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A BIBENZYL FROM *EMPETRUM NIGRUM*

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Key Word Index—*Empetrum nigrum*; Empetraceae; leaves; batatasin; bibenzyl; dihydrostrilbene; synthesis.

Abstract—A new bibenzyl, 1-(2-hydroxyphenyl)-2-(3-hydroxy-4,5-dimethoxyphenyl)-ethane, possessing similar germination inhibitory activity to batatasin III *in vitro*, was isolated from the leaves of *Empetrum nigrum*. The isolation, structural determination and synthesis of the new compound is reported. © 1998 Elsevier Science Ltd. All rights reserved

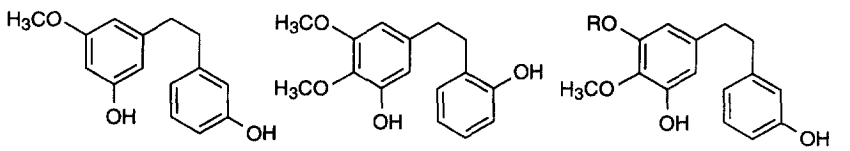
INTRODUCTION

The secondary metabolites of *Empetrum hermaphroditum* have attracted attention due to the apparent effect of these shrubs on inhibition of seed germination of Scots pine, as well as aspen [1]. Investigations of *E. hermaphroditum* have shown that it produces batatasin III (**1**), which also has been isolated as a growth inhibitor from yams [2]. Because *E. hermaphroditum* and *E. nigrum* are closely related and show very similar morphological and growth characteristics, the aim of the present study was to investigate the presence of batatasin and similar bibenzyls in *E. nigrum*.

RESULTS AND DISCUSSION

Comparison between the extracts of *E. hermaphroditum* and *E. nigrum* by various analytical techniques suggested that the two species do not produce the same aromatic secondary metabolites. Extracts of *E. hermaphroditum* yielded as expected batatasin III (**1**), while *E. nigrum* produces a similar but distinctly different metabolite. This was isolated, the structure determined by spectroscopic techniques (see Experimental), and shown to be 1-(2-hydroxyphenyl)-2-(3-hydroxy-4,5-dimethoxyphenyl)ethane (**2**), a new bibenzyl. The ^{1}H-^{13}C long-range correlations observed in the HMBC spectrum (the pertinent correlations are summarised in Fig. 1), clearly demonstrated that the hydroxyl group in the hydroxphenyl moiety of compound **2** is positioned at carbon 2, not carbon 3 as in batatasin III (**1**). This was somewhat surprising, as a previous investigation of *E. nigrum* yielded compounds **3a** and **3b** [3]. However, the NMR data reported for compound **3b** are significantly different from those obtained with compound **2**, and it is possible that the *E. nigrum* collected in Germany (yielding **3a** and **3b**) differs from *E.nigrum* collected in Sweden. In order to determine the structure of compound **2** unambiguously, and at the same time to obtain larger quantities for biological experiments, a synthetic route was explored.

The synthesis of compound **2** is outlined in Scheme 1 and essentially follows the reported synthesis of batatasin III (**1**) [2]. (2-Benzyloxy)-benzyltri-



