Antioxidative Compounds Isolated from Safflower (*Carthamus tinctorius* L.) Oil Cake

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Seven antioxidative serotonin derivatives were isolated from safflower (*Carthamus tinctorius* L.) oil cake. Their structures were established as *N*-[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]ferulamide (1), *N*-[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]-*p*-coumaramide (2), *N*,*N*′-[2,2′-(5,5′-dihydroxy-4,4′-bi-1*H*-indol-3,3′-yl)diethyl]-di-*p*-coumaramide (3), *N*-[2-[3′-[2-(*p*-coumaramido)ethyl]-5,5′-dihydroxy-4,4′-bi-1*H*-indol-3-yl]ethyl]ferulamide (4), and *N*,*N*′-[2,2′-(5,5′-dihydroxy-4,4′-bi-1*H*-indol-3,3′-yl)diethyl]-diferulamide (5), *N*-[2-[5-(β-D-glucosyloxy)-1*H*-indol-3-yl)ethyl]-*p*-coumaramide (6), and *N*-[2-[5-(β-D-glucosyloxy)-1*H*-indol-3-yl)ethyl]ferulamide (7). Antioxidative activities of the compounds were measured by the ferric thiocyanate method and the α,α-diphenyl-β-picrylhydrazyl (DPPH) method, and compounds 1—5 were found to have relatively strong antioxidative activity.

Key words safflower; *Carthamus tinctorius*; antioxidant; serotonin derivative

Polyunsaturated fatty acids in vegetable oils are important for human nutrition; linoleic acid is essential for the maintenance of growth and α-linolenic acid for neural functions. However, lipid peroxidation is one of the major factors causing food deterioration, and breakdown products of lipid peroxides, such as aldehydes, hydroperoxides, alkoxyl radical, and alkylperoxyl radical, are highly toxic and may cause disease.[1—3] Active oxygen species and free radicals are known to attack biological molecules under certain pathological conditions, exacerbating cancer, inflammation, atherosclerosis and aging.[4] For these reasons, antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), α-tocopherol, and ascorbic acid are widely used as food additives and the latter two play major roles *in vivo* in the defense of biological cell membranes against oxidative damage. However, synthetic antioxidants are often associated with problems of carcinogenicity and toxicity,[5,6] and it remains desirable to find more potent and safer antioxidants, especially from natural sources.

In the course of our investigation on natural antioxidants, we focused on oil seeds,[7] because they should contain antioxidants to protect their polyunsaturated fatty acids from oxidative damage. However, it is generally troublesome to separate oil (triglycerides) from desired compounds, so we chose to use oil cake as the source of antioxidants. It is noteworthy that making use of oil cake would help to solve ecological problems in part, because oil cake is a waste product of the oil industry. In a previous paper, we reported briefly three novel serotonin dimeric derivatives.[8] In this paper, we report in detail the identification of four known serotonin derivatives (**1**, **2**, **6**, **7**)[9,10] and three new serotonin derivatives (**3**, **4**, **5**) from safflower (*Carthamus tinctorius* L.) oil cake and the determination of their antioxidative activities.

Isolation and Identification Safflower oil cake was extracted with MeOH. After filtration, the MeOH solution was evaporated to give a residue. This residue was dissolved in MeOH and the solution was washed with iso-octane. The MeOH layer was partitioned between *n*-hexane and 80% MeOH. The 80% MeOH layer was further partitioned with EtOAc and H_2O. The EtOAc extract was subjected to SiO_2 column chromatography, and eluted with a $CHCl_3$–MeOH system to give seven fractions (A—G). The antioxidative active fractions D—F were further separated by SiO_2 column chromatography and HPLC to yield the five compounds (**1**—**5**). On silica gel TLC, fraction G afforded similar spots to those of **1**—**5**, suggesting that fraction G included compounds similar to **1**—**5**. The non-antioxidative fraction G was subjected to Diaion HP-20 and SiO_2 column chromatography followed by HPLC to give compounds **6** and **7**.

Compound **1** showed a pseudo-molecular ion peak at *m/z* 353 ($M+H^+$) in the FAB-MS spectrum. The IR spectrum of **1** showed the presence of NH, OH (3400 cm^{-1}) and NHCO (1650 cm^{-1}) groups. The ^{13}C-NMR spectrum showed three sp^3 carbon signals (one methyl group and two methylene groups), and seventeen sp^2 carbon signals (nine methine and eight quaternary carbons) in the range of 100—170 ppm.

