A Facile Synthesis of Mollugin

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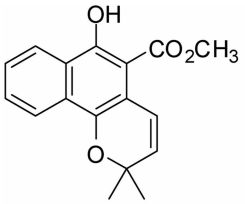
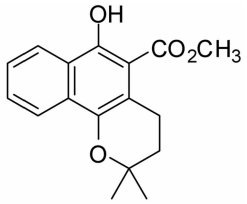
Mollugin (**1**) is one of the major 2*H*-naphtho[1,2-*b*]pyran component isolated from Rubiaceae species such as *Putoria calabrica* and *Rubia cordifolia* L. (Rubiaceae),[1] a herbal medicine used for the treatment of arthritis, dysmenorrhea, hematorrhea, and hemostasis, as a tonic, and for wound healing in China[2] and used for menstrual pain, rheumatism, and urinary disorders in India.[3] Antitumor activity,[4] antimutagenic activity,[5] antiviral activity against hepatitis B virus,[1c] and strong inhibitory activity on arachidonic acid and collagen-induced platelet aggregation[6] of mollugin have been reported. In addition, mollugin inhibited cell adhesion between angioendothelial cells and lymphocytes thus having potentials for cosmetic additives with hair growth stimulant activity[7] and anti-aging activity.[8]



1 (mollugin)



2 (dihydromollugin)

The intriguing structure and the various biological activities of **1** have led to introduce a series of total synthesis. The first synthesis was achieved from 1,4-dihydroxy-2-naphthoic acid and 3-chloro-3-methylbut-1-yne in 23% over all yield.[9] Reaction of methyl 1,4-dihydroxy-2-naphthoate and 3-methylbut-1-en-3-ol afforded dihydromollugin (**2**),[10] which was then dehydrogenated by DDQ to lead **1**, but no yield was given. Lumb and Trauner employed oxa 6π-electrocyclization of 2-methoxycarbonyl-3-prenyl-1,4-naphthoquinone.[11] Recently, Claessens, *et al.* reported a 9-step synthetic sequence from 1,4-naphthoquinone *via* selective prenylation and oxa 6π-electrocyclization.[12] Biosynthesis of **1** by using *Galium mollugo* L. cell cultures has also been reported.[13]

On the other hand, 2,2-dimethylchromen (2,2-dimethyl-2*H*-1-benzopyran) ring has been constructed by a couple of synthetic methods. The older methods included reaction of coumarins with methylmagnesium halides,[14] reaction of phenols with 2-methylbut-3-yn-2-ol,[15] pyridine-catalyzed condensation of phenols with 3-methyl-2-butenal diacetal.[16] However, pyridine-catalyzed condensation limited only to phenols with *meta*-pair hydroxy groups.[16b] Recently, phenylboric acid has been used for the *ortho*-specific attack on phenols by α,β-unsaturated aldehydes and ketones to lead to a protonated quinonemethides which are readily undergone electrocyclization to construct benzopyran skeletons.[17] Such methods have also been applied to construct 2*H*-naphtho-[1,2-*b*]pyran skeleton.[16c,17]

As a part of our continuing interest in the establishment of synthetic method for nature-originated biologically active compounds[18] and of pursuing further biological properties of mollugin on experimental animals, a practical synthetic method is required to secure sufficient quantity of mollugin. We, herein, described a facile synthesis of **1** from readily available 1,4-dihydroxy-2-naphthoic acid and 3-methyl-2-butenal by an intermolecular phenylboronic acid-mediated cyclization.

We reasoned that the intermolecular phenylboronic acid-mediated cyclization of **4a** with 3-methyl-2-butenal would afford mollugin. We thus attempted direct esterification of **3** with CH_3OH in the presence of H_2SO_4. The reaction, however, afforded methyl 1-hydroxy-4-methoxynaphthoate (**4b**) and 4-methoxy-1-hydroxy-2-naphthoic acid (**4c**) instead of desired **4a** in a ratio of 98:2 with 94% conversion. Subsequent $AlCl_3$-catalyzed demethylation of **4b** afforded desired methyl 1,4-dihydroxy-2-naphthoate (**4a**) in quantitative yield. Although **4a** could be prepared by selective esterification of **3** by employing previously reported method,[19] the present method is more cost-effective. Subsequent intermolecular phenylboronic acid-mediated cyclization of **4a** with 3-methyl-2-butenal yielded mollugin (**1**) in 87% yield. Structures of **4b** were confirmed by spectroscopic methods including HMBC (Figure 1).

