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Communications

Isolation and Structure Elucidation of a Catechin Glycoside with Phospholipase A_2 Inhibiting Activity from Ulmi cortex

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Extract of Ulmi cortex, dried root bark of *Ulmus pumila*[1] is known to possess antiinflammatory activity.[2] Several compounds have been isolated from Ulmi cortex,[3,4,5] but the biologically active principles have not been investigated. When we examined the extract for inhibition against phospholipase A_2 (PLA_2, E.C. 3.1.1.4), it showed strong activity. Thus, we tried to isolate the inhibitory principle from Ulmi cortex. PLA_2 catalyzes hydrolysis of phosphatidylcholines to produce arachidonic acid and lysophosphatidylcholines which can be transformed to inflammatory mediators such as prostaglandins, leukotrienes or a platelet activating factor. So inhibitors of PLA_2 may show antiinflammatory activity. Here, we wish to report isolation of (+)-catechin-7-*O*-β-D-apiofuranoside (**1**) for the first time from a higher plant as a PLA_2 inhibitory principle.

Ulmi cortex (200 g), purchased from a folk medicine market in Seoul, was extracted with dichloromethane-methanol (1 : 1) and the extract was concentrated under reduced pressure to give a red oily residue (34 g). The ethyl acetate soluble components (4.8 g) of the extract was subjected to silica gel column chromatography with $CHCl_3$-MeOH (3 : 1) to give PLA_2 inhibitory fractions. The PLA_2 inhibitory component was purified by Sephadex LH-20 (methanol) column chromatography and by preparative TLC. A yellowish amorphous solid (10 mg)[6] which showed inhibition against PLA_2 was identified to be (+)-catechin-7-*O*-β-D-apiofuranoside (**1**).

The compound showed strong absorption at 279 nm in its UV spectrum which implied the presence of phenolic aromatic rings. The doublets at 6.023 ppm (J=2.4 Hz) and 6.080 ppm (J=2.4 Hz) in its ^1H NMR spectrum were suggestive of two aromatic protons existing at the *meta* positions. The signals at 6.661 ppm (dd, J=2.0, 8.0 Hz), 6.781 ppm (d, J=2.0 Hz), and 6.712 ppm (d, J=8.0 Hz) inferred the presence of another set of aromatic protons existing at 1, 3, 4 positions. These signals, the two double doublets at 2.485 ppm (J=16.0, 7.6 Hz) and 2.803 ppm (J=16.0, 5.0 Hz), and the double double doublet at 3.945 ppm (J=7.6, 7.2, 5.0 Hz) suggested that the compound should be a flavan-3-ol derivative.[7] Furthermore, the ^1H NMR spectrum of the compound showed characteristic signals for (+)-*trans*-flavan-3-ol at 4.549 ppm (a doublet) with a coupling constant of 7.2 Hz.[8] Also the fragmentation ion at m/z 290 in its mass spectrum inferred that the compound should have (+)-catechin as an aglycone. Existence of a sugar in its structure was evidenced by the anomeric proton signal which appeared as a doublet at 5.429 ppm (J=3.0 Hz). Comparison of the ^{13}C NMR data of the compound with those of other flavonoid glycosides[9] indicated that the sugar attached to (+)-catechin was D-apiofuranose. The coupling constant (J=3.0 Hz) of the anomeric proton of the sugar suggested that the phenoxy group be attached to the anomeric carbon atom by β-configuration. Thus, we concluded that the isolated compound was (+)-catechin-*O*-β-D-apiofuranoside.

The sugar-attached site in the aromatic ring was figured out by HMBC technique (Figure 1 and 2). Since, among the signals of C-5, C-7, and C-9 in ring A, which were observed at higher values than 150 ppm, the signal at 158.127 ppm only showed correlations with those of H-6 and H-8 (at 6.080 ppm and at 6.023 ppm, respectively) as shown in Figure 1, we assgined it to C-7. The signals at 157.552 ppm and 156.816 ppm showed correlations with those of H-6 and H-8, respectively, so we assigned them to C-5 and C-9, respectively. The assignments were confirmed further from the correlations of the signal of C-9 with those of H-2 at 4.549 ppm, and C-5 and C-9 with those of H-4 at 2.485 and 2.803 ppm. The carbon signal of C-7 did not show correlations with those of H-2 or H-4. The correlation of the anomeric proton signals at 5.429 ppm with that of C-7 at 158.127 ppm suggested that the sugar should be attatched at the C-7 position of the aromatic ring A. From these data we concluded

