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Studies on the Constituents of the Water Extract of the Root of Mulberry Tree (*Morus bombycis* KOIDZ)

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From the water extract of the root bark of *Morus bombycis* KOIDZ, seven compounds (**1**—**7**) were isolated: betaine (**1**), 1-deoxynojirimycin (**2**), γ-aminobutyric acid (**3**), L-asparagine (**4**), L-arginine (**5**), L-lysine (**6**) and choline (**7**). Compound **2**, unlike nojirimycin, did not show antibacterial activity and was identified as the component with sweet flavor. Although **2** has been chemically derived from nojirimycin, this is the first report of the isolation of **2** as a natural product.

Keywords——*Morus bombycis* KOIDZ; betaine; 1-deoxynojirimycin; γ-aminobutyric acid; L-asparagine; L-arginine; L-lysine; choline; nojirimycin

The root bark of the mulberry tree (*Morus bombycis* KOIDZ) and other plants of the genus *Morus*, Moraceae), so-called "Mori Cortex," is used as an antiphlogistic, diuretic, and expectorant in tranditional medicine. There have been many reports[1-6] on the constituents of this root bark, and many biologically active substances have been isolated, such as kuwanons G[7,8] and H[7,9] with hypotensive effect, and moracins A—Z[10-12] (phytoalexins) and albanins A—H[13-15] with antimicrobial activity. However, there have been no detailed analysis of the constituents of the water extract of this root. In this study, we examined the constituents of the water extract of the root bark of *M. bombycis* KOIDZ and isolated seven substances (**1**—**7**) according to the method shown in Chart 1. Among them, compounds **1**, **3**, **4**, **5**, **6** and **7** were identified as betaine, γ-aminobutyric acid, L-asparagine, L-arginine, L-lysine and choline by direct comparison with authentic samples.

Compound **2**, mp 195—197 °C, $[\alpha]_D^{25}$ +54.1° (c=1.045, H_2O) $C_6H_{13}O_4N$, was positive in the ninhydrin reaction (yellow) and was identified as the component with sweet flavor. All physico-chemical and spectral data of **2** were similar to those of 1-deoxynojirimycin.[16] Through direct comparison of the physico-chemical and spectral data, **2** was shown to be identical with 1-deoxynojirimycin (3,4,5-trihydroxy-2-piperidinemethanol, Chart 2) chemically derived from nojirimycin (Chart 2).[17] Nojirimycin is a monosaccharide antibiotic produced by some strains of *Streptomyces*[18] and has antibacterial activity against drug-resistant strains of *Shigella flexneri*, *Sarcina lutea* and *Xanthomonas oryzae*. On the other hand, **2** did not show antibacterial activity. As mentioned above, **2** has been chemically derived from nojirimycin. However, this is the first report of the isolation of **2** from a natural source. It is worth noting that **2** was obtained from a higher plant.

Experimental

All melting points are uncorrected. The following instruments were used for obtaining physico-chemical data:

