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Polyphenols based on isoflavones as inhibitors of *Helicobacter pylori* urease

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Abstract—Twenty polyphenols were synthesized and evaluated for their effect on *Helicobacter pylori* urease. Among these compounds, 4-(*p*-hydroxyphenethyl)pyrogallol (**15**) (IC_{50} = 0.03 mM) and 7,8,4′-trihydroxyisoflavone (**19**) (IC_{50} = 0.14 mM) showed potent inhibitory activities, and inhibited *Helicobacter pylori* urease in a time-dependent manner. The structure–activity relationship of these polyphenols revealed: the two ortho hydroxyl groups were essential for inhibitory activity of polyphenol. When the C-ring of isoflavone was broken, the inhibitory activity markedly decreased. As for deoxybenzoin, the carboxyl group was clearly detrimental.
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1. Introduction

Urease (urea amidohydrolase; E.C.3.5.1.5) is widely distributed in a variety of bacteria, fungi, algae, and plants. People infected by these bacteria characterized by urease activity such as *Helicobacter pylori* (*H. pylori*)and *Proteus mirabilis* are exposed to a high risk for chronic atrophic gastritis, peptic ulcer[1], and urolithiasis.[2] Structural studies of the enzymes from *Klebsiella aerogenes*, *Bacillus pasteurii*, and *H. pylori* have revealed a dinuclear Ni active site with a carbamylated lysine residue that bridges the deeply buried metal atoms.[3–5] Urease catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide, and to protect the bacteria in the acidic environment through the elevation in pH.[6] Many urease inhibitors have been described in the past decades, like fluorofamide, hydroxyureas, and hydroxamic acids, but part of them were prevented from using in vivo because of their toxicity or instability. For instance, acetohydroxamic acid was demonstrated to be teratogenic in rats.[7] Thus, current efforts are focused on seeking novel urease inhibitors with good bioavailability and low toxicity.

Keywords: Urease inhibitors; Isoflavones; Deoxybenzoins; Polyphenols.
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Polyphenols, such as flavones and isoflavones, constitute one of the most represented classes of compounds in higher plants including medicinal and edible plants. Extensive epidemiological and animal studies and in vitro experiments with polyphenols have indicated their broad variety of biological activities, including anticancer,[8] anti-inflammatory,[9] antibacterial,[10] cardioprotective,[11] anti-osteoporotic[12], and enzyme-inhibitory[13] activities. More attention has been focused on exploring novel biological properties of polyphenols. As an example, tea polyphenols and flavones as urease inhibitors were reported by Wotherspoon et al.[14] and Tamura,[15] respectively. In general, isoflavones exhibited similar biological activities as flavones,[10,12,16–19] which inspired us to screen isoflavones and their bioisosteres for urease inhibitors. Twenty compounds were designed to test for urease inhibitory activities against *H. pylori* urease. To our knowledge, this is the first report on the screening of isoflavone-based compounds for their urease inhibitory activities.

2. Results and discussion

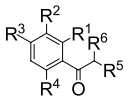
2.1. Chemistry

A series of isoflavone-based polyphenols with structural diversity were designed for urease inhibitors. Inspection

of the chemical structure of isoflavone suggested that the compound could be divided into three subunits: A-, B-, and C-rings (Fig. 3). Initial structure–activity relationship (SAR) studies were performed by modification of the parent compound to determine if any of the subunits displayed urease inhibitory activity. Based on this consideration, we further designed some deoxybenzoins by breaking C-ring of the corresponding isoflavones. The deoxybenzoins (**4**, **5**, **6**, **7**, **8**, and **9**) were successfully synthesized by the method of Wähälä,[20] while compounds **13** and **14** were produced in the presence of gas HCl by treating phloroglucinol and 1,3,5-trihydroxy-2-methoxybenzene with *p*-hydroxyphenylacetonitrile in dry ether, respectively.[21] Compounds **1** and **14** were transformed into **17**, **18**, and **19** by CH_3SO_2Cl in anhydrous DMF in the presence of $BF_3{\cdot}Et_2O$. All compounds were fully characterized by spectroscopic methods and elemental analysis. Out of the compounds, four (compounds **11**, **12**, **15**, and **16**) were reported for the first time.

2.2. Biological activity

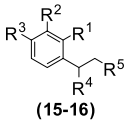
Twenty polyphenols **1**–**20** (Figs. 1–3) were evaluated against *H. pylori* urease. Percent inhibition at a 400 μg/mL concentration of compounds **1**–**20** was initially determined, and the results are reported in Table 1. Compounds **1**, **3**, **4**, **5**, **9**, **15**, and **17** (with catechol skeleton) exhibited potent inhibitory activities, especially **15** (IC_{50} = 0.03 mM) and **17** (IC_{50} = 0.14 mM) which demonstrated excellent in vivo activities. However, replacing one or all of the hydroxyl groups on the catechol skeleton by methoxy produced a partial or complete loss in activity (**10**, **11**, **12**, **14**, **18**, and **19**). So the two ortho hydroxyl groups presented on aromatic ring of polyphenol molecule may be responsible for inhibitory activity and coordinate with the nickel (active site) of enzyme. Any compounds (**2**, **6**, **7**, **8**, and **13**) with resorcinol skeleton showed no or extremely weak inhibitory activity. Probably, the long distance between the two hydroxyl groups at 1 and 3 positions on benzene ring counted for less inter-



(1-14)

