Chromenone Glucosides Acylated with Monoterpene Acids from the Leaves of *Eucalyptus camaldulensis* var. *obtusa*

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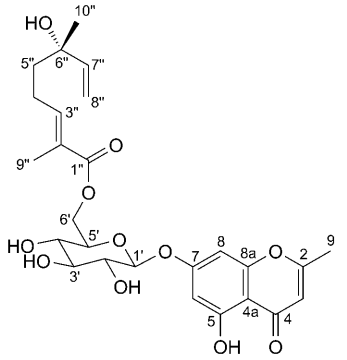
Three new chromenone glucosides acylated with monoterpene acids, eucamaldusides A (**1**), B (**2**), and C (**3**), were isolated from the leaves of *Eucalyptus camaldulensis* var. *obtusa*, together with the five known compounds ursolic acid lactone, obtusilin, β-sitosterol glucoside, 4-hydroxybenzoic acid, and cypellocarpin C. The structures of the new compounds were established by exhaustive 1D- and 2D-NMR spectroscopic studies. Their configuration was determined by measuring the $[\alpha]_D$ of the known methyl esters of the monoterpene acids obtained by methanolysis of **1**–**3**.

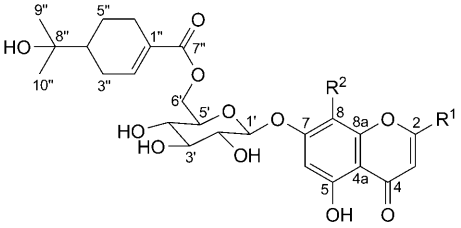
Introduction. – The genus *Eucalyptus* (Myrtaceae) comprising more than 700 species is native to Australia. The plants of this genus are mainly cultivated for paper, pharmaceutical, and cosmetic industries. Several species of *Eucalyptus* have been utilized for the treatment of cold, influenza, toothache, fever, diarrhoea, and other diseases [1–5]. *Eucalyptus camaldulensis* DEHNH. also known as 'Red River Gum' is regarded as one of the most widely planted *Eucalyptus* in the world. In the course of our phytochemical and biological investigation of *E. camaldulensis* DEHNH. var. *obtusa*, we reported the isolation and characterization of several triterpenoid constituents from the leaves of this plant [6][7]. In this article, we describe the isolation of three new acylated chromenone glucosides, eucamaldusides A[1]) (**1**), B[1]) (**2**), and C[1]) (**3**), together with the four known compounds ursolic acid lactone [8], obtusilin [9][10], β-sitosterol glucoside [11], and 4-hydroxybenzoic acid [12]. In addition, the epimer mixture **4** of cypellocarpin C (= 5-hydroxy-7-{{6-*O*-{[(4*R*)-4-(1-hydroxy-1-methylethyl)cyclohex-1-en-1-yl]carbonyl}-β-D-glucopyranosyl}oxy}-2-methyl-4*H*-1-benzopyran-4-one) [13] and its (4″*S*)-epimer was also obtained for the first time in the present study. The structures of the new compounds were established with the help of 1D- and 2D-NMR spectroscopy. The known compounds were identified by spectroscopic comparison with reported data.

Results and Discussion. – The MeOH extract of the leaves of *E. camaldulensis* var. *obtusa* was partitioned between AcOEt and H_2O. The AcOEt fraction was successively extracted with hexane, Et_2O, and AcOEt. The AcOEt portion was subjected to further fractionation by vacuum liquid chromatography (VLC). The fractions were purified by

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*



1[1])



	R^1	R^2	Configuration at C(4")
2[1])	i-Pr	H	(*R*/*S*)
3[1])	Me	Me	(*R*/*S*)
4[1])	Me	H	(*R*/*S*)

column chromatography (CC; silica gel), TLC, and HPLC (*GS 320* and ODS column) to yield eight compounds.

