Isolation and Synthesis of Methyl Bovinate, an Unusual Pulvinic Acid Derivative from *Suillus bovinus* (Basidiomycetes)

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Cultures and fruit bodies of *Suillus bovinus* produce the pulvinic acid derivative methyl bovinate (**4**), which contains an extra carbonyl group that bridges ring A of methyl variegatate with the hydroxy group at the central butenolide ring. This unprecedented structure was deduced from the spectroscopic data and confirmed by total synthesis *via* a grevillin intermediate. In this synthesis, the menthyl group was used for carboxyl protection.

Key words: Suillus, Boletales, Pulvinic Acids, Mushroom Pigments, Synthesis

Introduction

Suillus bovinus (L.: Fr.) Roussel (Kuhröhrling) is a common bolete found in sandy pine forests in autumn. The fruit bodies contain boviquinone-4 as the main pigment [1], in addition to smaller quantities of bovilactone-4,4 [2], concentrated in the pink mycelium at the base of the fruit bodies. Atromentin [1] and the characteristic Boletales pigments atromentic acid (**1**), xerocomic acid (**2**), variegatic acid (**3**), and variegatorubin represent a second set of coloured metabolites found in *S. bovinus* [3, 4] (Fig. 1). These common pulvinic acids are also produced in mycelial cultures [5], in addition to an orange pigment, methyl bovinate (**4**) [3], which can be detected on TLC plates by its colour change to red-violet on exposure to gaseous ammonia. In this communication we report on the isolation, structural elucidation and synthesis of this unique fungal metabolite.

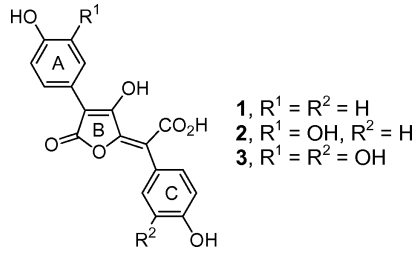


Fig. 1. Pulvinic acids from mycelial cultures of *S. bovinus*.

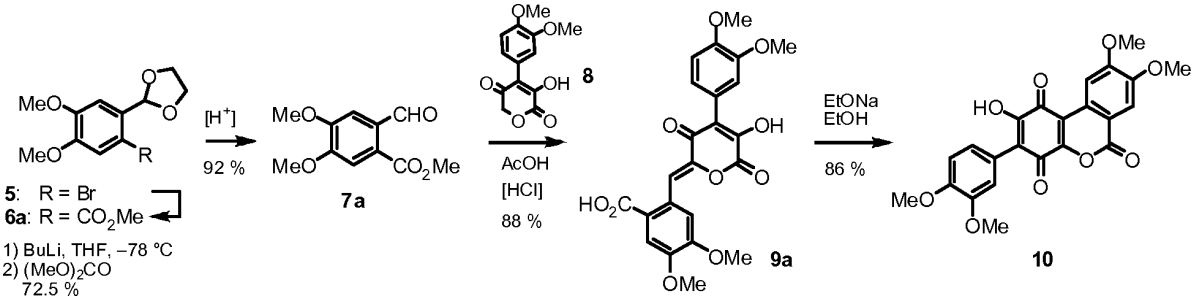
Results and Discussion

Isolation

The new pigment was isolated from mycelial cultures of *S. bovinus*, grown on Moser B fluid medium for 25 d. The cultures were extracted with acidified acetone, and the concentrated extracts were partitioned between water and EtOAc. Chromatography of the organic phase on a polyamide column with acetone as eluent yielded an orange-yellow pigment fraction, which was further purified by preparative TLC on silica gel. Methyl bovinate (**4**) was obtained pure after column chromatography on acetylated polyamide followed by repeated chromatography on Sephadex LH-20. The compound was also detected in the methanol extract of the fruit bodies by analytical HPLC.

Structural elucidation

Methyl bovinate was obtained as an orange powder, which exhibited UV/Vis maxima at λ_{max} = 206, 246, 270, and 408 nm. The high-resolution mass spectrum shows a strong molecular ion at *m/z* = 412.0434 corresponding to the molecular formula $C_{20}H_{12}O_{10}$. In the ^{1}H NMR spectrum a methyl ester signal at



Scheme 1. Attempted synthesis of methyl bovinate (**4**).

Table 1. ^{1}H-coupled ^{13}C NMR spectrum of compound **4**[a].

Position	δ_c	Multiplicity[b], *J* (Hz), coupling partner[c]
1	124.0	d, 7.6 (5-H)
2	108.1	D, 164 (2-H)
3	154.2	dd, 7.6 (5-H), 2.4 (2-H)
4	148.2	dd, 6.4 (2-H), 2.4 (5-H)
5	115.5	D, 164 (5-H)
6	110.9	dd, 8.8 (2-H), 1.4 (5-H)
7	158.6	dd, 4.8 (5-H), 1.4 (2-H)
1′	121.3	d, 9.0 (3′-H)
2′	116.4	Dd, 160 (2′-H), 6.8 (6′-H)
3′	145.6	ddd, 6.6 (5′-H), 3.8 (2′-H), 1.2 (6′-H)
4′	148.1	ddd, 7.0 (2′-H), 7.0 (6′-H), 3.0 (5′-H)
5′	116.1	D, 162 (5′-H)
6′	121.9	Dd, 164 (6′-H), 7.6 (2′-H)
1″	162.7[d]	s
2″	102.0	dd, 4.6 (2-H), 1.4 (5′-H)
3″	157.1[d]	s
4″	135.4[d]	s
5″	117.7	dd, 4.8 (2′-H), 4.8 (6′-H)
6″	165.9	q, 3.8 (OMe)
OMe	53.0	Q, 149 (OMe)

[a] 100.6 MHz, in [D$_6$]DMSO; [b] multiplets due to ^{1}J(C,H) couplings are indicated by capital letters; ^{3}J (C,H) and other couplings are in small letters; [c] confirmed by selective ^{1}H decoupling; [d] interchangeable. The height of the singlet at δ_C = 135.4 is half of that at 157.1 ppm.

