A New Dimeric Secoiridoid Glycoside from the Leaves of *Olea ferruginea* ROYLE

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A new dimeric secoiridoid glycoside, trivially named oleferrugine B (**1**), has been isolated from the AcOEt-soluble part of the MeOH extract of leaves of *Olea ferruginea* ROYLE. The structure of the isolated compound was established on the basis of ESI-MS fragmentation patterns, and 1D- and 2D-NMR techniques, including ^{1}H- and ^{13}C-NMR, HSQC, ^{1}H,^{1}H-COSY, HMBC, and NOESY experiments, and by comparison with literature data.

Introduction. – *Olea* is a small genus of the family Oleaceae which contains 40 widespread species occurring throughout warm temperate and tropical regions of Australasia, Africa, Southern Europe, and Southern Asia [1]. As one of the species, *Olea ferruginea* is a small evergreen tree found in Afghanistan, Pakistan, and Kashmir. In Pakistan, it is mostly found in the hilly areas of Khyber Pakhtunkhwa, Rawalpindi, and Azad Kashmir [2]. Various parts are used in folk medicine to cure a number of ailments. Local people use its stem bark to treat fever [3], and the fruit, leaves, and bark are used as antiperiodic, astringent, uretic, rubefacient, and antiseptic [4], to cure hoarseness of voice, diabetes, toothache, and asthma. *O. ferruginea* is also used as antimalarial, antileprosy, anthelmintic, and for dressing of wounds [5]. Rheumatism and dislocation of bones are treated by massage with oil extracted from fruits of *O. ferruginea*. Extracts of different parts of *O. ferruginea* have also been found to possess a number of *in vitro* activities [4–10]. Ripe fruits of *O. ferruginea* have been reported as good source of antioxidants [11]. Hexane and BuOH extracts of leaves of *O. ferruginea* have shown very good antibacterial and antifungal activities [12]. The genus *Olea* is a rich source of secoiridoids, secoiridoid glycosides, flavonoids, lignans, and other phenolic compounds [13–16]. However, few phytochemical investigations have been conducted on *O. ferruginea*; only oleanolic acid was reported from this plant in the past [17]. Previously, we reported the cytotoxic and alkaline phosphatase inhibitory activities of pure isolates (secoiridoids and triterpenoids) from *O. ferruginea* [18], as well as a flavanone which has not been reported from the genus *Olea* before [19], and a secoiridoid glycosidic lignan ester [20]. In continuation of our work, herein, we report

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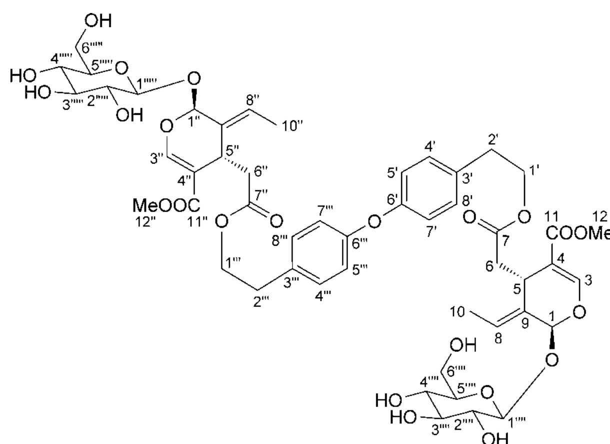

Fig. 1. *Structure of compound* **1**

the isolation of a new dimeric secoiridoid glycoside, trivially named oleferrugine B (**1**), from *O. ferruginea* (*Fig. 1*).

