Phytochemistry, Vol. 22, No. 7, pp. 1659–1661, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

FLAVANOL GLUCOSIDES FROM RHUBARB AND *RHAPHIOLEPIS UMBELLATA**

GEN-ICHIRO NONAKA, EMIKO EZAKI,† KATSUYA HAYASHI† and ITSUO NISHIOKA

Faculty of Pharmaceutical Sciences, Kyushu University 62, Maidashi, Higashi-ku, Fukuoka 812, Japan; †Department of Agricultural Chemistry, Faculty of Agriculture, Kyushu University 46, Hakozaki, Higashi-ku, Fukuoka 812, Japan

(*Received* 29 *November* 1982)

Key Word Index—Rhubarb; Polygonaceae; *Rhaphiolepis umbellata*; Rosaceae; flavan-3-ol glucosides; catechin.

Abstract—Investigations of rhubarb and the bark of *Rhaphiolepis umbellata* led to the isolation of new flavan-3-ol glucosides. Their structures were elucidated on the basis of ^1H and ^{13}C NMR analysis and hydrolytic studies as (+)-catechin 5-*O*-β-D-glucopyranoside and (−)-catechin 7-*O*-β-D-glucopyranoside.

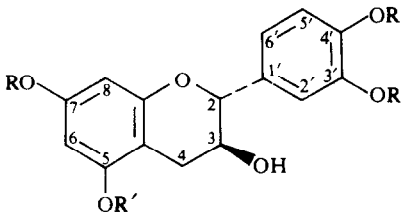
INTRODUCTION

The glycosides of flavan-3-ols are rarely found in nature, although flavan-3-ols are widely distributed in the plant kingdom as their free forms, associated in particular with condensed tannins. They have been assumed to be biosynthetically related to these flavonoids. Only three flavan-3-ols, namely, 3′-*O*-methyl-(−)-epicatechin 7-*O*-β-D-glucopyranoside (symplocoside) [1], (+)-catechin 7-*O*-α-L-arabinoside (polydine) [2] and (+)-catechin 7-*O*-β-D-xylopyranoside [3], have hitherto been isolated from *Symplocos uniflora*, *Polypodium vulgare* and *Ulmus americana*, respectively.

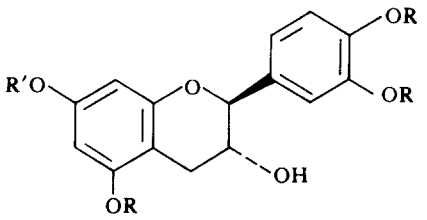
Our systematic chemical investigation of tannins and related compounds in crude drugs has led to the isolation of two new catechin glucosides (**1** and **2**) from commercial rhubarb and the bark of *Rhaphiolepis umbellata*, the latter being used in Japan as an astringent and a dyeing agent. This report describes the structures of these glucosides.

RESULTS AND DISCUSSION

Extraction of commercial rhubarb with aqueous acetone, followed by Sephadex LH-20 chromatography using a variety of solvent systems, afforded a crystalline flavanol glucoside (**1**), mp 203–204°, $[\alpha]_D$ −28.8° (MeOH). The molecular formula, $C_{21}H_{24}O_{11} \cdot 1/2H_2O$, was assigned to this compound on the basis of elemental analysis and field desorption mass spectrometry; the latter gave $[M+2H]^+$ at *m/z* 454 and $[M+Na]^+$ at *m/z* 475. The ^1H and ^{13}C NMR spectra revealed the presence of a flavan skeleton, and the former indicated a *trans* configuration of C-2 and C-3 (H-2: δ4.51, J = 7 Hz). The occurrence of a carbohydrate moiety was deduced from the appearance of the anomeric proton doublet at δ4.72 (J = 7 Hz) and also from six aliphatic carbon signals including the anomeric carbon signal at δ 100.3. Enzymatic hydrolysis of **1** with crude hesperidinase yielded a carbohydrate moiety and an aglycone (**3**), mp 177°, $[\alpha]_D$ +10.5° (Me_2CO). The sugar was identified as glucose by cellulose TLC. The identity of **3** with (+)-catechin was confirmed by direct comparison of the physical and spectral data with an authentic sample.



