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# BUDDLEJONE, A DITERPENE FROM *BUDDLEJA ALBIFLORA*

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**Key Word Index**—*Buddleja albiflora*; Loganiaceae; Buddlejaceae; diterpenoids; abietane derivatives; buddlejone.

**Abstract**—A new methylene quinone diterpene, buddlejone, was isolated from the roots of *Buddleja albiflora* and the roots of *B. globosa* Lam. The structure of the compound was established by spectroscopic means as 7-hydroxy-7,9,12-abietrien-14-one. This is the first reported diterpene to be isolated from the Buddlejaceae.

## INTRODUCTION

The roots of *Buddleja* species have been shown to produce sesquiterpenes with a caryophyllene ring system [1, 2]. As part of our programme of research into the constituents of this genus, chloroform extracts of the roots of *B. globosa* Lam. and *B. albiflora* Hemsl. were examined by TLC and found to contain compounds giving a strong reaction after spraying with acidic anisaldehyde and heating at 105°, a reagent commonly used for the detection of terpenes [3]. One of the zones observed had a yellow colour in daylight and this intensified to a bright orange after treatment with the reagent. This compound was isolated and named buddlejone after its structure was determined using spectroscopic data.

## RESULTS AND DISCUSSION

The accurate mass measurement of buddlejone gave a molecular formular $C_{20}H_{28}O_2$ which indicated that it could be a diterpene. The $^1H$ NMR and two-dimensional COSY spectra showed the presence of a single uncoupled methyl group at $\delta$ 1.18, two geminal methyl groups at $\delta$ 0.93 and 1.01 and also two methyl signals at $\delta$ 1.25 and 1.26 coupled with a methine at $\delta$ 3.42, characteristic of an isopropyl side-chain. These factors indicated that the compound could have an abietane skeleton but the colour of the compound and its UV spectrum showed that it possessed an extended conjugated $\pi$ electron system. Such compounds have been isolated from several plant families, especially the genera *Salvia* (Labiatae) and *Taxodium* (Taxodiaceae) and other sources [4–8]. The presence of a hydrogen-bonded phenolic or enolic hydroxyl signal at $\delta$ 17.75 in the $^1H$ NMR spectrum and signals over $\delta$ 160 in the $^{13}C$ NMR spectrum suggested that the $\pi$ electron system included at least one carbonyl group and one phenol which were H-bonded to one another. The strong hydrogen bonding observed precluded the possibility of buddlejone having the tropolone structure **1** (see Fig. 3), which would be a novel type of diterpene skeleton, since the hydroxyl H in the similar compounds salvidone and miltiplone show no signal lower than $\delta$ 10 in their $^1H$ NMR spectra [9].

The $^{13}C$ NMR and $^1H$ NMR signals for ring A of buddlejone conform very closely to those of the known abietane compounds, such as taxodone (**2**) [5], which possess an enol H-bonded to a carbonyl group. The chair configuration of this ring in buddlejone was deduced from the proximity of the $CH_3$-18 and $CH_3$-20 groups, demonstrated by a strong interrelationship seen in the NOESY spectrum. However, many of the $^1H$ and $^{13}C$ signals for rings B and C in buddlejone did not conform to those reported for taxodone (**2**) [5] and related compounds so a different pattern of oxygenation must exist.

The substitution pattern of rings B and C was established with the aid of two-dimensional COSY, NOESY and HETCOR $^1H$-range coupling spectra (Figs 1 and 2).

The $CH_2$-6 group giving a signal at $\delta$ 2.65 showed coupling with the H-5 signal at $\delta$ 1.86 and careful examination of the NOESY spectrum indicated the proximity of one component of the $CH_2$-6 signal to $CH_3$-10 while the other component was close to H-5, thus indicating a *trans* relationship between the $CH_3$-10 and H-5. The NOESY spectrum also showed that this methylene group was spatially close to H-bonded enolic H at $\delta$ 17.75 and the latter was therefore assumed to be attached to C-7. The chemical shifts for both $^{13}C$ and

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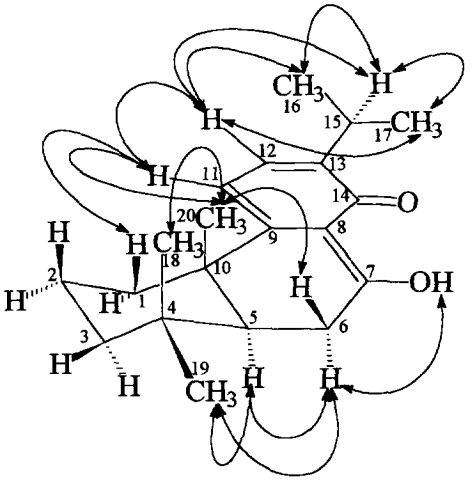

Fig. 1. Significant NOE relationships observed in buddlejone (**3**).

