PERGAMON

Phytochemistry 59 (2002) 889–895

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

A chlorinated monoterpene ketone, acylated β-sitosterol glycosides and a flavanone glycoside from *Mentha longifolia* (Lamiaceae)

Muhammad Shaiq Ali[a,*], Muhammad Saleem[a], Waqar Ahmad[a], Masood Parvez[b], Raghav Yamdagni[b]

[a]*H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan*
[b]*Department of Chemistry, University of Calgary, 2500 University Drive NW, Calgary, Alberta, Canada T2N IN4*

Received 4 June 2001; received in revised form 15 November 2001

Abstract

Mentha longifolia (Lamiaceae), an aromatic herb yielded a new halogenated chloro-derivative of menthone (longifone), two new derivatives of β-sitosterol glycoside (longiside-A and -B) and a new flavanone-glycoside (longitin). The β-sitosterol and flavanone glycosides were purified as their acetate derivatives. Structures of all the isolated constituents were elucidated with the aid of HMBC techniques. However, the structure of longifone was also determined through X-ray crystallography.

Keywords: *Mentha longifolia*; Lamiaceae; Halogenated-monoterpenoid; X-ray; Steroidal-glycosides; Flavanone-glycoside; Structure elucidation; Spectroscopy; 2D NMR

1. Introduction

The genus *Mentha* belongs to the family Lamiaceae (Labiatae) consisting of about 25–30 species (Ali and Nasir, 1990); most of them are found in temperate regions of Eurasia, Australia and South Africa. Although *Mentha* is a distinct genus of the family Lamiaceae, it has some taxonomic difficulties at species level. These difficulties arise from a very long history of cultivation and naturalization, vegetative plasticity and wide spread. The aromatic *Mentha* herbs are perennials found in damp or wet places. Members of this genus are the most important sources of essential oil production in the world. It has been estimated that the annual production of oils from three mint species, peppermint (*M. piperita*), cornmint (*M. arvensis*) and spearmint (*M. spicata*) is worth 186 million dollars (Laplinska, 1993). Some members of this genus are also used as herbal teas and condiments in both fresh and dried form due to their distinct aroma (Baser and Kurkcuoglu, 1999). The most common species of the genus *Mentha* found in Pakistan are: *M. pulegium, M. arevensis, M. spicata, M. longifolia, M. piperita* and *M. royleana* (Ali and Nasir, 1990).

Chemical literature surveys of the genus *Mentha* revealed that mostly it has been studied for the chemical composition of essential oils (Baser et al., 1997; Guido et al., 1997). Constituents of essential oils and their glycosides have been reported by several groups from various species of this genus. They include: menthone, menthol, menthyl acetate, *neo*-isomenthyl acetate, 1-menthyl-β-D-glucopyranosyl (Sakata and Mitsui, 1975) and 1-menthyl-6′-*O*-acetyl-β-D-glucopyranosyl (Sakata and Koshimizu, 1978). Other than essential oils, various species of this genus have been afforded steroidal glycosides (Shimizu et al., 1990), flavone (Ahmad et al., 1886) and flavone glycosides (Kamiya et al., 1979).

Mentha longifolia is distributed through out Eurasia and tropical Asia but uncommon in our region. Several essential oils (Ghoulami et al., 2001; Nori-Shargh et al., 2000; Abu-Al-Futuh et al., 2000), flavones and flavone-glycosides (Ghoulami et al., 2001; Sharaf et al., 1999) have recently been obtained from this species. In the present communication, we wish to report the isolation and structure elucidation of four new constituents: a new chloro-derivative of menthone (longifone, **1**), two

* Corresponding author. Tel.: +92-21-9243232; fax: +92-21-9243190-91.
E-mail address: shaiq303@super.net.pk shaiq303@hotmail.com (M. Shaiq Ali).

0031-9422/02/$ - see front matter
PII: S0031-9422(01)00490-3

new derivatives of β-sitosterol glucoside (longiside-A, **2** and longiside-B, **3**) and a new flavanone-glycoside (longitin, **4**).

2. Results and discussion

A methanolic extract of aerial parts of *M. longifolia* afforded compound **1** as colorless shiny crystals. The electron impact mass spectrum showed the molecular ion peak at *m/z* 204 together with an isotopic peak (M + 2) at *m/z* 206 indicating the presence of a halogen atom in the molecule. Finally, the presence of chlorine was identified in the molecule by observing the ratio between M^+ and M + 2 peaks and the peak at *m/z* 168 (48%) appeared due to the loss of HCl from the molecular ion. The presence of chlorine was further confirmed by fusing **1** with sodium metal. When the resulting fused sample was diluted with distilled water, white precipitates were obtained on addition of silver nitrate. The HRMS of **1** showed a molecular ion peak at *m/z* 204.0921 corresponding to the formula $C_{10}H_{17}O_2Cl$. The IR spectrum of the same showed two strong absorptions at 1735 and 3550 cm^{-1} due to the ketonic and hydroxyl functions in the molecule.