1 **2** **3**

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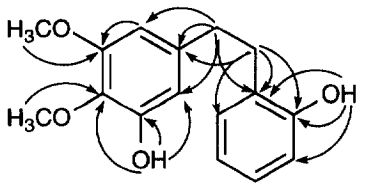
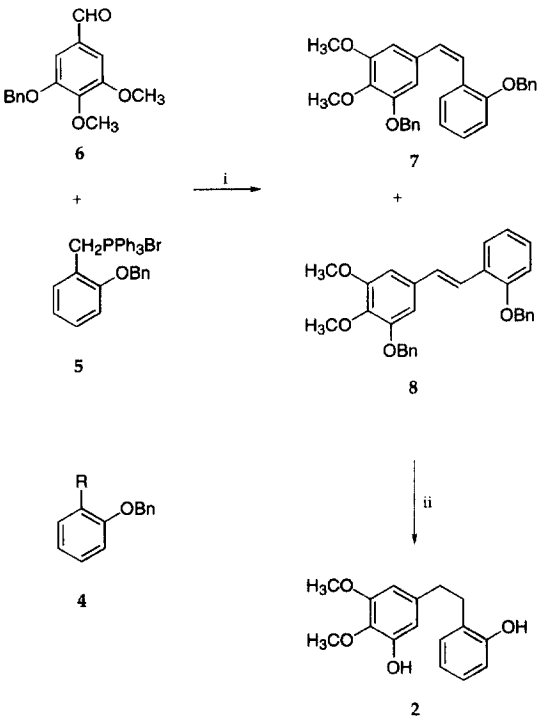


Fig. 1. Significant HMBC correlations observed for compound **2**.



Scheme 1. Synthesis of compound **2**. a: R = CHO; b: R = CH_2OH; c: R = CH_2Br. i: $(MeSi)_2NK$, THF; ii: H_2, Pd/C, EtOH.

phenylphosphonium bromide (**5**) was prepared from *o*-hydroxy-benzaldehyde and the corresponding ylide condensed with aldehyde **6** to produce the stilbenes **7** and **8**. Compound **2**, in all respects identical to the isolated product, was obtained by catalytic hydrogenation of stilbenes **7** and **8**; the total yield (starting from **4a**) was 51%. Preliminary biological characterisation [4] of compound **2** indicates that it possesses a similar inhibitory activity on the germination of seeds as batatasin III (**1**), although it appears to be slightly less potent. Results from the biological assays will be reported separately.

EXPERIMENTAL

Plant material

Leaves of *E. nigrum* L. were collected in September 1992 at Salmisjärvi (68°11'N, 21°50'E) and leaves of *E. hermaphroditum* Hagerup were collected in September 1993 at Rovågern outside Umeå (63°50'N, 20°15'E). Voucher specimens (No. 32 and 197, respectively) were authenticated by Prof. Olle Zackrisson, Department of Forest Vegetation Ecology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden, where they are deposited. Leaves were left to dry at 20° for ca 2 weeks and then stored at −18° prior to analysis. Leaves were analysed for batatasin-III by GC-MS according to Ref. [4].

Extraction and isolation

Pulverised dry leaves were extracted with EtOAc (40 ml per g leaves) and the extracts fractionated by chromatography on silica gel (eluted by EtOAc–heptane mixts) and by HPLC (Merck 250 × 10 mm LiCrospher 100 diol 5 μm, hexane–1-PrOH–H_2O–HOAc 52.2:40.3:3:1.5, flow rate 3.2 ml min^{-1}). The R_t for compound **2** in the latter system was 13.7 min, compared with 16.1 min for batatasin III (**1**). Dry leaves (1 g) yielded 9 mg of compound **2**.

Spectroscopy

^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temp. in $CDCl_3$, with an inverse 5 mm probe equipped with a shielded gradient coil. COSY, HMQC and HMBC expts were performed with gradient enhancements using sine-shaped gradient pulses and for 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH}$ = 145 Hz and $^2J_{CH}$ = 10 Hz. Chemical shifts are given in δ relative to TMS with the solvent signals [δ 7.26 (^1H) and δ 77.0 (^{13}C)] as ref.

Synthesis

CH_2Cl_2 was dist. from CaH_2 immediately before use, THF and toluene were dist. from Na-benzophenone ketyl, while DMF and hexane were dried over 4 Å molecular sieves. All other reagents were used as received. All reactions were carried out in septum-capped, oven- or flame-dried flasks under N_2.

