CHEMICAL TRANSFORMATION FROM DIHYDROISOCOUMARIN INTO BENZYLIDENEPHTHALIDE BY USE OF REGIOSPECIFIC OXIDATIVE LACTONIZATION MEDIATED BY COPPER CHLORIDE (II) – SYNTHESES OF THUNBERGINOL F AND HYDRAMACROPHYLLOL A AND B –

Masayuki YOSHIKAWA,*[,a] Emiko HARADA,[a] Nobuhiro YAGI,[a] Yasuhiro OKUNO,[a] Osamu MURAOKA,[b] Hiroshi AOYAMA,[b] and Nobutoshi MURAKAMI [a]

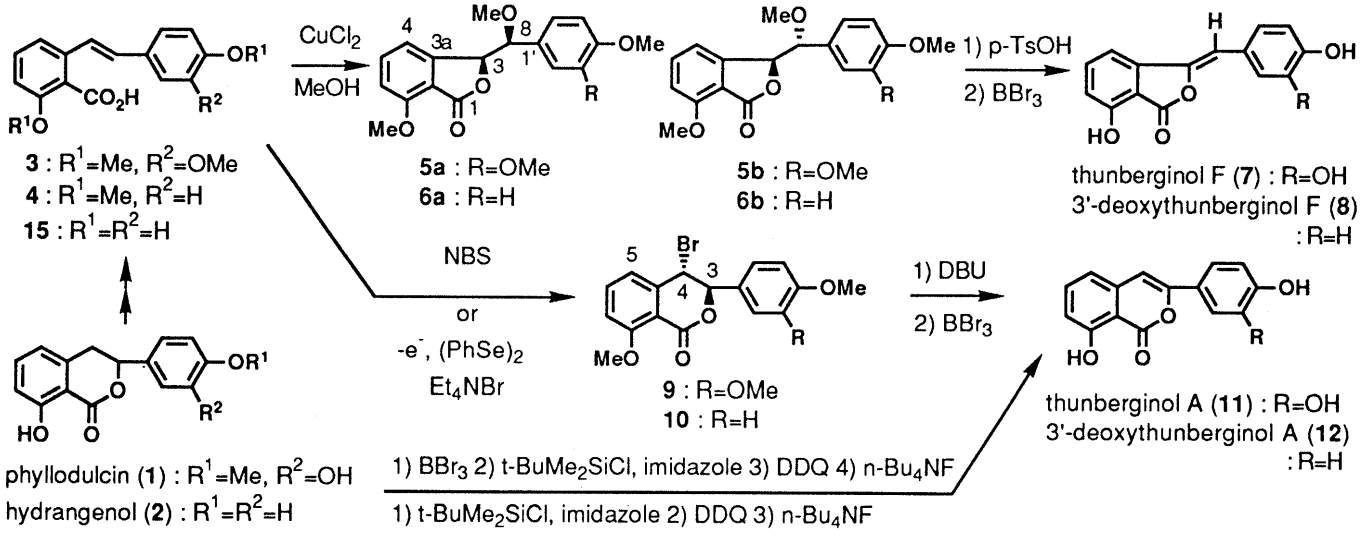
Kyoto Pharmaceutical University,[a] 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan and Faculty of Pharmaceutical Sciences, Kinki University,[b] Kowakae 3-4-1, Higashi-Osaka, Osaka 577, Japan

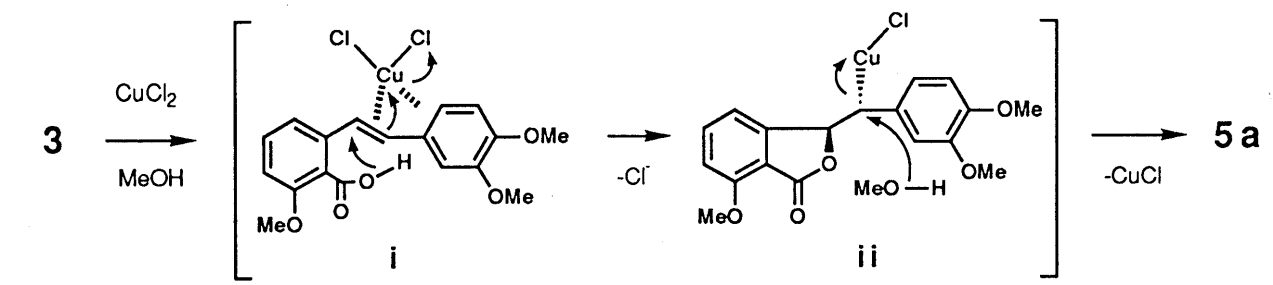
Oxidative lactonization of 2-carboxystilbene mediated by $CuCl_2$ proceeded regiospecifically to give the five-membered lactone. By utilizing this lactonization as a key reaction, chemical transformation from dihydroisocoumarine into benzylidenephthalide was accomplished, and it was applied to structural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

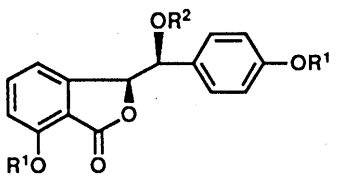
KEYWORDS dihydroisocoumarin; benzylidenephthalide; oxidative lactonization; copper chloride (II); thunberginol F; hydramacrophyllol

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 benzylidenephthalide 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 benzylidenephthalide 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 benzylidenephthalide utilizing copper chloride (II) ($CuCl_2$)-mediated regiospecific lactonization of 2-carboxystilbene.