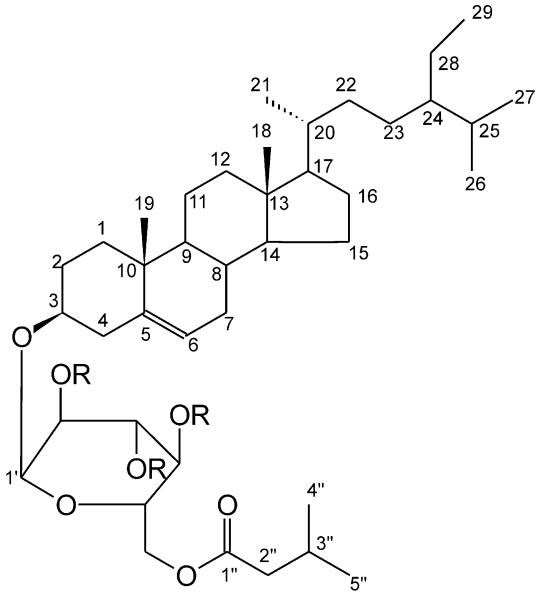
The ^1H NMR spectrum displayed a methyl singlet at δ 1.40 (H-7) which in ^{13}C spectrum appeared at δ 29.3. The chemical shifts of this methyl in NMR spectra showed its connectivity to a quaternary carbon bearing a hydroxyl function and this quaternary carbon in ^{13}C NMR spectrum resonated at δ 77.3 (C-6). Another downfield signal of a proton appeared at δ 4.53 as a doublet with very small coupling (H-1, *J* = 0.5 Hz). The carbon attached to this proton was located through 2D NMR experiments at δ 75.2 (C-1). The chemical shifts of this methine revealed the presence of chlorine atom. An isopropyl moiety was also observed in the NMR spectra and the presence of this moiety was attested to by the ^1H NMR spectrum which showed two doublets (three protons each) at δ 0.92 and 0.88 (H-9 and 10, 6.5 Hz each). The connectivities of these two methyls with the methine protons at δ 1.83 (H-3) was confirmed through a COSY 45° experiment. The resonance of a ketonic function was observed in the ^{13}C NMR spectrum at δ 200.1. In addition to this, DEPT experiments determined the presence of two methylenes which appeared at δ 24.0 (C-4) and 37.7 (C-5).

The presence of a ketonic function and a chlorine atom and the absence of an olefinic moiety in the molecule confirmed that **1** is a monocyclic compound. The spectral information concluded the structure of **1** as 1-chloro-6-hydroxymenthone and it was named longifone. Menthone, menthol and their derivatives have already been reported from the genus *Mentha* (Sakata and Mitsui, 1975; Sakata and Koshimizu, 1978). Finally, the shiny crystals were submitted for single crystal X-ray diffraction analysis for further confirmation of structure **1**. A computer-generated diagram is given as Fig. 1. The way of molecular aggregation is represented in Fig. 2.

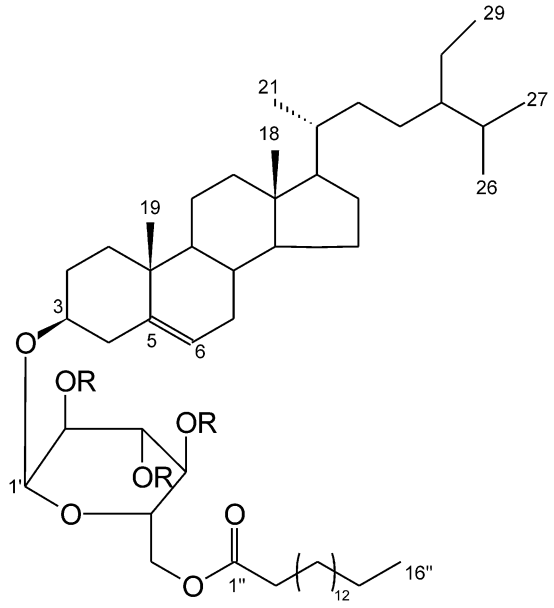
Halogen containing compounds frequently found in marine algae (Dembitskii and Tolstikov, 1999) but they are rare in terrestrial plants. The possibility of artefact for **1** has to be ruled out, as hydrochloric acid was not used during the isolation/purification process. On the other hand, there are many Asteraceous plants are known to have chloro-sesquiterpenoids, such as *Pluchea arguta* (Ahmad et al., 1992) and *Centaurea scoparia* (Youssef and Frahm, 1994). Some of these show anticancer activity (Gonzalez et al., 1980). Monoterpenoids are also derived from the same building block from which sesquiterpenoids are formed. Therefore, presence of chlorine in **1** is justified and this compound has not been reported so far from any natural source.