1-(2-*Hydroxyphenyl*)-2-(3-*hydroxy*-4,5-*dimethoxyphenyl*)*ethane* (**2**). Colourless oil. UV λ_{max}^{EtOH} nm (log ε): 275 (3.49). IR ν_{max}^{KBr} cm^{-1}: 3380, 2920, 2830, 1590, 1500, 1450, 1425, 1345, 1230, 1195, 1160, 1090, 985. EIMS (probe) 70 eV, *m/z* (rel. int): 274.1217 $[M]^+$, 33, $C_{16}H_{18}O_4$ requires 274.1205), 167 (100), 123 (5), 107 (8), 77 (5). ^1H NMR (500 MHz, $CDCl_3$): δ 7.09 (1H, *d*, $J_{5'-6'}$ = 7.4 Hz, H-6'), 7.07 (1H, *m*, H-4'), 6.86 (1H, *ddd*, $J_{3'-5'}$ = 1.0 Hz, $J_{4'-5'}$ = 7.4 Hz, H-5'), 6.74 (1H, *d*, $J_{3'-4'}$ = 7.5 Hz, H-3'), 6.49 (1H, *d*, $J_{2''-6''}$ = 1.8 Hz, H-2''), 6.25 (1H, *d*, H-6''), 5.70 (1H, *s*, 3''-OH), 4.60 (1H, *s*, 2'-OH), 3.87 (3H, *s*, 4''-OCH_3), 3.80 (3H, *s*, 5''-OCH_3), 2.88 (2H, *m*, H_2-1), 2.82 (2H, *m*, H_2-2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 153.5 (C-2'), 152.1 (C-5''), 149.1 (C-3''), 138.2 (C-1''), 133.8 (C-4''), 130.4 (C-6'), 127.8 (C-1'), 127.3 (C-4'), 120.9 (C-5'), 115.4 (C-

3′), 107.8 (C-2″), 104.5 (C-6″), 61.0 (4″-OCH_3), 55.8 (5″-OCH_3), 36.3 (C-2), 32.2 (C-1).

(*2-Benzyloxy)benzaldehyde* (**4a**). Prepared by adding *o*-hydroxybenzaldehyde (13.1 ml, 0.125 mol) dropwise to a cooled (0°) suspension of NaH (0.15 mol) in dry DMF (300 ml), heating to 50° for 2 h, cooling to 0° before the dropwise addition of benzyl bromide (17.8 ml, 0.15 mol), and stirring the reaction mixture at 70° overnight. The product (20.7 g, 78%) was obtained as a colourless oil after diln of the reaction mixt. with H_2O (300 ml) and extraction with Et_2O (5 × 100 ml), and flash CC with heptane–EtOAc (9:1). ^1H NMR (400 MHz, $CDCl_3$): δ 10.59 (2H, *d*, J = 0.7 Hz), 7.90–7.86 (1H, *m*), 7.58–7.53 (1H, *m*), 7.48–7.35 (5H, *m*), 7.09–7.04 (2H, *m*), 5.22 (2H, *s*). ^{13}C NMR (100.624 MHz, $CDCl_3$): δ 190.2, 136.5, 136.3, 129.2, 128.9, 128.7, 127.7, 125.6, 121.5, 113.5, 70.9.

(*2-benzyloxy)benzyl alcohol* (**4b**). Prepared by adding an Et_2O (20 ml) soln of aldehyde **4a** (8.57 g, 40.4 mmol) to a dispersion of $LiAlH_4$ (3.06 g, 80.7 mmol) in Et_2O (80 ml) at 0° and stirring the reaction mixt. overnight at room temp. The reaction was quenched by adding MeOH (1 ml) in Et_2O (4 ml) followed by 1 M H_2SO_4 (60 ml). The organic layer was washed with H_2O (80 ml), aq. $NaHCO_3$ (40 ml) and brine (40 ml), and the product (7.11 g, 82%) was obtained as a colourless oil by flash CC with heptane–EtOAc (5:1). ^1H NMR (400 MHz, $CDCl_3$): δ 7.47–7.25 (7H, *m*), 7.02–6.96 (2H, *m*), 5.15 (2H, *s*), 4.76 (2H, *d*, J = 6.5 Hz), 2.37–2.31 (1H, *m*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.0, 137.2, 129.9, 129.4, 129.3, 129.2, 128.6, 127.8, 121.5, 112.1, 70.5, 62.6.