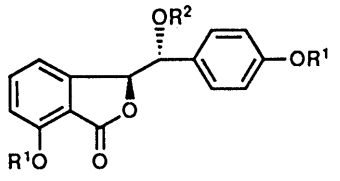
Isocoumarins (**11** and **12**)[5] were derived from **1** and **2** in 86% and 90% yields:1) demethylation of **1**, 2) t-butyldimethylsilylation of hydroxyl groups in demethylphyllodulcin and **2**, 3) dehydrogenation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 4) deprotection with n-Bu_4NF. In order to synthesize benzylidenephthalide, 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 **9**[7] as lactone derivatives in 66% yield. Bromonium cation, electrochemically generated with $(PhSe)_2$ 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 ^1H NMR spectrum, the signals due to one methine proton bearing a bromine at δ 5.49 (d, J=4.0) and another one adjacent to lactone-oxygen at δ 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**.







hydramacrophyllol A (**13**)
$R^1=R^2=H$
13a : R^1=Me, R^2=(*R*)-MTPA



hydramacrophyllol B (**14**)
$R^1=R^2=H$
14a : R^1=Me, R^2=(*R*)-MTPA

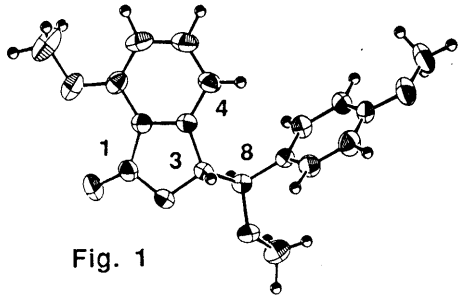


Fig. 1

Olefin-lactonization using oxdative 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=5.9Hz)], the other was lactone-oxygen [δ 5.58 (1H, d, J=5.9Hz)]. 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 regiospecificity in the lactonization to the five-membered lactone intermediate (**ii**). Nucleophilic substitution in S_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 A[13] (**13**, 0.00013%) and B[13] (**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**), $[\alpha]_D^{25}$ -5.9° (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 (3440 cm^{-1}), a γ-lactone (1742 cm^{-1}), and an aromatic ring (1617 cm^{-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, $[\alpha]_D^{25}$ 0° (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 phyllodulcin (**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.

REFERENCES AND NOTES

1) M. Yoshikawa, E. Harada, H. Matsuda, T. Murakami, J. Yamahara, N. Murakami, *Chem. Pharm. Bull.*, **41**, 2069 (1993).
2) M. Yoshikawa, E. Uchida, N. Chatani, N. Murakami, J. Yamahara, *Chem. Pharm. Bull.*, **40**, 3121 (1992).
3) M. Yoshikawa, E. Harada, H. Matsuda, N. Chatani, J. Yamahara, N. Murakami, Abstract of Papers, 9th Symposium on the Development and Application of Naturally Occurring Drug Materials Abstracts, Shizuoka, July 1993, P45.
4) a) C. E. Castro, E. J. Gaughan, D. C. Owsley, *J. Org. Chem.*, **31**, 4071 (1966); b) O. Villemin, D. Goussu, *Heterocycles*, **29**, 1255 (1989); c) S. Ohta, Y. Kamata, T. Inagaki, Y. Masuda, S. Yamamoto, M. Yamashita, I. Kawasaki, *Chem. Pharm. Bull.*, **41**, 1188 (1993).
5) **12** : pale yellow prisms (EtOH-AcOEt), mp 200-202°C, UV λmax (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-iPr_2O), mp 145-148°C, UV λmax (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, OMex3), 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.
8) a) M. Yoshikawa, H. K. Wang, V. Tosirisuk, I. Kitagawa, *Chem. Pharm. Bull.*, **30**, 3057 (1982); b) S Torii, K. Uneyama, M. Ono, *Tetrahedron Lett.*, **21**, 2653 (1980).
9) **5a** : colorless needles (EtOH), mp 170-173°C, UV λmax (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, OMex4), 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, $CDCl_3$) δc : 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 λmax (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, OMex4), 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 : $C_{18}H_{18}O_5$, M=314.34, triclinic, a=10.196 (1), b=10.724 (1), c=7.9454 (9)Å, α=102.492 (9), β=105.60(1), γ=98.62 (1)°, V=796.6 (2)$Å^3$, Z=2, space group p1, Dc=1.310g/cm^3. All data were collected on Rigaku AFC5R diffractometer with MoKa radiation and a graphite monochrometer. 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 λmax (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** : $C_{15}H_{12}O_5$, ^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** : $C_{15}H_{12}O_5$, 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.

(Received December 21, 1993; accepted January 29, 1994)