Compound **1** showed a pseudomolecular-ion peak at *m*/*z* 521.2060 ($[M+H]^+$) in the positive-ion-mode HR-FAB-MS corresponding to the molecular formula $C_{26}H_{32}O_{11}$. Its IR spectrum showed bands at 3386 (OH), 1703 (α,β-unsaturated ester C=O), 1658 (C=O), and 1624 (C=C) cm^{-1}. The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) indicated that **1** is an acylated chromenone glycoside with substitution at C(2) of the chromen-4-one moiety [14]. The ^{1}H-NMR (*Table 1*) displayed signals due to a Me group (δ(H) 2.39 (*s*, Me(9))), an olefinic H-atom (δ(H) 6.13 (*s*, H–C(3))), two *meta*-coupled aromatic H-atoms (δ(H) 6.46 (*d*, $J=2.0$ Hz, H–C(6)) and 6.60 (*d*, $J=2.0$ Hz, H–C(8))) as well as those characteristic of a β-glucopyranose unit. The UV spectrum showed maxima at λ_{max} 313, 284, 256, 249, and 225 nm which suffered a marked bathochromic shift on addition of $AlCl_3$ strongly suggesting the presence of a free OH group at C(5). The ^{1}H-NMR signal at δ(H) 13.47 in C_5D_5N as a one-H-atom broad *s* further supported the presence of a chelated OH group at C(5). These informations further suggested that the glucose unit was located at OH–C(7) of the chromenone. Significant peaks at *m*/*z* 192.0431 ($C_{10}H_8O_4^+$) and 354.0945 ($C_{16}H_{18}O_9^+$) in the HR-EI-MS due to the aglycone and glucoside supported these assignments. All assignments were confirmed by the ^{13}C-NMR (*Table 2*), HMQC, and HMBC (*Fig. 1*) experiments. The data indicated the presence of a partial structure reminiscent that of undulatoside (= 7-β-D-glucopyranosyloxy-5-hydroxy-2-methyl-4*H*-1-benzopyran-4-one) [8][15]. The ^{13}C-NMR spectral data of **1** further revealed that the acyl moiety was a monoterpene unit, exhibiting 26 signals in total, of which 16 were assigned to the chromenone and glucose units, and the remaining 10 to the monoterpene moiety. These 10 signals arose from two Me, three CH_2, and two CH groups (DEPT), and three quaternary C-atoms including one carboxyl C-atom, one olefinic C-atom, and one C-atom bearing an OH group in the monoterpene moiety (broad-band decoupled) (*Table 2*). This was further substantiated by a fragment ion which appeared at *m*/*z* 167.1073 ($C_{10}H_{15}O_2$) in the HR-EI-MS of **1**. The structure of the monoterpene moiety of **1** was established as 6-hydroxy-2,6-dimethylocta-2,7-dienoyl with the aid of 1D- and 2D-NMR experiments. A broad *t* at δ(H) 6.79 ($J=7.4$ Hz, H–C(3″)) in the ^{1}H-NMR

Table 1. *^{1}H-NMR Data* (600 MHz, CD_3OD) *of* **1**–**3**[1]). δ in ppm, J in Hz.

	1	**2**	**3**
H–C(3)	6.13 (*s*)	6.11 (*s*)	6.10 (*s*)
H–C(6)	6.46 (*d*, $J = 2.0$)	6.49 (*d*, $J = 1.8$)	6.56 (*s*)
H–C(8)	6.60 (*d*, $J = 2.0$)	6.63, 6.62 (1/2 H each, *d*, $J = 1.8$)	–
Me(9)	2.39 (*s*)	–	2.42 (*s*)
H–C(9)	–	2.89 (*sept.*, $J = 6.9$)	–
Me(10)	–	1.31 (*d*, $J = 6.9$)	2.23 (*s*)
Me(11)	–	1.31 (*d*, $J = 6.9$)	–
H–C(1′)	5.02 (*d*, $J = 7.5$)	5.03, 5.02 (2*d*, $J = 7.2$, 1/2 H each)	5.03, 5.02 (2*d*, $J = 7.3$, 1/2 H each)
H–C(2′)	3.48–3.55 (*m*)	3.48–3.56 (*m*)	3.53 (*t*, $J = 9.1$)
H–C(3′)	3.48–3.55 (*m*)	3.48–3.56 (*m*)	3.50 (*t*, $J = 9.1$)
H–C(4′)	3.34 (*t*, $J = 9.1$)	3.35 (*t*, $J = 9.0$)	3.34 (*t*, $J = 9.1$)
H–C(5′)	3.77–3.80 (*m*)	3.74–3.78 (*m*)	3.73–3.77 (*m*)
H_a–C(6′)	4.55 (*dd*, $J = 11.9$, 1.9)	4.54, 4.53 (2*dd*, $J = 11.8$, 2.5, 1/2 H each)	4.54, 4.53 (2*dd*, $J = 11.6$, 2.6, 1/2 H each)
H_b–C(6′)	4.18 (*dd*, $J = 11.9$, 7.5)	4.17, 4.15 (2*dd*, $J = 11.8$, 7.6, 1/2 H each)	4.17, 4.14 (2*dd*, $J = 11.8$, 8.2, 1/2 H each)
H–C(2″)	–	7.01–7.04 (*m*)	7.02–7.05 (*m*)
H_a–C(3″)	6.79 (*t*, $J = 7.4$)	2.28–2.35 (*m*)	2.28–2.34 (*m*)
H_b–C(3″)	–	1.99–2.05 (*m*)	1.98–2.04 (*m*)
CH_2(4″)	2.20–2.25 (*m*)	–	–
H–C(4″)	–	1.49–1.55 (*m*)	1.49–1.54 (*m*)
H_a–C(5″)	1.54–1.57 (*m*)	1.95–2.00 (*m*)	1.95–2.01 (*m*)
H_b–C(5″)	1.54–1.57 (*m*)	1.18–1.24 (*m*)	1.17–1.23 (*m*)
H_a–C(6″)	–	2.45–2.52 (*m*)	2.45–2.51 (*m*)
H_b–C(6″)	–	2.10–2.16 (*m*)	2.10–2.17 (*m*)
H–C(7″)	5.88 (*dd*, $J = 17.2$, 10.8)	–	–
H_a–C(8″)	5.20 (*dd*, $J = 17.2$, 1.4)	–	–
H_b–C(8″)	5.04 (*dd*, $J = 10.8$, 1.4)	–	–
Me(9″)	1.83 (*s*)	1.17, 1.16 (2*s*, 3/2 H each)	1.17, 1.16 (2*s*, 3/2 H each)
Me(10″)	1.23 (*s*)	1.16, 1.15 (2*s*, 3/2 H each)	1.17, 1.16 (2*s*, 3/2 H each)

