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Cyanogenic Glycosides and Glycosidase of *Sorbus* Species[1)]

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Cyanogenic glycosides–prunasin(I) and amygdalin(II)–were isolated in crysatalline from *Sorbus commixta*, *S. gracilis*, *S. sambucifolia*, and *S. matsumurana*; the glycosides were found in all tissues of the plant. The contents of the glycosides of *Sorbus* spp. distributed at higher elevation was more abundant than those of the *Sorbus* spp. at lower elevation. The contents of I and II were the most abundant in seeds (0.189%) and, among them, the content of I was ten times more than that of II in the plants.

The activity of the glycosidase in the seeds was about ten times as high as that in other tissues.

Keywords——cyanogenic glycoside; prunasin; amygdalin; gentiobiose; ultraviolet light; hydrolysis; glycosidase; *Sorbus* species

Sorbus species plants (Rosaceae) are widely distributed throughout Japan. Colour of their leaves and fruits is marked as a guide of autumnal red colouration in Japan, since the colour turns scarlet–tinged red in autumn and is the deepest in many plants. During our previous studies[3)] on autumnal red colour leaves of Japan, we noticed a smell of benzaldehyde on fresh barks when we crushed them followed by leaving for a few minutes. Then we isolated two cyanogenic glycosides–prunasin (I) and amygdalin (II)–from a methanolic extract of barks of *S. commixta* (*S. aucuparia* var. *japonica*) by a chromatography on silica gel with MeOH-AcOEt (1 : 4). I and II were also isolated from *S. gracilis*, *S. sambucifolia* and *S. matsumurana* in Japan (Table I). This is the first isolation of I and II in crystals from *Sorbus* species, though I was already detected from *S. aucuparia* in Europe by a paper partition chromatography (PPC).[4)] In Europe, *S. aucuparia* has been used as a folk medicine; thus, its juice, fruits and barks have been used as a diuretic-antiscorhutic, antidiarrheic and dyestuff, respectively.[5)]

The content of the glycosides (I and II) was most abundant in seeds (0.189%) and then in the order of barks, root barks, sarcoparps, woods, germs, root woods and leaves. The content of I was more than ten times that of II.

Cyanogenic glycosides have a wide distribution in plants but they are usually present in particular tissues of a plant such as in seeds in the case of apricot.[6)] In view of the above, our present finding that the glycosides were present in all the tissues of *Sorbus* species (Table I) would be our present finding that the glycosides were present in all the tissues of *Sorbus* species would be very interesting. Incidentally, *S. sambucifolia* (Japanese name, Takanenanakamado) and *S. matsumurana* (Urajironanakamado) distributed at higher elevation were found to contain more glucosides than other *Sorbus* species grown at lower elevation (Table I).

1) A part of this work was already presented as a short report by K. Takaishi, and H. Kuwajima in *Phytochemistry*, **15**, 1984 (1976).
2) Location: *Kowakae 3-4-1, Higashiosaka, Osaka, 577, Japan.*
3) K. Takaishi, *Phytochemistry*, **10**, 3302 (1971).
4) J. Sendra, M. Oswiecimska, and Z. Janeczko, *Dissert. Pharm. Pharmacol.*, **23**, 1 (1971).
5) K. Shibata,"A Cyclopedia of Useful Plants and Plant Products (Shigen Shokubutsu Jiten)," Hokuryukan, Tokyo, 1961, p. 525.
6) D.S. Seigler, *Phytochemistry*, **14**, 9 (1975).

Table I. Cyanogenic Glycosides of *Sorbus* spp. (%)

	Leaves	Bark	Wood	Seeds	Sarco-carp	Root bark	Root wood	Germ
Sorbus commixta[a] (Nanakamado)	0.009	0.181	0.095	0.189	0.095	0.146	0.017	0.034
S. gracilis[b] (Nankin Nanakamado)	0.009	0.198	0.026					
S.sambusifolia[c] (Takane Nanakamado)	0.069	0.310	0.413					
S.matsumurana[c] (Urajiro Nanakamado)	0.043	0.155	0.103					

a) Zaoo, Gassan, Yamagata Pref. Oct. 1976.
b) The experimental plantation of Kyoto University, Wakayama Pref. July. 1976.
c) Kisokomagatake, Nagano Pref. Aug. 1976.
Contents (%) were calculated from the amount of benzaldehyde obtained by a hydrolysis with β-glycosidase.