The ^1H-NMR spectrum of compound **1** exhibited twenty proton signals. The aromatic ABC-type proton signals at δ 7.03 (1H, d, $J=2.4$ Hz), 6.71 (1H, dd, $J=2.4$, 8.5 Hz), 7.19 (1H, d, $J=8.5$ Hz), 7.15 (1H, d, $J=1.8$ Hz), 7.05 (1H, dd, $J=1.8$, 8.5 Hz), and 7.03 (1H, d, $J=8.5$ Hz) were assignable to two 1,3,4-trisubstituted benzenes. The

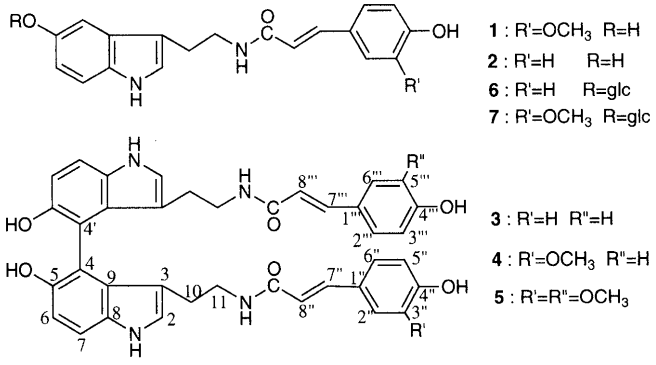

Chart 1

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proton signals at δ 6.52 (1H, d, J=15 Hz) and 7.47 (1H, d, J=15 Hz) suggested the presence of *trans* olefin. The signals at δ 7.72, 8.01, 7.26 and 9.75, which were exchangeable with D_2O, were assignable to OH or NH protons. In the ^1H–^1H COSY spectrum, the signal at δ 3.59 showed cross peaks with δ 2.91, 7.26, and the signal at δ9.75 showed a cross peak with δ 7.09, so the partial structures $-CH_2-CH_2-NH-$ and $-NH-CH=$ were inferred. In addition, the heteronuclear multiple bond correlation (HMBC) spectrum (J_{C-H}=8.3 Hz) showed long-range correlations from H-2 to C-8, C-9 and C-3, from H-10 to C-2, C-3 and C-9, and from H-11 to C-3 and CO, which indicated a serotonin group. The correlations from H-8′ to C-1′ and CO, from H-7′ to C-2′, C-6′ and CO, and from the methoxy protons at δ 3.87 to C-3′ revealed the connection of the feruloyl group with serotonin. Thus compound **1** was identified as *N*-[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]ferulamide.[9,10]

Compound **2** showed a pseudo-molecular ion peak at m/z 323 ($M+H^+$) in the FAB-MS. The absorptions of OH and NH groups at 3400 cm^{-1} and an amido group at 1650 cm^{-1} were observed in the IR spectrum. The ^1H- and ^{13}C-NMR spectra of 2 were very similar to those of **1**, except for the signals due to a *para*-substituted benzene moiety at δ 7.40 (2H, ddd, J=8.5, 3.0, 1.8 Hz), and δ 6.86 (2H, ddd, J=8.5, 3.0, 1.8 Hz) and the absence of the methoxyl signal. Thus, compound **2** was identified as *N*-[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]-*p*-coumaramide.[9,10]

The molecular formula of **3** was determined to be $C_{38}H_{34}N_4O_6$ from the high-resolution FAB-MS (HR-FAB-MS). The IR spectrum of **3** showed the presence of NH, OH and NHCO groups. The ^{13}C-NMR spectrum was very similar to that of **2**, except for one less methine signal and one more quaternary carbon signal relative to **2**, and showed only nineteen carbon signals, which suggested that **3** was a symmetrical dimer. The ^1H-NMR spectrum was also very similar to that of **2**, except for the signals due to one 1,2,3,4-tetrasubstituted benzene (δ 6.73 and 7.15, each 1H, d, J=8.5 Hz) observed in place of one 1,3,4-trisubstituted benzene signals. In the HMBC spectrum (J_{C-H}=8.3 Hz), long-range correlations from H-11 to C-3 and CO, and from H-7″ to CO indicated the connection of 4-substituted serotonin with the coumaroyl group, and **3** was clarified to be a 4-substituted *N*-(*p*-coumaroyl)serotonin. As the molecular formula of this partial structure was determined as $C_{19}H_{17}N_2O_3$, the structure of **3** was established as *N*,*N*′-[2,2′-(5,5′-dihydroxy-4,4′-bi-1*H*-indol-3,3′-yl)diethyl]-di-*p*-coumaramide, as shown in Chart. 1.

