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A Sesquiterpene Glycoside, Loquatifolin A, from the Leaves of *Eriobotrya japonica*

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The structure of a new sesquiterpene glycoside, loquatifolin A (**1**), isolated from the leaves of *Eriobotrya japonica* (THUNB.) LINDLE., was established to be α-L-rhamnopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranosyl-6,7-*trans*-neloridol on the basis of chemical and spectral studies.

Keywords——*Eriobotrya japonica*; Rosaceae; sesquiterpene glycoside; 6,7-*trans*-nerolidol; ^{13}C-NMR

The leaves of loquat, *Eriobotrya japonica* (THUNB.) LINDLE. (Rosaceae), have been used as a component of Chinese medical prescriptions as an antitussive and anti-inflammatory agent for chronic bronchitis, as well as in Japanese folk medicine as a diuretic, digestive and antipyretic agent. As volatile oil principles of the leaves, nerolidol and farnesol were found, and the occurrence of α- and β-pinene, camphene, β-myrcene, *p*-cymene, *cis*-β,γ-hexenol, *trans*-linalool oxide, *cis*-linalool oxide, linalool, camphor, α- and β-farnesene, nerol, geraniol and α-cardinol was also reported by Suemitsu *et al.*[1] They pointed out that although nerolidol accounts for 61—74% of the essential oil obtained from the leaves, the fresh leaves have no

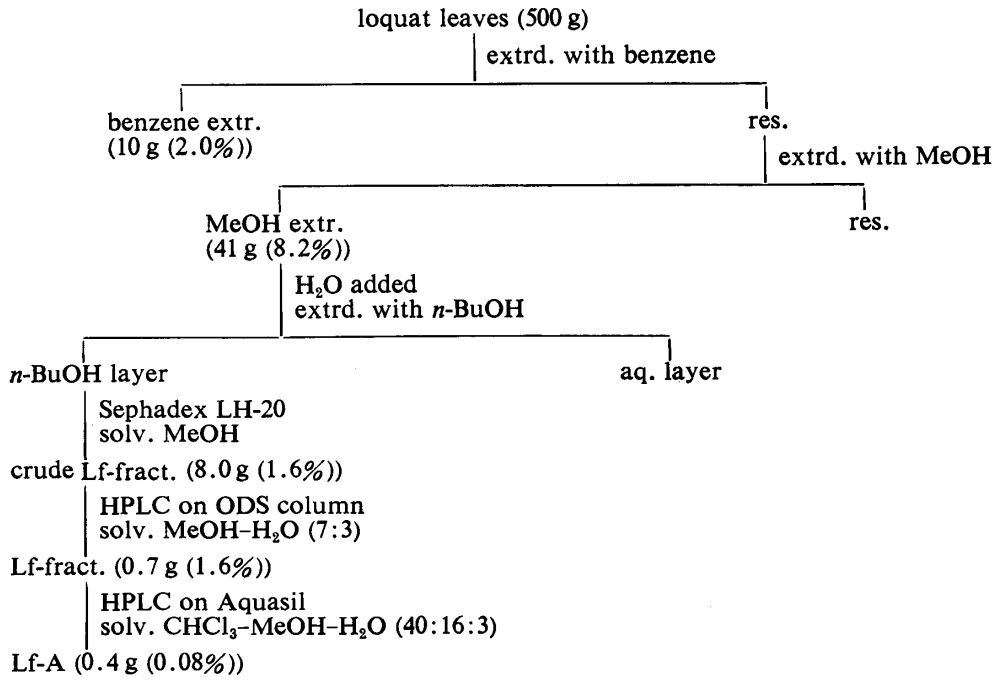


Chart 1

smell of nerolidol. Thus, nerolidol might be separated from a non-volatile conjugated form during the course of steam distillation. As non-volatile constituents of the leaves, vitamin B_1, vitamin C, ursolic acid, oleanolic acid[2] and quercetin 3-*O*-galactoside[3] have been described.

The present work deals with the isolation of a new sesquiterpene glycoside, named loquatifolin A (Lf-A) (**1**). Compound **1** was isolated from the leaves of loquat by the procedure indicated in Chart 1. The butanol-soluble fraction of the methanolic extracts was separated on a Sephadex LH-20 column to obtain fifteen fractions, and fractions 2—5 were separated as the crude Lf-fraction, which gave a spot of **1** as the main component on thin-layer chromatography (TLC).