1 R=H, R′ = β-D-glucopyranosyl
3 R=R′=H
4 R=Me, R′ = β-D-glucopyranosyl



2 R=H, R′ = β-D-glucopyranosyl
5 R=R′=H
6 R=Me, R′ = β-D-glucopyranosyl

The sugar was shown to be linked to a phenolic hydroxyl group by the appearance of three phenolic hydroxyl signals in the ^1H NMR spectrum of **1** (δ 8.81, 8.85 and 9.18, disappearing upon addition of D_2O). This was confirmed by formation of the trimethyl ether (**4**, mp 219–220°, $[\alpha]_D$ −3.7° (MeOH–pyridine), upon methylation of **1** with dimethyl sulfate and potassium carbonate in dry acetone. The location of the carbohydrate moiety was finally established at the C-5 position on the basis that

*Part 11 in the series "Tannins and Related Compounds". For part 10 see Nonaka, G. and Nishioka, I. *Chem. Pharm. Bull.* (*Tokyo*) (in press).

Table 1. ^{13}C NMR spectral data of **1**–**3***

		1†	2†	3‡
	C-2	80.9	80.8	82.3
	C-3	65.9	65.8	68.0
	C-4	27.3	27.3	27.5
	C-4a	100.3	100.5	100.2
	C-5	156.4	155.0	156.7§
	C-6	94.9	94.8	95.0
	C-7	154.7	156.7	156.3§
	C-8	96.0	96.1	95.9
	C-8a	156.1	155.7	157.1§
	C-1′	130.3	130.2	131.5
	C-2′	114.3	114.2	115.0
	C-3′	144.6	144.6	145.2
	C-4′	144.6	144.6	145.2
	C-5′	115.0	115.0	115.4
	C-6′	118.4	118.4	119.6
sugar	C-1	101.2	101.9	—
	C-2	73.1	72.9	—
	C-3	76.3	76.2	—
	C-4	69.4	69.4	—
	C-5	76.7	76.6	—
	C-6	60.4	60.4	—

*Run at 25.05 MHz with TMS as int. standard. Chemical shifts in δ-values (ppm).

†In DMSO-d_6 + D_2O solution.

‡In Me_2CO-d_6 + D_2O solution.

§Assignments may be interchanged.

the trimethylether (**4**) gave, on similar enzymatic hydrolysis, 7,3′,4′-trimethyl-(+)-catechin identical to the sample obtained by partial methylation of (+)-catechin [2]. Since the β-configuration of the anomeric carbon could be assigned on the basis of the coupling constant (J = 7 Hz) in the ^1H NMR spectra of **1** and **4**, the structure of the glucoside **1** is established as (+)-catechin 5-*O*-β-D-glucopyranoside.

The second flavanol glucoside (**2**), $[\alpha]_D$ −20.7° (MeOH), was obtained as an off-white amorphous powder from the aqueous acetone extracts of the bark of *Rhaphiolepis umbellata* by repeated Sephadex LH-20 chromatography. It gave ^1H and ^{13}C NMR spectra almost idential to those of **1**, indicating the presence of a sugar moiety and a flavan-3-ol skeleton with a similar substitution pattern. Enzymatic hydrolysis of **2** with crude hesperidinase furnished glucose and an aglycone (**5**), mp 175–178°. The mp and ^1H NMR spectrum of **5** coincided with those of (+)-catechin, but the optical rotation, although the value was less than that of (−)-catechin probably due to partial racemization, showed the negative sign [−4.1° (Me_2CO)], thus showing that **5** is (−)-catechin.

