

CHEMICAL TRANSFORMATION FROM DIHYDROISOCOUMARIN INTO BENZYLIDENE-PHTHALIDE BY USE OF REGIOSPECIFIC OXIDATIVE LACTONIZATION MEDIATED BY COPPER CHLORIDE (II) - SYNTHESIS OF THUNBERGINOL F AND HYDRAMACROPHYLLOL A AND B -

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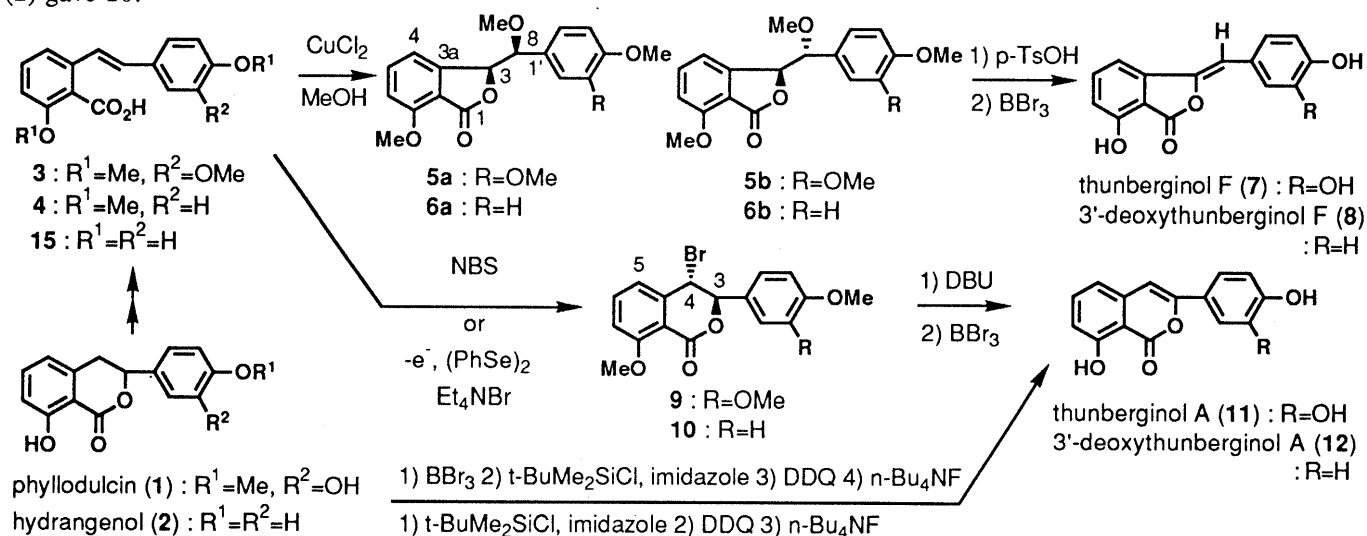
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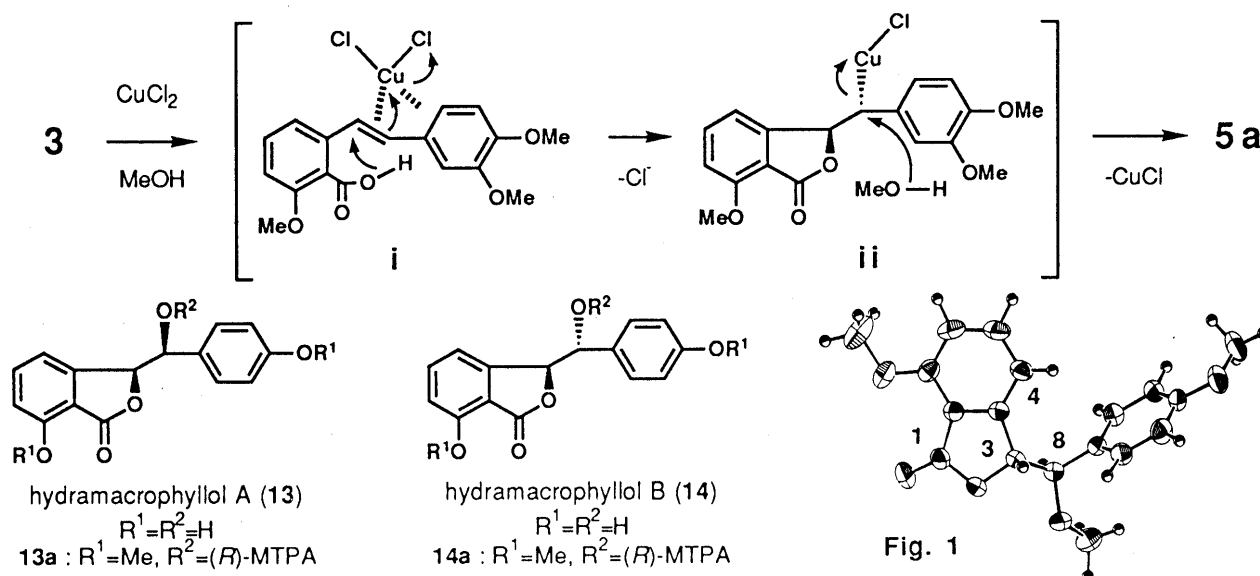
Oxidative lactonization of 2-carboxystilbene mediated by CuCl_2 proceeded regiospecifically to give the five-membered lactone. By utilizing this lactonization as a key reaction, chemical transformation from dihydroisocoumarin into benzylidene-phthalide was accomplished, and it was applied to structural elucidation of two new phthalide, hydramacrophyllols A and B.

