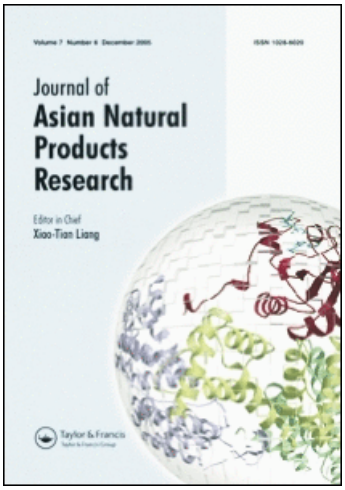

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CYCLOARTANE-TYPE TRITERPENOIDS FROM *SPHAEROPHYSA SALSULA*

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Four novel cycloartanes, named sphaerophysone A (**1**), B (**2**), C (**3**) and D (**4**), were isolated from the ethanol extract of *Sphaerophysa salsula* (Pall.) DC. The structures were elucidated on the basis of spectral evidence, and the stereochemistry of compound **1** was defined by X-ray crystallographic analysis. Sphaerophysone B (**2**) may be an artifact formed in the isolation procedure.

Keywords: Leguminosae; *Sphaerophysa salsula*; Whole herbs; Cycloartane; Sphaerophysone A–D

INTRODUCTION

Sphaerophysa salsula (Pall.) DC is widely distributed in Middle-Asian and northwest China. It has been used as a folk medicine to treat hypertension in China [1]. We previously reported the isoflavans [2] and stilbenes [3] from *S. salsula*, and in continuing our studies on the ingredients of *S. salsula* we have isolated cycloartanes from the whole herbs of the plant for the first time. This paper describes the isolation and structural elucidation of the compounds.

RESULTS AND DISCUSSION

Sphaerophysone A (**1**) [4] has a molecular formula $C_{30}H_{48}O_4$ based on ^{13}C NMR and positive HR-EIMS data. In the ^1H NMR spectrum of **1**, six singlet methyls at δ 0.86 (3H, s, H-30), 0.96 (3H, s, H-29), 0.98 (3H, s, H-18), 1.22 (3H, s, H-28), 1.51(3H, s, H-27) and 1.54 (3H, s, H-26) and one doublet methyl at δ 1.01 (3H, d, $J = 4.5$ Hz, H-21) were seen. Two olefinic protons appeared at δ 6.23 (1H, d, $J = 10.0$ Hz, H-2) and δ 6.88 (1H, d, $J = 10.0$ Hz, H-1), and in the ^{13}C NMR spectrum two carbon signals are at δ 127.2 and 153.4, as well as a carbonyl resonance at δ 204.0, which are characteristics of the conjugated 1-en-3-one system in the structure. The ^1H NMR spectrum also displayed two cyclopropane methylene

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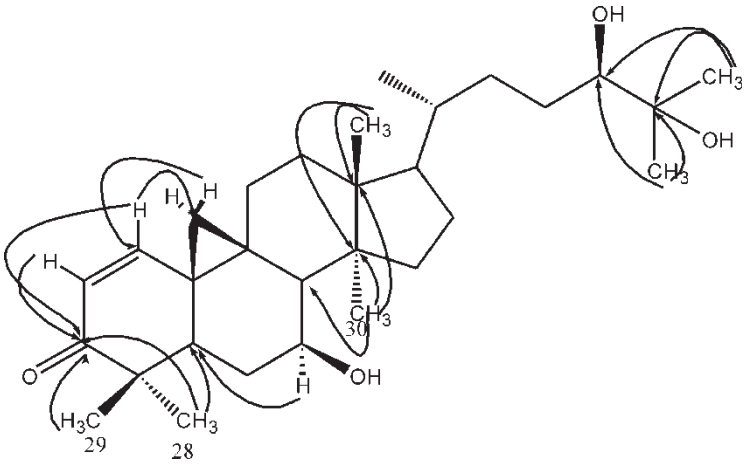


FIGURE 1 Significant HMBC correlations of compound **1**.

protons at δ 0.54 (1H, d, $J = 4.7$ Hz, H-19a) and δ 1.62 (1H, d, $J = 4.7$ Hz, H-19b)—not so evident as a cycloartane. This is because they are deshielded by the conjugated systems in the 1-en-3-one [5].

