Phytochemistry, Vol. 28, No. 2, pp. 607–609, 1989.
Printed in Great Britain.

0031–9422/89 $3.00+0.00

GALLOYLHOMOARBUTIN AND RELATED POLYPHENOLS FROM *PYROLA INCARNATA*

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(*Received* 26 *July* 1988)

Key Word Index—*Pyrola incarnata*; Pyrolaceae; polyphenols; 6-*O*-galloylhomoarbutin; 2″-*O*-galloylhyperin; procyanidins.

Abstract—A new polyphenol, 6-*O*-galloylhomoarbutin was isolated from *Pyrola incarnata*, along with (+)-catechin, (−)-epicatechin gallate, procyanidin B1, B3, B2-3′-*O*-gallate, B2-3,3′-di-*O*-gallate, hyperin and hyperin-2″-*O*-gallate. Hyperin-2″-*O*-gallate, one of the main components, showed strong tanning activity.

INTRODUCTION

The whole plant of *Pyrola incarnata* Fisch. (Pyrolaceae) have been used as a diuretic and a styptic, mixed indiscriminately with *P. japonica* Klenze and other species [1]. Although the presence of tannin in some *Pyrola* species [2], and isolation of phenolic glucosides [3, 4] and quinones [5] have been reported, the tannins in the species of this genus and also of Pyrolaceae are unknown. We have isolated a new polyphenol, along with other phenolics including a galloylated flavone which has strong tanning activity.

RESULTS AND DISCUSSION

The ethyl acetate extract from the total extract of dried whole plant of *P. incarnata* yielded a new compound (**1**) upon column chromatographic separation, together with known polyphenols, (+)-catechin, (−)-epicatechin gallate [6], procyanidins B1 (**2**), B3 (**3**) [7], B2-3′-*O*-gallate (**4**), B2-3,3′-di-*O*-gallate (**5**) [8] hyperin (**6**) [9] and hyperin-2″-*O*-gallate (**7**) [10], and fairly large amounts of homoarbutin [11]. Among them, the structure of **7** was confirmed by hydrolysis using tannase to give hyperin (**6**) and gallic acid, and by the decoupling experiment in ^{1}H NMR which proved the location of galloyl group at the 2″-OH of galactose. Finally, the identification of this compound was accomplished by the comparison of the data of its TMS ether [10].

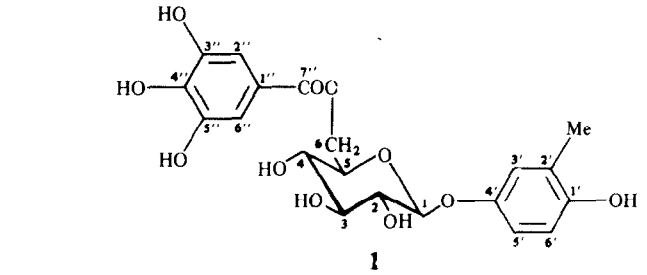
Compound **1**, an off-white amorphous powder, gave a dark blue colour with ferric chloride on TLC plate (cellulose). Its ^{1}H NMR spectrum showed ABX type signals at δ 6.81 (1H, *d*, $J=1.8$ Hz), 6.77 (1H, *dd*, $J=1.8$, 8.5 Hz), 6.65 (1H, *d*, $J=8.5$ Hz) assignable to 1,2,4-trisubstituted benzene ring, a 2H singlet at 7.17 ppm, and a doublet at 4.78 ppm ($J=7$ Hz), attributable to an anomeric proton of glucose, and a methyl group signal at 2.08 ppm (3H, *s*). They are closely related to the signals of homoarbutin except the presence of additional 2H singlet at 7.17 ppm, assignable to a galloyl group in the former. Acid hydrolysis of **1** with 0.5 M sulphuric acid gave two aromatic compounds, gallic acid and toluhydroquinone which were identified with the authentic samples by direct comparison. Glucose in the hydrolysate of **1** was identified by GLC. Partial hydrolysis of **1** in dilute ammonium hydroxide gave homoarbutin. These data indicate that **1** is a galloyl ester of homoarbutin. The location of galloyl group in **1** was established to be *O*-6 of the glucose moiety by the ^{1}H NMR analysis, as the signals of H-6 were shifted to lower field [δ 4.61 (1H, *dd*, $J=2$, 12 Hz), 4.38 (1H, *dd*, $J=7$, 12 Hz)] from that of homoarbutin, and all glucose signals were analogous to those of arbutin-6-*O*-gallate [12]. These data and the ^{13}C NMR spectrum described in the Experimental indicate that **1** is homoarbutin-6-*O*-gallate.

