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A new pyrrole alkaloid from seeds of *Castanea sativa*

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Abstract

A new pyrrole alkaloid, methyl-(5-formyl-1*H*-pyrrole-2-yl)-4-hydroxybutyrate (**1**), was isolated from sweet chestnut seeds and its structure elucidated on the basis of data from NMR spectroscopy and by comparison with synthetic analogues.

Keywords: *Castanea sativa*; Alkaloids; Methyl-(5-formyl-1*H*-pyrrole-2-yl)-4-hydroxybutyrate

1. Introduction

Seeds of *Castanea sativa* Mill. (Fagaceae), a deciduous tree growing in Southern Europe (especially in the Mediterranean region and the Balkans), are currently used in paediatrics for treatment of gastroenteritis and as a gluten-free diet in cases of coeliac disease [1,2]. Only few reports have concerned the phytochemistry of these seeds [3–6]. In the Middle Ages the raw seeds were disclosed by the German herbalist Hildegard von Bingen as useful in the treatment of heart disorders [7]. As part of our ongoing phytochemical studies on the sweet chestnut seeds we isolated a new pyrrole alkaloid **1**. According to our knowledge, no pyrrole

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derivative of a similar structure has been isolated from plants before. In this paper, we report on the isolation and structure elucidation of this compound.

2. Experimental

2.1. General

NMR spectra were recorded with a Bruker AMX 500 spectrometer equipped with a 5-mm inverse probe head at 300 K. TMS was used as internal standard. Before NOE experiments were performed, dissolved oxygen was removed by bubbling Ar through the solution. LSI-MS spectra were measured on a Mat 8500 (Finnigan), matrix glycerol, 4.5 kV Cs beam, negative and positive ion mode. UV/VIS spectra were recorded on a Shimazu UV160A and the IR measurements were performed on a Perkin-Elmer 1720-X. Elementary analysis was performed at the Microanalytical Laboratory, Institute of Physical Chemistry, University Vienna, Austria.

2.2. Plant material

Dried seeds (400 g) of *C. sativa* were collected from Grubberg, near Graz, Austria, in October 1992. The plant material was authenticated by macro- and microscopy. A voucher specimen (HIKED 1/92) is deposited at the Institute of Pharmacognosy at the University of Graz.

2.3. Extraction and isolation

Powdered seeds were Soxhlet extracted successively with petrol and MeOH. The MeOH extract was separated on a Sephadex LH 20 column (length, 80 cm; i.d., 5 cm) eluting with MeOH. Fractions of 100 ml were collected and monitored by TLC. Fractions 8–12 were combined, concentrated to dryness and the residue suspended in 40 ml H_2O. The aqueous phase was then subjected to CC on Polyamide (length, 40 cm; i.d., 3 cm) using a H_2O–MeOH step gradient (H_2O 1000 ml, 50% MeOH 2000 ml, MeOH 1000 ml). Each fraction was analysed by TLC (Si-gel 60 F_{254}, $CHCl_3$/MeOH/H_2O, 36:25:5, detection by spraying with 2,4-dinitrophenylhydrazine and potassium ferricyanide). Fractions of the 50% MeOH eluates containing compound **1** were pooled and further purified by preparative HPLC on Lichrosorb RP-18 (250 × 20 mm) 7 μm, MeOH/H_2O 15:85, UV 296 nm. The total isolation procedure was repeated ×4 to afford 4 mg of the oily compound **1**.

Treatment of **1** with excess diazomethane afforded the *N*-methyl derivative **2**.

