NOVEL ANTIOXIDANTS FROM SAFFLOWER (*CARTHAMUS TINCTORIUS L.*) OIL CAKE

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Three novel antioxidative serotonin derivatives were isolated from safflower oil cake (*Carthamus tinctorius L.*). Their structures were established as 4,4"-bis(*N*-*p*-coumaroyl)serotonin (**1**), 4-[*N*-(*p*-coumaroyl)serotonin-4"-yl]-*N*-feruloylserotonin (**2**), and 4,4"-bis(*N*-feruloyl)serotonin (**3**). Antioxidative activities of the compounds were measured by ferric thiocyanate method and DPPH method, and the compounds showed strong antioxidative activity.

KEY WORDS *Carthamus tinctorius L.*; antioxidant; 4,4"-bis(*N*-*p*-coumaroyl)serotonin; 4-[*N*-(*p*-coumaroyl)serotonin-4"-yl]-*N*-feruloylserotonin; 4,4"-bis(*N*-feruloyl)serotonin

Generation of active oxygen and free radical has been known as one of major factors in the deterioration of food. In addition, active oxygen and free radical attacks biological molecules, leading to cancer, inflammation, atherosclerosis and aging.[1] For these reasons, antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), α-tocopherol, and ascorbic acid were widely used for food protection, and some of them are also used for the defense of biological molecules against oxidative damage. However, synthetic antioxidants are suspected to be carcinogenic and toxic,[2,3] and use of natural antioxidants is desired. Thus continuous investigations are carried out in order to discover more potent and safer antioxidants, especially from natural sources.

In the course of our investigation on natural antioxidants, we focused on oil seeds.[4] Oil plants should have antioxidants in their seeds to protect their oil from oxidative damage. However, it is troublesome to separate oil (triglycerides) from desired compounds, thus we choose the oil cakes as the source of antioxidants. It is noteworthy that antioxidants from oil cakes are becoming useful in soving ecological problems in the oil industry, because oil cakes are the waste of the industry. In this paper, we report isolation and identification of three novel serotonin derivatives from safflower (*Carthamus tinctorius L.*) oil cake and elucidation of their antioxidative activity.

Safflower oil cake (300g) was extracted with hot methanol (500ml×3). After filtration, the methanol solution was evaporated under reduced pressure to give the residue (40g). The residue was dissolved in MeOH and the solution was washed with *iso*-octane. The methanol layer was partitioned between *n*-hexane and 80% methanol. The 80% methanol layer was further partitioned with EtOAc and H_2O. The EtOAc extract (7.9g) was subjected to SiO_2 column chromatography eluted with $CHCl_3$-MeOH system ($CHCl_3$-MeOH, 10:1→5:1→$CHCl_3$-MeOH-H_2O, 10:3:1) to give seven fractions. One of the antioxidative active fractions which include **1-3** was further separated by SiO_2 column chromatography ($CHCl_3$-EtOAc-MeOH, 4:5:1 and $CHCl_3$-MeOH-H_2O, 10:3:1) and HPLC to yield the three compounds **1** (12.4mg), **2** (18.1mg), and **3** (4.0mg), respectively.

Compound **1**[5] was isolated as a colorless powder. The molecular formula of **1** was determined as $C_{38}H_{34}N_4O_6$ (MH^+, *m/z* 642.2582, 0.2mmu error) by HRFAB-MS. The IR spectrum of **1** showed the presence of

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NH, OH (3400cm^{-1}) and NHCO (1650cm^{-1}) groups. The ^{13}C-NMR spectrum showed only 19 carbon signals *viz.* 2 sp^3 methylene, 9 methyne and 8 quaternary sp^2 carbon signals, and suggested that **1** was a symmetrical dimer structure. The ^{1}H-NMR spectrum[6] exhibited 4 exchangeable proton signals due to OH (δ7.75 and δ9.80) and NH (δ7.36 and δ10.4), a 1,2,3,4-substituted benzene (δ6.73 and δ7.15), a *para*-substituted benzene (δ7.30 and δ6.74), a *trans* olefin (δ6.35 and δ7.21), and two methylenes (δ2,06 and δ2.78). In HMBC spectrum (J_{C-H}=8.3Hz), long-rang correlations were observed between OH (δ7.75)/4-C, 5-C and 6-C, 6-H/4-C, 5-C and 7a-C, 7-H/3a-C, 5-C, and the NH (δ10.42)/2-C, 3-C, 3a-C, and 7a-C, which indicated the 5-OH-3,4-disubstituted indole. The correlation between 7'-H/1'-C, 2'-C, 6'-C, and 9'-C, NH (δ7.36)/9'-C, and 9-H/3-C and 9'-C exhibited the connection of *para*-substituted benzene↔olefin↔CO-NH↔two methylenes↔C-3 of indole, and **1** was clarified to consist of 4-substituted (*N*-*p*-coumaroyl)serotonin. As this partial structure was determined to be $C_{19}H_{17}N_2O_3$, the structure of **1** was established as 4,4"-bis(*N*-*p*-coumaroyl)serotonin as shown in Fig.1.