KEYWORDS dihydroisocoumarin; benzylidene-phthalide; oxidative lactonization; copper chloride (II); thunberginol F; hydramacrophyllol

In the course of our studies in the search for biologically active constituents from naturally occurring drug materials,¹⁾ we have clarified two isocoumarins and a benzylidene-phthalide named thunberginol A (11), B, and F (7) as anti-allergic and anti-microbial (against oral bacteria) principles from *Hydrangea Dulcis* Folium.²⁾ Furthermore, pharmacological assessment for 7 and 11 showed them to have much more potent anti-allergic activity than dihydroisocoumarins such as phyllodulcin (1) and hydrangenol (2), which are major constituents of the crude drug (ca. 21.4% from the MeOH extract).³⁾ Isocoumarins (ex. 11) were readily synthesized from dihydroisocoumarins (ex. 1) in high yields. On the other hand, a few methods for construction of a benzylidene-phthalide skeleton have been developed;⁴⁾ they, however, seem to be impractical for synthesizing highly oxygen-functionalized compounds or in taking total yield into consideration. Herein we describe the chemical transformation from dihydroisocoumarin into benzylidene-phthalide utilizing copper chloride (II) (CuCl_2)-mediated regiospecific lactonization of 2-carboxystilbene.

Isocoumarins (11 and 12)⁵⁾ were derived from 1 and 2 in 86% and 90% yields: 1) demethylation of 1, 2) *t*-butyldimethylsilylation of hydroxyl groups in demethylphyllodulcin and 2, 3) dehydrogenation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 4) deprotection with *n*- Bu_4NF . In order to synthesize benzylidene-phthalide, several lactonizations of 2-carboxystilbene readily prepared from dihydroisocoumarin were examined. Bromolactonization of 2-carboxy-3,3',4'-trimethoxystilbene (3)⁶⁾ derived from phyllodulcin (1) with *N*-bromosuccinimide (NBS) in DMF afforded only 9⁷⁾ as lactone derivatives in 66% yield. Bromonium cation, electrochemically generated with $(\text{PhSe})_2$ and Et_4NBr ,⁸⁾ induced lactonization of 3 to also give 9 exclusively (46%). The IR spectrum of 9 showed an absorption band due to a carbonyl group in a six-membered lactone at 1732 cm^{-1} . In the $^1\text{H NMR}$ spectrum, the signals due to one methine proton bearing a bromine at $\delta\ 5.49$ (d, $J=4.0$) and another one adjacent to lactone-oxygen at $\delta\ 5.76$ (d, $J=4.0$) appeared. Additionally, the NOE enhancements were observed in the following pairs of protons (3-H & 2', 6'-H, 4-H & 5-H) in the difference NOE spectra. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) treatment of 9 followed by demethylation with BBr_3 provided thunberginol A (11).²⁾ Based on the above findings, the structure of 9 was established as shown. In a similar manner, 4 obtained from hydrangenol (2) gave 10.