	R^1	R^2	R^3	R^4	R^5	R^6
1	OH	OH	OH	H	H	H
2	OH	H	OH	H	H	H
3	H	OH	OH	H	H	H
4	OH	OH	OH	H	4-hydroxyphenyl	H
5	OH	OH	OH	H	4-methoxyphenyl	H
6	OH	H	OH	H	4-hydroxyphenyl	H
7	OH	H	OH	H	4-methoxyphenyl	H
8	OH	H	OH	H	4-nitrophenyl	H
9	H	OH	OH	H	4-hydroxyphenyl	H
10	OCH_3	OCH_3	OCH_3	H	4-methoxyphenyl	H
11	OCH_3	OCH_3	OCH_3	H	4-methoxyphenyl	CH_3
12	OH	OCH_3	OCH_3	H	4-methoxyphenyl	H
13	OH	H	OH	OH	4-hydroxyphenyl	H
14	OH	OCH_3	OH	OH	4-hydroxyphenyl	H

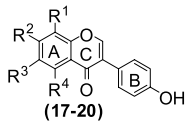
Figure 1. Chemical structures of compounds (**1**–**14**).



(15-16)

	R^1	R^2	R^3	R^4	R^5
15	OH	OH	OH	H	4-hydroxyphenyl
16	OH	OCH_3	OCH_3	OH	4-methoxyphenyl

Figure 2. Chemical structures of compounds (**15**–**17**).



(17-20)

	R^1	R^2	R^3	R^4
17	OH	OH	H	H
18	H	OH	OCH_3	OH
19	OCH_3	OH	H	OH
20	H	OH	H	OH

Figure 3. Chemical structures of compounds (**18**–**20**).

Table 1. Percent inhibition of compounds **1**–**20** against *H. pylori* urease at the concentration of 400 μg/mL

Compound	Percent inhibition (%)	Compound	Percent inhibition (%)
1	38	**11**	—
2	14	**12**	—
3	41	**13**	—
4	51	**14**	17
5	44	**15**	94.5
6	16	**16**	42
7	14	**17**	81.7
8	—	**18**	36
9	39	**19**	23
10	—	**20**	—

action with the nickel of the enzyme. Deoxybenzoins (**4**, **14**, and **13**) were regarded as products by breaking the C-rings of isoflavones (**17**, **18** (**19**), and **20**), respectively (Figs. 1 and 3). A comparison of the inhibitory activities of isoflavones with the corresponding deoxybenzoins, that is, comparing **4** and **17**, **14** and **18** (**19**), **13** and **20**, showed clear difference in their activities. This indicated that the C-ring of isoflavone was critical to its inhibitory effect. Changing the carbonyl group of **4** for a methylene (**15**) significant increased activity. Meanwhile replacing the carbonyl group of **12** by hydroxyl group (**16**) also provided significantly increase in activity. These result showed the carbonyl group was clearly detrimental to polyphenolic activity.

Generally, reversible inhibition is characterized by a definite degree of inhibition which is usually attained rapidly.[22] The depression by acetohydroxamic acid (AHA), a reversible urease inhibitor,[23] rapidly attained the maximal constant degree. Under the assay conditions, compounds **15** and **17** showed potent inhibitory activities with $IC_{50} = 0.03$ and 0.14 mM, respectively (Table 2). The inhibitory activities of compounds **15** and **17** were dependent on the length of preincubation with the urease. Three hours of preincubation with compounds **15** and **17** was indispensable for the maximum inhibition (Fig. 4), which indicated that compounds **15** and **17** were the irreversible inhibitors. These results agreed with published observations that the inhibitory actions of α,β-unsaturated ketones were dependent on the length of preincubation with urease.[24]

Table 2. Inhibition of *H. pylori* urease by compounds **15** and **17**

Compound	IC_{50}[a] (mM)
15	0.030 ± 0.0008
17	0.140 ± 0.003
Acetohydroxamic acid	0.017 ± 0.002

[a] Urease activity was assayed as described in Section 4 with 3 h preincubation. Each value is represented as means ± SD from triplicate measurements.

It was reported that some urease inhibitors depressed *H. pylori* urease activities by interacting with the sulfhydryl groups, especially the Cystein-592 residue, which is a key residue in the active sites.[25] The interaction of an isoflavone with the sulfhydryl group may be related to its inhibitory influence on urease, and the keto group may be responsible for the interaction. In fact, dithiothreitol (DTT) and 2-mercaptoethanol (2-ME) diminished the activity of **17** dose-dependently and even at low concentrations (Table 3, Figs. 5 and 6). These results, together with the SAR, suggested that the keto group and the two ortho hydroxyl groups in **17** hooked the active site of *H. pylori* urease concurrently. On the other hand, 2-ME and DTT showed weak effect on the inhibitory activity of **15** and AHA, in which no keto

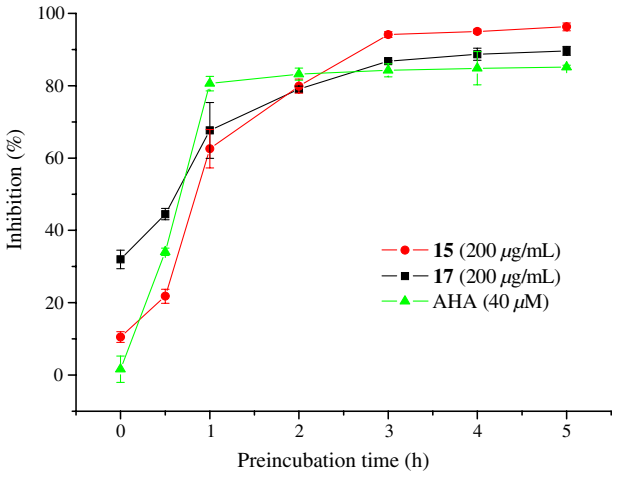


Figure 4. Time course of inhibition of *H. pylori* urease activity by acetohydroxamic acid (AHA) and compounds **15** and **17**. Urease activity was assayed as described in Section 4. Data are the average of three experiments and error bars indicate the standard deviations.