δ_H = 3.94 is present in addition to two aromatic singlets at δ_H = 7.45 and 7.52 and typical signals for a 3,4-dihydroxyphenyl residue, matching those observed for ring C of variegatic acid (**3**) [6]. The ^{1}H-coupled ^{13}C NMR spectrum (Table 1) contains 20 signals, which can be ascribed to 19 signals in the aromatic region and that of a methoxy group (δ_C = 53.0). An analysis of this spectrum, assisted by selective decouplings, allowed us to propose the 5*H*-furo[3,4-*c*] benzopyran structure **4** for methyl bovinate (Fig. 2a). Of special significance for this assignment are the $^{3}J_{H,C}$-couplings depicted in Fig. 2b. Unusual is the high-field position of the singlet at δ_c = 135.4, which must belong to one of the oxygen-carrying carbon atoms in the butenolide ring. Simple pulvinic acids

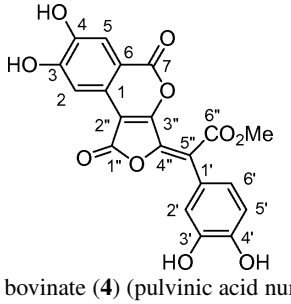


Fig. 2a. Methyl bovinate (**4**) (pulvinic acid numbering).

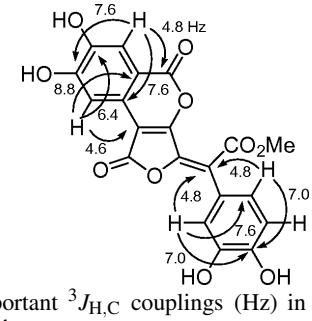


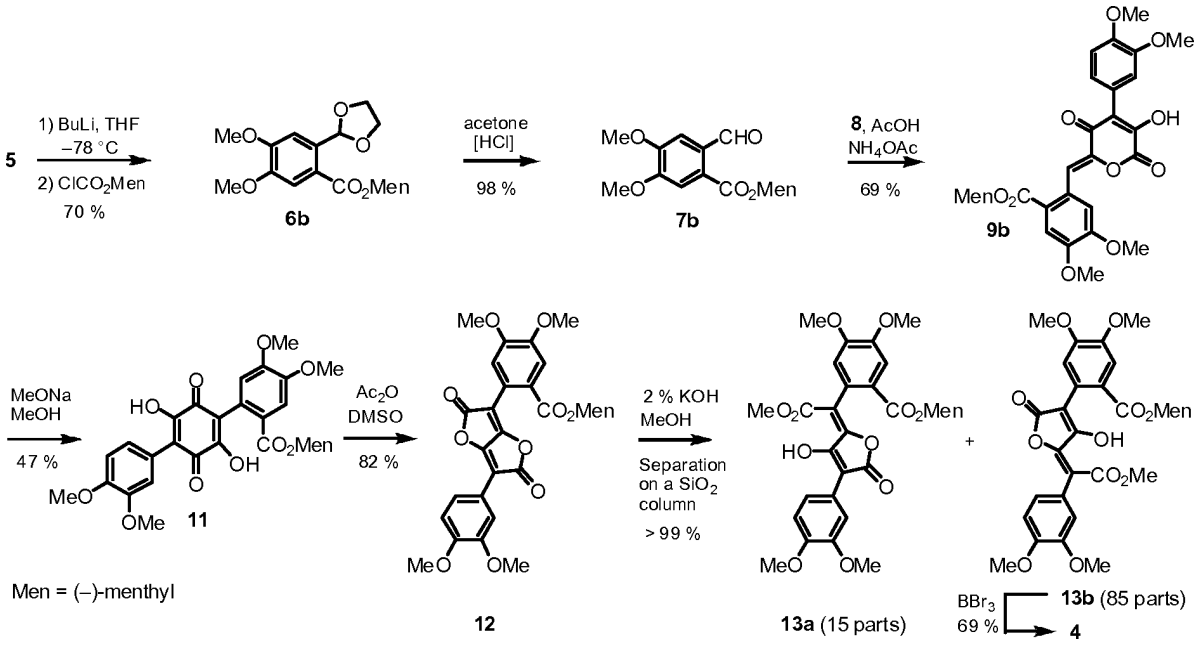
Fig. 2b. Important $^{3}J_{H,C}$ couplings (Hz) in the ^{13}C NMR spectrum of **4**.

show the corresponding signals in the range of 154 – 167 ppm [7].

In order to confirm this structure, we set out to undertake the total synthesis of methyl bovinate.

