82. Synthesis of Coumarins and Derivatives

Part 1

Biomimetic Synthesis of Esculetin and Halogenated Derivatives[1])

by **Fernanda Borges** and **Madalena Pinto***

Laboratório de Química Orgânica, Faculdade de Farmácia do Porto, Rua Aníbal Cunha, 4000-Porto, Portugal

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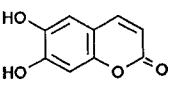
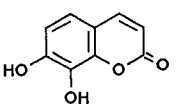
Esculetin (**1**) and the novel compounds 5-chloroesculetin (**5**) and 5-bromoesculetin (**6**) were obtained from a light-induced cyclization of *trans*-caffeic acid (**3**) catalyzed by [FeNa(edta)] and/or H_2SO_4, HCl, or HBr (*Scheme 1*). The experimental conditions for *trans-cis*-isomerization of the cinnamic-acid derivative **3** and subsequent non-enzymatic cyclization were described. The photoperiod and the presence of air and iron-chelate catalyst are shown to be important parameters that markedly affect yields. The reactions probably occur by a free-radical mechanism involving a photo-initiated one-electron redox process (*Scheme 2*).

1. Introduction. – During phytochemical studies of the phenolic content of several plant extracts, *van Sumere et al.* [1] found that the occurrence of certain coumarin derivatives could be due to an artefact. The presence of important quantities of esculetin (= 6,7-dihydroxy-2*H*-1-benzopyran-2-one; **1**) and sometimes also of small amounts of daphnetin (7,8-dihydroxy-2*H*-1-benzopyran-2-one; **2**) was attributed to a nonenzymatic process in which *trans*-caffeic acid (= (*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid; **3**) is involved.



1



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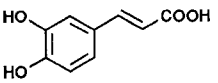

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For this reason, we tried to obtain hydroxylated coumarins by a biomimetic process in which the cinnamic-acid derivatives corresponding to the key building blocks of target coumarins were used as starting materials [2]. The possibility of obtaining coumarins by light induction [1] and the recent growth of interest in solar energy to assist and carry out chemical reactions [3–5] were some of the reasons for choosing daylight to induce the photochemical reaction required to synthesize the lactonic compounds.

In this biomimetic synthesis based on biosynthetic pathways to coumarins [6] [7], a *trans-cis*-isomerization of cinnamic-acid derivatives and nonenzymatic cyclization were

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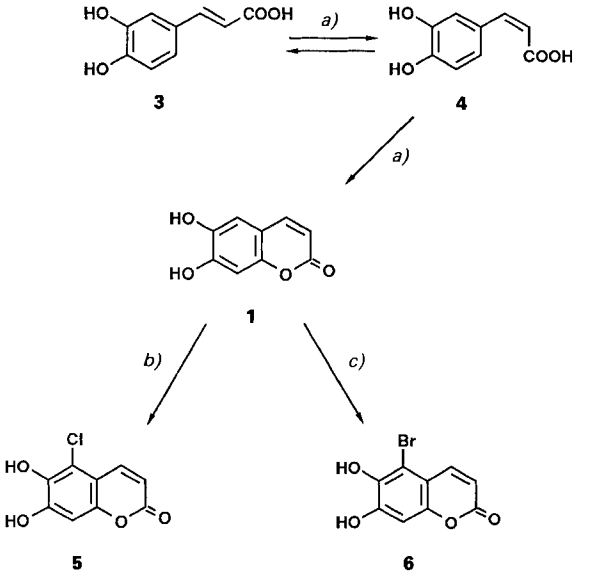
required to allow spontaneous lactonization. While some methods for such an isomerization were reported [8–11], relatively little is known about their photocyclization. *Pandey et al.* [12] proposed that a photo-induced single-electron transfer (SET), in which an electron acceptor is needed for the photoreaction, could lead to aromatic radical cations and provoke the intramolecular cyclization. This direct cyclization was also reported in enzymatic studies of cinnamic acids as a key step for their lactonization [13]. However, free-radical aromatic hydroxylation using H_2O_2 or O_2 and electron donors such as transition metals, which mimic the mixed-function oxidases, should also be considered since hydroxylation in position 2 or 6 of *cis*-cinnamic-acid derivatives could allow spontaneous lactonization [14–17].