The ethyl acetate extract showed tanning activity (RMBG = 0.22) [13], and among 10 compounds isolated, hyperin-2″-*O*-gallate (**7**) exhibited RMBG value (1.30) which is higher than that of tannic acid obtained from Chinese gall. The HPLC analysis of the total extract showed presence of three main components; homoarbutin (40.8 mg/g dry wt), hyperin (6.87 mg/g dry wt) and hyperin-2″-*O*-gallate (7.31 mg/g dry wt). Therefore, **7** can be regarded as contributing significantly to the tanning activity of this plant, since **6** and homoarbutin had almost no activity.

EXPERIMENTAL

^{1}H NMR spectra were measured at 90 or 300 MHz in acetone-d_6 with TMS as int. std. TLC was conducted on cellulose (Avicel, Funakoshi) using 7% HOAc as developer. Avicel microcrystalline cellulose (Funakoshi), Toyopearl HW-40 (coarse and fine, TOSOH) were used for CC, and Sep-pak C-18 (Waters) was used for the final purification. HPLC was conducted on a YMC-Pack A312 (ODS) (6 × 150 mm) column, developing with 0.01 M H_3PO_4–KH_2PO_4 in MeOH (7:3), at flow rate 1.3 ml/min, detecting at 254 nm. GLC was run on a column of 3% OV-1 (1 m), at 165°, detecting with FID.

Extraction and isolation. Dried whole plant of *Pyrola incarnata* was purchased in Okayama, and was identified with the standard specimen [1] by Prof. T. Namba. The plant materials (2 kg) were homogenized in 70% aq. Me_2CO (15 l), and the homogenate filtered and concd to give the total extract of *P. incarnata*. This extract was successively extracted with Et_2O,



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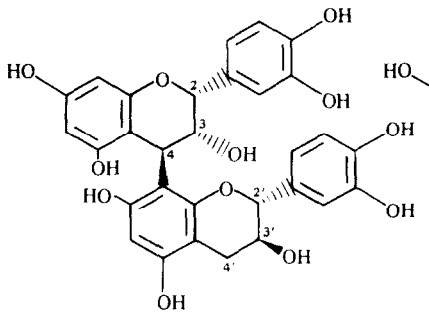

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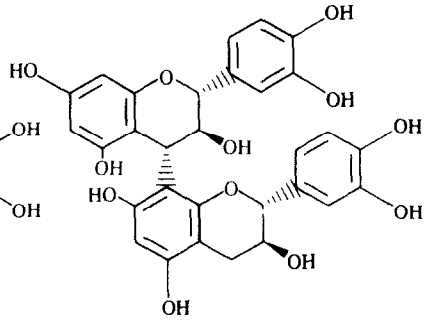

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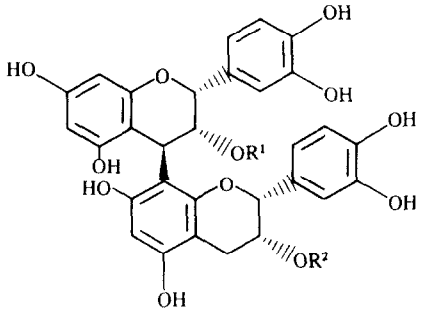
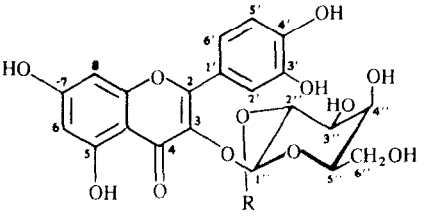