spectrum along with a broad *s* at δ(H) 1.83 (Me(9″)), correlated with C(1″) (δ(C) 169.5), C(2″) (δ(C) 128.4), and C(3″) (δ(C) 144.5) in the HMBC spectrum (*Fig. 1*), were in favor of a trisubstituted C=C moiety conjugated with the ester C=O. The (*E*)-geometry of the conjugated C=C bond was established by the chemical shift of the olefinic H-atom in β-position (δ(H) 6.79) [16] and also by a cross-peak between the signals of Me(9″) and CH_2(4″) in the NOESY plot. The ^{1}H-NMR spectrum further exhibited three downfield *ABC*-type *dd* at δ(H) 5.88 ($J = 17.2$, 10.8 Hz, H–C(7″)), 5.20 ($J = 17.2$, 1.4 Hz, H_a–C(8″)), and 5.04 ($J = 10.8$, 1.4 Hz, H_b–C(8″)) indicating the presence of a terminal C=C bond. A three-H-atom *s* resonating at δ(H) 1.23 was attributed to a Me(10″) at C(6″) bearing a tertiary OH group. The H-atoms H–C(7″), H_a–C(8″), and H_b–C(8″) correlated with C(6″) and H–C(3″) with C(1″), C(2″), C(4″), C(5″), and C(9″) of the acyl unit in the HMBC spectrum (*Fig. 1*). The location of the glucose unit at OH–C(7) of the chromenone skeleton of **1** and acylation at OH–C(6′) of the glucose unit were finally established by the HMBC experiments (*Fig. 1*). Thus,

Table 2. *^{13}C-NMR Data* (150 MHz, CD_3OD) *of* **1**–**3**. δ in ppm.

	1	**2**	**3**
C(2)	169.9	177.3	169.9
C(3)	109.4	106.6	108.9
C(4)	184.2	184.5	184.7
C(4a)	106.8	106.9	106.5[a])
C(5)	163.1	163.2	161.2
C(6)	100.7	100.5 (br.)	99.37, 99.35
C(7)	164.6	164.7	162.2
C(8)	96.1	96.3	106.6[a])
C(8a)	159.3	159.3	156.5
C(9)	20.5	34.5	20.4
C(10)	–	20.3[a])	7.9
C(11)	–	20.4[a])	–
C(1′)	101.3	101.26, 101.23	101.46, 101.44
C(2′)	77.6	77.6	78.0
C(3′)	74.6	74.6	74.7
C(4′)	71.8	71.9	72.0
C(5′)	75.7	75.7 (br.)	75.7
C(6′)	65.1	64.95, 64.91	65.00, 64.96
C(1″)	169.5	130.8	130.9
C(2″)	128.4	141.81, 141.79	142.00, 141.99
C(3″)	144.5	28.6	28.6
C(4″)	24.6	45.4 (br.)	45.5
C(5″)	41.6	24.54, 24.51	24.53, 24.50
C(6″)	73.6	26.3	26.4
C(7″)	145.9	168.7	168.8
C(8″)	112.5	72.8	72.8
C(9″)	12.6	27.11, 27.07[a])	27.11, 27.05[a])
C(10″)	27.9	26.49, 26.46[a])	26.46, 26.44[a])

[a]) Values in a vertical column may be interchanged.