In our work, various conditions for the photocyclization of *trans*-caffeic acid (**3**) were studied using EtOH as solvent and [FeNa(edta)] and/or H_2SO_4, HCl, or HBr as catalysts, resulting in the formation of esculetin (**1**) and of two halogenated derivatives **5** and **6**. The effectiveness of [FeNa(edta)] as a catalyst in these experiments was assessed by an increase of the reaction rates.

2. Results and Discussion. – 2.1. *Synthesis of Esculetin* (**1**), *5-Chloroesculetin* (**5**), *and 5-Bromoesculetin* (**6**). Ethanolic solutions of *trans*-caffeic acid (**3**) were submitted to diffuse daylight (see *Exper. Part*, solutions *A* and *B*). The *trans-cis*-isomerization **3** → **4** and formation of **1** could be observed by TLC, but a HPLC method was developed for rapid identification and quantification of the products [18]. Thus, the rapid isomerization equilibrium **3** ⇄ **4** seems to be affected by an increase in the yield of **1** during the

Scheme 1



a) *hv*, EtOH, r.t., without/with [FeNa(edta)].
b) *hv*, EtOH/1N HCl 1:1, r.t., without/with [FeNa(edta)].
c) *hv*, EtOH/1N HBr 1:1, r.t., without/with [FeNa(edta)].

photoreaction (*Fig. 1*). The yield of **1** was better in diluted than in concentrated solutions. *Fig. 2* shows the effect of the [FeNa(edta)] catalyst on the synthesis of **1** (solutions *C* and *D*): the catalyst is effective either in concentrated or diluted solution. Comparative data of esculetin yield in solutions *A–D* are shown in *Fig. 3*. To confirm the light-induced free-radical reaction, aliquots of the solutions of **3** were kept in the dark: neither *cis*-caffeic acid (**4**) nor esculetin (**1**) were detected, even not when [FeNa(edta)] was used as catalyst (*Figs. 1* and *2*). Exposure of deoxygenated solutions of **3**, to diffuse daylight, showed that the formation of **1** is more efficient in the presence of air and improved by an increase in the photoperiod.

Neither daphnetin (**2**) nor side-chain oxidation products were detected under all the experimental conditions.

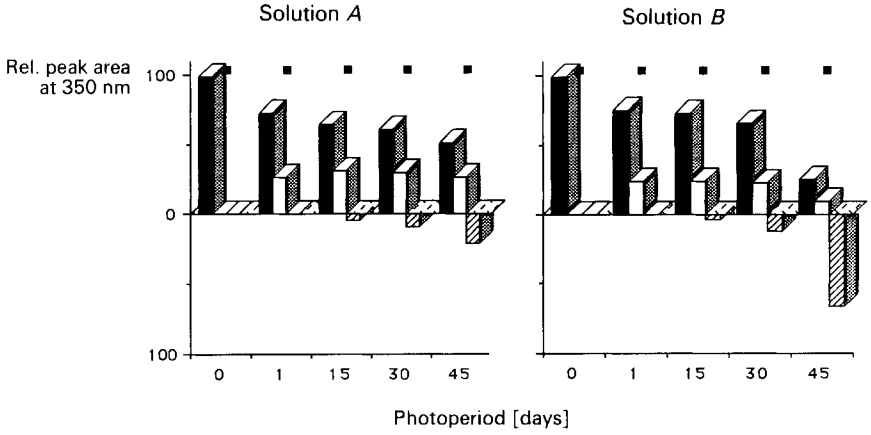


Fig. 1. *Product composition* vs. *photoperiod of solutions* A *and* B *of* **3** (10 and 1 mg/ml, resp., EtOH). ■ *trans*-Caffeic acid (**3**), □ *cis*-caffeic acid (**4**), ▨ esculetin (**1**). When the solutions were kept in the dark, only **3** was detected (▪).

