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Isomers of Shikonin, a Purple Pigment*1

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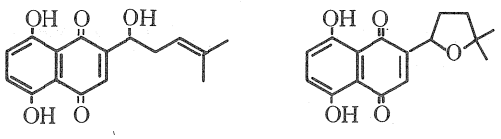
Two shikonin isomers which are 5, 8-dihydroxy-2-(1-hydroxy-2, 2-dimethyl-3-butenyl)-1, 4-naphthoquinone and (±)-cycloshikonin, were prepared to search new bioactive compounds. Treatment of 2-formyl-1, 4, 5, 8-tetramethoxynaphthalene with Grignard reagents prepared from 3-methyl-2-and-3-butenyl chlorides gave the corresponding tetramethoxynaphthalenes. The methoxynaphthalenes were subjected to two-step demethylation (oxidation with cerium ammonium nitrate to dimethoxy-1, 4-naphthoquinones and subsequent treatment with aluminum chloride-diethyl sulfide) to give the isomers.

1 Introduction

We have already showed the total synthesis of (±)-shikonin (1)[1] and new methods of (±)-cycloshikonin (2).[2] It is well-known that shikonin,[3] cycloshikonin, and their enantiomers (alkannin[4] and cycloalkannin) have antibacterial, antitumor, and wound healing activities.[4] Papageorgiou et al.[5] have reported that polymerization of naphthoquinone compounds such as shikonin and alkannin derivatives resulted in a complete loss of their antimicrobial activities and also the alkylation of the phenolic hydroxyl groups led to complete loss of them, while acylation didn't appear to influence their antibacterial properties. Tabata et al.[6] have examined an antibacterial activity of eleven quinone derivatives and concluded that the



1 2

Chart 1

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naphthoquinone skeleton itself showed the activity. Now we describe the synthesis of shikonin isomers to search new bioactive compounds.

2 Materials and Methods

2.1 Instrumentation and Chemicals

Tetrahydrofuran (Wako Chem. Co.) was distilled from sodium metal. Cerium(Ⅳ) ammonium nitrate, aluminum chloride, and diethyl sulfide were purchased from Kanto Chem. Co. 3-Methyl-2-butenyl bromide and chloride were taken from Tokyo Kasei Kogyo Co.

Melting points were determined with a Yanagimoto micromelting point apparatus and were uncorrected. ^{1}H NMR spectra were taken on a JEOL JNM-FX 60 spectrometer in $CDCl_3$ solution, unless otherwise specified. Mass spectra were obtained with a JEOL DX-300 spectrometer. IR spectra were measured using a Hitachi 260-30 and a Shimadzu IR-470 spectrometers. Column chromatography was carried out on silica gel (Wakogel C-200) or on alumina (Sumitomo, KCG-30). For thin-layer chromatography (TLC), silica gel 60 $PF_{254+366}$ (Merck) was employed.

2.2 Synthetic Reactions

Reaction of the Aldehyde (3) with a Grignard Reagent Prepared from 3-Methyl-2-butenyl Bromide. The bromide (0.5 g) was added to a suspension of magnesium (400 mg, 16.7 mmol) in tetrahydrofuran (THF) (20 m*l*) under a nitrogen atmosphere and a few crystal of iodine was added with stirring. The rest of the bromide (1.5 g) in THF (5 m*l*) was added dropwise to the solution about at 20 ℃. After 2 h, a solution of the aldehyde (3)[1] (500 mg, 1.81 mmol) in THF (10 m*l*) was added and the resulted mixture was refluxed for 2 h and left overnight at room temperature. The mixture was poured into aq. NH_4Cl and extracted with chloroform. The extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on alumina to give two products. An elution with chloroform afforded 2-hydroxymethyl-1, 4, 5, 8-tetramethoxynaphthalene (4)[9] (mp 99-100 ℃, 160 mg, 32% yield). An elution with chloroform-methanol (20 : 1) gave 1, 2-bis(1, 4, 5, 8-tetramethoxy-2-naphthyl) ethanediol (5) (240 mg, 48% yield). Recrystallization from hexane-ethanol afforded an analytical sample, light yellow crystals, mp 212-214 ℃. IR (KBr) 3480 (OH), 1085, and 1070 cm^{-1}; ^{1}H NMR δ 1.6 (broad, 2H, 2×OH), 3.73, 3.76, 3.86, 3.89 (each s, 6H, 2×OCH_3), 5.51 (s, 2H, 2×CH), 6.82(s, 4H, ArH), and 6.92 (s, 2H, ArH); MS, *m/z* 554(M^+), 536, and 277. Found: C, 64.73; H, 6.26%. Calcd for $C_{30}H_{34}O_{10}$: C, 64.97; H, 6.18%.

