Two New Aromatic Constituents from *Stocksia brahuica*

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The methanolic extract of *Stocksia brahuica* yielded two new aromatic constituents: diphenylacetic acid and brahene (2′,4′-dihydroxy-7-methoxy-4,5-methylenedioxyisoflavene). Their structures were characterized with the help of modern spectroscopic techniques including 2D-NMR.

Introduction

*Stocksia brahuica* belongs to the family Sapindaceae having 150 genera [1]. The phytochemical investigation on various plants of the family Sapindaceae shows the presence of flavones [2], diterpenes [3], indoles [4], triterpenes and sapogenins [5], as reflected by the chemical literature survey. Except our recent report [6], no other phytochemical work on the genus *Stocksia* has been found in the literature. This communication describes the isolation and characterization of two new aromatic compounds: diphenylacetic acid (1) and brahene (2′,4′-dihydroxy-7-methoxy-4,5-methylenedioxyisoflavene) (2).

![Structures of 1 and 2](image)

Results and Discussion

Compound 1 was obtained as a white amorphous substance from the ethyl acetate soluble part of the methanolic extract of *S. brahuica* and characterized spectroscopically. The IR spectrum of 1 showed a sharp absorption at 1695 cm⁻¹ due to the carboxylic function. The EI mass spectrum exhibited the molecular ion peak at m/z 212 along with the base peak at m/z 167 due to the loss of carboxyl function. The stability of fragment at m/z 167 (100%) was due to the generation of secondary carbonion which stabilized by two phenyl moieties. The HREI mass spectrum of 1 displayed the formulae of the ions at m/z 212 and 167 as C_{14}H_{12}O_2 (m/z 212.0843 requires 212.0837) and C_{13}H_{11}O_2 (m/z 167.0850 requires 167.0860) which were consistent with nine and eight degrees of unsaturations, respectively.

The ¹H NMR spectrum of 1 displayed only two signals. A multiplet having ten protons integration in the aromatic region at δ 7.22–7.42 showed the presence of two aromatic moieties. The ¹³C NMR (BB) also showed altogether six signals which were resolved through DEPT experiment and gave the information that molecule contains four methines and two quaternary carbons. The signals at δ 127.5, 127.7, 128.6 and 137.9 showed high intensities than the signals at δ 57.1 and 178.5. With the help of molecular formula established through HREI mass spectrum, it was concluded that the signals at δ 127.7 and 128.6 were due to four chemically equivalent carbon atoms (C-3′, C-5′, C-3″, C-5″) and (C-2′, C-6′, C-2″, C-6″), respectively. The signal at δ 127.5 was due to two equivalent methines (C-4′, C-4″). The most upfield and downfield signals in the broad-band spectrum of 1 appeared at δ 57.1 and 178.5 were attributed to C-2 and acidic carbonyl carbon (C-1). The signals at δ 128.6 and 127.7 were distinguished with the help of HMBC experiment (Fig. 1) and assigned to or-
tho and meta positions, respectively. With the help of spectral informations, 1 was assigned as diphenylacetic acid. This compound has not been isolated so far from any natural source however, the phenylacetic acid had been isolated from rose oil [7].

Fig. 1. HMBC (→) and COSY-45° (↔) interactions of 1 and 2.

Compound 2 was isolated from the chloroform soluble part of the methanolic extract and characterized by 2D-NMR and comparative spectral data.

The IR spectrum of 2 displayed a strong absorption at 3500–3400 cm⁻¹ due to the hydroxyl function in the molecule. The molecular weight was confirmed through EI mass spectrum and found as 314 Daltons. The formula was established as C₁₇H₁₄O₁₀ (m/z 314.0789 requires 314.0790) with the help of HREI mass spectrum and consistent with eleven degrees of unsaturation.

The ¹H NMR spectrum showed three methine signals in the aromatic region which appeared at δ 7.34 (H-6'), δ 6.57 (H-5') as a doublet (J = 8.4 Hz) and δ 6.49 (H-3') as a doublet (J = 2.4 Hz). With the help of HMOCQ spectrum, the protons at δ 7.34, 6.57 and 6.49 were correlated to their respective carbons which appeared in the ¹³C NMR spectrum at δ 121.4, 108.0 and 103.7 respectively. These chemical shifts were suitable for ring B provided, oxygenated substitutions at C-2' and C-4' [8]. The assignment of chemical shifts in ring B were further confirmed through HMBC and COSY-45° experiments (Fig. 1).

Two more aromatic methines appeared in the ¹H NMR spectrum at δ 6.94 (H-6) and 7.11 (H-8) as a pair of doublets (J = 1.7 Hz). The magnitude of coupling constants (J = 1.7 Hz) revealed the meta correlation among them. The carbons associated with these signals were located with the help of HMOCQ and found at δ 98.4 (C-6) and 94.5 (C-8), respectively. These carbon chemical shifts were assigned to C-6 and C-8 in ring A with the help of reported data [9] and also confirmed through HMBC experiment (Fig. 1). Apart from aromatic methines, two methylene signals, resolved through DEPT experiment resonated at δ 65.9 and 102.4. The methylenes at δ 65.9 and 102.4 were found to couple through HMBC experiment with the protons which appeared in the ¹H NMR spectrum at δ 5.55 and 6.03, respectively, as singlets of two protons integration each. The signal at δ 5.55 was assigned to H-2 due to its HMBC interactions with the carbons at δ 155.9 (C-9), 148.2 (C-3) and 120.0 (C-1'). A quaternary signal resonated at δ 148.2 (C-3) instead of δ 159.0, the reported value in 7,2'-dihydroxy-8,4'-dimethoxyisoflavene [10]. The shifting (10.8 ppm) towards upfield is due to substitution through oxygen atom at C-4. The signal of other methylene at δ 6.03 showed cross-peaks in the HMBC spectrum with the quaternary carbons resonated at δ 146.8 and 147.5 (C-4, C-5) and revealed the presence of methylenedioxy bridge in the molecule.