There are 30 carbon signals in the ^{13}C NMR spectrum of **1**, including three oxygen-bearing carbon signals. In the HMQC spectrum, the correlated peaks between proton δ 3.94 (1H, m, H-7) and carbon δ 67.6 (C-7), proton δ 3.71 (1H, br.d, $J = 9.3$ Hz, H-24) and carbon δ 80.0 (C-24) were presented, suggesting the presence of two hydroxyl groups. Conversely, another carbon signal, at δ 72.8 (C-25), suggested the presence of a tetra-substituted carbon bearing a hydroxyl group. In the HMBC spectrum of **1** (Fig. 1), the proton signals at δ 0.54 (1H, d, $J = 4.5$ Hz, H-19a) and δ 1.62 (1H, d, $J = 4.5$ Hz, H-19b) have long-range correlations with the carbon at δ 153.4 (C-1). In the NOESY spectra of **1**, correlated peaks between H-7 and H-30 were found; thus the configuration of the hydroxyl at C-7 is β. The configuration of hydroxyl group at C-24 was determined by X-ray crystallographic analysis.[†] The results of X-ray analysis (Fig. 2) also support the above conclusions. So the structure of **1** was established as 9,19-cycloart-7β,24β,25-triol-1-en-3-one (Fig. 3), named sphaerophysone A. On the basis of its ^{1}H–^{1}H COSY, NOESY, HMQC and HMBC spectra, all the ^{1}H and ^{13}C NMR signals were assigned as shown in Table I.

Sphaerophysone B (**2**) has a molecular formula $C_{33}H_{52}O_4$ based on ^{13}C NMR and the ion peak of HR-EIMS. The UV and ^{1}H and ^{13}C NMR spectra of **2** were almost the same as those of **1**. The difference between **1** and **2** was apparent only at the side chain. Analysis of the 2D NMR spectra indicated the presence of two more methyl groups and one more tetra-substituted carbon than in **1**. Analysis of the HMBC and HMQC data indicated that the chemical shifts of C-24 and C-25 were moved downfield to δ 84.3 and δ 80.3, respectively; however, that of C-23 was moved upfield to δ 27.2, suggesting that the hydroxyls at C-24 and C-25 have disappeared. In the HMBC spectra, H-2′ and H-3′ are correlated with C-1′ (δ 106.5), which is a ketal carbon signal. The configuration of 7-OH was determined by the NOESY spectra of **2**. Thus, the structure of **2** was formulated

[†]Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033) or e-mail: deposit@ccdc.cam.ac.uk).

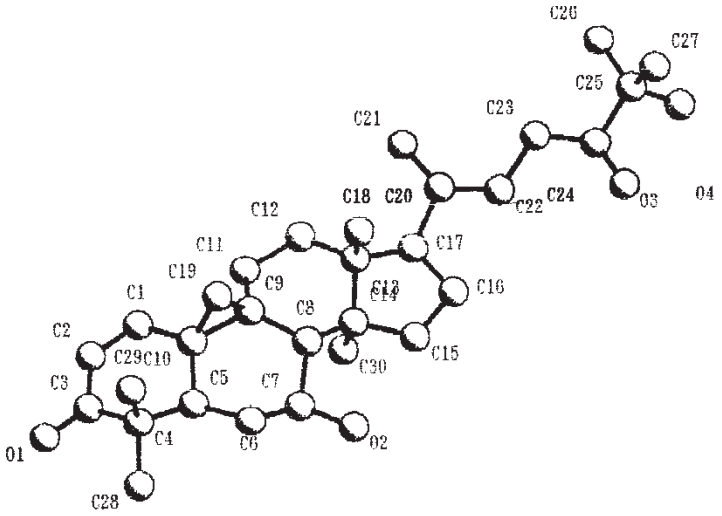
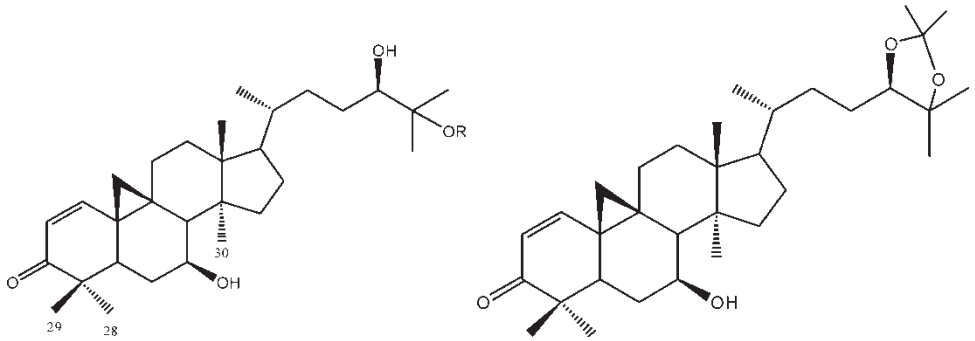