Table 3. Effects of additives on the inhibitory activity of compounds **15** and **17** against *H. pylori* urease

Compound	Additives	IC_{50}[a] (mM)
15	None	0.030
	+2-ME (0.4 mM)	0.071
	+DTT (0.4 mM)	0.053
17	None	0.14
	+2-ME (0.4 mM)	>1.48
	+DTT (0.4 mM)	>1.48
Acetohydroxamic acid	None	0.017
	+2-ME (0.4 mM)	0.019
	+DTT (0.4 mM)	0.018

[a] Urease activity was assayed as described in Section 4 with 3 h preincubation.

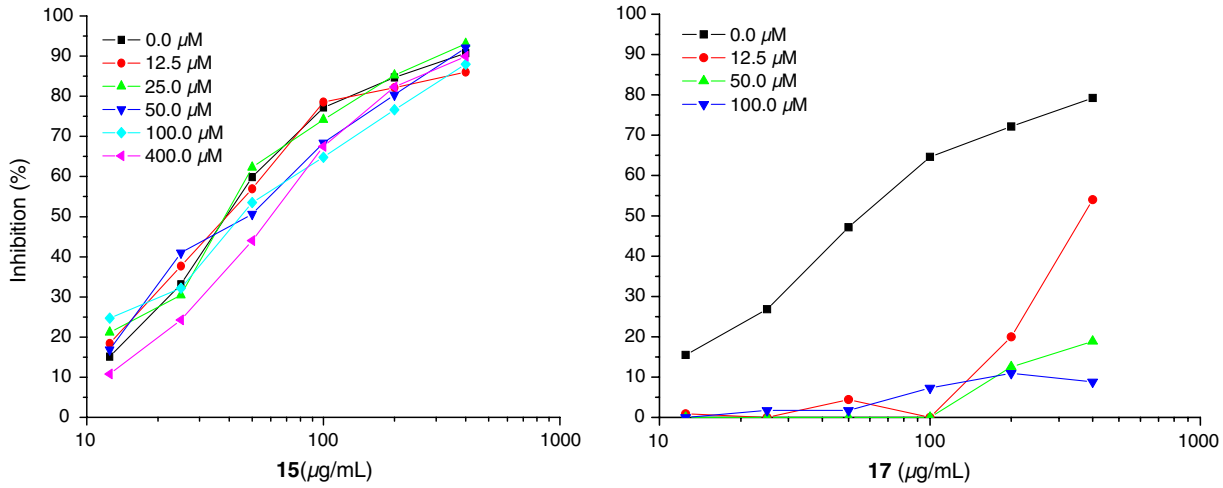


Figure 5. Effect of DTT on the inhibition of urease activity by compounds **15** and **17**. Urease activity was assayed as described in Section 4 with 3 h preincubation.

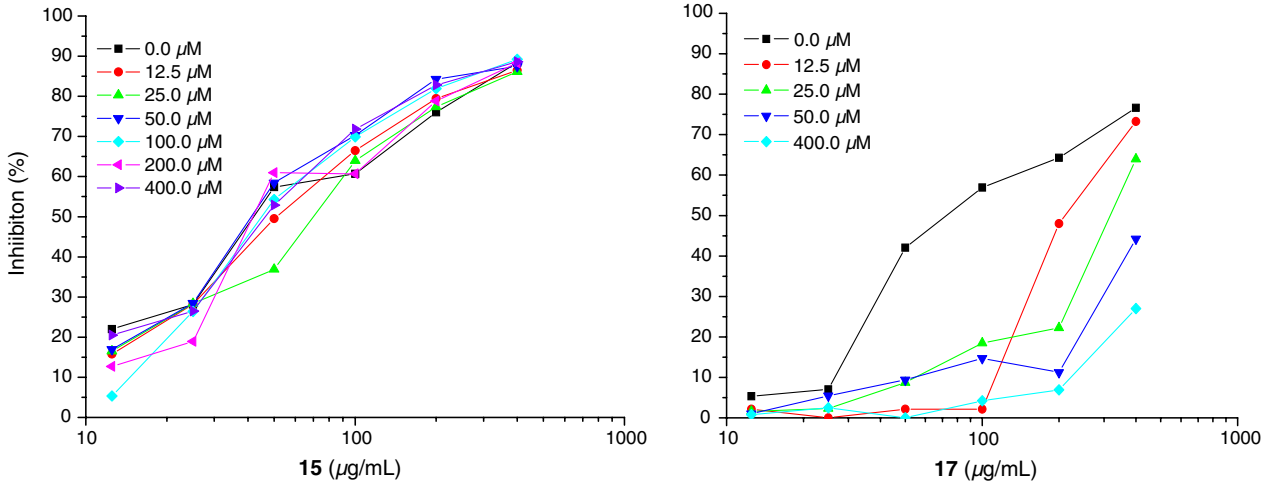


Figure 6. Effect of 2-ME on the inhibition of urease activity by compounds **15** and **17**. Urease activity was assayed as described in Section 4 with 3 h preincubation.

groups existed (Fig. 6, Table 3). This implied that the Cystein-592 residue of the active sites was unlikely to be involved in binding to **15**. Further studies are required to elucidate the mechanism by which polyphenols exhibit the urease inhibitory activity.

