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Synthesis, structural revision, and antioxidant activities of antimutagenic homoisoflavonoids from *Hoffmanosseggia intricata*

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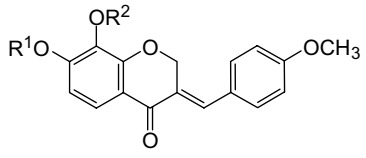
Abstract—Intricatinol and intricatin, the two homoisoflavonoids isolated from *Hoffmanosseggia intricata*, and two analogs have been synthesized from pyrogallol in three steps. The spectral data of synthetic intricatinol are in good agreement with those of natural metabolite, but the spectral data of intricatin are not corroborative with those of the natural product. The structure of intricatin has been thus revised to 8-methoxybonducellin, a compound isolated from *Caesalpinia pulcherrima*. The antioxidant activity of all the four homoisoflavonoids was determined by superoxide (NBT) and DPPH free radical scavenging methods. The synthetic analog 7,8-dihydroxy-3-[(3,4-dihydroxyphenyl)methylene]chroman-4-one displayed excellent activity in both methods.

Homoisoflavonoids (3-benzylidene-4-chromanones) are related to flavonoids and occur as natural products and found to exhibit antifungal,[1,2] hypocholesterolemic,[3] and antiviral activities.[4] 7,8-Dihydroxy-4′-methoxyhomoisoflavonoid (intricatinol, **1**) and 8-hydroxy-4′,7-dimethoxyhomoisoflavonoid (intricatin, **2**, Fig. 1) were isolated from *Hoffmanosseggia intricata*.[5] These are the first examples of antimutagenic homoisoflavonoids which displayed the inhibition of the mutagenicity of 2AN toward *Salmonella typhimurium* (T98). Due to our interest on homoisoflavonoids,[6,7] and the importance of dietary antioxidants in chemoprevention of degenerative diseases, such as cancer, Alzheimer's, Parkinson's, and cardiovascular diseases, we have synthesized these natural products along with two new structural analogs. In this paper, we report the details of synthesis, structural revision, and antioxidative activities of these homoisoflavonoids.



1. Intricatinol, R^1=R^2=H
2. Intricatin, R^1=CH_3, R^2=H
3. 8-Methoxybonducellin, R^1=H, R^2=CH_3

Figure 1. Chemical structures of natural homoisoflavonoids.

7,8-Dihydroxychroman-4-one (**8**), a key intermediate, was obtained by the reaction of pyrogallol (**6**) with acrylonitrile in the presence of sodium methoxide followed by cyclization with aqueous sulfuric acid. Base-catalyzed condensation of **8** with 4-methoxybenzaldehyde afforded intricatinol (**1**) in 60% yield (Scheme 1).[8] The spectral data of synthetic homoisoflavonoid **1** (Table 1) were in agreement with those of natural homoisoflavonoid **1**.[5] Selective methylation of homoisoflavonoid **1** with dimethyl sulfate using sodium bicarbonate as a base afforded intricatin (**2**) in 48% yield and dimethoxy derivative in 15% yield. The other analogs **4** and **5** were synthesized using appropriately substituted benzaldehydes as shown in Scheme 1 and were characterized by their