Alternatively, direct cyclization of **3** was also pursued to afford the corresponding 6-hydroxy-2,2-dimethyl-2*H*-

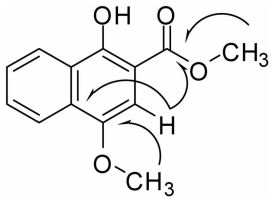
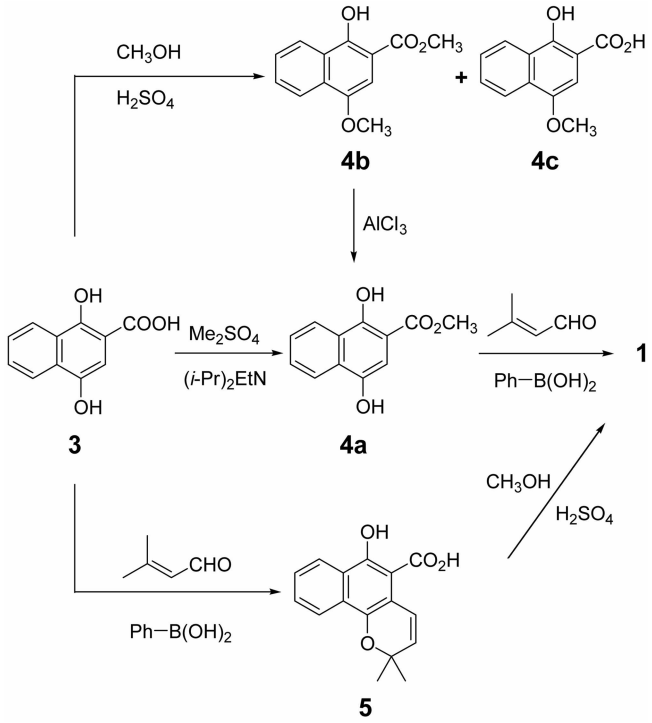


Figure 1. Selected ^1H-^{13}C long-range correlations in HMBC spectrum of **4b**.

naphtho[1,2-*b*]pyran-5-carboxylic acid (**5**) in 89% yield, which was then converted to mollugin by conventional esterification in 98% yield.

Catalytic hydrogenation of **1** by previously reported method[1c] quantitatively afforded corresponding dihydromollugin (**2**).



In conclusion, mollugin was prepared from 1,4-dihydroxy-2-naphthoic acid and 3-methyl-2-butenal by intermolecular phenylboronic acid-mediated cyclization. Alternative 2-step synthesis of mollugin from 1,4-dihydroxy-2-naphthoic acid was also successful. Studies on the synthesis of mollugin derivatives and their biological properties are in progress.

Experimental Section

Melting points were determined using a Fischer-Jones melting points apparatus and are not corrected. IR spectra were obtained using a Perkin-Elmer 1330 spectrophotometer. NMR spectra were obtained using a Bruker-250 spectrometer 250 MHz for ^{1}H NMR and 62.5 MHz for ^{13}C NMR and are reported as ppm from the internal standard TMS. Chemicals and solvents were commercial reagent grade and used without further purification. The starting compounds 1,4-dihydroxy-2-naphthoic acid[20] and 3-methyl-2-butenal[16] were prepared by employing previously reported methods. Electrospray ionization (ESI) mass spectrometry (MS) experiments were performed on a LCQ advantage-trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA). Elemental analyses were taken on a Hewlett-Packard Model 185B elemental analyzer.

Methyl 1,4-dihydroxy-2-naphthoate (4a)

Method A: To a prepared ethereal solution of diazomethane (*ca.* 15-16 mmol, 25 mL Et_2O) was added **3** (2.04 g, 10 mmol). The reaction mixture was allowed to stir for 30 min and evaporated under reduced pressure to give the residue. The residue was recrystallized from ethyl acetate to give **4a** (1.88 g, 86%) as pale yellow needles: mp 193-194 °C (lit.[19b] mp 192-193.5 °C); ^{1}H NMR (DMSO-d_6) δ 11.44 (s, 1H), 9.19 (s, 1H), 8.32 (d, 1H, J = 8.1 Hz), 8.18 (d, 1H, J = 8.1 Hz), 7.63-7.50 (m, 2H), 7.14 (s, 1H), 3.98 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 170.87, 153.04, 145.48, 129.37, 129.06, 126.75, 125.19, 123.54, 122.52, 105.08, 104.13, 52.90; ESI MS calcd for $C_{12}H_{10}O_4$ 219 $[M + H]^-$, found 219.