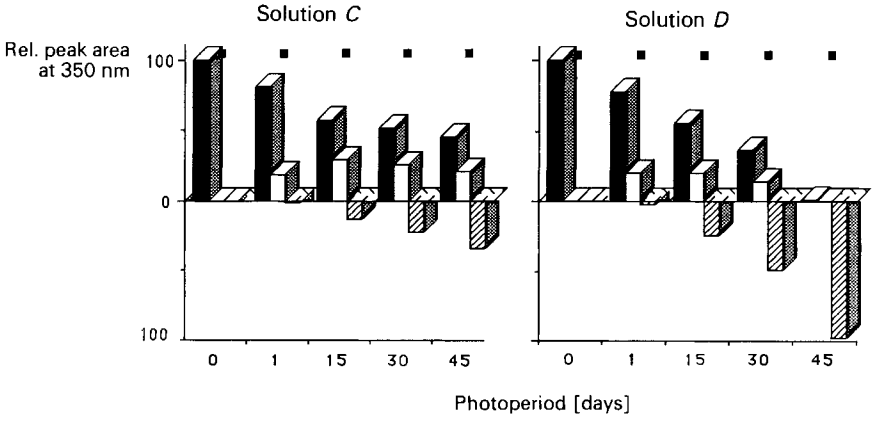


Fig. 2. *Product composition* vs. *photoperiod of solutions* C *and* D (10 mg of **3** and 0.368 mg of [FeNa(edta)]/ml and 1 mg of **3** and 0.0368 mg of [FeNa(edta)]/ml, resp.; EtOH). ■ *trans*-Caffeic acid (**3**), □ *cis*-caffeic acid (**4**), ▨ esculetin (**1**). When the solutions were kept in the dark, only **3** was detected (▪).

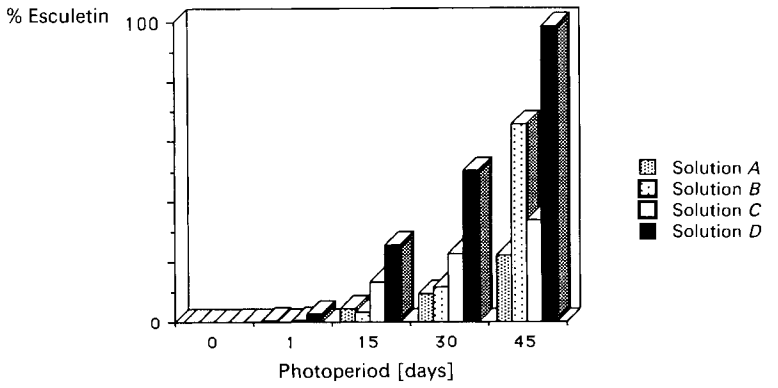


Fig. 3. *Effect of [FeNa(edta)] on the oxidation of* trans-*caffeic acid* (**3**): *Yields of esculetin* (**1**) *from solutions* A–D (see *Figs. 1* and *2*)

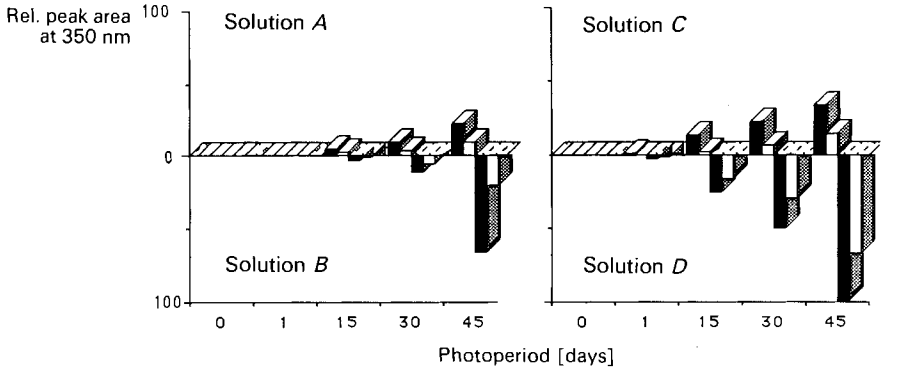


Fig. 4. *Effect of different storage conditions on the yield of esculetin* (**1**) *in solutions* A–D (see *Figs. 1* and *2*). ■ Air, □ N_2 atmosphere.

2.2. *Mechanistic Aspects.* According to the results described herein and data previously reported [19–21], the selective oxidation of *trans*-caffeic acid (**3**) to esculetin (**1**) is believed to be typically photochemical in origin and depending on *cis*-caffeic acid (**4**) formation. The dependence of the reaction on light and O_2 partial pressure and the ability of transition metals to act as catalysts show that the reaction could be, at least partly, an autooxidation. In this case, the O_2 required for the process could be derived from air in a subsequent stage.

As the reaction vessels were kept closed during photoreaction and analysis to prevent further oxygenation by atmospheric O_2 and as the yield of **1** improved with time, an autocatalytic cycle is thought to occur; *i.e.* on autooxidation of *cis*-caffeic acid (**4**), generated by photoinduction from *trans*-caffeic acid (**3**), a 'semiquinoid intermediate'[2]) and H_2O_2 could be produced (see *Scheme 2*). In the presence of transition metals such as

[2]) As daphnetin (**2**) was not found among the products, it is concluded that the more electrophilic position in the aromatic ring of caffeic acid [21] is not involved in its selective oxidation.