Results and Discussion. – Compound **1** was isolated as colorless gummy solid. HR-ESI-Q-TOF-MS (pos.) showed a *quasi*-molecular-ion peak at *m/z* 1031.3783 ($[M+H]^+$) indicating the molecular formula $C_{50}H_{62}O_{23}$. The IR spectrum displayed common absorption bands for OH groups (3371 cm^{-1}), and stretching vibrations of aliphatic C–H (2922 cm^{-1}), ester C=O (1706 cm^{-1}), alkene C=C (1629 cm^{-1}), aromatic C=C (1515 and 1439 cm^{-1}), and ether C–O bonds (1261, 1160, and 1076 cm^{-1}). UV Data of **1** showed typical absorption maxima for a tyrosol moiety at 230, 243, and 277 nm. ESI-MS/MS of **1** exhibited a *quasi*-molecular-ion peak at *m/z* 1031 ($[M+H]^+$) and provided useful information about the aglycone and glycone parts, with fragment-ion peaks at *m/z* 869 (loss of one glucosyl moiety), 689 (loss of both glucosyl moieties), 525 (loss of one monomeric unit with the bridging O-atom), 363 (loss of a monomeric fragment along with the bridging O-atom from the aglycone dimer at *m/z* 689), and the base peak at 345, which appeared due to cleavage of the ether linkage between two monomers of the aglycone dimer at *m/z* 689 composed of two equal monomers each with a mass of 344. ^{1}H- and ^{13}C-NMR data (*Table*) and their comparison with the literature revealed that the basic skeleton of **1** was that of a secoiridoid glycoside [21]. Analysis of BB, DEPT-90, and DEPT-135 experiments revealed the presence of 13 CH, four CH_2, and two Me groups, and six C_q-atoms. The ^{1}H-NMR spectrum of **1** also showed two *doublet*s of CH H-atoms (δ(H) 7.05 (*d*, $J=8.5$, 2 H) and 6.71 (*d*, $J=9.0$, 2 H)). The HSQC experiment revealed the direct attachment of these H-atoms to the C-atoms with signals at δ(C) 131.0 and 116.3, respectively, confirming the presence of a *para*-disubstituted benzene ring with C_q-atom signals at δ(C) 130.5 (C(3′)) and 157.0 (C(6′)). A CH_2 C-atom signal was observed at δ(C) 35.1 (C(2′)) with the corresponding H-atom signal at δ(H) 2.81 (*t*, $J=7.0$, 2 H) while another CH_2 C-atom resonated at δ(C) 66.9 (C(1′)) with two corresponding H-atom signals at δ(H) 4.21 (*dt*, $J=6.5$, 3.0, 1 H) and 4.10 (*dt*, $J=7.0$, 3.5, 1 H). The H-atoms at C(2′) and C(1′) showed HMBCs

