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ИССЛЕДОВАНИЕ БИОЛОГИЧЕСКИ АКТИВНЫХ ГЛИКОЗИДОВ ПОЛУЧЕННЫХ СПИРТОВОЙ ВЫТЯЖКОЙ ИЗ НЕПАЛЬСКОГО САНДАЛОВОГО ДЕРЕВА *OSYRIS WIGHTIANA* (НЕТРАДИЦИОННОЕ НАЗВАНИЕ WALL EX WIGHT)

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Спиртовые фракции сырой древесины сандалового дерева Osyris wightiana (непальское название Wall ex Wight) проанализированы с помощью хроматографических и спектральных методов на наличие биологически активных химических компонентов. С помощью колоночной хроматографии и ВЭЖХ из бутанольной фракции экстрактов выделены новые классы гликозидов. Структуры нескольких выявленных соединений охарактеризованы при помощи комплекса физико-химических методов: спектроскопических методик, ЯМР и масс-спектрометрических исследований.

Ключевые слова: биологически активные соединения, Osyris wightiana, хроматография, структура, сандаловое дерево, спиртовая экстракция.

Introduction

Osyris wightiana Wall. ex Wight is a shrub about 2–3 m tall. It is widely distributed in the tropical and temperate zones at the altitudes of 900 to 2,500 m from Simla to Bhutan, Myanmar, India, Nepal and China [1]. The different species of the genus *Osyris* are used for the gynecological complaints of menorrhagia and infertility in Africa. The root bark boiled in water, is given to women after childbirth to stop bleeding and to boost energy. The leaves, roots, woods, barks and fruits have been used medicinally in the traditional healing system, mostly in Asia and Africa [2]. The dried leaves of *Osyris* are commonly used as a substitute of tea in the central part of Nepal. Despite of its important potential uses, there are the limited phytochemical studies of the genus *Osyris*. These facts inspired us to undertake the present phytochemical investigation.

From the genus *Osyris*, phenyl propanoid, benzyl alcohol, iridoid and megastigmane, were isolated from the butanolic fractions as the new source. Volatile constituents of the genus *Osyris* have been mostly analysed due to its fragrance. Previous studies in the genus *Osyris* led to isolation of hexyl and hexenyl derivatives, sesquiterpenes, phenolic acids, flavonoids, pyrrolizidine and quinolizidine alkaloids, long chain hydrocarbons and fatty acids, triterpenes, dihydro-β-agarofuran sesquiterpenes, lignans and phenolics [3–13]. Syringin (**1**) belongs to phenyl propanoids and di-*O*-methylcrenatin (**2**) is benzyl alcohol derivative. They are shikimic acid derived products and have significant biological activities. 8-Epideoxyloganic acid (**3**) is iridoid belonging to the subclass of terpenoidal metabolites. Megastigmanes like citroside B (**4**) and roseoside (**5**) form a norisoprenoid class of compounds formed by the enzymatic degradation of carotenoids in plants biosynthesis. These compounds contribute to flavors of the fruits and wines. Here the detail reports of isolation and structure elucidation of megastigmanes, iridoid, benzyl alcohol and phenyl propanoid from the new source, *O. wightiana*, are presented.

Experimental

Plant Material

The aerial parts of *O. wightiana* were collected from Kavre district, Nepal, in August 2005 at the altitudes of 1,600 m to 1,700 m. It was identified by comparing with herbarium specimen at the National Herbarium laboratory, Department of Plant Resources, Godawari, Nepal.

Column Chromatography

Diaion HP-20 resin was used for fractionation of the crude *n*-butanolic extract. Polyamide was further used for preparing sub-fractions. Final purifications of com-

pounds were achieved by preparative recycling HPLC (LC-908, JAI) and the columns L-80 (YMC. Co. Ltd.). It was eluted with MeOH and H_2O system. Precoated silica gel TLC plates (E. Merck, F_{254}) were used for checking the purity of compounds.