During the column chromatography, a mixture of two compounds was obtained with 100% chloroform, which on acetylation resolved into two compounds (**2a** and **3a**) as colorless oil.

The mass fragmentation pattern of **2a** observed in EIMS clearly indicated the presence of sitosterol type skeleton, which was confirmed by comparing the ^1H and ^{13}C NMR data with that of reported data for β-sitosterol (Shameel et al., 1996; Ali et al., 1997). The ^1H NMR spectrum displayed olefinic proton (H-6) at δ 5.33 as a distorted triplet. The same spectrum showed signals for H-3 at δ 3.43 (*m*), Me-18 at δ 0.66 (*s*), Me-19 at δ 0.97 (*s*), Me-21 at δ 0.91 (*d*, *J* = 6.5 Hz), Me-26 and 27 at δ 0.80 (*d*, *J* = 6.8 Hz) and Me-29 at δ 0.83 (*t*, *J* = 6.5 Hz). The presence of sugar moiety in the molecule attested



	R
2	H
2a	Ac



R	
3	H
3a	Ac

due to the presence of an anomeric proton at δ 4.50 (7.0 Hz), other sugar-protons appeared between δ 5.00–5.21, methylene-6′ protons at δ 4.16–4.28 (*m*) and an anomeric carbon at δ 99.6 in NMR spectra.

In the ^{1}H NMR spectrum, only three acetate methyls instead of four were found at δ 1.98, 1.99 and 2.00, whereas the downfield chemical shifts of three sugar methine protons (except anomeric) revealed that they contain acetoxyl moieties. In addition to the signals due

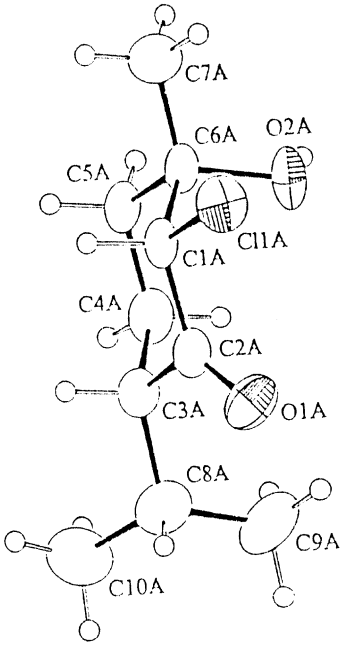
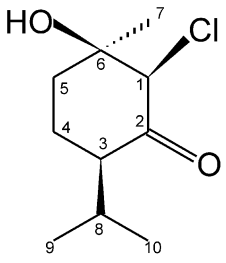


Fig. 1. A computer generated diagram of compound **1**.



1

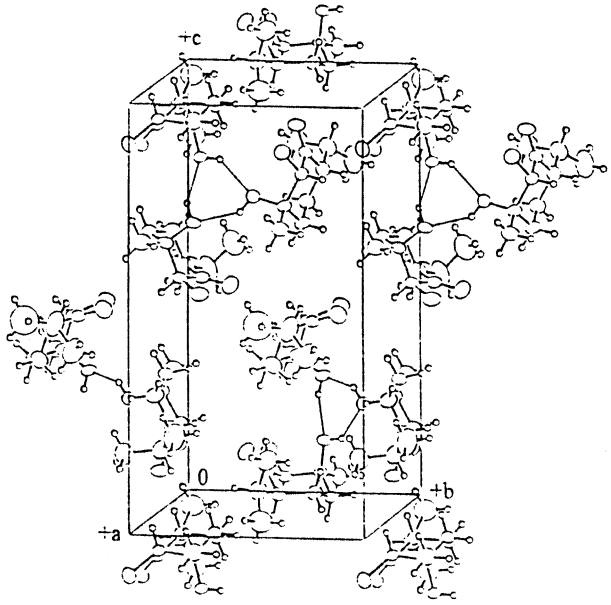


Fig. 2. Molecular aggregation in compound **1**.

to sitosterol and sugar moiety, additional signals appeared in the ^{1}H NMR spectrum at δ 0.82 as a doublet of six protons. The ^{13}C NMR also showed five extra carbons which appeared at δ 173.5 (C=O, ketone), 42.3 (CH_2), 26.3 (CH), 22.1 and 22.5 (2×Me). The presence of five extra carbons and a doublet of two methyls concluded that the molecule has a 3-methylbutanoate moiety, which through 2D NMR experiments (Fig. 3) was confirmed at methylene of sugar unit (C-6′).