(*2-Benzyloxy)benzyl bromide* (**4c**). Prepared by adding PBr_3 (2.69 ml, 28.6 mmol) in toluene (20 ml) to a soln of alcohol **4b** (5.58 g, 26.0 mmol) in toluene (100 ml) at 0°, and stirring the resulting mixt. at 0° for 20 min and at room temp. for 20 min. Dropwise addition of the reaction mixt. to H_2O (100 ml, 0°) and the washing of the organic layer with aq. $NaHCO_3$ (100 ml), H_2O (100 ml) and brine (50 ml), gave the crude bromide **4c** (6.97 g, 97%), which was used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$): δ 7.55–7.50 (2H, *m*), 7.45–7.34 (4H, *m*), 7.32–7.26 (1H, *m*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.0, 137.3, 131.4, 130.6, 129.5, 129.0, 128.4, 127.6, 126.9, 121.4, 112.7, 70.5.

(*2-Benzyloxy)benzyltriphenylphosphonium bromide* (**5**). Prepared by adding bromide **4c** (6.97 g, 25.2 mmol) in toluene (20 ml) to a soln of triphenyl phosphine (8.57, 32.7 mmol) in toluene (30 ml), refluxing for 1 h and cooling to room temp. The product (12.6 g, 93%) was obtained as a white ppt. which was filtered off and washed with toluene. ^1H NMR (400 MHz, $CDCl_3$): δ 7.76–7.71 3H, *m*), 7.56–7.46 (12H, *m*), 7.41–7.36 (3H, *m*), 7.27–7.23 (1H, *m*), 7.19–7.15 (1H, *m*), 7.14–7.10 (2H, *m*), 6.87 (1H, *t*, J = 7.5 Hz), 6.69 (1H, *d*, J = 8.3 Hz), 5.25 (2H, *d*, J = 8.3 Hz), 4.47 (2H, *s*). ^{13}C NMR (100 Hz, $CDCl_3$): δ 157.0, 156.9, 136.3, 135.2, 134.7, 134.6, 133.0, 132.9, 130.7, 130.4, 130.3, 129.5, 129.1, 128.9, 128.6, 128.3, 122.1, 118.9, 118.1, 116.6, 116.5, 111.9, 111.8, 70.4, 26.6, 26.1.

(*3-Benzyloxy-4,5-dimethoxy)benzaldehyde* (**6**). Prepared by dropwise addition of benzyl bromide (213 µl, 1.79 mmol) to a stirred soln of 3,4-dimethoxy-5-hydroxybenzaldehyde (297 mg, 1.63 mmol), K_2CO_3 (248 mg, 1.79 mmol) and a catalytic amount of 18-crown-6 in THF (15 ml) at room temp. The reaction mixt. was refluxed overnight and the KBr filtered off. The product (440 mg, 99%) was obtained as a colourless oil by flash CC with heptane–EtOAc (4:1). ^1H NMR (400 MHz, $CDCl_3$): δ 9.82 (1H, *S*), 7.47–7.43 (2H, *m*), 7.41–7.30 (3H, *m*), 7.16 (2H, *AB-q*, J = 14.7 Hz, 1.7 Hz), 5.18 (2H, *s*), 3.96 (3H, *s*), 3.92 (3H, *s*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 191.4, 154.3, 153.1, 144.7, 136.9, 132.1, 129.1, 128.6, 127.8, 109.5, 107.0, 71.6, 61.4, 56.7.