Total synthesis

Our first synthetic approach to methyl bovinate (**4**) started from the known acetal **5**, which is easily available in two steps from veratraldehyde [8] (Scheme 1). Lithium-halogen exchange with *n*-butyllithium followed by addition of dimethyl carbonate afforded methyl ester **6a**, which was then converted into aldehyde **7a** by acidic cleavage of the acetal group. Condensation of aldehyde **7a** with pyrandione **8** [9] in acetic acid containing a catalytic amount of HCl gave grevillin carboxylic acid **9a** in high yield. Clearly, the



Scheme 2. Synthesis of methyl bovinate (**4**).

methyl ester group was not stable enough to survive the condensation conditions. Attempts to carry out this reaction under milder conditions (*e. g.*, AcOH, ammonium acetate, 20 °C) were unsuccessful, again yielding the free acid **9a**. The alcoholate-catalysed rearrangement of the latter into the corresponding terphenylquinone [10], followed by treatment with mineral acid, proceeded with concomitant lactone formation and delivered quinone **10** in excellent yield.

In order to avoid the instability problems of the methyl ester, we used the menthyl group for carboxyl protection. The desired (–)-menthyl ester **6b** could be easily prepared from bromo acetal **5** and (–)-menthyl chloroformate. After acidic cleavage of the acetal group, the resulting *m*-opianic acid menthyl ester **7b** was condensed with pyrandione **8** to yield grevillin carboxylic ester **9b** (Scheme 2). We were pleased to note that this reaction and the subsequent methoxide-catalysed rearrangement into terphenylquinone **11** proceeded with retention of the ester group. Oxidative cleavage of dihydroxybenzoquinone **11** with Ac_2O/DMSO according to Moore and Wikholm [11] then afforded the dilactone **12** in high yield.

Treatment of the latter with dilute KOH in methanol led to the formation of two regioisomeric monolactone diesters **13a** and **13b**, which could be easily separated on a silica gel column. The structural assignment is based on the chemical shifts of the *ortho* protons at the 3,4-dimethoxyphenyl ring, those of isomer **13a** appearing at lower field due to the deshielding effect of the neighbouring butenolide ring [6]. The desired diester **13b** was obtained in 85 % yield, and we were pleased to note that treatment of diester lactone **13b** with BBr_3 in CH_2Cl_2 resulted in cleavage of the menthyl ester [12] with concomitant lactone formation to give methyl bovinate (**4**), in every respect identical with the natural product. This confirmed the structure of this unusual fungal pulvinic acid derivative and demonstrated the advantageous use of a menthyl group for carboxyl protection. Methyl bovinate (**4**) was obtained from bromo acetal **5** in 7 steps with 11 % overall yield.

The co-occurrence of methyl bovinate (**4**) with variegatic acid (**3**) in fruit bodies and cultures of *S. bovinus* points to a close biosynthetic relationship. Our attempts to introduce the extra carbonyl group by treatment of methyl variegatate with phosgene were unsuccessful.

Experimental Section

General

Melting points (uncorrected): Reichert Thermovar hot stage microscope. UV/Vis: Hewlett-Packard 8452 diode ar-

ray spectrophotometer. IR: Perkin-Elmer 1420 Ratio Recording Infrared Spectrometer. Intensity of the bands: ss (very strong), s (strong), m (medium), and w (weak). NMR: Varian EM 390 with TMS as internal standard, Bruker AC 200 and AM 400 spectrometers, chemical shifts in δ [ppm] rel. to [D_6]DMSO (δ_H = 2.49, δ_C = 39.7), and $CDCl_3$ (δ_H = 7.26, δ_C = 77.1) as internal standard. MS: AEI MS 30 and MS 50 (direct inlet, 70 eV). TLC: Silica gel 60 F_{254} aluminium foils (Merck); solvent system A (v/v): toluene/HCO_2Et/HCO_2H, 10 : 5 : 3. Column chromatography: Silica gel 60 (40 – 63 μm, Merck) and acetylated polyamide-6 (MN Polyamide 6-AC, Macherey-Nagel). Gel chromatography: Sephadex LH-20 (Pharmacia). HPLC: Analytical HPLC: Waters M 6000A pump with gradient controller and photodiode array detector M 990 equipped with a Knauer Lichrosorb RP-18 column (7 μm, 250 × 4 mm). Solvent A (v/v): water/acetonitrile 9 : 1 with 0.5 % (v) CF_3CO_2H, solvent B: water/acetonitrile 1 : 9. Gradient: start 90 % A, 10 % B; 20 min: 60 % A, 40 % B; 25 min: 60 % A, 40 % B; 30 min: 90 % A, 10 % B; flow rate 1 mL min^{-1}, detection range 230 – 550 nm. The elementary analyses were performed by the microanalytical laboratory of the Institute for Organic Chemistry and Biochemistry, University of Bonn.

Mycelium culture of Suillus bovinus

S. bovinus (strain 349, derived from fruit bodies collected near Weiden, Oberpfalz, Germany) was kept for 25 days on 1.25 L Moser B fluid medium [13] at r. t. in the dark.