Reaction of the Aldehyde (3) with a Grignard Reagent Prepared from 3-Methyl-2-butenyl Chloride. The chloride (0.5 g) was added to a suspension of magnesium (263 mg, 10.8 mmol) in THF (20 m*l*) under a nitrogen atmosphere. After the reaction took place, the solution was cooled to −10 ℃ and the rest of the chloride (0.8 g) in THF (2 m*l*) was added and then the resulted mixture was stirred for 3 h. The Grignard solution was cooled to −70 ℃ and a solution of 3 (704 mg, 2.55 mmol) in THF (10 m*l*) was added and stirred at −10 ℃ for 3 h. The mixture was quenched with aq. NH_4Cl and extracted with chloroform. The usual work-up and chromatography on alumina with chloroform as an eluent gave 2-(1-hydroxy-2, 2-dimethyl-3-butenyl)-1, 4, 5, 8-tetramethoxynaphthalene (6) (860 mg, 97% yield). Recrystallization from hexane-ethanol afforded an analytical sample, light yellow crystals, mp 130.5-131 ℃. IR (KBr) 3503 (OH), 1601, 1260, 1080, 1070, 995 (vinyl), and 925 cm^{-1} (vinyl); ^{1}H NMR δ 1.00, 1.11 (each s, 3H, CH_3), 2.1 (broad, 1H, OH), 3.72, 3.89 (each s, 3H, OCH_3), 3.91 (s, 6H, 2×

OCH_3), 4.9-5.3 (m, 3H, -CH(OH)-, $=CH_2$), 6.10 (dd, $J=18.2$, 11.5 Hz, 1H, -CH$=CH_2$), 6.82 (s, 2H, ArH), and 6.97 (s, 1H, ArH); MS, *m/z* 346 (M^+), 328, 277, and 262. Found: C, 69.15; H, 7.54%. Calcd for $C_{20}H_{26}O_3$: C, 69.34; H, 7.56%.

General Procedure for Oxidative Demethylation of 2-Substituted 1, 4, 5, 8-Tetramethoxynaphthalenes (**6**, **11**) with Cerium(IV) Ammonium Nitrate (CAN). A solution of CAN (2.5 mmol) in water (5 m*l*) was added dropwise to a solution of 2-substituted 1, 4, 5, 8-tetramethoxynaphthalene (1 mmol) in acetonitrile (10 m*l*) or in a mixture of acetonitrile-chloroform (2:1 v/v). The mixture was stirred at room temperature for 30 min, diluted with water, and extracted with chloroform. The extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by chromatography on silica gel with chloroform as eluent and recrystallized.

7: orange crystals, mp 128.5-129 ℃ (hexane-ethanol); IR (KBr) 3480 (OH), 1650 (C=O), 1630 (vinyl), 1580, 1560, 1270, 1210, 1050, 970 (vinyl), and 915 cm^{-1} (vinyl); ^1H NMR δ 1.02, 1.06 (each s, 3H, CH_3), 2.6 (broad, 1H, OH), 3.93, 3.95 (each s, 3H, OCH_3), 4.7-5.2 (m, 3H, -CH(OH)-, $=CH_2$), 5.93 (dd, $J=16.6$, 11.5 Hz, 1H, -CH$=CH_2$), 6.17 (d, $J=0.8$ Hz, 1H, quinone ring H), 7.28 (s, 2H, benzene ring H); MS, *m/z* 316 (M^+), 248, and 233. Found: C, 68.26; H, 6.45%. Calcd for $C_{20}H_{20}O_5$: C, 68.34; H, 6.37%.

8: orange crystals, mp 118-119 ℃ (hexane-ethanol); IR (KBr) 3475 (OH), 1650 (C=O), 1618, 1580, 1555, 1250, 1060, 1055, 990 (vinyl), and 915 cm^{-1} (vinyl); ^1H NMR δ 0.97, 1.10 (each s, 3H, CH_3), 2.3 (broad, 1H, OH), 3.81, 3.97 (each s, 3H, OCH_3), 4.7-5.2 (m, 3H, -CH(OH)-, $=CH_2$), 6.78 (s, 2H, quinone ring H), and 7.45 (s, 1H, benzene ring H). Found: C, 68.33; H, 6.44%. Calcd for $C_{20}H_{20}O_5$: C, 68.34; H, 6.37%.

