Synthesis of Toddacoumaquinone, a Coumarin-Naphthoquinone Dimer, and Its Antiviral Activities

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Toddacoumaquinone (1), a coumarin-naphthoquinone dimer, was synthesized through Diels–Alder reaction between 8-(1-acetoxy-3-methyl-1,3-butadienyl)-5,7-dimethoxycoumarin (15) and 2-methoxy-1,4-benzoquinone (5). The activities of 1 against several viruses were examined. A weak activity (EC_{50} = 10 μg/ml) was observed against HSV-1 and HSV-2, but no activity was seen against HIV-1.

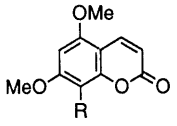
Key words coumarin-naphthoquinone dimer; synthesis; antiviral activity; Diels–Alder reaction

It is well known that a number of natural products containing a quinone moiety in the molecule, *e.g.*, antibiotics such as mitomycin C and anthracyclines, show very important biological activities. Toddacoumaquinone (**1**)[1] is a unique coumarin-naphthoquinone dimer isolated from *Toddalia asiatica* (L.) LAM. (*T. aculeata* PERS.) (Rutaceae) by us. Structurally related dimers, naphthohernianin (**2**)[2] and pummeloquinone (**3**),[3] also appear in Rutaceous plants. These dimers **1**—**3** may be biogenetically synthesized through Diels–Alder reaction between (3-methyl-1,3-butadienyl)coumarin derivatives (**4**) and 2-methoxy-1,4-benzoquinone (**5**), followed by oxidative aromatization (Chart 1). We were interested in the possible antiviral activity of **1** because of the presence of a naphthoquinone moiety[4] in it, but the small amounts of **1** available from the natural source were insufficient for testing. Furthermore, the structure of **1** has been deduced only by spectroscopic means. In this report we present a concise synthesis of **1** from commercially available limettin (5,7-dimethoxycoumarin) (**6**), and its antiviral activities against herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2) and human immunodeficiency virus type 1 (HIV-1).

Results and Discussion

At first we planned a [4+2] cycloaddition using gleinadiene (**4a**)[5] for the synthesis of **1**. The successful synthesis[3,6] of the related dimeric compounds **2** and **3** indicates that appropriate regioselectivity can be expected in the [4+2] cycloaddition. Reisch and Bathe[6] have reported the preparation of **4a** from 7-hydroxy-5-methoxycoumarin in five steps (iodination, methylation, Heck reaction using 1,1-dimethyl-2-propen-1-ol, and dehydration). Their method led us to examine 8-formyllimettin (**7**),[7] easily obtained by formylation of **6**. However, several trials, such as Wittig reaction using isobutyltriphenylphosphorus ylid, aldol condensation with acetone, and Heck reaction using either a triflate **8** or a phosphonate **9** derived from 8-hydroxycoumarin (leptodactylone) (**10**),[7] were unsuccessful. Furthermore, iodination of **6** resulted in ineffective formation of the 8-iodocoumarin **11**, a more promising candidate as a substrate for Heck reaction.

Thus, we focused on the preparation of a (3-methyl-1,3-butadienyl)coumarin derivative carrying an oxygen function on the dienyl side chain in place of gleinadiene (**4a**) as a diene unit, by direct introduction of a C_5 unit into **6**, which should directly produce **1** through spontaneous aromatization when subjected to Diels–Alder reaction. Vilsmeier–Haack reaction of **6** using *N*,*N*-dimethylamino-3,3-diethylacrylamide and phosphorus oxychloride gave an unexpected dibenzo[*c*,*e*]pyran-2(1*H*)-one de-