Compound **2**[5] was also isolated as a colorless powder. The molecular formula of **2** was determined from HRFAB-MS evidence (MH$^+$, *m/z* 672.2572, -1.2mmu error) to be $C_{39}H_{36}N_4O_7$. The IR spectrum of **2** was very similar to that of **1**. The ^{1}H- and ^{13}C-NMR spectra of **2**[7] suggested that **2** possessed methoxyl group and 1,2,4-substituted phenyl group, other than two serotonin moieties and *p*-coumaroyl group, by comparison with the spectra of **1**. C-H COSY and HMBC spectra exhibited long-rang correletions between 7'-H/6'-C and CH_3O-H/3'-C, which suggested the presence of a feruloyl group. Thus compound (**2**) was established as 4-[*N*-(*p*-coumaroyl)serotonin-4"-yl]-*N*-feruloylserotonin, as shown in Fig.1.

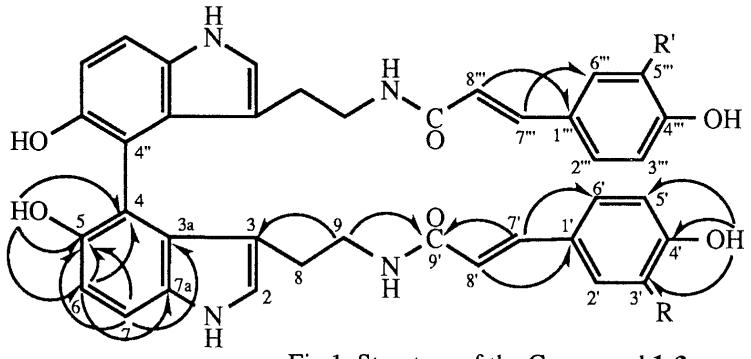

Fig.1. Structure of the Compound **1-3**

Compound **3**[5] showed its pseudo-molecular ion peak at 702 (M+H$^+$) in FAB-MS, which was 60 larger than that of **1**. The ^{13}C-NMR[8] spectrum exhibited 20 carbon signals and suggested that **3** was also the symmetrical dimer structure. In comparison of ^{1}H- and ^{13}C-NMR of **3** with those of **1** and **2**, compound **3** consisted of serotonine and feruloyl moieties. Thus the compound **3** was established as 4,4"-bis(*N*-feruloyl)serotonin, as shown in Fig.1.

The antioxidative activity of these novel serotonin dimers (**1-3**) was determined by the ferric thiocyanate method.[9] As shown in Fig. 2, the antioxidative activities of the compounds **1-3** were stronger than natural antioxidant (α-tocopherol) and comparable to BHA. The radical scavenging activity was determined using the stable α,α-diphenyl-β-picrylhydrazyl (DPPH) radical.[10] As shown in Fig. 3, compounds **1-3** scavenged the DPPH radical to the same degree as BHA and α-tocopherol. Further investigetions on biological activity of the compounds **1-3** as well as the isolation and identification of other antioxidants from safflower oil cake are now in progress.