Toxicity and rapid metabolic degradation prevented some efficient urease inhibitors in vitro from developing into therapeutic agents such as AHA. Heinonen et al. reported that compound **17** was one of the metabolites of daidzein.[26] Therefore, this result attracted our interest in further structural modifications of compound **17** as a leading compound which provided new template structures for the design of new inhibitors with an optimized pharmacological profile.

3. Conclusion

Twenty polyphenols were prepared and evaluated for their activity against *H. pylori* urease. Among these compounds, **15** (IC_{50} = 0.03 mM) and **17** (IC_{50} = 0.14 mM) showed potent inhibitory activities. The SAR of these polyphenols disclosed: the two ortho hydroxyl groups were essential for inhibitory activity of polyphenol. When the C-ring of isoflavone was broken, the inhibitory activity markedly decreased. As for deoxybenzoin, the carboxyl group was clearly detrimental. Further efforts to modify the potent urease inhibitors (**15** and **17**) are underway and will be reported in due course.

4. Experiments

4.1. Materials

Protease inhibitors (Complete mini EDTA-free) were purchased from Roche Diagnostics GmbH (Mannheim, Germany) and brucella broth was from Becton–Dickinson (Cockeysville, MD). Horse serum was from Hyclone (Utah, America).

4.2. Bacteria

Helicobacter pylori (ATCC 43504; American Type Culture Collection, Manassas, VA) was grown in brucella

broth supplemented with 10% heat-inactivated horse serum for 24 h at 37 °C under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), as previously described.[27]

4.3. Preparation of *H. pylori* urease

For urease inhibition assays, 50 mL broth cultures (2.0×10^8 CFU/mL) were centrifuged (5000*g*, 4 °C) to collect the bacteria, and after washing twice with phosphate-buffered saline (pH 7.4), the *H. pylori* precipitation was stored at −80 °C. *H. pylori* was returned to room temperature, and after addition of 3 mL of distilled water and protease inhibitors, sonication was performed for 60 s. Following centrifugation (15,000*g*, 4 °C), the supernatant was desalted through Sephadex G-25 column (PD-10 columns, Amersham–Pharmacia Biotech, Uppsala, Sweden). The resultant crude urease solution was added to an equal volume of glycerol and stored at 4 °C until use in the experiment.

4.4. Measurement of urease activity

The assay mixture, containing 25 μL (4 U) of *H. pylori* urease and 25 μL of the test compound, was preincubated for 3 h at room temperature in a 96-well assay plate. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn.[28]

4.5. Chemistry

The compounds previously reported were synthesized according to the methods of the references with appropriate modifications. Separation of the compounds by column chromatography was carried out with silica gel 60 (200–300 mesh ASTM, E. Merck). The quantity of silica gel used was 50–100 times the weight charged on the column. Then, the eluates were monitored using TLC. Melting points (uncorrected) were determined on a XT4 MP apparatus (Taike Corp., Beijing, China). ESI-mass spectra were obtained on a Mariner System 5304 mass spectrometer, and ^{1}H NMR spectra were recorded at DPX500 or DPX300 in DMSO-d_6 on a Bruker spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values.

4.5.1. Gallacetophenone (1).[29] Equimolar quantities of pyrogallol (2.5 g; 19.8 mmol) and Ac_2O (1.9 mL; 20.1 mmol) were refluxed with a drop of concentrated H_2SO_4 in a current of nitrogen on the oil-bath for about 3 h. The contents were cooled and 10 mL of water containing 2 mL of ethanol was added. Then two drops of concentrated HCl were added, and the solution was refluxed for 2 h to decompose the excess Ac_2O. The reaction mixture was cooled which was extracted thrice with EtOAc. The EtOAc solution was washed with aqueous saturated $NaHCO_3$ and brine, dried, and concentrated under reduced pressure to give the product as a dark gummy material which on crystallization from methanol to water gave compound **1** as pale yellow leaflets (3.2 g; 96%). Mp 169–171 °C (begin to decompose above 150 °C) (lit., 169–172 °C). ^{1}H NMR δ: 2.52 (s, 3H, CH_3), 6.39 (d, 1H, $J = 9$ Hz, H-5), 7.30 (d, 1H, $J = 9$ Hz, H-6), 8.59 (s, 1H, 3-OH), 10.06 (s, 1H, 4-OH), 12.59 (s, 1H, 2-OH). ESI-MS $C_8H_9O_4$ $[M+H]^+$ 169. Anal. Calcd for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 57.21; H, 4.77.

4.5.2. 2,4-Dihydroxyacetophenone (2).[30] To the solution of resorcinol (2.2 g; 20 mmol) in 6.3 mL of acetic acid was added 2.8 g (20 mmol) of anhydrous $ZnCl_2$. The mixture was heated under reflux on the oil-bath for 5 h. After cooling, the product was poured into 150 mL of ice-water. The precipitate was sucked and crystallized from water to give compound **2** (2.8 g; 92%) as dark red needles. Mp 145–146 °C (lit., 141–144 °C). ^{1}H NMR δ: 2.52 (s, 3H, CH_3), 6.24 (d, 1H, $J = 2$ Hz, H-3), 6.37 (d × d, 1H, $J = 9$ Hz, $J = 2$ Hz, H-5), 7.76 (d, 1H, $J = 9$ Hz, H-6), 10.60 (s, 1H, 4-OH), 12.60 (s, 1H, 2-OH). ESI-MS $C_8H_9O_3$ $[M+H]^+$ 153. Anal. Calcd for $C_8H_8O_3$: C, 63.15; H, 5.30. Found: C, 63.27; H, 5.26.