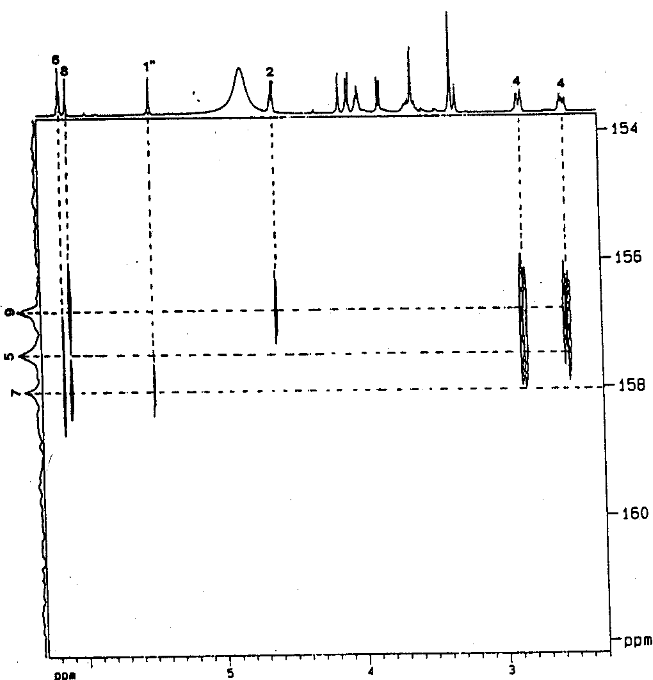


Figure 1. The HMBC spectrum of (+)-catechin-7-*O*-β-D-apiofuranoside (600 MHz, CD_3OD).

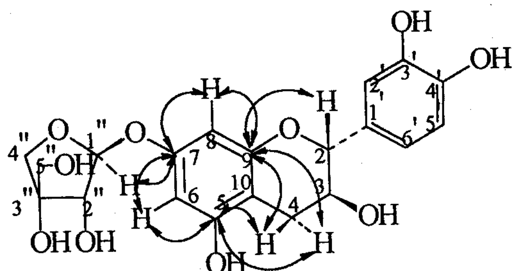
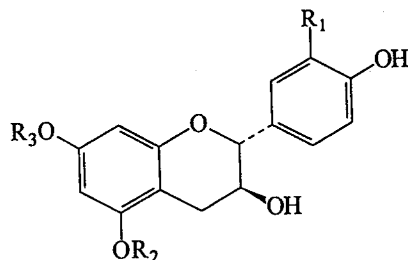


Figure 2. Correlations between carbon signals and proton signals of the A ring observed in the HMBC spectrum of (+)-catechin-7-*O*-β-D-apiofuranoside.

that the structure of the isolated flavanoid was (+)-catechin-7-*O*-β-D-apiofuranoside (**1**).



1 : R_1 = OH, R_2 = H, R_3 = β-D-apiofuranose
2 : R_1, R_2 = H, R_3 = β-D-apiofuranose
3 : R_1 = OH, R_2 = H, R_3 = α-L-arabinose
4 : R_1 = OH, R_2 = H, R_3 = α-L-rhamnose
5 : R_1 = OH, R_2 = β-D-apiofuranose, R_3 = H

(+)-Catechin-7-*O*-β-D-apiofuranoside, which was first isolated by Karl *et al.*[10] from a fern *Polypodium vulgare*, is now isolated from a higher plant as a PLA_2 inhibitory principle.

Table 1. The chemical shifts assigned to the phenolic carbon atoms in A ring in (+)-catechin-7-*O*-β-D-apiofuranoside (**1**), (+)-afzelechin-7-*O*-β-D-apiofuranoside (**2**), (+)-catechin-7-*O*-α-L-arabinoside (**3**), and (+)-catechin-7-*O*-α-L-rhamnoside (**4**)*

Carbon Number	1	2	3	4
5	157.552	156.08	156.07	155.20
7	158.127	156.16	155.83	156.22
9	156.817	155.15	155.11	156.17

*NMR data were obtained at 125 MHz in CD_3OD (**1**), at 90.8 MHz in DMSO-d_6 (**2, 3**), or at 125 MHz in DMSO-d_6 (**4**). The assignments of chemical shifts to the carbon atoms were done by NMR techniques by us in the present paper for compound **1** and by other people for compound **2**[8], **3**[13] and **4**[13].

Table 2. Carbon atom chemical shifts of (+)-catechin-7-*O*-β-D-apiofuranoside (**1**; 125 MHz; TMS; CD_3OD) and (+)-catechin-5-*O*-β-D-apiofuranoside (**5**; 125 MHz; py-d_5; reported by Son *et al.*[5]) and their differences (Δδ)

Carbon Number	1	5	Δδ
2	82.885	82.9	0.015
3	68.616	67.8	0.816
4	28.386	29.2	0.814
5	157.552	157.8*	0.248
6	97.404	97.1	0.304
7	158.127	157.9*	0.227
8	96.950	96.1	0.850
9	156.817	156.8*	0.017
10	103.331	103.4	0.069
1′	132.137	131.8	0.337
2′	115.240	115.7	0.460
3′	146.275	146.9	0.625
4′	146.244	146.9	0.656
5′	116.147	116.1	0.047
6′	119.961	119.4	0.561
1″	108.758	108.8	0.042
2″	78.305	77.8	0.505
3″	80.272	80.0	0.272
4″	75.449	75.4	0.049
5″	65.068	64.5	0.568

*The assignments of these aromatic signals of **5** were not done clearly in the original paper and adjusted by us to fit best to those of **1**.