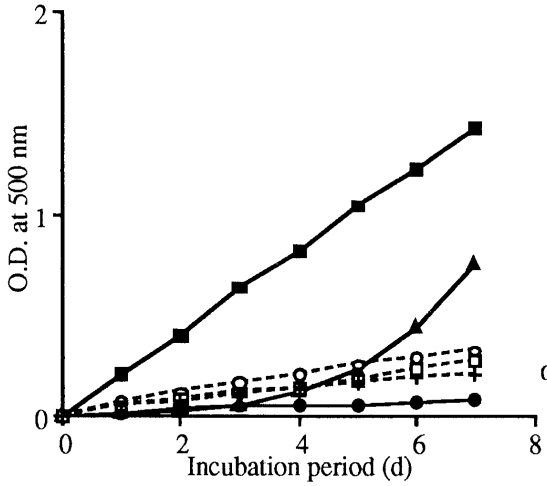

Fig.2. Antioxidative Activity of **1-3** Determined by The Ferric Thiocyanate Method

control, BHA, **2**, α-tocopherol, **1**, **3**

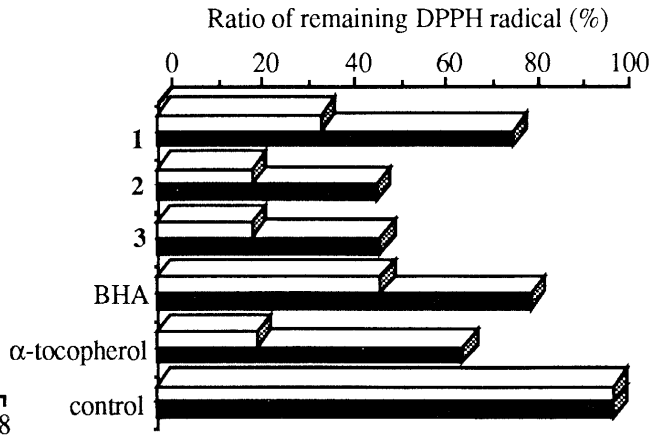

Fig.3. Scavenging Effect of Isolated Compounds (**1-3**) on DPPH Radical (1.5×10^{-5} M)