4 R^1 = H, R^2 = galloyl
5 R^1 = R^2 = galloyl



6 R = H
7 R = galloyl

EtOAc and *n*-BuOH. A part (45 g) of the EtOAc extract (98.8 g in total) was subjected to Toyopearl HW-40 (coarse) CC eluted stepwise with 20, 30 and 50% aq. EtOH. Fraction I was crystallized from EtOH–Me_2CO to give homoarbutin (6.21 g) as colourless crystals. Fraction II was crystallized from EtOH–H_2O to give yellow crystals (2.2 g) which was identified with hyperin by direct comparison with authentic sample. Crystallization of fraction III and IV from EtOH–H_2O gave hyperin-2″-*O*-gallate (**7**) as yellow needles (4.68 g). The mother liquor of fraction II was further purified with cellulose CC using H_2O, and then with Sep-pak C-18 to yield 6-*O*-galloylhomoarbutin (246 mg). The mother liquor of fraction III contained hyperin-2″-*O*-gallate (**7**), (+)-catechin and procyanidin B1 (**2**), which were isolated by Tyopearl HW-40 (fine) CC and cellulose CC eluted with H_2O. The identification of (+)-catechin (580 mg) and procyanidin B1 (**2**, 8.2 mg) was achieved by direct comparison with authentic specimens. Procyanidin B3 (**3**, 269 mg) was isolated from fraction V by cellulose CC eluted with H_2O, and finally purified with Sep-pak C-18. Fraction VI was re-chromatographed on cellulose column as above to give (−)-epicatechin gallate (108 mg) and procyanidin B2-3′-*O*-gallate (**4**, 150 mg). Procyanidin B2-3,3′-di-*O*-gallate (**5**, 707 mg) was isolated from fraction VII in the same manner as applied to fraction V. These compounds were identified with authentic samples by direct comparison.

Hyperin-2″-O-gallate (**7**). Yellow needles, mp 185–187°, $[\alpha]_D$ −91.6° (MeOH; *c* 0.5). UV λ_{max}^{MeOH} (log ε): 212 (4.67), 258 (sh. 4.35), 268 (4.36), 365 (sh. 4.20). TLC: R_f 0.30. ^{1}H NMR: δ 7.82 (1H, *d*, *J* = 2 Hz, H-2′), 7.71 (1H, *dd*, *J* = 2, 8.5 Hz, H-6′), 7.23 (2H, *s*, galloyl), 6.93 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.45 (1H, *d*, *J* = 2 Hz, H-8), 6.23 (1H, *d*, *J* = 2 Hz, H-6), 5.93 (1H, *d*, *J* = 8 Hz, H-1″), 5.49 (1H, *dd*, *J* = 8, 10 Hz, H-2″), 4.06 (1H, *d*, *J* = 3.5 Hz, H-4″), 4.00 (1H, *ddd*, *J* = 3.5, 5, 10 Hz, H-3″), 3.77–3.67 (3H, *m*, H-5″, 6″ × 2).

Degalloylation of **7**. A soln of **7** (2 mg) in H_2O 2 ml was treated with tannase at 37°. The reaction was monitored by HPLC. After 2 hr, most of **7** was hydrolysed to give gallic acid and hyperin.

Trimethylsilylation of **7**. To a mixture of hexamethyldisilazane (0.2 ml), trimethylchlorosilane (0.1 ml) and dry pyridine (1 ml), **7** (10 mg) was added. After standing for 30 min at room temp., the solvent was evapd to dryness. The ^{1}H NMR (in CCl_4) of the residue was identical with that of trimethylsilylated hyperin-2″-*O*-gallate [10].

6-*O*-*Galloylhomoarbutin* (**1**). An off-white amorphous powder, $[\alpha]_D$ −39.2° (EtOH; *c* 0.6). UV λ_{max}^{MeOH} nm (log ε): 216 (4.50), 280 (4.10). TLC: R_f 0.56. ^{1}H NMR: δ 7.17 (2H, s, H-2″, 6″), 6.81 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.77 (1H, *dd*, *J* = 1.8, 8.5 Hz, H-6′), 6.65 (1H, *d*,

