References and Notes

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Synthesis of Antibacterial *p*-Quinols from Marine Sponges. Synthetic Applications of "Masked" Quinones

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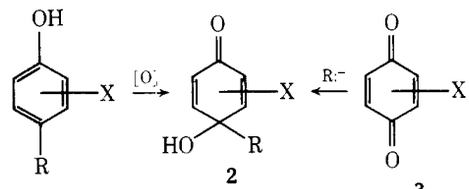
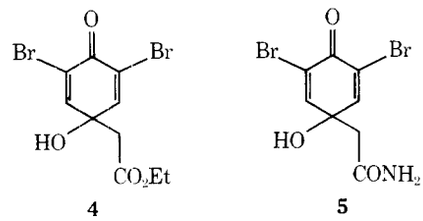
Received June 18, 1976

o- and *p*-quinols, including closely related derivatives, are important intermediates in the biosynthesis and metabolism of phenolic natural products.[2] Recently, research in this laboratory[3] and elsewhere[4] has been directed toward exploiting such versatile intermediates in the synthesis of naturally occurring quinones and alkaloids.

In principle, there are two potentially attractive routes to the synthesis of *p*-quinols such as **2**, one being the oxidation of the appropriately substituted phenol **1**, and the other being the regioselective nucleophilic addition of a carbon nucleophile ($R:^-$) to the quinone moiety **3**. Historically, the use of

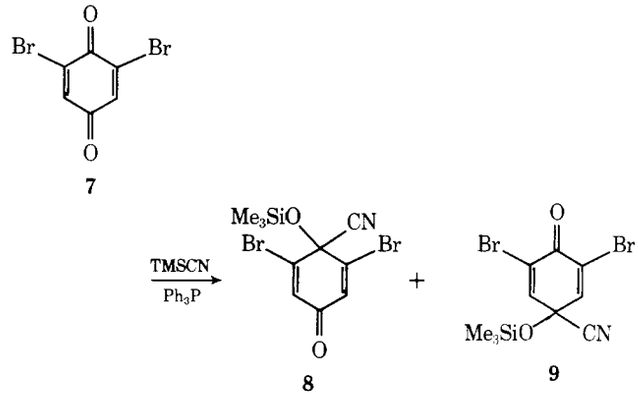


phenol oxidation techniques as an effective means of producing *p*-quinols has been quite disappointing;[5] however, with the recent development of selective oxidants such as thallium(III),[6] these phenol oxidations may now be considered as viable synthetic processes. Recently, we have developed the capability of regioselectively monoprotecting substituted *p*-quinones **3**, and have demonstrated that such substrates are excellent general precursors to *p*-quinols.[7] The purpose of this note is to report on the application of such methodology to the synthesis of *p*-quinol antibiotic metabolites **4** and **5**



recently isolated from the mollusk *Tylodina fungina*, and marine sponges of the genus *Verongia*, respectively.[8]

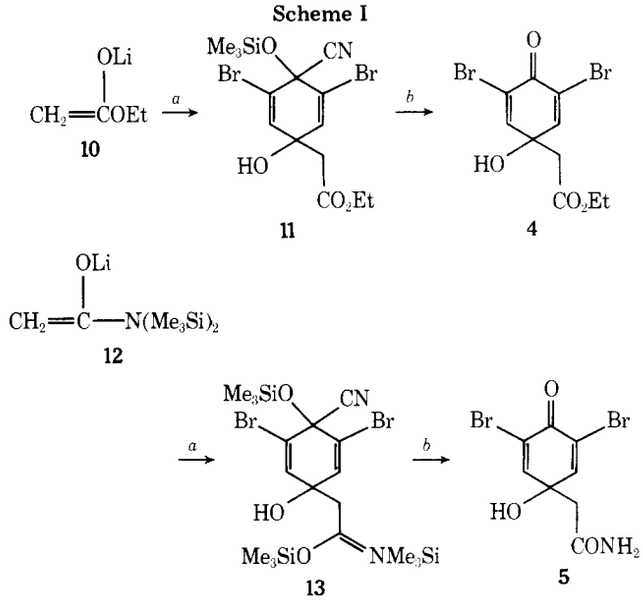
The requisite monoprotected *p*-quinone **8** was readily prepared in two steps from commercially available precursors. 2,4,6-Tribromophenol (**6**) was oxidized with thallium tris(trifluoroacetate), according to the method of Taylor,[9] to 2,6-dibromo-*p*-benzoquinone (**7**) in 67% yield.[10] Regiospecific carbonyl protection of the requisite quinone carbonyl was realized upon treatment of **7** with 1 equiv of trimethylsilyl cyanide (TMSCN) and a catalytic amount of triphenylphosphine in acetonitrile at 0 °C. *Under these conditions the adduct* **8** *was produced in essentially quantitative yield uncontaminated by the isomeric quinone adduct* **9**. A change