Method B: A solution of 30 g (0.15 mol) of **3**, 20.9 g (0.16 mol) ethyldiisopropylamine, and 20.8 mL (0.22 mol) of dimethyl sulfate in 200 mL of dry DMF was stirred at 85 °C for 1 h. The reaction mixture was cooled, poured to aq. $NaHCO_3$, and worked up to afford 25.2 g (79%) of **4a**: mp 192-193 °C. The spectral data were identical to those described above in Method A.

Method C: To a cooled (0 °C) solution of methyl 1-hydroxy-4-methoxy-2-naphthoate (**4b**) (23.2 g, 0.1 mol) in dry CH_2Cl_2 (200 mL) was added $AlCl_3$ (26.2 g, 0.2 mol) and the reaction mixture was stirred for 4 h in a flask fitted with a $CaCl_2$ tube. The reaction mixture was quenched by the addition of water (200 mL) (exothermic!) and the aqueous solution was further diluted with 1 *N* HCl (500 mL), extracted with CH_2Cl_2 (100 mL × 3), dried over $MgSO_4$ and evaporated *in vacuo*. Flash chromatography on silica gel using EtOAc/petroleum ether (1:19) afforded a yellow powder (21.5 g, 99%), which was recrystallized from methanol to give **4a** as pale yellow needles: mp 193-194 °C. The spectral data were identical to those described above in Method A.

Methyl 1-hydroxy-4-methoxy-2-naphthoate (4b). To a solution of 1,4-dihydroxy-2-naphthoic acid (20.4 g, 0.1 mol) in CH_3OH (100 mL) was slowly added H_2SO_4 (5 mL) at 0 °C and resulting mixture was refluxed for 2 h. Excess CH_3OH (~70 mL) was distilled off and additional CH_3OH (50 mL) was added. Resulting mixture was refluxed for 8 h. The white precipitate formed was collected and washed with CH_3OH, 5% aq. NaOH and water to give **4b** (21.3 g, 92%) as white crystals after recrystallization from CH_3OH:EtOAc (1:1): mp 136-137 °C (lit.[19b] 137-138 °C). Unreported spectral data are as follows: IR (KBr) υ 1660, 1640, 1605, 1395, 1360, 1255, and 775 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 11.59 (s, 1H exchanged with D_2O), 8.36 (d, 1H, J = 8.1 Hz), 8.16 (d, 1H, J = 8.1 Hz), 7.60 (td, 1H, J = 8.1, 0.8 Hz), 7.53 (td, 1H, J = 8.1, 0.8 Hz), 6.95 (s, 1H), 3.95 (s, 3H), 3.92 (s, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 171.22, 155.43, 147.49, 129.69, 128.90, 126.30, 125.34, 123.64, 121.77, 104.13, 100.19, 55.52, 52.18; MS (m/e) 232, 200, 129, 102, and 101. Anal. Calcd for $C_{13}H_{12}O_2$: C, 67.23; H, 5.21. Found: C, 67.27; H, 5.22. The resulting reaction mixture was evaporated and dissolved in EtOAc (100 m) and extracted with 5% NaOH (3 × 50 mL). The combined aq. layers were washed with EtOAc and made acidic (pH = ~1). The resulting solution was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over

$MgSO_4$ and evaporation of the solvent afforded 4.40 g (2%) of 1-hydroxy-4-methoxy-2-naphthoic acid (**4c**): mp 199-200 °C [lit.[19b] 196-198 °C (dec)]. ^{1}H NMR (250 MHz, $CDCl_3$) δ 11.41 (s, 1H exchanged with D_2O), 8.39 (d, 1 H, J = 8.1 Hz), 8.16 (d, 1H, J = 8.1 Hz), 7.65 (td, 1H, J = 8.1, 0.8 Hz), 7.56 (td, 1H, J = 8.1, 0.8 Hz), 7.05 (s, 1H), 3.98 (s, 3H).