FIGURE 2 Structure of **1**.

as 24,25-acetonide-sphaerophysone A. Because acetone was used as an eluent, compound **2** may be an artificial product derived from **1**.

Sphaerophysone C (**3**) showed an $[M + Na]^+$ ion peak at *m/z* 657 in the positive FAB mass spectrum, and the molecular formula was determined as $C_{36}H_{58}O_9$ on the basis of ^{13}C NMR and HR-FABMS. The ^1H NMR of **3** showed an AB quartet signal at δ 0.54 (1H, d, $J = 4.5$Hz, H-19a) and δ 1.62 (1H, d, $J = 4.5$ Hz, H-19b). In the ^{13}C NMR spectrum of **3** six more signals appeared than for compound **1** (δ 98.7, 78.7, 78.3, 75.4, 71.7 and δ 62.7). Upon hydrolysis with 0.5 M HCl, **3** afforded **1** and glucose; the glucose was identified by direct comparison with authentic samples by high-performance thin-layer chromatography (HPTLC) and HPLC. The ^1H NMR spectrum of **3** showed an anomeric proton signal at δ 5.25 (1H, d, $J = 7.5$ Hz), so the configuration of the sugar is β on the basis of the coupling constant. The ^{13}C NMR spectrum of **3** showed the corresponding signals due to



1. R = H **2**
3. R = -Glc
4. R = -Glc-Ara (4→1)

FIGURE 3 Structures of **1**–**4**.

TABLE I ^{13}C NMR data of **1**–**4**

Position	***1***	***2***	***3***	***4***	*Position*	***1***	***2***	***3***	***4***
1	153.4	153.3	153.4	153.5	22	30.5	30.5	30.5	30.4
2	127.2	127.2	127.2	127.3	23	29.4	27.2	29.4	29.3
3	204.0	204.0	204.0	204.2	24	80.0	84.5	78.5	78.6
4	45.7	45.7	45.7	45.7	25	72.8	80.3	81.0	81.2
5	42.2	42.2	42.1	42.2	26	26.0	26.4	24.3	24.5
6	34.6	34.4	34.7	34.6	27	26.0	23.4	21.3	21.1
7	67.6	67.6	67.6	67.7	28	21.8	21.8	21.8	21.9
8	51.1	51.2	51.1	51.1	29	19.1	19.3	19.1	19.1
9	26.4	26.4	26.4	26.5	30	18.8	18.7	18.7	18.8
10	30.5	30.5	30.5	30.5	1′		106.5	98.7	98.5
11	28.3	28.2	28.2	28.3	2′		29.0	75.4	75.1
12	32.4	32.4	32.3	32.4	3′		27.0	78.7	76.5
13	45.7	45.7	45.7?	45.7	4′			71.7	80.8
14	49.4	49.4	49.4?	49.5	5′			78.3	76.3
15	34.4	34.0	34.4	34.4	6′			62.7	61.7
16	28.3	28.2	28.2	28.3	1″				105.4
17	52.0	52.0	52.1	52.2	2″				74.9
18	15.7	15.7	15.7	15.7	3″				78.3
19	25.2	25.2	25.2	25.2	4″				70.7
20	37.2	37.0	37.1	37.2	5″				67.5
21	19.4	18.7	19.3	19.4					