Methyl-(5-formyl-1H-pyrrole-2-yl)-4-hydroxybutyrate (**1**). TLC: R_f 0.66 ($CHCl_3$/MeOH/H_2O 36:25:5); UV max (MeOH): 291, 255 sh nm; ^{1}H and ^{13}C-NMR: see Tables 1 and 2, respectively; LSI-MS negative ion mode *m/z*: 210

Table 1
^{1}H-NMR spectral data of compounds **1** (CD_3OD), **3**, **5**, **7** and **9** (DMSO-*d6*)

H	**3**	**1**	**5**	**7**	**9**
2′	4.49 (*s*)	4.64 (*s*)	5.11 (*s*)	5.11 (*s*)	5.05 (*s*)
3	6.22 (*d*)[a]	6.25 (*d*)	6.31 (*d*)	6.30 (*d*)	6.27 (*d*)
4	6.93 (*d*)	6.97 (*d*)	6.94 (*d*)	6.92 (*d*)	6.90 (*d*)
5′	9.42 (*s*)	9.41 (*s*)	9.41 (*s*)	9.40 (*s*)	9.38 (*s*)
NH	11.93 (*s br*)	nd[b]	nd	11.62 (*s br*)	11.56 (*s br*)
2″		2.24 (*t*)	2.43 (*t*)	2.49 (*t*)	2.35 (*t*)
3″		1.98 (*m*)	1.95 (*m*)	2.08 (*m*)	1.85 (*m*)
4″		4.37 (*t*)	4.06 (*t*)	3.41 (*t*)	3.42 (*t*)
5″					4.38 (*s*)
6″			1.99 (*s*)		
7″–9″					7.25 (*m*)

[a] $J_{3,4} \approx 4$ Hz, $J_{2'',3''} \approx 7$ Hz, $J_{3'',4''} \approx 7$ Hz for all cases.
[b] nd, not detected.

($M - H^+$); LSI-MS positive ion mode *m/z*: 212 (MH^+), 194 ($MH^+ - H_2O$), 166 ($MH^+ - C_2H_6O$). Calculated: $C_{10}H_{13}NO_4$.

Methyl-(5-formyl-1-methyl-1H-pyrrole-2-yl)-4-hydroxybutyrate (**2**). ^{1}H-NMR (C_6D_6): δ 1.50 (1H, *m*, H-3″), 1.95 (1H, *t*, *J* 7.3 Hz, H-2″), 3.20 (3H, *s*, N-CH_3), 4.15 (1H, *t*, *J* 7.6 Hz, H-4″), 4.20 (1H, *s*, H-5′), 5.90 (1H, *d*, *J* 4.0 Hz, H-4), 6.50 (1H, *d*, *J* 4.0 Hz, H-3), 9.45 (1H, *s*, CHO); LSI-MS negative ion mode *m/z*: 224 ($M - H^+$). Calculated: $C_{11}H_{15}NO_4$.

Table 2
^{13}C-NMR spectral data of compounds **1**, **3**, **5**, **7** and **9**

C	**3**[a]	**1**[b]	**5**[a]	**7**[a]	**9**[a]	**9**[b]
2′	56.11	56.38	61.44	61.60	61.37	59.12
2	132.09	136.51	136.53	136.47	136.47	136.64
3	108.81	111.41	114.44	114.61	114.49	112.21
4	120.89	126.09	124.35	124.57	124.48	122.24
5	142.44	nd[c]	138.90	138.89	139.02	134.10
5′	178.61	180.81	182.87	183.03	182.88	182.77
1″		175.85	176.05	175.79	176.55	174.31
2″		32.05	33.22	35.16	33.70	31.59
3″		28.56	26.87	30.87	27.95	25.75
4″		61.12	66.45	35.45	72.05	69.99
5″			174.71		75.68	73.31
6″			22.64		141.54	139.28
7″					130.67	128.97
8″					131.26	128.30
9″					130.53	128.20

[a] Solvent: DMSO-*d6*.
[b] Solvent: CD_3OD.
[c] nd, not detected.