*Isolation of methyl bovinate (**4**)*

The culture broth together with the mycelium was extracted with acidified acetone (2 × 1 L). The extracts were concentrated under reduced pressure, and the residue was partitioned between water and EtOAc. The combined organic extracts were dried (Na_2SO_4) and concentrated. Chromatography of the residue on a polyamide column with acetone as eluent yielded the crude pigments, which were separated by preparative TLC on silica gel (solvent system A). The yellow band with R_f = 0.27 (reversible red-violet colour change with gaseous NH_3!) was purified by column chromatography on acetylated polyamide (acetone/MeOH, 1 : 1 v/v). Further chromatography on Sephadex LH-20 (acetone/MeOH, 4 : 1 v/v) yielded **4** (10 mg) as an orange powder. – UV (MeOH): λ_{max} (lg ε) = 206 (4.52), 246 (4.47), 270 (4.36), 408 (4.40) nm. – IR (KBr): ν = 3400 (s, br.), 2950 (m), 1750 (ss), 1730 (ss, br.), 1640 (m), 1595 (m), 1520 (s), 1445 (m), 1430 (m), 1280 (ss), 1190 (s), 1150 (s), 1145 (m), 1045 (m), 1020 (w), 980 (m), 910 (w), 890 (w), 820 (w), 800 (w), 780 (w), 680 (w) cm^{-1}. – ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 3.94 (s, 3H), 6.85 (d, J = 8.4 Hz, 1H), 6.95 (dd, J = 8.4, 2 Hz, 1H), 7.22 (d, J = 2 Hz, 1H), 7.45 (s, 1H), 7.52 (s, 1H). – ^{1}H-coupled ^{13}C NMR see Table 1. – MS (DI, 250 °C): *m/z* (%) = 412 (100) [M]$^+$, 366 (50), 354 (60), 353 (85), 326 (35), 279 (38), 235 (30), 120 (70), 44 (75). – HRMS: *m/z* = 412.0434 (calcd. 412.0431 for $C_{20}H_{12}O_{10}$, [M]$^+$).

*Detection of methyl bovinate (**4**) in the fruit bodies*

Freeze-dried, defatted fruit bodies of *S. bovinus* (10 g) were extracted in a shaking apparatus for 1 h with MeOH (2 × 250 mL), containing a few drops of 2 N HCl and 50 mg of ascorbic acid. The extracts were concentrated, and the residue was distributed between EtOAc and diluted HCl (pH = 3). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure, and the residue was dissolved in a small amount of MeOH. After filtration over a SepPak C_{18}-cartridge, an aliquot of 10 μL was used for the HPLC investigation. The separation was carried out with a LiChrosorb RP-18 column (7 μm) from Knauer, Berlin, and diode array detection: t_R (**3**) = 13.48, t_R (**2**) = 16.83, t_R (**1**) = 21.18, t_R (**4**) = 23.88, t_R (variegatorubin) = 27.13. The identity of methyl bovinate (**4**) was established by co-chromatography with an authentic sample (identical t_R values and UV spectra).

*2-[1,3]Dioxolan-2-yl-4,5-dimethoxybenzoic acid methyl ester (**6a**)*

To a solution of acetal **5** [8] (10.0 g, 35 mmol) and TMEDA (N,N,N',N'-tetramethylethylenediamine) (5 mL) in anhydrous THF (50 mL) at −78 °C was added dropwise *n*-BuLi (1.6 M in pentane, 24 mL, 38.5 mmol) under an argon atmosphere. After warming to 0 °C, the mixture was stirred for 5 min, cooled to −78 °C, and added to a solution of dimethyl carbonate (10 mL, 110 mmol) in anhydrous THF (60 mL). The resulting mixture was stirred for 1 h at r. t. and then quenched with saturated aqueous NH_4Cl (100 mL) and water (200 mL). After extraction of the aqueous phase with EtOAc (4 × 100 mL), the combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography on silica gel (CH_2Cl_2/EtOAc, 2 : 1 v/v) and recrystallisation of the product from CH_2Cl_2/*n*-hexane yielded **6a** (6.73 g, 72.5 %) as cream-coloured crystals. M. p. 96 °C. – R_f = 0.72 (CH_2Cl_2/EtOAc, 2 : 1). – IR (KBr): ν = 3090 (w), 2990 (m), 2930 (m), 2880 (m), 2820 (m), 1690 (ss), 1600 (s), 1510 (s), 1465 (m), 1450 (m), 1430 (s), 1410 (s), 1340 (s), 1275 (ss), 1250 (s), 1210 (s), 1190 (m), 1160 (s), 1090 (ss), 1050 (m), 1030 (m), 1000 (s), 980 (s), 955 (m), 930 (m), 875 (m), 855 (m), 780 (s), 760 (m), 720 (m), 670 (w), 640 (w) cm^{-1}. – ^{1}H NMR (90 MHz, $CDCl_3$): δ = 3.87, 3.90, 3.93 (each s, 3H), 4.03 (m, 4H), 6.57 (s, 1H), 7.29 (s, 1H), 7.45 (s, 1H). – MS (DI, 180 °C): *m/z* (%) = 268 (5) [M]$^+$, 253 (20) [M−CH_3]$^+$, 240 (100) [M−C_2H_4]$^+$, 237 (12) [M−OCH_3]$^+$, 223 (24), 209 (58), 196 (19), 193 (25), 181 (7), 165 (33), 73 (15), 45 (8). – $C_{13}H_{16}O_6$ (268.27): calcd. C 58.20, H 6.01; found C 58.02, H 6.07.

