Published in final edited form as:

J Org Chem. 2006 April 14; 71(8): 3314–3316. doi:10.1021/jo060059+.

Synthesis of a Novel Dicyano Abietane Analogue—a Potential Anti-inflammatory Agent

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Abstract

From a structure–activity relationship perspective, the new abietane **5** having cyano groups at C-2 and C-13 and a phenolic ring C has been synthesized and evaluated biologically because the related compound **4** has high potency in inflammation models in vitro and in vivo. Compound **5** was synthesized from **8**, which was obtained in five steps from the known compound **9**, via an unexpected aromatization caused by the addition of PhSeCl and subsequent oxidation/elimination of the selenated intermediate **14** with H₂O₂.

The abietane-type diterpenoids with 20 carbon atoms are believed to result from the pyrophosphate ester of geranyl-geraniol functioning as a leaving group in a complete cyclization sequence.¹ A large number of abietane-type diterpenoids having phenol [e.g., 1,2-dehydrohinokione (**1**)²] and quinone methide [e.g., taxodione (**2**)³ and taxodone (**3**)³] structures in ring C has been reported.¹ Amongst them, **2** and **3**, isolated from *Taxodiaceae* species, exhibit anti-tumor potency.³

We have previously shown that tricyclic compound ($\pm$)-**4** with cyano–enone functionalites in rings A and C is a novel and highly active inhibitor of nitric oxide (NO) production in mouse macrophages.⁴ Compound ($\pm$)-**4** is also orally active in a preliminary in vivo inflammation model.⁴ In addition, we have found that (+)-**4** having the opposite configuration to those of naturally occurring diterpenoids **1–3** shows 10 times higher inhibitory activity than (–)-**4** on NO production in mouse macrophages. To the contrary, (–)-**4** is active against MCF-7 mouse breast cancer cell lines, whilst (+)-**4** is inactive.⁵

Thus, from the perspective of structure–activity relationships, we envisioned the synthesis of a new abietane analogue **5**, which has two cyano groups at C-2 and C-13 positions⁶ and a

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Supporting Information Available: General experimental procedures, experimental procedures for **10–12**, UV, IR, and MS spectra for **8** and **13**, and ¹H and ¹³C NMR spectra for **5** and **10–13**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

phenolic ring C. Analogue **5** is similar in structure to that of the naturally occurring abietane, 1,2-dehydrohinokione (**1**). Analogue **5** differs from **4** in that **5** has only one Michael acceptor whereas **4** has three. Side effects and/or toxicity, which might be caused by Michael acceptors, may be reduced.

We herein describe the synthesis of compound **5** via an unexpected aromatization caused by the addition of phenylselenenyl chloride (PhSeCl) and subsequent oxidation/elimination of the selenated intermediate with H₂O₂.⁷ Although various syntheses of the abietane skeletons having a phenolic ring C have been reported,^{1b,1c} the construction of the skeleton by our synthetic sequence including this novel aromatization represents a new route to abietanes. We also briefly report the inhibitory activity of **5** against NO production in RAW cells.

Initially, we intended to synthesize **5** from acid **6** by decarboxylation at the C-8 position, but, due to unsuccessful conversion of the corresponding methyl ester⁸ to **6** under various conditions,⁹ we were not able to obtain **6**. However, when we attempted to synthesize the new tricyclic-*bis*-enone **7** with a methoxymethyl group at C-8 from **8** by addition with PhSeCl, followed by oxidation/elimination of the selenated intermediate with H₂O₂, the desired compound **5** was unexpectedly obtained from **8** in moderate yield. This transformation and the synthesis of **8** are shown in Scheme 1.

