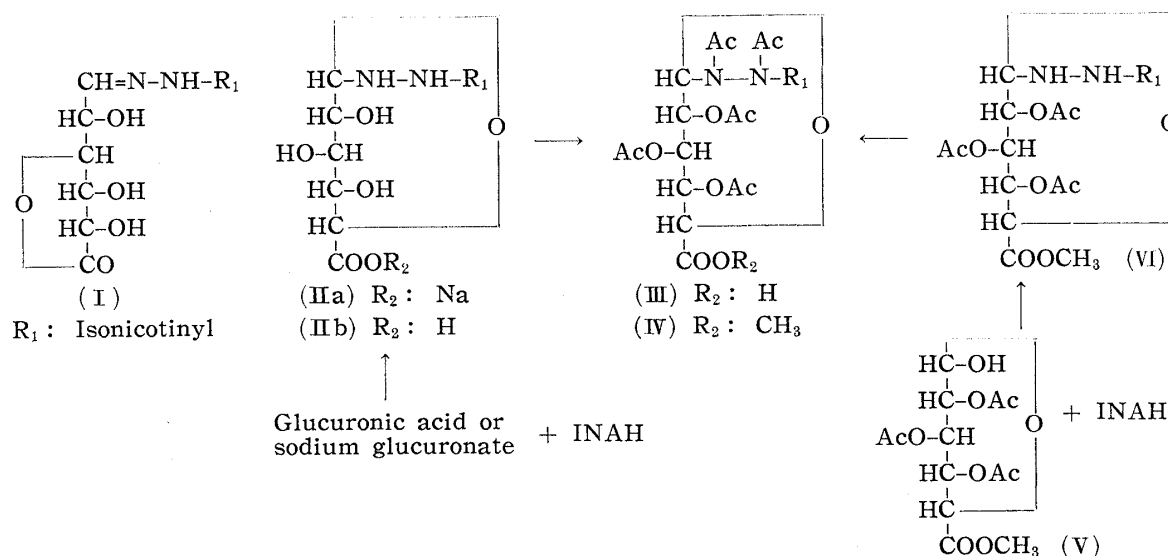
It remains in question whether the new condensation products, the salt and the free acid, have a hydrazone structure (acyclic form) or whether they are hydrazino-N-glucuronoside (cyclic form).

Since the infrared spectra of the salt and the acid gave a similar absorption band in the region between 1200 and 750  $\text{cm}^{-1}$ , both must have the same structure, but the spectra were found to be ambiguous in indicating the presence or absence of  $\text{N}=\text{C}$  bond. To clarify this point further study is required.

The reaction product (IIb), when acetylated with acetic anhydride in pyridine, gave a pentaacetyl derivative (III).

The methyl ester of the acetyl compound (III), which was obtained by reaction with methanol and hydrochloric acid, was isolated as a crystalline picrolonate (m.p. 202°) after purification by means of column chromatography using calcium hydrogen phosphate. The same pentaacetyl methyl ester (IV) was obtained by condensation of methyl glucuronate with isonicotinyldiazine and by subsequent acetylation. Furthermore, this compound (IV) was synthesized by another method, e.g. by the reaction of methyl 2,3,4-tri-O-acetyl-D-glucopyranuronate (V), derived from the corresponding 1-bromo derivative, with isonicotinyldiazine and by subsequent acetylation. All these three substances were found to be identical with each other in all respects. The infrared absorption spectra (Fig. 2) of the compounds which are considered to have an ether linkage (pyranoside form) exhibited a characteristic absorption at 1040  $\text{cm}^{-1}$ , which is not found in the compound (I) (No. 1) and in the reference compound, sodium glucuronate diethylmercaptal (No. 7). In addition the absorption band corresponding to the two NH groups is observed distinctly in the region of 3400  $\text{cm}^{-1}$  as double peaks in the compound (VI) (No. 5) (3375, 3320  $\text{cm}^{-1}$ ) and in the reference compound, anilino-2,3,4-tri-O-acetyl-(glucopyranosid)uronic acid anilide (3400, 3365  $\text{cm}^{-1}$ ), while the corresponding absorption is missing in other N-acetylated derivatives as well as in methyl anilino-2,3,4-O-acetyl-(glucopyranosid)uronate (No. 10) (3375  $\text{cm}^{-1}$ ).