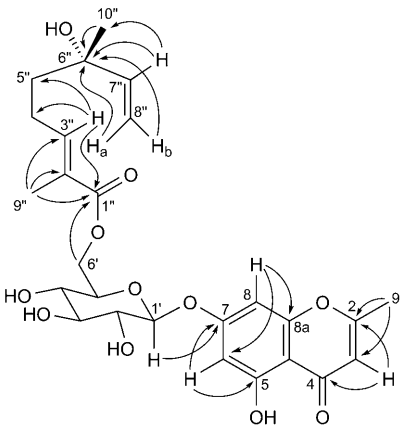


Fig. 1. *Significant HMBC* (H → C) *interactions* of **1**

H_a–C(6′) and H_b–C(6′) (δ(H) 4.55 and 4.18) showed a 3-bond correlation with the ester C=O at δ(C) 169.5, and the anomeric H–C(1′) exhibited a 3-bond correlation with C(7) of the chromenone unit. It may further be noted that, due to the effect of acylation, the signals of C(6′) and C(5′) were shifted paramagnetically (by *ca.* 2 ppm) and diamagnetically (by *ca.* 3 ppm), respectively, as observed in the case of related glycosides [17]. The configuration at C(6″) of **1** was established as (*S*) by methanolysis of **1** with 1% MeONa in MeOH which furnished (+)-(2*E*,6*S*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid methyl ester of $[\alpha]_D = +12$ ([18]: $[\alpha]_D = +15.8$ for the corresponding ethyl ester). The sugar liberated on acid hydrolysis of the gluco side obtained by methanolysis of **1** was identified as D-glucose by the sign of its optical rotation and comparative TLC with an authentic sample (see *Exper. Part*). In the light of the above discussion, the structure of **1** was assigned to eucamalduside A[1]).

Compound **2** showed a pseudomolecular-ion peak at *m*/*z* 549.2303 ($[M+H]^+$) in the positive-ion-mode HR-FAB-MS, corresponding to the molecular formula $C_{28}H_{36}O_{11}$ which was further confirmed by ^{13}C-NMR data (broad-band decoupled and DEPT) and mass fragments in the HR-EI-MS. Compound **2** gave a single spot on TLC (silica gel and reversed-phase) as well as a single peak on reversed-phase HPLC; however, several signals in the ^{1}H- and ^{13}C-NMR were duplicated in a ratio of *ca.* 1:1 implying that **2** exists as a mixture (*Tables 1* and *2*). The ^{1}H- and ^{13}C-NMR data indicated that **2** was also an acylated 2-substituted chromenone glucoside. The chromenone moiety was different from that found in **1** in that it had an i-Pr group at position 2 instead of a Me group as evident from a broad six-H-atom *d* and a one-H-atom *sept.* resonating at δ(H) 1.31 ($J = 6.9$ Hz, Me(10) and Me(11)) and 2.89 ($J = 6.9$ Hz, H–C(9)), respectively, in the ^{1}H-NMR spectrum. Its location at C(2) was confirmed by the HMBC H–C(9)/C(2) (δ(C) 177.3) and C(3) (δ(C) 106.6) (*Fig. 2*). All these data were comparable with those of takanechromenone C (= 7-(β-D-glucopyranosyloxy)-5-hydroxy-2-(1-methylethyl)-4*H*-1-benzopyran-4-one) [17]. This partial structure was also substantiated by the ^{13}C-NMR spectrum showing 28 signals, 18 of which were assignable to chromenone and glucose moieties (*Table 2*). The remaining 10 C-atom signals corresponded to two Me, three CH_2, and two CH groups (DEPT), and three quaternary C-atoms including one ester C=O C-atom, one olefinic C-atom, and one C-atom bearing an OH group in the monoterpene moiety (broad-band decoupled) (*Table 2*). Furthermore, the HMBC plot of **2** showed that H–C(2″) (δ(H) 7.02) correlated with C(1″) (δ(C) 130.8) and C(7″) (δ(C) 168.7), and Me(9″) and Me(10″) correlated with C(4″) (δ(C) 45.4) and C(8″) (72.8). This was also supported by the appearance of peaks at *m*/*z* 167.1071 ($C_{10}H_{15}O_2^+$) and 59.0660 ($C_3H_7O^+$) in the HR-EI-MS. Thus, these data established that **2** had an oleuropeic acid (= 4-(1-hydroxy-1-methylethyl)cyclohex-1-ene-1-carboxylic acid) moiety [19]. It was further corroborated by connectivities observed between H–C(2″), 2 H–C(3″), H–C(4″), 2 H–C(5″), and 2 H–C(6″) in a total-correlation spectrum (TOCSY). Moreover, the correlation of the anomeric H–C(1′) (δ(H) 5.03 and 5.02) with C(7) (δ(C) 164.7) of the chromenone nucleus and the correlations between the two H–C(6′) (δ(H) 4.54, 4.53, 4.17, and 4.15) and C(7″) (δ(C) 168.7) in the HMBC spectrum (*Fig. 2*) led to place the glucose unit at OH–C(7) of the chromenone and the oleuropeoyl group at OH–C(6′) of the glucose moiety. Methanolysis of **2** with MeONa in MeOH gave oleuropeic acid methyl ester