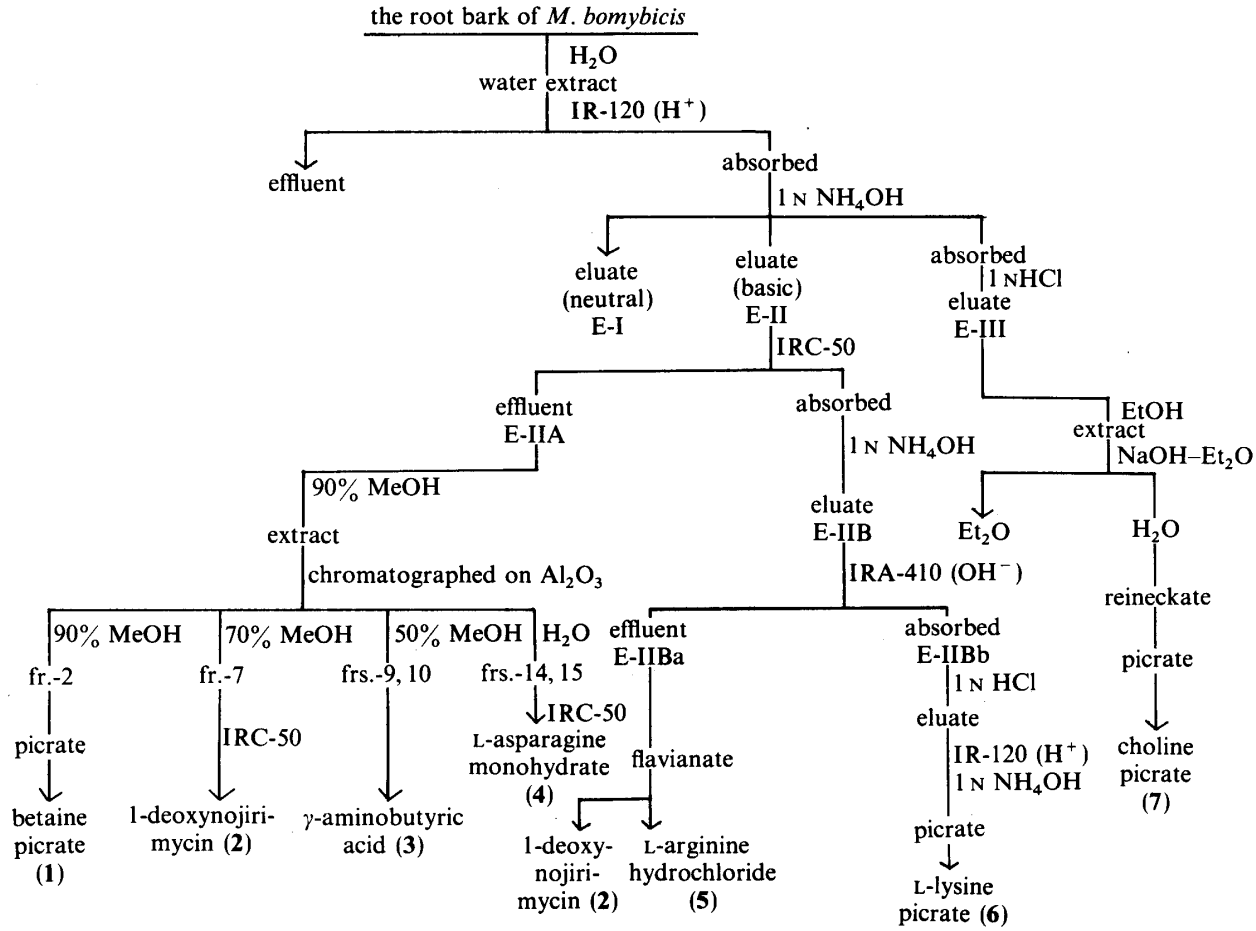

Chart 1

1-deoxynojirimycin (2) nojirimycin

Chart 2. Chemical Structures of 1-Deoxynojirimycin (2) and Nojirimycin

melting points, Yanagimoto micromelting point apparatus; infrared (IR) spectra, JASCO IR-180; proton nuclear magnetic resonance (^{1}H-NMR) spectra, Varian HA-100; paper electrophoresis, Toyo paper electrophoresis apparatus, model C. Paper chromatography (PPC) was performed on Toyo Roshi No. 51 and paper electrophoresis (PEP) was carried out on Toyo Roshi No. 50. Column chromatography was carried out on activated alumina (Wako 300 mesh), Amberlite IR-120 (Rohm & Haas), Amberlite IRC-50 (Rohm & Haas) and Amberlite IRA-410 (Rohm & Haas).

