



Typhaphthalide and typharin, two phenolic compounds from *Typha capensis*

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Abstract

Two new phenolics, named typhaphthalide (a benzylphthalide) and typharin (an isocoumarin) plus sitosterol were isolated and identified from the hexane extract of the rhizomes of *Typha capensis*. The acetone extract yielded afzelechin, epiafzelechin, and catechin.

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1. Introduction

Typha capensis (Rohrb.) N.E.Br, commonly referred to as bulrush, is a robust, reed-like plant sparsely distributed in the North Western Cape region but very common in South Africa (Van Wyk et al., 1997). The rhizomes are used in traditional medicine during pregnancy to ensure easy delivery, for venereal diseases, dysmenorrhea, diarrhoea, dysentery, and to enhance the male potency and libido, amongst others (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996). The phytochemistry of several species of the genus *Typha* has been documented (Chapman and Hall, 2000). Several flavones and other phenolic compounds, long chain hydrocarbons as well as various triterpenoids with a steroidal skeleton have been isolated (Chapman and Hall, 1996).

Examination of a sample of rhizomes of *T. capensis* collected in Pinetown near Durban, S.A. has yielded the novel compounds typhaphthalide (**1**), typharin (**2**), as well as sitosterol, afzelechin (**3**), epiafzelechin (**4**), (+)-catechin (**5**), and (–)-epicatechin (**6**). The structures of compounds **1** and **2** were elucidated using spectroscopic techniques.

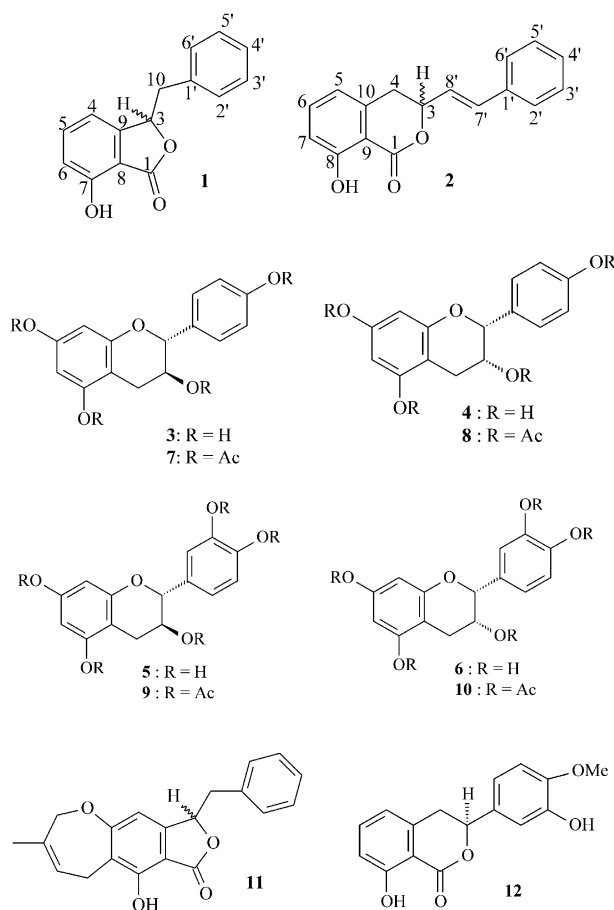
2. Results and discussion

A n-hexane extract of rhizomes of *T. capensis* deposited crystals of compound **1** on standing for several days. This compound had the molecular formula $C_{15}H_{12}O_3$ as determined by high resolution mass spectrometry and ^{13}C NMR analysis. The IR spectrum of **1** showed the presence of a chelated phenolic hydroxyl group (3544 cm^{-1} , 3437 cm^{-1}), a carbonyl group (1714 cm^{-1}), a methylene group (2922 cm^{-1}), and aromatic ring (1624 cm^{-1} , 1607 cm^{-1} , 1471 cm^{-1}). The NMR spectra (Table 1) of compound **1** showed the presence of one monosubstituted benzene ring (δ_H 7.30–7.19 m 5H; δ_C 129.7 d, 128.6 d, 127.3 d, 5C), a trisubstituted benzene ring [δ_H 6.87 d, 1H ($J=8.2\text{ Hz}$), 7.46 t 1H ($J=7.9\text{ Hz}$), 6.63 d 1H ($J=7.4\text{ Hz}$); δ_C 115.5 d, 136.6 d, 113.7 d, 3C], a benzylic methylene at δ_H 3.22 dd ($J=6.7\text{ Hz}$, 14 Hz); δ_C 40.7 t, and a broad singlet at δ_H 7.71 1H; δ_C 156.5 s 1C indicated the presence of a phenolic proton which attenuated in D_2O . The benzylic methylene at δ_H 3.22 coupled with one methine [δ_H 5.68 t ($J=6.5\text{ Hz}$, 6.3 Hz); δ_C 82.6 d 1C] bearing an ether oxygen. The above spectral evidence and the molecular formula indicated the presence of a γ - or δ -lactone in compound **1**. The presence of a 7-hydroxyphthalide group in **1** was suspected and confirmed by comparison of its spectral data (IR and NMR) with known compounds such as **11** (Asakawa et al., 1982; Asakawa et al., 1987; Asakawa et al., 1986), which have similar skeletal structure. This compound **1**, which was named

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typhaphthalide, was determined to be 7-hydroxy-3-benzylphthalide.