which was optically inactive confirming that **2** is a (4″*S*)/(4″*R*) mixture of epimers in a 1:1 ratio, thus justifying the doubled signals of some of the nuclei in the NMR spectra [20]. The sugar unit was identified as D-glucose as discussed above for compound **1**. Based on these observations, eucamalduside B[1]) was characterized as structure **2** (1:1 epimer mixture at the C(4″) position).

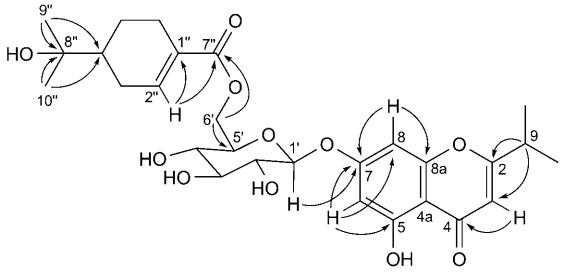


Fig. 2. *Significant HMBC* (H → C) *interactions* of **2**

The molecular formula $C_{27}H_{34}O_{11}$ of compound **3** was established from a pseudomolecular-ion peak at *m*/*z* 535.2152 ($[M+H]^+$) in the HR-FAB-MS. Its IR spectrum showed bands at 3419 (OH), 1701 (α,β-unsaturated ester C=O), 1659 (C=O), and 1624 cm^{-1} (C=C), and the UV spectrum exhibited maxima at 313, 285, 255, 249, and 226 nm. Analysis of the ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) suggested that **3** is also an acylated chromenone glucoside in which only the chromenone moiety is different from that of **2**. Several peaks in the ^{1}H- and ^{13}C-NMR spectra of **3** were also duplicated (ratio *ca.* 1:1) suggesting that **3** is also an epimer mixture at the C(4″) position. The ^{1}H-NMR spectrum of **3** showed a signal due to a H-bonded OH group (δ(H) 13.40 (*s*), observed in C_5D_5N). Two Me groups appeared as *s* at δ(H) 2.42 (δ(C) 20.4; HMQC) and 2.23 (δ(C) 7.9; HMQC) and were placed at C(2) and C(8), respectively. An olefinic and an aromatic H-atom resonated as *s* at δ(H) 6.10 (δ(C) 108.9; HMQC) and 6.56 (δ(C) 99.37 and 99.35 HMQC) and were assigned to H–C(3) and H–C(6), respectively. All these assignments were confirmed by HMBCs (*Fig. 3*), *i.e.*, H–C(3)/C(2), C(4), and C(4a), H–C(6)/C(4a), C(5), C(7), and C(8), Me(9)/C(2) and C(3), and Me(10)/C(6), C(7), C(8), and C(8a). These data indicated the presence of a 2,8-dimethylchromenone aglycone in the molecule [21]. The chromenone moiety

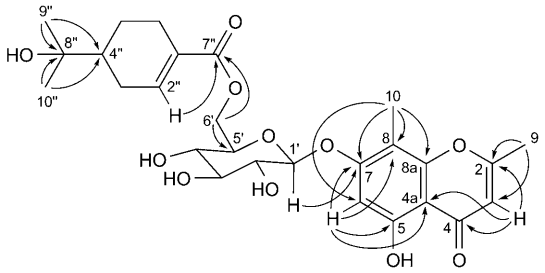


Fig. 3. *Significant HMBC* (H → C) *interactions* of **3**

was substantiated by a strong peak at *m/z* 206.0571 in the HR-EI-MS. Characteristic signals in the NMR spectra for *O*-oleuropeoyl-*β*-glucopyranose (*Tables 1* and *2*) were also noted, as observed in **2**. The positions of the ester and glycosidic linkages in **3** were established by HMBC data. Thus H_a–C(6′) and H_b–C(6′) of the glucose residue were correlated through a three-bond coupling with the ester C=O. A correlation between the anomeric H–C(1′) and the chromenone C(7) was also observed. Upon methanolysis with MeONa in MeOH, **3** also gave racemic methyl oleuropeate. Subsequent acid hydrolysis furnished D-glucose which was confirmed by the procedures described for compound **1**. These observations established the structure of eucamalduside C[1]) as **3**.