The methylenedioxy bridge is normally found either in ring A or B [11], but chemical shifts of ring B and three chemical shifts (C-6, C-8 and C-9) of ring A have been already fully satisfied, therefore, methylenedioxy moiety has only possibility to serve as a bridge between C-4 and C-5.

A singlet of three protons integration in the ¹H NMR spectrum at δ 3.80 and its associated carbon in the ¹³C NMR spectrum at δ 55.7 (through HMQC spectrum) showed the presence of methoxyl group in the molecule. The methoxyl group was found to be attached to the quaternary carbon resonated at δ 162.0 (through HMBC experiment). Due to the confirmation of all quaternary carbons through HMBC experiment (Fig. 1) the chemical shift, δ 162.0 has only possibility to occupy position C-7.

With the help of spectroscopic evidence, 2 was characterized as brahene (2',4'-dihydroxy-7-methoxy-4,5-methylenedioxyisoflavene).

Experimental

The NMR (¹H, ¹³C) were recorded on Bruker AM-300, AM-400, respectively. The IR spectra were recorded on a Shimadzu IR-460. The UV spectra were recorded on a Shimadzu UV-240. The mass spectra were recorded on a Jeol-JMX HX-110 mass spectrometer.
The aerial parts of Stocksia brahuica (13 Kg) were collected from Baluchistan (Pakistan) and identified by Dr. Rasool Bakhsh Tareen, Department of Botany, Baluchistan University, Quetta, Pakistan, where the voucher specimen (No. 535) of the identified material is deposited in the herbarium.

The collected aerial parts of S. brahuica were air dried in the shade for fifteen days and then chopped into small pieces. The dried and chopped material was soaked in methanol (10 L) for a period of ten days. The extraction was performed two times in the same manner and the combined extracts were concentrated at low pressure and temperature into a thick gummy mass (674.7 g). The later was portioned into hexane, chloroform, ethyl acetate and butanol soluble parts. The crude CHCl$_3$ (105.6 g) and EtOAc (155.2 g) extracts were subjected to column chromatography one by one. The fraction eluted with MeOH–CHCl$_3$ (5:95) from the EtOAc crude extract afforded compound 1 in the impure form which was further purified (14.9 mg) by preparative TLC developed in MeOH–CHCl$_3$ (8:92). The fraction eluted with MeOH–CHCl$_3$ (4:96) from the column loaded with CHCl$_3$ crude extract containing compound 2 as an impure sample which was further purified (11.2 mg) by preparative TLC developed in MeOH–CHCl$_3$ (6:94).

**Diphenylacetic acid (1):** m.p. 141–143 °C; UV $\lambda_{max}$ (MeOH) nm 258, 201; IR $\nu_{max}$ (CHCl$_3$) cm$^{-1}$ 3060–2600 (O–H, C–H), 1695 (C=O), 1595 (C=C), 1490, 1445, 1405, 1310, 1220, 1070, 1025, 930, 920; EIMS $m/z$ 212 [M$^+$] and 167 [M–CO$_2$H]$^+$; HREIMS [M]$^+$ $m/z$ 212.0843 for C$_{14}$H$_{12}$O$_2$ requires 212.0837, [M–CO$_2$H]$^+$ $m/z$ 167.0850 for C$_{13}$H$_{11}$ requires 167.0860; $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ 5.13 (1H, $\text{H-2}$) and 7.22–7.42 (10H, m, aromatic); $^{13}$C NMR (CDCl$_3$, 100 MHz) 57.1 (C-2), 127.5 (C-4, C-4”), 127.7 (C-3’, C-3”, C-5’, C-5”), 128.6 (C-2’, C-2”, C-6’, C-6”), 137.9 (C-1’, C-1”) and 178.5 (C-1).

**Brahene (2):** m.p. 181–183 °C; IR $\nu_{max}$ (CHCl$_3$) cm$^{-1}$ 3500–3100 (O–H), 1615 1460 (C=C), 1145; EIMS $m/z$ 314; HREIMS $m/z$ [M]$^+$ 314.0789 for C$_{17}$H$_{14}$O$_6$ requires 314.0790; $^1$H NMR (CD$_3$D$_6$, 400 MHz) $\delta$ 5.55 (2H, $\text{H-2}$), 6.94 (1H, d, $J=1.7$ Hz, H-6), 7.11 (1H, d, $J=1.7$ Hz, H-8), 6.49 (1H, d, $J=2.4$ Hz, H-3”), 6.57 (1H, dd, $J=8.4$, 2.4 Hz, H-5”), 7.34 (1H, d, $J=8.4$ Hz, H-6”), 6.03 (2H, s, O–CH$_2$–O), 3.80 (3H, s, OMe); $^{13}$C NMR (CD$_3$D$_6$, 75 MHz) $\delta$ 65.9 (C-2), 148.2 (C-3), 146.8, 147.5 (C-4, C-5), 98.4 (C-6), 162.0 (C-7), 94.5 (C-8), 155.9 (C-9), 103.7 (C-10), 120.0 (C-1’), 150.5 (C-2’), 103.7 (C-3’), 155.0 (C-4’), 108.0 (C-5’), 121.4 (C-6’), 55.7 (OMe), 102.4 (O–CH$_2$–O).