in solvent (C_6H_6, CCl_4, $CHCl_3$) or increased reaction temperatures resulted in a loss in regioselectivity of the reaction. For example, the same reaction carried out in chloroform (25 °C, 1 h) afforded an **8**:**9** ratio of 38:62. Tentative conclusions relating to the relative stabilities of the isomeric adducts were obtained from an equilibrium experiment. Treatment of **8** with a catalytic amount of triphenylphosphine at 40 °C (240 h) in acetonitrile resulted in an apparent isomerization to **9** ($K_{eq} \geq 10$).

With the requisite "masked" quinone **8** in hand, the *p*-quinol ester **4** and amide **5** were readily prepared via the enolate carbonyl addition processes outlined in Scheme I. A tetrahydrofuran solution of lithioethyl acetate (**10**) was gen-

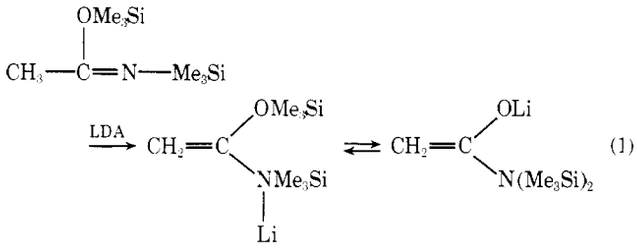
Scheme I



a, **8**, −100 °C, NH_4Cl-H_2O; b, AgF, H_2O-THF.

erated according to established procedures,[11] and then cooled to −100 °C, whereupon a tetrahydrofuran solution of blocked quinone **8** was added over a period of a few seconds. The reaction mixture was allowed to warm to 0 °C over a 2-h period, and quenched with 1 equiv of ammonium chloride. The resultant dark crude blocked quinol **11** was immediately deblocked with 1 equiv of silver fluoride in THF–water (10:1).[3] The flocculent mixture was stirred at room temperature for 2.5 h to yield the desired quinol **4**[8b] in a 77% overall yield from masked quinone **8**.

Owing to the general acid and base lability of *p*-quinols,[12] a simple acetamide enolate equivalent was desired for the construction of quinol acetamide **5**. Toward this end the lithiation of *N,O*-bis(trimethylsilyl)acetamide (BSA)[13] with lithium diisopropylamide (LDA) was investigated (eq 1). Treatment of BSA with 1 equiv of LDA (−78 °C) under



(1)

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standard conditions[11] afforded the enolate **12** which can presumably exist as either of the two tautomeric species shown above. Addition of the masked quinone **8** to enolate **12** under conditions identical with those followed by ester enolate **10** afforded the adduct **13**. Quinone deprotection with aqueous silver fluoride with concomitant hydrolysis of the amide-protecting silyl ligands afforded the quinol acetamide **5** in 37% overall yield. The physical and spectroscopic data reported for both **4** and **5** are identical with those obtained for the compounds prepared via this route.[8b,14]

Experimental Section

Melting points are uncorrected. Infrared spectra were taken on a Beckman infrared spectrophotometer Model 4210. Nuclear magnetic resonance spectra were taken on a Varian Associates A-60D spectrometer. The mass spectra were recorded on a Du Pont MS 21-492B double focusing mass instrument at an ionizing voltage of 70–75 eV. Ultraviolet spectra were recorded on a Cary 14 instrument.