In this way, appearance of only three acetates and the downfield shifts of sugar protons could be justified. A number of methylene substituted glycosides have been reported in the literature (Garcia et al., 1989; Gournelis et al., 1989; Park et al., 2000). Chemical shifts (^{1}H and ^{13}C) of sugar unit were exactly matched with the reported data of acetylated glucose (Jensen et al., 1981). Finally, the structure of discussed compound was established as 3-*O*-β-D-[6′ (3″-methylbutanoate) glucopyranosyl-β-sitosterol (**2**) and named longiside-A. Sitosteryl glycosides have been reported form *M. arvensis* (Shimizu et al., 1990). However, this compound is a new addition in natural products having substituted glucose methylene.

The electron impact mass and ^{1}H NMR spectral data of **3a** were exactly matched with data of **2a** except that the absence of a doublet of two methyls at δ 0.82 and instead of that a triplet of methyl at δ 0.86 and a triplet of methylene (CH_2-2″) at δ 2.20 were observed in the ^{1}H NMR spectrum of **3a**. In the ^{13}C NMR spectrum, this methyl resonated at δ 14.5. This information revealed that methylene-6′ of glucose unit was coupled with a fatty acid instead of 3-methylbutanoate as was observed in **2a**. On the bases of mass spectral information, the length of carbon chain of fatty moiety was deduced as palmitic acid. The structure of above discussed compound was thus elucidated as **3a**. Therefore, the original

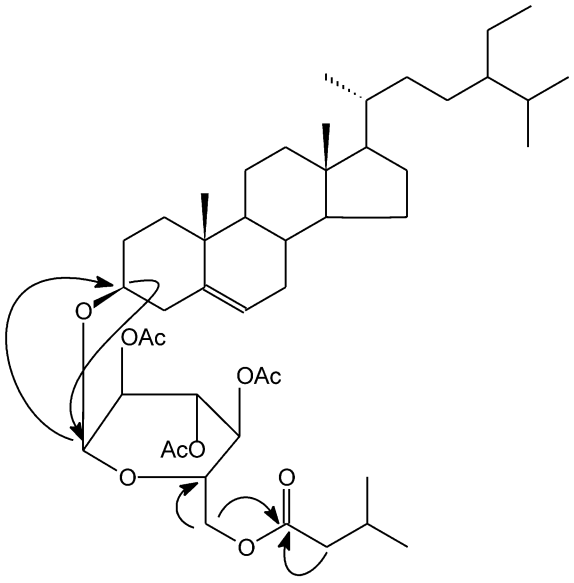


Fig. 3. Important HMBC interactions in compound **2a**.

compound (natural metabolite) would have the structure **3** and named longiside-B. This is also a new addition in the constituents of *M. longifolia*.

Fraction eluted with addition of methanol in chloroform (5%) was subjected to acetylation. Compound **4a** was obtained as a yellowish gum. The EIMS of **4a** showed a peak at *m/z* 286 after the lose of sugar moiety. Therefore, in order to get the complete mass of **4a**, the FAB–MS in positive mode of the sample was scanned, which showed the pseudo-molecular ion peak at *m/z* 847 (M + H). The formula, ($C_{40}H_{46} + HO_{20}$) of molecular ion peak was obtained with the aid of HRMS in +ve mode showing the presence of eighteen degrees of unsaturation. The infrared spectrum of **4a** displayed four significant absorptions at 3520, 1740, 1660 and 1635 cm^{-1} due to the hydroxyl, carbonyls (ester and ketonic) and olefinic functions, respectively.

The proton NMR spectrum of **4a** exhibited a bunch of singlets between δ 2.05–1.98 of altogether eighteen protons integration confirming the presence of six methyls of acetyl moieties, which were further confirmed through carbon spectrum showing six signals between δ 20.8–20.5 and their corresponding carbonyl-carbons appeared between δ 170.1–169.1. In addition to six acetyl moieties, the NMR spectra of **4a** displayed a doublet of secondary methyl and a singlet of methoxyl at δ 1.14 (*d*, *J* = 6.3 Hz, H-6‴) and 3.82, their associated carbons showed their presence at δ 18.2 and 55.3, respectively, in the carbon spectrum. The proton NMR spectrum also showed four aromatic protons at δ 6.11 (1H, *d*, *J* = 2.4 Hz, H-6), 6.07 (1H, *d*, *J* = 2.4 Hz, H-8), 7.38 (2H, *d*, *J* = 9.0 Hz, H-2′ and H-6′) and 6.95 (2H, *d*,