Extraction and Separation of the Compound (1—7)——The dry root bark (2 kg) was finely cut and extracted with water (2 l) at room temperature for 2 d. The extracted solution was filtrated and the filtrate (2 l) was passed through a column of Amberlite IR-120 (H^+, 1.5 l). The resin was washed with water, and the absorbed substances were eluted with 1 N NH_4OH. The eluate (7 l) was evaporated to dryness under reduced pressure. The residue, E-II, was dissolved in water (500 ml) and passed through a column of Amberlite IRC-50 (H^+, 500 ml). The effluent was evaporated to dryness under reduced pressure, and the residue, E-IIA, was dissolved in 90% MeOH and filtered. The filtrate was chromatographed on activated alumina (500 g) with 90% MeOH, 70% MeOH, 50% MeOH and H_2O as eluents to give **1** (betaine picrate, 30 mg), **2** (1-deoxynojirimycin, 21 mg), **3** (γ-aminobutyric acid, 220 mg) and **4** (L-asparagine, 32 mg). On the other hand, the substances absorbed on the Amberlite IRC-50 column were eluted with 1 N NH_4OH.

The eluate, E-IIB (2 l), was evaporated to dryness, then the residue was dissolved in water (100 ml) and passed through a column of Amberlite IRA-410 (OH^-, 500 ml). The effluent (500 ml), E-IIBa, was evaporated to a small volume (10 ml), and 10% flavianic acid in water was added to give a precipitate (37 mg). The precipitate, after being heated with conc. HCl, was worked up to give **5** (L-arginine hydrochloride, 450 mg) according to the usual method. The mother liquor of L-arginine flavianate was passed through a column of Amberlite IRA-410 (OH^-, 200 ml). The effluent, after being treated with active carbon, was concentrated to a small volume, and a small amount of water (*ca.* 5 ml) was added to give (1-deoxynojirimycin, 540 mg). On the other hand, the substances absorbed (E-IIBb) on the Amberlite IRA-410 column were eluted with 1 N HCl. The eluate was evaporated to dryness under reduced pressure, then the residue was dissolved in AcOH (10 ml), and 10% picric acid in AcOH was added to give **6** (L-lysine picrate, 32 mg). Furthermore, the Amberlite IR-120 resin was washed with water and the absorbed material was eluted with 1 N HCl. The eluate (7.2 l) was evaporated to dryness under reduced pressure. The residue, E-III, was dissolved in EtOH (300 ml) and filtered, then the filtrate was evaporated to dryness under reduced pressure. The residue, after being made basic with 10% NaOH, was extracted with ether. The aqueous layer was adjusted to pH 6.0 with 10% HCl, and 5% ammonium reineckate (10 ml) in MeOH was added to give a precipitate (460 mg), which was dissolved in 50% acetone (50 ml) and passed through a column of Amberlite IRA-410 (OH^-, 50 ml). The effluent was evaporated to dryness under reduced pressure. The residue was dissolved in EtOH and a solution of saturated picric acid in EtOH was added to give **7** (choline picrate, 200 mg).

Betaine Picrate (1)——**1** was recrystallized from 50% EtOH as fine needles, mp 183 °C (dec.). PPC: *Rf*: 0.14 (*n*-BuOH : AcOH : H_2O = 4 : 1 : 5), 0.86 (PhOH : H_2O = 4 : 1). PEP: migrat. distance (mm): −34 (1 N AcOH, 1000 V, 40 min). *Anal.* Calcd for $C_5H_{12}NO_2 \cdot C_6H_2N_3O_7$: C, 38.15, H, 4.07, N, 16.08. Found: C, 37.73; H, 4.34; N, 16.39. All physico-chemical and spectral data of **1** were identical with those of an authentic sample of betaine picrate.