2.4. General procedure for synthesis of **5**, **7** and **9**

One of the *O*-substituted 4-hydroxybutyric acids **4**, **6** and **7** (see Fig. 1) (0.02 mol) and 0.02 mol of *N*,*N*′-carbonyldiimidazole was dissolved in 60 g anhydrous DMF and the mixture was stirred at room temperature for 1 h, 2.5 g of 5-hydroxymethyl-pyrrole-2-carbaldehyde (**3**) were suspended with 60 g of anhydrous DMF and catalytic amounts of NaH in another flask and the mixture was stirred at 40°C for 1 h. Then, both mixtures were pooled and kept under stirring at 40°C overnight. The separated imidazole was filtered off and the filtrate was evaporated in high vacuum. The residue was dissolved in a mixture of Et_2O and water. The aqueous layer was extracted with Et_2O and the organic layer was washed several times with water and sodium hydrogen carbonate. The organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated to yield the reaction product (**5**, **7** and **9**, respectively; yield: 32, 19 and 34%, respectively).

Methyl-(5-formyl-1H-pyrrol-2-yl)-4-acetoxybutyrate (**5**). Yellow-brown oil; TLC: R_f 0.81 ($CHCl_3$/EtOAc 4:1); UV max (MeOH): 290, 204 nm; IR bands (KBr): 3236 (s), 3132 (w), 2968 (w), 1734 (s), 1640 (s) cm^{-1}; ^1H and ^{13}C-NMR: see Tables 1 and 2, respectively. (Found: C, 56.94; H, 5.92; N, 5.48. Calculated for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53.)

Methyl-(5-formyl-1H-pyrrol-2-yl)-4-bromobutyrate (**7**). Yellow oil; TLC: R_f 0.88 ($CHCl_3$/EtOAc 4:1); IR bands (KBr): 3236 (s), 3132 (w), 2968 (w), 1734 (s), 1640 (s) cm^{-1}; ^1H and ^{13}C-NMR: see Tables 1 and 2, respectively. (Found: C, 48.75; H, 4.47; N, 5.09. Calculated for $C_{10}H_{12}BrNO_3$: C, 48.82; H, 4.41; N, 5.11.)

Methyl-(5-formyl-1H-pyrrol-2-yl)-4-benzyloxybutyrate (**9**). Yellow-brown oil; TLC: R_f 0.71 ($CHCl_3$/EtOAc 4:1); UV max (MeOH): 291, 207 nm; IR bands (KBr): 3264 (s), 3140, 3065, 3040 (w), 2940, 2860 (w), 1738 (s), 1646 (s) cm^{-1}; ^1H and ^{13}C-NMR: see Tables 1 and 2, respectively. (Found: C, 67.71; H, 6.39; N, 4.72. Calculated for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.35; N, 4.65.)

3. Results and discussion

A methanolic extract of defatted *C. sativa* seeds was worked up by CC on Sephadex LH-20 and polyamide as well as preparative RP-HPLC to give the pyrrole alkaloid **1**, which is present in very low concentration. The structure of **1** was determined by ^1H and ^{13}C-NMR as well as by comparison with synthetically obtained derivatives.

The ^1H-NMR spectrum of **1** showed a pair of coupled olefinic protons at 6.25 and 6.97 ppm. Their assignment as H-4 and H-3, respectively, was made possible by the analysis of the HMBC spectrum of compound **9** as well as by a NOE experiment performed with the title compound. This experiment resulted in the signal enhancement of H-3 upon irritation of the aldehyde proton. The vicinity of the aldehyde group and the NH of the pyrrol ring was proved by NOE contacts between CHO and NCH_3 in the *N*-methylated derivative **2**. The position of the