Preparative high performance liquid chromatography (HPLC) of Lf-fraction gave **1** as a white powder, $C_{39}H_{66}O_{18} \cdot 1.5H_2O$, and the molecular formula was confirmed by the elemental analysis and field desorption mass spectrum (FD-MS) (*m/z* $M^+ + Na$ 845; $M^+ + K$ 861; M^+ 822). The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** was measured by the complete decoupling method as shown in Tables I-A and -B. The existence of thirty-nine carbon atoms in the molecule of **1** was supported by thirty-nine carbon signals.

The four anomeric carbon signals at 97.0, 100.3, 101.1 and 102.1 ppm (Table I-B) indicated that the sugar part consists of four monosaccharide residues. The complex profile of proton nuclear magnetic resonance (^{1}H-NMR) signals in the anomeric proton region was difficult to analyze.

Compound **1** was hydrolyzed with 2 N H_2SO_4 in ethanol to identify the sugar components and the aglycone. The aglycone portion of the hydrolyzate gave multiple spots on TLC, while the sugar portion was separated into three spots on TLC, two of which were identified as glucose and rhamnose. By gas-liquid chromatography (GLC) after trimethylsilylation, the sugar components were confirmed to be glucose and rhamnose, and the third spot on the TLC was concluded to be ethyl rhamnopyranoside from the ^{13}C-NMR spectrum.

In order to determine the sugar linkages of **1**, enzymatic degradation using hesperidinase[4] and naringinase[5] was attempted. On hydrolysis with naringinase, an oily aglycone was obtained which gave a violet-red-colored spot at *Rf* 0.5 on TLC developed with *n*-hexane–EtOAc (5 : 1) and sprayed with 10% H_2SO_4. The aglycone part of **1** was suggested to be a sesquiterpene from the remaining fifteen carbon numbers, and the presence of three double

TABLE I-A. ^{13}C-NMR Chemical Shifts of Nerolidol, Lf-A and Proloquatifoligenins in d_5-Pyridine (ppm from TMS) (Aglycone Part)

	a	b	c	d	e	f
C-1	110.0	113.6	113.9	114.3	114.7	114.0
C-2	145.7	143.4	143.0	143.0	144.2	142.8
C-3	71.1	78.9	79.2	79.5	80.4	79.3
C-4	42.0	41.1	41.0	41.2	41.8	40.8
C-5	25.9	25.9	25.7	25.9	27.1	26.0
C-6	125.1	123.8	123.6	123.8	125.1	123.9
C-7	133.5	133.8	133.7	133.9	135.2	133.9
C-8	38.8	38.9	38.7	38.9	40.0	38.8
C-9	22.0	21.8	21.8	21.9	23.0	21.9
C-10	124.2	124.1	123.9	124.0	123.9	123.9
C-11	130.0	130.1	129.8	130.0	130.9	129.8
C-12	16.5	16.6	16.4	16.6	17.7	16.6
C-13	24.6	24.6	24.5	24.6	25.7	24.6
C-14	14.8	15.0	14.9	15.0	16.2	15.0
C-15	22.4	22.4	21.3	21.4	22.7	21.4

a, nerolidol. b, proloquatifoligenin-III (**2**). c, proloquatifoligenin-II (**3**). d, proloquatifoligenin-I (**4**). e, proloquatifoligenin-IV (**5**). f, loquatifolin A (**1**).

TABLE I-B. ^{13}C-NMR Chemical Shifts of Lf-A and Proloquatifoligenins in d_5-Pyridine (ppm from TMS) (Sugar Part)

		b	c	d	e	f
Glc	C-1	98.6	96.8	96.9	96.4	97.0
	C-2	74.3	78.6	78.9	80.9	78.6
	C-3	77.0[a]	76.0[a]	76.2[a]	77.0[a]	75.3[a]
	C-4	70.7	71.7[b]	71.1[b]	72.0[b]	71.0[b]
	C-5	77.7[a]	76.5[a]	75.4[a]	77.7[a]	75.9[a]
	C-6	61.9	61.5	67.1	62.8	67.0
x-Rha	C-1		100.5	100.8	103.0	100.3[f]
	C-2		71.0[b]	71.3[b]	72.7[b]	71.5[b]
	C-3		71.4[b]	71.6[b]	72.5[b]	71.6[b]
	C-4		72.8	72.9[e]	79.7	79.8
	C-5		68.2	68.4[d]	67.4	68.6[d]
	C-6		17.4	17.5[c]	19.0[c]	17.9[c]
y-Rha	C-1			101.3		102.1
	C-2			71.2[b]		71.1[b]
	C-3			71.6[b]		71.4[b]
	C-4			73.0[e]		72.8[e]
	C-5			68.6[d]		66.4[d]
	C-6			17.6[c]		17.2[c]
z-Rha	C-1				101.2	101.1[f]
	C-2				72.5[b]	72.0[b]
	C-3				73.1[b]	72.6[b]
	C-4				73.8	72.8[e]
	C-5				70.0	69.0[d]
	C-6				18.4[c]	17.4[c]