The position of the sugar moiety was determined in the same way as for **1**. Since three phenolic hydroxyl signals were observed at δ 8.82, 8.86 and 9.40 in the ^1H NMR spectrum of **2**, the sugar should be attached to a phenolic hydroxyl group. Methylation of **2** in the same manner as described before yielded a trimethyl ether (**6**), $[\alpha]_D$ −41.8° (DMSO), which was subsequently hydrolysed with crude hesperidinase giving 5,3′,4′-trimethyl-(−)-catechin (**6**) [2]. From these results the sugar could be placed on the C-7 position. The β-configuration was assigned to the anomeric center based upon the coupling constant (J = 7 Hz) of the anomeric proton signal in **2** and **6**. Consequently, the glucoside, **2**, is identified as (−)-catechin 7-*O*-β-D-glucopyranoside.

EXPERIMENTAL

Mps are uncorr. ^1H and ^{13}C NMR spectra were recorded at 100 and 25.05 MHz, respectively, and chemical shifts are given in the δ (ppm) scale with TMS as int. standard. TLC was performed on Si gel and Avicel SF cellulose, and spots were detected by spraying $FeCl_3$ or anisaldehyde-H_2SO_4 (for phenolics) and aniline hydrogen phthalate (for sugars) reagents.

Isolation of catechin glucoside (**1**). Finely powdered rhubarb (commercial name: Ya-huang in Chinese) (3 kg) purchased from the market in Hong Kong was extracted × 3 at room temp. with 80% aq. Me_2CO. The combined extracts were concd under red. pres. to *ca* 5 l. and partitioned against EtOAc. The aq. layer, after removal of the solvent, was subjected to Sephadex LH-20 chromatography. Elution with H_2O gave uncharacterized substances negative to the $FeCl_3$ reagent. Successive elution with H_2O–EtOH (1:1) yielded a mixture consisting of low MW phenolics. Repeated chromatography of this fraction using a variety of solvents (EtOH, Me_2CO and 60% aq. MeOH) afforded a catechin glucoside, **1** (260 mg).

(+)-*Catechin 5-O-β-D-glucopyranoside* (**1**). Colorless needles from H_2O–MeOH, mp 203–204°, $[\alpha]_D^{20}$ −28.8° (MeOH; *c* 1.0). ^1H NMR (DMSO-d_6): δ 2.38 (1H, *dd*, J = 16, 8 Hz, H-4), 2.90 (1H, *dd*, J = 16, 4 Hz, H-4), 4.51 (1H, *d*, J = 7 Hz, H-2), 4.72 (1H, *d*, J = 7 Hz, anomeric H), 5.19 (1H, *d*, J = 2 Hz, H-6), 6.12 (1H, *d*, J = 2 Hz, H-8), 6.9–7.1 (3H, aromatic H). (Found: C, 56.81; H, 6.10. $C_{24}H_{30}O_{11} \cdot 1/2H_2O$ requires: C, 57.25; H, 6.21%). aromatic OH). ^{13}C NMR: see Table 1. (Found: C, 54.91; H, 5.41. $C_{21}H_{24}O_{11} \cdot 1/2H_2O$ requires: C, 54.66; H, 5.46%.) FDMS *m/z* (rel. int.): 475[M + Na]$^+$ (100), 454[M + 2H]$^+$ (18), 291 (2).

Enzymatic hydrolysis of **1**. Glucoside **1** (22 mg) was dissolved in H_2O (2 ml) and crude hesperidinase was added. The mixture was maintained at 37° for 30 min with agitation. The solvent was concd *in vacuo* and the residue treated with MeOH. The MeOH soluble portion was subjected to CC over Sephadex LH-20. Elution with MeOH afforded glucose identified by cellulose TLC (R_f: 0.40; solvent: BuOH–pyridine–H_2O, 6:4:3) and (+)-catechin (**3**) (13 mg), colorless needles from H_2O, mp 177°, $[\alpha]_D^{20}$ +10.5° (Me_2CO; *c* 0.4). ^1H NMR (Me_2CO-d_6): δ 2.52 (1H, *dd*, J = 16, 8 Hz, H-4), 2.93 (1H, *dd*, J = 16, 6 Hz, H-4), 4.00 (1H, *m*, H-3), 4.56 (1H, *d*, J = 8 Hz, H-2), 5.87 (1H, *d*, J = 2 Hz, H-6), 6.02 (1H, *d*, J = 2 Hz, H-8), 6.7–6.9 (3H, aromatic H). ^{13}C NMR: see Table 1.