*2-Formyl-4,5-dimethoxybenzoic acid methyl ester (**7a**)*

A suspension of **6a** (6.7 g, 25 mmol) in Et_2O (100 mL) was treated with 4 N HCl (100 mL). After the mixture was stirred for 20 min at r. t., the phases were separated, and the aqueous phase was extracted with Et_2O (3 × 100 mL). The combined organic phases were subsequently washed with saturated aqueous $NaHCO_3$ (2×) and H_2O (1×), dried (Na_2SO_4) and concentrated under reduced pressure to yield **7a** as a white solid (5.44 g, 92 %). M. p. 95 °C (lit. 93 – 95 °C [14]).

*2-[4-(3,4-Dimethoxyphenyl)-5-hydroxy-3,6-dioxo-3,6-dihydropyran-2-ylidenemethyl]-4,5-dimethoxybenzoic acid (**9a**)*

A suspension of **7a** (3.50 g, 15.6 mmol) and 4-(3,4-dimethoxyphenyl)-3-hydroxypyran-2,5-dione (**8**) [9] (4.29 g, 16.2 mmol) in acetic acid (180 mL) was warmed to 60 °C. After addition of conc. HCl (1 mL), the mixture was heated to 90 °C for 3 h, then cooled to r. t. and kept overnight to complete the precipitation. The product was collected by filtration and washed with Et_2O until the filtrate remained colourless. Drying under high vacuum yielded **9a** (6.30 g, 88 %) as red crystals. M. p. 279 °C. – R_f = 0.38 (solvent system A), yellow spot, which turns violet on addition of conc. H_2SO_4. – UV/Vis (THF): $\lambda_{max}(\lg\varepsilon)$ = 248 (4.39), 264 (4.29), 290 (4.26), 378 (4.15) nm. – IR (KBr): ν = 3250 (s, br.), 3000 (m), 2960 (m), 2920 (m), 1690 (ss, br.), 1660 (m), 1600 (s), 1590 (s), 1560 (w), 1515 (ss), 1465 (m), 1445 (s), 1410 (s), 1370 (ss), 1340 (s), 1315 (s), 1280 (ss), 1260 (ss), 1210 (ss), 1180 (s), 1160 (s), 1145 (s), 1075 (m), 1025 (s), 1010 (m), 990 (m), 890 (w), 880 (w), 850 (w), 790 (m), 775 (s), 745 (m), 705 (w) cm^{-1}. – ^1H NMR (200 MHz, [D_6]DMSO): δ = 3.73, 3.79 (each s, 3H), 3.87 (s, 6H), 7.00 ('s', 3H), 7.50 (s, 1H), 7.64 (s, 1H), 7.74 (s, 1H). – MS (DI, 180 °C): m/z (%) = 456 (16) $[M]^+$, 412 (7) $[M-CO_2]^+$, 411 (18) $[M-CO_2H]^+$, 384 (7) $[M-CO_2-CO]^+$, 194 (12), 193 (100) $[M-C_{13}H_{11}O_6]^+$, 177 (15), 151 (26), 149 (10), 44 (18). – HRMS: m/z = 456.1065 (calcd. 456.1057 for $C_{23}H_{20}O_{10}$, $[M]^+$).

*3-(3,4-Dimethoxyphenyl)-2-hydroxy-8,9-dimethoxybenzo[c]chromene-1,4,6-trione (**10**)*

To a suspension of **9a** (470 mg, 1.03 mmol) in anhydrous EtOH (40 mL) at r. t. was added a solution of sodium (450 mg, 19.6 mmol) in anhydrous EtOH (40 mL). The solution was stirred for 1 h, whereby it gradually turned red-violet. Then, the solution was poured into 1 N HCl (100 mL), and the mixture was stirred for an additional 1 h. For completion of the reaction, the resulting flocky precipitate was collected by filtration and refluxed for 2 h in a mixture of MeOH (70 mL) and 98 % H_2SO_4 (1 mL). After cooling to r. t., the brown precipitate was collected by filtration, washed with water and dried in a vacuum desiccator over KOH and P_4O_{10} to furnish **10** (387 mg, 86 %) as a bright brown solid. M. p. 297 °C. – R_f = 0.54 (solvent system A), turns violet with NH_3. – UV/Vis (1,4-dioxane): $\lambda_{max}(\lg\varepsilon)$ = 254 (4.59), 278 (4.39), 316 (4.18), 416 (3.60) nm. – IR: ν = 3580 (w, br.), 3510 (w), 3350 (w, br.), 2960 (w, br.), 2940 (w), 2840 (w), 1755 (s), 1735 (s), 1660 (s), 1650 (s), 1600 (s), 1510 (ss), 1460 (s), 1420 (m), 1390 (s), 1365 (m), 1340 (s), 1330 (s), 1260 (ss), 1215 (m), 1140 (m), 1105 (m), 1025 (ss), 980 (w), 885 (w), 850 (w), 810 (w), 795 (w), 770 (m) cm^{-1}. – ^1H NMR (200 MHz, $CDCl_3$/[D_6]DMSO): δ = 3.77, 3.81, 3.93, 3.98 (each s, 3H), 6.87 (AB-d, J = 8.4 Hz, 1H), 6.96 ('s', 1H), 6.98 (AB-'d', br., J = 8.4 Hz, 1H), 7.62, 8.52 (each s, 1H), 10.6 – 10.9 (br, 1 OH). – MS (DI, 180 °C): m/z (%) = 440 (27.5) $[M+2H]^+$, 439 (19) $[M+H]^+$, 438 (100) $[M]^+$, 411 (17.5), 410 (99) $[M-CO]^+$, 395 (18) $[M-CH_3-CO]^+$, 367 (8), 339 (8), 149 (7), 44 (9). – HRMS: m/z = 438.0966 (calcd. 438.0951 for $C_{23}H_{18}O_9$, $[M]^+$).

