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Chemical constituents of Centella asiatica

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Studies on the chemical constituents of the aerial part of *Centella asiatica* have led to the isolation of three new compounds, named centellin (1), asiaticin (2), and centellicin (3). Their structures have been elucidated through spectral studies including 2D NMR experiments (HMQC, HMBC, ${}^{1}H{-}^{1}H$ COSY, NOESY and *J* resolved).

Keywords: Centella asiatica; Umbelliferae; Polyacetylene; Centelin; Asiaticin; Centellicin

1. Introduction

Centella asiatica (syn. *Hydrocotyle asiatica*), a plant of the family Umbelliferae, is a weakly scented species occurring in parts of India, Sri Lanka, China, Indonesia, Malaysia, Australia and Southern and Central Africa. It has been used in traditional medicine in India for the treatment of leprosy, varicose veins, ulcers, lupus and certain eczemas and mental retardation. Clinical trials have shown that extracts of *C. asiatica* heal wounds, burns and ulcerous abnormalities of the skin [1-2]. The chemical constituents of *Centella* include polyacetylenes, triterpenoids, asiaticosides. Asiatiocides are useful antileproic agents. The present studies undertaken on *C. asiatica* in view of its attributed medicinal significance have resulted in the isolation and structure elucidation of three new compounds named as centellin, asiaticin and centellicin. Their structures have been elucidated as 6-acetoxy-trideca-1,7-dien-4-yn-3-ol (1), *p*-benzoyloxy methyl-butyl benzoate (2), and 1-(2',3'-dihydroxypropyl)-2-en-3-methyl-6-hydroxy-9-yn-undecanoate (3).

2. Results and discussion

The molecular formula of compound **1** was established as $C_{15}H_{22}O_3$ by M⁺ ion in the HR– EI–MS at m/z 250.3325. The IR spectrum showed characteristic absorption bands caused by

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acetylenic group at 2145, hydroxyl group at 3241 and ester carbonyl group at 1744 cm^{-1} . The UV spectrum showed absorption maxima at 202 (log ε 4.9) nm. These UV and IR data indicated that 1 has ene and yne-chromophores. The NMR spectra (table 1) revealed the presence of two double bonds, one hydroxy containing carbon, one acetoxy carbonyl carbon, one triple bond comprising two quaternary C-atoms, four ordinary methylenes, and two methyl groups which accounted for 15 carbon atoms in the molecule. The structure was derived from the ¹H NMR and ¹³C NMR data as well as the proton-proton and protoncarbon connectivities observed in the COSY, HMOC and HMBC spectra. In the ¹H NMR spectrum a pair of double doublets of olefinic protons was present at $\delta 5.41(J = 16.0, 1.0 \text{ Hz},$ H-1a) and 5.23 (J = 10.1, 1.0 Hz, H-1b). One proton showed up as a ddd at δ 5.90 (J = 16.0, 1.0 Hz, H-1b). 10.1 and 5.3 Hz, H-2), while another proton showed a doublet at δ 4.91 (J = 5.3 Hz, H-3). The ${}^{1}\text{H}-{}^{1}\text{H}$ COSY spectrum indicated that the proton at δ 5.90 (H-2) was coupled with olefinic protons at δ 5.41 and 5.23 (H-1a, H-1b) as well as with the proton at δ 4.91 (H-3). The proton at δ 4.91 (H-3) was coupled with only one proton (H-2) and connected with a carbon at δ 63.5 in the HMQC identified as CH in the DEPT. Further H-3 showed connections in the HMBC spectrum with quarternary carbons at δ 70.2 (C-4) and 78.4 (C-5) which were assigned to acetylenic carbons [3-5]. The C-4 and C-5 also had long-range connectivity with a proton at δ 6.11 (HMBC) which appeared as a doublet (J = 8.7 Hz, H-6) and showed correlation with a carbon at δ 60.1 CH in DEPT. In the COSY plot H-6 was related with a proton at δ 5.47 (dt, J = 10.4, 8.7 Hz, H-7), H-7 with δ 5.63 (dt, J = 10.4, 7.5, 7.5H-8), H-8 with δ 2.13 (t, J = 7.5 Hz, H₂-9), H-9 with δ 2.10 (m, H₂-10), H-10 with δ 1.38 (m, H₂-11), H-11 with δ 1.26 (m, H-12), H-12 with δ 0.86 (t, J = 6.8 Hz, H₃-13). The C-9 to C-12 were methylenes while C-13 was a methyl (DEPT) showing correlation with a carbon at δ 13.9 in the HMQC. These data exhibited that **1** is a straight chain compound with two double and one triple bonds placed in the order drawn in the structure 1. The chemical shifts of H-3 and H-6 and their connected carbons further showed that they have oxygen substituents. One of these was decided as acetoxy group in light of presence of a three-proton singlet at $\delta 2.05$ and ¹³C NMR signal (δ_{CO} 169.4 and δ_{CH3} 20.8 singlet) and placed at C-6 due to downfield resonance of H-6 as compared to that of H-3. These features indicated that the second oxygen

