Notes

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Drug Absorption, Metabolism, and Excretion. VI.[1] Preparation of Ether-type Glucuronide of 4-Hydroxyacetanilide

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Ether-type glucuronide of 4-hydroxyacetanilide (*p*-acetamidophenyl-β-D-glucopyranosiduronic acid (I)) is the major metabolite in man and rabbits dosed with 4-hydroxyacetanilide.[3] Furthermore, analgesic and antipyretic anilides such as acetanilide,[3] phenacetin,[3] and bucetin[4] have been known as metabolic precursors of I.

During the course of the pharmacokinetic studies on the anilides in this laboratory, it became necessary to know detailed aspects of the excretion of I after it is administered *per se* and accordingly to prepare I.

Smith and Williams isolated crude I from each urine of rabbits receiving acetanilide,[5a] 4-hydroxyacetanilide,[5a] and phenacetin,[5b] respectively, but the attempt for recrystallization was unsuccessful. They confirmed the structure of I by converting it to methyl–acetyl derivative (methyl (*p*-acetamidophenyl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (II)) and benzylamine salt (III). Another method employed by them for the preparation of I was acetylation of *p*-aminophenyl-β-D-glucopyranosiduronic acid (IV), which had been previously isolated and recrystallized from the urine of rabbits after the administration of *p*-aminophenol[6] and they reported that I thus obtained was purified to crystalline form.

Recently, Cummings, *et al.*[7] prepared potassium salt of I from the urine of rabbits dosed with 4-hydroxyacetanilide by the method reported by Smith and Williams.[5a] Little attention was, however, paid to the purity of I.

The experiments described below deal with reexamination of preparative methods of I reported by Smith and Williams[5a] and synthetic preparation of I.

Preparation of Metabolic I[8] from the Urine of Rabbits dosed with 4-Hydroxyacetanilide

When I isolated from the urine of rabbits dosed with 4-hydroxyacetanilide by the method reported by Smith and Williams[5a] was detected by thin-layer chromatography (TLC), it was found that the product was contaminated slightly with IV and unchanged 4-hydroxyacetanilide. Therefore, the crude glucuronide was, after the treatment of ethyl acetate, submitted to acetylation by the manner described in the following section to yield a substance free of the impurities, which, however, could not be recrystallized because of its hygroscopicity. Its infrared spectrum was proved to be essentially identical with that of synthetic I, but the

1) Part V: T. Koizumi and J. Shibasaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1218 (1969).
2) Location: *a*) *1–14, Bunkyo-machi, Nagasaki*; *b*) *3150, Gofuku, Toyama.*
3) R.T. Williams, "Detoxication Mechanisms," John Wiley and Sons Co., New York, 1959, pp. 432—436.
4) J. Shibasaki, T. Koizumi, T. Tanaka, and M. Nakatomi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1726 (1968).
5) *a*) J.N. Smith and R.T. Williams, *Biochem. J.*, **42**, 538 (1948); *b*) *Idem, ibid.*, 44, 239 (1949).
6) R.T. Williams, *Biochem. J.*, **37**, 329 (1943).
7) A.J. Cummings, M.L. King, and B.K. Martin, *Brit. J. Pharmacol.*, **29**, 150 (1967).
8) Hereafter, I due to metabolism of 4-hydroxyacetanilide, due to acetylation of IV, and due to totally synthetic method will be referred to metabolic, semi-synthetic, and synthetic I, respectively.

former contains some crushed peaks of absorbance band, which suggests that it is less pure than the synthetic I, as shown in Fig. 1. To obtain further evidence for the structure of the metabolic I, II and III were prepared after the method of Smith and Williams,[5a] and they were proved by mixed melting point test and the comparison of infrared spectra to be identical respectively with those derived from synthetic I.

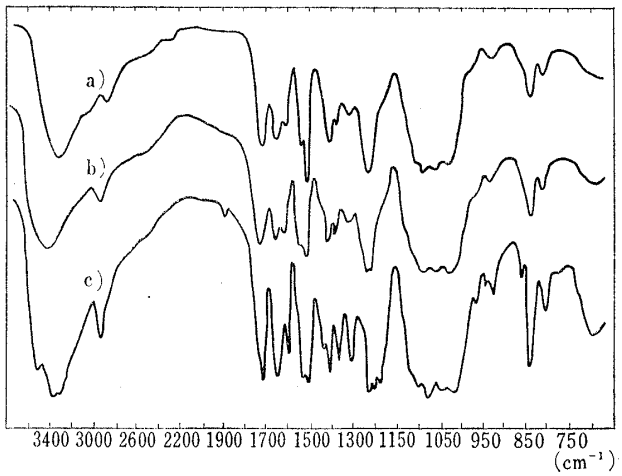

Fig. 1. Infrared Spectra of *p*-Acetamidophenyl-*β*-D-Glucopyranosiduronic Acid (I) in KBr
a) metabolic I, b) semi-synthetic I, c) synthetic I.