4.5.3. 3,4-Dihydroxyacetophenone (3).[31,32] A similar treatment of catechol (2.2 g; 20 mmol) in 6.3 mL of acetic acid with 2.8 g (20 mmol) of anhydrous $ZnCl_2$ gave the product as dark gummy material which was purified by chromatography (petroleum ether/EtOAc = 2:1) to yield compound **3** (2.2 g; 72%) as orange red powder. Mp 117–119 °C (lit., 116, 91, 184, 180 °C). ^{1}H NMR δ: 2.43 (s, 3H, CH_3), 6.80 (d, 1H, $J = 8$ Hz, H-5), 7.33 (s, 1H, H-2), 7.34 (d, 1H, $J = 8$ Hz, H-6), 9.32 (s, 1H, 3-OH), 9.83 (s, 1H, 4-OH). ESI-MS $C_8H_9O_3$ $[M+H]^+$ 153. Anal. Calcd for $C_8H_8O_3$: C, 63.15; H, 5.30. Found: C, 63.30; H, 5.21.

4.5.4. 2,3,4,4′-Tetrahydroxydeoxybenzoin (4).[33] 0.63 g (5 mmol) of pyrogallol and 0.76 g (5 mmol) of *p*-hydroxyphenylacetic acid were dissolved into 5 mL of fresh distilled $BF_3{\cdot}Et_2O$. The mixture was stirred and heated on the oil-bath at 80–85 °C for about 3 h. After cooling, the contents were poured into 150 mL of ice-cold aqueous sodium acetate (wt% = 10%) with stirring. Then, the precipitate was filtered and washed thrice with water. Crystallization from methanol to water gave compound **4** (1.15 g; 89%) as pale yellow crystals. Mp 206–208 °C (lit., 190–192 °C). ^{1}H NMR δ: 4.12 (s, 2H, $ArCH_2$), 6.40 (d, 1H, $J = 9$ Hz, H-5), 6.69 (d, 2H, $J = 8$ Hz, H-3′, 5′), 7.07 (d, 2H, $J = 8$ Hz, H-2′, 6′), 7.48 (d, 1H, $J = 9$Hz, H-6), 8.64 (s, 1H, 3-OH), 9.30 (s, 1H, 4′-OH), 10.12 (s, 1H, 4-OH), 12.59 (s, 1H, 2-OH). ESI-MS $C_{14}H_{13}O_5$ $[M+H]^+$ 261. Anal. Calcd for $C_{14}H_{12}O_5$: C, 64.61; H, 4.65. Found: C, 64.68; H, 4.58.

4.5.5. 2,3,4-Trihydroxy-4′-methoxydeoxybenzoin (5). A similar treatment described for **4** of pyrogallol (0.63 g; 5 mmol) with *p*-methoxyphenylacetic acid (0.83 g; 5 mmol) afforded compound **5** (1.03 g; 76%) as nearly colorless crystals. An analytical sample was prepared by crystallization from methanol to water. Mp 145–147 °C. ^{1}H NMR δ: 3.72 (s, 3H, OCH_3), 4.20 (s, 2H, $ArCH_2$), 6.40 (d, 1H, $J = 9$ Hz, H-5), 6.87 (d, 2H, $J = 9$ Hz, H-3′, 5′), 7.19 (d, 2H, $J = 9$ Hz, H-2′, 6′), 7.49 (d, 1H, $J = 9$ Hz, H-6), 8.63 (s, 1H, 3-OH), 10.11 (s, 1H, 4-OH), 12.54 (s, 1H, 2-OH). ESI-MS $C_{15}H_{15}O_5$

$[M+H]^+$ 275. Anal. Calcd for $C_{15}H_{14}O_5$: C, 65.69; H, 5.14. Found: C, 65.75; H, 5.17.

4.5.6. 2,4,4′-Terthydroxydeoxybenzoin (6).[34] A similar treatment described for **4** of resorcinol (0.55 g; 5 mmol) with *p*-hydroxyphenylacetic acid (0.76 g; 5 mmol) afforded compound **6** (1.0 g; 82%) as colorless crystals. An analytical sample was prepared by crystallization from methanol to water. Mp 188–190 °C (lit., 189–191 °C). ^{1}H NMR δ: 4.13 (s, 2H, $ArCH_2$), 6.24 (s, 1H, H-3), 6.37 (d, 1H, J = 9 Hz, H-5), 6.90 (d, 2H, J = 8 Hz, H-3′, 5′), 7.07 (d, 2H, J = 8 Hz, H-2′, 6′), 7.92 (d, 1H, J = 9 Hz, H-6), 9.29 (s, 1H, 4′-OH), 10.67 (s, 1H, 4-OH), 12.60 (s, 1H, 2-OH). ESI-MS $C_{14}H_{13}O_4$ $[M+H]^+$ 245. Anal. Calcd for $C_{14}H_{12}O_4$: C, 68.85; H, 4.95. Found: C, 68.89; H, 4.93.