Table 1. ¹H NMR and ¹³C NMR and HMBC spectral data for compound **1** in CDCl₃[†].

| Position | δ_H | (mult., J in Hz) | δ_C | C/H long-range correlations |
|----------|------------|-----------------------|------------|-----------------------------|
| 1a | 5.41 | dd (16.0, 1.0) | 117.3 | C-2, C-3 |
| 1b | 5.23 | dd (10.1, 1.0) | _ | _ |
| 2 | 5.90 | ddd (16.0, 10.1, 5.3) | 135.7 | C-3, C-4 |
| 3 | 4.91 | d (5.3) | 63.5 | C-1, C-2, C-4, C-5 |
| 4 | _ | _ | 70.2 | _ |
| 5 | _ | _ | 78.4 | _ |
| 6 | 6.11 | d (8.7) | 60.1 | C-4, C-5, C-1' |
| 7 | 5.47 | dt (10.4, 8.7) | 123.7 | C-8, C-9 |
| 8 | 5.63 | dt (10.4, 7.5) | 136.4 | C-6, C-7, C-9 |
| 9 | 2.13 | t (7.5) | 27.8 | C-7, C-8, C-10 |
| 10 | 2.10 | m | 28.8 | C-9, C-11, C-12 |
| 11 | 1.38 | m | 22.4 | C-10, C-12, C-13 |
| 12 | 1.26 | m | 31.3 | C-11, C-13 |
| 13 | 0.86 | t (6.8) | 13.9 | C-11, C-12 |
| 1' | _ | _ | 169.4 | _ |
| 2' | 2.05 | S | 20.8 | C-1′ |

[†] Chemical shift values are in ppm, and J values in parentheses are in Hz. The assignments are based on ${}^{1}H{-}^{1}H$ COSY, J-resolved and HMQC spectra.



Figure 1. 6-Acetoxy-trideca-1,7-dien-4-yn-3-ol (1).

is a hydroxyl group, which was placed at C-3. In light of these observations, the structure of **1** has been elucidated as 6-acetoxy-trideca-1,7-dien-4-yn-3-ol (figure 1). The structure was fully supported by HMBC correlations (table 1) and other 2D NMR techniques (HMQC, ${}^{1}\text{H}{-}^{1}\text{H}$ COSY).

