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New Anthracene Derivatives from *Coussarea macrophylla*

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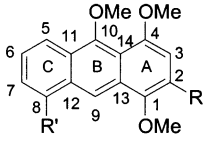
Five new naturally occurring anthracene derivatives, 1,4,10-trimethoxyanthracene-2-carbaldehyde (**1**), (1,4,10-trimethoxy-2-anthracen-2-yl)methanol (**2**), 1,4,8,10-tetramethoxyanthracene-2-carbaldehyde (**3**), 1,4,10-trimethoxyanthracene-2-carboxylic acid (**4**), and 1,3-dimethoxy-2-methoxymethylanthraquinone (**5**), were isolated from *Coussarea macrophylla* along with three known compounds, 3-hydroxy-1-methoxy-2-methoxymethylanthraquinone (**6**), scopoletin, and 3-*epi*-pomolic acid. The structures of **1**−**5** were determined on the basis of analysis of their spectroscopic data and by total synthesis.

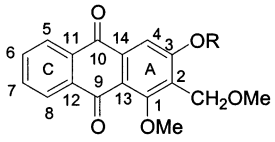
About 150 plants of the genus *Coussarea* grow in Central and South American countries, between Cuba and the equatorial regions;[1] however, despite the wide occurrence of *Cousarrea* species, their secondary metabolites are almost unknown.[2] *Coussarea macrophylla* Muell. Arg. (Rubiaceae) is a woody plant, occurring in the tropical swamp forests of Ecuador. It usually stands upright, reaching a height of 4 m. Upon cutting, the fruits emanate a pleasant peppermint fragrance. Although this species is not used in traditional medicine in Ecuador, during a general screening of Ecuadorian plants for new bioactive compounds, we considered this plant worthy of phytochemical investigation.

Results and Discussion

The ground bark of *C. macrophylla* was extracted with EtOAc, followed by 95% EtOH. The first extract was partitioned between hexane and MeCN to remove most of the chlorophylls, and the polar phase was then submitted to conventional purification procedures, resulting in the isolation of four new anthracene derivatives (**1**−**3** and **5**), along with scopoletin and 3-*epi*-pomolic acid. The EtOH extract was submitted to extraction with Na_2CO_3, yielding a new naturally occurring anthracenecarboxylic acid (**4**), along with the known anthraquinone **6**.



1 R = CHO R' = H
2 R = CH_2OH R' = H
3 R = CHO R' = OMe
4 R = COOH R' = H
4a R = COOMe R' = H



5 R = Me
6 R = H

Compound **1** was detected as a highly yellow fluorescent spot on TLC plates and gave a molecular ion peak at *m*/*z* 296.1046 in the HRMS, which, in accordance with data obtained from the NMR spectra, corresponded to the molecular formula $C_{18}H_{16}O_4$. The UV absorption curve showed multiple absorption maxima between 220 and 450 nm, with those at higher wavelength (>330 nm) being weak as in anthracene. Four of the 10 hydrogens of the nucleus were substituted by three methoxy groups [δ 4.07, 4.15, and 4.20 (3H each, 1s each)] and one formyl group (*C*HO δ_C 189.1; C*HO* δ 10.64). The ^{1}H NMR spectrum displayed a pattern of four aromatic protons assignable to an unsub-

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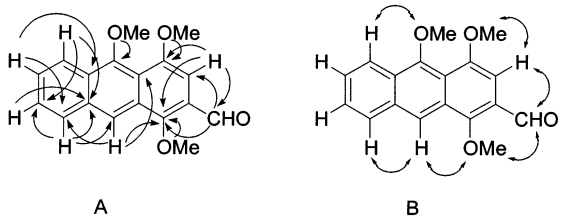


Figure 1. Selected HMBC (A) and NOESY (B) correlations of compound **1**.