$^1$H for $CH_2$-6 are similar to those observed for sugiol ($\delta_C$ 36.37 and $\delta_H$ 2.55, respectively) [6], cyrtophyllone B ($\delta_C$ 34.9 and $\delta_H$ 2.48 and 2.56) [7] and margocin ($\delta_C$ 36.0 and $\delta_H$ 2.66 and 2.72) [8], which all have a 7-keto group.

If an enol 7-OH exists in a highly conjugated abietane skeleton, H-bonding can only occur with a carbonyl group at C-14. Dreiding models demonstrated that if this were the case, the carbonyl and hydroxyl groups are spatially close and this would account for the very low shift for the hydroxyl, a feature often seen in flavonoids and chromones where a similar relationship occurs [10]. The two signals at $\delta$ 193.15 and $\delta$ 189.09 in the $^{13}$C NMR spectrum in $CDCl_3$ would indicate the presence of two carbonyl groups but the accurate mass measurement confirms that only two oxygen atoms are present in the molecule and one of these must be the hydroxyl as this is clear in the $^1$H spectrum. Similar signals were observed when benzene and DMSO were used as solvents. The presence of two carbonyl signals in the $^{13}$C NMR spectrum is therefore considered to be due to rapid intramolecular keto–enol transformation between the two forms **3** and **4** and the signals correspond to the relevant keto rather than enol states at C-7 and C-14 because these have a lower relaxation time and are more easily detected. Attempts to clarify the situation by the formation of acyl or methyl derivatives failed and this lends further weight to the presence of a strongly hydrogen-bonded hydroxyl group. The attachment of the isopropyl group at C-13 conforms with the pattern observed in the majority of compounds belonging to the abietane series and is confirmed by the long-range coupling observed between the $C{=}O_{14}$ at $\delta_C$ 193.2 and the $CH_3$ signals at $\delta_H$ 1.25 and 1.26.

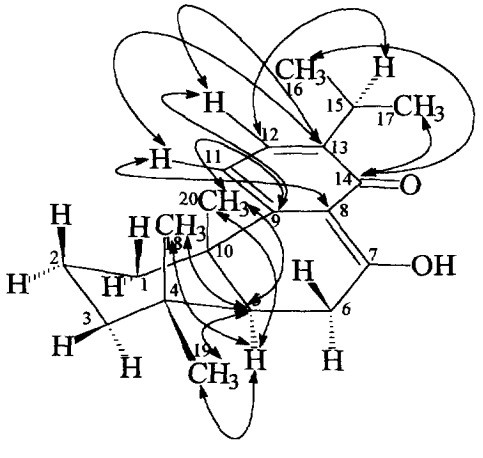

Fig. 2. Significant HMBC couplings observed in compound **3**.

The two-dimensional COSY spectrum showed that the two double bond methine signals at $\delta$ 7.31 and 6.21 are coupled only with each other and also demonstrate close proximity in the NOESY spectrum. They were considered to be in an *ortho* relationship even though the coupling constant of 4.1 Hz is lower than the value of about 8 Hz that is usually observed for two ortho aromatic protons; however, it should be noted that ring C is not strictly a true aromatic system. The signal at $\delta$ 7.31 in the $^1$H NMR spectrum was ascribed to H-12 because it showed long-range coupling with the C-15 C signal at $\delta$ 33.9 whereas the signal at $\delta$ 6.21 did not. The $\delta$ 7.31 signal also gave a NOESY correlation with H-15 at $\delta$ 3.43 and the H-1 axial at $\delta$ 2.14 but no similar effect was noted for the signal at $\delta$ 6.21. The allocation of shifts in the $^{13}$C NMR spectrum for the quaternary double bond atoms C-8, C-9 and C-13 was also based on long-range coupling characteristics since the $\delta$ 119.2 signal ascribed to C-13 showed fairly strong coupling with both the =CH signals whereas the signal at $\delta$ 120.9 showed a strong coupling only with H-11 at $\delta$ 6.21 and not H-12 at $\delta$ 7.31 which is four bonds away. The signal at $\delta$ 167.6 was ascribed to C-9 on the basis of the long-range coupling observed with $CH_3$-20 at $\delta$ 1.18 and H-12 at $\delta$ 7.31. The shift seems very downfield but similar values have been reported for sugiol ($\delta$ 158) [7] and related molecules where an extended oxygenated $\pi$ electron system exists [6, 8]).