The molecular formula of compound 2 was established as $C_{19}H_{20}O_4$ by the M⁺ ion in the HREI-MS at m/z 312.3645. The IR spectrum showed characteristic bands caused by ester absorption (C=O) at 1730 and benzene ring by absorptions between 1631 and 1459 cm^{-1} . The UV spectrum showed absorption maxima at 215 (log $\varepsilon = 4.4$) and 275 (log $\varepsilon = 4.5$) nm. The NMR spectra (table 2) revealed the presence of one disubstituted and one monosubstituted benzene ring, two ester carbonyl carbons, four methylenes and one methyl group which accounted for the 19 carbon atoms in the molecule. In the ¹H NMR spectrum a triplet of methylene proton was present at $\delta 4.16$ (J = 6.8 Hz H-1"), indicating its link with an ester group, which was substantiated by carbons resonating at δ 65.6 (C-1["]) and 167.4 (C-1). The ${}^{1}H-{}^{1}H$ COSY spectrum revealed correlation of this methylene with methylene protons at δ 1.60 (H-2"). Further, H-1" showed correlations in the HMBC spectrum with quarternary carbon at δ 167.4 (C-1) and methylene carbons at δ 30.5 (C-2") and 19.1 (C-3"). A singlet of two-protons was noted at δ 5.31 (H₂-8) which was connected with a carbon at δ 67.5 (C-8) in the HMQC identified as CH₂ in the DEPT. Furthermore, H-8 showed correlations with quarternary carbons at δ 167.6 (C-9), 135.5 (C-1'), 131.8 (C-5), and 128.4 (C-4/C-6). In the downfield region a multiplet of four protons was present at δ 7.74 which was attributed to (H-3, H-7, H-2' and H-6'). These protons were connected with carbons at δ

Table 2. ¹H NMR and ¹³C NMR and HMBC spectral data for compound **2** in CDCl₃[†].

| Position | δ_H | (mult., J in Hz) | δ_C | C/H long range correlations |
|----------|------------|------------------|------------|-----------------------------|
| 1 | _ | _ | 167.4 | |
| 2 | _ | _ | 132.5 | |
| 3/7 | 7.74 | m | 131.1 | C-1, C-2, C-4/C-6 |
| 4/6 | 7.39 | m | 128.4 | C-2, C-3/C-7 |
| 5 | _ | _ | 131.8 | _ |
| 8 | 5.31 | S | 67.5 | C-1′, C-4/C-6 |
| 1' | _ | _ | 167.6 | _ |
| 2' | _ | _ | 135.1 | _ |
| 3'/7' | 7.74 | m | 130.9 | C-1', C-2', C-4' |
| 4' | 7.31 | m | 128.6 | _ |
| 5' | 7.31 | m | 129.0 | _ |
| 6' | 7.31 | m | 129.6 | _ |
| 7'/4' | 7.74 | m | 130.9 | C-1', C-2', C-4' |
| 1″ | 4.16 | t (6.8) | 65.6 | C-1, C-2", C-3" |
| 2" | 1.60 | dd (14.4, 6.8) | 30.5 | C-3", C-4" |
| 3″ | 1.35 | dd (14.6, 7.3) | 19.1 | C-2", C-4" |
| 4″ | 0.89 | t | 13.1 | C-2", C-3" |

[†] Chemical shift values are in ppm, and J values in parentheses are in Hz. The assignments are based on ${}^{1}\text{H} - {}^{1}\text{H}$ COSY, J-resolved and HMQC spectra.

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131.1 (C-3, C-7/C-2', C-6') and 130.9 (C-2', C-6'/C-3, C-7) in the HMQC identified as CH in the DEPT. In the HMBC spectrum their interaction was observed with quaternary carbons at δ 167.4 (C-1), 132.5 (C-2), 167.6 (C-9) and 135.1 (C-1'). Another five-proton multiplet was present at δ 7.31-7.39 (5H, m) attributable to (H-4, H-6, H-3', H-4', H-5'). These protons were connected with carbons present at δ 128.4 and 129.0 in the HMQC identified as CH in DEPT. The structure of **2** was also supported by mass fragment at *m*/*z* 256.2424 (C₁₅H₁₂O₄) resulting from McLaferty rearrangement (figure 2). Further fragmentation gave base peak at *m*/*z* 149.0256 (C₈H₅O₃)⁺ and another important fragment at *m*/*z* 105.0378 (C₇H₅O)⁺. In light of these observations, the structure of **2** has been elucidated as *p*-benzoyloxy methyl butylbenzoate. The structure was fully supported by HMBC correlations (table 2) and other 2D NMR techniques (HMQC and ¹H–¹H COSY).





Figure 2. Mass fragmentation of 2.