Fe^{3+} or its chelated form, a second catalytic cycle could operate to generate OH· or OOH· radicals (whose formation is pH dependent) from H_2O_2 produced during autooxidation. These radicals could oxidize caffeic acid leading to its radical intermediate which, in turn, could consume O_2 and produce $O_2^{\cdot-}$. The former radical can trigger the reaction of autooxidation and the latter can reduce Fe^{3+} to Fe^{2+} which can further generate OH· or OOH· *via* either *Haber-Weiss* reaction or a superoxide *Fenton* reaction [22]. In this way, a pool of powerful one-electron oxidizing agents may be obtained which could act as important driving force in the production of **1**.

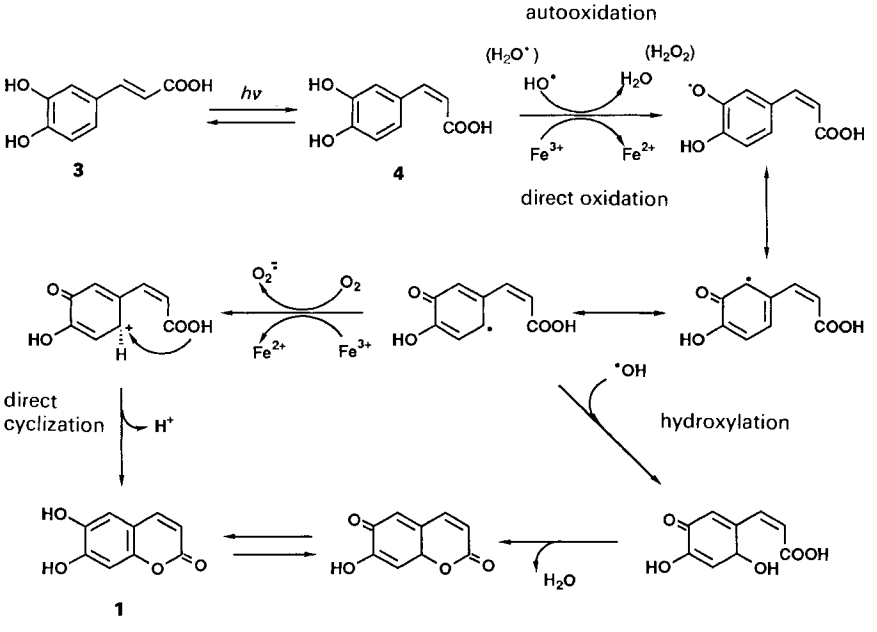
As yields of **1** in the reaction without [FeNa(edta)] and in those with [FeNa(edta)] but without O_2 are almost equal to the yields of **1** in the reaction with the presence of both [FeNa(edta)] and atmospheric O_2 (*Table 1*), a direct oxidation is thought to occur besides autooxidation, which could play an important role in the redox system described herein.

Table 1. *Yields of Esculetin* (**1**) *Obtained on Oxidation under Various Conditions of* trans-*Caffeic Acid* (**3**) *in EtOH, after a Photoperiod of 15 Days* (solutions *B* and *D*, see *Exper. Part*)

Conditions	Yield[a]) [%]
Soln. purged with N_2	1.7
Presence of air	3.3
[FeNa(edta)] added, soln. purged with N_2	17.1
[FeNa(edta)] added, presence of air	23.2

[a]) Determined by HPLC [18].

Scheme 2



Thus, on the way to esculetin (**1**), the autooxidation and direct oxidation are regarded as key steps which can contribute to the formation of the proposed radical intermediate[3]) (*Scheme 2*). During this process, further oxidation of the radical intermediate is thought to occur, leading to the quinone form of caffeic acid, which, in a one-electron process assisted by one-electron oxidants, could also produce the semiquinone radical.

2.3. *Effect of Inorganic pH Modifiers.* To obtain more information about the photooxidation of **3** in EtOH, the pH of the solutions was modified by addition of 1N HCl, 1N HBr, or 1N H_2SO_4: again *trans-cis* isomerization and formation of **1** were observed by TLC [23].

In solutions *G* and *H*, catalyzed by HCl/[FeNa(edta)] (see *Exper. Part*), a great improvement of the yield of **1** was observed, as compared to the solutions *E* and *F* catalyzed by HCl alone for the same period. Thus, the reaction rate depends only on the addition of [FeNa(edta)].