Buddlejone is therefore considered to have structure **3**. This is the first report of a diterpene from the Buddlejaceae.

## EXPERIMENTAL

*Plant material.* Roots of *B. albiflora* (3.5 kg) were collected from Qin Ling mountains in western China. The identification and authentication of the plant material was carried out by Li Shangxiao and a voucher specimen (no. LS115) is deposited in the Herbarium of The Institute of Materia Medica, Shardong Academy of Medical Sciences. *Buddleja globosa* root bark was collected from a specimen growing in a London garden. The material was authenticated by Dr Peter Houghton and a voucher specimen (Bg003) is deposited in the

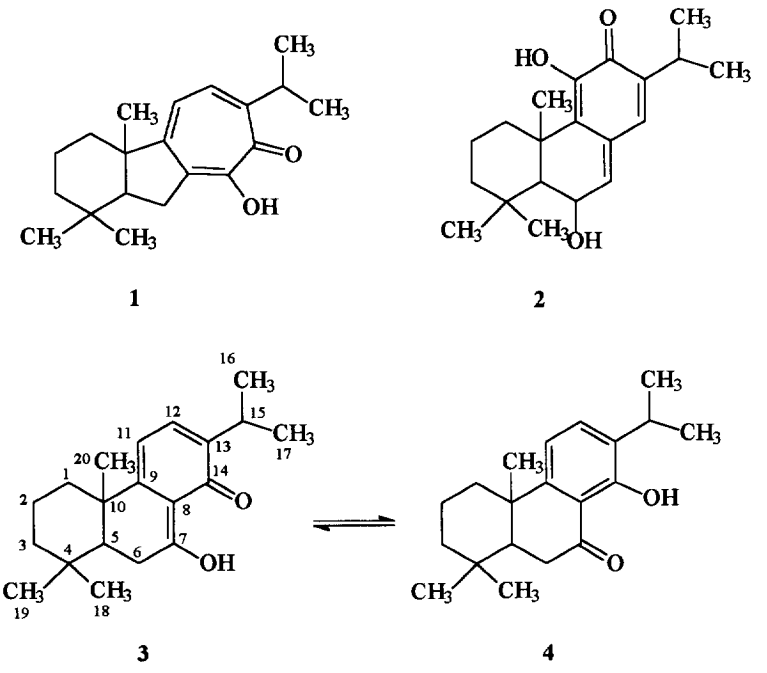


Fig. 3. Structures of possible tropolone (**1**), taxodone (**2**) and keto-enol tautomers of buddlejone (**3** and **4**).

Herbarium of the Department of Pharmacy, King's College London.

*TLC.* A 5 g sample of the dried plant material was extracted with $CH_2Cl_2$ (50 ml) for 15 min at 100° under reflux. The mixt. was filtered and the filtrate concd under red. press. and made up to 10 ml with $CH_2Cl_2$. This extract was applied to a 20 × 20 cm silica gel $GF_{254}$ (Merck) TLC plate. The mobile phases used were (a) Toluene–EtOAc (9:1) (b) Hexane–$CHCl_3$ (1:1) (c) Hexane–EtOAc (9:1). Detection: UV light 254 nm; anisaldehyde 0.5% w/v in AcOH–$H_2SO_4$–MeOH (10:5:85) followed by heating at 105° for 10 min.

*NMR.* $^1$H NMR, $^{13}$C NMR, $^1$H–$^{13}$C short- and long-range correlation HETCOR and HMBC spectra were obtained at 400/100 MHz on an AMX 400 NMR spectrometer using $CDCl_3$, benzene-$d_6$ and DMSO-$d_6$ as solvents with TMS as int. standard. Standard programs from the library; $^nJ_{C,H} = 7$ Hz was used for the long-range experiments.

*Isolation of buddlejone.* Fresh roots of *B. albiflora* (1.0 kg) were dried in the shade and coarsely powdered. Extraction was carried out with $CHCl_3$. (1.5 l) at room temp. for 24 hr. The extraction process was repeated twice and the combined extracts were concd under red. press. to a viscous residue (12.2 g) which was subjected to VLC (40 g silica gel, petrol 40–60° with increasing amounts of $CHCl_3$). Residues from yellow fractions obtained with 10% $CHCl_3$ in petrol 40–60° (115 mg) were subjected to prep. silica gel TLC using *n*-hexane–$CHCl_3$ (4:1). The yellow band was eluted to yield buddlejone (**3**) (65 mg).