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The molecular formula of compound **3** was established as $C_{15}H_{24}O_5$ by the M⁺ ion in the HREI-MS at m/z 284.3500. The IR spectrum showed characteristic bands caused by ester group at 1734, hydroxyl group at 3394 and acetylenic group at 2145 cm⁻¹. The UV spectrum showed absorption maxima at 227 (log $\varepsilon = 5.0$) and 207 (log $\varepsilon = 4.9$) nm. The NMR spectra (table 3) revealed the presence of one trisubstituted double bond, four oxygen containing carbons, 6-methylenes including a carbinylic methylene, two methines and two methyl carbons. The ¹³C NMR further showed two quaternary carbons attributable to C \equiv C. The structure of 3 was derived from the spectral data as described below. In the ¹H NMR spectrum a pair of double doublets of methylene protons was present at δ 4.20 $(J_{1'a,1'b} = 11.4, J_{1'a,2'} = 5.6 \text{ Hz}, \text{ H-1'a})$ and $4.14 (J_{1'b,1'a} = 11.4, J_{1'b,2'} = 5.6 \text{ H-1'b})$ indicating its link with an ester group which was substantiated by a carbon resonating at δ 65.1 (C-1) and 174.3 (C-1). Both H $- 1'_{a}$ and H $- 1'_{b}$ showed correlations in the HMBC spectrum with δ 174.3 (C-1), 112.9 (C-2), 70.2 (C-2') and 63.1 (C-3'). A one-proton quintet was present at $\delta 3.90 (J = 5.6 \text{ Hz}, \text{H-2'})$, which was connected with a carbon at $\delta 70.2 (\text{C-2'})$ in the HMQC identified as CH in the DEPT spectrum. Another pair of double doublets was present at δ 3.69 ($J_{3'a,3'b} = 11.4$, $J_{3'a,2'} = 5.6$ Hz, H-3a') and 3.57 ($J_{3'b,3'a} = 11.4$, $J_{3'b,2'} = 5.6$ Hz, H-3'b) which was connected with a carbon at δ 63.1 (C-3') in the HMQC identified as CH₂ in the DEPT spectrum. The ¹H-¹H COSY spectrum indicated that the proton at $\delta 3.90$ (H-2') was coupled with protons present at $\delta 4.20$ (H-1a') and 4.14 (H-1b') as well as with protons at δ 3.69 (H-3'a) and 3.57 (H-3'b). A singlet of an olefinic proton was present at δ 5.66 (H-2), which was connected with a carbon at δ 112.9 in the HMQC identified as CH in the DEPT spectrum. H-2 showed connections in the HMBC spectrum with quaternary carbons at δ 174.3 (C-1) and 136.8 (C-3). A singlet of three-protons was present at δ 1.73 (H-1") showing that this methyl was present at an olefinic carbon (C-3). A two-proton triplet was present at $\delta 2.98 (J = 7.4 \text{ Hz}, \text{H}_2\text{-}4)$ connected with a carbon at $\delta 47.2$ (C-4) in the HMQC identified as CH_2 in DEPT. Further, a multiplet was observed at δ 1.90 (H-5) which was connected with a carbon at δ 31.9 in the HMQC identified as CH₂ in the DEPT spectrum. The ${}^{1}H - {}^{1}H COSY$ spectrum indicated that these protons (H-4 and H-5) are coupled with each other. In the HMBC spectrum the methyl protons (H-4) showed

Table 3. ¹H and ¹³C NMR and HMBC spectral data for compound **3** in CDCl₃[†].

