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Antifungal constituents of the stem bark of *Bridelia retusa*

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Abstract

Antifungal activity guided fractionation of solvent extracts of the stem bark of *Bridelia retusa* of the family Euphorbiaceae against *Cladosporium cladosporioides*, furnished new bisabolane sesquiterpenes, (*E*)-4-(1,5-dimethyl-3-oxo-1-hexenyl)benzoic acid, (*E*)-4-(1,5-dimethyl-3-oxo-1,4-hexadienyl) benzoic acid, (*R*)-4-(1,5-dimethyl-3-oxo-4-hexenyl)benzoic acid and (−)-isochaminic acid, together with the known (*R*)-4-(1,5-dimethyl-3-oxohexyl)benzoic acid (ar-todomatuic acid), 5-allyl-1,2,3-trimethoxybenzene (elemicin), (+)-sesamin and 4-isopropylbenzoic acid (cumic acid). All these compounds showed fungicidal activity on TLC bioautography method at very low concentrations except elemicin.
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Keywords: *Bridelia retusa*; Euphorbiaceae; Antifungal activity; *Cladosporium cladosporioides*; Bisabolane sesquiterpene; Isochaminic acid

1. Introduction

In a continuation of our work on search for bioactive compounds from Sri Lankan plants, present investigation was carried out on *Bridelia retusa* (L.) Spreng. of the family Euphorbiaceae. *B. retusa* is a tree of moderate size growing in Sri Lanka. Roots and stem bark of this plant used in the indigenous system of medicine for the treatment of rheumatism and as an astringent (Jayaweera, 1982). No previous phytochemical work has been reported on this plant. In this paper we report the isolation of eight compounds from the stem bark of *B. retusa* and structure elucidation of these compounds by spectral methods. Out of the eight compounds, seven compounds showed antifungal activity against *Cladosporium cladosporioides*.

2. Results and discussion

Cold *n*-hexane, dichloromethane, ethyl acetate, 15% methanol in ethyl acetate and methanol extracts of stem bark of *B. retusa* were screened for antifungal activity against *C. cladosporioides* by TLC bioautography method wherein spore germinates as black zones and antifungal compounds appear as white zones (Homans and Fuchs, 1970). Except the *n*-hexane and methanol extracts, other three extracts showed an antifungal active broad band on the TLC in the range of R_f 0.3–0.5, and some of the antifungal constituent appeared to be present in all the three extracts. All the antifungal active extracts (dichloromethane, ethyl acetate and 15% methanol in ethyl acetate) were combined and chromatographed over silica gel followed by activity guided fractionation. All the antifungal active fractions were combined and attempted to separate by silica gel gravity column, preparative thin layer chromatography and Sephadex LH-20. However, the mixture could not be separated effectively into constituents by these methods due to their close polarity and properties of tailing nature. Finally the isolation of pure compounds **1**–**8** (Fig. 1) was achieved by reversed phase HPLC.

Compound **1** was assigned the molecular formula $C_{15}H_{18}O_3$ (EI-MS *m*/*z* 246 $[M]^+$). The ^1H NMR spectrum showed signals of an olefinic methyl [δ 2.55 (*d*, $J = 1.2$ Hz)], an isopropyl methyl doublet [(δ 0.97 (6H)],