Methylation of **1**. Glucoside **1** (100 mg) was methylated for 3 hr with Me_2SO_4 (1.2 ml) and K_2CO_3 (1.5 g) in dry Me_2CO (15 ml). After filtration of the inorganic ppt, the soln was concd to a syrup which was purified by CC over Si gel. Elution with EtOAc–MeOH–H_2O (20:2:1) gave the trimethyl ether (**4**) (15 mg), colorless needles from MeOH, mp 219–220°, $[\alpha]_D^{20}$ −3.7° (MeOH–pyridine, 1:1; *c* 0.43). ^1H NMR (DMSO-d_6): δ 3.68 (3H, *s*, OMe), 3.76 (6H, *s*, OMe), 4.65 (1H, *d*, J = 8 Hz, H-2), 4.78 (1H, *d*, J = 7 Hz, anomeric H), 6.11 (1H, *d*, J = 2 Hz, H-6), 6.32 (1H, *d*, J = 2 Hz, H-8), 6.9–7.1 (3H, aromatic H). (Found: C, 56.81; H, 6.10. $C_{24}H_{30}O_{11} \cdot 1/2H_2O$ requires: C, 57.25; H, 6.21%).

Enzymatic hydrolysis of **4**. Compound **4** (51 mg) in H_2O (8 ml) was incubated at 37° with crude hesperidinase. After 1 hr the mixture was concd *in vacuo* to dryness. The residue was treated with MeOH and the soluble portion subjected to CC on Si gel. Elution with C_6H_6–Me_2CO (17:3) yielded 7,3′,4′-trimethyl-(+)-catechin (14 mg), colorless needles from MeOH, mp 207–208°,

$[\alpha]_D^{20}$+ 42.6° (pyridine; *c* 0.39) (lit. [2] mp 203–204°). ^{1}H NMR (DMSO-d_6): δ 2.38 (1H, *dd*, *J* = 16, 8 Hz, H-4), 2.77 (1H, *dd*, *J* = 16, 6 Hz, H-4), 3.63 (3H, *s*, OMe), 3.76 (6H, *s*, OMe), 3.95 (1H, *m*, H-3), 4.62 (1H, *d*, *J* = 8 Hz, H-2), 4.62 (1H, *d*, *J* = 8 Hz, H-2), 5.91 (1H, *d*, *J* = 2 Hz, H-6), 6.02 (1H, *d*, *J* = 2 Hz, H-8), 6.9–7.0 (3H, aromatic H). (Found: C, 64.89; H, 6.15, Calc. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07%.)

Isolation of catechin glucoside (**2**). Fresh bark (5.0 kg) of *R. umbellata* (Thumb.) Makino, collected in July 1981 in the campus of Kyushu University, was extracted at room temp. with 80% aq. Me_2CO. Evaporation of Me_2CO *in vacuo* gave an aq. soln which was successively extracted with C_6H_6 and EtOAc. The aq. layer, after concn *in vacuo* to *ca* 1 l., was subjected to CC on Sephadex LH-20. Elution first with H_2O and then with H_2O containing an increasing amount of MeOH yielded a catechin glucoside, **2** (78 mg).