| Position | δ_H | (mult., J in Hz) | δ_C | C/H long range correlations |
|----------|------------|------------------|------------|-----------------------------|
| 1 | _ | _ | 174.3 | _ |
| 2 | 5.66 | S | 112.9 | C-1, C-3 |
| 3 | _ | _ | 136.8 | _ |
| 4 | 2.98 | t (7.4) | 47.2 | C-2, C-3, C-5 |
| 5 | 1.90 | m | 31.9 | _ |
| 6 | 4.29 | q (5.0) | 66.8 | C-5, C-4, C-8 |
| 7 | 1.67 | m | 29.4 | C-6, C-8, C-9 |
| 8 | 2.34 | t (7.4) | 45.2 | _ |
| 9 | _ | _ | 86.0 | _ |
| 10 | _ | _ | 78.5 | _ |
| 11 | 1.66 | S | 26.4 | C-9, C-10 |
| 1′a | 4.20 | dd (11.4, 5.6) | 65.1 | C-1, C-2', C-3' |
| 1′b | 4.14 | dd (11.4, 5.6,) | _ | |
| 2' | 3.90 | q (5.6) | 70.2 | C-1′, C-3′ |
| 3′a | 3.69 | dd (11.4, 5.6,) | 63.1 | C-1', C-2' |
| 3′b | 3.57 | dd (11.4, 5.6) | _ | _ |
| 1″ | 1.73 | S | 27.4 | C-2, C-3, C-4 |

[†] Chemical shift values are in ppm, and J values in parentheses are in Hz. The assignments are based on ${}^{1}H-{}^{1}H$ COSY, J-resolved and HMQC spectra.



Figure 3. 1-(2',3'-Dihydroxypropyl)-2-en-3-methyl-6-hydroxy-yn-undecanoate (3).

correlations with C-1, C-2, C-3 and C-5. A one-proton quintet at δ 4.29 (J = 5.0 Hz, H-6) was connected with a carbon at δ 66.8 (C-6) in the HMQC and identified as CH in the DEPT. The ¹H-¹H COSY spectrum indicated that this proton was coupled with methylene protons present at δ 1.90 (H-5) and 1.67 (H-7). A triplet of two-protons was present at δ 2.34 (J = 7.4, H₂-8), which was connected with a carbon at δ 45.2 (C-8) in the HMQC identified as CH₂ in DEPT. This methylene proton showed connectivity with acetylenic carbons C-9 and C-10. Another singlet of three-protons was present at δ 1.66 (H-11) which showed connectivity with a carbon at δ 26.4 in the HMQC spectrum. This methyl showed correlations in the HMBC plot with C-8, C-9, C-10. In light of these observations, the structure of **3** has been elucidated as 1-(2',3'-dihydroxypropyl-2-en-3-methyl-6-hydroxy-9-yn-undecaoate (figure 3). The structure was fully supported by HMBC correlations and other 2D NMR techniques (HMQC and ¹H-¹H COSY).

3. Experimental

3.1 General experimental procedure

For flash column chromatography silica gel 9385 (Merck, 0.040–0.063 mm) was used. TLC was performed on silica gel 60 PF₂₅₄ (Merck) and the spots were visualised under a UV lamp (254 nm) and with iodine spray. UV spectra were recorded in MeOH on a Hitachi-U-3200 spectrophotometer and IR spectra were recorded in CHCl₃ on a JASCO-A-302 spectrophotomer. ¹H NMR, COSY, NOESY and *J*-resolved spectra were recorded on Bruker Avance 300 and 400 spectrometers and ¹³C NMR spectra were run on the same instruments operating at 75 MHz and 100 MHz, respectively. Chemical shifts are in δ (ppm) and coupling constants (*J*) are in Hz. The spectra were referenced to the residual solvent signal. EI-MS spectra were obtained on a Finnigan-Mat-311A mass spectrometer. EI source at 250° and 70 eV; *m/z* (rel. %). HREI-MS spectra were recorded on Jeol JMS-600H. Petrol used was of boiling range 60–80°C.

3.2 Plant material

The aerial parts of *Centella asiatica* were collected from Karachi region during December 2001 and were identified by Professor Dr S.I. Ali, Department of Botany, University of

Karachi. A voucher specimen (GH. No. S.N.68315) has been deposited in the Herbarium of the Department of Botany, University of Karachi, Karachi.