*Buddlejone* (**3**). Orange oil, $[\alpha]_D^{Nujol}$ −28.8°, h$R_f$ values (a) 80 (b) 72 (c) 78; UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 389 (3.96), 331 (4.04), 274 (sh) 245 (4.11); (0.1 M NaOH) 371 (4.06), 335 (3.98); 250 (4.09); IR (Nujol) $\nu_{max}$ cm$^{-1}$: 3425, 2924, 1731, 1631, 1559, 1430, 1374; EI-MS (probe) 70 eV *m/z* (rel. int.): 300 (97) $[M]^+$ ($C_{20}H_{28}O_2$ measd 300.2053, calcd 300.2089.), 285 (100), 257 (82), 215 (17), 177 (24), 149 (30), 129 (23), 113 (20); $^1$H NMR ($CDCl_3$): $\delta$ 17.82 (1H, *s*, C-7, OH), 7.31 (1H, *d* $J = 4.1$ Hz, H-12), 6.20 (1H, *d* $J = 4.1$ Hz, H-11), 3.43 (1H, *septet* $J = 6.8$ Hz, H-15), 2.66 (1H, *d* $J = 2.8$ Hz, $H_e$-6), 2.65 (1H, *s*, $H_a$-6), 2.14 (1H, *m*, $H_a$-1), 1.86 (1H, *m*, H-5), 1.77 (1H, *m*, $H_a$-2), 1.61 (1H, *m*, $H_e$-2), 1.52 (1H, *m*, $H_e$-1), 1.50 (1H, *m*, $H_a$-3), 1.26 (1H, *m*, $H_e$-3), 1.26 (3H, *d* $J = 4.1$ Hz, $CH_3$-17), 1.25 (3H, *d* $J = 4.1$ Hz, $CH_3$-16), 1.18 (3H, *s*, $CH_3$-20), 1.01 (3H, *s*, $CH_3$-18), 0.95 (3H, *s*, $CH_3$-19); $^{13}$C NMR: Table 1. Fresh roots of *B. globosa* (250 g) were treated in the same way to yield a compound (33 mg) that was identical in all respects to that isolated from *B. albiflora.*

*Reaction of buddlejone with acetyl chloride.* Acetyl chloride (20 drops) was added to a stirred soln of buddlejone (4 mg) in $CHCl_3$ (25 ml), and the reaction mixture was allowed to reflux with magnetic stirring for 5 hr. TLC was carried out at regular intervals on samples of the reaction mixture against reference buddlejone. TLC examination (silica gel, chloroform–petrol (40–60°), 1:1) showed that no appreciable reaction had occurred and, after cooling of the reaction mixture, the solvent and acetyl chloride were evaporated *in vacuo* to leave unreacted buddlejone.

*Reaction of buddlejone with MeI and $Ag_2O$.* To a hot, stirred soln of buddlejone (4 mg) in MeI (5 ml by micropipette) was added powdered $Ag_2O$ (0.25 g). The reaction mixture was allowed to reflux with magnetic

Table 1. $^{13}C$ NMR signals for buddlejone in different solvents

| C | $CDCl_3$ | Benzene-$d_6$ | DMSO-$d_6$ |
|---|---|---|---|
| 1 | 37.3 | 37.6 | 36.7 |
| 2 | 19.1 | 19.4 | 18.6 |
| 3 | 42.0 | 42.1 | 41.4 |
| 4 | 33.6 | 33.4 | 36.7 |
| 5 | 53.1 | 52.9 | 52.5 |
| 6 | 32.4 | 32.4 | 31.8 |
| 7 | 189.1 | 188.9 | 188.8 |
| 8 | 119.2 | 119.8 | 120.2 |
| 9 | 167.6 | 167.3 | 167.8 |
| 10 | 36.9 | 34.3 | 33.1 |
| 11 | 116.2 | 116.1 | 116.7 |
| 12 | 135.9 | 136.1 | 137.0 |
| 13 | 120.9 | 121.8 | 120.2 |
| 14 | 195.2 | 193.7 | 192.3 |
| 15 | 33.9 | 33.1 | 32.9 |
| 16 | 21.1 | 21.1 | 20.9 |
| 17 | 20.8 | 20.9 | 20.7 |
| 18 | 33.3 | 34.3 | 32.9 |
| 19 | 21.9 | 21.8 | 21.6 |
| 20 | 22.1 | 22.3 | 21.8 |

stirring for 5 hr. TLC (as above) was carried out at 10 min, 30 min, 1 hr, 3 hr and 5 hr from the start of reflux. No reaction was observed other than the gradual decomposition of **3** to give baseline detritus.

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