(−)-*Catechin 7-O-β-*D*-glucopyranoside* (**2**). An off-white amorphous powder, $[\alpha]_D^{20}$ − 20.7° (MeOH; *c* 1.0). ^{1}H NMR (DMSO-d_6): δ 2.38 (1H, *dd*, *J* = 16, 8 Hz, H-4), 2.70 (1H, *dd*, *J* = 16, 6 Hz, H-4), 4.52 (1H, *d*, *J* = 8 Hz, H-2), 4.66 (1H, *d*, *J* = 7 Hz, anomeric H), 5.98 (1H, *d*, *J* = 2 Hz, H-6), 6.10 (1H, *d*, *J* = 2 Hz, H-8), 6.5–6.9 (3H, aromatic H), 8.82, 8.86, 9.40 (1H, each *s*, aromatic OH). ^{13}C NMR: see Table 1. (Found: C, 51.56; H, 5.88. $C_{21}H_{24}O_{11} \cdot 2H_2O$ requires: C, 51.64; H, 5.78%.)

Enzymatic hydrolysis of **2**. Glucoside **2** (20 mg) in H_2O was incubated at 37° for 1 hr with crude hesperidinase. Work-up in the same way as for **1** yielded glucose and (−)-catechin, colorless needless from H_2O, mp 175–178°, $[\alpha]_D^{21}$ − 4.1° (Me_2CO; *c* 0.41). The ^{1}H NMR spectrum of the latter was identical with that of (+)-catechin.

Methylation of **2**. Glucoside **2** (53 mg) was methylated in the same way as described before to give the trimethyl ether (**6**) (19 mg), colorless needles from MeOH $[\alpha]_D^{20}$ − 41.8° (DMSO; *c* 0.16). ^{1}H NMR (DMSO-d_6): δ 2.40 (1H, *dd*, *J* = 16, 8 Hz, H-4), 2.80 (1H, *dd*, *J* = 16, 6 Hz, H-4), 4.67 (1H, *d*, *J* = 8 Hz, H-2), 4.75 (1H, *d*, *J* = 7 Hz, anomeric H), 6.15 (1H, *d*, *J* = 2 Hz, H-6), 6.27 (1H, *d*, *J* = 2 Hz, H-8), 6.9–7.1 (3H, aromatic H). (Found: C, 56.06; H, 6.34. $C_{24}H_{30}O_{11} \cdot H_2O$ requires: C, 56.24; H, 6.29%.)

Enzymatic hydrolysis of **6**. Compound **6** (10 mg) was hydrolysed with crude hesperidinase in the same way as for **4** giving 5,3′,4′-trimethyl-(−)-catechin (1.6 mg), colorless needles from MeOH, mp 255–260°. ^{1}H NMR (DMSO-d_6): δ 2.38 (1H, *dd*, *J* = 16, 8 Hz, H-4), 2.74 (1H, *dd*, *J* = 16, 6 Hz, H-4), 3.70 (3H, *s*, OMe), 3.75 (6H, *s*, OMe), 3.94 (1H, *m*, H-3), 4.59 (1H, *d*, *J* = 8 Hz, H-2), 5.86 (1H, *d*, *J* = 2 Hz, H-6), 5.98 (1H, *d*, *J* = 2 Hz, H-8), 6.9–7.1 (3H, aromatic H).

Acknowledgements—We are indebted to Mr. T. Tanaka of Tanabe Seiyaku Co., Ltd for the generous supply of crude hesperidinase. Our thanks are also due to Mr. Y. Tanaka, Miss K. Soeda and Mr. I. Maetani for the measurements of FDMS, ^{1}H and ^{13}C NMR spectra, and to the staff of the Central Analysis Room of this University for microanalysis.

REFERENCES

1. Tschesche, R., Braun, T. M. and Sassen, W. V. (1980) *Phytochemistry* **19**, 1925.
2. Weinges, K. and Wild, R. (1970) *Justus Liebig's Ann. Chem.* **734**, 46.
3. Doskotch, R. W., Mikhail, A. A. and Chatterji, S. K. (1973) *Phytochemistry* **12**, 1153.