The term "dry tetrahydrofuran" refers to purification of the commercial material by distillation from lithium aluminum hydride under anhydrous conditions. "Dry acetonitrile" and "dry diisopropylamine" were obtained by distillation of the solvent from calcium hydride. TMSCN and BSA were purchased from Silar Laboratories Inc.

3,5-Dibromo-4-cyano-4-trimethylsilyloxy-2,5-cyclohexadienone (8). A 25-ml flask equipped with magnetic stirring bar and drying tube was charged with 102 mg (0.38 mmol) of 2,6-dibromo-*p*-benzoquinone (**7**) in 5 ml of dry acetonitrile, and the solution cooled to 0 °C. To the cold, stirred, yellow solution was added 0.05 ml of TMSCN (distilled from CaH_2) and 3 mg of triphenylphosphine. Stirring was allowed to continue at 0 °C for 1 h.

The solvent was removed in vacuo to yield 144 mg (100%) of a crude oil. Molecular distillation yielded a clear yellow oil: bp 110 °C (bath temperature) (0.022 mm); ir ($CHCl_3$) 3000 (methyls), 1665 cm^{-1}; NMR ($CDCl_3$) δ 0.35 (s, 9 H, $-Me_3Si$), 6.75 (s, 2 H, vinyl CH). The undistilled adduct **8** is quite pure and may be utilized without further purification.

Anal. Calcd for $C_{10}H_{11}Br_2NO_2Si$: C, 32.89; H, 3.04; Br, 43.77; N, 3.84; O, 8.76; Si, 7.69. Found: C, 32.98; H, 3.02.

General Procedure for the Preparation of Lithium Diisopropylamide. A solution of dry diisopropylamine in dry tetrahydrofuran was placed under nitrogen and cooled to between −50 and −20 °C (dry ice–2-propanol). To the clear, colorless solution was added 1 equiv of *n*-butyllithium (Alfa Inorganics) maintaining the temperature in the above range. The mixture was allowed to stir for an additional 5-min, and finally cooled to −78 °C (dry ice–2-propanol).

General Procedure for the Synthesis of Masked Quinols. To a cooled (−78 °C) solution of lithium diisopropylamide (1 equiv) in tetrahydrofuran was added 1 equiv of ethyl acetate or BSA. After stirring at −78 °C for 30 min, the enolate solution was then cooled to −100 °C (ether–liquid nitrogen). A solution of blocked quinone **8** in dry tetrahydrofuran was then added, and the reaction allowed to warm to 0 °C over a 2-h period, and quenched with 1 equiv of ammonium chloride in a minimum volume of water. The reaction was finally transferred onto sodium sulfate with dichloromethane. After filtration through Celite–sodium sulfate, the solvent was removed in vacuo to yield the crude masked quinol.

2,6-Dibromo-4-carboethoxymethyl-4-hydroxy-2,5-cyclohexadienone (4). A solution of lithium diisopropylamide (1.41 mmol) was prepared from 0.20 ml (1.41 mmol) of dry diisopropylamine and 0.58 ml (1.41 mmol) of *n*-butyllithium (2.44 M, hexane) in 10 ml of dry tetrahydrofuran. Reaction of 513 mg (1.41 mmol) of 2,6-dibromo blocked quinone **8** with 0.14 ml (1.41 mmol) of ethyl acetate under the above conditions yielded 602 mg of the corresponding masked quinol **11** as a crude, dark brown oil: NMR ($CDCl_3$) δ 0.30 (s, 9 H, Me_3SiO-), 1.43 (t, J = 7 Hz, 3 H, $-CO_2CH_2CH_3$), 2.69, 2.80 (s, 2 H, epimeric $-CH_2CO_2Et$), 4.20, 4.22 (q, J = 7 Hz, 2 H, epimeric $-CO_2CH_2CH_3$), 6.77 (s, 2 H, vinyl CH).