4.5.7. 2,4-Dihydroxy-4′-methoxydeoxybenzoin (7). [35] A similar treatment described for **4** of resorcinol (0.55 g; 5 mmol) with *p*-methoxyphenylacetic acid (0.83 g; 5 mmol) afforded compound **7** (1.1 g; 85%) as pale yellow crystals. An analytical sample was prepared by crystallization from methanol to water. Mp 160–162 °C. ^{1}H NMR δ: 3.71 (s, 3H, OCH_3), 4.20 (s, 2H, $ArCH_2$), 6.24 (d, 1H, J = 2 Hz, H-3), 6.39 (d × d, 1H, J = 2 Hz, J = 9 Hz, H-5), 6.87 (d, 2H, J = 9 Hz, H-3′, 5′), 7.19 (d, 2H, J = 9 Hz, H-2′, 6′), 7.94 (d, 1H, J = 9 Hz, H-6), 10.65 (s, 1H, 4-OH), 12.55 (s, 1H, 2-OH). ESI-MS $C_{15}H_{15}O_4$ $[M+H]^+$ 259. Anal. Calcd for $C_{15}H_{14}O_4$: C, 69.76; H, 5.46. Found: C, 69.71; H, 5.41.

4.5.8. 2,4-Dihydroxy-4′-nitrodeoxybenzoin (8). A similar treatment described for **4** of resorcinol (0.55 g; 5 mmol) with *p*-methoxyphenylacetic acid (0.90 g; 5 mmol) afforded the crude product of compound **8** as dark oil which was extracted with EtOAc and dried. After removal of solvent, the residue was purified by a flash chromatography with EtOAc–petroleum ether (1:3) to afford brown oil which crystallized from methanol to water to give pale yellow crystals (0.80 g; 59%). Mp 206–208 °C. ^{1}H NMR δ: 4.54 (s, 2H, $ArCH_2$), 6.28 (s, 1H, H-3), 6.41 (d, 1H, J = 8 Hz, H-5), 7.56 (d, 2H, J = 8 Hz, H-2′, 6′), 7.94 (d, 1H, J = 8 Hz, H-6), 9.19 (d, 2H, J = 8 Hz, H-3′, 5′), 10.72 (s, 1H, 4-OH), 12.25 (s, 1H, 2-OH). ESI-MS $C_{14}H_{12}NO_5$ $[M+H]^+$ 274. Anal. Calcd for $C_{14}H_{11}NO_5$: C, 61.54; H, 4.06; N, 5.13. Found: C, 61.57; H, 4.01; N, 5.15.

4.5.9. 3,4,4′-Terthydroxydeoxybenzoin (9).[20] A similar treatment described for **4** of catechol (0.55 g; 5 mmol) with *p*-hydroxyphenylacetic acid (0.76 g; 5 mmol) afforded the crude product of compound **9** as dark oil which was extracted with EtOAc and dried. After removal of solvent, the residue was purified by a flash chromatography with EtOAc–petroleum ether (1:1) to afford pale brown solid (0.74 g; 62%). Mp 207–209 °C (lit., 211 °C). ^{1}H NMR δ: 4.05 (s, 2H, $ArCH_2$), 6.67 (d, 2H, J = 8 Hz, H-3′, 5′), 6.80 (d, 1H, J = 8 Hz, H-5), 7.02 (d, 2H, J = 8 Hz, H-2′, 6′), 7.37 (s, 1H, H-2), 7.44 (d, 1H, J = 8 Hz, H-6), 9.22 (s, 1H, 3-OH), 9.30 (s, 1H, 4′-OH), 9.82 (s, 1H, 4-OH). ESI-MS $C_{14}H_{13}O_4$ $[M+H]^+$ 245. Anal. Calcd for $C_{14}H_{12}O_4$: C, 68.85; H, 4.95. Found: C, 68.79; H, 4.92.

4.5.10. 2,3,4,4′-Tetramethoxydeoxybenzoin (10) and 2-(4-methoxyphenyl)-1-(2,3,4-trimethoxyphenyl)-propan-1-one (11). 0.78 g (3 mmol) of compound **4** and 0.90 mL (15 mmol) of CH_3I were dissolved into 15 mL of dry acetone. To the solution, 2.1 g (15 mmol) of potassium carbonate was added. The mixture was stirred and heated under reflux for 24 h. After cooling, the contents were poured into 80 mL of water with stirring. Then, the mixture was extracted with EtOAc and dried. After removal of solvent, the residue was purified by a flash chromatography with EtOAc–petroleum ether (1:10) to afford two fractions. The first fraction gave compound **11** as colorless oil (0.53 g). ^{1}H NMR δ: 1.34 (d, 3H, J = 7 Hz, $ArCHCH_3$), 3.68 (s, 3H, 3-OCH_3), 3.71 (s, 3H, 4′-OCH_3), 3.76 (s, 3H, 2-OCH_3), 3.84 (s, 3H, 4-OCH_3), 4.63 (q, 1H, J = 7 Hz, ArCH), 6.80 (d, 1H, J = 9 Hz, H-5), 6.82 (d, 2H, J = 9 Hz, 3′, 5′-H), 7.10 (d, 2H, J = 9 Hz, 2′, 6′-H), 7.24 (d, 1H, J = 9 Hz, H-6). ESI-MS $C_{19}H_{23}O_5$ $[M+H]^+$ 331. Anal. Calcd for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71. Found: C, 68. 69.11; H, 6.68. The second fraction gave compound **10** as yellowish solid (0.36). Mp 51–53 °C. ^{1}H NMR δ: 3.71 (s, 3H, 4′-OCH_3), 3.76 (s, 3H, 3-OCH_3), 3.85 (s, 3H, 2-OCH_3), 3.87 (s, 3H, 4-OCH_3), 4.16 (s, 2H, $ArCH_2$), 6.85 (d, 2H, J = 8 Hz, H-3′, 5′), 6.89 (d, 1H, J = 9 Hz, H-5), 7.11 (d, 2H, J = 8 Hz, H-2′,6′), 7.39 (d, 1H, J = 9 Hz, J = 9Hz, H-6). ESI-MS $C_{18}H_{20}O_5Na$ $[M+Na]^+$ 339. Anal. Calcd for $C_{18}H_{20}O_5$: C, 68.34; H, 6.37. Found: C, 68.38; H, 6.34.