UV and IR Spectrophotometer

Shimadzu UV 240 spectrophotometer was used for determination of λ_{max} for isolated compounds. The IR spectra of pure compounds were recorded by using JASCO A-320 spectrophotometre.

^{1}H NMR and ^{13}C NMR

^{1}H NMR spectra were recorded on Bruker AC-300 and AMX-500 MHz instrument, while ^{13}C NMR spectra were recorded at 100 and 125 MHz. ^{1}H-NMR and ^{13}C-NMR chemical shifts were reported here in δ (ppm) and coupling constant values (*J*) were measured in Hz. Multiplicities of carbon signals were determined by DEPT 90^o and 135^o experiments. 2D NMR spectra were recorded on a Bruker Avance AMX 500 NMR spectrometer.

Mass Spectrometry

The EI MS spectra were recorded on mass spectrometer Varian MAT 312. FAB MS and HREI MS experiments were performed on Jeol HX 110 mass spectrometer. The ion peaks are presented in *m/z* (%).

Extraction and Isolation

The air dried aerial parts of the plant (6.3 Kg) was first extracted with 80% ethanol/water. Then it was successively extracted by *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. The *n*-butanol fraction (64.5 g) was first fractionated by column chromatography (Diaion HP-20, 200 g) by using H_2O/MeOH as the solvent system. Five sub-fractions were obtained by gradual elution with increasing methanol in H_2O/MeOH system. The sub-fraction OB-52 (15 g, eluted with 5% to 10% of MeOH in H_2O) and OB-53 (11 g, eluted with 10% to 20% MeOH in H_2O) were further subjected to the polyamide column chromatography with $CHCl_3$-MeOH as the solvent. 8-Epideoxyloganic acid (**3**) was obtained from the sub-fraction OB-52, on eluting through recycling HPLC (using L-80 column) at 1:1 MeOH: H_2O solvent system. Syringin (**1**), di-*O*-methylcrenatin (**2**), citroside B (**4**) and roseoside (**5**) were isolated from sub-fraction OB-53 on eluting through recycling HPLC (using L-80 column) at 1:1 MeOH: H_2O solvent system.

Syringin (1). Crystalline needles, M. p.: 192–193^{o}C; UV (EtOH) λ_{max} nm: 265, 222; IR (KBr) λ_{max} cm^{-1}: 3400 (OH), 1585 (C=C); EI MS *m/z* (rel. int. %): 210 (100), 196 (2), 182 (20), 167 (22), 154 (7), 139 (4); ^{1}H NMR, (500 MHz, MeOH-d4): δ_H 6.74 (*s*, 2H, H-2 and H-6), 6.52 (*d*, $J_{1',2'}$ =15.8 Hz, 1H, H-1′), 6.33 (*dt*, $J_{2',1'}$ = 15.8 Hz, $J_{2',3'}$ = 5.5 Hz, 1H, H-2′), 4.21 (*d*, $J_{3',2'}$ = 5.5 Hz, 2H, H-3′), 4.86 (*d*, $J_{1'',2''}$ = 7.5 Hz, 1H, H-1′′), 3.85 (*s*, 6H, 2×OCH_3), 3.78 (*dd*, $J_{6''a,6''b}$ = 11.9 Hz, $J_{6''a,5''}$ = 2.2 Hz, 1H, H6′′a), 3.66 (*dd*, $J_{6''b',6''a}$ = 11.9 Hz, $J_{6''b,5''}$ = 5.1 Hz, 1H, H6′′b), 3.46 (*m*, 1H, H-2′′), 3.40 (*m*, 1H, H-4′′), 3.39 (*m*, 1H, H-5′′), 3.20 (*m*, 1H, H-3′′); ^{13}C-NMR (125 MHz, MeOH-d4): δ_C 154.4 (C-3 and C-5), 135.9 (C-4), 135.3 (C-1), 131.3 (C-1′), 130.1 (C-2′), 105.5 (C-2 and C-6), 105.4 (C-1′′), 78.3 (C-3′′), 77.8 (C-5′′), 75.7 (C-2′′), 71.4 (C-4′′), 63.5 (C-3′), 62.6 (C-6′′), 57.0 (2×CH_3O).