Deblocking of 602 mg (1.33 mmol) of **11** with 169 mg (1.33 mmol) of silver fluoride in 10 ml of THF–H_2O (10:1) was accomplished by stirring at room temperature for 2.5 h. The reaction mixture was diluted with dichloromethane and filtered, and the filtrate was washed successively with water and brine and dried (Na_2SO_4). The solvent was removed in vacuo to yield 400 mg of a clear, crude, dark brown oil established as 90% pure by gas chromatography (77% yield based on masked quinone **8**). Preparative thin layer chromatography on silica gel (ether eluent) gave a light tan solid, which was sublimed, and finally recrystallized twice from hexane–dichloromethane to yield analytically pure white needles: sublimed at 70 °C (bath temperature) (0.017 mm); mp 127.0–127.5 °C (lit. 121 °C);[8b] ir ($CHCl_3$) 3570 (free OH), 3470 (H-bonded OH), 3030, 2980, 1705 (ester C=O), 1685 cm^{-1} (dienone C=O); NMR ($CDCl_3$) δ 1.29 (t, J = 7 Hz, 3 H, $-CO_2CH_2CH_3$), 2.76 (s, 2 H, $-CH_2CO_2Et$), 4.18 (s, 1 H, OH), 4.25 (q, J = 7 Hz, 2 H, $-CO_2CH_2CH_3$), 7.41 (s, 2 H, vinyl CH); uv (CH_3OH) λ_{max} 257 nm (ϵ 9596); *m/e* 351.895 ± 0.003 (calcd for $C_{10}H_{10}Br_2O_4$, 351.895).

Anal. Calcd for $C_{10}H_{10}Br_2O_4$: C, 33.93; H, 2.85; Br, 45.15; O, 18.08. Found: C, 33.68; H, 2.82.

2,6-Dibromo-4-(carbamoylmethyl)-4-hydroxy-2,5-cyclohexadienone (5). A solution of lithium diisopropylamide was prepared from 0.52 ml (3.71 mmol) of dry diisopropylamine and 1.52 ml (3.71 mmol) of *n*-butyllithium (2.44 M, hexane) in 25 ml of dry tetrahydrofuran. Reaction of 1.35 g (3.71 mmol) of masked quinone **8** with 755 mg (3.71 mmol) of BSA under the above conditions yielded 1.53 g of the corresponding masked quinol **13** as a dark green foam: NMR ($CDCl_3$) δ 2.52 (s, 2 H, CH_2), 6.47 (s, 2 H, vinyl CH). Without purification **13** was treated with 338 mg (2.66 mmol) of silver fluoride in 16 ml of THF–H_2O (15:1) at room temperature for 6 h. Isolation of quinol acetamide **5** according to the procedure described for **4** afforded 1.06 g a dark foam which was purified by preparative thick layer chromatography on silica gel (ethyl acetate eluent). The resultant light tan solid, 441 mg, mp 188–190 °C (37%), was recrystallized three times from acetone–ether to give analytically pure colorless needles: mp 194–195 °C (lit. 195–196 °C);[8b] NMR (acetone-d_6) δ 2.79 (s, 2 H, CH_2), 7.59 (s, 2 H, vinyl C-H); uv (CH_3OH) λ_{max} 257 nm (ϵ 8315); *m/e* 322.881 ± 0.003 (calcd for $C_8H_7Br_2NO_3$, 322.879).

Anal. Calcd for $C_8H_7Br_2NO_3$: C, 29.57; H, 2.17; Br, 49.18; N, 4.31; O, 14.77. Found: C, 29.71; H, 2.30.

Acknowledgment. This work has been supported by the National Institutes of Health, Grant CA-17904.

Registry No.—**4**, 24744-57-8; **5**, 17194-81-9; **7**, 19643-45-9; **8**, 60498-69-3; **11**, 60498-70-6; **13**, 60498-71-7; TMSCN, 7677-24-9; lithium diisopropylamine, 4111-54-0.