Flash column chromatography over silica gel of the filtrate obtained from compound **1** gave compound **2** and sitosterol. Compound **2**, which was isolated as non-crystalline yellow compound, had molecular formula $C_{17}H_{14}O_3$ as determined by high resolution mass spectrometry and ^{13}C NMR analysis. The IR spectrum of compound **2** showed the presence of a methylene group (3063 cm^{-1}), a chelated aromatic hydroxyl group (3440 cm^{-1}), a carbonyl group (1690 cm^{-1}), aromatic ring (1600 cm^{-1} , 1580 cm^{-1} , 1475 cm^{-1}) and a *trans*-alkene group (980 cm^{-1}). The NMR spectra (Table 2) of compound **2** showed high degree of similarities with the spectra of compound **1**. These include the presence of one mono-substituted benzene ring (δ_H 7.45–7.20 *m* 5H; δ_C 128 *d*, 126.8 *d*, 128.5 *d* 5C), a trisubstituted benzene ring [δ_H 6.72 *d* 1H ($J=8.1\text{ Hz}$), 7.42 *dd* 1H ($J=8.0\text{ Hz}$, 8.1 Hz), 6.90 *d* 1H ($J=8.4\text{ Hz}$); δ_C 118.1 *d*, 136.3 *d*, 116.5 *d*, 3C], a benzylic methylene (δ_H 3.12 *m* 2H; δ_C 33.5 *t* 1C), two vinylic protons at *trans* configuration [δ_H 6.78 *d* 1H ($J=16\text{ Hz}$), 6.30 *dd* 1H ($J=6.4\text{ Hz}$, 16 Hz); δ_C 125.1 *d*, 134.0 *d*, 2C], a hydrogen-bonded phenolic proton (δ_H 10.97 *s* 1H; δ_C 162.3 *s* 1C), a methine [δ_H 5.23 *q* 1H ($J=6.2\text{ Hz}$, 14.3 Hz); δ_C 79.7 *d* 1C] bearing an ether oxygen, and a lactone ring. The comparison of compound **2** spectral data with

Table 1

1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data and coupling constants (in Hz in parentheses)^a for compound **1**

H (C)	δ_H	δ_C
1		171.9 <i>s</i>
3	5.68 <i>t</i> (6.5, 6.3)	82.6 <i>d</i>
4	6.87 <i>d</i> (8.2)	115.5 <i>d</i>
5	7.46 <i>t</i> (7.9)	136.6 <i>d</i>
6	6.63 <i>d</i> (7.4)	113.7 <i>d</i>
7	7.71 <i>br s</i>	156.5 <i>s</i>
8	—	111.0 <i>s</i>
9	—	149.4 <i>s</i>
10A/B	3.22 <i>dd</i> (6.7, 14)(2H)	40.7 <i>t</i>
1'	—	134.8 <i>s</i>
2', 6'	7.30–7.19 <i>m</i> (5H)	129.7 <i>d</i> ^b
3', 5'		128.6 <i>d</i> ^b
4'		127.3 <i>d</i>

^a Assignments were aided by 2D NMR 1H – 1H COSY, DEPT, and 1H – ^{13}C COSY.

^b Assignments may be interchanged.

known dihydroisocoumarins such as (**12**) (Takeuchi et al., 1983), confirmed its structure as 8E-hydroxy-3-[2-(phenyl)ethenyl]dihydro - isocoumarin. The obvious similarities in the structures of typhaphthalide and typharin, suggest a definite biogenetic relationship between phthalides and isocoumarins in this plant.

Flash column chromatography of a portion of the acetone extract over silica gel with gradient elution using benzene/ethyl acetate/acetone mixtures gave two major fractions **A** and **B**. Both fraction **A**, which was a mixture of afzelechin (**3**) and epiafzelechin (**4**), and fraction **B**, which was a mixture of catechin (**5**) and epicatechin (**6**), were acetylated and isolated as acetates from the respective mixtures by preparative TLC.