Olefin-lactonization using oxidative metal salts would introduce a nucleophilic substituent with construction of a lactone ring involving reductive elimination of metal salts. Thus, lactonizations of **3** with various oxidative metal salts were examined to prepare desired five-membered lactones, which would be precursors of benzylidenephthalide. Heating under reflux of **3** with CuCl_2 in MeOH facilitated oxidative lactonization to provide **5a**⁹⁾ and **5b**⁹⁾ in 70% and 15% yields. The ^1H NMR spectrum of **5a** showed the signals ascribable to one aliphatic methoxyl group (δ 3.35) and two methine protons linked to oxygen functions of which one was a methoxyl group [δ 4.46 (1H, d, $J=5.9\text{Hz}$)], the other was lactone-oxygen [δ 5.58 (1H, d, $J=5.9\text{Hz}$)]. In the IR spectrum, a carbonyl absorption band appeared at 1767cm^{-1} , indicative of a five-membered lactone. Furthermore, the different NOE spectra exhibited the enhancements in the pairs of protons (3-H & 4-H, 8-H & 2', 6'-H). The plane structure of **5a** was finally confirmed by the ^{13}C - ^1H correlation *via* long-range coupling (COLOC) spectrum, in which 3-H and 8-H were correlated with 3a-C and 1'-C, respectively. Similarity in the physicochemical properties of **5a** and **5b** except for the coupling constants between 3-H and 8-H in the ^1H NMR spectra showed them to be stereoisomers. The presumption was verified by the following chemical conversion. Both **5a** and **5b** by acidic treatment with *p*-TsOH in benzene completely afforded the more stable (*Z*)-benzylidenephthalide which was subjected to demethylation with BBr_3 furnished thunberginol F (**7**) in 61% yield from **5a** and **5b**.

With CuBr_2 in the place of CuCl_2 in the present lactonization, **5a** and **5b** were similarly prepared from **3** in 40% and 11% yields, respectively. No reaction was observed when CuCl was used. It therefore seems that the lactonization involves an oxidation process. For the purpose of confirming the structures of **5a** and **5b** and investigating reaction mechanism in the CuCl_2 -mediated lactonization, a single-crystal X-ray analysis of **5a** was carried out.¹⁰⁾ A perspective view as shown in Fig.1 disclosed the major product (**5a**) to be a 3,8-*syn*-lactone. On the basis of this finding, a plausible reaction mechanism of the lactonization is shown. Initial nucleophilic attack by a carboxyl group on α -carbon by chelation of copper (II) between the olefin and the electron-rich B-ring in stilbene (i) would generate regioselectivity in the lactonization to the five-membered lactone intermediate (ii). Nucleophilic substitution in $\text{S}_{\text{N}}2$ mode of MeOH to ii concomitant with reductive elimination of copper (II) would yield 3,8-*syn*-methoxylactone (**5a**). The *anti*-lactone (**5b**) was presumed to be similarly formed from (*Z*)-2-carboxystilbene, which was readily given by isomerism of **3** in the reaction medium.¹¹⁾ Application of the above described chemical transformation to hydrangenol (**2**), 3'-deoxyanalog (**8**)¹²⁾ of thunberginol F (**7**) was similarly synthesized *via* **6a** and **6b** in 71% yield from **4**.

On the other hand, two new phthalides named hydramacrophyllols A¹³⁾ (**13**, 0.00013%) and B¹³⁾ (**14**, 0.00047%) were isolated from the AcOEt-soluble portion of the MeOH extract in the course of the search for biologically active constituents of *Hydrangeae Dulcis Folium*. Hydramacrophyllol A (**13**), $[\alpha]_{\text{D}}^{25} -5.9^\circ$ (EtOH), UV [EtOH, nm (ϵ)] : 225 (17000), 301 (4800), was obtained as a white powder. The IR spectrum of **13** exhibited the presence of hydroxyl groups (3440cm^{-1}), a γ -lactone (1742cm^{-1}), and an aromatic ring (1617cm^{-1}), while the ^1H NMR spectrum showed the signals ascribable to a 1,2,3-trisubstituted benzene ring and a 1,4-disubstituted one. Furthermore, the coupled signals due to two methine protons adjacent to the hydroxy group and the lactone-oxygen also appeared in it. In the difference NOE experiments, the two pairs of protons, 3-H & 4-H, 8-H & 2', 6'-H, showed NOE enhancements. Hydramacrophyllol B (**14**), a white powder, $[\alpha]_{\text{D}}^{25} 0^\circ$ (EtOH), UV [EtOH, nm (ϵ)] : 226 (15000), 300 (4700), had spectral features fairly similar to **13** except for the coupling constants between the two oxymethine protons. Thus, hydramacrophyllol B (**14**) was assumed to be a stereoisomer of **13**. Finally, CuCl_2 -mediated lactonization of hydrageic acid (**15**)²⁾ obtained from **2** by alkaline treatment in 80% aq. acetone gave **13** (11%) and **14** (5%)¹⁴⁾. Respective conversion of **13** and **14** to **6a** and **6b** by treatment with CH_3I and NaH in DMF as well as