(Z)-2,3′-*Dibenzyloxy*-4′,5′-*dimethoxystilbene* (**7**) *and* (E)-2,3′-*dibenzyloxy*-4′,5′-*dimethoxystilbene* (**8**). Prepared by dropwise addition of a 1.60 M soln of potassium *bis*(trimethylsilyl)amide [5] in THF (2.90 ml, 4.63 mmol) to a slurry of **5** (2.65 g, 4.94 mmol) in toluene (20 ml) at −20°, stirring for 45 min at room temp., cooling to −20° and adding the aldehyde **6** (421 mg, 1.54 mmol) in THF (10 ml) dropwise. The reaction mixt. was stirred for 30 min at room temp., partitioned between Et_2O (50 ml) and H_2O (50 ml), whereafter the organic layer was washed with H_2O (100 ml) and brine (75 ml). The products **7** (319 mg, 45%) and **8** (367 mg, 52%) were obtained as colourless oils by flash CC with heptane–EtOAc (8:1). **7**. ^1H NMR (400 MHz, $CDCl_3$): δ 7.39–7.37 (2H, *m*), 7.37–7.36 (2H, *m*), 7.36–7.34 (3H, *m*), 7.34–7.32 (1H, *m*), 7.32–7.30 (1H, *m*), 7.30–7.28 (1H, *m*), 6.97 (1H, *d*, J = 7.9 Hz), 6.86 (1H, *t*, J = 7.5 Hz), 6.73 (1H, *d*, J = 12.1 Hz), 6.55 (1H, *d*, J = 1.8 Hz), 6.52 (1H, *d*, J = 12.2 Hz), 6.49 (1H, *d*, J = 1.8 Hz), 5.10 (2H, *s*), 4.86 (2H, *s*), 3.87 (3H, *s*), 3.63 (3H, *s*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.6, 153.3, 152.4, 137.7, 137.6, 133.0, 130.9, 130.6, 129.0, 128.9, 128.8, 128.2, 128.1, 127.8, 127.7, 127.6, 127.5, 126.1, 121.1, 113.0, 108.6, 106.9, 71.2, 70.6, 61.4, 56.2. **8**. ^1H NMR (400 MHz, $CDCl_3$): δ 7.61 (1H, *dd*, J = 7.7, 1.6 Hz), 7.53–7.48 (4H, *m*), 7.42 (1H, *d*, J = 16.7 Hz), 7.41 (2H, *t*, J = 7.3 Hz), 7.40 (2H, *t*, J = 8.0 Hz), 7.37–7.30 (2H, *m*), 7.24 (1H, *dt*, J = 7.5, 1.5 Hz), 7.09 (1H, *d*, J = 16.4 Hz), 7.04–6.96 (2H, *m*), 6.80 (1H, *d*, J = 1.8 Hz), 6.76 (1H, *d*, J = 1.8 Hz), 5.19 (2H, *s*), 5.18 (2H, *s*), 3.93 (3H, *s*), 3.92 (3H, *s*). ^{13}C NMR (100 Mhz, $CDCl_3$): δ 156.6, 153.9, 152.9, 139.0, 137.7, 137.6, 134.1, 129.6, 129.0, 128.9, 128.4, 128.3, 127.8, 127.7, 127.6, 127.2, 127.1, 123.6, 121.7, 113.3, 106.4, 104.4, 71.6, 71.0, 61.5, 56.5.

1-(*2-Hydroxyphenyl*)-2-(*3-hydroxy*-4,5-*dimethoxyphenyl)ethane* (**2**). Prepared by stirring a mixt. of **7** and **8** (294 mg, 0.65 mmol) in EtOH (20 ml) and heptane (5 ml) in the presence of catalytic amounts of 10% Pd/C and H_2 (atm. pres.) at room temp. Filtration of the slurry through a pad of Celite followed by flash CC with heptane–EtOAc (2:1) gave a product

(103 mg, 71%) which in all respects was identical with the natural product.

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