Phytochemistry, Vol. 24, No. 10, pp. 2438–2439, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

CYANOGENIC GLYCOSIDES IN LEAVES OF *PERILLA FRUTESCENS* VAR. *ACUTA**

MASAKAZU ARITOMI†, TETSUYA KUMORI‡ and TOSHIO KAWASAKI§

†Faculty of Education, Kumamoto University, Kurokami, Kumamoto, 860 Japan; ‡Faculty of Pharmaceutical Sciences, Kyushu University, Maidashi, Fukuoka, 812 Japan; §Faculty of Pharmaceutical Sciences, Setsunan University, Nagaotoge-cho, 45-1, Hirakata, Osaka, 573-01 Japan

(*Revised received* 25 *March* 1985)

Key Word Index—*Perilla frutescens* var. *acuta*; Labiatae; cyanogenic glycoside; (*R*)-2-(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyloxy)-phenylacetonitrile; prunasin; 7-(2-*O*-β-D-glucuronyl-β-D-glucuronyloxy)-5,3′,4′-trihydroxyflavone; scutellarin; rosmarinic acid; caffeic acid.

Abstract—Besides 7-(2-*O*-β-D-glucuronyl-β-D-glucuronyloxy)-5,3′,4′-trihydroxyflavone, scutellarin, rosmarinic acid and caffeic acid, two cyanogenic glycosides have been isolated from the dried leaves of *Perilla frutescens* var. *acuta*. One of them is prunasin and the other is (*R*)-2-(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyloxy)-phenylacetonitrile, a new isomer of amygdalin.

Perilla frutescens Britt. var. *acuta* Kudo (Japanese name; Shiso), an annual medicinal herb of Labiatae [1, 2], is important in Japanese cooking, as one of the popular garnishes and as a food colourant. A tradition that, prior to the application as a colourant, the leaves are treated with table-salt to remove the harshness, suggests the existence of some water-soluble principles in addition to flavonoids which have already been studied by Ishikura [3].

On chromatographic isolation, the methanolic extract of the dried leaves provided two non-phenolic compounds, **1** and **2**, besides four phenolics, i.e. 7-(2-*O*-β-D-glucuronyl-β-D-glucuronyloxy)-5,3′,4′-trihydroxyflavone [4], scutellarin, rosmarinic acid and caffeic acid. The elementary analyses, FDMS [5] and FABMS [6] assigned the formulae $C_{20}H_{27}O_{11}N$ for **1** and $C_{14}H_{17}O_6N$ for **2**. The intense fragment ion at *m/z* 116 in FDMS and FABMS of both compounds might well be ascribed to $[C_6H_5CHCN]^+$. In harmony with the IR (C–O at around 1000–1100 cm^{-1}) and ^1H NMR data [five aromatic, one benzylic and fourteen (seven in **2**) sugar protons], this indicates that **1** and **2** are di- and mono-hexosides of the same aglycone. Indeed **1** was hydrolysed with N-hydrochloric acid to **2** and D-glucose.

Compound **2** was further hydrolysed with concentrated hydrochloric acid to (*R*)-mandelic acid, ammonium salt and glucose. Enzymic hydrolysis of **2** with β-glucosidase provided benzaldehyde, hydrogen cyanide and glucose. From these results and by comparison with literature data [7–9], **2** was identified as (*R*)-2-(β-D-glucopyranosyloxy)-phenylacetonitrile (prunasin).

In the ^{13}C NMR spectrum of **1** the signals due to the aglycone were practically superimposable with those of **2**. Of sugar signals, the ones assignable to C-2 and C-1 of the inner glucose were respectively shifted downfield by +8.5 ppm and upfield by −1.1 ppm than those in **2**, indicating the 1 → 2 linkage between the two glucose moieties. Two anomeric proton signals were detected in the ^1H NMR spectrum of **1** as two doublets at δ4.96 and 5.12, whose coupling constants (J = 6 Hz) indicated the β-configuration for both glycosidic linkages. Since on acidic hydrolysis of amygdalin and prunasin the steric configuration at C-2 remained unchanged [7], it is not unreasonable to assume that **1** has the same chiral center as **2**. Compound **1** is that (*R*)-2-(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyloxy)-phenylacetonitrile, a position isomer of amygdalin. This constitutes the first report of cyanogenic glycosides in the Labiatae.