1-Deoxynojirimycin (2)——**2** was recrystallized from H_2O–EtOH as colorless needles, mp 195—197 °C, $[\alpha]_D^{25}$ +54.1° (*c* = 1.045, H_2O). PPC: *Rf*: 0.43 (*n*-BuOH : AcOH : H_2O = 4 : 1 : 5), 0.27 (PhOH : H_2O = 4 : 1). PEP: migrat. distance (mm): −68 (1 N AcOH, 1000 V, 40 min). *Anal.* Calcd for $C_6H_{13}NO_4$: C, 44.16; H, 8.03; N, 8.58. Found: C, 44.32; H, 7.93; N, 8.79. All physico-chemical and spectral data of **2** were identical with those of an authentic sample of 1-deoxynojirimycin. **2** was identified as the component with sweet flavor.

γ-Aminobutyric Acid (3)——**3** was recrystallized from H_2O–EtOH as pale yellow needles, mp 203 °C (dec.). PPC: *Rf*: 0.30 (*n*-BuOH : AcOH : H_2O = 4 : 1 : 5), 0.76 (PhOH : H_4O = 4 : 1). PEP: migrat. distance (mm): −126 (1 N AcOH, 1000 V, 40 min). *Anal.* Calcd for $C_4H_9NO_2$: C, 46.59; H, 8.80; N, 13.58. Found: C, 46.45; H, 9.30; N, 13.13. All physico-chemical and spectral data of **3** were identical with those of an authentic sample of γ-aminobutyric acid.

L-Asparagine Monohydrate (4)——**4** was recrystallized from H_2O as colorless needles, mp 232—234 °C. PPC: *Rf*: 0.43 (*n*-BuOH : AcOH : H_2O = 4 : 1 : 5), 0.27 (PhOH : H_2O = 4 : 1). PEP: migrat. distance (mm): −58 (1 N AcOH, 1000 V, 40 min). *Anal.* Calcd for $C_4H_8N_2O_3 \cdot H_2O$: C, 32.00; H, 6.71; N, 18.66. Found: C, 31.84, H, 7.01, N, 18.38. All physico-chemical and spectral data of **4** were identical with those of an authentic sample of L-asparagine monohydrate.

L-Arginine Hydrochloride (5)——**5** was recrystallized from H_2O–EtOH as colorless plates, mp 216—217 °C. PPC: *Rf*: 0.10 (*n*-BuOH : AcOH : H_2O = 4 : 1 : 5), 0.65 (PhOH : H_2O = 4 : 1). PEP: migrat. distance (mm): −110 (2 N AcOH, 1000 V, 40 min). *Anal.* Calcd for $C_6H_{14}N_4O_2 \cdot HCl$: C, 34.20; H, 7.17; N, 26.59. Found: C, 34.08; H, 7.41; N, 26.59. All physico-chemical and spectral data of **5** were identical with those of an authentic sample of L-arginine hydrochloride.

L-Lysine Picrate (6)——**6** was recrystallized from H_2O as yellow needles, mp 252 °C (dec.). PPC: *Rf*: 0.14 (*n*-BuOH : AcOH : H_2O = 4 : 1 : 5), 0.86 (PhOH : H_2O = 4 : 1). PEP: migrat. distance (mm): −116 (1 N AcOH, 1000 V, 40 min). *Anal.* Calcd for $C_6H_{15}N_2O_2 \cdot C_6H_2N_3O_7$: C, 38.40, H, 4.57, N, 18.66. Found: C, 38.12; H, 4.71; N, 18.38. All physico-chemical and spectral data of **6** were identical with those of an authentic sample of L-lysine picrate.

Choline Picrate (7)——**7** was recrystallized from EtOH as yellow needles, mp 242—243 °C. PPC: *Rf*: 0.20 (*n*-BuOH : AcOH : H_2O = 4 : 1 : 5), 0.79 (PhOH : H_2O = 4 : 1). PEP: migrat. distance (mm): −136 (1 N AcOH, 1000 V, 40 min). *Anal.* Calcd for $C_5H_{14}NO \cdot C_6H_2N_3O_7$: C, 39.76; H, 4.85: N, 16.86. Found: C, 39.72, H, 5.08, N, 17.14. All physico-chemical and spectral data of **7** were identical with those of an authentic sample of choline picrate.

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