cyclopropane methylene at δ 25.2 (C-19). Three oxygen-bearing carbons at δ 67.6 (C-7), 78.5 (C-24), 81.0 (C-25) and an anomeric carbon signal at δ 98.7 (C-1) were also present, as well as two olefinic carbon signals at δ 153.4 (C-1), 127.2 (C-2) and one carbonyl signal at δ 204.0 (C-3). The HMBC of **3** showed that H-1′ of the glucopyranosyl moiety was correlated with carbon C-25 (δ 81.0), indicating that the sugar moiety is linked to the C-25 hydroxy group of **3**. The chemical shift and the shape of H-7 and H-24 signals are similar to those in the ^1H NMR of **1**, and so the configurations of 7- and 24-OH are β; the configuration of 7-OH was also confirmed by a NOESY spectra. Thus the structure of **3** was configured as sphaerophysone A 25-*O*-β-D-glucopyranoside.

Sphaerophysone D (**4**) was a white powder from CH_3OH. After hydrolysis with 0.5 M HCl, glucose, arabinose and **1** were afforded. The ^1H NMR spectrum showed AB quartet signals at δ 0.50 (1H, d, $J = 4.5$ Hz, H-19) and δ 1.63 (1H, d, $J = 4.5$ Hz, H-19). Two anomeric protons were seen at δ 5.19 (1H, d, $J = 8.0$ Hz, H-1′) and δ 5.08 (1H, d, $J = 7.5$ Hz,H-1″), and, on the basis of the coupling constants, the configuration of glucose is β and that of arabinose is α. The ^{13}C NMR spectrum of **4** exhibited 41 carbon signals, 11 more than in **1** (*viz.* δ 105.4, 98.5, 80.8, 78.3, 76.5, 76.3, 75.1, 74.9, 70.7, 67.5 and 61.7). Two anomeric carbon signals were also present, at δ 98.5 (C-1′) and 105.4 (C-1″). 2D NMR (HMBC, HMQC) was applied to determine the connection sequence between the aglycon and the sugar moiety, and the sequence between the two sugars was determined by HMBC correlations, so that **4** was formulated as 25-*O*-β-D-arabinopyranosyl-(1→4)-β-D-glucopyranosyl sphaerophysone A.

EXPERIMENTAL

General Experimental Procedures

NMR data, including DEPT90, DEPT135, HMQC, HMBC, ^1H–^1H COSY and NOESY experiments, were recorded on a Bruker-AMX-300 spectrometer. All spectra were recorded

in pyridine-d_5. HR-EIMS and FABMS spectra were recorded using a JEOL HX110 spectrometer. Optical rotations were performed on a Perkin-Elmer 241 polarimeter. UV spectra were recorded with a SHIMAZU UV-2201. The X-ray analysis was determined with a MAC DIP-2030k diffractometer with graphite monochromator.

Plant Material

Whole herbs of *S. salsula* were collected in the west of Inner Mongolia and identified by Professor Kang Shuanglong in August 1999. A specimen has been deposited in the Herbarium of the Department of Natural Medicines, Shenyang Pharmaceutical University.

Extraction and Isolation

Dried whole herbs of the plant (1.8 kg) were extracted with ethanol. After filtration, the extract was concentrated to 1.8 L, and then fractioned with $CHCl_3$, EtOAc and n-BuOH successively. The $CHCl_3$ extract (15 g) was subjected to column chromatography over silica gel, eluting with a light petroleum–acetone mixture. Compound **1** (20 mg) was obtained when eluted with light petroleum–acetone (1:1). When the eluent was light petroleum–acetone (4:1), **2** (6 mg) was obtained. The EtOAc extract was also subjected to column chromatography on silica gel and eluted with a $CHCl_3$–MeOH gradient system to produce 12 fractions, of which fraction 8 was subjected to HPLC with a TOSOH C18 column and UV detector, with 80% MeOH–H_2O as an eluent, to afford **3** (17 mg, 120 min) and **4** (19 mg, 87 min).