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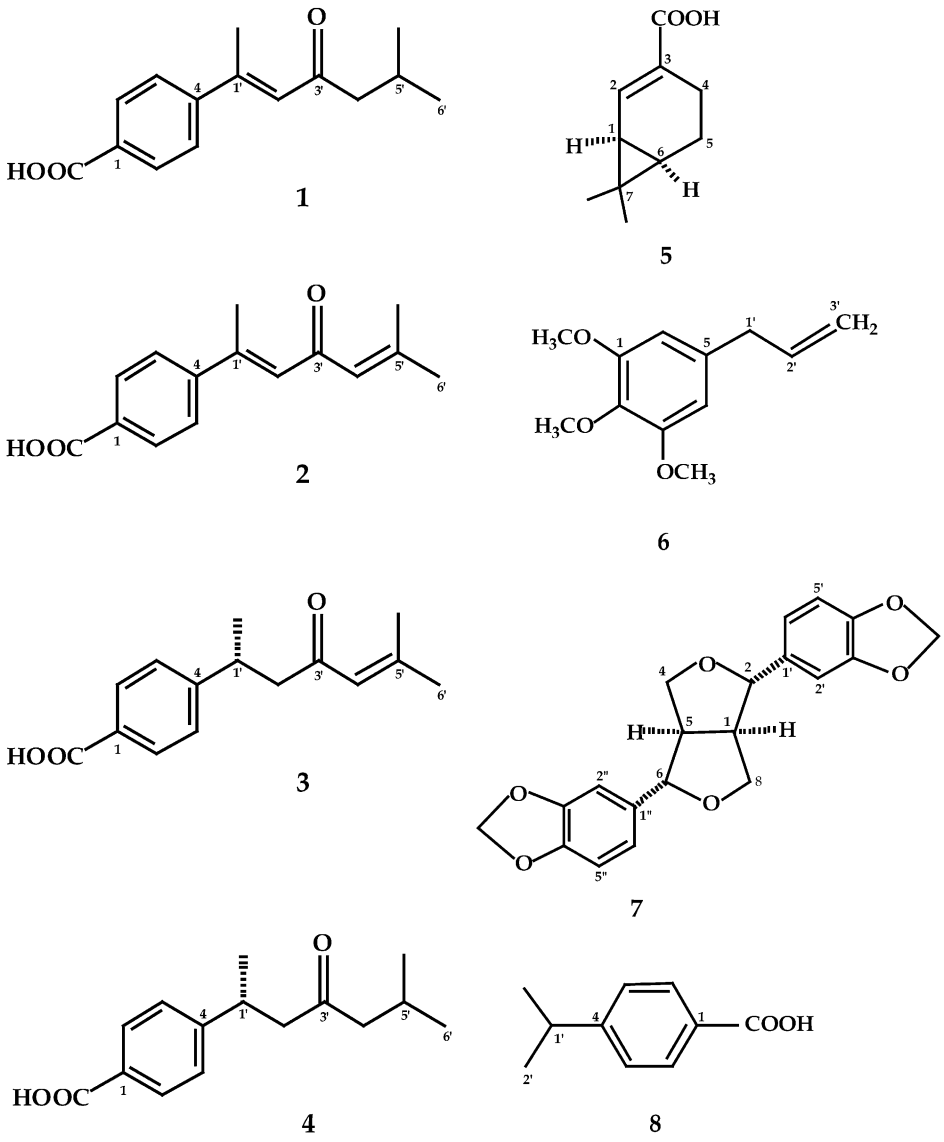

Fig. 1. Compounds **1**–**8**.

an isopropyl methine (δ 2.21), a set of methylene protons [δ 2.44 (2H)] and an olefinic proton [δ 6.52 (*d*, $J=1.2$ Hz)] in addition to two *ortho*-coupled hydrogens [δ 8.11 (2H, *d*, $J=8.6$ Hz) and 7.56 (2H, *d*, $J=8.6$ Hz)] characteristic for a *para*-substituted benzene. The ^{13}C NMR spectrum showed signals of three methyls [(δ 18.3, 22.7×2)], a methine (δ 25.2), a methylene (δ 54.0), two olefinic (δ 126.0, 152.0), a carboxyl (δ 170.4) and a carbonyl (δ 201.4), in addition to signals of *para*-substituted benzene (δ 129.6, 126.6×2, 130.4×2, 147.9). The isopropyl and the methylene groups were joined to form an isobutyl group because of the presence of spin–spin couplings between these protons as evidenced by decoupling experiments. The olefinic methyl group was correlated to the olefinic proton to form another partial structure, –CH=CMe–, due to the presence of a small allylic coupling (1.2 Hz) which was confirmed by decoupling experiments. The HMBC experiments showed long-range correlations as depicted in Fig. 2, which permitted us to assemble the partial structures including the carbonyl and carboxyl groups. The (*E*)-geometry of the double bond was deduced from observation of a NOE between the olefinic proton and one (δ 7.56) of the benzene ring protons. No NOE was

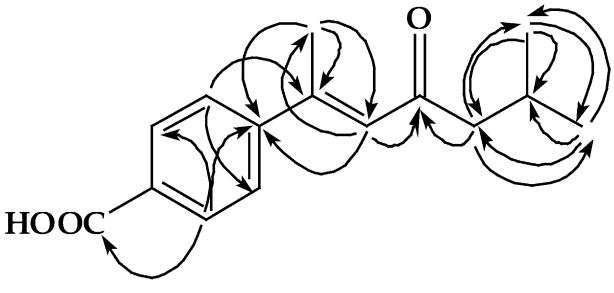

Fig. 2. HMBC correlations from H to C for compound **1**.

