STRUCTURE OF NOVEL ANTIOXIDATIVE LIGNAN TRIGLUCOSIDE ISOLATED FROM SESAME SEED

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Abstract-A new lignan triglucoside was isolated from sesame seed. This compound had branched (1→2)- and (1→6)-glucosidic linkage, and showed antioxidative activity.

We recently reported the structural determination of lipid-soluble antioxidative lignans isolated from sesame seed, sesamolinol[1] and sesaminol,[2] in the course of isolation of novel antioxidants from plant material.[3] We have now succeeded in further purification of the other water-soluble fraction which also has strong antioxidative activity. A new lignan glucoside composed of three D-glucoses and (+)-pinoresinol was also isolated. This pinoresinol triglucoside, whose structure was fully determined by instrumental analyses including methylation analysis,[4] was a characteristic compound for its sugar component and characteristic linkage pattern. In this paper, we describe isolation and structural determination of the new pinoresinol triglucoside.

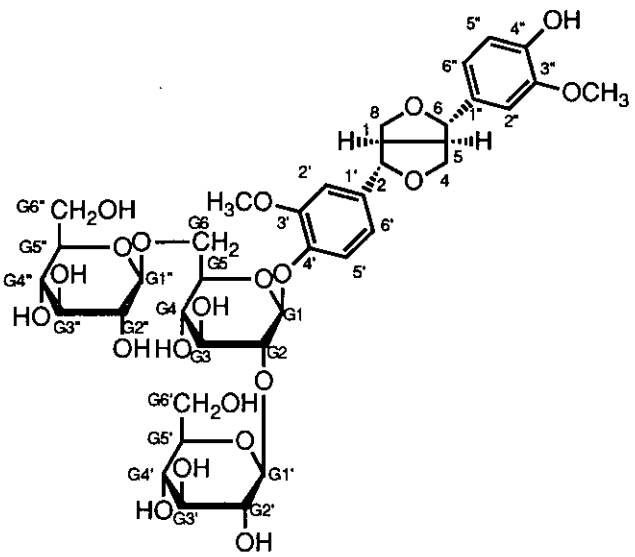
Sesame seed (500 g) was ground and defatted with *n*-hexane, and extracted with 80% ethanol. The extract was charged on an amberlite XAD-2 column.[5] The 50% methanol fraction had antioxidative activity. Then, this fraction was purified by preparative hplc using three reverse-phase columns: ODS-10 (r.t. 22 min),[6] ODS-5 (r.t. 16.5 min),[7] and ph-7 (r.t. 16.5 min),[8] and pure KP3 (26.1 mg) was finally isolated.

KP3 showed $[\alpha]_D^{25}$ -12.0 (c 0.136, H_2O). Uv spectrum showed, λ_{max} (ε_{max}) at 278 nm (5790) and 228 nm (15820) in H_2O. This data suggests that KP3 has phenolic residue as the partial structure. An aglycone and a sugar component were generated from KP3 by treatment with β-glucosidase. Aglycone moiety was identified as (+)-pinoresinol by comparison of the ms, nmr, uv, ir and $[\alpha]_D$ data with those of an authentic sample.[9] The suagr component observed as one peak by hplc analysis[10] was identified as D-glucose by its retention time. After methanolysis of KP3 was carried out, α- and β-methylglucoside TMS derivatives were analyzed by glc.[11] The FAB-ms spectrum of KP3 showed m/z 845 as a $[M+H]^+$ peak and m/z 867 as a $[M+Na]^+$ peak. The results of hydrolysis and FAB-ms spectroscopy indicate that KP3 is constituted from one (+)-pinoresinol and three D-glucoses.

In the ^{1}H-nmr spectrum of KP3, chemical shifts of anomeric proton showed at 5.18 ppm (H-G1 J = 7.3 Hz), 4.68 ppm (H-G1' J = 7.9 Hz) and 4.26ppm (H-G1" J = 7.6 Hz), respectively. The anomeric configurations were deduced from the homonuclear vicinal coupling constants. Values obtained for the ^{1}H homonuclear coupling constants of the D-glucose moieties (7.3 Hz, 7.9 Hz and 7.6 Hz) were characteristic of β-configuration. In the ^{13}C-nmr spectrum of KP3,[12] the C-4' at 136.5 ppm showed a dwonfield shift in comparison with C-4" at 133.8 ppm. This data indicate that the three sugar residues are linked at the C-4' position of the pinoresinol. One of the 2-position carbon signal of D-glucosyl residue at 82.1 ppm showed a downfield shift in comparison with the other 2-position carbon signals of D-glucosyl residues observed at 74.9 ppm and 74.4 ppm. The same downfield shift was also observed in the case of one of 6-position carbon signal of D-glucosyl residue. One of the 6-carbon signal at 69.5 ppm showed a downfield shift in comparison with the other 6-carbon signals at 62.0 ppm and 60.9 ppm. These results suggest that KP3 has (1→2)-linkage and (1→6)-linkage for sugar moiety. From these results, three glucosidic linkages pattern are possible;