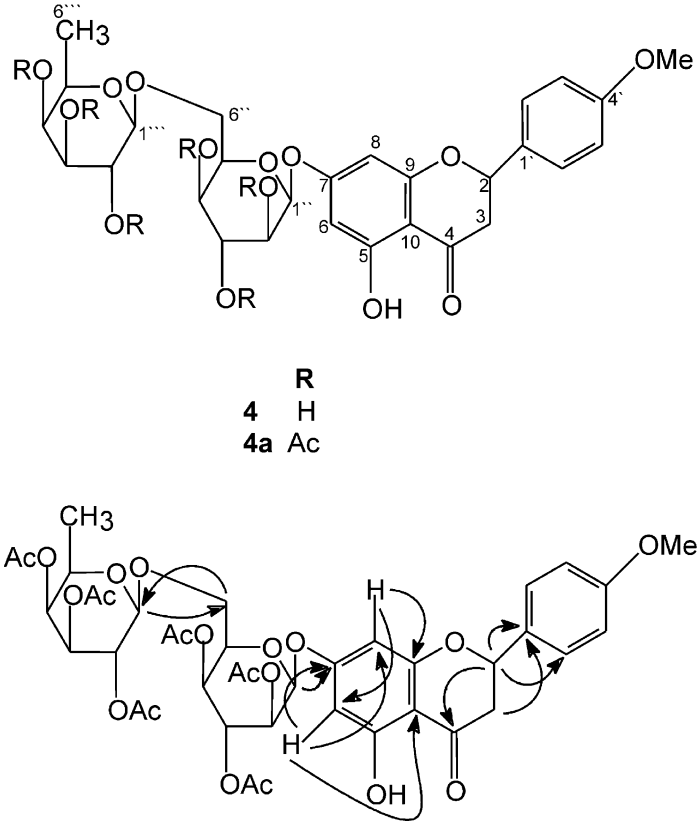


Fig. 4. HMBC connectivities in compound **4a**.

$J = 9.0$ Hz, H-3′ and H-5′). Their corresponding carbons appeared in the carbon spectrum at δ 97.3 (C-6), 96.0 (C-8), 127.7 (C-2′ and 6′) and 114.2 (C-3′ and 5′). Presence of six acetyl moieties and two anomeric signals [δ 5.34 ($J = 7.8$ Hz) (100.4) and 5.85 ($J = 7.6$ Hz) (99.8)] in the NMR spectra showed that **4a** having two hexose units.

The above-discussed data gave an idea of flavanone type skeleton. However, the signals at δ 2.81–2.76 (2H, *dd*, $J = 12.9$, 3.2 Hz, H-3), 5.44 (1H, *dd*, $J = 12.9$, 3.0 Hz, H-2) and δ 43.1 (C-3), 78.9 (C-2) in the NMR spectra further helped to characterize the discussed compound as **4a** containing fucopyranosyl 1→6 glucopyranosy moiety at C-7. The characterization and position of glyco-moiety in **4a** were determined with the aid of spectral data and HMBC connectivities (Fig. 4). The structure was finally confirmed with the aid of reported data of similar type of compounds (Jayaprakasam et al., 2000; Vasconcelos et al., 1998) as naringenin 4′-methyl ether 7-*O*-fucoparanosyl-1→6 glucoside. C-7 glycosides-flavones (Kamiya et al., 1979) and flavones (Ahmad et al., 1986) are common metabolites of the genus *Mentha*. In conclusion, the natural compound would have the structure **4**. This is also a new addition in natural products obtained from *M. longifolia* and named longitin.

3. Experimental

3.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300, 500 MHz and 100, 125 MHz, respectively.

3.2. Collection, identification and extraction

The plant material (aerial parts) was collected from Northern areas of Pakistan and identified by Dr. Abdul Rasheed, Department of Botany, Peshawar University, Peshawar (Pakistan) where the voucher specimen has been deposited in the herbarium (No. 240-PHU1998).

The collected plant material was dried under shade for one week. The dried material (8 kg) was then soaked in methanol for 10 days. The resulted extract was concentrated (230 g) by means of evaporation and re-diluted with distilled water. The organic material was recovered in ethyl acetate. The ethyl acetate soluble part after concentration (198 g), subjected to silica gel column chromatography using hexane, hexane: chloroform, chloroform and chloroform: methanol as mobile phase.

3.3. Isolation, purification and characterization

Fraction eluted with 20% chloroform in hexane yielded colorless shining crystals which on washing with methanol yielded **1** (30.0 mg).

