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TWO NEW IRIDOID GLUCOSIDES FROM *OSMANTHUS FRAGRANS**

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Key Word Index—*Osmanthus fragrans*; Oleaceae; esterified iridoid glucosides; 10-acetoxyligustroside; 10-acetoxyoleuropein.

Abstract—Two new iridoid glucosides, 10-acetoxyligustroside and 10-acetoxyoleuropein, along with two known glucosides, acteoside and phillyrin, have been isolated from the leaves of *Osmanthus fragrans* Lour. and their structures have been determined.

INTRODUCTION

We have previously elucidated the structure of oleuropein and other iridoids present in members of the Oleaceae [1–4]. We have now examined the constituents of the leaves of *Osmanthus fragrans* Lour. (Japanese name: Kimmokusei). Although the occurrence of phillyrin, a lignan glucoside, in this plant has already been reported [5], we have isolated, besides this substance, acteoside [6] which had been isolated from *Syringa vulgaris* L. of the same family and two new secoiridoid glucosides and undertaken their structure elucidation.

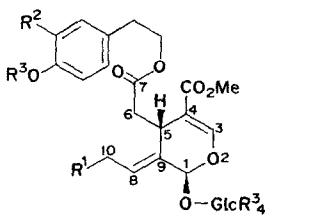
RESULTS AND DISCUSSION

Hot H_2O extract of the leaves of *O. fragrans* was fractionated by repeated chromatography (see Experimental), giving two new glucosides along with phillyrin and acteoside.

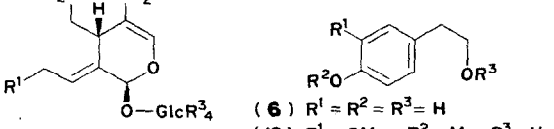
The new glucoside **1** is a white powdered bitter principle, formula $C_{27}H_{34}O_{14}.2/3\ H_2O$, $[\alpha]_D = -143{\cdot}9°$ (MeOH), which gives a positive $FeCl_3$ test (bluish-green) and yields D-glucose on hydrolysis with β-glucosidase. Substance **1** shows UV absorption (MeOH) at 228, 279 and 285 (sh) nm (log ϵ 4·26, 3·37 and 3·32) and IR bands (nujol) especially at 1730, 1705 and 1635 cm^{-1}. Its NMR spectrum (CD_3OD) exhibits, besides a singlet characteristic of the olefinic proton on C-3 of the secoiridoid glucoside at δ 7·43, $A'_2B'_2$ pattern signals assigned to four aromatic protons centered at δ 6·90, a triplet (J 7·0 Hz) of the other olefinic proton on C-8 at δ 6·10, a broad singlet of the acetal proton on C-1 at δ 5·98, a singlet of a methoxycarbonyl group at δ 3·72 and a singlet of an alcoholic acetoxy group at δ 2·01. Thus **1** appears to be a secoiridoid glucoside composed of an oleoside (**3**) type element and an aromatic portion. Acetylation of **1** gave the hexaacetate (**4**), $C_{37}H_{44}O_{19}$, NMR signals and IR spectrum of which are superimposable with those of the hexaacetate of hydroxyligustroside (**5**) isolated first from *Ligustrum obtusifolium* Sieb. et Zucc. [7]. On the other hand, the 100 MHz NMR spectrum (DMSO-d_6) of **1** shows a broad doublet at δ 4·67 which is attributable to two protons on the acetoxy-bearing C-10 atom. Irradiation of the olefinic proton on C-8 appearing as a broad triplet at δ 5·98 caused the collapse of this doublet to a singlet. This decoupling experiment together with the fact that **1** can be hydrolysed with β-glucosidase unequivocally indicates that the acetoxy group is not attached to glucose but to the C-10 position. Therefore, **1** is 10-acetoxyligustroside. The stereochemistry of **1** was resolved by the following series of reactions. Hydrolysis of **1** with

* Part 29 in the series Studies on monoterpene glucosides and related natural products. For part 28 of this series, see Takeda, Y., Nishimura, H. and Inouye, H. (1975) *Phytochemistry* **14**, in press.

aq NaOH gave the *p*-hydroxy-β-phenethyl alcohol (**6**) and the secoiridoid glucoside moiety. Methylation of the latter with diazomethane followed by acetylation gave the pentaacetate dimethyl ester (**7**), $C_{28}H_{36}O_{17}.1/2\ H_2O$, which has UV and IR absorption characteristic of secoiridoid glucoside and several NMR features assignable to the same glucoside moiety of **1**. Catalytic hydrogenation of **7** over a Pd-C catalyst in AcOH afforded, as the main product, oleoside dimethyl ester tetraacetate (**8**) [1], mp 113–114°, which is also derivable from oleuropein (**9**). The absolute structure of **1** is thus established by this conversion.