1. Glcβ1→6Glcβ1→2Glcβ1→4'Pinoresinol
2. Glcβ1→2Glc1β→6Glcβ1→4'Pinoresinol
3. $\begin{matrix} \text{Glc}\beta1\rightarrow2 \\ \text{Glc}\beta1\rightarrow6 \end{matrix}$ Glcβ1→4'Pinoresinol

Since the H-G1 at 5.18 ppm showed a downfield shift in comparison with H-G1' at 4.68 ppm and H-G1" at 4.26 ppm, H-G1 was linked to pinoresinol. The H-G2 at 3.70 ppm was correlated with the H-G1 at 5.18 ppm by ^{1}H-^{1}H COSY spectrum, and the H-G2 at 3.70ppm was correlated with the C-G2 at 82.1 ppm by ^{13}C-^{1}H COSY spectrum. Hence, it was found that the C-G2 at 82.1 ppm is located at 2-position of the D-glucosyl residue which is linked to pinoresinol. This result eliminates the second possible KP3 structure given above. Numbers one and three possible structures were then distinguished by methylation analysis. Two peaks were observed by methylation analysis using GC-ms.[13] The first peak was determined as 1,5-*O*-di-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, second peak was confirmed as 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-D-glucitol from mass fragment.[14] Since the O-2 and O-6 positions of D-glucosyl residue were not methylated, the pinoresinol was found to linked with D-glucosyl residues that had the branched (1→2)- and (1→6)-glucosidic linkages. From these results, the structure of KP3 was confirmed as (+)-pinoresinol 4'-*O*-β-D-glucopyranosyl(1→2) -*O*-[β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside.



KP3

KP3 has strong antioxidative activity using the rabbit erythrocyte membrane ghost system.[15] Moreover KP3 can be classified as pro-antioxidant, because KP3 has the potentiality to produce lipid-soluble antioxidative pinoresinol by hydrolysis with β-glucosidase of intestinal bacteria after ingestion.[16] Therefore, KP3 may play an important protective role from oxidative damage in two different mechanisms. A direct inhibitory effect on lipid peroxidation in the form of glucoside in foods and a inhibitory effect on lipid peroxidation in the membrane lipid of cells and erythrocytes by the lipid-soluble aglycone after digestion. Details of the antioxidative mechanism and other biological activities will be reported soon.

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5. Bed volume 1l and eluted with H_2O (3l), 50% methanol (3l), methanol (3l) and acetone (3l).
6. Column, Develosil ODS-10 (20 i.d. x 250 mm); mobile phase, methanol/H_2O = 3:7; flow rate, 6 ml/min.

7. Column, Develosil ODS-5 (10 i.d. x 250 mm); mobile phase, methanol/H_2O = 3:7; flow rate, 2.5 ml/min.

8. Column, Develosil ph-7 (8 i.d. x 250 mm); mobile phase, methanol/H_2O = 3:7; flow rate, 3 ml/min.

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10. Column, Develosil NH_2-5(4.6 i.d. x 250 mm); mobile phase, Acetonitrile/H_2O = 7:3; flow rate, 1 ml/min, r.t. 7 min.

11. Column, silicone GE SE-52 (0.4 i.d. x 200 cm); carrier gas, N_2; flow rate, 40 ml/min; column temp., 180 °C; injection temp., 240 °C, r.t. 9.1 min and 9.7 min.

12. ^{13}C-Nmr(67.5 MHz) data of KP3 (D_2O) and pinoresinol ($CDCl_3$).

	KP3	pinoresinol		KP3
1	54.4	54.5	G1	99.6
2	86.8	85.8	G2	82.1
4	72.5	71.7	G3	76.3
5	54.3	54.5	G4	70.1
6	87.1	85.8	G5	76.7
8	72.7	71.7	G6	69.5
1'	146.2	145.2	G1'	103.9
2'	111.8	108.6	G2'	74.4
3'	149.8	146.7	G3'	77.0
4'	136.5	132.9	G4'	70.2
5'	115.6	114.3	G5'	77.0
6'	120.3	118.7	G6'	60.9
1"	146.1	145.2	G1"	103.9
2"	112.0	108.6	G2"	74.9
3"	148.9	146.7	G3"	76.9
4"	133.8	132.9	G4"	70.9
5"	116.8	114.3	G5"	77.2
6"	120.3	118.7	G6"	62.0
OMe	57.0	55.9		
OMe'	57.3	55.9		

13. GC-ms data for alditol acetate derived from both forms of methylated KP3.

Methylated sugar (as alditol acetate)	r.t. (min)	Main-mass fragment m/z(relative intensity)
2,3,4,6-Me_4-Glc	4.9	101(100), 117(71), 129(68), 145(59), 161(77), 205(30)
3,4-Me_2-Glc	6.5	129(100), 189(60)

Column, DB-1 (0.25 mm id x 15 m); carrier gas, N_2; column temp., 180-240 °C (4 °C/min).

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Received, 18th November, 1992