The molecular formula of **4** was determined to be $C_{39}H_{36}N_4O_7$ from the HR-FAB-MS. The ^{13}C-NMR spectrum of **4** showed twenty-five carbon signals, *viz.* one methyl and two methylene signals, twelve methine and ten quaternary sp^2 carbon signals, thirteen carbon signals which were observed as overlapped signals of two serotonin moieties (20C), 3″-C, two carbonyl groups (2C) and *para*-substituted benzene (4C). The ^1H-NMR and C–H COSY spectra suggested that compound **4** possessed a methoxyl group and a 1,2,4-trisubstituted phenyl group, besides two serotonin moieties and a *p*-coumaroyl group, by comparison with the spectrum of **3**. The HMBC spectrum exhibited long-range correlations from H-7″ to C-2″, C-6″ and CO, from H-CH_3O to C-3″ and from H-7‴ to C-2‴, C-6‴ and CO, which suggested the connection of a feruloyl group and a coumaroyl group with two serotonin groups. Thus, compound 4 was concluded to be an unsymmetrical dimer, *N*-[2-[3′-[2-(*p*-coumaramido)ethyl]-5,5′-dihydroxy-4,4′-bi-1*H*-indol-3-yl]ethyl]-ferulamide.

Compound **5** showed a pseudo-molecular ion peak at m/z 703 ($M+H^+$) in the FAB-MS, *i.e.*, 60 mass units larger than that of **3**. The ^{13}C-NMR spectrum exhibited twenty carbon signals, suggesting that **5** is also a symmetrical dimer. Based on a comparison of the ^1H- and ^{13}C-NMR spectra of **5** with those of **1**, compound **5** was determined to consist of serotonin and feruloyl moieties. Thus, compound **5** was established as *N*,*N*′-[2,2′-(5,5′-dihydroxy-4,4′-bi-1*H*-indol-3,3′-yl)diethyl]-diferulamide. Compounds **3**—**5** were optically inactive.

Compound **6** showed a pseudo-molecular peak at m/z 485 ($M+H^+$) in the FAB-MS. A comparison of the ^{13}C-NMR spectra of **6** and **2**, showed one additional methylene and five methine carbon signals at 60—102 ppm, which suggested that **6** is a glycoside of **2**. The ^1H-NMR spectrum of **6** exhibited a signal at δ 4.76 (1H, d, J=7 Hz), which was supposed to be an anomeric proton and correlated with 5-C in the HMBC spectrum. These results indicated that the sugar moiety of **6** was connected to 5-C.

Table 1. ^{13}C-NMR Spectra for **1**—**7** (125 MHz, δ, ppm)

C	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[b]	**8**[c]	**9**[c]
C-2	124.0	124.1	123.2	123.1	123.9	123.4	123.1	123.9	124.2
C-3	112.6	112.4	112.5	112.5	113.2	111.4	111.2	112.5	112.3
C-4	103.5	103.5	113.7	113.7	114.0	105.1	104.7	103.5	103.5
C-5	151.6	151.5	147.6	147.6	148.4	151.0	150.6	151.0	151.5
C-6	112.3	112.4	110.9	110.9	111.6	112.5	112.1	112.3	112.4
C-7	112.5	112.5	110.3	110.2	111.0	111.5	111.0	112.6	112.6
C-8	132.5	132.2	131.0	131.0	131.7	132.3	131.8	133.0	132.4
C-9	129.4	129.3	127.1	127.1	127.9	127.3	127.0	129.4	129.3
C-10	26.4	26.4	25.0	25.0	25.7	26.2	24.8	26.2	26.6
C-11	40.8	40.8	39.1	39.0	40.7	39.3	40.0	41.2	40.6
C-1″	128.3	127.7	125.8	125.8	125.0	125.8	125.9	129.4	128.9
C-2″	111.3	130.2	129.0	110.6	111.3	129.1	110.2	130.3	129.9
C-3″	148.6	116.6	115.6	147.6	148.4	115.7	147.3	116.2	115.0
C-4″	149.1	159.8	158.6	148.0	148.8	158.6	147.7	156.7	161.6
C-5″	116.1	116.6	115.6	115.4	116.2	115.7	115.1	116.2	115.0
C-6″	122.6	130.2	129.0	121.3	122.0	129.1	121.0	130.3	129.5
C-7″	140.3	140.4	138.2	138.1	139.2	138.5	138.5	32.2	139.6
C-8″	120.2	119.6	118.7	118.8	129.8	118.6	118.6	39.5	120.7
C-1‴				126.3					
C-2‴,6‴				128.9					
C-3‴,5‴				115.6					
C-4‴				158.6					
C-7‴				138.4					
C-8‴				119.1					
CO	166.5	165.1	165.1	165.1	165.8	165.4	165.4	175.3	166.2
OCH_3	56.2			55.3	56.1		55.0		55.1
glc-1						102.3	101.9		
glc-2						73.4	73.0		
glc-3						76.8	76.3		
glc-4						71.9	69.5		
glc-5						76.7	76.5		
glc-6						60.8	60.4		

a) In CD_3COCD_3, *b*) in DMSO-d_6, *c*) in CD_3OD.