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; in CD_3OD) *for* **1**. δ in ppm, J in Hz. Trivial atom numbering as indicated in *Fig. 1*.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
Secoiridane I			7′′	–	173.2
1	5.90 (br. *s*)	95.1	8′′	6.07 (*dq*, $J = 6.0$, 1.0)	124.9
2	–	–	9′′	–	130.0
3	7.50 (*s*)	155.1	10′′	1.63 (*dd*, $J = 7.5$, 1.0)	13.5
4	–	109.4	11′′	–	168.7
5	3.95 (*dd*, $J = 9.0$, 4.5)	31.8	12′′	3.70 (*s*)	51.9
6	2.69 (*dd*, $J = 14.0$, 4.5),	41.2			
	2.43 (*dd*, $J = 14.5$, 9.0)		p-*Tyrosol II*		
7	–	173.2	1′′′	4.21 (*dt*, $J = 6.5$, 3.0),	66.9
8	6.07 (*dq*, $J = 6.0$, 1.0)	124.9		4.10 (*dt*, $J = 7.0$, 3.5)	
9	–	130.0	2′′′	2.81 (*t*, $J = 7.0$)	35.1
10	1.63 (*dd*, $J = 7.5$, 1.0)	13.5	3′′′	–	130.5
11	–	168.7	4′′′	7.05 (*d*, $J = 8.5$)	131.0
12	3.70 (*s*)	51.9	5′′′	6.71 (*d*, $J = 9.0$)	116.3
			6′′′	–	157.0
p-*Tyrosol I*			7′′′	6.71 (*d*, $J = 9.0$)	116.3
1′	4.21 (*dt*, $J = 6.5$, 3.0),	66.9	8′′′	7.05 (*d*, $J = 8.5$)	131.0
	4.10 (*dt*, $J = 7.0$, 3.5)				
2′	2.81 (*t*, $J = 7.0$)	35.1	*Glc I*		
3′	–	130.5	1′′′′	4.79 (*d*, $J = 8.0$)	100.8
4′	7.05 (*d*, $J = 8.5$)	131.0	2′′′′	3.39–3.31 (*m*)	74.7
5′	6.71 (*d*, $J = 9.0$)	116.3	3′′′′	3.39–3.31 (*m*)	78.4
6′	–	157.0	4′′′′	3.39–3.31 (*m*)	71.5
7′	6.71 (*d*, $J = 9.0$)	116.3	5′′′′	3.40 (*dd*, $J = 9.0$, 9.0)	77.9
8′	7.05 (*d*, $J = 8.5$)	131.0	6′′′′	3.87 (*dd*, $J = 11.5$, 1.0),	62.7
				3.66 (*dd*, $J = 12.0$, 5.5)	
Secoiridane II			*Glc II*		
1′′	5.90 (br. *s*)	95.1	1′′′′′	4.79 (*d*, $J = 8.0$)	100.8
2′′	–	–	2′′′′′	3.39–3.31 (*m*)	74.7
3′′	7.50 (*s*)	155.1	3′′′′′	3.39–3.31 (*m*)	78.4
4′′	–	109.4	4′′′′′	3.39–3.31 (*m*)	71.5
5′′	3.95 (*dd*, $J = 9.0$, 4.5)	31.8	5′′′′′	3.40 (*dd*, $J = 9.0$, 9.0)	77.9
6′′	2.69 (*dd*, $J = 14.0$, 4.5),	41.2	6′′′′′	3.87 (*dd*, $J = 11.5$, 1.0),	62.7
	2.43 (*dd*, $J = 14.5$, 9.0)			3.66 (*dd*, $J = 12.0$, 5.5)	

with C(3′) (δ(C) 130.5), confirming the presence of a *p*-tyrosol moiety in **1** [22–24]. The aglycone moiety showed some characteristic ^{1}H-NMR signals of secoiridoids. These were the CH signals at δ(H) 5.90 (br. *s*), 7.50 (*s*), 3.95 (*dd*, $J = 9.0$, 4.5), and 6.07 (*dq*, $J = 6.0$, 1.0), two CH_2 signals at 2.69 (*dd*, $J = 14.0$, 4.5, 1 H) and 2.43 (*dd*, $J = 14.5$, 9.0, 1 H), and a Me signal at 1.63 (*dd*, $J = 7.5$, 1.0). The ^{13}C-NMR spectrum showed the corresponding C-atom resonances at δ(C) 95.1 (C(1)), 155.1 (C(3)), 31.8 (C(5)), 124.9 (C(8)), 41.2 (C(6)), and 13.5 (C(10)), respectively, plus two C=O signals at 168.7 (C(11)) and 173.2 (C(7)), and two C_q-atom signals at 109.4 (C(4)) and 130.0 (C(9)). The ^{1}H- and ^{13}C-NMR spectra indicated the presence of a sugar residue whose anomeric CH group resonated at δ(H) 4.79 (*d*, $J = 8.0$), with the corresponding C-atom