The direct synthesis of methyl ether **12** from keto-alcohol **9**, whose synthesis¹⁰ we explored for our projected synthesis of C-8 functionalized tricyclic-*bis*-enone compounds, with CH₃I in the presence of NaH in DMF was unsuccessful because **12** was only obtained in low yield along with several by-products, one of which is **12** with a C-2 methyl group. Therefore, we considered that protection of C-3 carbonyl group of **9** is necessary for the synthesis of **12** in good yield. Ketal **10** was synthesized in 99% yield from **9** with ethylene glycol in the presence of pyridinium *p*-toluenesulfonate (PPTS) in benzene.¹¹ Methyl ether **11** was obtained in 90% yield from **10** using the same conditions as for converting **9** to **12**. Deketalization of **11** using PPTS in aqueous acetone¹¹ produced **12** in 99% yield. Thus, **12** was obtained from **9** in good overall yield (88%). A chromium-mediated allylic oxidation¹² of **12** with 70% *tert*-butyl hydroperoxide gave enone **13** in 77% yield. Double cyanation of the *bis*-enolate of **13** with *p*-toluenesulfonyl cyanide (*p*-TsCN) in THF¹³ afforded dinitrile **8**¹⁴ in 82% yield. The desired compound **5** was synthesized in 45% yield (overall yield from **9**, 25%) by treatment of **8** with PhSeCl in the presence of pyridine in CH₂Cl₂⁷ and followed by treatment with 30% aqueous H₂O₂ solution.^{7,15} The structure of compound **5** is fully characterized by ¹H and ¹³C NMR, low-and high-resolution MS, UV, IR and elemental analysis (see Experimental section).

For confirmation about which step causes aromatization of ring C, the addition step with PhSeCl or the oxidation/elimination step of phenylselenenyl group with H₂O₂, we attempted to isolate the selenated intermediate **14**. Treatment of **8** with PhSeCl gave the four possible isomers, **14a–d** in 50% yield.¹⁶ The major pair, **14a** and **14b** (38% yield) and the minor pair, **14c** and **14d** (12% yield) were separated by flash column chromatography. The isolated yields and ¹H NMR of each pair showed that the ratio of four isomers (**14a:14b:14c:14d**) is approximately 5:5:2:1. The oxidation/elimination of each pair with 30% aqueous H₂O₂ solution gave **5** in the same yield (65%). Therefore, we believe that ring C is simultaneously aromatized following oxidation/elimination of the C-2 and C-13 phenylselenenyl groups, and the resulting unstable dienone **7** fragments immediately by a vinylogous retro aldol type pathway. Possible mechanisms are shown in Scheme 2. Our literature survey disclosed that ester **15** having a hydroxymethyl group protected with an allyl group gives phenol **16** under various ester hydrolysis conditions.¹⁷ However, our work seems to be the first example where aromatization is accomplished from a γ -methoxymethyl- α,β -unsaturated ketone by oxidation/elimination of the selenated intermediate with H₂O₂.

We have evaluated the inhibitory activity of abietane analogue **5** and hydrocortisone, which is clinically used as an anti-inflammatory drug, on NO production in RAW 264.7 cells stimulated with interferon- γ .¹⁸ Interestingly and importantly, we have found that **5** ($IC_{50} = 0.12 \mu M$) is two times more potent than hydrocortisone ($IC_{50} = 0.28 \mu M$). Thus, the abietane analogue **5** represents a new class of drug candidates for inflammatory diseases.

Experimental Section

($\pm$)-(4a β ,8a β ,10a α)-4,4a,7,8,8a,9,10,10a-Octahydro-8a-methoxymethyl-1,1,4a-trimethylphenanthrene-2,6(1*H*,3*H*)-dione (**13**)