Preparation of Semi-synthetic I by Acetylation of *p*-Aminophenyl-*β*-D-glucopyranosiduronic Acid (IV)

It was reported by Smith and Williams[5a] that IV was selectively acetylated to I by acetic anhydride in sodium carbonate solution. The authors followed as in the literature with a little modification, which included the procedure that the reaction mixture after acetylation was passed through ion exchange resin column developing with water and acidic eluate was submitted to freeze-drying. The resulting substance appeared to be almost pure, but was unable to be recrystallized because of its hygroscopicity. After drying over phosphorus pentoxide *in vacuo*, it afforded mp 105—125° (decomp.) and $[\alpha]_D^{22.5}$ −77.8° (c=1.0) and the results of elemental analysis coincided with anhydrous $C_{14}H_{17}O_8N$.[9] Further evidence for the structure of the semi-synthetic I was provided by the fact that the infrared spectrum was identical with that of synthetic I as shown in Fig. 1.

Preparation of Synthetic I

Methyl (*p*-nitrophenyl 2,3,4-tri-O-acetyl-*β*-D-glucopyranosid)uronate (V) prepared by condensing *p*-nitrophenol with methyl (2,3,4-tri-O-acetyl-*β*-D-glucopyranosylbromid)uronate as in the literature[10] was hydrogenated using palladium-on-charcol as a catalyst to methyl (*p*-aminophenyl 2,3,4-tri-O-acetyl-*β*-D-glucopyranosid)uronate (VI), which was treated with acetic anhydride in pyridine to yield methyl (*p*-acetamidophenyl 2,3,4-tri-O-acetyl-*β*-D-glucopyranosid)uronate (II). When II was treated with sodium methoxide at room temperature, hydrolyzation took place selectively at three O-acetyl groups and a esteric methyl group, keeping intact with N-acetyl group, to afford I. The synthetic I thus obtained was recrystallized successfully from methanol as crystals, mp 132—133°, $[\alpha]_D^{27.5}$ −79.6° (c=2.5), which was analyzed well for anhydrous $C_{14}H_{17}O_8N$ as the case of semi-synthetic I. Its infrared spectrum was found to be identical with that of metabolic or semi-synthetic I as mentioned above (Fig. 1). Further it was confirmed that III derivable from the synthetic I was identical with III from metabolic I as described above.

The synthetic method seems to be most suitable for the preparation of I, since the yield of each step of the synthesis described above is more than 60% of theoretical amount and the synthetic I shows more excellent purity than I through the other two methods. The three preparative methods of I are represented in Chart 1.

9) Smith and Williams[5a] reported that I obtained by this method was able to be recrystallized from 95% ethanol as a crystalline form, mp 193° (decomp.), $[\alpha]_D^{22}$ −63.9° (c=7.5) and the analytical results were coincided with $C_{14}H_{17}O_8N \cdot 3/2H_2O$.

10) K. Kato, K. Yoshida, H. Tsukamoto, M. Nobunaga, T. Masuya, and T. Sawada, *Chem. Pharm. Bull.* (Tokyo), **8**, 239 (1960).