Table 1
Fungitoxic activity of the compound **1**–**8**

Compound no.	Minimum amount for activity (μg)
1	50
2	25
3	5
4	25
5	10
6	No activity
7	25
8	10

The table shows the minimum amount of compounds needed to inhibit the growth of *Cladosporium cladosporioides* on TLC plate.

observed between the olefinic methyl and olefinic proton. Hence, the structure of compound **1** was elucidated as a new bisabolane sesquiterpene, (*E*)-4-(1,5-dimethyl-3-oxo-1-hexenyl)benzoic acid.

Compound **2** was assigned the molecular formula $C_{15}H_{16}O_3$, (EI-MS *m*/*z* 244 $[M]^+$, two mass units less than **1**). The ^1H NMR signals of **2** were similar to those of **1** except for the presence of two olefinic methyls (δ 2.23 and 1.93) and an olefinic proton (δ 6.21) and the absence of the signals due to the isobutyl group of **1**. These data clearly indicate that compound **2** has a $-CH{=}CMe_2$ group instead of isobutyl group of **1**. Hence, the structure of compound **2** was established as (*E*)-4-(1,5-dimethyl-3-oxo-1,4-hexadienyl) benzoic acid. This is the first report of the compound **2** as a natural product. It has been previously synthesized (Mane and Rao, 1973).

Compound **3** was assigned the molecular formula $C_{15}H_{18}O_3$ (EI-MS *m*/*z* 246 $[M]^+$). The ^1H NMR spectrum of **3** was similar to that of **2** except for the presence of a methyl doublet (δ 1.29), a set of methylene protons (δ 2.72 *dd*) and a multiplet proton (δ 3.42) and the absence of the olefinic methyl and the olefinic proton of **2**. Decoupling experiments allowed connecting these protons to form a partial structure, $-CH_2CHMe-$. It is therefore clear that the –CH=CMe– moiety in **2** is replaced with $-CH_2CHMe-$ in **3**. The ^{13}C NMR signals supported this alternation. Hence, compound **3** was established as 4-(1,5-dimethyl-3-oxo-4-hexenyl)benzoic acid. This is the first report of the compound **3** as a natural product. It has been previously synthesized (Mane and Rao, 1974).

Compound **4** was assigned the molecular formula $C_{15}H_{20}O_3$ (EI-MS *m*/*z* 248 M^+). On the basis of spectral data, compound **4** was elucidated as 4-(1,5-dimethyl-3-oxohexyl)benzoic acid. This bisabolane sesquiterpene acid, ar-todomatuic acid, was previously isolated from the volatile wood oil of *Pseudotsuga menziesii* (Sakai and Hirose, 1973) and was synthesized (Mane and Rao, 1973). (*R*)-Configuration was assigned for compound **4** on the basis of the $[\alpha]_D$ data of the methyl ester prepared from **4**, which were essentially identical to those of the methyl ester of (*R*)-ar-todomatuic acid (Sakai and Hirose, 1973). The configuration of **3** was not determined and the same (*R*)-configuration as for **4** was assigned for compound **3** tentatively. Antifungal activity of todomatuic acid, a non-aromatic analog of **4** was previously reported (Aoyama et al., 1991).