Di-*O*-methylcrenatin (2). Crystalline needles, M. p.: 175–177^{o}C, EI MS *m/z* (rel. int. %): 184 (100), 167 (13), 155 (5), 123 (11), 109 (11), 73 (21); ^{1}H-NMR (500 MHz, MeOH-d4): δ_H 6.69 (*s*, 2H, H-2 and H-6), 4.83 (*d*, $J_{1',2'}$ = 7.5 Hz, 1H, H-1′), 4.54 (*s*, 2H, H-1a), 3.84 (*s*, 6H, 2×OCH_3), 3.77 (*dd*, $J_{6'a,6'b}$ =11.9 Hz, $J_{6a',5'}$ = 2.1 Hz, 1H, H-6′a), 3.66 (*dd*, $J_{6'b,6'a}$ = 11.9 Hz, $J_{6'b,5'}$ = 5.1 Hz, 1H, H-6′b), 3.47 (*m*, 1H, H-2′), 3.41 (*m*, 1H, H-4′), 3.40 (*m*, 1H, H-5′), 3.19 (*m*, 1H, H-3′); ^{13}C-NMR (125 MHz, MeOH-d4): δ_C 154.2 (C-3 and C-5), 139.7 (C-1), 135.4 (C-4), 105.8 (C-2 and C-6), 105.5 (C-1′), 78.3 (C-3′), 77.8 (C-5′), 75.7 (C-2′), 71.3 (C-4′), 65.0 (C-1a), 62.5 (C-6′), 57.0 (2×OCH_3).

8-Epideoxyloganic acid (3). Colorless crystalline needles, M. p.: 106^{o}C; UV (EtOH) λ_{max} nm (log ε): 235 (4.0); IR (KBr) λ_{max} cm^{-1}: 3300 (OH), 1658, 1635 (C=C); EI MS *m/z* (rel. int. %): 198 (21), 180 (29), 154 (45), 137 (37), 125 (40), 81 (99), 57 (100); ^{1}H-NMR (300 MHz, MeOH-d4): δ_H 7.29 (*s*, 1H, H-3), 5.41 (*d*, $J_{1,9}$ = 5.0 Hz, 1H, H-1), 4.68 (*d*, $J_{1',2'}$ = 7.8 Hz, 1H, H-1′), 3.91 (*br. d*, $J_{6'a,6'b}$ = 11.7 Hz, 1H, H-6′a), 3.63 (*dd*, $J_{6'b,6'a}$ = 11.7 Hz, $J_{6'b,5'}$ = 5.8 Hz, 1H, H-6′b), 3.36 (*m*, 1H, H-3′), 3.23 (*m*, 1H, H-4′), 3.27 (*m*, 1H, H-5′), 3.18 (*m*, 1H, H-2′), 2.91 (*m*, 1H, H-5), 2.24 (*m*, 1H, H-9), 2.17 (*m*, 1H, H-8), 2.02 (*m*, 1H, H-6a), 1.77 (*m*, 1H, H-7a), 1.61 (*m*, 1H, H-6b), 1.34 (*m*, 1H, H-7b), 1.06 (*d*, $J_{10,8}$ = 6.3 Hz, 3H, CH_3); ^{13}C-NMR (125 MHz, MeOH-d4): δ_C 173.2 (CO_2H), 150.6 (C-3), 115.9 (C-4), 99.6 (C-1′), 95.8 (C-1), 78.3 (C-5′), 77.9 (C-3′), 74.8 (C-2′), 71.7 (C-4′), 62.9 (C-6′), 44.4 (C-9), 37.5 (C-8), 34.9 (C-5), 33.4 (C-7), 32.3 (C-6), 16.8 (CH_3).