*2-[1,3]Dioxolan-2-yl-4,5-dimethoxybenzoic acid (-)-menthyl ester (**6b**)*

To a solution of **5** (6.0 g, 20.8 mmol) in THF (60 mL), maintained under argon at −78 °C, was added dropwise *n*-BuLi (1.6 M solution in pentane, 24 mL, 38.5 mmol). The mixture was stirred for 30 min at −78 °C, and the resulting suspension was slowly added to a solution of menthyl chloroformate (5 mL, 22.8 mmol) in THF (10 mL). The reaction mixture was stirred for 1.5 h, then it was warmed to r. t., quenched with saturated aqueous NH_4Cl (60 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography of the crude product on silica gel (CH_2Cl_2/MeOH, 100 : 2, v/v) yielded **6b** (5.7 g, 70 %) as a colourless oil. – R_f = 0.75 (CH_2Cl_2/MeOH, 100 : 2). – ^1H NMR (90 MHz, $CDCl_3$): δ = 0.70 – 2.30 (m, 18H), 3.90 (s, 3H), 3.95 (s, 3H), 4.15 (m, 4H), 4.91 (td, J = 11.7, 4 Hz, 1H), 6.55 (s, 1H), 7.28 (s, 1H), 7.43 (s, 1H). – MS (DI, 180 °C): m/z (%) = 392 (1.3) $[M]^+$, 364 (3) $[M-C_2H_4]^+$, 254 (25), 253 (100), 227 (20), 226 (60), 218 (8), 208 (35), 193 (20), 182 (10). – HRMS: m/z = 392.2197 (calcd. 392.2198 for $C_{22}H_{32}O_6$, $[M]^+$). – $C_{22}H_{32}O_6 \times 2\ H_2O$: calcd. C 61.66, H 8.47; found C 61.41, H 8.36.

*2-Formyl-4,5-dimethoxybenzoic acid (-)-menthyl ester (**7b**)*

A solution of **6b** (6.7 g, 25 mmol) in acetone (400 mL), kept in an ice bath, was treated with 4 N HCl (30 mL). The mixture was stirred for 1 h at 0 °C and 3 h at r. t., then it was poured into water (300 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (2×) and H_2O (1×), dried (Na_2SO_4) and concentrated under reduced pressure to yield **7b** (4.80 g,

98 %) as a colourless oil. – R_f = 0.81 (CH_2Cl_2/MeOH, 100 : 2, v/v). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.70 – 2.30 (m, 18H), 3.96, 3.98 (each s, 3H), 4.99 (td, *J* = 10.5, 4.7 Hz, 1H), 7.42, 7.48, 10.62 (each s, 1H). – MS (DI, 180 °C): *m/z* (%) = 348 (7) $[M]^+$, 211 (14), 210 (64), 209 (100), 193 (39), 182 (45), 95 (12), 83 (25), 81 (10), 69 (14). – HRMS: *m/z* = 348.1935 (calcd. 348.1936 for $C_{20}H_{28}O_5$, $[M]^+$).

*2-[4-(3,4-Dimethoxyphenyl)-5-hydroxy-3,6-dioxo-3,6-dihydropyran-2-ylidenemethyl]-4,5-dimethoxybenzoic acid (-)-menthyl ester (**9b**)*

A mixture of **7b** (1.16 g, 3 mmol), pyrandione **8** (1.06 g, 4 mmol) [9], ammonium acetate (460 mg), and acetic acid (12 mL), maintained under argon, was stirred for 15 h at 50 °C. The mixture was then cooled to r. t., poured into water and extracted with Et_2O (3 × 100 mL). The combined organic phases were subsequently washed with dilute HCl and water, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by chromatography on acetylated polyamide with toluene as eluent to yield **9b** (1.23 g, 69 %) as an orange-red solid. M. p. 134 °C. – R_f = 0.58 (solvent system A), yellow spot which turns violet on addition of conc. H_2SO_4. – UV/Vis (1,4-dioxane): $\lambda_{max}(\lg\varepsilon)$ = 248 (4.43), 282 (4.36), 380 (4.15) nm. – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.70 – 2.30 (m, 18H), 3.89, 3.92, 3.96, 3.99 (each s, 3H), 4.94 (td, *J* = 11.5, 4.4 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 7.16 (d, *J* = 2.3 Hz, 1H), 7.22 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.48 (s, 1H), 7.56 (s, 1H), 8.06 (s, 1H). – MS (DI, 210 °C): *m/z* (%) = 594 (1.3) $[M]^+$, 456 (16), 412 (23), 411 (100), 410 (26), 196 (10), 165 (20), 95 (22), 83 (14), 81 (17), 69 (10). – HRMS: *m/z* = 594.2457 (calcd. 594.2465 for $C_{33}H_{38}O_{10}$, $[M]^+$).