EXPERIMENTAL

Extraction and isolation. Perilla frutescens var. *acuta* cv. akachirimen-shiso, cultivated in the farm of Kumamoto Univ. and harvested in July, was used. The air-dried leaves (650 g) were twice percolated with MeOH. The percolate was evaporated to dryness, digested with hot H_2O and filtered. The aq. soln was acidified with HOAc (about pH 4) and filtered through a column of Amberlite XAD-2. The column was successively washed with 2% HOAc, H_2O and MeOH. The MeOH eluate was subjected to DCCC (ascending method, $CHCl_3$–*n*-BuOH–MeOH–H_2O, 9:2:12:8) monitoring with TLC (microcrystalline cellulose, 15% HOAc, UV light 366 nm) to give 11 fractions. Fraction 1 was chromatographed over the column of Diaion HP20AG (a highly porous co-polymer of styrene and divinylbenzene) with gradient concentration of MeOH (H_2O → 40% MeOH). The TLC homogeneous fraction crystallized (from 50% dioxan) to 7-(2-*O*-β-D-glucuronyl-β-D-glucuronyloxy)-5,3′,4′-trihydroxyflavone (yield 0.01% of the dried leaves), mp 177–178°, $[\alpha]_D^{21}$ −89.2° (MeOH; *c* 0.12). CC over Diaion HP20AG (H_2O → 50% MeOH) of fraction 2 furnished **1** (yield 0.05%). Fraction 4

*Part 4 in the series "Chemical Studies on the Constituents of Edible Plants". For Part 3, see *Phytochemistry* (1984) **23**, 2043.

crystallized from H_2O to give scutellarin (yield 0.01%), mp > 200°. CC (Diaion HP20AG, $H_2O \rightarrow 70\%$ MeOH) of fraction 6 furnished the faster moving and slower moving fractions, from the latter of which rosmarinic acid [10], mp 167–168°, $[\alpha]_D^{23}$ +85.0° (MeOH; *c* 0.12), was provided. The faster moving fraction was re-chromatographed over silica gel ($CHCl_3$–MeOH, 7:3, satd. with H_2O), giving **2** (yield 0.13%). Fraction 7 was chromatographed over Diaion HP20AG ($H_2O \rightarrow 70\%$ MeOH) to give an additional crop of rosmarinic acid (total yield 0.19%) and caffeic acid (yield 0.005%), mp 191–195° (decomp.).

Compound **1**. Recrystallized from H_2O as colourless needles, mp 193–195°, $[\alpha]_D^{22}$ −22.6° (MeOH; *c* 0.14), $[\alpha]_D^{25}$ −9.7° (H_2O; *c* 0.11). (Found: C, 52.50; H, 5.92; N, 3.26. $C_{20}H_{27}O_{11}N$ requires: C, 52.51; H, 5.95; N, 3.06%.) FDMS *m/z* (rel. int.): 496 $[M+39]^+$ (62), 480 $[M+23]^+$ (100), 458 $[M+1]^+$ (14), 396 $[M+1-26-2\times18]^+$ (8), 163 (11), 116 $[C_6H_5CHCN]^+$ (19); FABMS *m/z* (rel. int.): 458 $[M+1]^+$ (1), 116 $[C_6H_5CHCN]^+$ (78), 325 $[163+162]^+$ (8), 163 (25), 145 $[163-18]^+$ (37). IR ν_{max}^{KBr} cm^{-1}: 3300, 2925, 2880, 1500, 1455, 1410, 1150–1000, 740, 690. ^1H NMR (60 MHz, C_5D_5N): δ3.5–4.5 [12H on sugar moiety except H-1 (2x)], 4.96, 5.12 (each 1H, *d*, *J* = 6 Hz, H-1′, 1″), 6.13 (1H, *s*, H-2), 7.0–7.6, 7.8–8.0 (3H and 2H, *m*, H-5, 6, 7 and H-4, 8). ^{13}C NMR (68 MHz, DMSO-d_6): δ118.6 (C-1), 67.1 (C-2), 133.7 (C-3), 127.0 (C-4, 8), 128.7 (C-5, 7), 129.2 (C-6), 100.0 (C-1′), 81.6 (C-2′), 76.1 (C-3′, 3″), 69.3 (C-4′ or C-4″), 76.8 (C-5′ or C-5″), 60.8 (C-6′, 6″), 104.0 (C-1″), 74.7 (C-2″), 69.6 (C-4″ or C-4′), 77.0 (C-5″ or C-5′). Acetylation of **1** with Ac_2O–pyridine and subsequent purification by prep. TLC (silica gel, C_6H_6–Me_2CO, 8:2) provided an acetate as amorphous powder, $[\alpha]_D^{26}$ −16.9° (MeOH; *c* 0.12). ^1H NMR (60 MHz, $CDCl_3$): δ1.97, 1.99, 2.07, 2.10 (21H, OAc), 3.5–5.2 (14H on sugar moiety), 5.54 (1H, *s*, H-2), 7.37, 7.50 (5H, *m*, H-4, 5, 6, 7, 8).