To a solution of **12** (320 mg, 1.10 mmol) in CH_2Cl_2 (5.4 mL) was added *t*-BuOOH (70% aqueous solution, 1.5 mL). To the mixture was added CrO_3 (131 mg, 1.31 mmol) under cooling in an ice-water bath. The mixture was stirred at rt for 70 min. The reaction mixture was diluted with CH_2Cl_2 - Et_2O (1:2, 30 mL). It was washed with 5% aqueous NaOH solution (2×20 mL). The basic washings were extracted with CH_2Cl_2 - Et_2O (1:2, 2×20 mL). After the combined organic layer was washed with 5% aqueous HCl solution (twice), it was worked up by the standard method to give a residue as a brown oil. The residue was purified by flash column chromatography [hexanes-ethyl acetate (1:1)] to give **13** as a crystalline solid (259 mg, 77%). mp 123–125 °C. 1H NMR ($CDCl_3$) δ 5.95 (1H, s), 3.54 (1H, *d*, $J = 9.15$ Hz), 3.41 (1H, *d*, $J = 9.15$ Hz), 3.33 (3H, s), 2.59 (3H, m), 2.30 (1H, m), 2.11 (3H, m), 1.95–1.55 (6H, m),¹⁹ 1.24, 1.11, 1.09 (each 3H, s); ^{13}C NMR ($CDCl_3$) δ 215.7, 201.5, 173.6, 125.4, 76.0, 59.2, 52.7, 47.9, 40.4, 40.0, 37.0, 36.7, 36.2, 34.3, 34.2, 26.2, 21.9, 21.8, 19.5. HRMS (ESI+) calcd for $C_{19}H_{28}O_3 + H$ 305.2117. Found: 305.2131. Anal. Calcd for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.68, H, 9.24.

($\pm$)-(4a β ,8a β ,10a α)-1,2,3,4,4a,6,7,8,8a,9,10,10a-Dodecahydro-8a-methoxymethyl-1,1,4a-trimethyl-2,6-dioxophenanthrene-3,7-dicarbonitrile (**8**)

To a solution of **13** (139 mg, 0.46 mmol) in THF (5.1 mL) was added LDA (2M in THF/heptane, 633 μL , 1.27 mmol) at $-78^\circ C$. The mixture was stirred at rt for 20 min. Then, it was cooled at $-78^\circ C$ for 10 min. To the mixture was added a cloudy solution of *p*-TsCN (95%, 348 mg, 1.82 mmol) in THF (3.9 mL) at $-78^\circ C$. The mixture was stirred at the same temperature for 30 min. To the mixture was added saturated aqueous NH_4OH solution (2.7 mL). The mixture was allowed to reach rt. The aqueous layer was acidified with 10% aqueous HCl solution. The acidic aqueous solution was extracted with ethyl acetate (3×30 mL). The extract was worked up by the standard method to give an amorphous solid (197 mg). The solid was purified by flash column chromatography [hexanes-ethyl acetate (1:1.5)] to give **8** (133 mg, 82%) as an amorphous solid. 1H NMR ($CDCl_3$) major tautomer **8a**, δ 6.50 (1H, brs), 6.02 (1H, s), 4.21 (1H, dd, $J = 14.3$ and 4.76 Hz), 3.60 (1H, dd, $J = 9.52$ and 1.29 Hz), 3.50 (1H, d, $J = 9.52$ Hz), 3.32 (3H, s), 2.40 (1H, ABq, $J = 14.6$ Hz), 2.34 (1H, ABq, $J = 14.6$ Hz), 1.21, 1.17, 1.15 (each 3H, s); minor tautomer **8b**, δ 6.00 (1H, s), 4.26 (1H, dd, $J = 15.0$ and 4.39 Hz), 4.06 (1H, dd, $J = 13.9$ and 5.13 Hz), 3.69 (1H, dd, $J = 9.52$ and 1.83 Hz), 3.49 (1H, d, $J = 9.52$ Hz), 3.33 (3H, s), 1.21, 1.20, 1.16 (each 3H, s). HRMS (ESI+) calcd for $C_{21}H_{26}N_2O_3 + H$ 355.2022. Found: 355.2012. Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.29; H, 7.47; N, 7.89.