From these results, it was concluded that the condensation product of sodium glucuronate or glucuronic acid with isonicotinyldiazine definitely possesses an N-glucopyranuronoside structure (II), and possibly that of  $\beta$ -configuration, judging from the optical rotation as well as the reaction process.



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### Experimental

**Sodium (Isonicotinylhydrazino-D-N-glucopyranosid)uronate (IIa)**—(1) One g. each of sodium D-glucuronate and isonicotinylhydrazine were dissolved in 1.5 cc. of water and heated for 30 mins. After cool, 4.5 cc. of EtOH was added to the solution and cooled overnight. Fine needles separated and were recrystallized from dil. EtOH; yield, 1.5 g., m.p. 169~176°(decomp.);  $[\alpha]_D^{20}$   $-52^\circ$  (c=1.0, H<sub>2</sub>O), and  $-13^\circ$ , after 24 hrs.

(2) D-Glucuronolactone isonicotinylhydrazone, m.p. 169~170°(decomp.) (2 g.), was dissolved in 1.5 cc. of water and treated with an equimolar amount of NaOH solution. The solution was poured into 5 cc. of EtOH or acetone. The following day, fine needles precipitated out, which were recrystallized from 75% EtOH, m.p. 170~176°(decomp.). *Anal.* Calcd. for C<sub>12</sub>H<sub>14</sub>O<sub>7</sub>N<sub>3</sub>Na·H<sub>2</sub>O: C, 40.9; H, 4.51. Found: C, 40.5; H, 4.85.

**Isonicotinylhydrazino-D-N-(glucopyranosid)uronic Acid (Isonicotinylhydrazino-D-N-glucuronide) (IIb)**—(1) To an aqueous solution (1 cc.) of (IIa) (1 g.), 0.5 cc. of 4N HCl was added dropwise under cooling. Fine colorless needles that separated were recrystallized from water; yield, 0.5 g., m.p. 178°(decomp.). It is hardly soluble in cold water or EtOH.

(2) An aqueous solution (2 cc.) of glucuronic acid (2 g.) and isonicotinylhydrazine (1.4 g.) was heated at 80° for 20 mins. After cool, 10 cc. of water was added, and separated needle crystals were recrystallized from hot water; yield, 1.5 g., m.p. 178°, no depression upon admixture with the preparation from (1) procedure. *Anal.* Calcd. for C<sub>12</sub>H<sub>15</sub>O<sub>7</sub>N<sub>3</sub>: C, 46.39; H, 4.95. Found: C, 46.01; H, 4.83.

**N,N'-Diacetyl-N'-isonicotinylhydrazino-2,3,4-tri-O-acetyl-D-N-(glucopyranosid)uronic Acid (III)**—In a mixture of pyridine (250 cc.) and Ac<sub>2</sub>O (150 cc.), 20 g. of (IIb) was suspended and stirred for several days at room temperature. The yellowish colored solution was concentrated in vacuum and filtered after addition of EtOH (50 cc.). The filtrate was added with 250 cc. of cold water to separate needles on standing, which were recrystallized from EtOH; yield, 14 g., m.p. 230°(decomp.);  $[\alpha]_D^{20}$   $-75^\circ$  (c=1, CHCl<sub>3</sub>). *Anal.* Calcd. for C<sub>22</sub>H<sub>25</sub>O<sub>12</sub>N<sub>3</sub>(dried for 3 hrs. at 100°): C, 50.40; H, 4.27. Found: C, 50.45; H, 4.78.