Compound **5** was assigned the molecular formula $C_{10}H_{14}O_2$ (EI-MS *m*/*z* 166 $[M^+]$). The ^{13}C NMR and DEPT spectra of **5** indicated the presence of two methyl carbons (δ 15.9, 29.3), two methylene carbons (δ 17.0, 21.3), two methine carbons (δ 24.4, 26.3) and a quaternary carbon (δ 29.8) in aliphatic region, in addition to down-field carbons at δ 126.1 and 142.7 due to a trisubstituted olefin and at δ 171.7 presumably due to carboxyl group. These data and HMQC data suggested **5** could be a cyclopropane-containing monoterpene acid possessing two carbocycles. Literature survey showed isochaminic acid, an isomer of chaminic acid, satisfied this structure requirement. The ^1H and ^{13}C NMR data reported for (−)-isochaminic acid was in excellent agreement with those of compound **5**. Thus, compound **5** was identified with (−)-isochaminic acid. (−)-Isochaminic acid was previously described as a microbial oxidation product of (+)-carene (Stumpf et al., 1990). This paper is the first report on the isolation of **5** from a plant.

The EI-MS of compound **6** showed M^+ at *m*/*z* 208, consistent with the molecular formula $C_{12}H_{16}O_3$. The ^1H and ^{13}C NMR data established the structure of **6** as 5-allyl-1,2,3-trimethoxybenzene (elemicin). Identification of **6** was further confirmed by the direct comparison of the reported spectral data of elemicin from *Anthriscus sylvestris* of the family Umbelliferae (Ikeda et al., 1998).

The EI-MS of compound **7**, showed M^+ at *m*/*z* 354 consistent with the molecular formula $C_{20}H_{18}O_6$. Compound **7** was identified as (+)-sesamin by comparing the NMR (Rana et al., 2000) and $[\alpha]_D$ (Atal et al., 1967) data with those published.

The EI-MS of compound **8** showed M^+ at *m*/*z* 164, consistent with the molecular formula $C_{10}H_{12}O_2$. On the basis of the NMR data, compound **7** was identified as 4-isopropylbenzoic acid (cumic acid), often found in essential oils of various plants (Ding et al., 1994).

All these compounds **1**–**8** were subjected to the antifungal bioassay against *C. cladosporioides* by TLC bioautography method (Homans and Fuchs, 1970). The observed minimum inhibition concentrations are summarized in Table 1. Compound **3** showed the most potent antifungal activity.

3. Experimental

3.1. General

Mps were determined by Gallenkamp apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were

recorded on a Jeol LA 400 (400/100 MHz for $^{1}H/^{13}C$ NMR) spectrometer in $CDCl_3$ solution with tetramethylsilane as an internal reference. EI-MS (70 eV) were obtained on a Jeol JMS-AX505HA spectrometer. HPLC analyses were carried out on Shimadzu LC-6A apparatus equipped with UV detector under a reversed phase C_{18} column and isocratic solvent condition.

3.2. Plant material

Stem bark of the *B. retusa* (L.) Spreng. was collected from the University premises, Peradeniya, Central Province of Sri Lanka in May 2001 and identified by comparison with herbarium sample available at Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen (IFS/2001/BR1) is deposited at the Institute of Fundamental Studies.

3.3. Extraction, isolation and antifungal bioassay

Dried, grounded stem bark of *B. retusa* (2.0 kg) was sequentially extracted with cold *n*-hexane, dichloromethane, ethyl acetate, 15% methanol in ethyl acetate and methanol. Each extract was evaporated to dryness to give *n*-hexane extract (5 g), dichloromethane extract (4.8 g), ethyl acetate extract (2.3 g), 15% methanol in ethyl acetate extract (72 g) and methanol extract (102 g). Each extract was subjected to antifungal activity test on TLC bioautography method against *C. cladosporioides* (Homans and Fuchs, 1970). Inhibition zones appeared in the range of R_f 0.31–0.5 for dichloromethane and ethyl acetate extracts and 0.22–0.44 for 15% methanol in ethyl acetate extract (TLC aluminium sheets Merck 1.05554 silica gel 60 F_{254}; eluant: 5% MeOH in $CHCl_3$). The antifungal active three extracts (CH_2Cl_2, EtOAc and 15% MeOH in EtOAc) were combined and a portion (70 g) was chromatographed over a column of silica gel (Merck Art. 7734) eluting with *n*-hexane, dichloromethane and methanol to give seven fractions. The TLC bioautography studies revealed that all the antifungal compounds were eluted with 5% methanol in dichloromethane (900 mg). Further purification of this fraction by HPLC (STR Prep-ODS 20×250 mm column, 20% H_2O–MeOH, 5ml/min; UV detection 254 nm) furnished compounds **1** (39 mg), **2** (13 mg), **3** (20 mg), **4** (14 mg), **5** (5 mg), **6** (5 mg), **7** (20 mg) and **8** (6 mg).