stituted ring of the anthracene moiety. This comprised two sets of double doublets at δ 8.10 and 8.45, each integrating for one proton, attributed to H-8 and H-5, respectively, and a multiplet centered at δ 7.59, integrating for two protons, assigned to H-6 and H-7. In addition, there were two singlets at δ 7.05 and 8.65, which were allocated to H-3 and H-9, respectively. The deshielded signals observed for H-5 and H-9 could be attributed to the occurrence of an oxygen atom in a peri position (C-10 and C-1, respectively).[3] Each carbon atom was correlated to the respective one-bond linked proton(s) by interpretation of the FGHMQC NMR spectrum, so that assignment of the structure of **1** was then carried out by interpretation of the FGHMBC NMR spectrum (Figure 1A). Particularly diagnostic for establishing the substitution pattern of ring A were the H−C long-range correlations of the formyl proton at δ 10.64 with the signals of an aromatic methine carbon at δ 96.6 (C-3) and an aromatic MeO-*C* carbon at δ 158.0 (C-1), and the correlations of an aromatic proton at δ 7.05 (s, H-3) with the signals of two carbons each bearing a MeO group (δ 158.0 and 153.1; C-1 and C-4, respectively). Further, the aromatic proton at δ 8.65 (s, H-9) showed long-range correlations with the carbon signals at δ 158.0 (C-1), 123.0 (C-14), and 128.6 (C-8), indicating that this singlet aromatic proton occurred at C-9 in ring B.

These results, as well as other C−H correlations observed between protons in ring A and the respective carbons, and NOESY correlations (Figure 1B) strongly supported the proposed structure of **1** as the new compound 1,4,10-trimethoxyanthracene-2-carbaldehyde.

Compound **2** gave a molecular ion at *m*/*z* 298.1206 in the HRMS, which, in accordance with the NMR spectra, corresponded to the molecular formula $C_{18}H_{18}O_4$. The ^{1}H NMR spectrum of compound **2** was similar to that of **1** with the exception of a singlet peak integrating for two protons at δ 4.90, representative of a benzylic hydroxymethyl group, replacing the signal of the formyl proton in **1**. Consistent with the electronic effects exerted by the different substituents at C-2, the singlet aromatic protons H-3 and H-9 of compound **2** moved upfield with respect to the corresponding signals of **1**, whereas the proton pattern in ring A remained unchanged. Compound **2** was also identical to the product obtained by reduction of **1** with $NaBH_4$; it was thus identified as the new compound (1,4,10-trimethoxyanthracen-2-yl)methanol.

The structure of compound **3** contained an additional OMe group with respect to **1**, as indicated by the molecular ion peak at *m*/*z* 326.1156 in the HRMS and an additional singlet at δ 4.10, integrating for three protons, in the ^{1}H NMR spectrum. Consequently, there were five aromatic protons as indicated by the ^{1}H NMR spectrum, and their pattern clearly showed that ring C of the anthracene moiety was monosubstituted by an OMe group which could be located either at C-5 or at C-8. The substituent was eventually placed on C-8 on the basis of NOESY evidence (Figure 2) and the deshielded signal observed at δ 9.10 for H-9. This proton was shifted downfield compared to the

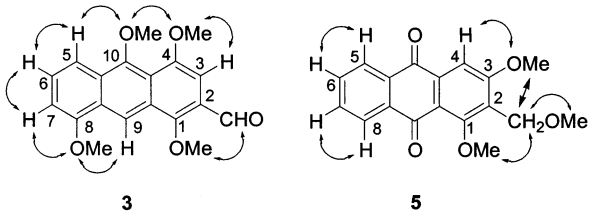


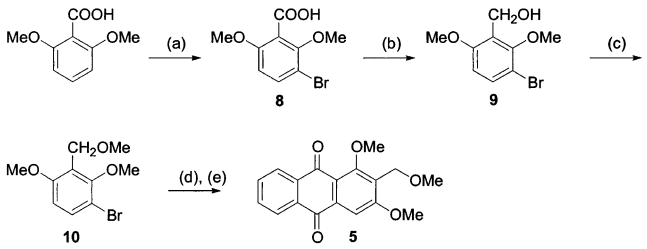
Figure 2. Selected NOESY correlations of compounds **3** and **5**.

corresponding signal in the ^{1}H NMR spectrum of **1**, due to the occurrence of an additional oxygen atom at its proximity (C-8).[3] Compound **3** was thus identified as the new compound 1,4,8,10-tetramethoxyanthracene-2-carbaldehyde.