($\pm$)-(4a β ,10a α)-1,2,4a,9,10,10a-Hexahydro-6-hydroxy-1,1,4a-trimethyl-2-oxophenanthrene-3,7-dicarbonitrile (**5**)

To a solution of PhSeCl (155 mg, 0.81 mmol) in CH_2Cl_2 (3.6 mL) was added a solution of pyridine (68 mg, 0.86 mmol) in CH_2Cl_2 (0.9 mL) in an ice bath. The mixture was stirred in the same bath for 15 min. To the mixture was added a solution of **8** (70 mg, 0.20 mmol) in CH_2Cl_2 (1.5 mL) in an ice bath. The mixture was stirred in the same bath for 1 h. After the mixture was washed with 10% aqueous HCl solution (2×3.6 mL), 30% H_2O_2 (155 μL) was

added in an ice bath. The mixture was stirred for 10 min in the same bath. Subsequently, 30% H₂O₂ (90 μ L) was added and the mixture was stirred for additional 10 min. The reaction mixture was worked up by the standard method to give a residue (64 mg). The residue was purified by flash column chromatography [hexanes–ethyl acetate (1:1)], followed by preparative TLC [hexanes–ethyl acetate (3:2)] to give **5** (27 mg, 45%) as an amorphous solid. The solid was treated with hexanes to give crystals. mp > 220 °C (dec.). UV (EtOH) λ_{max} (log ϵ) 213 (4.32), 240 (4.06), 311 (3.43) nm. IR (KBr) 3304, 2976, 2930, 2228, 1672, 1615, 1507, 1417, 1388, 1286 cm⁻¹. ¹H NMR (CDCl₃) δ 8.31 (1H, s), 7.31 (1H, s), 7.14 (1H, brs), 7.10 (1H, s), 2.94 (2H, m), 2.19 (1H, dd, J = 12.1 and 2.9 Hz), 1.96 (2H, m), 1.50, 1.30, 1.25 (each 3H, s); ¹³C NMR (CD₃OD) δ 199.4, 169.7, 160.0, 150.3, 135.8, 128.8, 117.7, 116.2, 115.9, 113.0, 100.0, 47.8, 46.3, 43.2, 29.5, 27.7, 27.3, 21.8, 19.9. MS (ESI+) m/z 307 [M+H]⁺. HRMS (ESI+) calcd for C₁₉H₁₈N₂O₂+H 307.1447. Found: 307.1454. Anal. Calcd for C₁₉H₁₈N₂O₂: C, 74.49; H, 5.92; N, 9.14. Found: C, 74.38; H, 5.90; N, 9.20.

Isolation of Phenylselenyl Adducts **14** for Confirmation of Mechanism

To a solution of PhSeCl (86 mg, 0.44 mmol) in CH₂Cl₂ (2.0 mL) was added a solution of pyridine (38 mg, 0.48 mmol) in CH₂Cl₂ (0.5 mL) in an ice bath. The mixture was stirred in the same bath for 15 min. To the mixture was added a solution of **8** (39 mg, 0.11 mmol) in CH₂Cl₂ (0.9 mL) in an ice bath. The mixture was stirred in the same bath for 1 h. After the mixture was washed with 10% aqueous HCl solution (2 $\times$ 2 mL), it was worked up by the standard method to give a yellow residue (77 mg). The residue was purified by flash column chromatography [hexanes–ethyl acetate (3:2)] to give a mixture of two isomers **14a** and **14b** (27.6 mg, 38%) and a mixture of two isomers **14c** and **14d** (8.5 mg, 12%) as a yellow amorphous solid, respectively.¹⁶ The ratio of four isomers (**14a**:**14b**:**14c**:**14d**) was approximately 5:5:2:1. A mixture of **14a** and **14b**: ¹H NMR (CDCl₃) **14a**, δ 6.17 (1H, s), 3.46 (1H, dd, J = 9.16 and 1.46 Hz), 3.37 (1H, d, J = 9.16 Hz), 3.27 (3H, s), 1.65, 1.22, 1.18 (each 3H, s); **14b**, δ 6.90 (1H, s), 3.27 (3H, s), 3.19 (1H, d, J = 14.8 Hz), 3.11 (1H, d, J = 14.8 Hz), 1.70, 1.29, 1.19 (each 3H, s). MS (ESI+) m/z 667 [M+H]⁺. HRMS (ESI+) calcd for C₃₃H₃₄N₂O₃Se₂+H 667.0978, Found: 667.0974. A mixture of **14c** and **14d**: ¹H NMR (CDCl₃) **14c**, δ 6.29 (1H, s), 3.54 (1H, dd, J = 9.15 and 1.47 Hz), 3.36 (1H, d, J = 9.15 Hz), 3.27 (3H, s), 1.45, 1.26, 1.17 (each 3H, s); **14d**, δ 7.00 (1H, s), 3.35 (3H, s), 3.26 (1H, d, J = 14.3 Hz), 3.09 (1H, d, J = 14.3 Hz), 1.47, 1.20, 1.18 (each 3H, s). MS (ESI+) m/z 667 [M+H]⁺. HRMS (ESI+) calcd for C₃₃H₃₄N₂O₃Se₂+H 667.0978, Found: 667.0975.