3.3.1. (*E*)-4-(1,5-Dimethyl-3-oxo-1-hexenyl)benzoic acid (**1**)

Mp 122 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.97 (6H, *d*, $J=6.6$ Hz, Me-5′ and H_3-6′), 2.21 (1H, *m*, H-5′), 2.44 (2H, *d*, $J=7.1$ Hz, H-4′), 2.55 (3H, *d*, $J=1.2$ Hz, Me-1′), 6.52 (1H, *d*, $J=1.2$ Hz, H-2′), 7.56 (2H, *d*, $J=8.6$ Hz, H-3 and H-5), 8.11 (2H, *d*, $J=8.6$ Hz, H-2, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.3 (Me-1′), 22.7 (C-6′), 22.7 (Me-5′), 25.2 (C-5′), 54.0 (C-4′), 126.0 (C-2′), 126.6 (C-3 and 5), 129.6 (C-1), 130.4 (C-2 and C-6), 147.9 (C-4), 152.0 (C-1′), 170.4 (COOH), 201.4 (C-3′); EI-MS *m*/*z* (%): 246 $[M]^+$(25), 231 [M^+-15] (10), 203 (15), 189 (100), 171 (12), 162 (8), 159 (12), 149 (12), 145 (12), 131 (5), 117 (25), 115 (30), 105 (5), 91 (10), 83 (15), 77 (5), 57 (10).

3.3.2. (*E*)-4-(1,5-Dimethyl-3-oxo-1,4-hexadienyl)benzoic acid (**2**)

Mp. 156 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.93 (3H, *s*, Me-5′) 2.23 (3H, *s*, H_3-6′), 2.57 (3H, *s*, Me-1′), 6.21 (1H, *s*, H-4′), 6.52 (1H, *s*, H-2′), 7.57 (2H, *d*, $J=8.3$ Hz, H-3, H-5), 8.10 (2H, *d*, $J=8.3$ Hz, H-2, H-6); EI-MS *m*/*z* (%): 244 $[M]^+$ (30), 229 (100), 211 (15), 199 (10), 189 (10), 185 (12), 171 (8), 167 (12), 157 (8), 150 (15), 141 (7), 132 (7), 129 (12), 123 (12), 115 (22), 105 (8), 96 (12), 91 (8), 83 (30), 77 (5), 73 (5), 69 (7), 60 (5), 55 (18).

3.3.3. (*R*)-4-(1,5-Dimethyl-3-oxo-4-hexenyl)benzoic acid (**3**)

Mp. 83–85 °C; $[\alpha]_D^{25}$ −68.1 ($CHCl_3$; *c* 0.34); ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.29 (3H, *d*, $J=7.1$ Hz, Me-1′), 1.86 (3H, *d*, $J=1.0$ Hz, Me-5′), 2.10 (3H, *d*, $J=1.0$ Hz, H_3-6′), 2.67 (1H, *dd*, $J=16.4$, 7.8 Hz, H_a-2′), 2.72 (1H, *dd*, $J=16.4$, 7.9 Hz, H_b-2′), 3.42 (1H, *q*, $J=7.2$ Hz, H-1′), 6.02 (1H, *t*, $J=1.2$ Hz, H-4′), 7.32 (1H, *d*, $J=8.3$ Hz, H-3, H-5), 8.02 (1H, *d*, $J=8.3$ Hz, H-2, H-6); ^{13}C NMR($CDCl_3$, 400 MHz): δ 20.8 (C-6′), 21.7 (Me-1′), 27.7 (Me-5′), 35.7 (C-1′), 52.0 (C-2′), 123.9 (C-4′), 127.0 (C-3 and C-5), 127.3 (C-1), 130.4 (C-2 and C-6), 153.0 (C-4), 155.8 (C-5′), 170.7 (COOH), 199.0 (C-3′); EI-MS *m*/*z* (%): 246 $[M]^+$ (30), 231 (15), 189 (7), 162 (8), 146 (7), 131 (7), 115 (50), 105 (10), 98 (10), 91 (5), 83 (100), 77 (10), 69 (12), 58 (25), 55 (30).