12: orange oil: IR (neat) 3470 (OH), 1645 (C=O), 1580, 1560, 1275, 1255, 1210, and 1050 cm^{-1}; ^1H NMR δ 1.75 (s, 3H, CH_3), 1.8-2.4 (m, 4H, $2\times CH_2$), 2.7 (broad, 1H, OH), 3.96 (s, 6H, $2\times OCH_3$), 4.74 (m, 3H, -CH(OH)-, $=CH_2$), 6.78 (d, $J=1.2$ Hz, 1H, quinone ring H), and 7.31 (s, 2H, benzene ring H); MS, *m/z* 316 (M^+), 298, 283, 247, and 233; HRMS, Found: *m/z* 316.1310. Calcd for $C_{18}H_{20}O_5$: M, 316.1310.

13: orange oil: IR (neat) 3470 (OH), 1650 (C=O), 1620, 1585, 1555, 1250, and 1050 cm^{-1}; ^1H NMR δ 1.76 (s, 3H, CH_3), 1.8-2.7 (m, 5H, $2\times CH_2$, OH), 3.82, 3.99 (each s, 3H, OCH_3), 4.78 (m, 2H, $=CH_2$), 5.09 (broad, 1H, CH), 6.77 (s, 2H, quinone ring H), and 7.54 (s, 1H, benzene ring H); MS, *m/z* 316 (M^+), 260, 247, and 204. HRMS, Found: *m/z* 316.1284. Calcd for $C_{18}H_{20}O_5$: M, 316.1310.

General Procedure for Demethylation of Dimethoxy-1, 4-naphthoquinones (**7**, **12**) with Aluminum Chloride-Diethyl Sulfide ($AlCl_3$-Et_2S). To a stirred solution of the dimethoxy-1, 4-naphthoquinone (0.5 mmol) in dry dichloromethane (2 m*l*) at 0 ℃ was added a dichloromethane solution (2 m*l*) containing $AlCl_3$ (5 mmol) and Et_2S (15 mmol). After stirring at room temperature for 2 h, the mixture was poured into an ice-cold 1% HCl and the resulted mixture was extracted with dichloromethane. The extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by TLC (**10**: $CHCl_3$: $Et_2O=3:1$, **2**: CH_2Cl_2).

10: brown crystals, mp 80.5-83 ℃; IR (KBr) 3480(OH), 1608 (C=O), 1570, 1200, 1065, and 910 cm^{-1} (vinyl); ^1H NMR δ 1.05, 1.10 (each s, 3H, CH_3), 2.3 (broad, 1H, OH), 4.8-5.2 (m, 3H, -CH(OH)-, -CH$=CH_2$), 5.99 (dd, $J=$ 16.8, 11.3 Hz, 1H, -CH $=CH_2$), 7.12 (s, 1H, quinone ring H), 7.19 (s, 2H, benzene ring H), 12.45 and 12.67 (each s, 1H, ArOH); MS, *m/z* 288 (M^+), 271, 220, and 191. HRMS, Found: *m/z* 288.0969. Calcd for $C_{16}H_{16}O_5$: M, 288.0987.

3 Results and Discussion

3.1 A Shikonin Isomer from 2-(1-Hydroxy-2, 2-dimethyl-3-butenyl)-1, 4, 5, 8-tetramethoxynaphthalene (6).

Treatment of 2-formyl-1, 4, 5, 8-tetramethoxynaphthalene (**3**)[1] with a Grignard reagent prepared from 3-methyl-2-butenyl bromide in tetrahydrofuran under reflux gave two products. One was 2-hydroxymethyl-1, 4, 5, 8-tetramethoxynaphthalene (**4**) (30% yield) which was formed by reduction of the carbonyl group in **3** with the Grignard reagent. The other product was 1, 2-bis (1, 4, 5, 8-tetramethoxy-2-naphthyl)-1, 2-ethanediol (**5**) (48% yield). The same reaction at room temperature or −70 ℃ gave only the alcohol **4**. A Grignard reaction of the aldehyde **3** with 3-methyl-2-butenyl chloride successfully afforded only 2-(1- hydroxy-2, 2-dimethyl-3-butenyl)-1, 4, 5, 8-tetramethoxynaphthalene (**6**) in 97% yield (Chart 2).

Oxidative demethylation[1] of **6** with CAN gave three quinones, **7** (31% yield), **8** (50%), and **9**[7] (14%). The quinone **9** was formed by the oxidative cleavage[8] of C-C bond in the bulky side-chain. Identifications of **7** and **8** were easily carried out by the ^{1}H NMR.[9]

Further demethylation of **7** using AgO-40% HNO_3[1] was unsuccessful, but the treatment with $AlCl_3$-Et_2S[10, 11] gave a shikonin isomer (**10**) in 35% yield (Scheme 1).