Table 2. ^{1}H-NMR Spectra for **1**—**7** (500 MHz, δ, ppm)

H	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[b]	**8**[c]	**9**[c]
2	7.09 (d, J=1.8 Hz)	7.09 (d, J=2.4 Hz)	6.89 (d, J=1.8 Hz)	6.89 (d, J=1.8 Hz)	6.90 (d, J=1.8 Hz)	7.13 (d, J=2.4 Hz)	7.13 (d, J=1.8 Hz)	6.91 (d, J=2.4 Hz)	7.07 (d, J=2.4 Hz)
4	7.03 (d, J=2.4 Hz)	7.05 (d, J=2.4 Hz)				7.26 (d, J=1.8 Hz)	7.26 (d, J=1.8 Hz)	6.91 (d, J=2.4 Hz)	7.02 (d, J=2.4 Hz)
6	6.71 (dd, J=2.4, 8.5 Hz)	6.73 (dd, J=2.4, 8.5 Hz)	6.73 (d, J=8.5 Hz)	6.73 (d, J=8.5 Hz)	6.74 (d, J=8.5 Hz)	6.87 (d, J=1.8, 8.5 Hz)	6.86 (d, J=1.8, 8.5 Hz)	6.86 (d, J=2.4, 8.5 Hz)	6.70 (dd, J=2.4, 8.5 Hz)
7	7.19 (d, J=8.5 Hz)	7.20 (d, J=8.5 Hz)	7.15 (d, J=8.5 Hz)	7.15 (d, J=8.5 Hz)	7.14 (d, J=8.5 Hz)	7.23 (d, J=8.5 Hz)	7.23 (d, J=8.5 Hz)	7.14 (d, J=8.5 Hz)	7.18 (d, J=8.5 Hz)
10	2.91 (m)	2.94 (m)	2.06 (m)	2.06 (m)	2.07 (m)	2.96 (m)	2.83 (m)	2.80 (m)	2.96 (m)
11	3.59 (m)	3.63 (m)	2.78 (m)	2.78 (m)	2.80 (m)	3.40 (m)	3.40 (m)	3.40(m)	3.61 (m)
2″	7.15 (d, J=1.8 Hz)	7.40 (ddd, J=1.8 3.0 8.5 Hz)	7.30 (d, J=8.5 Hz)	7.03 (d, J=1.8 Hz)	7.02 (d, J=1.8 Hz)	7.39 (d, J=8.5 Hz)	7.11 (d, J=1.8 Hz)	7.25 (dd, J=2.4, 8.5 Hz)	7.50 (ddd, J=1.8, 2.4 8.5 Hz)
3″		6.86 (ddd, J=1.8, 3.0 8.5 Hz)	6.74 (d, J=8.5 Hz)			6.78 (d, J=8.5 Hz)		6.68 (dd, J=2.4, 8.5 Hz)	6.86 (ddd, J=1.8, 2.4 8.5 Hz)
5″	6.83 (d, J=8.5 Hz)	6.86 (ddd, J=1.8, 3.0 8.5 Hz)	6.74 (d, J=8.5 Hz)	6.74 (d, J=8.0 Hz)	6.74 (d, J=7.93 Hz)	6.78 (d, J=8.5 Hz)	6.78 (d, J=7.93 Hz)	6.68 (dd, J=2.4, 8.5 Hz)	6.86 (ddd, J=1.8, 2.4 8.5 Hz)
6″	7.05 (dd, J=1.8, 8.5 Hz)	7.40 (tt, J=1.8, 3.0 8.5 Hz)	7.30 (d, J=8.5 Hz)	6.91 (dd, J=1.8, 8.0 Hz)	6.90 (dd, J=1.8, 7.93 Hz)	7.39 (d, J=8.5 Hz)	6.98 (dd, J=1.8, 7.93 Hz)	7.25 (dd, J=2.4, 8.5 Hz)	7.50 (ddd, J=1.8 2.4 8.5 Hz)
7″	7.47 (d, J=15 Hz)	7.52 (d, J=15 Hz)	7.21 (d, J=15 Hz)	7.21 (d, J=15 Hz)	7.21 (d, J=15 Hz)	7.33 (d, J=15 Hz)	7.33 (d, J=15 Hz)	2.79 (t, J=7.3 Hz)	7.48 (d, J=15 Hz)
8″	6.52 (d, J=15 Hz)	6.52 (d, J=15 Hz)	6.35 (d, J=15 Hz)	6.36 (d, J=15 Hz)	6.40 (d, J=15 Hz)	6.42 (d, J=15 Hz)	6.45 (d, J=15 Hz)	2.38 (t, J=7.3 Hz)	6.53 (d, J=15 Hz)
2‴,6‴				7.30 (d, J=8.5 Hz)					
3‴,5‴				6.74 (d, J=8.5 Hz)					
7‴				7.21 (d, J=15 Hz)					
8‴				6.40 (d, J=15 Hz)					
OCH_3	3.87 (s)			3.73 (s)	3.72 (s)		3.80 (s)		3.82 (s)
NH	9.75 (s)	9.78 (s)	10.4 (s)	10.2 (s)	10.4 (s)	10.6 (s)	10.6 (s)		
CONH	7.26 (t)	7.43 (t)	7.36 (t)	7.38 (t)	7.38 (t)	8.09 (t)	8.04 (t)		
5-OH			7.75 (s)	7.75 (s)	7.75 (s)				
4″-OH			9.80 (br)	9.40 (br)	9.40 (br)	9.86 (br)	8.28 (br)		
4‴-OH				9.80 (br)					
glc-1						4.76 (d, J=7.93 Hz)	4.76 (d, J=7.32 Hz)		
glc-2						3.26 (m)	3.26 (m)		
glc-3						3.31 (m)	3.31 (m)		
glc-4						3.19 (m)	3.19 (m)		
glc-5						3.31 (m)	3.31 (m)		
glc-6						3.46 (m)	3.46 (m)		
						3.76 (m)	3.74 (m)		