(**1**) R^1 = OAc, R^2 = R^3 = H
(**2**) R^1 = OAc, R^2 = OH, R^3 = H
(**4**) R^1 = OAc, R^2 = H, R^3 = Ac
(**5**) R^1 = OH, R^2 = R^3 = H
(**9**) R^1 = R^3 = H, R^2 = OH
(**10**) R^1 = R^2 = OAc, R^3 = Ac
(**11**) R^1 = H, R^2 = OAc, R^3 = Ac
(**14**) R^1 = R^2 = R^3 = H



(**3**) R^1 = R^2 = R^3 = H
(**7**) R^1 = OAc, R^2 = Me, R^3 = Ac
(**8**) R^1 = H, R^2 = Me, R^3 = Ac

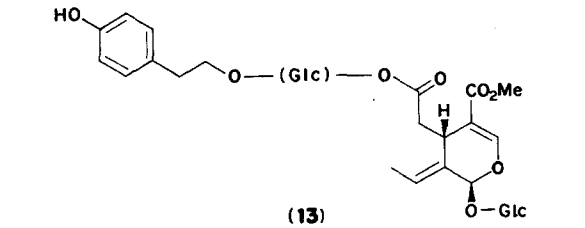
(**6**) R^1 = R^2 = R^3 = H
(**12**) R^1 = OMe, R^2 = Me, R^3 = H

Scheme 1.

The glucoside **2** is a white powdered bitter principle, $C_{27}H_{34}O_{15}.2\ H_2O$, $[\alpha]_D$ = −191·0° (MeOH), which gives positive $FeCl_3$ and Gibbs' test (bluish green and bluish violet, respectively) and yields D-glucose on hydrolysis with β-glucosidase. The glucoside (**2**) shows UV absorption (MeOH) at 233·5 and 283·5 nm (log ε 4·20 and 3·47) as well as IR bands (nujol) at 1740, 1705 and 1635 cm^{-1}. The NMR spectrum (CD_3OD) of **2**, which is very similar to that of **1** except signals in the aromatic region, exhibits a singlet characteristic of the proton on C-3 of the secoiridoid at δ 7·50, a multiplet of three aromatic protons at δ 6·48–6·88, a broad triplet (*J* 7·0 Hz) of the olefinic proton on C-8 at δ 6·12, a broad singlet of the acetal proton on C-1 at δ 5·95, a singlet of a methoxycarbonyl group at δ 3·71 and a singlet of an alcoholic acetoxy group at δ 2·02. Acetylation of **2** gave the heptaacetate (**10**), $C_{39}H_{46}O_{21}$, which displays IR bands at 1745, 1700, 1630, 1605 and 1500 cm^{-1}. Although the NMR spectrum of **10** resembles that of **4**, the former shows two phenolic acetoxy signals at δ 2·28 as expected from the difference between **1** and **2**. The signal patterns of **2** and **10** in the aromatic region are the same as those of oleuropein (**9**) and its hexaacetate (**11**), respectively. Taking into account all this evidence **2** must be 10-acetoxyoleuropein. Hydrolysis of **2** with aq NaOH afforded a phenethyl alcohol and secoiridoid glucoside moiety. The former was methylated with diazomethane giving rise to 3,4-dimethoxy-β-phenethyl alcohol (**12**). On the other hand, methylation of the latter with diazomethane followed by acetylation gave the **7**. Consequently, the absolute structure of **2** is also established.