3.3 Extraction and isolation

Aerial parts (4 kg) of C. asiatica were extracted repeatedly (four times) with MeOH at room temperature. The combined extract was concentrated under vacuum to give a thick syrup which constituted crude methanolic extract. The methanolic extract was partitioned between ethyl acetate and water. The ethyl acetate phase was dried (anhyd. Na_2SO_4), treated with charcoal, filtered and treated with 4% Na₂CO₃ to separate neutral and acidic fractions. The neutral fraction (40 g) was subjected to gravity column chromatography (hexane; hexane/EtOAc 9:1 to 7:3; CHCl₃; CHCl₃/MeOH 9.5:0.5 to 8.0:2.0). As a result 200 fractions were obtained and combined on the basis of TLC to ultimately afford 12 fractions (A–L). Fraction A (2.26 g) was again subjected to gravity column chromatography hexane; hexane/EtOAc 9:1 to 7:3; CHCl₃; CHCl₃/MeOH 9.5:0.5 to 8.0:2.0). The fractions obtained were combined on the basis of TLC to ultimately afford 8 fractions (A'-H'). Fraction C' (128 mg) was purified over prep. TLC (hexane/EtOAc; 8:2) to furnish compound 1 (20 mg). Fraction C (1.48 g) of the first column was also subjected to gravity column chromatography (hexane; hexane/EtOAc, 9.5:0.5 to 7.0:3.0; CHCl₃; CHCl₃/MeOH, 9.5:0.5 to 8.0:2.0). As a result 116 fractions were obtained and combined on the basis of TLC to ultimately afford 10 fractions (C-A' to C-J'). Fraction C-A' on purification through prep. TLC (hexane; hexane/EtOAc; 9:1) furnished compound 2 (7.5 mg). Fraction F of the first column was also subjected to gravity column chromatography (hexane; hexane/EtOAc 9:1 to 7:3; CHCl₃; CHCl₃/MeOH 9.5:0.5 to 8.0:2.0). As a result 73 fractions were obtained and combined on the basis of TLC to ultimately afford 7 fractions (F-A' to F-G'). Fraction F-E' on purification through prep. TLC (hexane; hexane/EtOAc; 7:3) furnished compound 3 (17 mg).

3.3.1 Centellin (1). Amorphous powder UV (MeOH) λ_{max} (log ε) nm: 202 (4.9) nm. $[\alpha]_D^{25}$ +11 (CHCL₃; *c* 0.15). IR (KBr) ν_{max} (cm⁻¹): 3241.0 (OH), 2144.9 (C=C) and 1743.9 (C=O) cm⁻¹. EI-MS *m/z*: 250 [M]⁺ and HREI-MS: *m/z* 250.3325 (calcd for C₁₅H₂₂O₃, 250.3334). ¹H NMR and ¹³C NMR: see table 1.

3.3.2 Asiaticin (2). Amorphous powder. UV (MeOH) λ_{max} (log ε) nm: 214.9 (4.4) and 275.1 (4.5) nm. IR (KBr) ν_{max} (cm⁻¹): 1730.0 (C=O), 1631.3 and 1459.6 (C=C, benzene) cm⁻¹. EI-MS m/z (RA %): 312.1 [M]⁺(8.9) and HREI-MS: m/z 312.3645 (10.5) (calcd for C₁₉H₂₀O₄, 312.3654; for C₁₅H₁₂O₄, 256.2424, 3.8; for C₈H₅O₃, 149.0256, 100; for C₇H₅O, 105.0378, 38.9). ¹H NMR and ¹³C-NMR: see table 2.

3.3.3 Cenetellicin (3). Amorphous powder. UV (MeOH) λ_{max} (log ε) nm: 227 (5.0) and 207 (4.9) nm. $[\alpha]_D^{25} - 8$ (CHCl₃; *c* 0.25). IR (KBr) ν_{max} (cm⁻¹): 3394.0 (OH), 2144.9 (C=C) and 1734.4 (C=O) cm⁻¹. EI-MS *m/z*: 284 [M]⁺ and HREI-MS: *m/z* 284.3500 (calcd for C₁₅H₂₄O₅, 284.3520). ¹H NMR and ¹³C NMR: see table 3.

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