Table II. Activity of β-glycosidase in *Sorbus commixta*

Names of tissues	Amount of benzaldehyde (mg) released
Seeds	0.839
Bark	0.082
Germ	0.102
Leaves	0.000
Wood	0.000
Sarcocarp	0.000
Root wood	0.000
Root bark	0.000

As the glycosides have been found in all tissues of the plant, activity of the glycosidase in various tissue were compared. Thus, amygdalin (10 mg) was hydrolyzed with an aqueous extract of the fresh tissue (2 g) of the plant and the resulting benzaldehyde released from amygdalin was determined by a gas liquid chromatography (GLC). It was thereby found that the glycosidase was present in seeds, barks and germs but not in other tissues and that the activity of the glycosidase in seeds was about ten times higher than that in barks or germs (Table II).

Experimental

The melting points were taken on a Yanagimoto micro melting points apparatus and were uncorrected. Infrared (IR) spectra were recorded on a Hitachi Model 323-S, and nuclear magnetic resonance (NMR) spectra on a Hitachi Perkin-Elmer Model 20A High Resolution NMR using tetramethylsilane as an internal reference. The chemical shifts were reported in δ and the solvents used were indicated. Gas liquid chromatography used was a Hitachi Model 063 with a hydrogen flame ionization detector. GLC condition A; column packing 2% OV-17 on chromosorb W(AW-DMCS) 3 mm×2 m; column temp: 90—150°, 7.5°/min; inject. temp.: 220°; detect. temp.: 200°; carrier gas: N_2, 30 ml/min. condition B; column packing 3% SE-52 on chromosorb W, 3 mm×2 m; column temp.: 150—250°, 10°/min; inject. and detect. temp.: 250°; carrier gas: N_2, 30 ml/min. The *Rf* values were determined by thin-layer chromatography (TLC) on Kiesel Gel nach Stahl using solvent A; $CHCl_3$–MeOH–H_2O (30: 10: 1), and the spots were detected by spraying with a 2% α-naphthol in EtOH, then H_2SO_4 followed by heating. Toyo Roshi No. 51 was used for PPC using solvent B; *n*-BuOH–AcOH–H_2O (4: 1: 5, the upper phase), and spots were detected by spraying with aniline hydrogen phthalate followed by heating.

Extraction——The fresh barks (3.7 kg) of *Sorbus commixta* collected in Ashiu experimental forest station of Kyoto University (Kyoto Pref.) on July 1974 were treated with steam so that the enzymes were inactivated and then extracted twice with 80% MeOH (20 l). The MeOH extract was evaporated *in vacuo* and the residue was extracted with water (the same volume as the residue) twice. The aqueous extract was treated with $Pb(OAc)_2$ and PbOAc·OH and the resulting precipitate was filtered off. The filtrate was extracted with $CHCl_3$ and then with ether for further one week according to an Asahina's method. The ether layer was evaporated *in vacuo* to give a residue (5 g)–ether soluble fraction–and the aqueous layer of the filtrate was evaporated *in vacuo* to give another residue (72 g)-H_2O soluble fraction-.

Detection of Hydrogen Cyanide——A paper previously impregnated with a 1% EtOH solution of picric acid and dried was sprayed with 2% Na_2CO_3 solution and the paper was hung in a test tube, which was tightly stopped with a cork. An aqueous solution containing cyanogenic glycoside and β-glycosidase was placed into the tube and was heated for 10 min on a water bath. The paper was coloured in brown with CN gas.