Compound **4** gave a molecular ion peak at *m*/*z* 312.1150 by HRMS and showed the same pattern of aromatic protons and methoxy groups as compound **1**; moreover, it gave a methyl ester (**4a**; IR: 1737 cm^{-1}; ^{1}H NMR: δ 3.89 (s, 3H) for COO*Me*) upon exposure to diazomethane. These results firmly established the structure of **4** as the new compound 1,4,10-trimethoxyanthracene-2-carboxylic acid.

Finally, an anthraquinone $C_{18}H_{16}O_5$ (M^+: *m*/*z* 312) was obtained, showing two signals of two carbonyl groups at δ 181.0 and 183.1 in the ^{13}C NMR spectrum and two multiplets and one singlet in the aromatic portion of the ^{1}H NMR spectrum, which were assigned to four protons of an unsubstituted ring and to an isolated proton, respectively. Three methoxy singlets at δ 3.48, 4.02, and 4.09 and one methylene signal at δ 4.64, along with NOESY correlations (Figure 2), clearly indicated a 1,2,3-trisubstituted ring A, with the methoxymethyl group being in the C-2 position. The resulting structure was 1,3-dimethoxy-2-methoxymethylanthraquinone (**5**). This compound was previously obtained by methylation of lucidin[4,5] and of damnacanthol;[6] however, it has not yet been reported to occur in nature. Moreover, spectroscopic data have not been published so far. To confirm the structure and to secure a larger amount for biological tests, we completed a short unambiguous synthesis of **5** (Scheme 1), based on the reaction, developed by Sammes[7] and Biehl,[8] of arynes with 3-lithiated phthalides. As anticipated, the synthetic material was identical to the natural compound.

Scheme 1. Synthesis of Anthraquinone **5**[a]



[a] Reagents and conditions: (a) Br_2, $CHCl_3$, 0 °C, 80%; (b) BH_3, THF, reflux, 77%; (c) $CH(OMe)_3$, cat. *p*-TsOH, MeOH, reflux, 78%; (d) 1(3*H*)-isobenzofuranone, lithium 2,2,6,6-tetramethylpiperidide, THF, −60 °C, 20 min; then add **10**, −40 °C, 15 min, 20 °C, 1 h; (e) air, 15 h, 10% overall from **10**.

The identity of the known compounds 3-hydroxy-1-methoxy-2-methoxymethylanthraquinone[9−11] (**6**), scopoletin,[12] and 3-*epi*-pomolic acid[13] were determined on the basis of spectroscopic data and comparison with the data in the literature. They were further confirmed by 2D NMR spectra. The ^{13}C NMR data of anthraquinone **6** and of 3-*epi*-pomolic acid have not been reported in the literature before. It has been suggested that 2-methoxymethylanthraquinones of type **5** and **6**, typical of several Rubiaceae, might be artifacts formed from the corresponding 2-hydroxymethyl

derivatives by contact with methanol.[9−11] In the present investigation, compounds **5** and **6** were not exposed to MeOH; therefore, we consider the two anthraquinones as naturally occurring metabolites of *C. macrophylla*. Anthracene derivatives **1**−**4** belong to a rare class of natural products; as far as we know, oruwal and oruwalol (5- or 8-hydroxyoruwal) are the only related compounds isolated so far from the plant kingdom and occur in *Morinda lucida* Benth., another member of the Rubiaceae.[14] The oxidation pattern of the two groups of anthraquinols is, however, significantly different; compounds **1**−**4** exhibit two OMe substituents at C-1 and C-4 of the anthracene nucleus, whereas these positions are unsubstituted in oruwal derivatives. It has been suggested that the isolation of anthracene derivatives such as oruwal is of biogenetic interest, since it may indicate the operation of the shikimic acid−mevalonate pathway[15] in the biosynthesis of co-occurring anthraquinones.[14] The isolation of the six compounds **1**−**6** from *C. macrophylla* strongly corroborates this hypothesis and indicates that oxidation of the ring A of anthracene derivatives may occur at a later step of the biosynthesis.