Osmanthus fragrans belongs to the tribe Oleeae which comprises 17 genera including *Ligustrum*, *Olea* and *Syringa* [8]. The iridoid and secoiridoid glucosides found in these plants are oleuropein (**9**), nuezhenide (**13**) [2], ligustroside (**14**) [7] and 10-hydroxyligustroside (**5**) [7] in several species of genera *Ligustrum* and *Olea* as well as syringopicroside (**15**) [9] in *Syringa* species. The occurrence of oleuropein (**9**) in the species of *Fraxinus*, tribe Fraxineae, has also been confirmed [4]. Syringopicroside (**15**) possessing 7-dehydrologanic acid as the structural element is biogenetically closely related to the oleoside (**3**) type glucoside [10]. Quite recently, oleuropein (**9**) and ligustroside (**14**) have also been isolated in *Syringa vulgaris* L. [11]. Thus secoiridoids based on oleoside (**3**) or 10-hydroxyoleoside (**16**) are quite common constituents in the Oleoideae.

In the Jasminoideae, jasminin (**17**) [12] which is also based on oleoside (**3**) has been isolated from the tribe Jasmineae, but glucosides of this type have not yet been detected anywhere else in the subfamily.



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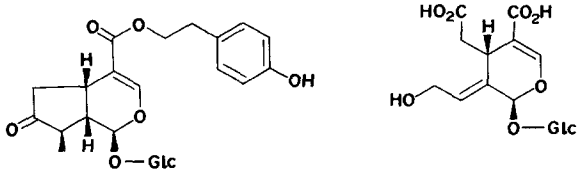
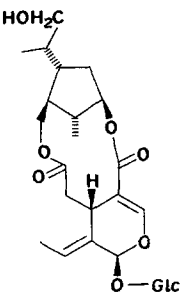


(15) (16)



(17)

Scheme 2.

EXPERIMENTAL

Mps are uncorrected. NMR spectra were usually taken in $CDCl_3$ and TMS as internal standard; the spectra of **1** and **2** were measured in CD_3OD with the same standard. 100 MHz spectra of these glucosides were obtained in DMSO-d_6 with DSS as internal standard. Si gel (100 mesh) and polyamide C-200 were employed for column chromatography. Si gel G was used for TLC and spots were detected with iodine vapour. Si gel GF_{254} was used for preparative TLC and spots were visualized in the UV.

Extraction and isolation of glucosides. Fresh leaves (4·2 kg) of *O. fragrans*, collected in Kyoto in December, were extracted with hot H_2O (6 × 5 l.) and the aq extract was concentrated *in vacuo* to 4 l. The resulting soln was extracted successively with $CHCl_3$ (4 × 0·5 l.) and EtOAc (6 × 0·5 l.). The EtOAc layer was concentrated *in vacuo* and the residue was chromatographed on Si gel (700 g) eluted first with $CHCl_3$ (6 l.), then with MeOH–$CHCl_3$ with an increasing MeOH content (28 l.) and finally with MeOH (4 l.). The 3% MeOH–$CHCl_3$ eluate was evaporated *in vacuo* and residue was crystallized from MeOH giving phillyrin (594 mg) as needles, mp 153–154° $[\alpha]_D^{23}$ + 47·0° (*c* 0·51, MeOH); IR ν_{max}^{nujol} cm^{-1}: 1590, 1520. (Found: C, 59·50; H, 6·66. Calc. for $C_{27}H_{34}O_{11}.1/2\ H_2O$: C, 59·66; H, 6·49%). This substance showed no depression in mp on admixture with an authentic sample. In addition, the physical properties of its acetate and the aglucone (epipinoresinol) obtained by hydrolysis of it with β-glucosidase were in accord with the reported data. The 4–6% MeOH–$CHCl_3$ eluates were collected and evaporated *in vacuo* to give a residue (17·5 g), a 5 g aliquot of which was rechromatographed on Si gel (150 g) and eluted with MeOH–$CHCl_3$ with an increasing MeOH content (14 l.) and finally with MeOH (2 l.). The 3% MeOH–$CHCl_3$ eluate was further purified by preparative TLC [(MeOH–$CHCl_3$, 1:3) R_f 0·50] giving 10-acetyligustroside (**1**) (704 mg) as a powder, $[\alpha]_D^{18}$ −143·9° (*c* 1·0, MeOH). (Found: C, 54·34; H, 6·12. $C_{27}H_{34}O_{14}.2/3\ H_2O$ requires: C, 54·63; H, 5·83%). The 4–5% MeOH–$CHCl_3$ eluates were combined and evaporated *in vacuo* to afford a residue (4·20 g), a 300 mg aliquot of which was further purified by preparative TLC giving 10-acetoxyoleuropein (**2**) (160 mg) as a white powder, $[\alpha]_D^{21}$ −191·1° (*c* 1·1, MeOH). NMR (100 MHz, DMSO-d_6) δ: 4·67 (*dd*, *J* 7·0 & 3·0 Hz, 10-H_2), 6·00 (*br t*, *J* 7·0 Hz, 8-H). Coupling between these protons was demonstrated by the decoupling experiments. TLC (MeOH–$CHCl_3$, 3:7) R_f 0·46. (Found: C, 51·39; H, 6·13. $C_{27}H_{34}O_{15}.2\ H_2O$ requires: C, 51·10; H, 6·03%). The MeOH eluate of the original chromatographic separation was evaporated *in vacuo* to leave a residue (7·3 g), a 2 g aliquot of which was chromatographed on polyamide (30 g) eluted first with H_2O (500 ml) and then MeOH–H_2O with an increasing MeOH content. The fraction obtained by eluting with 50% MeOH–H_2O was further chromatographed on cellulose powder (115 g). Elution with EtOAc saturated with H_2O followed by crystallization from EtOH gave acteoside (1·09 g) as needles, mp 148–150°, which were found to be identical to an authentic specimen (mmp and comparisons of TLC, IR and NMR spectra).