Citroside B (4). Colorless amorphous solid, UV ($CHCl_3$) λ_{max} nm (log ε): 230 (4.17); IR (KBr) λ_{max} cm^{-1}: 3400 (OH), 1945 (C=C=C), 1675 (C=O); EI MS *m/z* (rel. int. %): 206 (36), 191 (48), 173 (89), 162 (16), 147 (39), 119 (60), 91 (100); ^{1}H-NMR (500 MHz, MeOH-d4): δ_H 5.88 (*s*, 1H, H-8), 4.52 (*d*, $J_{1',2'}$ = 7.6 Hz, 1H, H-1′), 4.31 (*tt*, $J_{3,2b/3,4b}$ = 11.5 Hz, $J_{3,2a/3,4a}$ = 1.7 Hz, 1H, H-3), 3.81

(*dd*, $J_{6'a,6'b}$ = 11.7 Hz, $J_{6'a,5'}$ = 2.0 Hz, 1H, H-6′a), 3.61 (*dd*, $J_{6'b,6'a}$ = 11.7 Hz, $J_{6'b,5'}$ = 5.3 Hz, 1H, H-6′b), 3.34 (*m*, 1H, H-3′), 3.30 (*m*, 1H, H-5′), 3.21 (*m*, 1H, H-4′), 3.15 (*m*, 1H, H-2′), 2.48 (*dd*, $J_{4a,4b}$ = 11.5 Hz, $J_{4a,3}$ =1.6 Hz, 1H, H-4a), 2.18 (*s*, 3H, H-10), 1.92 (*dd*, $J_{2a,2b}$ = 11.5 Hz, $J_{2a,3}$ = 1.7 Hz, 1H, H-2a), 1.45 (*s*, 3H, CH_3-13), 1.36 (*s*, 3H, CH_3-12), 1.33 (Overlapped, H-4b), 1.30 (Overlapped, H-2b), 1.14 (*s*, 3H, CH_3-11); ^{13}C-NMR (125 MHz, MeOH-d4): δ_C 212.9 (C-7), 200.6 (CO), 119.1 (C-6), 101.3 (C-8), 98.6 (C-1′), 78.7 (C-1), 78.6 (C-3′), 77.8 (C-5′), 75.2 (C-2′), 71.7 (C-4′), 63.8 (C-3), 62.9 (C-6′), 49.9 (C-2), 48.0 (C-4), 37.0 (C-5), 32.5 (CH_3-12), 30.6 (CH_3-11), 26.7 (CH_3-13), 26.6 (CH_3-10).

Roseoside (5). Colorless amorphous soild, UV (MeOH) λ_{max} nm (log ε): 237 (4.06), 316 (2.55); IR (KBr) λ_{max} cm^{-1}: 3390 (OH), 1648 (enone); EI MS *m/z* (rel. int. %): 387 (3), 225 (13), 207 (57), 191 (5), 150 (100), 124 (71), 95 (21); HREI MS *m/z*: 386.4367 ($C_{19}H_{30}O_8$); ^{1}H-NMR (500 MHz, MeOH-d4): δ_H 5.86 (Overlapped, H-8), 5.85 (Overlapped, H-7), 5.84 (*s*, 1H, H-4), 4.41 (*m*, 1H, H-9), 4.34 (*d*, $J_{1',2'}$ = 7.8 Hz, 1H, H-1′), 3.84 (*dd*, $J_{6'a,6'b}$ = 11.8 Hz, $J_{6'a,5'}$ = 1.7 Hz, 1H, H6′a), 3.62 (*dd*, $J_{6'a,6'b}$ = 11.8 Hz, $J_{6'b,5'}$ = 5.4 Hz, 1H, H6′b), 3.31 (Overlapped, H-5′), 3.30 (Overlapped, H-3′), 3.23 (*m*, 1H, H-4′), 3.16 (*m*, 1H, H-2′), 2.52 (*d*, $J_{2a,2b}$ = 16.8 Hz, 1H, H2a), 2.15 (*d*, $J_{2b,2a}$ =16.8 Hz, 1H, H2b), 1.91 (*s*, 3H, CH_3-13), 1.27 (*d*, $J_{10,9}$ = 6.4 Hz, 3H, CH_3-10), 1.03 (*s*, 3H, CH_3-12), 1.02 (*s*, 3H, CH_3-11); ^{13}C-NMR (125 MHz, MeOH-d4): δ_C 201.1 (CO), 167.2 (C-5), 135.2 (C-8), 131.5 (C-7), 127.2 (C-4), 102.7 (C-1′), 80.0 (C-6), 78.1 (C-3′), 78.0 (C-5′), 77.2 (C-9), 75.2 (C-2′), 71.6 (C-4′), 62.8 (C-6′), 50.7 (C-2), 42.4 (C-1), 24.6 (CH_3-12), 23.4 (CH_3-11), 21.1 (CH_3-10), 19.5 (CH_3-13).