X-ray Crystal Structure Analysis of 1

Crystal data for **1**: $(C_{30}H_{48}O_4)_3 \cdot H_2O$, MW = 472.21, monoclinic, space group $P2_1$, with $a = 11.0360$ (2) Å, $b = 19.5970$ (8) Å, $c = 19.8170$ (7) Å, $\beta = 103.537$ (2) Å, $V = 4166.81$ (24) Å^3, $Z = 6$ and $D_{calc} = 1.145\ g\,cm^{-3}$ A crystal of **1** (0.20 × 0.40 × 0.40 mm) was selected for analysis and the X-ray intensity data measured on a MAC DIP2030K X-ray diffractometer with Mo Kα radiation. The detector-to-crystal distance was 100 mm. A total of 6902 unique reflections were recorded, of which 6889 were considered on the basis of $|F|^2 \geq 8\sigma|F|^2$. The structure was solved using direct methods, all the hydrogen and non-hydrogen atoms were located from a difference Fourier map and the parameters were refined. Final R factors were $R = 0.073$ and $Rw = 0.072$.

Sphaerophysone A (1)

Colorless needles from MeOH; $[\alpha]_D$ −21.6 (20°C, $c = 0.14$, CH_3OH); UV λ_{max} (MeOH) 203, 267 nm; ^{1}H NMR (300.0 MHz, pyridine-d_5) δ (ppm): 6.88 (1H, d, $J = 10.0$ Hz, H-1), 6.23 (1H, d, $J = 10.0$ Hz, H-2), 2.25 (1H, dd, $J = 13.7, 3.8$ Hz, H-5), 2.22 (1H, m, H-6a), 1.51 (1H, m, H-6b), 3.94 (1H, m, H-7), 2.43 (1H, d, $J = 4.3$ Hz, H-8), 2.02 (2H, m, H-11), 1.62 (1H, m, H-17), 0.98 (3H, s, H-18), 0.54, 1.62 (each 1H, d, $J = 4.7$ Hz, H-19), 1.51 (1H, m, H-20), 1.01 (3H, d, $J = 4.5$ Hz, H-21), 1.83 (2H, m, H-23), 3.71 (1H, br.d, $J = 9.3$ Hz, H-24), 1.54 (3H, s, H-26), 1.51 (3H, s, H-27), 1.22 (3H, s, H-28), 0.96 (3H, s, H-29), 0.86 (3H, s, H-30); ^{13}C NMR, see Table I; HR-EIMS m/z 472.3546 (calcd for $C_{30}H_{48}O_4$, 472.3552).

Sphaerophysone B (2)

Colorless needles from MeOH; $[\alpha]_D$ −37.3 (20°C, *c* 0.05, CH_3OH); UV λ_{max} (MeOH) 203, 265 nm; ^1H NMR (300.0 MHz, pyridine-d_5) δ (ppm): 6.87 (1H, d, $J = 10.0$ Hz, H-1), 6.23 (1H, d, $J = 10.0$ Hz, H-2), 2.28 (1H, dd, $J = 13.5, 3.5$ Hz, H-5), 2.07 (1H, m, H-6a), 1.50 (1H, m, H-6b), 3.94 (1H, m, H-7), 2.43 (1H, d, $J = 4.3$ Hz, H-8), 1.88 (2H, m, H-11), 1.57 (1H, m, H-17), 0.98 (3H, s, H-18), 0.57, 1.64 (each 1H, d, $J = 4.8$ Hz, H-19), 1.43 (1H, m, H-20), 0.96 (3H, d, $J = 6.6$ Hz, H-21), 1.85 (2H, m, H-23), 3.80 (1H, m, H-24), 1.30 (3H, s, H-26), 1.19 (3H, s, H-27), 1.28 (3H, s, H-28), 1.01 (3H, s, H-29), 0.89 (3H, s, H-30); ^{13}C NMR, see Table I; HR-EIMS *m/z* 512.3862 (calcd for $C_{33}H_{52}O_4$, 512.3865).

Sphaerophysone C (3)