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Secalonic Acids D and F Are Toxic Metabolites of *Aspergillus aculeatus*

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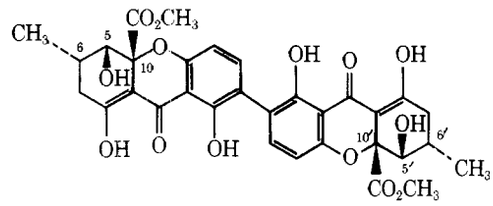
Received July 30, 1976

Continuing our studies of toxic substances produced by food spoilage fungi, we have investigated the metabolites of *Aspergillus aculeatus* Iizuka grown on white corn. Purification of the crude methylene chloride extracts by petroleum ether precipitation and subsequent preparative thin layer chromatography on silica gel yielded two toxic metabolites, secalonic acid D and F. Secalonic acid D has previously been isolated from *Penicillium oxalicum*.[3] Other secalonic acids are known metabolites of *Aspergillus ochraceus* (secalonic acid A),[4] *Claviceps purpurea* (secalonic acids, A, B, C)[5] and *Phoma terrestris* (secalonic acids, A, E).[6] Secalonic acid F is a new member of this group. In the following, we report the chemical identification, preparation and biological activity of the two toxins of *A. aculeatus* Iizuka. The more polar compound, obtained as yellow needles, was identical with secalonic acid A[7] as judged by comparison of infrared, ultraviolet, mass, and proton magnetic resonance spectra. Its optical rotation, $[\alpha]^{20}D$ +64° (*c* 0.14, $CHCl_3$), however, was opposite in sign from that of secalonic acid A and hence the toxin



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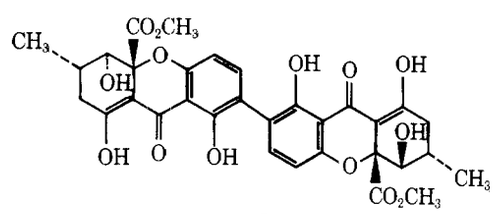

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is secalonic acid D (**1**)[3] (ergochrome EE), the enantiomer of secalonic acid A.

The second toxin crystallized from benzene–cyclohexane in the form of yellow needles, $[\alpha]^{20}D$ +190° (*c* 0.131, pyridine), *m/e* 638.15987 (calcd for $C_{32}H_{30}O_{14}$, 638.16356).[8] It was soluble in aqueous potassium carbonate, gave a positive ferric chloride test, and exhibited ultraviolet and infrared absorptions typical for secalonic acids.[5,9] The aromatic region of the proton magnetic resonance spectrum displayed four one-proton doublets at δ 6.51, 6.55, 7.30, and 7.33 each with a coupling constant of 8 Hz indicating that the new substance was an unsymmetrical dimer.[10] This hypothesis received further support from the appearance of the carbinol proton signals at C-5 and C-5′. A broad singlet at δ 4.08 and a doublet at δ 3.88, J = 11 Hz, suggest that hydroxyl and methyl groups are cis and trans oriented, respectively, in the two structural moieties. The circular dichroism spectrum was found to exhibit a large positive Cotton effect at 333 nm and comparison with values obtained for other secalonic acids[11] left no doubt that both C-10 and C-10′ have the *R* configuration. If it is assumed, in analogy to all known secalonic acids and ergochromes, that the C-6 and C-6′ methyl groups are trans to the C-10 and C-10′ carbomethoxy groups, respectively,[11-13] the new toxin, which we have named secalonic acid F (ergochrome BE),[14] should have structure **2**. This was confirmed as follows. When submitted to oxidation with potassium permanganate secalonic acid F (**2**) gave (*S*)-(−)-methylsuccinic acid[11] identical with a sample obtained by analogous oxidation of secalonic acid D (**1**). The two metabolites showed antimicrobial activity against *Bacillus megaterium*,[15] secalonic acid F being somewhat less active than secalonic acid D. Secalonic acid A had been reported to inhibit *Bacillus subtilis* and *Piricularia oryzae* but not other tested microorganisms.[4] Toxicity data will be presented in a forthcoming paper by Professor Gerald N. Wogan, Department of Nutrition and Food Science, M.I.T.