Result and Discussion

Spectral data of compounds **1-5** are given in the experimental section. The spectral data of compounds were compared with the reported data for the identification.

Phenyl propanoid

The phenyl propanoid glycoside, syringin (**1**) was isolated as the crystalline needles (Fig. 1). The mass spectrum showed its base peak at *m/z* 210.1 ($C_{11}H_{14}O_4$) due to cleavage of glycoside moiety from the C-4 (OH). The ^{13}C NMR spectrum contains 17 carbon signals. It includes 2 methyl, 2 methylene, 9 methine, and 4 quaternary carbons. The downfield signals at δ_C 105.5 (C-2 and C-6), 154.4 (C-3 and C-5) and 57.0 (two methoxy groups) were equivalent. The downfield singlet at δ_H 6.74 (H-2 and H-6) corresponds to two equivalent aromatic protons. It showed that the aromatic ring was tetrasubstituted. The methyl singlet at δ_H 3.85 was assigned to methoxy groups. The ^{1}H NMR and ^{13}C NMR spectra contain characteristic signals for glucose moiety. The anomeric proton signal appeared at δ_H 4.86 (*d*, $J_{1'',2''}$ = 7.5 Hz, H-1″). The signals for remaining protons of glucose moiety appeared at δ_H 3.46 (*m*, H-2″), 3.20 (*m*, H-3″), 3.40 (*m*, H-4″), 3.39 (*m*, H-5″), 3.78 (*dd*, $J_{6''a,6''b}$ = 11.9 Hz, $J_{6a'',5''}$ = 2.2 Hz, H6″a), and 3.66 (*dd*, $J_{6''b,6''a}$ = 11.9 Hz, $J_{6''b,5''}$ = 5.1 Hz, H6″b). The downfield doublet at δ_H 6.52 ($J_{1',2'}$ = 15.8 Hz, H-1′) and doublet of triplet at δ_H 6.33 ($J_{2',1'}$ = 15.8 Hz, $J_{3',2'}$ = 5.5 Hz, H-2′) were assigned to olefinic protons which are in *trans* disposition, what is evident from their *J* values. The downfield methylene doublet at δ_H 4.21 ($J_{3',2'}$ = 5.5 Hz) was assigned to H-3′ geminal to hydroxyl group [14, 15].

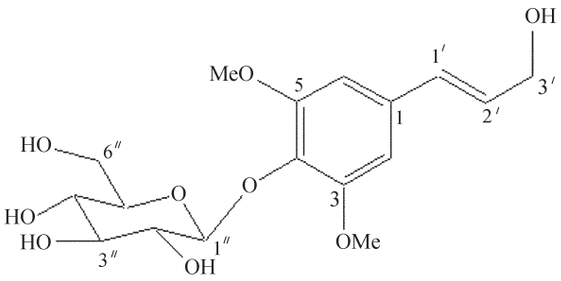

Fig. 1. Syringin (**1**)