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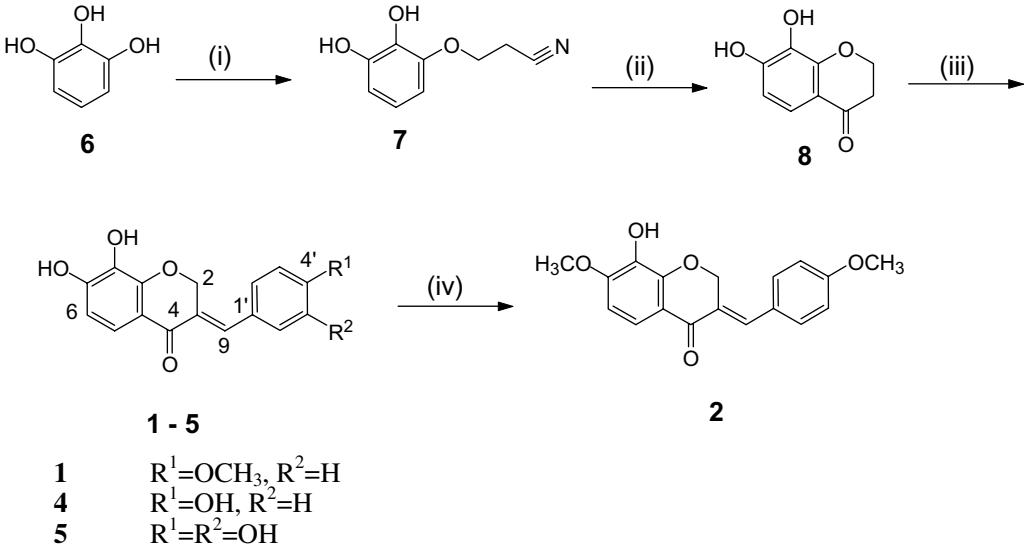

1 $R^1=OCH_3$, $R^2=H$
4 $R^1=OH$, $R^2=H$
5 $R^1=R^2=OH$

Scheme 1. Reagents and conditions: (i) acrylonitrile, NaOMe, 70–80 °C, 7 h, 35%; (ii) aq H_2SO_4, 95–100 °C, 3 h, 54%; (iii) substituted benzaldehyde, piperidine, 70–80 °C, 2 h, 53–60%; (iv) DMS, $NaHCO_3$, acetone, rt, 36 h, 48%.

Table 1. ^{1}H NMR data of synthetic and natural homoisoflavonoids

Assignment	Synthetic intricatinol (**1**)[a]	Natural intricatinol (**1**)[a]	Synthetic intricatin (**2**)[b]	Natural intricatin (**2**)[b]	8-Methoxy bonducellin (**3**)[b]
H-2	5.36 (s)	5.39 (d, 2)	5.41 (d, 2)	5.48 (d, 2)	5.49 (d, 1.8)
H-5	7.27 (d, 8.3)	7.27 (d, 8.5)	7.46 (d, 8.8)	7.61 (d, 8.8)	7.63 (d, 8.8)
H-6	6.57 (d, 8.3)	6.57 (d, 8.5)	6.79 (d, 8.8)	6.65 (d, 8.8)	6.66 (d, 8.8)
H-9	7.64 (s)	7.65 (br s)	7.71 (s)	7.72 (br t)	7.73 (t, 1.8)
H-3′,5′	7.03 (d, 8.3)	7.06 (d, 8.5)	7.04 (d, 8.6)	7.06 (d, 8.8)	7.08 (d, 9)
H-2′,6′	7.40 (d, 8.3)	7.42 (d, 8.5)	7.40 (d, 8.6)	7.43 (d, 8.8)	7.45 (d, 9)
Ar-OMe	3.80 (s)	3.83 (s)	3.85 (s)	3.82 (s)	3.84 (s)
			3.91 (s)	3.88 (s)	3.89 (s)

[a] NMR in DMSO-d_6.
[b] NMR in acetone-d_6.

spectral data.[9] In all cases, a single geometrical isomer (*E*) was obtained. The stereochemistry at the double bond was confirmed by the characteristic ^{1}H NMR data.[7,10]

Structural revision. The spectral data of synthetic homoisoflavonoid **2** were not in agreement with those reported for natural homoisoflavonoid **2**.[5] In the ^{1}H NMR data of natural **2** (Table 1), the aromatic proton adjacent to a methoxyl group was reported to resonate at δ 6.65 (d). However, in the ^{1}H NMR spectrum of synthetic homoisoflavonoid **2**, the proton adjacent to a methoxyl group appeared at δ 6.79 (d). Thus, it appears that the natural intricatin (**2**) may be an isomeric compound, 8-methoxybonducellin (**3**). In fact, careful literature survey revealed that the spectral data of natural intricatin corroborated with those reported for 8-methoxybonducellin (**3**), isolated from *Caesalpinia pulcherrima*[11] and *Caesalpinia sappan*.[12] From the foregoing, structure of intricatin, isolated from *Hoffmanosseggia intricata*, has been revised into 8-methoxybonducellin (**3**), a known natural homoisoflavonoid.