**Methyl 2,3,4-Tri-O-acetyl-D-glucopyranuronate (V)**—A mixture of methyl 2,3,4-tri-O-acetyl-1-bromo- $\alpha$ -D-glucopyranuronate (2 g.) and Ag<sub>2</sub>CO<sub>3</sub> (3 g.) dissolved in 6 cc. of water and 10 cc. of dioxane was left at room temperature for 48 hrs. The solution filtered from AgBr was concentrated in vacuum to syrup, which was dissolved in 10 cc. of xylene, dried, and evaporated again in vacuum. It was crystallized by addition of ether and recrystallized from benzene-petr. ether mixture; m.p. 113°, yield, 1.2 g.  $[\alpha]_D^{20}$   $+72^\circ$  (c=1.0, CHCl<sub>3</sub>);  $[\alpha]_D^{20}$   $+75^\circ$  (c=1.0, H<sub>2</sub>O). *Anal.* Calcd. for C<sub>13</sub>H<sub>18</sub>O<sub>10</sub>: C, 45.80; H, 5.37. Found: C, 45.52; H, 5.37. The structure of the substance is considered to be methyl 2,3,4-tri-O-acetyl- $\alpha$ -D-glucopyranuronate.

**Methyl (Isonicotinylhydrazino-2,3,4-tri-O-acetyl-D-N-glucopyranosid)uronate (VI)**—A solution of 5 g. of (V) and 2.7 g. of isonicotinylhydrazine in 30 cc. of dioxane was heated in an oil bath for 4 hrs., concentrated in vacuum, and allowed to stand after addition of a small amount of EtOH and 10 cc. of water. The needle crystals so obtained were recrystallized from a mixture of ethylene glycol monomethyl ether and ether; yield, 4.8 g., m.p. 105°.  $[\alpha]_D^{20}$   $-69^\circ$  (c=1, CHCl<sub>3</sub>). *Anal.* Calcd. for C<sub>19</sub>H<sub>23</sub>O<sub>10</sub>N<sub>3</sub>: C, 49.62; H, 5.17. Found: C, 49.87; H, 5.47.

**Methyl (N,N'-Diacetyl-N'-isonicotinylhydrazino-2,3,4-tri-O-acetyl-D-N-glucopyranosid)uronate Picrolonate**—(1) A solution of 0.4 g. of the triacetyl derivative (VI) in pyridine (4 cc.) was stirred with 2 cc. of Ac<sub>2</sub>O at 40° for 3 hrs., evaporated in vacuum, and dissolved in CHCl<sub>3</sub>. The CHCl<sub>3</sub> solution was purified by chromatography on Al<sub>2</sub>O<sub>3</sub> and the eluate was evaporated in vacuum. The treatment of the residue with 10.2 g. of picrolonic acid in MeOH gave a yellow precipitate, which was dissolved in CHCl<sub>3</sub> and chromatographed on CaHPO<sub>4</sub>. The product obtained from the first eluate was recrystallized from MeOH; yield, 0.17 g., m.p. 202°. *Anal.* Calcd. for C<sub>33</sub>H<sub>35</sub>O<sub>17</sub>N<sub>7</sub>: C, 49.45; H, 4.48. Found: C, 49.22; H, 4.09.

(2) By the methylation of (III): A solution of 4 g. of (III) in MeOH (20 cc.) was mixed with MeOH saturated with HCl. The mixture was allowed to stand for 24 hrs. under cooling, neutralized with Ag<sub>2</sub>CO<sub>3</sub>, and filtered. The filtrate was evaporated to dryness. The product was treated with pyridine (15 cc.) and Ac<sub>2</sub>O (10 cc.) for 24 hrs., the solvent was evaporated, and treated with a small amount of water. The crude substance was mixed with 2 g. of picrolonic acid in MeOH (40 cc.). The crude picrolonate obtained was chromatographed on CaHPO<sub>4</sub> in the manner described above. Recrystallization from MeOH gave crystals of m.p. 202°; yield, 2 g., identical with a sample prepared as in (1). *Anal.* Found: C, 49.87; H, 4.49.