Compounds **1**, **2**, and **5** were tested in standard filter paper disk assays[16] against *Staphylococcus aureus* and *Escherichia coli*; only aldehyde **1** showed an inhibition zone against *S. aureus* (inhibition diameter = 12 mm for 19 μg/disk). In addition, compounds **1** and **5** were inactive (LC_{50} > 280 ppm) in the brine shrimp (*Artemia salina*) lethality assay.[17] By contrast, the triterpene 3-*epi*-pomolic acid has been shown by Xu et al. to exhibit a significant anti-HIV-1 protease activity.[18] Compounds **3**, **4**, and **6** were not obtained in sufficient quantity for testing.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns hot-stage apparatus and are uncorrected. UV (in 95% EtOH) and IR (neat or in mini KBr disks) spectra were recorded on a Kontron Uvikon 941 and an FT-IR Perkin-Elmer Paragon 1000 PC spectrometer, respectively. ^{1}H and ^{13}C NMR spectra ($CDCl_3$) were determined on a Bruker CXP 300 spectrometer at 300 MHz (^{1}H) and 75.47 MHz (^{13}C), respectively. ^{1}H and ^{13}C chemical shifts (δ, ppm) are relative to residual $CHCl_3$ signals [δ_H 7.26, δ_C (central line of t) 77.1, respectively); the abbreviations s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad are used throughout; coupling constants (*J*) are reported in Hz. The spectra were interpreted by the aid of the standard FGCOSY, FGHMBC, and FGHMQC techniques. EIMS and HREIMS were recorded using a Finnigan MAT 8222 spectrometer, with ionization being induced by electron impact at 70 eV. Thin-layer chromatography was performed on silica gel 60 F_{254} Al sheets (Merck) and RP-18 HPTLC F_{254} glass-backed plates (Merck). Compounds were visualized under UV light (254 and 366 nm) and by spraying with 0.5% vanillin solution in H_2SO_4−EtOH (4:1) followed by heating. Flash column chromatography was performed with Merck Kieselgel 60 (40−63 μm) and Merck LiChroprep RP-18 (25−40 μm).

Plant Material. A sample of the bark of *C. macrophylla* was collected and identified by one of the authors (X.C.) in May 1998 in a tropical damp forest at an altitude of 250 m near the village Santa Ana, in the Provincia de Sucumbios, district Cascales, Ecuador. The material was dried, ground, and kept in the dark at room temperature. A voucher specimen, registered as Efrain Freire no. 2242, has been deposited in the Nacional Herbarium of Quito, Ecuador.

Extraction and Isolation. The plant material, 750 g of bark, was extracted with EtOAc (2 L) at room temperature three times with occasional stirring and filtered. The macerate was then extracted three times with 96% EtOH (2 L each) for 24 h each time. The extracts were combined and evaporated in vacuo to give 9.5 and 2.2 g of oily materials, extracts A and B, respectively.

Extract A was partitioned between MeCN and hexane to give 4.5 g of the MeCN extract (A_1) and 3.7 g of the hexane extract (A_2) after evaporation in vacuo. A quantity (1.1 g) of insoluble material (A_3) was also recovered. A sample of A_1 (4 g) was chromatographed on a silica gel (250 g) column, eluted with a hexane−EtOAc mixture (gradient of increasing polarity from 70:30 to 100% EtOAc) followed by EtOAc−MeOH, 4:1. The eluted fractions (15−20 mL each) were evaluated by TLC to give nine main fractions, CMA_1-1 to CMA_1-9.

Chromatography of fraction CMA_1-2 (50 mg) on a silica gel column (3.5 g, hexane−EtOAc, 93:7) yielded 15 mg of compound **1**. Chromatography of fraction CMA_1-3 (110 mg) on a silica gel column (10 g, hexane−EtOAc, 95:5) gave 10 major subfractions, CMA_1-3-1 to CMA_1-3-10. CMA_1-3-5 corresponded to compound **3**, while yellow fine crystals of anthraquinone **5** (4 mg) slowly separated from an EtOAc−hexane solution of CMA_1-3-3 kept at −20 °C. Chromatography of fraction CMA_1-4 (216 mg) on two consecutive silica gel columns (15 and 7 g, respectively), each eluted with hexane−EtOAc mixtures (gradient of increasing polarity from 85:15 to 0:100), afforded 3-*epi*-pomolic acid (16.4 mg). Chromatography of fraction CMA_1-5 (670 mg) on a silica gel column (50 g, hexane−EtOAc, gradient of increasing polarity from 40% to 100% EtOAc) yielded 15 mg of compound **2**. Chromatography of fraction CMA_1-9 (1.18 g) on a silica gel column (70 g, hexane−EtOAc + 1% AcOH, gradient of increasing EtOAc from 50% to 100%) yielded 12 major subfractions. Separation of fraction CMA_1-5-9 (6 mg) on a RP-18 column (MeOH−H_2O, 4:1) afforded scopoletin (1.8 mg).