signal at δ(C) 100.8. The coupling constant suggested β-configuration for the anomeric C-atom. Other ^{1}H-NMR signals of the sugar residue were observed at δ(H) 3.39–3.31 (*m*, H–C(2'''''), H–C(3'''''), and H–C(4''''')), 3.40 (*dd*, *J* = 9.0, 9.0, H–C(5''''')), 3.87 (*dd*, *J* = 11.5, 1.0, 1 H of CH_2(6''''')), and 3.66 (*dd*, *J* = 12.0, 5.5, 1 H of CH_2(6''''')). The corresponding ^{13}C-NMR signals of the sugar moiety were observed at δ(C) 74.7 (C(2''''')), 78.4 (C(3''''')), 71.5 (C(4''''')), 77.9 (C(5''''')), and 62.7 (C(6''''')), in line with the presence of a glucosyl residue. The connectivity of the glucose moiety to the aglycone was confirmed by HMBC experiment. The anomeric H-atom of the sugar resonating at δ(H) 4.79 showed HMBC (*Fig. 2*) with C(1) (δ(C) 95.1) of the aglycone while H–C(1) (δ(H) 5.90) showed inverse HMBC with the anomeric C-atom (δ(C) 100.8) of the sugar moiety. All these observations and comparison with the literature indicated the presence of an oleoside methyl ester moiety in **1** [25][26].

The connectivity of the oleoside methyl ester moiety with *p*-tyrosol was confirmed by a HMBC experiment. The H-atoms at C(1') of the *p*-tyrosol moiety (δ(H) 4.21 and 4.10) showed strong HMBCs with C(7)=O of the oleoside moiety (δ(C) 173.2) which confirmed its position. The above NMR data were in good agreement with those reported for the known secoiridoid glucoside ligstroside [16]. However, the MS data (*m/z* 1031 ($[M+H]^+$)) were inconsistent with the structure of ligstroside (mass 524). Furthermore, **1** had a t_R value of 41 min in HPLC, while ligstroside previously isolated by our group had a t_R value of 30.5 min under the same conditions [18]. MS/MS Data suggested that **1** is a symmetrical secoiridoid glycoside dimer, derived from two ligstroside monomers, with the molecular formula $C_{50}H_{62}O_{23}$. The connection of the two monomers was found to be as depicted in *Fig. 1* which was also supported by ESI-MS/MS data. Dimerization *via* the sugar residues was ruled out, as no C-atom of the glucose moieties was downfield-shifted. Furthermore, the linkage of the two monomeric moieties *via* the sugar units was excluded on the basis of the loss of one sugar moiety from the molecular ion ($[M+H-162]^+$) in the MS/MS experiment. On the basis of all the above evidences, the structure of **1** was elucidated as shown in *Fig. 1*.

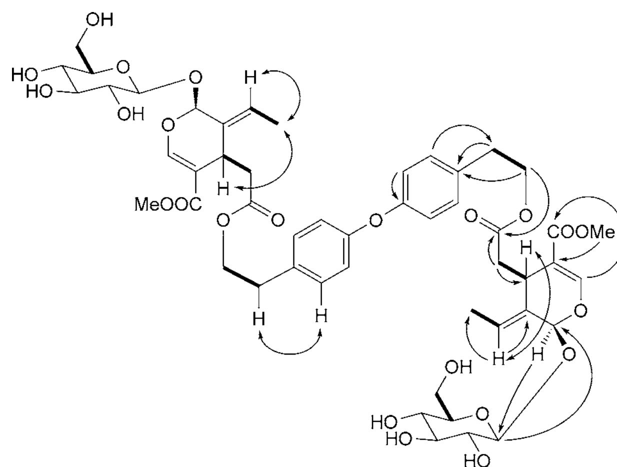

Fig. 2. *^{1}H,^{1}H-COSY* (—), *HMB* (H → C), *and NOESY* (H ↔ H) *correlations of* **1**

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Experimental Part

General. TLC: *RP-18-F$_{254s}$* plates (*Merck*); visualization with UV light at 254/366 nm. Column chromatography (CC): silica gel *60* (SiO_2; 230–400 mesh). Recycling prep. HPLC: *LC-908W* instrument (*Japan Analytical Industries* (*JAI*) *Co., Ltd.*), *Hibar LiChrosorb RP-18* column (7 µm, 250 × 25 mm; *Merck*), *JAI RI-5* refractive index, and *JAI UV-310* (254 nm) detectors; flow rate, 3 ml min^{-1}. UV Spectra: *Shimadzu UV-240* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *JASCO 302-A* IR spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker Avance AV-500 MHz* instrument (500 and 125 MHz, resp.); in CD_3OD; δ in ppm rel. to residual solvent peaks (δ(H) 3.31 and δ(C) 49.0), *J* in Hz. ESI-Q-TOF-MS/MS and HR-ESI-Q-TOF-MS (pos.): *QStar XL Hybrid* LC/MS/MS spectrometer (*Applied Biosystems*); collision gas, N_2; in *m/z*.