3.3.4. (*R*)-4-(1,5-Dimethyl-3-oxohexyl)benzoic acid (ar-todomatuic acid) (**4**)

Mp 62 °C; $[\alpha]_D^{25}$ −26.3 ($CHCl_3$; *c* 0.63); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.84 (3H, *d*, $J=6.6$ Hz, Me-5′), 0.85 (3H, *d*, $J=6.6$ Hz, H_3-6′), 1.28 (3H, *d*, $J=6.8$ Hz, Me-1′), 2.08 (1H, *m*, H-5′), 2.17 (1H, *dd*, $J=15.8$, 6.7 Hz, H_a-4′), 2.22 (1H, *dd*, $J=15.8$, 7.1 Hz, H_b-4′), 2.64 (1H, *dd*, $J=16.8$, 7.8 Hz, H_a-2′), 2.73 (1H, *dd*, $J=16.8$, 7.6 Hz, H_b-2′), 3.42 (1H, *m*, H-1′), 7.32 (2H, *d*, $J=8.5$ Hz, H-3, H-5), 8.03 (2H, *d*, $J=8.3$ Hz, H-2, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.7 (Me-1′), 22.5 (C-6′ and Me-5′), 24.5 (C-5′), 35.3 (C-1′), 51.1 (C-2′), 52.5 (C-4′), 127.0 (C-3 and 5), 127.4 (C-1), 130.5 (C-2 and C-6), 152.8 (C-4), 171.3 (COOH), 209.1 (C-3′); EI-MS *m*/*z* (%): 248 $[M]^+$(40), 233 (35), 206 (10), 191 (25), 177 (10), 163 (10), 149 (100), 146 (40), 135 (10), 133 (25), 131 (24), 121 (10), 117 (11), 105 (15), 103 (13), 89 (70), 87 (25), 85 (37), 77 (12), 73 (9), 57 (45).

*3.3.5. (−)-Isochaminic acid (**5**)*

Mp 60 °C; $[\alpha]_D^{25}$ −10.2 (ether; *c* 1.3); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (3H, *s*, Me-7), 1.17 (3H, *s*, Me′-7), 1.18 (1H, *m*, H-6), 1.31 (1H, *m,* H-1), 1.78 (1H, *m*, H_a-5), 1.91 (2H, *m*, H_a-4, H_b-5), 2.41 (1H, *m*, H_b-4), 7.40 (1H, *d*, $J=5.6$ Hz, H-2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.9 (Me-7), 17.0 (C-5), 21.3 (C-4), 24.4 (C-1), 26.3 (C-6), 29.3 (Me′-7), 29.8 (C-7), 126.1 (C-3), 142.7 (C-2), 171.7 (COOH).

*3.3.6. 5-Allyl-1,2,3-trimethoxybenzene (elemicin) (**6**)*

Oily; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.34 (2H, *brd*, $J=6.7$ Hz, H-1′), 3.83 (3H, *s*, 2-OCH_3), 3.85 (6H, *s*, 1 and 3-OCH_3), 5.08 (1H, *brd*, $J=10.0$ Hz, H_a-3′), 5.10 (1H, *brd*, $J=17.0$ Hz, H_b-3′), 5.95 (1H, *m*, H-2′), 6.41(2H, *m*, H-4, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 40.5 (C-1′), 56.1 (C-3-O$\underline{C}H_3$ and 1-O$\underline{C}H_3$), 60.9 (2-O$\underline{C}H_3$), 105.5 (C-4 and 6), 116.0 (C-3′), 130.8 (C-5), 135.8 (C-2), 137.2 (C-2′), 153.2 (C-1 and 3); EIMS *m*/*z* (%): 208 $[M]^+$ (100), 193 (70), 177 (15), 165 (15), 161 (10), 149 (35), 133 (15), 118 (10), 105 (10), 95 (5), 91 (10), 84 (30), 69 (15), 57 (15).