predominant formation of **13** in the lactonization established the relative stereochemistry of hydramacrophyllol A (**13**) and B (**14**). The optical purities of **13** and **14** were determined by HPLC analysis of the corresponding MTPA esters (**13a** and **14a**), which were prepared by methylation with CH_2N_2 followed by esterification with (*R*)- α -methoxy- α -trifluorophenyl-acetic acid (MTPA), dicyclohexylcarbodiimide, and 4-dimethylaminopyridine. The HPLC chromatogram of **13a** showed two peaks in a ratio of 62 : 38, while two peaks with nearly equal area were observed in the chromatogram of **14a**. This finding suggested that a part of **13** would be biogenetically synthesized in plants and **14** would be formed during the processing of the crude drug.

In the present chemical transformation, thunberginol F (**7**) was facilely synthesized from phyllo dulcin (**1**) in seven steps in 40.4% total yield. It is noted that individual employment of CuCl_2 -mediated lactonization and bromo-lactonization using the common 2-carboxystilbenes selectively lead to benzylidenephthalides and isocoumarins.

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- 5) **12** : pale yellow prisms (EtOH-AcOEt), mp 200-202°C, UV λ_{max} (EtOH) nm (ϵ) : 263 (20000), 310 (24000), 366 (23000), IR (KBr, cm^{-1}) : 3412, 1676, ^1H NMR (270MHz, CD_3OD) δ : 6.52 (1H, s, 4-H), 6.82 (2H, d, J=8.6, 3', 5'-H), 6.86 (1H, d, J=8.3, 5-H), 7.33 (1H, d, J=7.6, 7-H), 7.56 (1H, dd, J=7.6, 8.3, 6-H), 7.69 (2H, d, J=8.6, 2', 6'-H).
- 6) 2-Carboxy-3,3',4'-trimethoxystilbene (**3**) readily prepared from **1** by way of demethylation (BBr_3 in CH_2Cl_2), alkaline treatment (NaHCO_3 in aq. MeOH), methylation (CH_3I , K_2CO_3 , DMF), and saponification (KOH, EtOH) in 78% yield. Compound **4** was similarly prepared from **2** in 98% yield in three steps except for demethylation.
- 7) **9** : pale yellow needles (EtOH- $i\text{Pr}_2\text{O}$), mp 145-148°C, UV λ_{max} (EtOH) nm (ϵ) : 285 (4200), 309 (4800), IR (KBr, cm^{-1}) : 1732, ^1H NMR (270MHz, CDCl_3) δ : 3.80, 3.82, 3.96 (3H each, all s, OMe_3), 5.49 (1H, d, J=4.0, 4-H), 5.76 (1H, d, J=4.0, 3-H), 6.76 (3H, br s, 2', 3', 6'-H), 7.00 (1H, d, J=8.6, 5-H), 7.01 (d, J=7.6, 7-H), 7.50 (1H, dd, J=7.6, 8.6, 6-H), EI-MS m/z : 394 (M^+ , 1.1), 392 (M^+ , 1.2). The relative configuration of **9** was deduced by the reaction mechanism of bromolactonization.