White powder from MeOH; $[\alpha]_D$ −32.3 (20°C, *c* 0.11, CH_3OH); UV λ_{max} (MeOH) 204, 263 nm; ^1H NMR (300 MHz, pyridine-d_5) δ (ppm): 6.85 (1H, d, $J = 10.0$ Hz, H-1), 6.22 (1H, d, $J = 10.0$ Hz, H-2), 2.25 (1H, m, H-5), 2.11 (1H, m, H-6a), 1.50 (1H, m, H-6b), 3.97 (1H, m, H-7), 2.48 (1H, d, $J = 4.4$ Hz, H-8), 1.86 (2H, m, H-11), 1.57 (1H, m, H-17), 0.95 (3H, s, H-18), 0.54, 1.62 (each 1H, d, $J = 4.5$ Hz, H-19), 0.98 (3H, d, $J = 7.0$ Hz, H-21), 1.11 (1H, m, H-22), 2.19 (1H, br.t, $J = 12.5$ Hz, H-22), 1.83 (2H, m, H-23), 3.85 (1H, br.d, $J = 10.0$ Hz, H-24), 1.55 (3H, s, H-26), 1.53 (3H, s, H-27), 1.23 (3H, s, H-28), 1.00 (3H, s, H-29), 0.86 (3H, s, H-30), 5.25 (1H, d, $J = 7.5$ Hz, H-1′), 4.03 (1H, t, $J = 8.0$ Hz, H-2′), 4.26 (1H, m, H-3′), 4.23 (1H, m, H-4′), 3.98 (1H, m, H-5′), 4.42 (1H, dd, 5.5, 11.0, H-6′a), 4.54 (1H, dd, 11.0, 2.0, H-6′b); ^{13}C NMR, see Table I; positive HR-FABMS *m/z* 657.3950 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_9Na$, 657.3979).

Sphaerophysone D (4)

White powder from MeOH; $[\alpha]_D$ −47.4 (20°C, *c* 0.08, CH_3OH); UV λ_{max} (MeOH) 206, 260 nm; ^1H NMR (300.0 MHz, pyridine-d_5) δ (ppm): 6.85 (1H, d, $J = 10.0$Hz, H-1), 6.22 (1H, d, $J = 10.0$ Hz, H-2), 2.24 (1H, dd, $J = 3.5, 13.5$ Hz, H-5), 2.07 (1H, m, H-6), 1.50 (1H, m, H-6), 3.91 (1H, m, H-7), 2.43 (1H, d, $J = 4.0$ Hz, H-8), 1.56 (1H, m, H-17), 0.94 (3H, s, H-18), 0.54, 1.63 (each 1H, d, $J = 4.5$ Hz, H-19), 0.94 (3H, d, $J = 7.0$ Hz, H-21), 1.08 (1H, m, H-22), 2.05 (1H, br.t, $J = 12.5$ Hz, H-22), 1.84 (2H, m, H-23), 3.80 (1H, br.d, $J = 10.0$ Hz, H-24), 1.54 (3H, s, H-26), 1.48 (3H, s, H-27), 1.21 (3H, s, H-28), 0.99 (3H, s, H-29), 0.85 (3H, s, H-30), 5.19 (1H, d, $J = 8.0$ Hz, H-1′), 4.03 (1H, t, $J = 8.0$ Hz, H-2′), 4.25 (1H, m, H-3′), 4.29 (1H, m, H-4′), 3.90 (1H, m, H-5′), 3.90 (1H, m, H-6′a), 4.48 (1H, m, H-6′b), 5.08 (1H, d, $J = 7.5$ Hz, H-1″), 4.04 (1H, m, H-2″), 4.10 (1H, m, H-3″), 4.15 (1H, m, H-4″), 4.20 (1H, dd, $J = 11.0, 6.5$ Hz, H-5″a), 3.63 (1H, t, $J = 6.5$ Hz, H-5″b); ^{13}C NMR, see Table I; positive HR-FABMS *m/z* 789.4408 $[M + Na]^+$ (calcd for $C_{41}H_{66}O_{13}Na$ 789.4401).

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References

[1] Zhou, M., Tao, Y.J. and Xu, S.Y. (1987), *Chin. J. Pharm. Toxicol.* **1**, 258–261.
[2] Ma, Z.J., Hou, B.L., Wang, J.H. and Li, X. (2002), *Pharmazie* **57**, 75–76.
[3] Ma, Z.J., Li, X., Li, N. and Wang, J.H. (2002), *Fitoterapia* **73**, 313–315.
[4] Ma, Z.J., Li, X., Lu, Y., Wang, C. and Zheng, Q.T. (2003), *Chin. Chem. Lett.* **14**, 594–596.
[5] Atta-Ur-Rahman, Nasir, H. and Iqbal, Z. (1994), *Phytochemistry* **35**, 993–1000.