Isolation of Prunasin (I)——The ether soluble fraction (5 g) was dissolved in hot EtOAc and kept overnight to give crude I which was recrystallized from EtOAc to give I as white crystals (0.79 g), mp 149—150°, $[\alpha]_D$ −27.2° (c=0.5, H_2O). *Anal.* Calcd. for $C_{14}H_{17}NO_6$: C, 56.94; H, 5.80; N, 4.74. Found: C, 56.52; H, 5.69; N, 4.74. UV $\lambda_{max}^{H_2O}$ nm: 251, 257, 262, 268. IR ν_{max}^{Nujol} cm^{-1}: 2250 (–CN, quenched), 3220 (–OH). NMR (in D_2O) δ: 5.8 (1H, C_6H_5–C<u>H</u>), 3.4—4.8 (7H, glucosyl). I was identified with an authentic sample.

Isolation of Amygdalin (II)——The H_2O soluble fraction (72 g) dissolved in water (720 ml) was placed on a top of a column packed with an activated charcoal (Wako, 1 kg) and crude II (0.2 g) was obtained from an elute of 10—20% EtOH (12 l). It was chromatographed on silica gel (Wakogel, C-200) with a mixture of EtOAc and MeOH (4: 1), then on a preparative silica gel plate using solvent A (Rf 0.3) and was recrystallized from EtOAc saturated with water to give II as white crystals, mp 216°. The resulting II was identified by a mixed mp, UV and IR spectra with an authentic sample.

Hydrolysis of I and II with Emulsin——I (10 mg) dissolved in McIlvaine's buffer solution[7] (1 ml, pH 5.8) was mixed with emulsin (10 mg, Sigma Co.) and the solution was then put into a test tube in which a sheet of paper previously soaked in a picric acid solution was hung. The solution was incubated at 36° for 2 hr whereupon the yellow colour of the paper turned reddish brown due to HCN released from I. Benzaldehyde released from I was identified with an authentic sample by GLC (condition A, t_R 4.7 min). The aqueous solution was neutralized and evaporated *in vacuo* to a residue. Glucose in the residue was detected by GLC (condition B, t_R 6.2, 7.6 min) as its TMS derivative with an authentic sample. Similarly, benzaldehyde and glucose were obtained from II.

Isolation of Gentiobiose by UV Light Irradiation Hydrolysis——II (10 mg) was dissolved in 0.2 N H_2SO_4 (20 ml) at room temperature (20°). The solution was put into a quartz tube, irradiated by UV light (low voltage mercury lamp, 120 watts, 254 nm, Eikosha Co.) from a distance of 10 cm at room temperature for 3 hr, neutralized with an ion exchange resin (Dowex 2×8), and evaporated *in vacuo* to give gentiobiose (III) (about 1 mg). III was identified by PPC (solvent B, Rf 0.1) and GLC (condition B, t_R 34 min) as TMS derivative with an authentic sample.

Assay of Cyanogenic Glycoside by GLC——Seeds (5 g) of *S. commixta* was extracted with water and the aqueous solution was evaporated *in vacuo*. The residue was dissolved in McIlvaine's buffer solution (1 ml, pH 5.8) together with emulsin (1 mg, Sigma Co.) and the solution was hydrolyzed at 36° for 15 hr and extracted with $CHCl_3$ twice. To the $CHCl_3$ solution was added acetophenone (10 μl) as an internal standard and benzaldehyde in it was detected and determined by GLC (condition A).

Assay of β-Glycosidase in *S. commixta*——Fresh tissues (2 g) of *S. commixta* was extracted with water (10 ml) at room temperature (20°) for 15 min. The aqueous solution was added amygdalin (10 mg) and the mixture was hydrolyzed at 36° for 15 hr. Benzaldehyde formed from I and II by the hydrolysis was detected and determined by GLC (condition A). When the aqueous solution was hydrolyzed without the addition of amygdalin, formation of benzaldehyde by the hydrolysis was very little.

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7) Pharmaceutical Society of Japan, Standards Methods of Analysis for Hygienic Chemists—with Commentary—, 1965, p. 1101.