Compound **2**. Recrystallized from *n*-BuOH as colourless needles, mp 143–145°, $[\alpha]_D^{25}$ −57.4° (MeOH; *c* 0.15), $[\alpha]_D^{25}$ −29.5° (H_2O; *c* 0.11) [lit. [8] mp 148–151°, $[\alpha]_D^{20}$ −30.1° (H_2O)]. (Found: C, 56.91; H, 5.95; N, 4.79. Calc. for $C_{14}H_{17}O_6N$: C, 56.94; H, 5.80; N, 4.74%.) FDMS *m/z* (rel. int.): 318 $[M+23]^+$ (10), 296 $[M+1]^+$ (100), 269 $[M-26]^+$ (16), 234 $[M+1-26-2\times18]^+$ (30), 116 $[C_6H_5CHCN]^+$ (50). FABMS *m/z* (rel. int.): 296 $[M+1]^+$ (2), 116 $[C_6H_5CHCN]^+$ (100), 325 (3), 163 (18), 145 $[163-18]^+$ (29). IR ν_{max}^{KBr} cm^{-1}: 3300, 2950, 2920, 2880, 1500, 1450, 1435, 1405, 1100–1000, 745, 690. ^1H NMR (60 MHz, C_5D_5N): δ3.6–4.7 (6H on sugar moiety except H-1), 4.96 (1H, *d*, *J* = 6 Hz, H-1′), 6.23 (1H, *s*, H-2), 7.2–7.5, 7.6–7.9 (3H and 2H, *m*, H-5, 6, 7 and H-4, 8). ^{13}C NMR (68 MHz, DMSO-d_6): δ118.7 (C-1), 66.5 (C-2), 133.6 (C-3), 127.3 (C-4, 8), 128.9 (C-5, 7), 129.5 (C-6), 101.1 (C-1′), 73.1 (C-2′), 76.4 (C-3′), 69.8 (C-4′), 77.1 (C-5′), 61.0 (C-6′). Compound **2** provided an acetate, mp 136–137° (from MeOH), $[\alpha]_D^{25}$ −17.8° (MeOH; *c* 0.10), $[\alpha]_D^{26}$ −22.7° (EtOAc; *c* 0.13) [lit. [7] mp 139–140°, $[\alpha]_D^{20}$ −24.01° (EtOAc)].