Acetylation of 10-*acetoxyligustroside* (**1**). The crude acetylated product (from Ac_2O–C_5H_5N) was chromatographed on Si gel in Et_2O to give a white powder (**4**), $[\alpha]_D^{24}$ −128·6° (*c* 1·0, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 234·5 (4·12), 272 (2·94); IR ν_{max}^{nujol} cm^{-1}: 1745, 1705 (sh), 1640, 1510; NMR δ: 2·90 (*t* *J* 7·0 Hz, Ph–C$\underline{H}_2$$CH_2$O–), 4·24 (*t* *J* 7·0 Hz, Ph–CH_2C$\underline{H}_2$O–), 4·72 (*br d*, *J* 6·0 Hz, 10-H_2), 5·72 (*br s*, 1-H); 6·00 (*br t*, *J* 6·0 Hz, 8-H), 7·10 ($A'_2B'_2$ *q*, 4 arom. protons), 7·45 (*s*, 3-H). (Found: C, 56·14; H, 5·89. Calc. for $C_{37}H_{44}O_{19}$: C, 56·06; H, 5·60%). TLC: (Me_2CO–C_6H_6, 1:4) R_f 0·43. This substance was identical with an authentic sample of hexaacetate of 10-hydroxyligustroside (IR, UV, TLC).

Acetylation of 10-*acetoxyoleuropein* (**2**). The crude acetate was chromatographed on Si gel in Et_2O to give (**10**) as a white powder, $[\alpha]_D^{23}$ −117·4° (*c* 1·0, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 234·5 (4·11), 272 (2·92); NMR δ: 2·03, 2·04, 2·06 (each *s*, 5 alcoholic OCOMe), 2·90 (*t*, *J* 7·0 Hz, Ph–C$\underline{H}_2$$CH_2$O–), 3·72 (*s*, COOMe), 4·25 (*t*, *J* 7·0 Hz, Ph–CH_2C$\underline{H}_2$O–), 4·73 (*br d*, *J* 6·0 Hz, 10-H_2), 5·71 (*br s*, 1–H), 6·00 (*br t*, *J* 6·0 Hz, 8–H), 6·83–7·15 (3 arom. protons), 7·42 (*s*, 3–H); TLC: (Me_2CO–C_6H_6, 1:4) R_f 0·33. (Found: C, 55·09; H, 5·33. $C_{39}H_{46}O_{21}$ requires: C, 55·06; H, 5·45%).

Alkaline hydrolysis of 10-*acetoxyligustroside* (**1**). **1** (704 mg) was heated with 0·5 N aq NaOH at 90° for 1 hr. The cooled mixture was neutralized with Amberlite IR-120 (H^+ form), extracted with EtOAc (4 × 50 ml), and this layer concentrated *in vacuo* to afford a crystalline residue (152 mg) which was purified by preparative TLC (MeOH–$CHCl_3$, 3:7) R_f 0·59 and crystallization from Me_2CO–Et_2O, giving needles (**6**) (88 mg), mp 91–92°, which were found to be identical to *p*-hydroxy-β-phenethyl alcohol (mmp, IR, TLC). IR ν_{max}^{nujol} cm^{-1}: 3350, 3100, 1600, 1510 and 820. (Found: C, 69·60; H, 7·25. Calc. for $C_8H_{10}O_2$: C, 69·54; H, 7·30%). The aq layer was concentrated to dryness to give a residue (527 mg) which had R_f 0·50 on TLC (Si gel G containing 0·25% phosphoric acid, 50% MeOH–$CHCl_3$). Methylation of the residue with CH_2N_2–Et_2O and the subsequent acetylation gave crude acetate (722 mg) which was chromatographed on Si gel (35 g).