4.5.11. 2-Hydroxy-3,4,4′-trimethoxydeoxybenzoin (12).[36] 4-Methoxyphenacetyl chloride was prepared by treating 4-methoxyphenacetic acid with thionyl chloride at room temperature for 24 h. The solvent was removed in vacuo and further purification was not necessary. To a mixture of pyrogallol trimethoxyl ether (2 g; 11.9 mmol) and 4-methoxyphenacetyl chloride (2.3 g; 12.2 mmol) in dried carbon disulfide (4 mL) was added anhydrous aluminum chloride (2.8 g) with stirring at room temperature overnight. Then the mixture was heated under reflux for 0.5 h. After removal of the solvent by decantation, the residue was poured into ice-cold diluted hydrochloric acid. The precipitate was sucked and crystallized from methanol to give compound **12** as yellow crystals (3.3 g; 92%). Mp 122–123 °C (lit., 121–122 °C). ^{1}H NMR δ: 3.68 (s, 3H, 3-OCH_3), 3.72 (s, 3H, 4′-OCH_3), 3.88 (s, 3H, 4-OCH_3), 4.27 (s, 2H, $ArCH_2$), 6.70 (d, 1H, J = 9 Hz, H-5), 6.87 (d, 2H, J = 8 Hz, H-3′, 5′), 7.20 (d, 2H, J = 8 Hz, H-2′, 6′), 7.87 (d, 1H, J = 9 Hz, H-6). 12.32 (s, 3H, 2-OH). ESI-MS $C_{17}H_{19}O_5$ $[M+H]^+$ 303. Anal. Calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.50; H, 6.03.

4.5.12. 2,4,4′,6-Tetrahydroxydeoxybenzoin (13).[21] A solution of *p*-hydroxyphenylacetonitrile (1 g; 7.5 mmol) and anhydrous phloroglucinol (0.94 g; 7.5 mmol) in dry ether (8 mL) was saturated with hydrogen chloride at 0 °C and set aside for 24 h. The solvent was removed by decantation. Fifteen microliters of water was added and heated under reflux for 1 h with stirring. After cooling, yellow needles were separated and washed with water. Further purification was not necessary (1.4 g; 72%). Mp 258–260 °C with decomposition (lit., 259 °C). ^{1}H NMR δ:

4.21 (s, 2H, $ArCH_2$), 5.81 (s, 2H, H-3,5), 6.67 (d, 2H, $J = 8$ Hz, H-3′, 5′), 7.00 (d, 2H, $J = 8$ Hz, H-2′, 6′), 9.16 (s, 1H, 4′-OH), 10.34 (s, 1H, 4-OH), 12.21 (s, 2H, 2,6-OH). ESI-MS $C_{14}H_{13}O_5$ $[M+H]^+$ 260. Anal. Calcd for $C_{14}H_{12}O_5$: C, 64.61; H, 4.65. Found: C, 64.67; H, 4.62.

4.5.13. 2,4,4′,6-Tetrahydroxy-3-methoxyphenyldeoxybenzoin (14).[37] A similar treatment described for **13** of *p*-hydroxyphenylacetonitrile (266 mg, 2 mmol) with anhydrous 1,3,5-trihydroxy-2-methoxybenzene (312 mg, 2 mmol) afforded compound **14** as yellow needles. Further purification was not necessary (380 mg, 66%). Mp 224–225 °C (lit., 228 °C). ^{1}H NMR δ: 3.59 (s, 3H, 3-OH), 4.22 (s, 2H, $ArCH_2$), 5.91 (s, 1H, H-5), 6.67 (d, 2H, $J = 8$ Hz, H-3′, 5′), 7.01 (d, 2H, $J = 8$ Hz, H-2′, 6′), 9.21 (s, 1H, 4′-OH), 10.37 (s, 1H, 4-OH), 11.54 (s, 1H, 2-OH), 12.50 (s, 1H, 6-OH). ESI-MS $C_{15}H_{15}O_6$ $[M+H]^+$ 291. Anal. Calcd for $C_{15}H_{14}O_6$: C, 62.07; H, 4.86. Found: C, 62.11; H, 4.82.

4.5.14. 4-(*p*-Hydroxyphenethyl)pyrogallol (15). $NaBH_4$ (304 mg, 8 mmol) was added to a solution of pyrogallol (260 mg, 1 mmol) in 1.22 M NaOH (1.64 mL, 2 mmol) and water (5 mL), and the mixture was heated under reflux for 3 h. After cooling, 5 M hydrochloric acid was added to decompose the excess $NaBH_4$ on an ice-water bath. The mixture was extracted with EtOAc. After removal of the solvent, the resulting residue was purified over a silica gel column eluting with isopropanol–petroleum ether (1:4) to give compound **15** as yellowish oil which was crystallized from 5 M hydrochloric acid (pale brown needles, 210 mg, 85%). Mp 181–183 °C (begin to decompose above 160 °C). ^{1}H NMR δ: 2.62 (s, 4H, $2ArCH_2$), 6.17 (d, 1H, $J = 8$ Hz, ArH-6), 6.30 (d, 1H, $J = 8$ Hz, H-5), 6.64 (d, 2H, $J = 8$ Hz, H-3′, 5′), 6.97 (d, 2H, $J = 8$ Hz, H-2′, 6′), 8.04 (br s, 2H, 2,3-OH), 8.74 (s, 1H, 1-OH), 9.06 (s, 1H, 4′-OH). ESI-MS $C_{14}H_{14}O_4Na$ $[M+Na]^+$ 269. Anal. Calcd for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.23; H, 5.69.