Hydrolysis of **1** *with* HCl *to* **2**. A soln of **1** in N HCl was heated at 100° for 1 hr. The reaction mixture was filtered through a column of Diaion HP20AG. The column was successively washed with H_2O and MeOH. The filtrate and aq. washings were combined, neutralized with Amberlite IR 45 (OH^-), and evaporated to dryness. The residue showed one spot of glucose on TLC (microcrystalline cellulose, *n*-BuOH–pyridine–H_2O, 3:2:1), and on treatment with phenylhydrazine, it gave D-glucose phenylosazone, mp 202–204° (decomp.), $[\alpha]_D^{27}$ −68.3 → 38.6° (pyridine–EtOH, 2:3; *c* 0.59) [lit. [11] mp 205° (decomp.), 208° (decomp.), 210°, α_D: −0.62 → 0.35° (pyridine–EtOH, 2:3; *c* 2, *l* 0.5 dm)]. The MeOH eluate was chromatographed over silica gel ($CHCl_3$–EtOH, 8:2) and crystallized from *n*-BuOH to colourless needles, mp 143–144°, $[\alpha]_D^{23}$ −59.5° (MeOH; *c* 0.11). R_f value, IR and ^1H NMR data were identical with those of **2**.

Hydrolysis of **2** *with conc* HCl. A soln of **2** in conc HCl was heated at 100° for 10 min. The mixture was treated with H_2O and Et_2O. The Et_2O soln provided (*R*)-mandelic acid, mp 128–129° (from C_6H_6–hexane), $[\alpha]_D^{25}$ −145.4° (H_2O; *c* 0.12) [lit. [12] mp 133°, $[\alpha]_D^{24}$ −158.2° (H_2O)]. The aq. soln was positive to Nessler reagent and showed one spot of glucose on TLC.

Enzymic hydrolysis of **2**. To a soln of **2** (120 mg) in acetate buffer (pH 4.75, 20 ml) was added β-glucosidase (from almond, 17.5 μ/mg, 10 mg). After standing at 30° for 24 hr, the reaction mixture was extracted with Et_2O. The half of the Et_2O extract was treated with 2,4-dinitrophenylhydrazine in 5% H_2SO_4 to give benzaldehyde 2,4-dinitrophenylhydrazone, mp 236–237°. The other half provided (±)-mandelic acid, mp 113–114°, by heating with conc HCl. Half of the aq. soln was acidified, and the volatile compounds were collected in an ice-cold 10% NaOH by bubbling with N_2. The alkaline soln gave ferrocyanide by refluxing with $FeSO_4$. The other half gave one spot of glucose on TLC. Compound **1** was treated just in the same manner as above. The reaction mixture showed the spot of only **1** and did not have the odour of benzaldehyde.

Acknowledgements—The writers are deeply indebted to Dr. R. Mues, Fachrichtung Botanik, Universitat des Saarlandes, for the authentic 7-(2-*O*-β-D-glucuronyl-β-D-glucuronyloxy)-5,3′,4′-trihydroxyflavone, and to Dr. Y. Itakura, Wakunaga Pharmaceutical Ltd., for ^{13}C NMR spectra.

REFERENCES

1. Izawa, B. (1980) *Illustrated Cyclopedia of Medicinal Plants of Japan*. Seibundo-Shinkosha, Tokyo.
2. Morita, N. (1976) *Yakuyoshokubutsu*. Shufu no Tomo, Tokyo.
3. Ishikura, N. (1981) *Agric. Biol. Chem.* **45**, 1855.
4. Mues, R. (1983) *Biochem. Syst. Ecol.* **11**, 261.
5. Dreifuss, P. A., Wood, G. E., Roach, J. A. G., Brumley, W. C., Andrzejewski, D. and Sphon, J. A. (1980) *Biomed. Mass Spectrom.* **7**, 201.
6. Mellon, F. A. and Fenwick, G. R. (1984) *Biomed. Mass Spectrom.* **11**, 375.
7. Rosenthaler, L. (1932) *Handbuch der Pflanzen-Analyse*, Bd. III, 2 Halfte. Julius Springer, Wien.
8. Seigler, D. S. (1975) *Phytochemistry* **14**, 9.
9. Hubel, W., Nahrstedt, A. and Wray, V. (1981) *Arch. Pharm.* **314**, 609.
10. Aritomi, M. (1982) *Kaseigaku Zasshi* **33**, 353.
11. *Beilstein's Handbuch der Organischen Chemie* (1938) Bd. XXXI, p. 350.
12. *Beilstein's Handbuch der Organischen Chemie* (1949) Zweites Erganzungs Werk, Bd. X, p. 114.