Plant Material. Leaves of *O. ferruginea* were collected in May 2011 from the hilly areas of Abbottabad, Pakistan, and the plant was identified by a taxonomist, Dr. *Gul Jan*, at the Department of Botany, Hazara University, Mansehra, Pakistan. A voucher specimen (No. 3544) was deposited with the herbarium of the same department.

Extraction and Isolation. Plant material was air-dried in the dark and ground to a fine powder. The powdered material (16 kg) was soaked in MeOH (45 l) for 10 d, and the filtrate was concentrated with a rotary evaporator. This procedure was repeated three times to yield a brownish gum (1.28 kg). The gum was then suspended in dist. H_2O, and partitioned successively between hexane (250 g), $CHCl_3$ (37.5 g), AcOEt (162 g), and BuOH (280 g). The resulting extracts were concentrated and dried under reduced pressure. The AcOEt-soluble extract (162 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100:0 → 0:100) to yield 14 major fractions, *Frs. 1–14*. Of these fractions, *Fr. 5* (30 g) was further subjected to CC (SiO_2; $CHCl_3$/MeOH 95:5 → 0:100) to produce nine subfractions, *Frs. 5.1–5.9*. *Fr. 5.8* (56 mg) was seperated by repeated CC (SiO_2; $CHCl_3$/MeOH 100:0 → 0:100) to give five subfractions, *Frs. 5.8.1–5.8.5*. *Fr. 5.8.4* (39 mg) was then purified by recycling prep. HPLC (MeCN/H_2O 1:1) to yield **1** (t_R 41 min; 24 mg).

Oleferrugine B (= *Dimethyl* (2S,3E,4S,2′S,3′E,4′S)*-4,4′-{Oxybis[benzene-4,1-diylethane-2,1-diyloxy(2-oxoethane-2,1-diyl)]}bis[3-ethylidene-2-(β-*D*-glucopyranosyloxy)-3,4-dihydro-2*H*-pyran-5-carboxylate]*; **1**). Colorless gum. $[\alpha]_D^{24} = -0.070$ ($c = 0.07$, MeOH). UV (MeOH): 230 (4.48), 243 (4.35), 277 (3.58). IR: 3371 (ν OH), 2922 (C–H, alkane), 1706 (C=O, ester), 1629 (C=C), 1515 (arom. C=C), 1439 (arom. C=C), 1261 (ether linkage), 1160 (ether C–O), 1076 (ether C–O). ^{1}H- and ^{13}C-NMR: *Table*. ESI-Q-TOF-MS/MS (pos.): 1031 ($[M+H]^+$), 869 ($[M+H-162]^+$), 689 ($[M+H-(162+180)]^+$), 525 ($[M+H-(162+344)]^+$), 363 ($[M+H-(162+180+326)]^+$), 345 ($[M+H-(162+180+344)]^+$). HR-ESI-Q-TOF-MS: 1031.3783 ($[M+H]^+$, $C_{50}H_{63}O_{23}^+$; calc. 1031.3755).