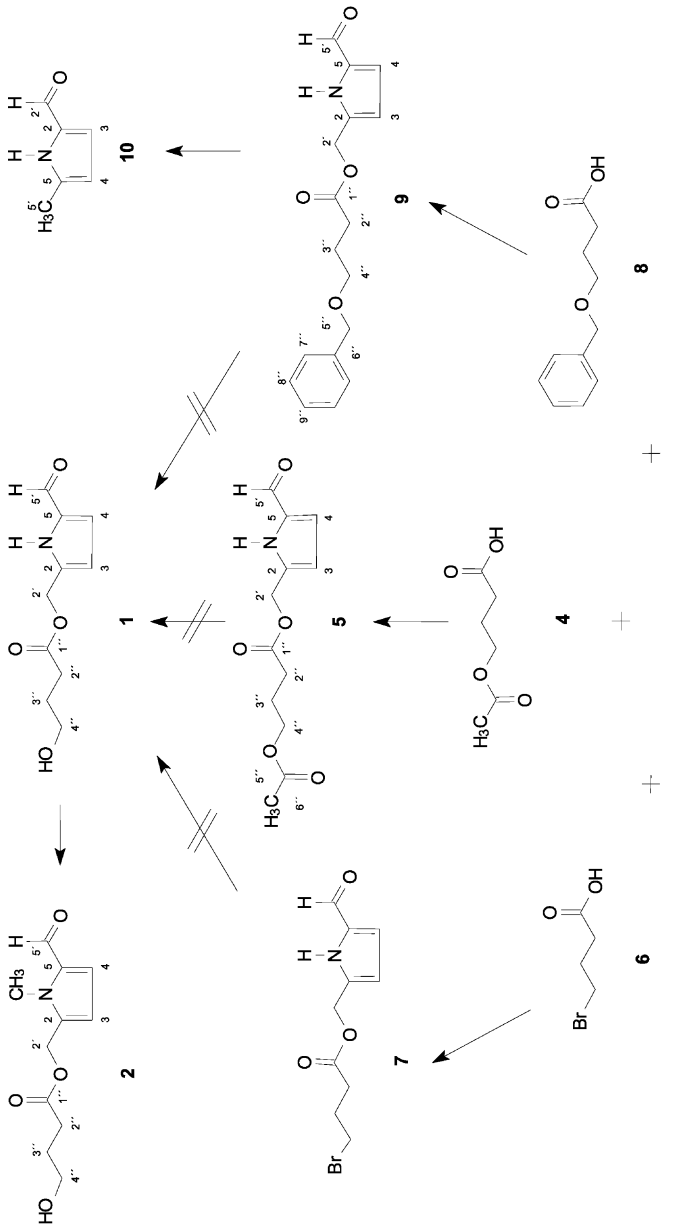

Fig. 1. Scheme of synthesis.

attachment of the CH_2O side chain to the pyrrol ring was proved by a HMBC contact from H-2′ to C-2 of the core structure [11,12].

To confirm the structure of **1** independently, we tried to prepare it or a related compound by total synthesis (Fig. 1). The starting material 5-hydroxymethyl-pyrrole-2-carbaldehyde **(3)**, which can be obtained in a well documented four step synthesis [8,9], was reacted in a one-pot reaction with ω-protected 4-hydroxybutyric acids **4**, **6**, **8** and *N,N′*-carbonyl-diimidazole [10] in the presence of catalytic amounts of sodium hydride in DMF. Thus, the synthesis of methyl-(5-formyl-1*H*-pyrrole-2-yl)-4-acetoxybutyrate **(5)**, methyl-(5-formyl-1*H*-pyrrole-2-yl)-4-bromobutyrate **(7)** and methyl-(5-formyl-1*H*-pyrrole-2-yl)-4-benzyloxybutyrate **(9)** was achieved. Hydrolysis from **5** and **7**, carried out by using various methods, did not result in the expected methyl-(5-formyl-1*H*-pyrrole-2-yl)-4-hydroxybutyrate **(1)**, only 5-hydroxymethyl-pyrrole-2-carbaldehyde **(3)** being recovered. Hydrogenation of **9** with palladium on charcoal under different conditions yielded exclusively 5-methyl-pyrrole-2-carbaldehyde **(10)**.

The chemical shifts in the ^{1}H- (Table 1) and in the ^{13}C-NMR spectra (Table 2) of the starting material **3**, the isolated compound **1** and the protected synthetic analogues **5**, **7** and **9** were very similar. The assignment of the ^{13}C shift values was supported by the CH correlation analysis (HMQC, HMBC) of compound **9**.

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