It is important to note that compound **4** was shown to be a mixture of cypellocarpin C and its (4″*S*)-epimer through exhaustive NMR analysis (*Table 3*) and chemical degradation. It is further noted that cypellocarpin C has been reported earlier from *E. camaldulensis* with the name camaldulenside [22][23], whereas the existence of the (4″*S*)-epimer was established here for the first time from a natural source.

Table 3. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; CD_3OD) *of* **4**. *δ* in ppm, *J* in Hz.

	δ(H)	*δ*(C)	HMBC (H → C)
C(2)	–	169.9	–
H–C(3)	6.12 (*s*)	109.3	C(2), C(4), C(4a), C(9)
C(4)	–	184.2	–
C(4a)	–	106.4	–
C(5)	–	163.1	–
H–C(6)	6.48 (*d*, *J* = 1.8)	100.7	C(4a), C(5), C(7), C(8)
C(7)	–	164.6	–
H–C(8)	6.61, 6.60 (2*d*, *J* = 1.8, 1/2 H each)	96.3	C(4a), C(6), C(7), C(8a)
C(8a)	–	159.3	–
Me(9)	2.39 (*s*)	20.5	C(2), C(3)
H–C(1′)	5.04, 5.03 (2*d*, *J* = 7.3, 1/2 H each)	101.29, 101.27	C(7)
H–C(2′)	3.48 – 3.51 (*m*)	77.7	C(1′), C(3′)
H–C(3′)	3.49 – 3.53 (*m*)	74.6	C(1′), C(2′), C(4′)
H–C(4′)	3.36 (*t*, *J* = 9.2)	71.9	C(2′), C(3′), C(5′), C(6′)
H–C(5′)	3.76 – 3.80 (*m*)	75.6	C(4′)
H_a–C(6′)	4.54, 4.52 (2*dd*, *J* = 11.5, 2.5, 1/2 H each)	64.95, 64.91	C(5′), C(7″)
H_b–C(6′)	4.18, 4.16 (2*dd*, *J* = 11.5, 7.6, 1/2 H each)		C(5′), C(7″)
C(1″)	–	131.0	–
H–C(2″)	7.01 – 7.03 (*m*)	141.75, 141.73	C(3″), C(4″), C(6″), C(7″)
H_a–C(3″)	2.29 – 2.34 (*m*)	28.6	C(1″), C(2″)
H_b–C(3″)	1.99 – 2.03 (*m*)		
H–C(4″)	1.50 – 1.55 (*m*)	45.5	C(6″), C(8″)
H_a–C(5″)	1.95 – 1.99 (*m*)	24.55, 24.53	C(3″), C(4″), C(6″)
H_b–C(5″)	1.19 – 1.25 (*m*)		C(2″), C(3″), C(4″), C(6″)
H_a–C(6″)	2.44 – 2.49 (*m*)	26.3	C(1″), C(4″)
H_b–C(6″)	2.10 – 2.16 (*m*)		–
C(7″)	–	168.8	–
C(8″)	–	72.8	–
Me(9″)	1.160, 1.157 (2*s*, 3/2 H each)[a])	27.08, 27.04[a])	C(4″), C(8″), C(10″)
Me(10″)	1.157, 1.153 (2*s*, 3/2 H each)[a])	26.52, 26.50[a])	C(4″), C(8″), C(9″)

[a]) Values in a vertical column may be interchanged.