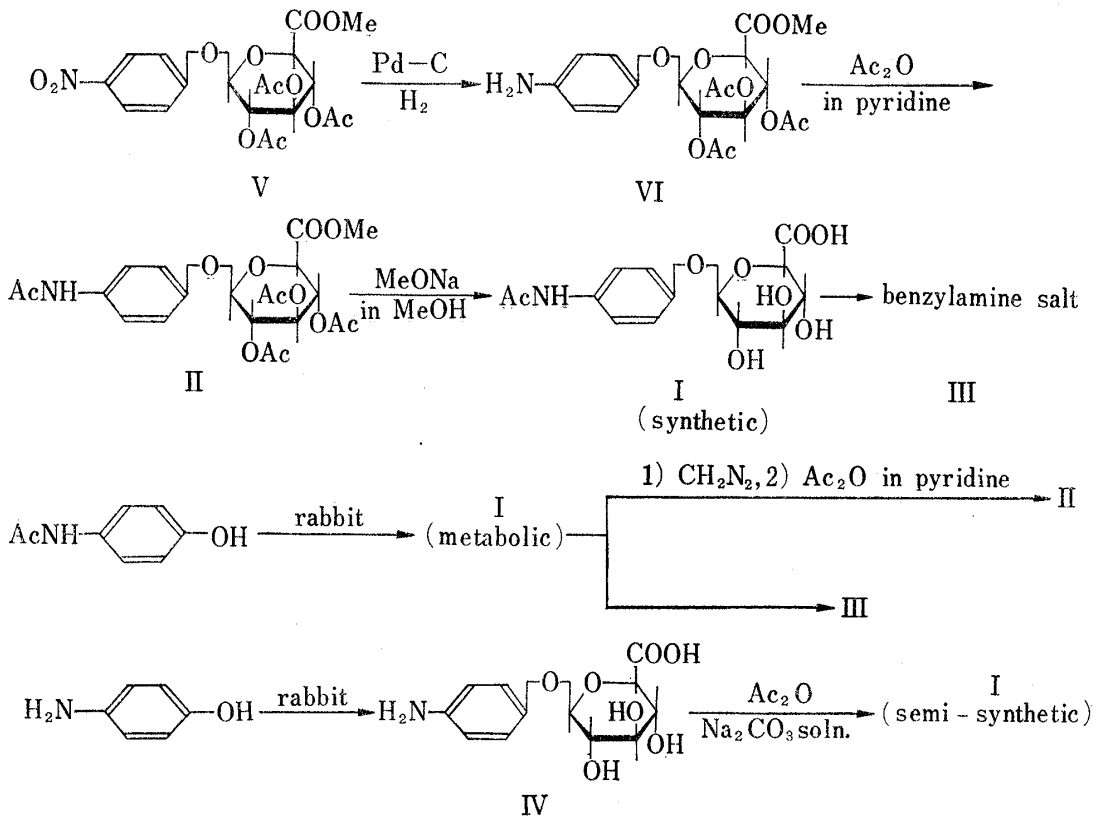

Chart 1

Experimental

Isolation of *p*-Acetamidophenyl-*β*-D-glucopyranosiduronic Acid (I) from the Urine Dosed with 4-Hydroxyacetanilide——4-Hydroxyacetanilide (400 mg/kg, total dose: 4.4 g) was fed to three rabbits and urine was collected for 24 hour. The urine was treated according to the conventional procedure using lead acetate[5a] to give pale brown powder, which by TLC was found to be contaminated slightly with IV and unchanged 4-hydroxyacetanilide. Therefore, the crude powder was dissolved in water (40 ml) and to the solution, after shaking with AcOEt, was added 10% Na_2CO_3 solution (8 ml) and Ac_2O (8 ml), pH being 4—5. On standing overnight at room temperature, the mixture was subjected to a column chromatography of Dowex 50W-X8 (50—100 mesh, H-form, 100 ml) developing with water. The acidic eluate (pH 2—4) was submitted to freeze-drying to give 1.72 g of pale brown powder (18.1%). Further purification was, however, unsuccessful. The infrared spectrum is shown in Fig. 1.

Methyl–acetyl derivative (II) and benzylamine salt (III) were prepared according to the method reported previously.[11]

II: Prepared by the treatment with diazomethane and successively with Ac_2O in pyridine. mp 207—207.5° (from EtOH). The identity with synthetic II was confirmed by mixed melting point test and the comparison of infrared spectra. *Anal.* Calcd. for $C_{21}H_{25}O_{11}N$: C, 53.96; H, 5.39; N, 3.00. Found: C, 54.27; H, 5.41; N, 3.10.

III: Prepared by the treatment of I with benzylamine. mp 197—198° (decomp.) (from H_2O–EtOH–AcOEt), $[\alpha]_D^{13.5}$ −35° (c=1.0). The identity with III derived from synthetic I was confirmed by mixed melting point test and the comparison of infrared spectra. *Anal.* Calcd. for $C_{21}H_{26}O_8N_2$: C, 58.06; H, 6.03; N, 6.45. Found: C, 58.09; H, 6.13; N, 6.12.

Acetylation of *p*-Aminophenyl-*β*-D-glucopyranosiduronic Acid (IV)——The procedure was same as in the literature[5a] except slight modification. IV ($C_{12}H_{15}O_7N \cdot H_2O$, 0.8 g) isolated from the urine of rabbits dosed with *p*-aminophenol[6] was dissolved in 3% Na_2CO_3 solution (10 ml) and to the solution was added Ac_2O (0.5 ml), pH being 4—5. The mixture was allowed to stand at room temperature for 24 hr, while the starting substarce became undetectable by TLC, and then submitted to a column chromatography of Dowex 50W-X8 (50—100 mesh, H-form, 20 ml) developing with water. The acidic eluate (pH 2—4) was subjected

11) The properties of II and III reported by Smith and Williams[5a] are as follows. II: $C_{21}H_{25}O_{11}N$, mp 200—205°, $[\alpha]_D^{25}$ −22.1° (c=7 in $CHCl_3$). III: $C_{21}H_{26}O_8N_2$, mp 195—197°, $[\alpha]_D^{25}$ −64.1° (c=4.4).

to freeze-drying to afford 0.8 g of colorless powder (92.7%), recrystallization of which was not successful. After drying over P_2O_5 at 60° for 24 hr *in vacuo* it had mp 105—125° (decomp.), $[\alpha]_D^{22.5}$ −77.8° (c=1.0). *Anal.* Calcd. for $C_{14}H_{17}O_8N$: C, 51.38; H, 5.24; N, 4.28. Found: C, 51.03; H, 5.59; N, 4.11. The infrared spectrum is identical with that of synthetic I (Fig. 1).