Antioxidant activity. In the present study, two commonly used antioxidant evaluation methods, superoxide radical scavenging (NBT method) and the DPPH free radical scavenging methods, were chosen to determine the antioxidant potential of the compounds. Both the methods measure the efficacy of a hydrogen atom transfer from a phenol to a radical.

Superoxide radical scavenging activity. Superoxide radicals were generated in vitro by non-enzymatic system and determined spectrophotometrically (560 nm) by nitro blue tetrazolium (NBT) photoreduction method of McCord and Fridovich.[13,14] The antioxidant activity of homoisoflavonoids (**1**–**5**) was expressed as 50% inhibitory concentration (IC_{50} in μM) and the values are incorporated in Table 2. From the superoxide scavenging activity data, 7,8-dihydroxy-3-[(3,4-dihydroxyphenyl)methylene]-chroman-4-one (**5**, IC_{50}: 8.5 μM) having two catechol moieties has shown highest activity and was several fold more potent activity in comparison with the commercially available antioxidants like BHA (IC_{50}: 966 μM) and vitamin C (IC_{50}: 852 μM). The superior scavenging ability of **5** lends further support to the fact that the catechol system enhances the antioxidant activity.[15]

Table 2. Antioxidant activity of homoisoflavonoids

Compound	(IC_{50}[a] (μM)	
	NBT superoxide scavenging activity	DPPH free radical scavenging activity
Intricatinol (**1**)	30.1	10.1
2	>200	>200
4	26.5	8.3
5	8.5	4.5
BHA	966	34
Vitamin C	852	25.1

BHA, butylated hydroxyanisole.
The lower the IC_{50} values, the higher the antioxidant activity.
[a] This indicates the significant difference at the level of $P < 0.05$, $n = 3$.

DPPH radical scavenging activity. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of homoisoflavonoids was determined by the method described by Lamaisen et al.[16,17] based on the reduction of methanolic solution of the colored DPPH radical. From the IC_{50} values (Table 2), again 7,8-dihydroxy-3-[(3,4-dihydroxyphenyl)methylene]-chroman-4-one (**5**, IC_{50}: 4.5 μM) was found powerful scavenger of DPPH free radicals.

In summary, we have accomplished the synthesis of intricatinol (**1**) and the proposed structure for intricatin (**2**), two plant antimutagenic homoisoflavonoids isolated from *Hoffmanosseggia intricata* in three steps, with two synthetic analogs. The structure of natural intricatin has been revised into 8-methoxybonducellin, a homoisoflavonoid isolated earlier from *Caesalpinia pulcherrima* and *Caesalpinia sappan*. These homoisoflavonoids were evaluated for their antioxidative potential by two commonly used methods, the superoxide (NBT) and DPPH free radical scavenging methods. Homoisoflavonoid **5** was found to be a potent antioxidant.