(3) A solution of methyl D-glucuronate (10 g.) and isonicotinylhydrazine (10 g.) in 100 cc. of EtOH was heated for 30 mins. The product separated as a syrup was washed with EtOH and the dried product was treated with pyridine and Ac<sub>2</sub>O. The crude acetylated product was purified as picrolonate by chromatography by the same procedure as described above. The picrolonate (1.5 g.), repeatedly recrystallized from MeOH (m.p. 203°), was identical with an authentic sample.

### Summary

1) The reaction of isonicotinyldiazine with glucuronic acid or its sodium salt gave isonicotinyldiazino-N-glucuronide and not isonicotinyldiazone derivative.

2) The evidence that the N-glucuronide is in  $\beta$ -N-(glucopyranosid)uronic acid form was established by preparation of the pentaacetyl derivative of the glucuronide and its identification with the compound synthesized from isonicotinyldiazine and methyl 2,3,4-tri-O-acetyl- $\alpha$ -D-glucopyranuronate as well as by comparison of their infrared spectra with that of the related compounds.

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### 85. Ken'ichi Takeda, Tokuo Kubota, and Yoshiki Matsui : Bile Acids and Steroids. X. On $\beta$ -Spinasterol.

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$\beta$ -Spinasterol was first isolated together with  $\alpha$ -spinasterol by Heyl and Larsen<sup>1)</sup> in 1933 from spinach fat, and later by King and Ball<sup>2)</sup> from alfalfa seed oil. The sterol had been presumed as a double-bond isomer of  $\alpha$ -spinasterol. Barton<sup>3)</sup> suggested location of its two double bonds at C-7 and at C-24(25) on the basis of the molecular rotatory power, but this has not been confirmed yet. The present paper describes some results of experiments on  $\beta$ -spinasterol.

Previously,  $\alpha$ -spinasterol was isolated from the root of *Bupleurum falcatum* L.<sup>4)</sup> From a more soluble fraction of this  $\alpha$ -spinasterol, another sterol corresponding to  $\beta$ -spinasterol (m.p. 147~149°,  $[\alpha]_D = +6.8^\circ$ ) was obtained by fractional crystallization of its dinitrobenzoate. The mixed melting point determination of the free sterol and Ball's  $\beta$ -spinasterol (m.p. 148~150°,  $[\alpha]_D = +5.9^\circ$ ) from alfalfa seed oil showed no depression, and the infrared spectra<sup>5)</sup> of these two samples were almost identical only differing slightly in the relative intensity of the bands at 9.51 and 9.59  $\mu$ , and in the fine structures of the band at around 10.32  $\mu$  (Fig. 1). Also an absorption band at 12.01  $\mu$  in Ball's sample probably corresponds to the band at 12.04  $\mu$  in the present sample.

When the samples of the two  $\beta$ -spinasterols were dried over P<sub>2</sub>O<sub>5</sub> at 80° *in vacuo* for one week, the infrared spectra of both samples changed and differences between the two spectra appeared at the following points :

1) An absorption maximum corresponding to hydroxyl group absorption appeared at 2.92  $\mu$  in Ball's sample, but at 2.83  $\mu$  in the present sample (Fig. 2).

2) An absorption band at 6.26  $\mu$  appeared only in Ball's sample.

3) A very weak absorption appeared at 8.41  $\mu$  in the present sample, but not in Ball's sample.

4) The intensity of the band at 10.32  $\mu$  was higher in Ball's sample than in the present sample (Fig. 2).

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1) F. W. Heyl, D. J. Larsen : J. Am. Pharm. Assoc., **22**, 510(1933).

2) L. C. King, C. D. Ball : J. Am. Chem. Soc., **64**, 2488(1942).

3) D. H. R. Barton : J. Chem. Soc., **1945**, 813; **1946**, 512.

4) K. Takeda, K. Hamamoto, T. Kubota : Yakugaku Zasshi, **73**, 272(1953).

5) Infrared spectra discussed in this paper were determined in Nujol using a Parkin-Elmer Single-beam Infrared Spectrophotometer, Model 12 C.