3.3.7. (+)Sesamin (7)

Mp 124 °C; $[\alpha]_D^{25}$ +63.2 ($CHCl_3$; *c* 0.34); ^{1}H NMR($CDCl_3$, 400 MHz): δ 3.05 (2H, *m*, H-1α and H-5α), 3.87 (2H, *dd*, $J=9.3$, 3.7 Hz, H-4β,H-8β), 4.23 (2H, *dd*, $J=9.3$, 6.8 Hz, H-4α,H-8α), 4.71 (2H, *d*, $J=4.4$ Hz, H-2β, H-6β), 5.95 (4H, *s*, 2x-O$\underline{C}H_2$O), 6.77 (2H, *d*, $J=7.9$ Hz, H-5′,H-5″), 6.80 (2H, *dd*, $J=7.9$, 1.5 Hz, H-6′, H-6″), 6.84 (2H, *d*, $J=1.2$ Hz, H-2′, H-2″); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 54.3 (C-1, C-5), 71.7 (C-4, C-8), 85.6 (C-2, C-6), 101.1 (–O$\underline{C}H_2$O–), 106.5 (C-2′, C 2″), 108.2 (C-5′, C-5″), 119.4 (C-6′, C-6″), 135.0 (C-1′, C-1″), 147.1, (C-4′, C-4″), 148.0 (C-3′, C-3″); EI-MS *m*/*z* (%): 354 (100), 323 (15), 219 (10), 203 (35), 189 (10), 178 (20), 161 (70), 148 (35), 135 (45), 131 (30), 122 (15), 105 (8), 71 (5), 57 (7).

*3.3.8. 4-Isopropylbenzoic acid(cumic acid) (**8**)*

Oily; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.28 (6H, *d*, $J=7.1$ Hz, Me-1′, H_3-2′), 2.98 (1H, *m*, H-1′), 7.32 (2H, *d*, $J=7.8$ Hz, H-3, H-5), 8.02 (2H, *d*, $J=7.3$ Hz, H-2, H-6); EIMS *m*/*z*: 164, 149.

References

Atal, C.K., Dhar, K.L., Pelter, A., 1967. Isolation and structure determination of (+)-diaeudesmin, the first naturally occurring diaxially substituted 3,7-dioxabicyclo[3, 3, 0]octane lignan. J. Chem. Soc. (C) 2228–2229.

Aoyama, M., Togashi, I., Yoneyama, S., Doi, S., 1991. Antifungal activity of (+)-juvabione and (+)-todomatuic acid against wood-destroying fungi. Bokin Bobai 19, 463–465.

Ding, J., Yu, X., Ding, Z., Cheng, B., Yi, Y., Wu, Y., Hayashi, N., Komae, H., 1994. Essential oils of some Lauraceae species from the south-western parts of China. J. Essent. Oil Res. 6, 577–585.

Homans, A.L., Fuchs, A., 1970. Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. J. Chromatogra. 51, 327–329.

Ikeda, R., Nagao, T., Okabe, H., Nakano, Y., Matsunaga, H., Katano, M., Mori, M., 1998. Antiproliferative constituents in Umbelliferae plants. Constituents in the root and the ground part of *Anthriscus sylvestris*. Chem. Pharm. Bull. 46, 871–874.

Jayaweera, D.M.A., 1982. Medicinal Plants (Indigenous and Exotic) Used in Ceylon, Part II. The National Science Council of Sri Lanka, Colombo.

Mane, R.B., Rao, G.S.K., 1973. Synthesis of ar-juvabione, its *o*- & *m*-isomers and of its other analogues. Ind. J. Chem. 11, 615–618.

Mane, R.B., Rao, G.S.K., 1974. Studies in terpenoids: Part XXVIII—Synthesis of dehydro-ar-juvabione. Ind. J. Chem. 12, 936–937.

Rana, K.K., Guin, C., Roy, S.C., 2000. A short and stereoselective total synthesis of (+/−)-sesamin by radical cyclisation of an epoxide using a transition-metal radical source. Tetrahedron Lett. 41, 9337–9338.

Sakai, T., Hirose, Y., 1973. Structures of new sesquiterpenes related to pseudotsugonal and todomatuic acid isolated from Douglas-fir wood. Chemistry Letters, 825–828.

Stumpf, B., Wray, V., Kieslich, K., 1990. Oxidation of carens to chaminic acids by *Mycobacterium smegmatis* DSM 43061. Appl. Microbiol. Biotechnol. 33, 251–254.