a) in CD_3COCD_3, *b*) in DMSO-d_6, *c*) in CD_3OD.

The acid hydrolysis of compound **6** gave compound **1** and glucose, which was confirmed to be D-glucose by a GLC method.[11] Thus, the compound **6** was identified as a known compound, *N*-[2-[5-(β-D-glucosyloxy)-1*H*-indol-3-yl)ethyl]-*p*-coumaramide.[9,10]

Compound **7** showed a pseudo-molecular ion peak at m/z 515 ($M+H^+$). By comparison of the NMR spectra with those of **1**, **7** was identified as *N*-[2-[5-(β-D-glucosyloxy)-1*H*-indol-3-yl)ethyl]ferulamide.[9,10]

Antioxidative Activity of 1—7 and Their Derivatives The antioxidative activities of **1**—**7** were determined by a ferric thiocyanate method using linoleic acid as a substrate for autoxidation in the ethanol–water system.[12] (Fig. 1-A) As radical-scavenging is also an important antioxidative mechanism, the radical-scavenging activities of **1**—**7** were determined by using a stable free radical, α,α-diphenyl-β-picrylhydrazyl (DPPH) (Fig. 2).[13]

The antioxidative activities of compounds **1**—**5** were stronger than that of α-tocopherol, a widely used natural antioxidant, and comparable to that of BHA, a commonly used synthetic antioxidant, but compounds **6** and **7** were inactive (Fig. 1-A, 2).

In order to determine the effect of the olefinic part and the 4′-OH group on the antioxidative activity of serotonin derivatives, compounds **8** and **9** were prepared. Namely, compound **2** was treated in the presence of PtO_2 under a hydrogen atmosphere to give **8**. Compound **9** was obtained from **6** by methylation of 4′-OH using CH_2N_2, followed

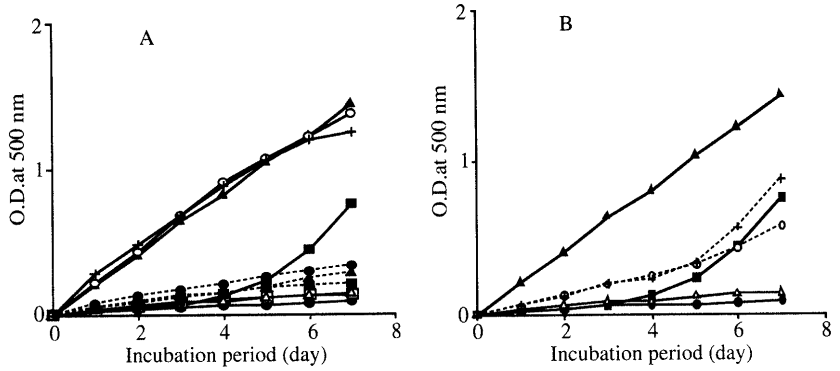

Fig. 1. A, B. Antioxidative Activity Determined by a Ferric Thiocyanate Method
—▲—, Control; —●—, BHA; —■—, α-tocopherol; —□—, 1; —△—, 2; ---●---, 3; ---▲---, 4; ---■---, 5; —○—, 6; —+—, 7; ---+---, 8; ---○---, 9.