Extract B was dissolved in Et_2O (200 mL) and extracted with four portions (50 mL each) of saturated aqueous Na_2CO_3. The basic layers were collected, acidified to pH 2 with 20% aqueous H_2SO_4, and extracted with three portions (50 mL each) of EtOAc. The organic layers were collected, washed with brine, and dried with anhydrous Na_2SO_4. Removal of solvent in vacuo afforded a sticky residue (165 mg), which was chromatographed on a silica gel (15 g) column. Elution with a hexane−EtOAc mixture (gradient of increasing polarity from 50:10 to 0:100), followed by an EtOAc−MeOH mixture (gradient of increasing polarity from 90:10 to 85:15), yielded five major fractions, CMB-1 to CMB-5. The first one (4.3 mg) corresponded to anthraquinone **6**, while fraction CMB-4 (44.2 mg) was further separated on a silica gel (12 g) column. Elution with CH_2Cl_2−EtOAc, 2:1, yielded five subfractions, CMB-4-1 to CMB-4-5. Chromatography of CMB-4-4 (2.8 mg) on a RP-18 column (eluent: MeCN−H_2O, 1:1) finally afforded compound **4** (0.8 mg).

1,4,10-Trimethoxyanthracene-2-carbaldehyde (1): pale yellow crystals (CH_2Cl_2−hexane); mp 145 °C; UV (EtOH) λ_{max} (log ϵ) 243 (4.51), 266 (4.75), 299 (4.44), 360 (3.62), 379 (3.76), 411 (3.75), 433 (3.78) nm; IR (KBr) ν_{max} 1675 (C=O), 1612, 1458, 1400, 1357, 1212, 1143, 1067, 976 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 296 (M^+, 100), 281 (72), 253 (24), 238 (20), 221 (6), 209 (15); HREIMS [M^+] *m/z* 296.1046 (calcd for $C_{18}H_{16}O_4$, 296.1049).

(1,4,10-Trimethoxyanthracen-2-yl)-methanol (2): pale yellow crystals (CH_2Cl_2−hexane); mp 116 °C; UV (EtOH) λ_{max} (log ϵ) 244 (4.50), 260 (4.62), 354sh (3.50), 370 (3.72), 384 (3.60), 402sh (3.53) nm; IR (KBr) ν_{max} 3392 (OH), 2916 (C−H), 1621, 1454, 1348, 1137, 1036 cm^{-1}); ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 298 (M^+, 100), 283 (33), 268 (40), 255 (35), 239 (28), 224 (26), 211 (20); HREIMS [M^+] *m/z* 298.1206 (calcd for $C_{18}H_{18}O_4$, 298.1205).

The same compound was obtained by reduction of aldehyde **1** with $NaBH_4$ according to a standard procedure.[19]

1,4,8,10-Tetramethoxyanthracene-2-carbaldehyde (3): pale yellow sticky solid; UV (EtOH) λ_{max} (log ϵ) 238 (4.62), 268 (4.81), 298sh (4.50), 344 (3.76), 364 (3.91), 382 (4.06), 422sh (4.02), 440 (4.06) nm; IR (film) ν_{max} 2933 (C−H), 1673 (C=O), 1613, 1459, 1353, 1067 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 326 (M^+, 100), 311 (60), 296 (13), 283 (22), 268 (28), 253 (15); HREIMS [$M]^+$ *m/z* 326.1156 (calcd for $C_{19}H_{18}O_5$, 326.1154).