However, in solutions *F*, *H*, and *J*, catalyzed by HCl or HBr, other compounds exhibiting a yellow fluorescence appeared in the reaction mixture, their increasing amount matching the disappearance of **1**. After determinate photoperiod (*Table 2*), no cinnamic-acid derivatives and esculetin (**1**) were observed by TLC[4]). The only remaining compounds were identified as 5-chloroesculetin (**5**) and 5-bromoesculetin (**6**).

These results suggest that **5** and **6** were derived directly from the first formed dihydroxycoumarin **1**. Indeed when **1** was used as starting material (data not reported), these derivatives were obtained in a few days.

In all acidic *trans*-caffeic-acid solutions (see *Table 2*) that were kept in the dark, another compound was detected by TLC which was identified as ethyl *trans*-caffeate (see *Exper. Part*). This compound and its geometrical isomer were also found in the acidic *trans*-caffeic-acid solutions exposed to light, but after the disappearance of the parent acids **3** and **4**, these esters were transformed into esculetin (**1**).

Table 2. *TLC Detection of the Products from Acidic* trans-*Caffeic-Acid* (**3**) *Solutions, after a Photoperiod of 90 Days*

Solution	Catalyst	pH	Main products (by TLC)				
			3[a])	**4**	**1**	**5**	**6**
E	HCl	1–2	+	+	+	–	–
F[b])	HCl	3–4	+	+	+	–	–
G	HCl/[FeNa(edta)]	1–2	+	+	+	–	–
H	HCl/[FeNa(edta)]	3–4	–	–	–	+	–
I	HBr/[FeNa(edta)]	0–1	+	+	+	–	–
J[c])	HBr/[FeNa(edta)]	1–2	+	+	+	–	+
L	H_2SO_4/[FeNa(edta)]	1–2	+	+	+	–	–
M	H_2SO_4/[FeNa(edta)]	3–4	–	–	+	–	–

[a]) Unreacted substrate.

[b]) After a longer photoperiod, formation of 5-chloroesculetin (**5**) was detected.

[c]) After a longer photoperiod, the only compound in solution was 5-bromoesculetin (**6**).

[3]) As the reaction occurs at different pH (see *Table 2* and *Exper. Part*), caffeic acid and its semiquinone radical are represented in their neutral form, for simplification.

[4]) The comparative experimental data between solutions *F* and *H* allowed us to conclude that the rate of halogenation depends only on the addition of [FeNa(edta)], for the same photoperiod.

The formation of the halogenated derivatives can be explained by a photochemical process, since no halogenated compounds were detected in the dark. In this process, the generation of $Cl^{\cdot}$ and $Br^{\cdot}$ radicals in the reaction medium may occur [24] [25]. Since halogen radicals are electrophilic, they could, under the above experimental conditions, be responsable for the formation of the 5-halogenated aromatic derivatives.

3. Conclusion. – This work shows that the photoperiod and the presence of air and iron-chelate catalyst play an important role in the biomimetic formation of esculetin (**1**) from *trans*-caffeic acid (**3**). The dependence of the reaction on light and on O_2 partial pressure and the ability of transition-metal ions to act as catalysts show that the transformation is, at least partly, an autooxidation. The mechanism by which caffeic acid promotes oxygen-radical reactions is not entirely clear but may well involve its ability for autooxidation, catalyzed by transition-metal chelates. The detailed investigation of this oxidative transformation establishes the optimum conditions for the conversions **3** → **1** → **5** or **6**. The results obtained so far give an interesting clue to a promising biomimetic method for the obtention of coumarins and derivatives.

The light-induced halogenation of the aromatic ring, achieved by [FeNa(edta)] at room temperature, is currently tested with other heterocyclic compounds [26].

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Experimental Part

1. *General.* For TLC, HPLC, and reagents, see [18] [23]. Solvents were evaporated in a *Büchi Rotavapor*. ^{1}H-NMR Spectra ($(D)_6$DMSO): *Bruker* pulse NMR sprectrometer *C.P.* (300 MHz); chemical shifts δ in ppm rel. to TMS as internal reference and coupling constants J in Hz. MS: *Hitachi-Perkin-Elmer-RMU-6M* apparatus; m/z (% of most important fragments).