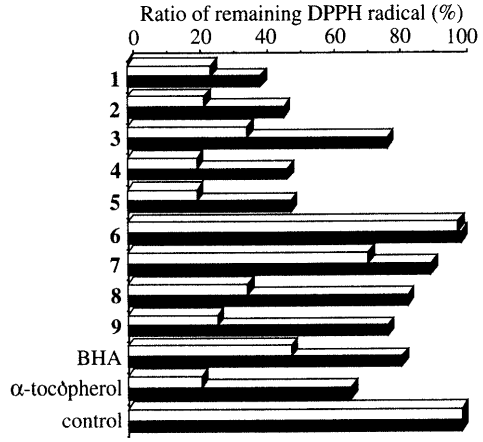

Fig. 2. Scavenging Effect of Isolated Compounds **1**—**9** on DPPH Radical (1.5×10^{-4} M)
■, 1×10^{-4} M; □, 5×10^{-4} M.

The ratio of remaining DPPH radical (%) was calculated as follows : residual ratio (%) = O.D. 520 nm (sample)/O.D. 520 nm (control) × 100. Control: in the absence of sample.

by β-glucosidase treatment (Chart. 2). The antioxidative and radical scavenging activities of **8** and **9** are shown in Figs. 1-B and 2. Compounds **8** and **9** had weaker antioxidative and radical scavenging activities than compound **2**.

Discussion

Serotonin monomers (**1** and **2**) and the glucosides (**6** and **7**) were isolated as major constituents, as well as small amounts of the dimers (**3**—**5**) as new compounds. Although the glucosides (**6** and **7**) have no significant antioxidative activity, they may be useful substrates for preparing **1** and **2**. The antioxidative activity decreased in the order of BHA > **1**, **2**, **3**, **4**, **5** > α-tocopherol > **9** > **8** > **6**, **7** (no activity). In a comparison of monomers (**1** and **2**) and dimers (**3**, **4** and **5**), these two groups showed almost the same antioxidative activity per weight, indicating that the substitution at the 4 position has no effect on the antioxidative activity of the derivatives. As compounds **6** and **7** exhibited no antioxidative activity, the presence of free 5-OH group appears to be essential for the antioxidative activity. In a comparison of **8** and **9** with **2**, saturation of the double bond and the methylation of the 4″-OH group significantly reduce the antioxidative activity, indicating that they are requisite functional groups for the antioxidative activity.

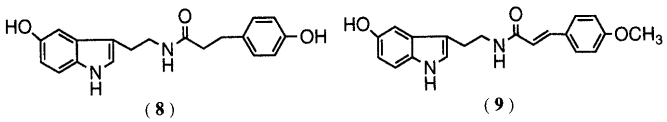

Chart 2

In summary, we isolated seven serotonin derivatives (**1**—**7**) from safflower oil cake and compounds **1**—**5** were found to have a relatively strong antioxidative activity. The hydroxy group at the **5** position of serotonin and the 4″ position of coumaroyl or feruloyl group, and the olefinic bond were clarified to be important for the activity. Investigation of other biological activities of **1**—**7** is now in progress.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The ultraviolet (UV) spectra were recorded with a JASCO V-530 spectrophotometer. FAB-MS and HR-FAB-MS were measured with a JEOL SX-102A mass spectrometer and IR spectra with a JASCO IR-810 spectrometer. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were obtained using a JEOL JNM-EX-270 (^{1}H, 270 MHz; ^{13}C, 68 MHz), JEOL JNM-GSX-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) and JEOL JNM-α-500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer. Chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard. HPLC was performed using a JASCO 880-PU pump and 830-RI differential refractometer. TLC was performed on Merck precoated Kieselgel 60 F_{254} and the spots were detected by spraying 5% vanillin-concentrated H_2SO_4, followed by heating. Column chromatography was carried out on silica gel BW-200 (Fuji Davison Chemicals Co., Ltd.) and highly porous synthetic resin Diaion HP-20 (Mitsubishi Kagaku Co., Ltd.). GLC was carried out on a Shimadzu GC-8A.