Benzyl alcohol

Di-*O*-methylcrenatin (**2**) was obtained as the needle-like crystalline compound (Fig. 2). Its base peak was observed at *m/z* 184.0 ($C_9H_{12}O_4$) due to cleavage of glycoside moiety from C-4 (OH) in its mass spectrum. The ^{13}C NMR and its DEPT spectra showed 2 methyl, 2 methylene, 7 methine, and 4 quaternary carbons. The chemical shift values of ^{1}H NMR and ^{13}C NMR were similar to those in syringin (**1**), however, signals for olefinic protons are absent and singlet signal at δ_H 4.54 (2H, H-1a) was observed for methylene protons [16].

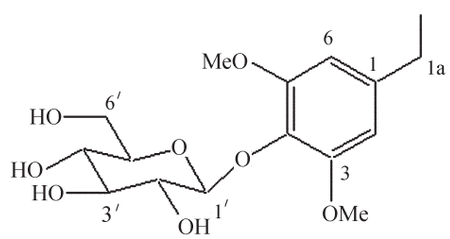

Fig. 2. Di-*O*-methylcrenatin (**2**)

Iridoids

8-Epideoxyloganic acid (**3**) was obtained as the colorless crystalline compound. (Fig. 3) The EI MS showed a prominent peak at *m*/*z* 198.1 ($C_{10}H_{14}O_4$) due to the cleavage of glycoside moiety from C-1 (OH). The molecular composition of compound (**3**) was deduced from FAB $^+$ve (*m*/*z* 361) and FAB $^-$ve (*m*/*z* 359) MS. The ^{13}C NMR (broad-band decoupled) spectrum of compound (**3**) showed a total 16 carbon signals, including 1 methyl, 3 methylene, 10 methine, and 2 quaternary carbons. The downfield singlet at δ_H 7.29 (H-3) was assigned to olephinic proton. It was showed by HMQC correlation with methine carbon at δ_C 150.6 (C-3). The H-3 proton showed HMBC correlations with carbons at δ_C 95.8 (C-1), 115.9 (C-4), 34.9 (C-5), and 173.2 (CO_2H). The downfield doublet at δ_H 5.41($J_{1,9}$ = 5.0 Hz) was assigned to H-1 acetal proton. The H-1 proton showed COSY coupling network to H-9 (*m*, 2.24), H-8 (*m*, 2.17), H-7a (*m*, 1.77), H-7b (*m*, 1.34), H-6a (*m*, 2.02), H-6b (*m*, 1.61) and H-5 (*m*, 2.91). It indicated the cyclopentane ring fused to six-membered heterocyclic ring system. The upfield methyl doublet signal at δ_H 1.06 ($J_{10,8}$ = 6.3 Hz, CH_3-10) was attached to C-8 (δ_C 37.5) according to its HMBC correlation. The stereochemistry was confirmed by comparing with the literature [17]. A doublet at δ_H 4.68 ($J_{1',2'}$ = 7.8 Hz) was assigned to anomeric proton H-1′ of glucose. Remaining proton signals of glucose were at δ_H 3.18 (*m*, H-2′), 3.36 (*m*, H-3′), 3.23 (*m*, H-4′), 3.27 (*m*, H-5′), 3.91 (*br. d*, $J_{6'a,6'b}$ = 11.7 Hz, H-6′a), and 3.63 (*dd*, $J_{6'b,6'a}$ = 11.7 Hz, $J_{6'b,5'}$ = 5.8 Hz, H-6′b).