Karl *et al.* deduced the sugar-attached site in (+)-catechin-apiofuranoside from comparison of its ^{13}C NMR data with those of other 4-ketoflavonoids.[11] However, (+)-catechin-apiofuranoside is a flavan-3-ol-glycoside, and currently, NMR data of flavan-3-ol-glycosides are limited. So, unambiguous determination of the sugar-attached phenolic carbon atom in flavan-3-ol glycosides from comparison of the NMR data with those of other flavonoids[12] was not easy and we could not see any rules in chemical shifts of C-5, C-7 and C-9

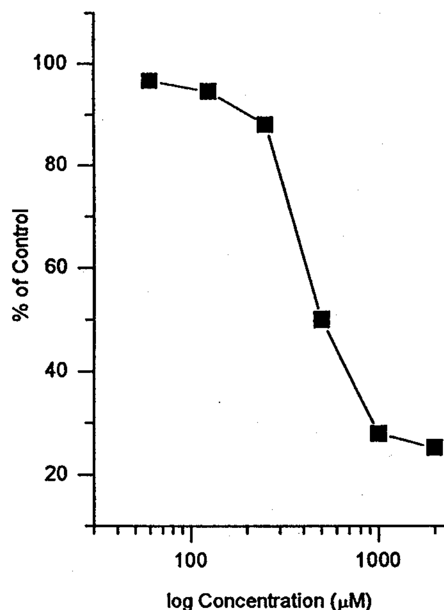


Figure 3. Inhibition of PLA_2 by (+)-catechin-7-*O*-β-D-apiofuranoside. PLA_2 activity was measured by the method of Tanaka *et al.*[16] with a slight modification using 1-stearoyl-2-[1-^{14}C]arachidonyl-L-3-phosphatidylcholine as the substrate. The standard assay mixture contained Tris-HCl buffer (100 mM, pH 7.4), $CaCl_2$ (3.0 mM), the substrate (20 μM) and an enzyme catalyzing hydrolysis of the substrate less than 20% in 40 min. The mixture was incubated at 37 ℃ for 40 min. and the reaction was terminated by addition of Dole's reagent (1.25 mL). Free fatty acid was extracted with *n*-heptane and the radioactivity of the extract was counted with a liquid scintillation counter.

of the aromatic ring A in flavan-3-ol-7-*O*-glycosides (**1, 2, 3, 4**),[8,13] which are summarized in Table 1. HMBC technique was the most reliable method to figure out the sugar attached site in flavan-3-ol-glycosides.

Son *et al.* reported isolation of (+)-catechin-5-*O*-β-D-apiofuranoside (**5**) from *Ulmus davidiana*.[5] But the isolated compound seems to be the same as ours. They elucidated the sugar attached site by Gibbs' test.[14] However, when we compared the NMR data (Table 2), both compounds showed almost identical chemical shifts.

The methanol extract of Ulmi cortex was reported to have antiinflammtory activity by Hong *et al.*[2] They obtained the result from carageenin paw edema swelling and leukocyte emigration experiments. Di Rosa *et al.*[15] observed the increase in prostaglandin production in the carageenin-induced paw edema inflammation site. When we examined the inhibitory activity of the isolated compound against *Crotalus adamanteus* PLA_2, it showed weak inhibition (IC_{50}, 500 μM; Figure 3). Since it showed weak inhibition against PLA_2, we are still looking for other antiinflammatory principles in Ulmi cortex.

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6. $[\alpha]_D$ −36° (MeOH, c 0.52); UV λ_{max} (MeOH) nm: 279; IR ν_{max} (KBr) cm^{-1}: 3420, 1620, 1610, 1510, 1040; FAB-MS m/z: 423$(M+H)^+$; EI-MS(70 eV) m/z(rel. int.): 290 (20), 152(53), 139(100); ^{1}H NMR (400 MHz, CD_3OD): δ 2.485 (1H, dd, J=16.0, 7.6 Hz, 4-H_{axial}), 2.803 (1H, dd, J=16.0, 5.0 Hz, 4-$H_{equatorial}$), 3.566 (2H, m, 5″-H_2), 3.795, 4.032 (2H, AB system, J_{AB}=10 Hz, 4″-H2), 3.945 (1H, ddd, J=7.6, 7.2, 5.0 Hz, 3-H), 4.086 (1H, d, J=3.0 Hz, 2″-H), 4.549 (1H, d, J=7.2 Hz, 2-H), 5.429 (1H, d, J=3.0 Hz, 1″-H), 6.023 (1H, d, J=2.4 Hz, 8-H), 6.080 (1H, d, J=2.4 Hz, 6-H), 6.661 (1H, dd, J=8.0, 2.0 Hz, 6′-H), 6.712 (1H, d, J=8.0 Hz, 5′-H), 6.781 (1H, d, J=2.0 Hz, 2′-H); ^{13}C NMR (125 MHz, CD_3OD): See Table 2.
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