Extraction and Isolation The dried safflower oil cake (300 g) was extracted with hot methanol three times, each for 5 h. The MeOH solutions were concentrated under reduced pressure to give an MeOH extract (40 g). The methanol extract was partitioned between iso-octane and MeOH. The MeOH layer was further partitioned between *n*-hexane and 80% MeOH to give a hexane extract (2.8 g). The 80% MeOH extract was subsequently partitioned between EtOAc and H_2O to yield an H_2O extract (20.5 g) and an EtOAc extract (7.9 g). The EtOAc extract was subjected to SiO_2 column chromatography, and eluted with $CHCl_3$–MeOH (10 : 1 → 5 : 1) and $CHCl_3$–MeOH–H_2O (10 : 3 : 1) to give seven fractions (Frs. A—G). Fraction D was chromatographed repeatedly on SiO_2 using $CHCl_3$–EtOAc–MeOH (4 : 6 : 1) and *n*-hexane–EtOAc–MeOH (4 : 8 : 1) to give four fractions (Frs. D1—4). Fraction D2 was further purified by HPLC on a YMC AQ-314 ODS column [MeOH–H_2O

(1 : 1)] to yield **1** (322 mg). Fraction E was subjected to repeated chromatography on an SiO_2 column [$CHCl_3$–EtOAc–MeOH (4 : 6 : 1) and *n*-hexane–EtOAc–MeOH (4 : 8 : 1)] followed by HPLC on a YMC AQ-314 ODS column [MeOH–H_2O (1 : 1)] to afford **2** (371 mg). The fraction F was chromatographed on an SiO_2 column [$CHCl_3$–EtOAc–MeOH (4 : 6 : 1)] to give three fractions (Frs. F1—3). Chromatography of fraction F2 on an SiO_2 column [$CHCl_3$–MeOH–H_2O (10 : 3 : 1)] yielded three fractions (Frs. F2-1, F2-2, F2-3). Fractions F2-1, F2-2 and F2-3 were purified by HPLC [YMC AQ-314 ODS column, MeOH–H_2O (1 : 1)] to give **3** (12.4 mg), **4** (18.1 mg) and **5** (4.0 mg), respectively. Fraction G was subjected to Diaion HP-20 column chromatography [MeOH–H_2O (30%→50%→70%)] to give three fractions (Frs. G1—3). Fraction 2 was repeatedly chromatographed on SiO_2 column [*n*-hexane–EtOAc–MeOH (4 : 8 : 1) and $CHCl_3$–MeOH–H_2O (10 : 3 : 1)] to give a mixture of **6** and **7**, which was further purified by HPLC [YMC AQ-314 ODS column, MeOH–H_2O (2 : 3)] to afford **6** (396 mg) and **7** (184 mg).

Compound **1**: colorless needles, mp 115—117 °C. IR (KBr, cm^{-1}): 3400, 1650, 1600. FAB-MS *m/z*: 353 $[M+H]^+$. ^{1}H-NMR (CD_3COCD_3) δ ppm: Table 2. ^{13}C-NMR (CD_3COCD_3) δ_C ppm: Table 1.

Compound **2**: colorless needles, mp 195—196 °C. IR (KBr, cm^{-1}): 3400, 1650, 1600. FAB-MS *m/z*: 323 $[M+H]^+$. ^{1}H-NMR (CD_3COCD_3) δ ppm: Table 2. ^{13}C-NMR (CD_3COCD_3) δ_C ppm: Table 1.

Compound **3**: colorless powder, mp 180—182 °C. UV λ_{max} (MeOH) nm (ε): 305 (34700), 294 (35000), 221 (43000). IR (KBr, cm^{-1}): 3400, 1650, 1600. HR-FAB-MS *m/z*: Calcd for $C_{38}H_{34}N_4O_6$: 642.2580 $[M+H]^+$, Found: 642.2582. ^{1}H-NMR (DMSO-d_6) δ ppm: Table 2. ^{13}C-NMR (DMSO-d_6) δ_C ppm: Table 1.

Compound **4**: colorless powder, mp 179—181 °C. UV λ_{max} (MeOH) nm (ε): 309 (52700), 292 (51000), 221 (70500). IR (KBr, cm^{-1}): 3400, 1650, 1600. HR-FAB-MS *m/z*: Calcd for $C_{39}H_{36}N_4O_7$: 672.2584 $[M+H]^+$, Found: 672.2572. ^{1}H-NMR (DMSO-d_6) δ ppm: Table 2. ^{13}C-NMR (DMSO-d_6) δ_C ppm: Table 1.

Compound **5**: colorless powder, mp 158—160 °C. UV λ_{max} (MeOH) nm (ε): 317 (38800), 289 (35400). IR (KBr, cm^{-1}): 3400, 1650, 1600. FAB-MS *m/z*: 703 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6) δ ppm: Table 2. ^{13}C-NMR (DMSO-d_6) δ_C ppm: Table 1.

Compound **6**: colorless needles, mp 240—242 °C. $[\alpha]_D^{25.5}$ −14.9° (c=0.72, MeOH). IR (KBr, cm^{-1}): 3400, 1650. FAB-MS *m/z*: 485 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6) δ ppm: Table 2. ^{13}C-NMR (DMSO-d_6) δ_C ppm: Table 1.