Conversion of a Mixture of **14a** and **14b** to **5**

To a solution of **14a** and **14b** (13.6 mg, 0.02 mmol) in CH₂Cl₂ (0.6 mL) was added 30% H₂O₂ (16 μ L) in an ice bath. The mixture was stirred in the same bath for 10 min. The reaction mixture was diluted with CH₂Cl₂. The solution was worked up by the standard method to give a residue (6.4 mg). The residue was purified by preparative TLC [hexanes–ethyl acetate (3:2)] to give **5** (4.1 mg, 65%) as an amorphous solid.

Conversion of a Mixture of **14c** and **14d** to **5**

A mixture of **14c** and **14d** gave **5** (65%) as an amorphous solid according to the same procedure as for the mixture of **14a** and **14b**.

Acknowledgment

We thank Drs. Karen T. Liby and Michael B. Sporn (Dartmouth Medical School) for the evaluation of biological potency of compound **5**. This investigation was supported by funds from NIH Grant 5R03-CA105294.

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- ¹H NMR (CDCl₃) showed that dinitrile **8** exists as an inseparable mixture of two tautomers **8a** and **8b** at a 2:1 ratio in CDCl₃. Because a hydroxyl group and an α-proton of cyano group of **8a** were observed at δ 6.50 as a broad singlet and 4.21 as a double doublet (*J* = 14.3 and 4.76 Hz), respectively, although we concluded that **8a** has one of two possible structures as shown below, we could not determine which structure corresponds to **8a**. Because the two α-protons of the two cyano groups of **8b** were observed at δ 4.26 (*J* = 15.0 and 4.39 Hz) and 4.06 (*J* = 13.9 and 5.13 Hz) as double doublets, respectively, we concluded that **8b** has the structure as shown below with equatorial cyano groups.

15. DDQ oxidation of **8** did not give **5** and **7** but a complex mixture product.
16. Structure of each isomer cannot be assigned. Analytically pure sample of **14** could not be obtained.
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19. Overlapped signals which cannot be assigned.

SCHEME 1a.

^aKey: (a) ethylene glycol, PPTS, PhH, 99%; (b) CH₃I, NaH, DMF, 90%; (c) PPTS, aq acetone, 99%; (d) CrO₃, *t*-BuOOH, CH₂Cl₂, 77%; (e) *p*-TsCN, LDA, THF, 82%; (f) PhSeCl, pyr., CH₂Cl₂; 30% H₂O₂, 45%; and (g) DDQ, 1,4-dioxane.

SCHEME 2a.

^aKey: (a) PhSeCl, pyr., CH₂Cl₂, 50%; (b) 30% H₂O₂, 65%.