■, 1×10^{-4} M □, 5×10^{-4} M

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5) Compounds **1-3** are not optically active.
6) **1**: UV λ_{max} nm (ε) (MeOH) : 305 (34700), 294 (35000), 221 (43000). IR (KBr, cm^{-1}): 3400, 1650, 1600. ^{1}H-NMR (500 MHz, DMSO-d_6, δppm) : 2.06 (4H, m, 8-H, 8"-H), 2.78 (2H, m, 9-H, 9"-H), 2.84 (2H, m, 9- H, 9"H), 6.35 (2H, d, *J*=15 Hz, 8'-H, 8"'-H), 6.73 (2H, d, *J*=8.5 Hz, 6-H, 6"-H), 6.74 (4H, d, *J*=8.5 Hz, 3'-H, 5'-H, 3"'-H, 5"'-H), 6.89 (2H, d, *J*=1.8, 2-H, 2"-H), 7.15 (2H, d, *J*=8.5, 7-H, 7"-H), 7.21 (2H, d, *J*=15 Hz, 7'-H, 7"'-H), 7.30 (4H, d, *J*=8.5, 2'-H, 6'-H, 2"'-H, 6"'-H), 7.75 (2H, s, 5-OH, 5"-OH), 7.36 (2H, br, -NH-CO-), 9.80 (2H, br, 4'-OH, 4"'-OH), 10.4 (2H, s, -NH-). ^{13}C-NMR (125 MHz, DMSO-d_6, δCppm) : 25.0 (8-C, 8"-C), 39.1 (9-C, 9"-C), 110.3 (7-C, 7"-C), 110.9 (6-C, 6"-H), 112.5 (3-C, 3"-C), 113.7 (4-C, 4"-C), 115.6 (3'-C, 5'-C, 3"'-C, 5"'-C), 118.7 (8'-C, 8"'-C), 123.2 (2-C, 2"-C), 125.8 (1'-C, 1"'-C), 127.1 (3a-C, 3a"-C), 129.0 (2'-C, 6'-C, 2"'-C, 6"'-C), 131.0 (7a-C, 7a"-C), 138.2 (7'-C, 7"'-C), 147.6 (5-C, 5"-C), 158.6 (4'-C, 4"'-C), 165.1 (C=O).
7) **2**: UV λ_{max} nm (ε) (MeOH) : 309 (52700), 292 (51000), 221 (70500). IR (KBr, cm^{-1}): 3400, 1650, 1600. ^{1}H-NMR (500 MHz, DMSO-d_6, δppm) : 2.07 (4H, m, 8-H, 8"-H), 2.80 (4H, m, 9-H, 9"-H), 3.73 (3H, s, -OCH_3), 6.36 (1H, d, *J*=15.0, 8'-H), 6.40 (1H, d, *J*=15.0, 8"'-H), 6.74 (2H, d, *J*=8.5, 6-H, 6"-H), 6.74 (1H, d, *J*=7.94, 5'-H), 6.73 (2H, d, *J*=8.5, 3"'-H, 5"'-H), 6.89 (2H, d, *J*=2.4, 2-H, 2"-H), 6.91 (1H, dd, *J*=7.94, 1.84, 6'-H), 7.03 (1H, d, *J*=1.84, 2'-H), 7.13 (2H, d, *J*=8.5, 7-H, 7"-H), 7.21 (2H, d, *J*=15.0, 7'-H, 7"'-H), 7.30 (2H, d, *J*=8.5, 2"'-H, 6"'-H), 7.38 (2H, br, -NH-CO-), 7.75 (2H, s, 5-OH), 9.40 (1H, br, 4'-OH), 9.80 (1H, br, 4"'-OH), 10.23 (2H, s, -NH-). ^{13}C-NMR (125 MHz, DMSO-d_6, δCppm) : 25.0 (8-C, 8"-C), 39.0 (9-C, 9"-C), 55.3 (3'-OCH_3), 110.2 (7-C, 7"-C), 110.6 (2'-C), 110.9 (6-C, 6"-C), 112.5 (3-C, 3"-C), 113.7 (4-C, 4"-C), 115.4 (5'-C), 115.6 (3"'-C, 5"'-C),' 118.8 (8'-C), 119.1 (8"'-C), 121.3 (6'-C), 123.1 (2-C, 2"-C), 125.8 (1'-C), 126.31 (1"'-C), 127.1 (3a-C, 3a"-C), 128.9 (2"'-C, 6"'-C), 131.0 (7a-C, 7a"-C), 138.1 (7'-C), 138.4 (7"'-C), 147.6 (5-C, 5"-C, 3'-C), 148.0 (4'-C), 158.6 (4"'-C), 165.0 (C=O).
8) **3**: UV λ_{max} nm (ε) : 317 (38800), 289 (35400). IR (KBr, cm^{-1}): 3400, 1650, 1600. ^{1}H-NMR (DMSO-d_6, δppm) : 2.07 (4H, m, 8-H, 8"-H), 2.80 (4H, m, 9-H, 9"-H), 3.72 (6H, s, -OCH_3×2), 6.40 (2H, d, *J*=15.8 Hz, 8'-H,8"'-H) 6.74 (4H, dd, *J*=8.5, 7.93 Hz, 6-H, 6"-H, 5'-H, 3"'-H), 6.90 (4H, d,*J*=7.93, 6'-H, 2"'-H, 2-H, 2"-H), 7.02 (2H, d, *J*=1.83 Hz, 2'-H, 6"'-H), 7.14 (2H, d, *J*=8.5, 7-H, 7"-H), 7.21 (2H, d, *J*=15.8, 7'-H, 7"'-H), 7.38 (2H, br, -NH-CO-), 7.77 (2H, s, 5-OH, 5"-OH), 9.42 (2H, br, 4'-OH, 4"'-OH), 10.43 (2H, s, -NH-). ^{13}C-NMR (DMSO-d_6, δCppm) : 25.7 (8-C, 8"-C), 40.7 (9-C, 9"-C), 56.1 (OCH_3×2), 111.0 (7-C, 7"-C), 111.3 (2'-C, 6"'-C), 111.6 (6-C, 6"-C), 113.2 (3-C, 3"-C), 114 (4-C, 4"-C), 116.2 (5'-C, 3"'-C), 119.8 (8'-C, 8"'-C), 122.0 (6'-C, 2"'-C), 123.9 (2-C, 2"-C), 125.0 (1'-C, 1"'-C), 127.9 (3a-C, 3a"-C), 131.7 (7a-C, 7a"-C), 139.2 (7'-C, 7"'-C), 148.4 (5-C, 5"-C, 3'-C, 5"'-C), 148.8 (4'-C, 4"-C), 165.8 (C=O).
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