3.3.1. Longifone (*1*)

$[\alpha]_D$: +100° (chloroform, *c* 0.032); m.p.: 100.5–101 °C; IR ($CHCl_3$): 3550 (OH), 1735 (C=O, ketone) cm^{-1}; EIMS: *m/z* 204 $[M]^+$, 206 $[M+2]^+$, 189 $[M–Me]^+$, 168 $[M–HCl]^+$; HRMS: *m/z* 204.012187 (calc. *m/z* 204.016992 for $C_{10}H_{17}O_2Cl$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.53 (1H, *d*, $J = 0.5$ Hz, H-1), 2.12 (1H, *m*, H-3), 1.83 (1H, *m*, H-8), 1.40 (3H, *s*, H-7), 0.92 and 0.88 (3H each, *d*, $J = 6.5$ Hz, H-9 and 10); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 75.2 (C-1), 200.1 (C-2), 56.4 (C-3), 24.0 (C-4), 37.7 (C-5), 77.3 (C-6), 29.3 (C-7), 27.03 (C-8), 19.1 (C-9) and 21.5 (C-10); X-ray: the X-ray data are deposited with the X-ray Crystallographic Center, Cambridge, UK (CCDC 151302).

Further elution with 100% chloroform a fraction obtained, which showed two very close spots on TLC. After evaporation the material was re-dissolved in dry pyridine and acetic anhydride was added. This mixture was left overnight at room temperature. The resulted product was chromatographed on silica gel to yield compounds **2a** (15.0 mg) and **3a** (13.2 mg) as oily masses.

3.3.2. Longiside-A (*2a*)

$[\alpha]_D$: $+79.9^0$ (chloroform, *c* 0.082); EIMS *m/z:* 414 [M-sugar], 396 (100%), 275, 255; (+ve) FAB–MS: 787 (M + H); (+ve) FAB–HRMS: *m/z* 787.537561 (calc. *m/z* 787.53598 for $C_{46}H_{75}O_{10}$); IR ($CHCl_3$): 1740 (C=O, ester), 1610 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.33 (1H, distorted triplet, H-6), 5.21 (1H, *t*, $J = 8.0$ Hz, H-3′), 5.00 (2H, *m*, H-4′ and 5′), 4.50 (1H, *d*, $J = 7.0$ Hz, H-1′), 4.16–4.28 (2H, *m*, H-6′), 3.43 (1H, *m*, H-3), 2.00, 1.99, 1.98 (3×OAc), 0.97 (3H, *s*, H-19), 0.91 (3H, *d*, $J = 6.5$ Hz, H-21), 0.83 (3H, *t*, $J = 6.5$ Hz, H-29), 0.82 (6H, *d*, $J = 6.7$ Hz, H-4″ and 5″), 0.80 (6H, *d*, $J = 6.8$ Hz, H-26 and 27) and 0.66 (3H, *s*, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 37.2 (C-1), 29.4 (C-2), 80.1 (C-3), 38.9 (C-4), 140.3 (C-5), 122.1 (C-6), 31.9 (C-7), 31.8 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.7 (C-12), 42.2 (C-13), 56.7 (C-14), 24.2 (C-15), 28.2 (C-16), 56.0 (C-17), 11.9 (C-18), 19.5 (C-19), 36.1 (C-20), 18.8 (C-21), 33.9 (C-22), 26.0 (C-23), 45.8 (C-24), 29.1 (C-25), 19.8 (C-26), 19.0 (C-27), 23.0 (C-28), 12.0 (C-29) 99.6 (C-1′), 71.5 (C-2′), 71.7 (C-3′), 68.5 (C-4′), 72.9 (C-5′), 61.9 (C-6′), 173.5 (C-1″), 42.3 (C-2″), 26.3 (C-3″), 22.1 and 22.5 (C-4″ and 5″), 170.3–169.8 (3×CH_3**C**O), and 21.0–20.7 (3×CH_3CO).

3.3.3. Longiside-B (*3a*)

$[\alpha]_D$: +61.9° (chloroform, *c* 0.069); EIMS: *m/z* 414 [M-sugar], 396 (100%), 275, 255; (+ve) FAB–MS: *m/z* 941 $[M+H]^+$; (+ve) FAB–HRMS: *m/z* 941.6984 (calc. *m/z* 941.70812 for $C_{57}H_{97}O_{10}$); IR ($CHCl_3$): 1735 (C=O, ester), 1620 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.33 (1H, distorted triplet, H-6), 5.24 (1H, *t*, $J = 8.0$ Hz, H-3′ and 5′), 5.01–4.91 (2H, *m*, H-4′ and