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- 9) **5a** : colorless needles (EtOH), mp 170-173°C, UV λ_{max} (EtOH) nm (ϵ) : 233 (11000), 286 (3900), 300 (3700), IR (KBr, cm^{-1}) : 1767, ^1H NMR (270MHz, CDCl_3) δ : 3.35, 3.71, 3.85, 3.91 (3H each, all s, OMe_4), 4.46 (1H, d, J=5.9, 8-H), 5.58 (1H, d, J=5.9, 3-H), 6.61 (1H, br s, 6'-H), 6.66 (1H, d, J=8.2, 4-H), 6.78 (2H, br s, 2', 3'-H), 6.83 (1H, d, J=8.2, 6-H), 7.45 (1H, dd, J=8.2, 8.2, 5-H), EI-MS m/z : 181 (100), ^{13}C NMR (68MHz, CDCl_3) δ_c : 168.1 (1-C), 81.2 (3-C), 149.1 (3a-C), 115.5 (4-C), 135.4 (5-C), 110.7 (6-C), 158.2 (7-C), 114.3 (7a-C), 83.8 (8-C), 127.3 (1'-C), 129.3 (2', 6'-C), 113.6 (3', 5'-C), 159.8 (4'-C), **5b** : colorless needles (EtOH), mp 172-175°C, UV λ_{max} (EtOH) nm (ϵ) : 231 (6900), 286 (2700), 297 (2600), IR (KBr, cm^{-1}) : 1767, ^1H NMR (270MHz, CDCl_3) δ : 3.32, 3.86, 3.88, 3.96 (3H each, all s, OMe_4), 4.42 (1H, d, J=5.3, 8-H), 5.47 (1H, d, J=5.3, 3-H), 6.83 (2H, br s, 2', 3'-H), 6.84 (1H, br s, 6'-H), 6.86 (1H, d, J=7.6, 4-H), 6.89 (1H, d, J=8.2, 6-H), 7.52 (1H, dd, J=7.6, 8.2, 5-H), EI-MS m/z : 181 (100). Physical data of **6a**, **6b**, and **10** will be presented in a full paper.
- 10) crystal data : $\text{C}_{18}\text{H}_{18}\text{O}_5$, $M=314.34$, triclinic, $a=10.196$ (1), $b=10.724$ (1), $c=7.9454$ (9) \AA , $\alpha=102.492$ (9), $\beta=105.60$ (1), $\gamma=98.62$ (1) $^\circ$, $V=796.6$ (2) \AA^3 , $Z=2$, space group $p1$, $D_c=1.310\text{g/cm}^3$. All data were collected on Rigaku AFC5R diffractometer with MoKa radiation and a graphite monochromator. The structure was solved by direct methods and refined by full-matrix least-squares to an R factor of 0.042 for 2168 reflections.
- 11) After reflux of **3** in MeOH for 1h, ca. 20% of recovered substrate was isomerized.
- 12) **8** : pale yellow needles (EtOH-AcOEt), mp 193-195°C, UV λ_{max} (EtOH) nm (ϵ) : 231 (13000), 306 (8700), 319 (9600), 371 (18000), IR (KBr, cm^{-1}) : 1749, ^1H NMR (270MHz, CD_3OD) δ : 6.52 (1H, s, 8-H), 6.82 (2H, d, J=8.6, 3', 5'-H), 6.86 (1H, d, J=8.3, 4-H), 7.33 (1H, d, J=7.6, 6-H), 7.56 (1H, dd, J=7.6, 8.3, 5-H), 7.69 (2H, d, J=8.6, 2', 6'-H), EI-MS m/z : 254 (M^+ , 100). Full details on anti-allergic activity of thunberginols and their analogs will be published elsewhere.
- 13) **13** : $\text{C}_{15}\text{H}_{12}\text{O}_5$, ^1H NMR (270MHz, d_6 -acetone) δ : 5.00 (1H, d, J=4.8, 8-H), 5.65 (1H, d, J=4.8, 3-H), 6.71 (1H, d, J=7.3, 4-H), 6.77 (2H, d, J=8.6, 3', 5'-H), 6.87 (1H, d, J=7.6, 6-H), 7.20 (2H, d, J=8.6), 7.47 (1H, dd, J=7.3, 7.6, 5-H), EI-MS m/z : 123 (100). **14** : $\text{C}_{15}\text{H}_{12}\text{O}_5$, IR (KBr, cm^{-1}) : 3424, 3301, 1736, 1620, ^1H NMR (270MHz, d_6 -acetone) δ : 5.12 (1H, d, J=4.3, 8-H), 5.67 (1H, d, J=4.3, 3-H), 6.63 (1H, d, J=7.6, 4-H), 6.81 (1H, d, J=7.9, 6-H), 6.88 (2H, d, J=8.0, 3', 5'-H), 7.26 (2H, d, J=8.0, 2', 6'-H), 7.45 (1H, dd, J=7.6, 7.9, 5-H), EI-MS m/z : 123 (100).
- 14) Most of **15** was easily converted to **2** under this reaction condition, because of preferential acidic lactonization.

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