Pergamon

0031-9422(94)00655-5

Phytochemistry, Vol. 38, No. 3, pp. 711–713, 1995

0031-9422/95 $9.50 + 0.00

BIBENZYLS FROM *STEMONA TUBEROSA*

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(*Received* 17 *May* 1994)

Key Word Index—*Stemona tuberosa*; Stemonaceae; roots; bibenzyls.

Abstract—Three new bibenzyls were isolated from the roots of *Stemona tuberosa*. Their structures were identified by spectroscopic methods as 3,5-dihydroxy-4-methylbibenzyl, 3,5-dihydroxy-2′-methoxy-4-methylbibenzyl and 3-hydroxy-2′,5-dimethoxy-2-methylbibenzyl.

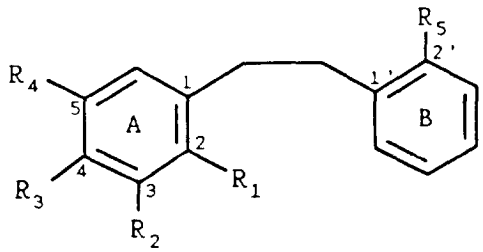
INTRODUCTION

The roots of *Stemona sessilifoloa*, *S. japonica* and *S. tuberosa* have long been prescribed in Chinese medicine as insecticides and anticough agents. Pharmacological studies showed that an ethanolic extract of *S. tuberosa* can inhibit the growth of many kinds of bacteria and fungi [1]. In the course of our studies, we described the isolation and structural determination of several *Stemona* alkaloids [2–4], but no relevant bioactivities were found for these alkaloids. For the purpose of identifying the active principle related to the medicinal utilities of *Stemona* plants, we further studied the nonalkaloid components. In the present paper, we wish to report the isolation and structural elucidation of three new bibenzyls from the roots of *S. tuberosa*.

RESULTS AND DISCUSSION

An ethanolic extract of *S. tuberosa* was extracted with chloroform. The chloroform-soluble fraction was separated by repeated chromatographies on silica gel and Sephadex LH-20 columns to give three new compounds **1–3**.

Compound **1**, crystals, mp 76°, gave an EI mass spectrum which exhibited a $[M]^+$ at *m/z* 228. ^1H and ^{13}C NMR revealed the presence of one methyl, two methylenes, two hydroxyls and two benzene moieties. Because the signals of two methylenes appeared as a broad multiplet at δ2.81 ($W_{1/2}$ = 21.4 Hz) in the ^1H NMR, **1** was deduced as a bibenzyl derivative. Its ^1H NMR data showed the signals of one methyl (δ2.10) and two equivalent aromatic protons (δ6.23), and ^{13}C NMR showed four signals in the aromatic region (δ141.1, 108.1, 154.8, 119.1). This suggested that one aromatic moiety (ring A) was symmetrically substituted as 3,5-dihydroxy-4-methyl benzyl or 2,6-dihydroxy-4-methylbenzyl. Meanwhile, the other aromatic moiety



1 $R_1 = R_5 = H$, $R_2 = R_4 = OH$, $R_3 = Me$
2 $R_1 = H$, $R_2 = R_4 = OH$, $R_3 = Me$, $R_5 = OMe$
3 $R_1 = Me$, $R_2 = OH$, $R_3 = H$, $R_4 = R_5 = OMe$

(ring B) was proved to be unsubstituted by ^1H and ^{13}C NMR analyses (see Tables 1 and 2). For determination of the substitution pattern of ring A, a NOE difference experiment was carried out. When the singlet of the two equivalent protons at δ6.23 was irradiated, 4.49% enhancement of the methylene signal at δ2.81 was observed. Therefore, the two hydroxyls were determined to be at the C-3 and C-5 positions. This was also confirmed by the presence of the two ion fragments at *m/z* 91 (C_7H_7) and 137 ($C_8H_9O_2$) in EI mass spectrum. Therefore, **1** was identified as 3,5-dihydroxy-4-methylbibenzyl.