2′), 4.52 (1H, *d*, *J* = 7.9 Hz, H-1′), 4.20–4.05 (2H, *m*, H-6′), 3.42 (1H, *m*, H-3), 2.20 (2H, *d*, *J* = 7.1 Hz, H-2″), 2.01–1.98 (3×OAc), 0.97 (3H, *s*, H-19), 0.91 (3H, *d*, *J* = 6.5 Hz, H-21), 0.86 (3H, *t*, *J* = 7.0 Hz, H-16″) and 0.82–0.81 (9H, *d* and *t*, *J* = 6.5 Hz, H-26, 27 and 29); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 37.0 (C-1), 29.5 (C-2), 79.7 (C-3), 38.7 (C-4), 140.2 (C-5), 122.3 (C-6), 32.03 (C-7), 31.9 (C-8), 50.1 (C-9), 36.6 (C-10), 21.8 (C-11), 39.6 (C-12), 42.2 (C-13), 56.6 (C-14), 24.2 (C-15), 28.1 (C-16), 55.9 (C-17), 11.7 (C-18), 19.2 (C-19), 36.0 (C-20), 18.6 (C-21), 34.0 (C-22), 26.0 (C-23), 45.7 (C-24), 29.2 (C-25), 19.6 (C-26), 18.9 (C-27), 23.0 (C-28), 11.8 (C-29), 99.6 (C-1′), 71.4 (C-2′), 71.8 (C-3′), 68.7 (C-4′), 72.9 (C-5′), 61.1 (C-6′), 174.6 (C-1″), 170.1–169.5 (3×CH_3CO), 21.0–20.7 (3×CH_3CO), 32.5 (C-2″), 30.1 (C-3″), 29.1–28.2 (C-4″–C-15″) and 14.5 (C-16″).

An impure fraction eluted with 5% methanol in chloroform was treated with pyridine and acetic anhydride to give **4a** as a yellowish gum (20 mg).

3.3.4. Longitin (*4a*)

$[\alpha]_D$: +81° (chloroform, *c* 0.071); EIMS: *m*/*z* 286 [M-(fuc-glc)]$^+$, 269 (286-OH)$^+$, 134 (100%); (+ve) FAB–MS: *m*/*z* 847 $[M+H]^+$; (+ve) FAB–HRMS: *m*/*z* 847.26913 (calc. *m*/*z* 847.26603 for $C_{40}H_{47}O_{20}$); UV (MeOH): λ_{max} 290 (log ε 4.3), 325 (*sh*); IR ($CHCl_3$): 3520 (OH), 1740 (C=O, ester), 1660 (C=O, ketone), 1635 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.03 (1H, *s*, OH), 7.38 (2H, *d*, *J* = 9.0 Hz, H-2′ and 6′), 6.95 (2H, *d*, *J* = 9.01 Hz, H-3′ and 5′), 6.11 (1H, d, *J* = 2.4 Hz, H-6), 6.07 (1H, *d*, *J* = 2.4 Hz, H-8), 5.47–5.10 (*m*, sugar methines), 5.44 (1H, *dd*, *J* = 12.9,3.0 Hz,H-2), 5.34 (1H, *d*, *J* = 7.8 Hz, H-1″), 4.85 (1H, *d*, *J* = 7.6 Hz,H-1‴), 3.82 (3H, *s*, OMe), 2.81–2.76 (2H, *dd*, *J* = 12.9, 3.2 Hz, H-3), 2.05–1.98 (6×OAc) and 1.14 (3H, *d*, *J* = 6.3 Hz, H-6‴); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 78.9 (C-2), 43.1 (C-3), 195.4 (C-4), 160.1 (C-5), 97.3 (C-6), 166.5 (C-7), 96.0 (C-8), 162.0 (C-9), 104.4 (C-10), 130.1 (C-1′), 127.7 (C-2′ and 6′), 114.2 (C-3′ and 5′), 160.1 (C-4′), 100.4 (C-1″), 66.3 (C-6″), 99.8 (C-1‴), 18.2 (C-6‴), 73.4–68.8 (sugar methines), 20.8–20.5 (6×CH_3CO), 170.1–169.1 (6×CH_3CO) and 55.3 (OCH_3).

Acknowledgements

We are thankful to Mr. Taous Khan, Department of Chemistry, Peshawar University, for providing the plant material and Dr. Abdul Rasheed, Department of Botany of the same university for the identification of plant material; and also to Ms. Ishrat Sultana of Chemistry Department, University of Karachi, for the halogen test.

References

Abu-Al-Futuh, M.I., Abdelmageed, O.H., Jamil, R.M., Avato, P., 2000. Apiperitenone oxide chemotype of *Mentha longifolia* growing wild in Jordan. J. Essential Oil Res. 12, 530–532.

Ahmad, A.A., Norris, J.A., Mabry, T.J., 1986. Flavonoids of *Brickellia vernicosa*. Phytochemistry 25, 1501–1502.

Ahmad, V.U., Farooqui, T.A., Fizza, K., Sultana, A., Khatoon, R., 1992. Purification of three new eudesmane sesquiterpenes from *Pluchea arguta*. J. Nat. Prod. 55, 730–735.