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8. General procedure for the preparation of *E*-homoisoflavonoids: A mixture of chroman-4-one **8** (1.22 mmol), substituted benzaldehyde (1.76 mmol), and piperidine (five drops) was heated at 70–80 °C for 2 h. The cooled reaction mixture was diluted with water (50 mL), acidified with dil HCl, and extracted with ethyl acetate (3× 50 mL). The combined EtOAc layer was washed with water (30 mL), brine (30 mL) and dried over sodium sulfate. The residue obtained after evaporation of the solvent was chromatographed over silica gel column using mixtures of petroleum ether and ethyl acetate as eluents to give homoisoflavonoids.
9. Physical and spectral characteristics of compounds, 7,8-dihydroxy-3-[(4-methoxyphenyl)methylene]chroman-4-one (**1**): Light brown powder, mp 192–194 °C (lit.[5] mp 196–198 °C). IR (KBr): 3394, 1658, 1602, 1254, 1173, 1150, 1071, 1030, 963 cm^{-1}. For ^1H NMR see, Table 1. ^{13}C NMR (DMSO-d_6): δ 180.2, 160.3, 152.4, 150.4, 135.3, 132.7, 132.2, 129.2, 126.6, 118.4, 115.2, 114.3, 110.5, 67.6, 55.3. MS (ESI, negative ion mode): *m/z* 297 $(M–H)^-$.
8-Hydroxy-7-methoxy-3-[(4-methoxyphenyl)methylene]-chroman-4-one (**2**). Light yellow powder, mp 96–98 °C (lit.[5] mp 157–159 °C). IR (KBr): 3305, 2932, 1658, 1603, 1288, 1255, 1176, 1102, 1027, 933 cm^{-1}. For ^1H NMR see Table 1. ^{13}C NMR (DMSO-d_6): δ 181.5, 161.8, 153.9, 150.3, 136.7, 134.4, 133.1, 130.9, 128.0, 119.2, 117.9, 115.2, 106.9, 68.9, 56.7, 55.9. MS (ESI, positive ion mode): *m/z* 335 $(M+Na)^+$.
7,8-Dihydroxy-3-[(4-hydroxyphenyl)methylene]-chroman-4-one (**4**). Light brown powder, mp 246–248 °C. IR (KBr): 3371, 1662, 1618, 1244, 1173, 1068, 996 cm^{-1}. ^1H NMR (DMSO-d_6): δ 10.08 (2H, s, 2× Ar-OH), 8.76 (1H, s, Ar-OH), 7.60 (1H, s, H-9), 7.31 (2H, d, J = 8.3 Hz, H-2′,6′), 7.26 (1H, d, J = 8.6 Hz, H-5), 6.87 (2H, d, J = 8.3 Hz, H-3′,5′), 6.56 (1H, d, J = 8.6 Hz, H-6), 5.35 (2H, s, H-2). ^{13}C NMR (DMSO-d_6): δ 180.2, 158.9, 152.2, 150.4, 135.6, 132.7, 132.4, 128.3, 125.1, 118.3, 115.7, 115.2, 110.4, 67.7. MS (ESI, negative ion mode): *m/z* 283 $(M–H)^-$. Anal. Calcd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.26. Found: C, 67.55; H, 4.32.
7,8-Dihydroxy-3-[(3,4-dihydroxyphenyl)methylene]-chroman-4-one (**5**). Light yellow powder, mp 220 °C (dec). IR (KBr): 3458, 3402, 3200, 1653, 1600, 1272, 1194, 1075, 980 cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.31–9.81 (br m, 4 H, Ar-OH), 7.52 (1H, s, H-9), 7.25 (1H, d, J = 8.8 Hz, H-5), 6.83–6.85 (3H, m, H-2′,6′), 6.77 (1H, dd, J = 8.3, 1.5 Hz, H-5′), 6.55 (1H, d, J = 8.8 Hz, H-6), 5.34 (1H, d, J = 1.0 Hz, H-2). ^{13}C NMR (DMSO-d_6): δ 180.2, 152.2, 150.3, 147.4,145.4, 135.9, 132.7, 128.1, 125.5, 123.0, 118.3, 117.5, 115.9, 115.2, 110.4, 67.7. MS (ESI, negative ion mode): *m/z* 299 $(M–H)^-$. Anal. Calcd for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03. Found: C, 63.97; H, 4.06.
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