Compound **2** was obtained as an oil, whose EI mass spectrum gave a $[M]^+$ at *m/z* 258. From consideration of its ^1H NMR spectrum, **2** was also presumed to be bibenzyl. A singlet of two equivalent protons at δ6.26 and a methyl at δ2.10 were observed in the ^1H NMR spectrum, which suggested the existence of the same dioxygenated ring A as present in **1**. A methoxyl signal was observed at δ3.82 and, furthermore, there was one proton less in the aromatic region than that of **1**. Thus, the methoxyl should be substituted on ring B. This was confirmed by the presence of ion fragments at *m/z* 121 (C_8H_9O) and 137 ($C_8H_9O_2$). In the ^1H NMR spectrum, a proton signal arising from ring B at δ 7.18 exhibited *ddd* multiplicity (*J* = 7.9, 7.8, 1.2 Hz), which revealed that it should be *ortho* to two protons and *meta* to the third one. Thus, the

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Table 1. ^{1}H NMR data of **1**, **2** ($CDCl_3$) and **3** (C_6D_6)

H	1	2	3
2	6.23 *s*	6.26 *s*	—
4	—	—	6.01 *d* (2.4)
6	6.24 *s*	6.26 *s*	6.50 *d* (2.4)
2′	7.18 *m*	—	—
3′	7.27 *dd* (7.4; 7.0)	6.85 *m*	6.57 *dd* (7.7, 1.7)
4′	7.18 *m*	7.18 *ddd* (7.9; 7.8; 1.2)	7.09 *ddd* (7.7, 7.6, 1.7)
5′	7.27 *dd* (7.4; 7.0)	6.86 *m*	6.84 *ddd* (7.1, 7.6, 1.2)
6′	7.18 *m*	7.10 *br d* (7.8)	7.03 *dd* (7.1, 1.2)
$2\times CH_2$	2.81 *m* ($W_{1/2}$ = 21.4)	2.78 *m* ($W_{1/2}$ = 23.5)	2.94 *m* ($W_{1/2}$ = 21.5)
Me	2.10 *s*	2.10 *s*	2.16 *s*
MeO	—	3.82 *s*	3.37 *s* (5-OMe) 3.31 *s* (2′-OMe)

Table 2. ^{13}C NMR data of **1–3** ($CDCl_3$)

C	1	2	3
1	141.1 *s*	141.5 *s*	143.0 *s*
2	108.1 *d*	107.9 *d*	114.1 *s*
3	154.8 *s*	154.6 *s*	154.6 *s*
4	119.1 *s*	120.6 *s*	99.3 *d*
5	154.8 *s*	154.6 *s*	158.2 *s*
6	108.1 *d*	107.9 *d*	107.4 *d*
1′	142.0 *s*	130.2 *s*	130.4 *s*
2′	128.7 *d*	157.5 *s*	157.6 *s*
3′	128.6 *d*	110.3 *d*	110.4 *d*
4′	126.2 *d*	127.2 *d*	127.2 *d*
5′	128.6 *d*	120.6 *d*	120.4 *d*
6′	128.7 *d*	129.9 *d*	129.8 *d*
CH_2	37.6 *t*	35.7 *t*	43.4 *t*
	37.8 *t*	32.2 *t*	31.7 *t*
OMe	—	55.3 *q*	55.2 *q*
			55.2 *q*
Me	8.0 *q*	7.8 *q*	10.3 *q*

methoxyl could be located at C-2′. The result was further confirmed by NOE difference experiments. When the methylene signal at δ2.78 was irradiated, only one aromatic proton signal from ring B at δ7.10 (*br d*, J = 7.8 Hz) was enhanced, and when the methoxyl signal was irradiated, another proton signal from ring B at δ6.82 (*m*) was enhanced. In addition, when the singlet of two equivalent protons on ring A at δ6.26 was irradiated, the methylene signal was enhanced by 5.99%. Therefore, the structure of **2** was identified as 3,5-dihydroxy-2′-methoxy-4-methylbibenzyl.