Megastigmanes

Citroside B (**4**) was obtained as the colorless amorphous powder (Fig. 4). A prominent peak at *m*/*z* 206.1 ($C_{13}H_{18}O_2$) was observed in EI MS, due to the cleavage of glycoside moiety from the C-1 position. The ^{13}C NMR and its DEPT spectra showed 4 methyl, 3 methylene, 7

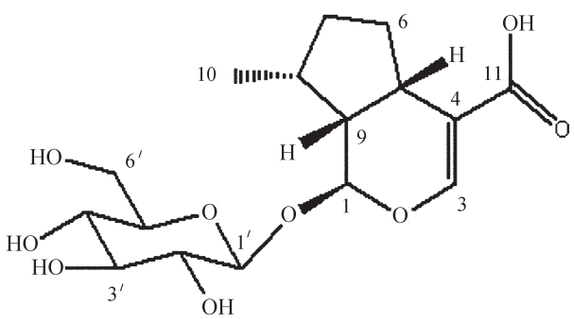

Fig. 3. 8-Epideoxyloganic acid (**3**)

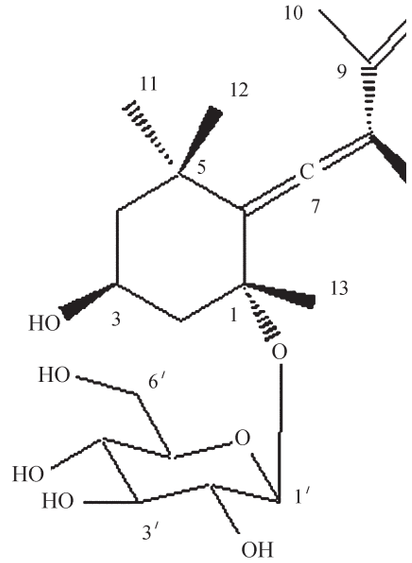

Fig. 4. Citroside B (**4**)

methine, and 5 quaternary carbons. The ^{13}C NMR and ^{1}H NMR spectral data showed the presence of signals at δ_C 101.3 and singlet at δ_H 5.88. It was assigned to methine H-8 of allenic moiety.[18] It showed HMBC correlation with C-7 (δ_C 212.9), C-9 (δ_C 200.6), and C-6 (119.1). The proton signal at δ_H 2.18 (*s*, 3H) was assigned to methyl adjacent to carbonyl group. Three more methyl signals were resonated at 1.14 (*s*, 3H, CH_3-11), 1.36 (*s*, 3H, CH_3-12) and 1.45 (*s*, 3H, CH_3-13). The triplet of a triplet at δ_H 4.31 ($J_{3,2b/3,4b}$ = 11.5 Hz, $J_{3,2a/3,4a}$ = 1.7 Hz) was assigned to carbinol H-3. Two sets of diastereotopic protons appeared at δ_H 1.92 (*dd*, $J_{2a,2b}$ = 11.5 Hz, $J_{2a,3}$ = 1.7 Hz, H-2a), 1.30 (overlapped, H-2b), 2.48 (*dd*, $J_{4a,4b}$ = 11.5 Hz, $J_{4a,3}$ = 1.6 Hz, H-4a) and 1.33 (overlapped, H-4b). A doublet at δ_H 4.52 ($J_{1',2'}$ = 7.6 Hz) was assigned to anomeric H-1′. Remaining glucose proton signals were at δ_H 3.15 (*m*, 1H, H-2′), 3.34 (*m*, 1H, H-3′), 3.21 (*m*, 1H, H-4′), 3.30 (*m*, 1H, H-5′), 3.81 (*dd*, $J_{6'a,6'b}$ = 11.7 Hz, $J_{6'a,5'}$ = 2.0 Hz, 1H, H-6′a) and 3.61 (*dd*, $J_{6'b,6'a}$ = 11.7 Hz, $J_{6'b,5'}$ = 5.3 Hz, 1H, H-6′b).