REFERENCES

[1] F. Medail, P. Quezel, G. Besnard, B. Khadar, *Bot. J. Linn. Soc.* **2001**, *137*, 249.
[2] M. A. Ginai, 'Alteratise on Horticulture', Bureau of Agriculture Information, Publication Division, Department of Agriculture, Government of West Pakistan, Lahore, 1968, p. 450.
[3] S. W. Khan, S. Khatoon, *Pakistan J. Bot.* **2007**, *39*, 699.
[4] A. Jabeen, M. A. Khan, M. Ahmad, M. Zafar, F. Ahmad, *Afr. J. Biotechnol.* **2009**, *8*, 763.
[5] A. Geyid, D. Abebe, A. Debella, Z. Makonnen, F. Aberra, F. Teka, T. Kebede, K. Urga, K. Yersaw, T. Biza, B. H. Mariam, M. Guta, *J. Ethnopharmacol.* **2005**, *97*, 421.
[6] F. Haq, H. Ahmad, M. Alam, *J. Med. Plants Res.* **2011**, *5*, 39.
[7] I. Haq, Z. Hussain, *Pakistan J. Plant Sci.* **1995**, *1*, 115.
[8] I. Haq, M. Rehman, *Hamdard Medicus* **1990**, *33*, 51.
[9] Z. K. Shinwari, M. I. Khan, *Hamdard Medicus* **1998**, *42*, 5.
[10] Q. Zabihullah, A. Rashid, N. Akhtar, *Pakistan J. Plant Sci.* **2006**, *12*, 115.

[11] R. K. Sharma, N. Sharma, S. S. Samant, S. K. Nandi, L. M. S. Palni, *Int. J. Biosci. Biochem. Bioinf.* **2013**, *3*, 154.
[12] A. Amin, M. A. Khan, S. Shah, M. Ahmad, M. Zafar, A. Hameed, *Pakistan J. Pharm. Sci.* **2013**, *26*, 251.
[13] A. Cárdeno, M. Sánchez-Hidalgo, M. A. Rosillo, C. Alarcón de la Lastra, *Nutr. Cancer* **2013**, *65*, 147.
[14] E. E. Rufino-Palomares, F. J. Reyes-Zurita, L. García-Salguero, K. Mokhtari, P. P. Medina, J. A. Lupiáñez, J. Peragón, *J. Proteomics* **2013**, *83*, 15.
[15] Y. Allouche, F. Warleta, M. Campos, C. Sánchez-Quesada, M. Uceda, G. Beltrán, J. J. Gaforio, *J. Agric. Food Chem.* **2011**, *59*, 121.
[16] S. Christophoridou, P. Dais, L.-H. Tseng, M. Spraul, *J. Agric. Food Chem.* **2005**, *53*, 4667.
[17] N. Sultana, A. Ata, *J. Enzyme Inhib. Med. Chem.* **2008**, *23*, 739.
[18] M. A. Hashmi, H. S. Shah, A. Khan, U. Farooq, J. Iqbal, V. U. Ahmad, S. Perveen, *Rec. Nat. Prod.* **2015**, *9*, 164.
[19] M. A. Hashmi, A. Khan, K. Ayub, U. Farooq, *Spectrochim. Acta, Part A* **2014**, *128*, 225.
[20] M. A. Hashmi, A. Khan, U. Farooq, M. Rani, V. U. Ahmad, A. R. Khan, *Magn. Reson. Chem.* **2015**, *53*, 163.
[21] A. Bianco, M. A. Chiacchio, G. Grassi, D. Iannazzo, A. Piperno, R. Romeo, *Food Chem.* **2006**, *95*, 562.
[22] M. Esti, L. Cinquanta, E. La Notte, *J. Agric. Food Chem.* **1998**, *46*, 32.
[23] A. Bianco, N. Uccella, *Food Res. Int.* **2000**, *33*, 475.
[24] R. W. Owen, R. Haubner, W. Mier, A. Giacosa, W. E. Hull, B. Spiegelhalder, H. Bartsch, *Food Chem. Toxicol.* **2003**, *41*, 703.
[25] S.-C. Ma, Z.-D. He, X.-L. Deng, P. P.-H. But, V. E.-C. Ooi, H.-X. Xu, S. H.-S. Lee, S.-F. Lee, *Chem. Pharm. Bull.* **2001**, *49*, 1471.
[26] H. Kuwajima, T. Uemura, K. Takaishi, K. Inoue, H. Inouyet, *Phytochemistry* **1988**, *27*, 1757.

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