Elution with Et_2O afforded 10-hydroxyoleoside dimethyl ester pentaacetate (**7**) (630 mg) as a powder, $[\alpha]_D^{23}$ −149·8° (*c* 1·3, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 236 (3·94); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1745, 1705, 1635; NMR δ: 2·02, 2·03, 2·06, 2·08 (each *s*, 5 alcoholic OCOMe), 3·65, 3·75 (each *s*, 2 × COOMe), 4·72 (*br d*, *J* 6·0 Hz, 10-H_2), 5·72 (*br s*, 1-H), 6·00 (*br t*, *J* 6·0 Hz, 8-H), 7·45 (*s*, 3-H). (Found: C, 51·45; H, 5·51. $C_{28}H_{36}O_{17}.1/2\ H_2O$ requires: C, 51·45; H, 5·71%).

Alkaline hydrolysis of 10-*acetoxyoleuropein* (**2**). Hydrolysis and the subsequent work up of **2** (700 mg), in the same way as described above, gave a substance showing the same behaviour on TLC as that of 3,4-dihydroxy-β-phenethyl alcohol. Methylation with CH_2N_2–Et_2O at 0° for 3 days gave, on crystallization from Et_2O, needles (**12**) (34 mg). mp 42–44°; IR ν_{max}^{nujol} cm^{-1}: 3220, 1600 and 1520. (Found: C, 65·75; H, 7·63. Calc. for $C_{10}H_{14}O_3$: C, 65·92; H, 7·74%). This substance was identical with an authentic sample of 3,4-dimethoxy-β-phenethyl alcohol (mmp, IR, TLC). Evaporation of the aq layer *in vacuo* gave a residue (594 mg) which was treated in the same manner as in the case of the hydrolysis of **1** to give 10-hydroxyoleoside dimethyl ester pentaacetate (**7**).

Catalytic hydrogenation of 10-*hydroxyoleoside dimethyl ester pentaacetate* (**7**). **7** (507 mg) in AcOH (30 ml) was hydrogenated over a Pd-C catalyst [prepared from 5% $PdCl_2$–HCl soln (2 ml) and activated charcoal (Darco 60, 500 mg)]. After an uptake of ca 1 mol of H_2, the catalyst was filtered off and the filtrate was concentrated to dryness leaving a residue which was dissolved in $CHCl_3$, washed successively with 5% aq $NaHCO_3$ soln and H_2O, dried and evaporated *in vacuo*. Separation of the residue by chromatography on Si gel (elution with Et_2O) gave, besides the starting material (**7**) (172 mg), a mixture of hydrogenation product (170 mg) (TLC: Et_2O, R_f 0·46), the NMR spectrum of which showed, in addition to signals based on oleoside dimethyl ester tetraacetate (**8**), weak signals at δ 7·43 (3-H, *br s*), δ 3·73 and 3·64 (2 × MeCOO, each *s*), δ 1·75 (8-H_2, 5-H, *m*) and δ 1·00 (10-H_3, *br t*) indicating the presence of dihydro derivative of **8** in a small amount. This product gave needles of **8** ex EtOH, mp 113–114°. $[\alpha]_D^{22}$ −123·3° (*c* 1·0, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 236·5 (4·02); IR ν_{max}^{nujol} cm^{-1}: 1750, 1705, 1635; NMR δ: 1·75 (*dd*, *J* 7·0 & 1·5 Hz, 10-H_2), 2·02, 2·04, 2·07 (each *s*, 4 alcoholic OCOMe), 3·64, 3·75 (each *s*, 2 × COOMe), 5·71 (*br s*, 1-H), 6·03 (*br q J* 7·0 Hz, 8-H), 7·45 (*s*, 3-H). (Found: C, 53·45; H, 5·92. Calc. for $C_{26}H_{34}O_{15}$: C, 53·24; H, 5·84%). This substance was identical with an authentic specimen of **8** (mmp, IR, NMR).

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