Roseoside (**5**) was obtained as the colorless amorphous powder (Fig. 5). The EI MS showed the peak [M-H$^+$] at *m*/*z* 387.3 ($C_{19}H_{30}O_8$). Its UV spectrum showed absorption bands at λ_{max} at 237 and 316 nm. The ^{13}C NMR and its DEPT spectra showed 4 methyl, 2 methylene, 9 methine, and 4 quaternary carbons. The singlet proton signal resonated at δ_H 5.84 was assigned to H-4 olefinic proton. The downfield carbon signals δ_C at 201.1 (C-3), 127.2 (C-4), and 167.2 (C-5) were characteristics of an enone

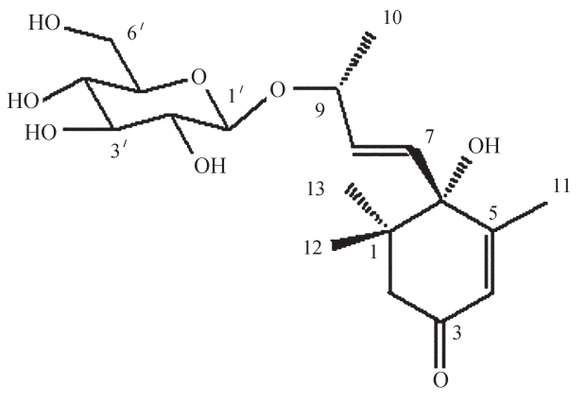

Fig. 5. Roseoside (**5**)

system [19]. The geminal coupled signals at δ_H 2.15 (*d*, $J_{2a,2b}$ = 16.8 Hz, H-2a) and 2.52 (*d*, $J_{2b,2a}$ = 16.8 Hz, H-2b) were assigned to CH_2 adjacent to carbonyl group. The ^1H NMR spectrum revealed overlapped signals assignable to olefinic protons at δ_H 5.85 (H-7) and 5.86 (H-8). The side chain was attached to C-6, what was deduced from HMBC correlation of olefinic H-7 with C-5 (δ_C 167.2) and C-6 (δ_C 80.0). The four methyl signals were resonated at δ_H 1.27 (*d*, $J_{10,9}$ = 6.4 Hz, CH_3-10), 1.02 (CH_3-11), 1.03 (CH_3-12) and 1.91 (CH_3-13). The carbinol methine proton appeared at δ_H 4.41 (*m*, H-9). A doublet at δ_H 4.34 ($J_{1',2'}$ = 7.8 Hz) was assigned to anomeric H-1′. The proton signals for remaining protons of glucose moiety appeared at δ_H 3.16 (*m*, H-2′), 3.30 (Overlapped, H-3′), 3.23 (*m*, H-4′), 3.31 (Overlapped, H-5′), 3.84 (*dd*, $J_{6'a,6'b}$ = 11.8 Hz, $J_{6'a,5'}$ = 1.7 Hz, H-6′a) and 3.62 (*dd*, $J_{6'b,6'a}$ = 11.8 Hz, $J_{6'b,5'}$ = 5.4 Hz, H-6′b).

Conclusion

Phenyl propanoid **1**, benzyl alcohol **2**, iridoid **3**, and megastigmanes **4** and **5** were for the first time reported from the genus *Osyris*. These compounds were identified on the basis of spectroscopic data. These classes of compounds are synthesized by different biosynthetic pathways and these results can be supportive to validate its medicinal claims.

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MEGASTIGMANE, IRIDOID, BENZYL ALCOHOL AND PHENYL PROPANOID GLYCOSIDES FROM THE NEPALESE SANDALWOOD *OSYRIS WIGHTIANA* WALL. EX WIGHT

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The crude ethanolic extract of the Nepalese sandalwood *Osyris wightiana* Wall. ex Wight, was analyzed for its bioactive chemical constituents by chromatographic and spectroscopic methods. Phenyl propanoid, benzyl alcohol, iridoid and megastigmanes, were isolated from the butanolic fraction by the successive use of column chromatography and HPLC. They were isolated as the new source from the genus *Osyris*. The structures of syringin (1) and di-*O*-methylcrenatin (2), 8-epideoxyloganic acid (3), citroside B (4) and roseoside (5) were characterized on the basis of extensive spectroscopic data analysis of NMR, mass and other spectroscopic techniques.

Key words: Osyris wightiana, phenyl propanoid, benzyl alcohol, iridoid, megastigmane, sandalwood.

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