6 : R=H
7 : R=CHO
8 : R=OSO_2CF_3
9 : R=$OP(O)(OEt)_2$
10 : R=OH
11 : R=I

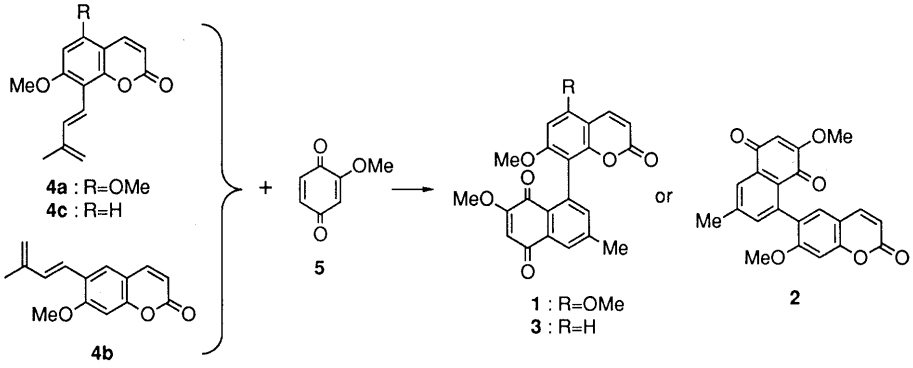


Chart 1

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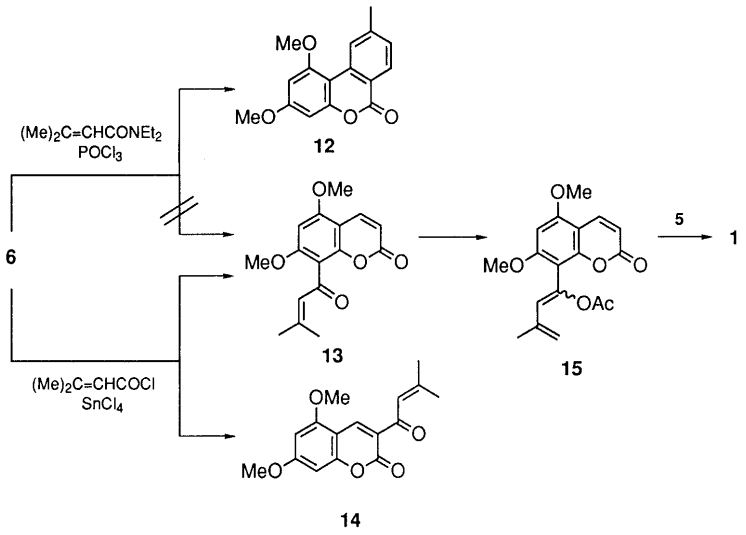

Chart 2

Table 1. Antiviral Activities of **1** and ACV[a)]

Virus	Compound	EC_{50} (μg/ml)
HSV-1	**1**	9.80
	ACV	0.13
HSV-2	**1**	10.0
	ACV	0.23

a) Plaque reduction assays for HSV-1 (strain KOS) and HSV-2 (strain 186) were performed with Vero cell monolayers by the reported procedure[12)] with a slight modification. Inhibition of plaque development for both viruses was evaluated on monolayers after 1 to 2 d incubation at 37 °C. EC_{50} values were determined from the drug concentration which conferred 50% plaque reduction compared to virus controls.

rivative **12**[8)] as the only isolable product (13%), in which substitution had occurred at the 3 position. When **6** was subjected to Friedel–Crafts acylation with 3,3-dimethylacryloyl chloride in the presence of tin(IV) chloride in a freezer (−22 °C), the desired 8-acylated product (glabralactone) **13**[9)] was obtained in 57% yield together with an isomeric 3-acylcoumarin **14** (23%). Treatment of **13** with isopropenyl acetate smoothly yielded a dienol acetate **15**. Heating of **15** in xylene with **5** at 140 °C for 5 days in a sealed tube directly produced a cycloadduct in 15% yield,[10)] through dehydroacetoxylation followed by aromatization, and this product was identical with natural **1** (Chart 2). An attempt to improve the yield of cycloaddition by elaboration of a ketonic function[11)] in **13**, such as preparation of more electrophilic alkyl or silyl dienol ethers or a dienamine using piperidine, was unsuccessful.

Thus, the structure of toddacoumaquinone was chemically established as **1** and the short synthesis from limettin (**7**) allowed us to synthesize sufficient **1** for biological examination. Synthetic **1** was tested for antiviral activities against HSV-1 and HSV-2 using acyclovir (ACV), an antiviral in clinical use, as a standard (Table 1). Toddacoumaquinone (**1**) showed a weak activity ($EC_{50}=10\,\mu$g/ml) against both virus strains. However, a test for activity against HIV-1 revealed no effect ($EC_{50}>10\,\mu$g/ml).

Experimental

All melting points were measured on a micro melting-point hot stage (Yanagimoto) and are uncorrected. IR spectra were recorded for KBr pellets on a Hitachi 260-10 or JASCO IR-700 spectrophotometer. ^{1}H-NMR spectra were recorded in $CDCl_3$ solution with a JEOL JNM GSX-500α spectrometer, unless otherwise stated, with tetramethylsilane as an internal reference. EI-MS (MS) and high resolution MS (HR-MS) were recorded on Hitachi M-60 and JEOL JMX-HX 110A spectrometers, respectively, with a direct inlet system. For column and flash chromatography, Silica gel 60 (70—230 mesh ASTM; Merck) and Silica gel 60 (230—400 mesh ASTM; Merck) were used, while for TLC and preparative TLC (PLC), DC-Fertigplatten SIL-G 25 UV254 (Macherey-Nagel) and Silica gel GF_{254} (Merck) were used. In general, extract solutions were washed with brine, dried over magnesium sulfate, then filtered, and the filtrate was evaporated to dryness under reduced pressure, unless otherwise stated.

Friedel–Crafts Reaction of Limettin (6) A solution of $SnCl_4$ (11.5 ml, 0.098 mol) in CH_2Cl_2 (15 ml) was added dropwise to a stirred solution of **6** (5.0 g, 0.024 mol) and 3,3-dimethylacryloyl chloride (6.0 ml, 0.055 mol) in CH_2Cl_2 (15 ml) at −70 °C. The reaction mixture was kept in a freezer (−22 °C) for 5 d and diluted with CH_2Cl_2. After work-up the residue was purified by columm chromatography using a mixed solvent of hexane and ethyl acetate (2 : 1) to give two fractions.