Table 2

1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data and coupling constants (in Hz in parentheses)^a for compound **2**

H (C)	δ_H	δ_C
1	—	169.5 <i>s</i>
3	5.23 <i>q</i> (6.2, 14.3)	79.7 <i>d</i>
4	3.12 <i>m</i>	33.5 <i>t</i>
5	6.72 <i>d</i> (8.1)	118.1 <i>d</i>
6	7.42 <i>dd</i> (8.0, 8.1)	136.3 <i>d</i>
7	6.90 <i>d</i> (8.4)	116.5 <i>d</i>
8	10.97 <i>s</i> (–OH)	162.3 <i>s</i>
9	—	109.3 <i>s</i>
10	—	138.9 <i>s</i>
1'	—	135.6 <i>s</i>
2' 6'	7.45–7.20 <i>m</i> (5H)	128.7 <i>d</i> ^b
3' 5'		126.8 <i>d</i> ^b
4'		128.5 <i>d</i>
7'	6.78 <i>d</i> (16)	125.1 <i>d</i>
8'	6.30 <i>dd</i> (6.4, 16)	134.0 <i>d</i>

^a Assignments were aided by 2D NMR 1H – 1H COSY, DEPT, and 1H – ^{13}C COSY.

^b Assignments may be interchanged.

3. Experimental

3.1. 3.1 General experimental procedures

Melting points (uncorrected) were determined on a Stuart Scientific SMP1 apparatus. IR spectra (KBr) were recorded on a Nicolet Impact 420 spectrophotometer. NMR spectra (both 1D and 2D) were obtained on a Varian 300 (300 MHz) spectrometer, using the residual solvent peaks as internal standards. HR-EIMS and LR-EIMS were determined on a Kratos 9/50 HRMS instrument and a MAT Finnigan GCQ spectrometer, respectively. Optical rotation was measured at ambient temperature using an Optical Activity AA-5 polarimeter. Column chromatography was carried out using Merck Si gel 60 (70–230 mesh). Flash chromatography was performed using Merck Si gel 60 (230–400 mesh) and preparative TLC was run using glass-coated silica gel F254 (0.20 mm thick) plates. Analytical TLC was carried out on precoated aluminum plates using Merck Si gel F254; the plates were visualized under UV light (λ_{254} and 366 nm) and by spraying with anisaldehyde/ H_2SO_4 reagent, followed by gentle heating.

3.2. Plant material

Fresh rhizomes were collected in March, 2000 from Pineridge near Pinetown, KwaZulu-Natal and identified by Mr. P. Poorun. A voucher specimen of the plant material was assigned collector's number (F.O. Shode/6), preserved and housed at Ward Herbarium, Department of Botany, Herbarium Unit, UDW.

3.3. Extraction and isolation

Air-dried and powdered rhizomes of *T. capensis* (300 g) were extracted with *n*-hexane (3×2l) at room temp. for 2 days. The combined extracts were evapd. under vacuum to a light yellow waxy residue (~1 g). It was redissolved in hexane and set aside with minimum evaporation for several days. Crystals (6.8 mg) of typhaphthalide (**1**) were deposited and filtered. M.p. 75–77 °C; $[\alpha]_D^{25}$ –9.2° (MeOH; *c* 0.136). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3544, 3437 (chelated OH, phenolic), 2922 (CH_2), 1714 ($\text{C}=\text{O}$), 1624, 1607, 1471 (aromatic). EI-MS *m/z* (rel int.): 240 $[\text{M}]^+$ (53.2), 149 (100), 121 (5.4), 91 (8.1). HR-MS: *m/z* 240.0786, $\text{C}_{15}\text{H}_{12}\text{O}_3$ requires 240.0786. ^1H NMR and ^{13}C NMR: see Table 1.

The filtrate from **1** was concd. and chromatographed [flash CC, silica gel 60 (230–400 mesh ASTM), 20 g; column dimensions 450×20 mm]. Gradient elution with hexane/EtOAc (9.5:0.5, 9:1) mixtures resulted into major fractions, I and II. Fraction I, a yellow solid, typharin (**2**) was insufficient in quantity to give an accurate melting

point. $[\alpha]_D^{25}$ +3.6 (MeOH; *c* 0.07). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3440 (chelated OH, phenolic), 3063 (CH_2), 1690 ($\text{C}=\text{O}$), 1600, 1580, 1475 (aromatic), 980 ($-\text{CH}=\text{CH}-$, *trans*). EI-MS *m/z* (rel. int.): 266 $[\text{M}]^+$ (85.5), 248 (100), 230 (63.8), 219 (40.9). HR-MS: 266.0943, $\text{C}_{17}\text{H}_{14}\text{O}_3$ requires 266.0944. ^1H NMR and ^{13}C NMR: see Table 2.

The marc was extracted with acetone (3×2 l) at room temp. for 2 days. The combined extracts were evapd under vacuum to a brown friable solid (12.5 g). A portion (2 g) of the solid was chromatographed [flash CC, silica gel 60 (230–400 mesh ASTM), 30 g; column dimension 650×20 mm]. Gradient elution with benzene/EtOAc/acetone (6:3:2, 6:3:3) mixtures resulted in fractions A and B. Fraction A was acetylated in the usual way and the acetates were separated by preparative TLC to give afzelechin tetraacetate (**7**) and epiafzelechin tetraacetate (**8**). Fraction B was acetylated as usual to give the acetates, which were separated by preparative TLC to give catechin pentaacetate (**9**) and epicatechin pentaacetate (**10**).

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