Table 1. ^{1}H NMR Data of Compounds **1**−**5** ($CDCl_3$, 300 MHz)[a]

proton	1	2	3	4	5
3	7.05 s	6.77 s	7.10 s	6.90 s	
4					7.71 s
5	8.45 (dd, 8.0, 1.5)	8.38 dd (dd, 8.0, 1.5)	7.98 (br d, 8.0)	8.40 (dd, 8.0, 1.5)	8.28 m
6	7.59 m	7.50 m	7.50 (t, 8.0)	7.66 m	7.77 m
7	7.59 m	7.50 m	6.87 (br d, 8.0)	7.66 m	7.77 m
8	8.10 (dd, 8.0, 1.5)	8.01 dd (dd, 8.0, 1.5)		8.10 (dd, 8.0, 1.5)	8.28 m
9	8.65 s	8.42 s	9.10 s	8.50 s	
15	10.64 s	4.93 s	10.60 s		4.64 s
OMe-1	4.20 s	4.10 s	4.25 s	4.21 s	4.02 s
OMe-3					4.09 s
OMe-4	4.15 s	4.08 s	4.13 s	4.13 s	
OMe-8			4.13 s		
OMe-10	4.07 s	4.03 s	4.05 s	4.06 s	
OMe-15					3.48 s

[a] Multiplicities and coupling constants (Hz) in parentheses.

Table 2. ^{13}C NMR Data of Compounds **1**−**3**, **5**, and **6** ($CDCl_3$, 75.47 MHz)[a]

carbon	1	2	3	5	6
1	158.0 s	146.7 s	153.3 s	162.0[b] s	160.2[b] s
2	120.4 s	126.7 s	120.7 s	136.8 s	136.8 s
3	96.6 d	103.2 d	97.0 d	163.3[b] s	163.0[b] s
4	153.1 s	152.8 s	153.0 s	105.3 d	112.4 d
5	123.4 d	122.8 d	115.0 d	126.6[c] d	127.4[c] d
6	126.1 d	125.4 d	127.2 d	133.1[d] d	133.0[d] d
7	127.1 d	126.2 d	103.3 d	134.3[d]d	134.6[d] d
8	128.6 d	128.7 d	155.9 s	127.1[c] d	128.1[c] d
9	118.9 d	116.6 d	113.8 d	181.0 s	182.2[e] s
10	153.8 s	152.8 s	140.0 s	183.1 s	183.5[e] s
11	128.4 s	128.7 s	129.5 s	132.4[e] s	132.5[f] s
12	132.7 s	135.1 s	124.3 s	134.8[e] s	135.1[f] s
13	128.4 s	132.6 s	127.7 s	120.0 s	122.5[g] s
14	123.0 s	118.1 s	118.0 s	127.3 s	127.7[g] s
15	189.1 d	60.7 t	189.1 d	62.6 t	69.5 t
OMe-1	66.0 q	62.5 q	64.1 q	63.2 q	62.1 q
OMe-3				58.6 q	
OMe-4	56.2 q	56.2 q	56.1 q		
OMe-8			56.7 q		
OMe-10	63.7 q	63.5 q	66.6 q		
OMe-15				56.4 q	59.3 q

[a] Signal multiplicities determined by DEPT experiments. [b−g] Assignments with the same superscript in the same column may be interchanged.

1,4,10-Trimethoxyanthracene-2-carboxylic acid (4): pale yellow sticky oil; UV (EtOH) λ_{max} (log ϵ) 226 (4.73), 261 (4.61), 292 (4.35) nm; IR (film) ν_{max} 3200 (OH), 2916 (C−H), 1723 (C═O), 1454, 1234, 1040 cm^{-1}; ^{1}H NMR data, see Table 1; MS data were collected for the corresponding methyl ester **4a**, obtained by exposure of **4** to diazomethane: APCIMS *m*/*z* 327 $[MH]^+$, 312 $[327 - Me]^+$, 297 $[312 - Me]^+$; HREIMS $[M]^+$ *m*/*z* 326.1150 (calcd for $C_{19}H_{18}O_5$, 326.1154).

1,3-Dimethoxy-2-methoxymethylanthraquinone (5): pale yellow needles (EtOAc−hexane); mp 155−157 °C (lit. 161−162 °C);[10] UV (EtOH) λ_{max} (log ϵ) 202 (4.50), 245 (4.15), 274 (4.59), 334 (3.73), 364 (3.60) nm; IR (KBr) ν_{max} 2907 (C−H), 1668 (C═O), 1574, 1463, 1326, 1283, 1133, 714 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m*/*z* 312 (M^+, 6), 297 (34), 281 (12), 267 (20), 251 (3), 237 (10), 223 (4), 209 (4), 195 (3), 181 (7). Compound **5** was identical to an authentic synthetic sample (see below).