Glabralactone (**13**): The first eluate gave **13** (4.0 g, 57%) as colorless prisms, mp 129—131 °C (lit.[9b)] mp 128—130 °C), which were recrystallized from CH_2Cl_2–hexane. IR ν_{max}: 1736, 1665 cm^{-1}. ^{1}H-NMR δ: 1.95 (3H, d, $J=1.2$ Hz, CH_3), 2.25 (3H, d, $J=0.9$ Hz, CH_3), 3.90, 3.96 (each 3H, s, OCH_3), 6.15 (1H, d, $J=9.8$ Hz, C_3-H), 6.30 (1H, m, COCH=CMe), 6.31 (1H, s, C_6-H), 7.95 (1H, d, $J=9.8$ Hz, C_4-H). MS *m/z*: 288 (M^+).

5,7-Dimethoxy-3-(3-methyl-1-oxo-2-butenyl)coumarin (**14**): Recrystallization of the product from the second eluate from CH_2Cl_2–hexane gave a 3-acrylated coumarin **14** (1.6 g, 23%) as colorless needles, mp 171—174.5 °C. IR ν_{max}: 1730, 1651 cm^{-1}. ^{1}H-NMR δ: 2.02 (3H, d, $J=0.9$ Hz, CH_3), 2.25 (3H, d, $J=1.2$ Hz, CH_3), 3.88, 3.91 (each 3H, s, OCH_3), 6.27 (1H, d, $J=2.1$ Hz, C_6-H), 6.42 (1H, $J=2.1$ Hz, C_8-H), 7.27 (1H, m, COCH=CMe), 8.79 (1H, s, C_4-H). MS *m/z*: 288 (M^+). *Anal.* Calcd for $C_{16}H_{16}O_5$: C, 66.67; H, 5.59. Found: C, 66.33; H, 5.44.

8-(1-Acetoxy-3-methyl-1,3-butadienyl)-5,7-dimethoxycoumarin (15) A solution of **13** (200 mg, 0.69 mmol) and $TsOH\cdot H_2O$ (25 mg, 0.13 mmol) in isopropenyl acetate (5 ml) was stirred at 85 °C for 5 h under argon, then cooled to room temperature and extracted with ethyl acetate. Evaporation of the extract afforded a green residue, and this was chromatographed using a mixed solvent of hexane and ethyl acetate (2 : 1) to give **15** (185 mg, 81%) as colorless prisms, mp 148—153 °C, which were recrystallized from AcOEt–hexane. IR ν_{max}: 1755, 1728 cm^{-1}. ^{1}H-NMR δ: 2.08 (3H, s, CH_3), 2.12 (3H, s, $OCOCH_3$), 3.94, 3.95 (each 3H, s, OCH_3), 5.01, 5.07 (each 1H, s, C=CH_2), 6.16 (1H, d, $J=9.8$ Hz, C_3-H), 6.33 (1H, s, C_6-H), 7.97 (1H, d, $J=9.8$ Hz, C_4-H). HR-MS *m/z*: 330.1089 (Calcd for $C_{18}H_{18}O_6$: 330.1101).

Toddacoumaquinone (1) A solution of **15** (50 mg, 0.15 mmol) and **5**[13] (28 mg, 0.20 mmol) in xylene (1.0 ml) was heated in a sealed tube at 140 °C under argon. During the reaction for 5 d, further **5** was added to maintain its concentration in the reaction mixture, because partial decomposition occurred under these conditions. Evaporation of the solvent gave **1** (9.3 mg, 15%) as orange prisms, mp 277—280 °C, after purification by preparative TLC using a mixed solvent of AcOEt and hexane (3 : 1) followed by washings with ether. *Anal.* Calcd for $C_{23}H_{18}O_7$: C, 67.98; H, 4.47. Found: C, 67.54; H, 4.41.

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References and Notes

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8) Brown prisms (from CH_2Cl_2–hexane), mp 190—193 °C. IR ν_{max}: 1728 cm^{-1}. ^{1}H-NMR δ: 2.53 (3H, s, CH_3), 3.87, 4.02 (each 3H, s, OCH_3), 6.45 (1H, d, J=2.5 Hz, C_6-H), 6.54 (1H, d, J=2.5 Hz, C_8-H), 7.29 (1H, diffused d, J=8.2 Hz, $C_{2'}$-H), 8.27 (1H, d, J=8.2 Hz, $C_{1'}$-H), 8.68 (1H, diffused s, $C_{4'}$-H). MS m/z: 270 (M^+, 100%).
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10) When the reaction was carried out in the presence of molecular sieves 4 A decomposition of **5** was suppressed, but the yield was not improved. Other trials using **15** as a diene were also unsuccessful.
11) Selective reduction of the ketonic function or allylic oxidation of the terminal methyl group in **13** could not be achieved.
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