6-Hydroxy-2,2-dimethyl-2*H*-naphtho[1,2-*b*]pyran-5-carboxylic acid (5). To a mixture of methyl 1,4-dihydroxy-2-naphthoate (**4a**) (20.4 g, 0.1 mol), 3-methyl-2-butenal (8.4 g, 0.1 mol), phenylboronic acid (37.6 g, 0.31 mol), and propionic acid (2 mL) in toluene (200 mL) was refluxed under Dean-Stark trap for 8 h. The reaction mixture was cooled to room temperature and diluted with ether (75 mL). The organic layer was separated, washed with brine (3 × 50 mL), dried, and evaporated *in vacuo*. The resultant residue was purified by column chromatography. The early fractions were recrystallized from pet. ether:EtOAc to afford 24.0 g (89%) of **5** as yellow-green flakes: mp 177-178 °C (lit.[9] mp 177 °C). Unreported spectral data are as follows: IR (KBr) υ 3400 cm^{-1}. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 12.16 (s, 1H, OH), 8.10-8.07 (m, 1H), 7.40-7.36 (m, 2H), 7.16 (d, 1H, J = 7.5 Hz, H7), 6.63 (d, 1H, J = 10.0 Hz, H4), 5.70 (d, 1H, J = 10 Hz, H3), 2.15 (s, 6H, CMe2). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 207.10, 141.78, 130.28, 129.85, 129.02, 128.21, 126.67, 125.55, 125.37, 125.27, 121.78, 118.78, 111.67, 30.95, 27.57. ESI MS: $[M+H]^+$ at *m/z* 271.

Mollugin (1)

Method A: To a mixture of methyl 1,4-dihydroxy-naphthoate (**4a**) (21.8 g, 0.1 mol), 3-methyl-2-butenal (8.4 g, 0.1 mol), phenylboronic acid (37.6 g, 0.31 mol), and propionic acid (2 mL) in toluene (200 mL) was refluxed under Dean-Stark trap for 8 h. The reaction mixture was cooled to room temperature and extracted with ether (75 mL). The organic phase was separated, washed with brine (3 × 50 mL), dried, and evaporated *in vacuo*. The resultant residue was purified by column chromatography. The early fractions were recrystallized from methanol to afford mollugin (**1**) as yellow-green flakes (24.5 g, 87%): mp 131-132 °C (lit.[1a] mp 129-130 °C). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 12.16 (s, 1H, OH), 8.38 (ddd, 1H, J = 8.3, 1.4, 0.7 Hz, H7), 8.18 (ddd, 1H, J = 8.3, 1.3, 0.7 Hz, H10), 7.62 (ddd, 1H, J = 8.3, 6.9, 1.4 Hz, H9), 7.51 (ddd, 1H, J = 8.3, 6.9, 1.3 Hz, H8), 7.12 (d, 1H, J = 10.1 Hz, H4), 5.68 (d, 1H, J = 10.1 Hz, H3), 4.00 (s, 3H, OMe), 1.51 (s, 6H, CMe2). ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 172.44, 156.46, 141.57, 129.28, 129.01, 128.81, 126.25, 125.09, 124.01, 122.28, 121.90, 112.54, 102.23, 74.63, 52.21, 26.86 (2 C's). ESI MS: $[M+H]^+$ at *m/z* 283.

Method B: To a solution of 6-hydroxy-2,2-dimethyl-2*H*-naphtho[1,2-*b*]pyran-5-carboxylic acid (**5**) (27.0 g, 0.1 mol) in CH_3OH (100 mL) was slowly added H_2SO_4 (5 mL) at 0 °C and resulting mixture was refluxed for 2 h. Excess CH_3OH (~70 mL) was distilled off and additional CH_3OH (50 mL) was added. Resulting mixture was refluxed for 8 h and poured to water. The white precipitate formed was collected and washed with CH_3OH and water to give **1** (27.8 g, 98%) as yellow flakes after recrystallization from CH_3OH:EtOAc (1:1): mp 131-133 °C. The spectral data are identical to those described above.

Dihydromollugin (2). A mixture of mollugin (100 mg, 0.35 mmol) and 5% Pd/C (25 mg) in methanol (15 mL) was stirred in a H_2 atmosphere for 5 h. The solution was then filtered through Celite. Evaporation of the solvent gave a yellow solid which was chromatographed on silica gel. The early fractions gave dihydromollugin as a yellow solid (96 mg, 99%): mp 96-97 °C (lit.[1e] mp 96-97 °C). ^{1}H NMR ($CDCl_3$) δ 12.1 (s, 1H), 8.35 (d, 1H, J = 8.1 Hz), 8.16 (d, 1H, J = 8.1 Hz), 7.58 (t, 1H, J = 7.8 Hz), 7.47 (t, 1H, J = 7.8 Hz), 3.97 (s, 3H), 3.04 (t, 2H, J = 6.6 Hz), 1.82 (t, 2H, J = 6.6 Hz), 1.39 (s, 6H). ESI MS calcd for *m/z* 287 $[M+H]^+$.

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