Ali, M.S., Shameel, S., Ahmad, V.U., Usmaghani, K., 1997. Chemical constituents of *Caesalpinia bonduc*. Pak. J. Sci. Ind. Res. 40, 20–22.

Ali, S.I., Nasir, Y.J., 1990. Flora of Pakistan No. 192, 256.

Baser, K.H.C., Kurkcuoglu, M., 1999. Essential oils of *Mentha* species from Northern Turkey. J. Essent. Oil Res. 11, 579–588.

Baser, K.H.C., Nuriddinov, Kh.R., Nigmatullaev, A.M., Aripov, Kh.N., 1997. Essential oils of *Mentha asiatica* from Uzbekistan. J. Essent. Oil Res. 9, 453–454.

Dembitskii, V.M., Tolstikov, G.A., 1999. Natural halogenated monoterpenoids. Khim. Interesakh Ustoich. Razvit. 7, 601–614.

Garcia, J., Mpondo, E.M., Nardin, R., 1989. Loganin and a new iridoid glucoside from *Gentiana pyrenaica*. J. Nat. Prod. 52, 423–425.

Ghoulami, S., Idrissi, A., Fkih-Tetouani, S., 2001. Phytochemical study of *Mentha longifolia* of Morocco. Fitoterapia 72, 596–598.

Gonzalez, A.G., Darias, A., Alonso, G., Estevenz, E., 1980. The cytostatic activity of the chlorohyssopifolins, chlorinated sesquiterpene-lactones from *Centaurea*. Planta Med. 40, 179–184.

Gournelis, D., Skaltsounis, A.L., Tillequin, F., Koch, M., 1989. Plantes de nouvelle-caledonie, CXXI. Ididoides et alcaloides de *Plectronia odorat*. J. Nat. Prod. 52, 306–316.

Guido, S., Alessandra, B., Guido, F., Luigi, C.P., Emilio, T.P., 1997. Variability of essential oil composition of *Mentha aquatica* collected in two different habitats of North Tuscany, Italy. J. Essent. Oil Res. 9, 455–457.

Jayaprakasam, B., Damu, A.G., Gunasekar, D., Bolond, A., Bodo, A., 2000. A biflavanone from *Cycas beddomei*. Phytochemistry 53, 515–517.

Jensen, S.R., Mikkelsen, C.B., Nielsen, B.J., 1981. Iridoid mono- and diglycosides in *Mentzelia*. Phytochemistry 20, 71–83.

Kamiya, S., Esaki, S., Konichi, F., 1979. Flavonoids in citrus and related genera. Part VII. Flavonoids in citrus hybrids. Agric. Biol. Chem. 43, 1529–1536.

Lapinskas, P., 1993. Factors affecting the commercial success of a novel crop. Acta Hort. 333, 72.

Nori-Shargh, D., NorouziArasi, H., Mohammad, S., Mirza, M., Jaimand, K., 2000. Volatile components of *Mentha longifolia* from Iran. J. Essent. Oil Res. 12, 111–112.

Park, E.J., Kim, Y., Kim, J., 2000. Acylated flavonol glycosides from the flower of *Inula britannica*. J. Nat. Prod 63, 34.

Sakata, I., Koshimizu, K., 1978. Constituents of Japanese peppermint. Part VII. Occurrence of 1-methyl-β-D-glucoside and methyl palmitate in rhizoma of Japanese peppermint. Agric. Biol. Chem. 42, 1959–1960.

Sakata, I., Mitsui, T., 1975. Constituents of Japanese peppermint. VI. Isolation and identification of 1-methyl-β-D-glucoside from Subi. Agric. Biol. Chem. 39, 1329–1330.

Shameel, S., Usmanghani, K., Ali, M.S., Ahmad, V.U., 1996. Chemical constituents from the seeds of *Pongamia pinnata*. Pak. J. Pharm. Sci. 9, 11–20.

Sharaf, M., ElAnsari, M.A., Saleh, N.A.M., 1999. Flavone glycosides from *Mentha longifolia*. Fitoterapia 70, 478–483.

Shimizu, S., Shibata, H., Maejima, S., 1990. A new monoterpene glucoside 1-methyl 6′-*O*-acetyl-β-D-glucoside in *Mentha arvensis*. (Studies on terpene glucosoides in *Mentha* plants. Part I). J. Essent. Oil Res. 2, 21–24.

Vasconcelos, J.M.J., Silva, A.M.S., Cavaleiro, J.A.S., 1998. Chromones and flavanones from *Artemisa campestris*. Phytochemistry 49, 1421–1424.

Youssef, D., Frahm, A.W., 1994. Constituents of the Egyptian *Centaurea scoparia*; chlorinated guaianolides of the aerial parts. Planta Med. 60, 267–271.