2. For photochemical studies several solns. were prepared: *Solutions* A–M *of* trans-*Caffeic Acid* (**3**). Soln. *A*: **3** (10 mg/ml), EtOH; soln. *B*: **3** (1 mg/ml), EtOH; soln. *C*: **3** (10 mg/ml), [FeNa(edta)] (0.368 mg/ml), EtOH; soln. *D*: **3** (1 mg/ml), [FeNa(edta)] (0.0368 mg/ml), EtOH; soln. *E*: **3** (10 mg/ml), EtOH/1N HCl 1:1; soln. *F*: **3** (1 mg/ml), EtOH/1N HCl; soln. *G*: **3** (10 mg/ml), [FeNa(edta)] (0.368 mg/ml), EtOH/1N HCl 1:1; soln. *H*: **3** (1 mg/ml), [FeNa(edta)] (0.0368 mg/ml), EtOH/1N HCl; soln *I*: **3** (10 mg/ml), [FeNa(edta)] (0.368 mg/ml), EtOH/1N HBr 1:1; soln. *J*: **3** (1 mg/ml), [FeNa(edta)] (0.0368 mg/ml), EtOH/1N HBr; soln. *L*: **3** (10 mg/ml), [FeNa(edta)] (0.368 mg/ml), EtOH/1N H_2SO_4 1:1; soln. *M*: **3** (1 mg/ml), [FeNa(edta)] (0.0368 mg/ml), EtOH/1N H_2SO_4. Solns. *F*, *H*, *J*, and *M* were obtained by diluting solns. *E*, *G*, *I*, and *L*, resp. with EtOH, resulting in a pH change (see *Table 2*). To increase the rate of dissolution of **3** and [FeNa(edta)] in the more concentrated solns. sonication (5 min) was used.

3. *Oxidative Transformation of* **3**. The reactions were performed with 1.0 ml of each soln. (see above) in thick-wall, 3.7-ml capacity, screw-stopped glass vials with *Teflon*-lined caps that were kept closed during the photoperiods and analysis. The solns. were all stored at r.t. on the laboratory bench, exposed to either artificial light or daylight from the windows (an aliquot part was kept in the dark). To prevent changes in composition, all manipulations of the solns. had to be carried out in subdued light. The compositions were determined TLC and HPLC. Results: *Figs. 1* and *2*.

Solns. were deoxygenated, when required, by degassing and flushing with a slow stream of N_2 during 10 min (syringe needles as glass inlet and outlet; inlet near the bottom of the tube, outlet above the surface of the liquid). The gas flow was such that solvent loss was negligible. The compositions were determined by HPLC: *Fig. 4*.

4. *5-Chloro-6,7-dihydroxy-2*H*-1-benzopyran-2-one* (**5**) *and 5-Bromo-6,7-dihydroxy-2*H*-1-benzopyran-2-one* (**6**) were obtained from solns. *H* and *J* resp., after the following workup: the soln. (100 ml) was diluted with AcOEt

(100 ml), the mixture washed with H_2O to neutral, the org. layer dried (Na_2SO_4) and evaporated, the dark oily residue purified by column chromatography (cellulose, AcOEt). The crude products were then recrystallized (EtOH) to give pale yellow needles.

Data of **5**: ^{1}H-NMR: 6.30 (*d*, $J = 9.7$, H–C(3)); 6.76 (*s*, H–C(8)); 8.01 (*d*, $J = 9.4$, H–C(4)). MS: 214 (28, $[M + 2]^+$), 212 (80, $M^{+\cdot}$), 186 (36) and 184 (100, $[M - CO]^+$), 149 (6, $[M - COCl]^+$).

Data of **6**: ^{1}H-NMR: 6.33 (*d*, $J = 9.7$, H–C(3)); 6.81 (*s*, H–C(8)); 8.08 (*d*, $J = 9.7$, H–C(4)). MS: 258 (96, $[M + 2]^+$), 256 (100, $M^{+\cdot}$), 230 (89) and 228 (92, $[M - CO]^+$), 149 (20, $[M - COBr]^+$).

5. *Ethyl* trans-*caffeate* (= *ethyl 3-(3,4-dihydroxyphenyl)prop-2-enoate*) was obtained from an acidic soln. (100 ml) after workup as described in *Exper.4*. The dark oily residue was purified by prep. TLC (cellulose, H_2O/AcOH 9:1). After separation from the cellulose with AcOEt and crystallization from aq. MeOH, light yellow crystals were obtained. The caffeate was identifical (chromatographic and spectral data) with a synthetic sample prepared by the method used for similar cinnamates [27].

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