Experimental Section

Melting points were measured on a Kofler hot stage or a Büchi SMP20 oil bath apparatus and are corrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. The following spectrometers were used: IR, Perkin-Elmer 567; ultraviolet, Cary 14; ^1H NMR, Hitachi Perkin-Elmer R22 90 MHz; CD, Cary 60; mass spectra, Hitachi Perkin-Elmer RMU-6L and CEC 110B (Du Pont Industries).

Aspergillus aculeatus Iizuka was screened on a variety of grains in 2.8-l. Fernbach flasks on the shaker for 10 days at 30 °C.[15] The best were white corn and minute rice. The more traditional procedure of unagitated fermentation on glutinous rice yielded no toxin. After growth on white corn, the cultures were homogenized in a blender with methylene chloride, the homogenate was filtered, and the methylene chloride filtrate was concentrated in vacuo. Precipitation with petroleum ether gave ~400 mg of toxic petroleum ether insolubles (PEI)/kg of substrate corn. The PEI (814 mg) was dissolved in 40 ml of hot methylene chloride and filtered to remove 50 mg of nontoxic precipitate. The filtrate was concentrated in vacuo and chromatographed on silica gel GF254 plates containing 6% tartatic acid (solvent 2-pentanone–chloroform, 2:8). Two yellow bands (R_f 0.17 and 0.29), which both gave red-brown ferric chloride tests, contained toxic substances.

Secalonic Acid D (1). The slow-moving band (R_f 0.17) yielded 94 mg of a yellow glass which was crystallized first from carbon tetrachloride and then from chloroform to give 74 mg (9.1% of PEI) of light yellow needles (mp 281–283 °C in evacuated capillary, 255–259 °C on hot stage). High-resolution mass spectrum M^+ 638.16088 (calcd for $C_{32}H_{30}O_{14}$, 638.16353); $[\alpha]^{25}D$ +64° (*c* 0.14, chloroform); UV max (ethanol) 236, 265, and 338 nm (ε 17 800, 15 100, and 37 800); ^1H NMR (Me_2SO-d_6–1% Me_4Si) δ 1.05 (d, 6 H, J = 4 Hz), 2.0–3.0 (m, 6 H), 3.63 (s, 6 H), 3.80 (d of d, 2 H, J = 6 and 10 Hz), 6.02 (d, 2 H, J = 6 Hz, exchanges), 6.64 (d, 2 H, J = 8 Hz), 7.47 (d, 2 H, J = 8 Hz), 11.70 (s, 2 H, exchanges), 13.72 (bs, 2 H, exchanges); CD (*c* 2.9 × 10^{-2} mg/ml dioxane) λ 400 (Δε 0), 332 (+13.5), 290 (0), 270 (−4), 260 (−1.5), 225 (−48), 215 (−23). Further, the compound was found to have TLC behavior (silica gel GF254 containing 6% tartaric acid, solvent 2-pentanone–chloroform, 2:8) and an IR spectrum [(KBr) 3505, 1735, 1610, 1585, 1432, 1232, and 1061 cm^{-1}] identical with those of an authentic sample of secalonic acid A.

Oxidation of Secalonic Acid D (1). Secalonic acid D (54 mg) was dissolved in 5 ml of 2 N sodium hydroxide. The solution was cooled to 0 °C and added to 5 ml of saturated potassium permanganate solution at 0 °C. The reaction mixture was maintained at 0 °C for 48 h and then clarified at 0 °C with sulfur dioxide. Extraction with ethyl acetate (3 × 50 ml) gave 40 mg of crude reaction products which were chromatographed on a 1-mm Avicel F plate (solvent ammonia–1-propanol, 3:7, developed twice). The desired band (R_f 0.37) was eluted