3-Hydroxy-1-methoxy-2-methoxymethylanthraquinone (6): pale yellow needles (EtOAc−hexane); mp 195−198 °C (lit. 204 °C);[9] UV, IR, ^{1}H NMR, EIMS, consistent with literature values;[9,11] ^{13}C NMR data, see Table 2.

Scopoletin and 3-*epi*-Pomolic Acid. Scopoletin was identical to an authentic sample. IR, ^{1}H NMR, and EIMS of 3-*epi*-pomolic acid were consistent with literature values;[13] ^{13}C NMR ($CDCl_3$) δ 14.9 (q, C-25), 16.1 (q, C-30), 16.8 (q, C-26), 18.2 (t, C-6), 22.1 (q, C-24),[a] 23.4 (t, C-11), 24.6 (q, C-29),[b] 25.0 (t, C-16),[c] 25.2 (t, C-21),[c] 25.8 (t, C-2),[c] 27.3 (q, C-27),[b] 28.0 (t, C-15), 28.1 (q, C-23),[a] 32.4 (t, C-7),[d] 32.7 (t, C-1),[d] 36.9 (s, C-10), 37.2 (t, C-22), 37.4 (s, C-4), 40.0 (d, C-20), 40.9 and 41.0 (2s, C-8 and C-14), 46.8 (d, C-9), 47.6 (s, C-17), 48.7 (d, C-5), 52.6 (d, C-18), 73.0 (s, C-19), 76.1 (d, C-3), 129.4 (d, C-12), 137.7 (s, C-13), 183.9 (s, C-28). ([a−d]Signal assignments may be interchanged.)

Synthesis of 1,3-Dimethoxy-2-methoxymethylanthraquinone (5). Br_2 (2.81 g, 17.6 mmol) was added dropwise to a solution of commercially available 2,6-dimethoxybenzoic acid (3 g, 16.5 mmol) in $CHCl_3$ (60 mL) cooled to 0 °C. The mixture was stirred for 90 min at 0 °C, then saturated aqueous $Na_2S_2O_5$ was added until decoloration. The two layers were separated, and the aqueous phase was acidified with 10% HCl and extracted exhaustively with Et_2O. The ether phase was dried ($MgSO_4$) and evaporated to give 3-bromo-2,6-dimethoxybenzoic acid (**8**)[20] as a pale yellow crystalline solid (3.44 g, yield = 80%). An analytical sample was obtained by recrystallization from EtOH−H_2O: mp 142−145 °C (lit.[20] 146−148 °C); IR (Nujol) ν_{max} 2924, 2854, 2630, 1711 (C═O), 1641, 1588, 1465, 1392, 1290, 1258, 1156, 1098, 1000, 911, 841, 804, 759, 714, 667, 636 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.90 (3H, s, OMe), 3.97 (3H, s, OMe), 6.67 (1H, d, J = 9.0 Hz, H-5), 7.58 (1H, d, J = 9.0 Hz, H-4); *anal*. C 41.35%, H 3.40%, calcd for $C_9H_9BrO_4$, C 41.41%, H 3.47%.

Borane−tetrahydrofuran complex (1.0 M in THF, 6 mL, 6 mmol) was added dropwise by syringe to a solution of compound **8** (0.59 g, 2.3 mmol) in THF (30 mL) at 0 °C under an argon atmosphere. After refluxing for 12 h, the solution was then cooled to 0 °C, and 6 M HCl (3 mL) was added, followed by 10% aqueous NaOH to basicity.[21] The aqueous phase was extracted exhaustively with Et_2O, and the collected organic layers were dried with $MgSO_4$. Evaporation of the solvent gave 3-bromo-2,6-dimethoxybenzyl alcohol (**9**) as a pale yellow oil (0.43 g, yield = 77%), which was immediately used in the following step. An analytical sample was obtained by filtration on a short silica gel column eluted with 1:1 hexane−EtOAc: IR (film) ν_{max} 3419 (OH), 3084, 2940, 2838; 1584, 1465, 1410, 1334, 1277, 1230, 1178, 1133, 1098, 1016, 895, 849, 800, 772, 682 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.40 (1H, br s, OH), 3.87 (3H, s, OMe), 3.89 (3H, s, OMe); 4.78 (2H, s, CH_2O), 6.61 (1H, d, J = 8.8 Hz, H-5), 7.45 (1H, d, J = 8.8 Hz, H-4); EIMS *m*/*z* 248 (^{81}Br M^+, 99), 246 (^{79}Br M^+, 100), 231 (80), 214 (75), 201 (30), 185 (15), 167 (20), 157 (10), 134 (40), 124 (20), 119 (15), 100 (20), 92 (20), 77 (50), 63 (55), 51 (35), 43 (8); HREIMS $[M]^+$ *m*/*z* 247.9867 (calcd for $C_9H_{11}{}^{81}BrO_3$, 247.9871).