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Experimental Part

General. Vacuum liquid chromatography (VLC): silica gel PF_{254} (SiO_2; *Merck*). Column chromatography (CC): SiO_2 *9385* (0.04–0.063 mm; *Merck*) and *Sephadex LH-20* (*Pharmacia Biotech AB*, Uppsala, Sweden). Anal. and prep. TLC: silica gel *Si* F_{254} precoated aluminium cards (0.2 mm thickness; *Merck*); spots visualized under UV light (254 and 365 nm) and by spraying with I_2 and aniline phthalate. Prep. recycling HPLC: *Jaigel LC 908W* equipped with a variable-wavelength UV detector *UV-S310A*, model *II*, and differential refractometer *RI-5*; *GS-320* and ODS (*M-80*) columns. UV Spectra: *Hitachi-U-3200* spectrophotometer; λ_{max} in nm. IR Spectra: *Bruker-Vector-22* spectrophotometer; in cm^{-1}. Optical rotations: *Jasco-P-1020* polarimeter; at 27°. 1D- and 2D-NMR Spectra: *Bruker-Avance* spectrometer; at 600 (^1H) and 150 MHz (^{13}C); δ in ppm. rel. to solvent peaks (CD_3OD), *J* in Hz. EI-MS: *Finnigan-MAT-311-A* mass spectrometer; at 70 eV; source at 250°; in *m*/*z* (rel. %). HR-EI-MS: *Jeol-JMS-HX-110* mass spectrometer; at 70 eV; source at 250°. HR-FAB-MS: *Jeol-JMS-HX-110* mass spectrometer; glycerol as matrix.

Plant Material. The leaves of *E. camaldulensis* var. *obtusa* were collected from the Karachi region. The plant was identified by Mr. *M. I. H. Brooker*, Eucalypt botanist, Center for Plant Biodiversity Research, Australian National Herbarium (CANB), Canberra, Australia, and a voucher specimen (G. H. No. 85851) was deposited with the Herbarium, Department of Botany, University of Karachi.

Extraction and Isolation. The fresh and uncrushed leaves (10 kg) were extracted with MeOH (3 × 20 l, 48 h each) at r.t. The concentrated extract obtained on removal of the solvent from the combined extracts under reduced pressure was partitioned between AcOEt (15 l) and H_2O (5 l). The AcOEt phase, after drying (Na_2SO_4) and passing through the activated charcoal, was concentrated. The charcoal bed was washed with MeOH/C_6H_6 1:1, and the solvent was evaporated. The AcOEt and MeOH/C_6H_6 fractions were combined on the basis of TLC and successively extracted with hexane, Et_2O, and AcOEt. The AcOEt-soluble fraction (10 g) was subjected to VLC ($CHCl_3$/MeOH 10:0 → 9:1): *Fractions 1–7*. *Fr. 2* (900 mg; from the $CHCl_3$ and $CHCl_3$/MeOH 9.9:0.1 eluates) was resubjected to CC (SiO_2, hexane/AcOEt 9:1 → 0:10). The residue obtained from the hexane/AcOEt 8:2 eluate furnished *ursolic acid lactone* (70.4 mg) on keeping in $CHCl_3$/MeOH 1:1 overnight at r.t. The residue obtained after combining the hexane/AcOEt 7:3 and 6:4 eluates afforded *obtusilin* (2.5 mg) after CC (SiO_2, hexane/AcOEt 9:1 → 0:10) followed by prep. TLC ($CHCl_3$/MeOH 9.8:0.2). *Fr. 3* (2.8 g), obtained after combining the $CHCl_3$/MeOH 9.9:0.1 → 9.6:0.4 eluates, gave *β-sitosterol glucoside* (200.0 mg) when kept overnight in $CHCl_3$/MeOH 1:1 at r.t. The mother liquor, after evaporation of the solvent (2.4 g), was subjected to CC (SiO_2, hexane/AcOEt 9:1 → 0:10 and then $CHCl_3$/MeOH 10:0 → 9:1): *Fr. 3.1–3.12* (by TLC). *Fr. 3.4* (hexane/AcOEt 6:4 eluate) afforded *4-hydroxybenzoic acid* (5.3 mg) on prep. TLC ($CHCl_3$/MeOH 9.5:0.5). *Fr. 3.6* (AcOEt eluate) gave a further quantity of *β-sitosterol glucoside* (20.4 mg) on keeping in $CHCl_3$/MeOH 1:1 overnight. The mother liquor was subjected to CC ($CHCl_3$/MeOH 10:0 → 9:1) which afforded several fractions. *Fr. 3.6.3* ($CHCl_3$/MeOH 9.5:0.5 eluate) was applied to HPLC (*GS-320* column, MeOH): *Fr. 3.6.3.1* and *Fr. 3.6.3.2* as main fractions. The two fractions were separately purified by HPLC (ODS, isocratic MeCN/H_2O 3:2, flow rate 3 ml/min). *Fr. 3.6.3.1* gave **1** (7.1 mg) and **2** (6.4 mg) after 9 recycling operations with t_R 28 and 32 min, resp., whereas *Fr. 3.6.3.2*, after 11 recycling operations, furnished **4** (12.2 mg) and **3** (6.6 mg) with t_R 22 and 25 min, resp. *Fr. 7* (AcOEt eluate) gave **4** (99.8 mg) upon crystallization from $CHCl_3$/MeOH 1:1.