*2-[4-(3,4-Dimethoxyphenyl)-2,5-dihydroxy-3,6-dioxocyclohexa-1,4-dienyl]-4,5-dimethoxybenzoic acid (-)-menthyl ester (**11**)*

To a solution of **9b** (577 mg, 0.97 mmol) in anhydrous MeOH (30 mL) was added a solution of NaOMe, prepared from Na (350 mg, 15.2 mmol) and MeOH (30 mL). The mixture was stirred at r. t. for 4 h and then poured into 1 N HCl (100 mL). The mixture was extracted with CH_2Cl_2 (3 × 100 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was digested with Et_2O and filtered to yield **11** (272 mg, 47 %). M. p. 235 °C. – R_f = 0.39 (solvent system A), turns violet with gaseous NH_3. – UV/Vis (1,4-dioxane): $\lambda_{max}(\lg\varepsilon)$ = 244 (4.44), 268 (4.45), 284 (4.39) nm. – IR (KBr): ν = 3300 (s), 2940 (m), 2920 (m), 2850 (m), 1700 (s), 1620 (s), 1610 (ss), 1590 (s), 1510 (ss), 1455 (s), 1325 (ss), 1260 (ss), 1205 (ss), 1170 (ss), 1145 (s), 1115 (m), 1060 (m), 1030 (s), 995 (ss), 870 (w), 850 (w), 795 (w), 770 (m), 760 (m) cm^{-1}. – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.70 – 2.30 (m, 18H), 3.89, 3.92, 3.93, 3.97 (each s, 3H), 4.79 (td, *J* = 8.4, 4.2 Hz, 1H), 6.70 (s, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 7.10 (d, *J* = 2.2 Hz, 1H), 7.15 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.63 (s, 1H). – MS (DI, 180 °C): *m/z* (%) = 594 (7) $[M]^+$, 457 (9), 456 (46), 441 (9), 440 (36), 439 (9), 438 (29), 412 (6), 411 (15), 410 (63), 203 (14), 181 (10), 165 (100) $[C_9H_9O_3]^+$, 138 (20), 123 (26), 96 (22), 95 (64), 83 (12), 82 (28), 81 (26), 71 (80), 69 (26), 67 (26). – HRMS: *m/z* = 594.2465 (calcd. 594.2465 for $C_{33}H_{38}O_{10}$, $[M]^+$). – $C_{33}H_{38}O_{10} \times H_2O$: calcd. C 64.69, H 6.58; found C 64.91, H 6.31.

*2-[6-(3,4-Dimethoxyphenyl)-2,5-dioxo-2,5-dihydrofuro[3,2-b]furan-3-yl]-4,5-dimethoxybenzoic acid (-)-menthyl ester (**12**)*

A mixture of **11** (210 mg, 0.35 mmol), Ac_2O (9 mL), and DMSO (9 mL) was kept at 60 °C for 1 h. After cooling to r. t., the solution was stirred for 14 h and then poured into water (80 mL). The reaction mixture was stirred for another 1 h and then extracted with Et_2O (3 × 100 mL). The combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure to yield **12** (175 mg, 84 %) as an orange solid. M. p. 67 °C. – R_f = 0.68 (solvent system A), orange fluorescence at 366 nm. – UV/Vis (1,4-dioxane): $\lambda_{max}(\lg\varepsilon)$ = 254 (4.22), 414 (4.15) nm. – IR ($CHCl_3$): ν = 2940 (m), 2920 (m), 1815 (s), 1785 (m, sh), 1690 (s), 1660 (s), 1595 (m), 1570 (w), 1510 (s), 1460 (m), 1415 (w), 1380 (w), 1345 (s), 1255 (ss), 1165 (m), 1135 (s), 1090 (m), 1060 (m), 1015 (m), 970 (w), 950 (w), 900 (ss), 850 (m), 805 (m), 710 (w), 645 (w), 610 (w) cm^{-1}. – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.70 – 2.30 (m, 18H), 3.93 (s, 3H), 3.94 (s, 6H), 3.96 (s, 3H), 4.86 (td, *J* = 11, 4.2 Hz, 1H), 6.91 (s, 1H), 6.94 (d, *J* = 8.3 Hz, 1H), 7.51 (d, *J* = 2.5 Hz, 1H), 7.54 (s, 1H), 7.67 (dd, *J* = 8.3, 2.5 Hz, 1H). – MS (DI, 180 °C): *m/z* (%) = 592 (2) $[M]^+$, 454 (100), 427 (8), 426 (50), 415 (7), 414 (42), 411 (6), 410 (27), 409 (7), 408 (11), 342 (8), 222 (14), 209 (2), 177 (16), 149 (7), 95 (10), 94 (20), 83 (20), 81 (10), 69 (15). – HRMS: *m/z* = 592.2302 (calcd. 592.2308 for $C_{33}H_{36}O_{10}$, $[M]^+$).