Trimethyl orthoformate (0.29 g, 2.73 mmol) and catalytic *p*-TsOH were added to a solution of compound **9** (0.43 g, 1.74 mmol) in MeOH (30 mL); the resulting solution was refluxed for 1 h and then cooled to room temperature. Aqueous Na_2CO_3 (15 mL; 10%) was added, and most of the MeOH was evaporated in vacuo. The remaining solution was extracted with three portions (25 mL each) of Et_2O, and the collected organic layers were dried (anhydrous Na_2SO_4) and evaporated in vacuo to yield 3-bromo-2,6-dimethoxybenzyl methyl ether (**10**) as a pale yellow oil (0.35 g, yield = 78%), which was immediately used in the following step. An analytical sample was obtained by filtration on a short silica gel column eluted

with 1:1 hexane−EtOAc: IR (film) ν_{max} 3084, 2938, 2838, 1584, 1469, 1409, 1383, 1279, 1230, 1189, 1106, 1017, 990, 951, 902, 800 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3,43 (3H, s, OMe), 3.85 (3H, s, OMe), 3.90 (3H, s, OMe), 4.54 (2H, s, CH_2O), 6.61 (1H, d, J = 9.1 Hz, H-5), 7.47 (1H, d, J = 9.1 Hz, H-4); EIMS *m/z* 262 (^{81}Br M^+, 49), 260 (^{79}Br M^+, 50), 245 (65), 229 (85), 215 (20), 201 (5), 101 (35), 171 (25), 159 (5), 150 (45), 149 (20), 134 (45), 120 (35), 105 (25), 91 (45), 77 (50), 63 (45), 51 (30), 45 (100); HREIMS $[M]^+$ *m/z* 262.0031 (calcd for $C_{10}H_{13}{}^{81}BrO_3$, 262.0028).

BuLi (2.5 M in hexane, 1.25 mL, 3.12 mmol) was added dropwise by syringe to a solution of dry 2,2,6,6-tetramethylpiperidine (0.42 g, 2.97 mmol) in dry THF (3 mL) at −60 °C (CH_2Cl_2−dry ice bath) under an argon atmosphere. The resulting yellow solution was stirred at −60 °C for 10 min, then a solution of 1(3*H*)-isobenzofuranone (0.12 g, 0.89 mmol) in dry THF (15 mL) was added. The mixture was stirred at −60 °C for an additional 20 min, during which time it turned yellow-orange, then warmed to −40 °C. Compound **10** (0.23 g, 0.88 mmol) in dry THF (10 mL) was added by syringe, and the resulting red solution was stirred at −40 °C for 15 min, then warmed to room temperature, where stirring was continued for an additional 1 h. Saturated aqueous NH_4Cl was added to neutrality, and the resulting mixture was stirred for 15 h under air. THF was evaporated in vacuo, and the remaining aqueous phase was extracted exhaustively with CH_2Cl_2. The organic layer was dried (anhydrous Na_2SO_4) and evaporated in vacuo to yield an oily residue. Chromatography on a silica gel column (20 g; eluent hexane−EtOAc, 3:1) yielded anthraquinone **5** (27.5 mg, yield = 10%), identical (mp, IR, ^1H NMR, ^{13}C NMR) to the natural sample. *anal*. C 69.30%, H 5.22%, calcd for $C_{18}H_{16}O_5$, C 69.22%, H 5.16%.

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