Compound **3** was obtained as an oil, whose EI mass spectrum gave a $[M]^+$ at *m/z* 272. Its ^{1}H NMR spectrum (in C_6D_6) exhibited the presence of six aromatic protons, one methyl, two methoxyls and two methylenes. By comparison of ^{1}H and ^{13}C NMR data (Tables 1 and 2) with those of **1** and **2**, **3** was also supposed to be a bibenzyl. The location of functional groups in **3** was determined by spectral methods as follows. Four aromatic protons in the ^{1}H NMR spectrum exhibited a similar resonance manner to those on ring B of **2**, so one aromatic moiety in **3** was an *ortho* disubstituted benzyl (ring B), like **2**. When the methoxyl signal at δ3.31 was irradiated, the one proton signal at δ6.57 (*dd*, J = 7.7, 1.7 Hz), which arises from one of the above four aromatic protons, was enhanced by 5.28%. Therefore, the methoxyl was considered to be substituted at C-2′ (ring B); the other groups should be located on ring A. This was confirmed on the basis of the presence of ion fragments at *m/z* 121 (C_8H_9O) and 151 ($C_9H_{11}O_2$) in the EI mass spectrum. Two protons on ring A resonated as two doublets coupled to each other, with J = 2.4 Hz at δ6.01 and 6.50 in the ^{1}H NMR, which meant that two aromatic protons were located at the *meta*-position. When the proton at δ6.50 was irradiated, the methoxyl at δ3.37 was enhanced by 5.97% and the methylene protons enhanced by 5.78%, whilst, when the methoxyl at δ3.37 was irradiated, the two aromatic protons were enhanced by 3.33 and 2.65%, respectively. The above results indicated that the methoxyl was substituted at the C-5 position, and the C-4 and C-6 positions were free. No NOE effects were observed between H-4 and the methyl in ring A, when the H-4 or methyl signals were irradiated. Thus, the methyl could only be substituted at C-2 with the hydroxyl at C-3. Consequently, the structure of **3** was established as 3-hydroxyl-2′,5-dimethoxy-2-methylbibenzyl.

It was reported that the crude extracts of some *Stemona* species showed antibacterial and antifungal activities [1]. Recently, a bibenzyl synthase has been isolated from rhizomes of the orchid, *Epipactis palustris*. Induction of this enzyme is dependent on wounding and subsequent infection from the mycorrhiza, and leads to the formation of bibenzyl and dihydrophenanthrene derivatives [5]. The above process might be a self-protective mechanism for plants when they meet harmful stimulation. Some pharmacological testing of bibenzyl derivatives has been done previously [6]. In the course of our study, **1** showed cytotoxicity toward murine cancer cell lines *in vitro*. At 10 $\mu g\,ml^{-1}$, it inhibited the growth rate of P388 leukaemic cells and hapatoma cells by 99.7 and 83.6%, respectively. Further biological tests on the bibenzyls from *S. tuberosa* will be undertaken.

EXPERIMENTAL

General. Mps: uncorr. ^{1}H NMR: 400 MHz, solvent as ref. ^{13}C NMR: 75 MHz, solvent as ref. EIMS: 70 eV, CC: silica gel, Lobar Si 60 column and Sephadex LH-20.

Extraction and isolation. Air-dried roots (20 kg) of *S. tuberosa* Loub. collected from Hainan province. South China were powdered and then percolated with 95% EtOH. After evapn of EtOH, 5 l residue was obtained. A portion of the residue (2 l) was extracted with $CHCl_3$ to give 200 g $CHCl_3$ extract. The extract was then chromatographed on a silica gel column, eluting with mixts of petrol and Me_2CO (4:1 → 1:1 → 100% Me_2CO). The (4:1) fr. was further chromatographed repeatedly on a silica gel column with petrol–EtOAC (5:1) to obtain **1** (13 mg) and **3** (7 mg). From the same fr., a mixt. containing **2** was subjected to CC on Lobar Si 60 with CH_2Cl_2 and then on Sephadex LH-20 with 80% EtOH to give pure **2** (10 mg).

3,5-*Dihydroxy*-4-*methylbibenzyl* (**1**). Crystals. Mp: 76°. MS (rel. int.) *m/z*: 228 (17, $[M]^+$), 137 (42), 121 (100), 91 (11). NMR data in Tables 1 and 2.

3,5-*Dihydroxy*-2′-*methoxy*-4-*methylbibenzyl* (**2**). Oil. MS (rel. int.) *m/z*: 258 (4, $[M]^+$), 137 (3), 121 (100), 91 (45), 86 (51), 84 (83). NMR data in Tables 1 and 2.

3-*Hydroxy*-2′,5-*dimethoxy*-2-*methylbibenzyl* (**3**). Oil. MS (rel. int.) *m/z*: 272 (19, $[M]^+$), 151 (8), 121 (50), 117 (82), 91 (27), 84 (100). NMR data in Tables 1 and 2.

Assay for growth inhibition in vitro. Murine P388 leukaemic and hapatoma cells were used for the assay. The bibenzl was cultured with the cells for 24 hr. Numbers of surviving cells were counted using the Trypanblau dye exclusion test.

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