Compound **7**: colorless needles, mp 143—145 °C. $[\alpha]_D^{25.5}$ −8.76° (c=1.25, MeOH). IR (KBr, cm^{-1}): 3400, 1650. FAB-MS *m/z*: 485 $[M+H]^+$. ^{1}H-NMR (DMSO-d_6) δ ppm: Table 2. ^{13}C-NMR (DMSO-d_6) δ_C ppm: Table 1.

Acid Hydrolysis of 6 and 7 Compound **6** (18 mg) in 1 N HCl/THF (5 ml) was heated at 60 °C for 1 h. The reaction mixture was diluted with water and extracted with EtOAc. The organic layer was concentrated under reduced pressure and the residue was subjected to HPLC [YMC AQ-314 ODS column, MeOH–H_2O (1 : 1)] to give **2** (7.5 mg). The water layer was also concentrated to give a crude sugar fraction. Compound **7** (18 mg) was similarly hydrolyzed to give **1** (6 mg) and a crude sugar fraction.

GLC Analysis of Sugar Moiety The analysis of sugar was performed essentially as described previously.[11] A solution of crude sugar fraction (7 mg) from **6** or **7** in pyridine (0.5 ml) was treated with trimethylchlorosilane (0.05 ml) and 1,1,1,3,3,3-hexamethyldisilazane (0.1 ml) to give silylated sugars. GLC analysis of silylated sugar was carried out on 3% OV-101 (stainless steel column, 0.3 mm i.d. × 2 m) using a Shimadzu GC-8A with a Shimadzu Chromatopac C-R6A.

Catalytic Hydrogenation of 2 A solution of compound **2** (11 mg) in MeOH (3 ml) was stirred with PtO_2 (2.5 mg) for 20 min at room temperature under an H_2 atmosphere. The reaction mixture was filtered and the filtrate was concentrated. The residue was chromatographed on SiO_2 [$CHCl_3$–MeOH–H_2O (10 : 3 : 1)] to give **8** (6 mg, 55%).

Compound **8**: colorless powder, mp 88—90 °C. UV λ_{max} (MeOH) nm (ε): 280 (36300). IR (KBr, cm^{-1}): 3400, 1650, 1600. FAB-MS *m/z*: 325 $[M+H]^+$. ^{1}H-NMR (CD_3OD) δ ppm: Table 2. ^{13}C-NMR (CD_3OD) δ_C ppm: Table 1.

Preparation of Compound 9 A solution of **6** (15 mg) in MeOH (3 ml) was treated with diazomethane in ether. The mixture was allowed to stand for 10 min at room temperature and the solvent was removed under reduced pressure. A mixture of the residue, β-glucosidase (Sigma Chemical Co. U.S.A., 1 mg) and Triton X-100 (2 mg) in 0.1 M acetate buffer (5 ml, pH 5.0) was stirred at 37 °C for 30 min. The reaction mixture was extracted with EtOAc, and the EtOAc layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was chromatographed on SiO_2 [$CHCl_3$–MeOH–H_2O (10 : 3 : 1)] to give **9** (12 mg, 80%).

Compound **9**: colorless powder, mp 88—90 °C. UV λ_{max} (MeOH) nm (ε): 291 (39100). IR (KBr, cm^{-1}): 3400, 1650, 1600. FAB-MS *m/z*: 337 $[M+H]^+$. ^{1}H-NMR (CD_3OD) δ ppm: Table 2. ^{13}C-NMR (CD_3OD) δ_C ppm: Table 1.

Antioxidation Assay Each compound (0.1 mg) dissolved in MeOH (0.1 ml) was added to a solution of linoleic acid (58 mg) in EtOH (5 ml) and 0.2 M phosphate buffer at pH 7.0 (5 ml), and the total volume was adjusted to 12 ml with distilled water. The solution in the test tube was incubated at 40 °C. At intervals of 24 h during incubation, an aliquot of each reaction mixture (0.1 ml) was added to a solution of $FeCl_2$ (0.02 M, 150 μl) and 30% ammonium thiocyanate (150 μl) in 75% EtOH (7 ml). The mixture was left standing for 3 min, and the absorbance at 500 nm was measured.[12]

Measurement of DPPH Radical-Scavenging Activity MeOH solutions of compounds **1**—**9** (1×10^{-4} M and 5×10^{-4} M, 100 μl) were each added to 1.5×10^{-5} M DPPH/EtOH solution (4 ml), and the absorbance of each reaction mixture was determined at 520 nm after 30 min.[13]

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