3.2 From 2-(1-Hydroxy-4-methyl-4-pentenyl)-1, 4, 5, 8-tetramethoxynaphthalene (11).

The tetramethoxynaphthalene **11**[2] was obtained by a reaction of the aldehyde **3** with a Grignard reagent from 3-methyl-3-butenyl chloride. Oxidative demethylation of **11** with CAN gave two 1, 4-naphthoquinones **12** (60% yield) and **13** (30%). Further demethylation of **12** with $AlCl_3$-Et_2S resulted in cyclization of the side-chain to afford (±)-cycloshikonin (**2**)[2] in 33% yield (Scheme 2).

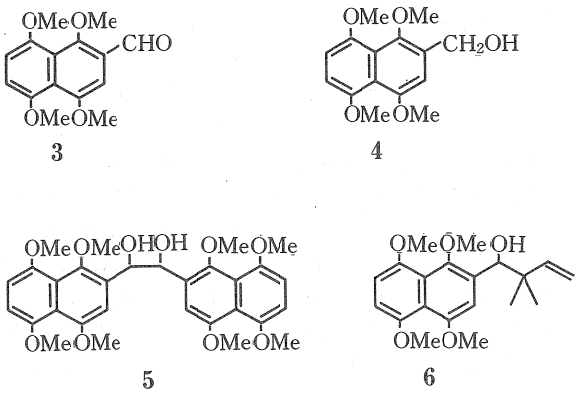

Chart 2

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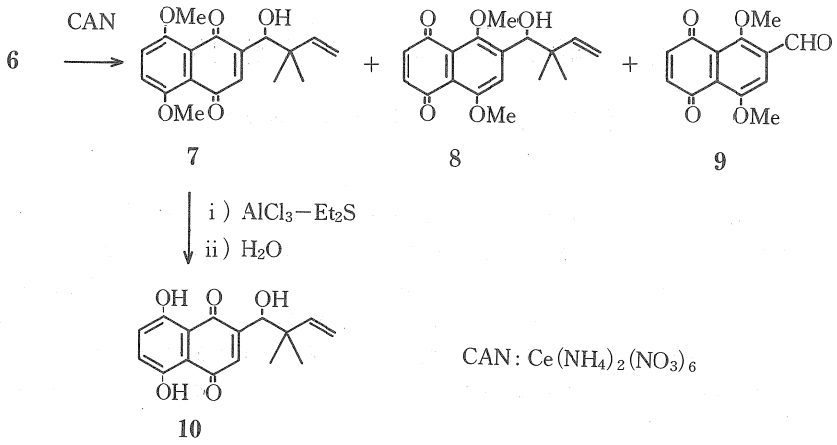
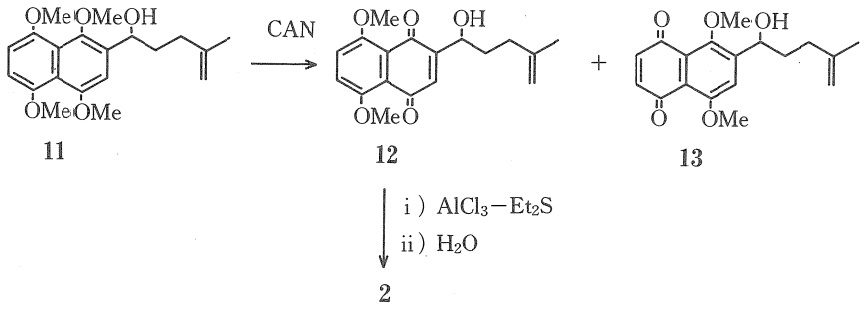


Scheme 1



Scheme 2

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紫色素シコニンの異性体

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新しい生理活性化合物を探す目的で，二つのシコニン異性体，5, 8-ジヒドロキシ-2-(1-ヒドロキシ-2, 2-ジメチル-3-ブテニル)-1, 4-ナフトキノンと(±)-シクロシコニンを合成した。3-メチル-2-ブテニルクロライドと3-メチル-3-ブテニルクロライドから得られたそれぞれのグリニャール試薬を2-ホルミル-1, 4, 5, 8-テトラメトキシナフタレンに反応させて，相当するテトラメトキシナフタレンが得られた。これらのテトラメトキシナフタレン化合物を二段階で脱メチル化(最初に硝酸セリウムアンモニウムで，続いて塩化アルミニウム－硫化ジエチルで脱メチル化)して，上述の二つの異性体が得られた。