4.5.15. 6-(1-Hydroxy-2-(4-methoxyphenyl)ethyl)-2,3-dimethoxyphenol (16). Acetic acid (0.1 mL) is added with cooling to $NaBH_4$ (25 mg, 0.6 mmol) in 1 mL THF. After the evolution of hydrogen has ceased (about 3 h) compound **12** is added at room temperature with stirring for 4 h. The reduction is interrupted by adding 1 mL of water, the mixture neutralized with aqueous $NaHCO_3$, and the reaction product isolated by extraction with EtOAc. After drying and evaporation, compound **16** is afforded as pale yellow solid (83 mg, 91%). Mp 87–89 °C. ^{1}H NMR δ: 2.60 (d × d, 1H, $J = 14$ Hz, $J = 8$Hz $ArCH_2$), 2.83 (d × d, 1H, $J = 14$ Hz, $J = 4$Hz, $ArCH_2$), 3.66 (s, 3H, 2-OCH_3), 3.70 (s, 3H, 1-OCH_3), 3.75 (s, 3H, 4′-OCH_3), 4.90 (q, 1H, Ar C(OH)H, $J = 8$ Hz, $J = 4$Hz), 6.46 (d, 1H, $J = 9$ Hz, H-5), 6.79 (d, 2H, $J = 9$ Hz, H-3′, 5′), 6.95 (d, 1H, $J = 9$ Hz, H-6), 7.07 (d, 2H, H-2′,6′, $J = 9$ Hz), 8.76 (s, H, 3-OH). ESI-MS $C_{17}H_{19}O_4$ $[M-H_2O+H]^+$ 287. Anal. Calcd for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62. Found: C, 667.11; H, 5.59.

4.5.16. 7,8,4′-Trihydroxyisoflavone (17). Compound **5** (260 mg, 1 mmol) in anhydrous DMF (1.6 mL) was treated cautiously with $BF_3{\cdot}Et_2O$ (0.4 mL, 3.3 mmol) on the ice-water bath. To this mixture was added a solution of CH_3SO_2Cl (0.3 mL, 3 mmol) in anhydrous DMF (0.7 mL) at 50 °C. The mixture was heated at 80 °C for 3 h. The cooling product was poured into ice-cold aqueous NaOAc (50 mL, wt% = 10%). The mixture was extracted with EtOAc. After removal of the solvent, the residue was purified via crystallization from aqueous methanol (v% = 85%) to give compound **17** as colorless needles (190 mg, 70%), which decomposed over 180 °C. ^{1}H NMR δ: 6.80 (d, 2H, $J = 7$ Hz, H-3′, 5′), 6.95 (d, 1H, $J = 8$ Hz, H-6), 7.38 (d, 2H, $J = 7$ Hz, H-2′, 6′), 7.46 (d, 1H, $J = 8$ Hz, H-5), 8.33 (s, 1H, H-2), 9.44 (s, 1H, 4′-OH), 9.52 (s, 1H, 8-OH), 10.31 (s, H, 7-OH). ESI-MS $C_{15}H_{11}O_5$ $[M+H]^+$ 271. Anal. Calcd for $C_{15}H_{10}O_5$: C, 66.67; H, 3.73. Found: C, 66.61; H, 3.75.

4.5.17. Tectorigenin (20) and ψ-tectorigenin (18).[37] A similar treatment described for **17** of compound **15** (203 mg, 0.7 mmol) in anhydrous DMF (1.6 mL) with CH_3SO_2Cl (0.3 mL, 3 mmol) in anhydrous DMF (0.7 mL) afforded a yellow precipitate. The precipitate was separated and purified by a flash chromatography with EtOAc–petroleum ether (1:1) to give two fractions. The first fraction gave compound **18** as pale yellow needles (76 mg, 36%). Mp 230–232 °C (lit., 228–230 °C). ^{1}H NMR δ: 3.75 (s, 1H, OCH_3), 6.50 (s, 1H, H-6), 6.81 (d, 2H, $J = 8$ Hz, H-3′, 5′), 7.37 (d, 2H, $J = 8$ Hz, H-2′, 6′), 8.34 (s, 1H, H-2), 9.60 (s, 1H, 4′-OH), 10.78 (s, 1H, 7-OH), 13.06 (s, H, 5-OH). ESI-MS $C_{16}H_{13}O_6$ $[M+H]^+$ 301. Anal. Calcd for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03. Found: C, 63.96; H, 4.05. The second fraction gave compound **19** as pale yellow needles (85 mg, 40%). Mp 241–243 °C (lit., 240 °C). ^{1}H NMR δ: 3.76 (s, 1H, OCH_3), 6.31 (s, 1H, H-8), 6.81 (d, 2H, $J = 8$ Hz, H-3′, 5′), 7.37 (d, 2H, $J = 8$ Hz, H-2′, 6′), 8.41 (s, 1H, H-2), 9.60 (s, 1H, 4′-OH), 10.83 (s, 1H, 7-OH), 12.65 (s, H, 5-OH). ESI-MS $C_{16}H_{13}O_6$ $[M+H]^+$ 301. Anal. Calcd for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03. Found: C, 64.05; H, 4.01.

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