$J = 8.5$ Hz, H-5′), 4.78 (1H, *d*, $J = 7$ Hz, H-1), 4.61 (1H, *dd*, $J = 2$, 12 Hz, H-6a), 4.38 (1H, *dd*, $J = 6$, 12 Hz, H-6b), 3.77 (1H, *dt*, $J = 2$, 6 Hz, H-5), 3.59–3.41 (3H, *m*, H-2, 3, 4), 2.08 (3H, *s*, Me), ^{13}C NMR: δ 166.63 (1C, ester C=O), 151.98 (1C, C4′), 151.37 (1C, C1′), 146.05 (2C, C3″, 5″), 138.79 (1C, C4″), 125.78 (1C, C3′), 121.79 (1C, C1″), 120.43 (1C, C5′), 115.88 (1C, C2′), 115.76 (1C, C6′), 109.96 (2C, C2″, 6″), 103.32 (1C, C1), 77.79 (1C, C3), 75.01 (1C, C2), 74.75 (1C, C5), 71.32 (1C, C4), 64.54 (1C, C6), 16.29 (1C, Me). IR ν_{max}^{KBr} cm^{-1}: 3400, 1690, 1670, 1505, 1350, 1210, 1070, 1040. Anal. calcd for $C_{20}H_{22}O_{11} \cdot 1/2H_2O$, C: 53.690, H: 5.182. Found C: 53.610, H: 5.329.

Acid hydrolysis of **1**. A soln of **1** (20 mg) in 5% H_2SO_4 (2 ml) was heated at 100° for 3 hr. After cooling, the hydrolysate was extracted with same vol. of $Et_2O \times 3$. The organic layer was dried, and crystallized from C_6H_6 to give gallic acid (3 mg) and toluhydroquinone as colourless needles which were identified with the standard specimens derived from homoarbutin. The aq. layer was evapd to dryness and the syrup trimethylsilylated by usual manner. Glucose was detected by GLC.

Partial hydrolysis of **1**. A soln of **1** (22 mg) in 0.1 M NH_4OH (2 ml) was kept at 37° overnight. The reaction mixture was concd *in vacuo*, and subjected to Toyopearl HW-40 (fine) CC with H_2O as the solvent to give homoarbutin as colourless crystals, which were identified by ^{1}H NMR, mp, and co-chromatography with the standard specimen.

Acknowledgement—We are grateful to Prof. T. Namba in Toyama Medical and Pharmaceutical University for the identification of the plant specimen.

REFERENCES

1. Namba, T., Miyake, M. and Nagae, K. (1980) *Shoyakugaku Zasshi* **34**, 97.
2. Gibbs, R. D. (1974) *Chemotaxonomy of Flowering Plants*, Vol. III, p. 1288. McGill-Queen's University Press, Montreal.
3. Inouye, H. (1954) *Pharm. Bull.* **2**, 359.
4. Averett, J. E. and Bohm, B. A. (1986) *Phytochemistry* **25**, 1955.
5. Inouye, H. (1956) *Yakugaku Zasshi* **76**, 976.
6. Okuda, T., Hatano, T., Kuwahara, M., Higashiyama, Y. and Maruyama, Y. (1981) Abstract Papers, 101st Annual meeting of the Pharmaceutical Society of Japan, p. 512, Kumamaoto.
7. Thompson, R. S., Jacque, D., Haslam, E. and Tanner, R. J. N. (1972) *J. Chem. Soc. Perkin Trans. I* 1387.
8. Hatano, T., Urita, K. and Okuda T. (1986) *J. Med. Pharm. Soc. Wakan-Yaku* **3**, 434.
9. Okuda, T. Mori, K., Terayama, K., Higuchi, K. and Hatano, T. (1979) *Yakugaku Zasshi* **99**, 543.
10. Nahrstedt, A., Dumkow, K., Janistym, B. and Pohl, R. (1974) *Tetrahedron Letters* 559.
11. Inouye, H. (1956) *Chem. Pharm. Bull.* **4**, 281.
12. Chen, X., Yoshida, T., Hatano, T., Fukushima, M. and Okuda, T. (1987) *Phytochemistry* **26**, 515.
13. Okuda, T., Mori, K. and Hatano, T. (1985) *Chem. Pharm. Bull.* **33**, 1424.