Synthesis of I——a) Methyl (*p*-Aminophenyl 2,3,4-Tri-O-acetyl-β-D-glucopyranosid) uronate (VI): A solution of methyl (*p*-nitrophenyl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (V) (3 g) in MeOH (150 ml) was shaken with H_2 in the presence of a catalyst prepared from activated charcoal (1.5 g) and $PdCl_2$ (75 mg) After the uptake of H_2 ceased, the catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was recrystallized from EtOH to give 2.3 g (82.1%) of IV as colorless needles, mp 154—156°, $[\alpha]_D^{15}$ −24° (c=5.0 in $CHCl_3$). *Anal.* Calcd. for $C_{19}H_{23}O_{10}N$: C, 53.65; H, 5.45; N, 3.29. Found: C, 54.07; H, 5.66; N, 3.36.

b) Methl (*p*-Acetamidophenyl 2,3,4-Tri-O-acetyl-β-D-glucopyranosid)uronate (II): VI (2.3 g) was dissolved in pyridine (10 ml) and to the solution was added Ac_2O dropwise under cooling with ice. After standing overnight, colorless needles deposited was collected by filtration and the filtrate was poured into ice-water (80 ml) to afford further crystals. Recrystallization from EtOH gave 2.25 g (89.0%) of colorless needles, mp 210—211°, $[\alpha]_D^{15}$ −22.0° (c=5.0 in $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{25}O_{11}N$: C, 53.96; H, 5.39; N, 3.00. Found: C, 54.03; H, 5.45; N, 3.28.

c) Synthesis of I by Deacetylation of II: II (2.14 g) was dissolved in MeOH (100 ml) under gentle warming and to the solution was added a mixture of Na (75 mg) and MeOH (15 ml). Allowing to stand the mixture for 48 hr at room temperature, the solvent was evaporated *in vacuo* below 30°. The pale yellow residue was dissolved in water (10 ml) and submitted to a column chromatography of Dowex 50W-X8 (50—100 mesh, H-form, 100 ml) developing with water. The acidic eluate (pH 2—4) was evaporated to dryness *in vacuo* below 30° to leave pale yellow powder, which was purified from MeOH to give 0.94 g (62.7%) of colorless crystals. After drying over P_2O_5 at 60° for 24 hr *in vacuo*, it gave mp 132—133° (decomp.), $[\alpha]_D^{27.5}$ −79.6° (c=2.5). *Anal.* Calcd. for $C_{14}H_{17}O_8N$: C, 51.38; H, 5.24; N, 4.28. Found: C, 51.33; H, 5.76; N, 3.63. The infrared spectrum is shown in Fig. 1.

Benzylamine Salt (III): Prepared as in the literature.[5a] mp 195—197°, $[\alpha]_D^{13.5}$ −38.0 (c=1.0). *Anal.* Calcd. for $C_{21}H_{26}O_8N_2$: C, 58.06; H, 6.03; N, 6.45. Found: C, 58.03; H, 6.17; N, 6.07.

Thin-Layer Chromatography——The procedure was as in the literature.[7] Silica gel HF_{254} according to Stahl was spread about 250 μ thick and dried at 110° for 1.5 hr. The solvent system was AcOH–H_2O–MeOH–AcOEt (1:9:30:60). All the compounds examined were revealed as dark spots against fluorescent background under ultraviolet light and the compounds containing free amino group were also detected by Ehrlich's reagent. *Rf* values of the compounds are as follows: *p*-aminophenyl-β-D-glucopyranosiduronic acid (IV), 0.2; *p*-acetamidophenyl-β-D-glucopyranosiduronic acid (I), 0.4; *p*-hydroxyacetanilide, 0.8; *p*-aminophenol, 0.85.

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Terpenoids. XVII.[1] Chemical Conversion of Trichokaurin into Isodocarpin *via* a Direct Pathway

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We have been investigating on diterpenoids of *Isodon* species. All of them belong to kaurene- and B-secokaurene-types. The former is biogenetically regarded as the precursor

1) Part XVI: E. Fujita, T. Fujita, and H. Katayama, *J. Chem. Soc.* (*C*), **1970**, 1681.
2) Location: *Uji, Kyoto.*