Eucamalduside A (= *5-Hydroxy-7-{{6-*O*-[(2*E*,6*S*)-6-hydroxy-2,6-dimethyl-1-oxoocta-2,7-dien-1-yl]-β-*D*-glucopyranosyl}oxy}-2-methyl-4*H*-1-benzopyran-4-one*; **1**): Amorphous solid. $[\alpha]_D = -126$ ($c = 0.01$, MeOH). UV (MeOH): 313 (3.28), 284 (3.42), 256 (3.88), 249 (3.88), 225 (4.00), 203 (4.15). IR (KBr): 3386, 2966, 2933, 1703, 1658, 1624, 1072. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 192 (100, aglycone), 167 (12), 164 (15), 152 (16), 149 (19), 121 (20), 111 (46), 108 (15), 93 (34), 59 (22), 55 (40). HR-FAB-MS: 521.2060 ($[M+H]^+$, $C_{26}H_{33}O_{11}^+$; calc. 521.2023).

Eucamalduside B (=*5-Hydroxy-7-{{6-*O*-{[(4R/S)-4-(1-hydroxy-1-methylethyl)cyclohex-1-en-1-yl]-carbonyl}-β-*D*-glucopyranosyl}oxy}-2-(1-methylethyl)-4*H*-1-benzopyran-4-one*; **2**): Amorphous solid. $[\alpha]_D = -111$ ($c = 0.02$, MeOH). UV (MeOH): 314 (3.28), 285 (3.42), 255 (3.88), 248 (3.90), 224 (4.00), 202 (4.16). IR (KBr): 3420, 2924, 2859, 1701, 1661, 1624, 1072. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 220 (77, aglycone), 167 (17), 152 (12), 149 (33), 121 (17), 111 (36), 108 (15), 93 (26), 59 (22), 55 (41). HR-FAB-MS: 549.2303 ($[M+H]^+$, $C_{28}H_{37}O_{11}^+$; calc. 549.2336).

Eucamalduside C (=*5-Hydroxy-7-{{6-*O*-{[(4R/S)-4-(1-hydroxy-1-methylethyl)cyclohex-1-en-1-yl]-carbonyl}-β-*D*-glucopyranosyl}oxy}-2,8-dimethyl-4*H*-1-benzopyran-4-one*; **3**): Amorphous solid. $[\alpha]_D = -104$ ($c = 0.02$, MeOH). UV (MeOH): 313 (3.27), 285 (3.41), 255 (3.88), 249 (3.90), 226 (4.01), 204 (4.16). IR (KBr): 3419, 2924, 2856, 1701, 1659, 1624, 1073. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 206 (18, aglycone), 177 (30), 167 (17), 149 (33), 138 (2), 84 (10), 64 (46), 56 (41). HR-FAB-MS: 535.2152 ($[M+H]^+$, $C_{27}H_{35}O_{11}^+$; 535.2179).

Methanolysis of **1**–**3**. To a soln. of **1** (5.0 mg) in MeOH (1 ml), 1% MeONa/MeOH (0.1 ml) was added and the mixture kept overnight at r.t. After neutralization with AcOH, the mixture was concentrated and purified by CC (*Sephadex LH-20*): (+)-(2*E*,6*S*)-6-hydroxy-2,6-dimethylocta-2,7-dienoic acid methyl ester (0.98 mg; $[\alpha]_D = +12$ ($c = 0.01$ $CHCl_3$)).

Similarly **2** and **3** (4.5 mg each) were subjected to methanolysis to yield 0.91 and 0.93 mg, resp., of (±)-oleuropeic acid methyl ester ($[\alpha]_D = \pm 0$ ($c = 0.01$, $CHCl_3$)).

The structures of the methyl esters obtained from **1**–**3** were confirmed by the comparison of their NMR data with those reported for the corresponding known methyl esters [13][19][24].

Acid Hydrolysis of Glycosides Obtained from **1**–**3**. The glycoside (2.5 mg) obtained from methanolysis of **1** was refluxed in 10% HCl soln. for 2 h. The mixture was cooled and extracted with AcOEt. The aq. phase was neutralized with Na_2CO_3 and concentrated; the sugar was identified as D-glucose by measuring its optical rotation ($[\alpha]_D = +52.6$) and by co-TLC (BuOH/AcOEt/AcOH/H_2O 12 : 2 : 2 : 2) with an authentic sample of D-glucose [25]. The sugar components in **2** and **3** were similarly identified as D-glucose.

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