*2-{[4-(3,4-Dimethoxyphenyl)-3-hydroxy-5-oxo-5H-furan-2-ylidene](methoxycarbonyl)methyl}-4,5-dimethoxybenzoic acid (-)-menthyl ester (**13a**) and 2-{5-[(3,4-Dimethoxyphenyl)(methoxycarbonyl)methylene]-4-hydroxy-2-oxo-2,5-dihydrofuran-3-yl}-4,5-dimethoxybenzoic acid (-)-menthyl ester (**13b**)*

A mixture of **12** (175 mg, 0.3 mmol) and 2 % methanolic KOH (30 mL) was stirred for 2 h at r. t., then poured into water (70 mL) and acidified with acetic acid. The intensely yellow solution was extracted with CH_2Cl_2 (3 × 100 mL), and the combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure. Isomers **13a** and **13b** were separated on a silica gel column: **13a** was eluted with EtOAc, and **13b** with acetone. Isomer **13a** (28 mg, 15 %) was obtained from the EtOAc fractions as an orange solid. The acetone fractions were concentrated, and the residue was dissolved

in Et_2O and filtered. Removal of the solvent from the filtrate yielded **13b** (157 mg, 85 %) as a lemon-yellow solid.

13a: M. p. 92 °C. – R_f = 0.71 (solvent system A). – UV/Vis (1,4-dioxane): $\lambda_{max}(\lg\varepsilon)$ = 238 (4.13), 268 (4.29), 286 (4.21), 418 (3.97) nm. – IR ($CHCl_3$): ν = 2950 (m), 2920 (m), 1750 (m), 1685 (s), 1595 (s), 1510 (s), 1460 (s), 1440 (m), 1350 (s), 1310 (s), 1260 (ss), 1170 (s), 1150 (s), 1095 (m), 1070 (m), 1040 (m), 1010 (m), 965 (w), 910 (m) cm^{-1}. – ^1H NMR (200 MHz, $CDCl_3$): δ = 0.70 – 2.30 (m, 18H), 3.78 (s, 3H), 3.90 (s, 3H), 3.91 (s, 6H), 3.95 (s, 3H), 4.85 (m, 1H), 6.73 (s, 1H), 6.93 (d, J = 8.4 Hz, 1H), 7.66 (s, 1H), 7.77 (m, 2H), 13.70 (s, 1H, OH). – MS (DI, 180 °C): m/z (%) = 626 (1.1), 625 (2.8), 624 (7.1) $[M]^+$, 553 (1.4), 552 (3.4), 486 (6), 455 (10), 454 (37), 440 (5.7), 426 (14.6), 415 (16.6), 414 (100), 411 (6), 410 (7.1), 369 (7), 342 (12), 222 (9), 209 (2), 177 (86), 149 (7), 95 (6), 83 (13), 69 (8). – HRMS: m/z = 624.2584 (calcd. 624.2571 for $C_{34}H_{40}O_{11}$, $[M]^+$).

13b: M. p. 73 °C. – R_f = 0.58 (solvent system A). – UV/Vis (1,4-dioxane): $\lambda_{max}(\lg\varepsilon)$ = 242 (4.17), 268 (4.27), 364 (4.05) nm. – IR ($CHCl_3$): ν = 3010 (w), 2950 (m), 2930 (m), 2860 (w), 1775 (m), 1695 (m), 1685 (s), 1600 (s), 1510 (s), 1460 (s), 1450 (s), 1440 (s), 1410 (w), 1370 (m), 1330 (m), 1300 (s), 1280 (s), 1260 (ss), 1175 (s), 1150 (m), 1130 (m), 1075 (m), 1060 (s), 1030 (m), 1000 (m), 950 (m), 910 (m), 885 (m) cm^{-1}. – ^1H NMR (200 MHz, $CDCl_3$): δ = 0.70 – 2.30 (m, 18H), 3.86, 3.88, 3.90, 3.92, 3.94 (each s, 3H), 4.82 (td, J = 11.5, 4.5 Hz, 1H), 6.77 (d, J = 1.5 Hz, 1H), 6.87 (m, 2H), 6.96 (s, 1H), 7.50 (s, 1H), 13.20 (s, 1H, OH). – MS (DI, 180 °C): m/z (%) = 624 (5) $[M]^+$, 502 (10), 469 (25), 468 (100), 454 (20), 440 (12), 426 (33), 410 (23), 409 (58), 408 (100), 397 (8), 281 (15), 265 (9), 263 (14), 236 (90), 222 (20), 221 (18), 210 (8), 209 (35), 177 (20), 165 (22), 95 (50), 83 (38), 81 (49), 71 (38), 69 (30), 55 (58), 44 (20), 43 (21). – HRMS: m/z = 624.2569 (calcd. 624.2571 for $C_{34}H_{40}O_{11}$, $[M]^+$).

*Methyl bovinate (**4**)*

To a solution of **13b** (94 mg, 0.15 mmol) in anhydrous CH_2Cl_2 (15 mL) at −78 °C was added a 1 M solution of BBr_3 (1 mL) in CH_2Cl_2, and the mixture was stirred for 24 h at r. t. Then the solvent was removed under reduced pressure, and the residue was treated with 1 N HCl (20 mL). The aqueous phase was extracted with EtOAc (3 × 100 mL), and the combined organic extracts were exhaustively washed with water and dried (Na_2SO_4). Removal of the solvent under reduced pressure and purification of the product by chromatography on acetylated polyamide (acetone/MeOH, 1 : 1, v/v) yielded **4** (43 mg, 69 %) as an orange solid. M. p. 295 °C. – R_f = 0.34 (solvent system A), + gaseous NH_3 colour change to red-brown. – The spectroscopic data (UV/Vis, IR, ^1H and ^{13}C NMR, MS) were in agreement with those of natural methyl bovinate.

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