a—f) These assignments might be interchanged in each column. b, proloquatifoligenin-III (**2**). c, proloquatifoligenin-II (**3**). d, proloquatifoligenin-I (**4**). e, proloquatifoligenin-IV (**5**). f, loquatifolin A (**1**).

bonds was revealed by six signals in the region of 114—124 ppm, of which the signal at 113.9 ppm gave a triplet in the off-resonance procedure, and was assigned to a terminal double bond (Table I-A). From the afore-mentioned NMR spectral data the aglycone was assumed to be nerolidol, and this has been confirmed by the retention time on GLC in comparison with an authentic sample of 6,7-*trans*-nerolidol. The commercially available sample of nerolidol (Tokyo Kasai Co.) gave two peaks on GLC, which were identified as 6,7-*cis*- and 6,7-*trans*-nerolidol, respectively.[6]

Besides the aglycone, three partially hydrolyzed glycosides, named proloquatifoligenin-I (**4**), -II (**3**) and -III (**2**), were isolated after enzymatic degradation. The aglycone and proloquatifoligenins were separated by means of HPLC and TLC.

Compound **2** gave ^{13}C-NMR spectral data as indicated in Table I-A, which also show those of nerolidol for comparison. The ^{13}C-NMR spectral signals assigned to the aglycone part of **2** were almost superimposable on those of 6,7-*trans*-nerolidol except C-3, which was shifted downward (71.1→78.9 ppm). This indicated that the sugar moiety is attached to the C-3 position of the aglycone. (Table I-A) The sugar moiety was confirmed to be glucose on TLC, and this was supported by the ^{13}C-NMR spectrum, in which the anomeric carbon signal appeared at 98.6 ppm without a glycosylation shift, indicating that the glucosyl group is attached to a tertiary hydroxyl.[7] The ^{1}H-NMR anomeric proton signal at 5.0 ppm (J= 7.6 Hz) showed β-linkage of the glucosyl moiety. If glucose is assumed to be the most commonly found D-form, **2** is β-D-glucopyranosyl-(1→3)-6,7-*trans*-nerolidol.

The molecular formula $C_{27}H_{46}O_{10}$ of **3** was supported by the elemental analysis and electron ionization mass spectrum (EI-MS) (m/z M^+ 530, 221, 204, 69). Two anomeric carbon

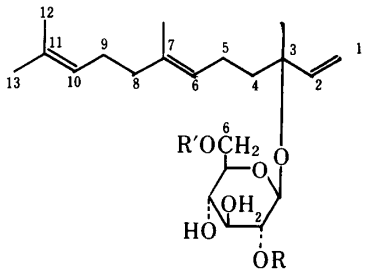
signals in the ^{13}C-NMR spectrum at 96.8 and 100.5 ppm were assigned to C-1 of glucose and rhamnose, respectively. The downfield shift of C-3 of nerolidol (71.1→79.2 ppm) indicated that the sugar moiety is attached there. The downfield shift of the glucopyranose C-2 signal (74.3→78.6 ppm), and the upfield shifts (Δ 1 ppm) of the C-1 and C-3 signals showed a linkage of Rha(1→2)Glc (Table I-B). If rhamnose is assumed to be a member of the commonly found L-series, **3** is α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl-(1→3)-6,7-*trans*-nerolidol (Table I-B). The configuration of the rhamnosyl group in **1** was assigned as α-L-Rha, as discussed later.

The molecular formula $C_{33}H_{56}O_{14}$ of **4** was supported by the elemental analysis and FD-MS (M^++Na 699; M^++K 715; M^+ 676). Three anomeric carbon signals were observed in the ^{13}C-NMR spectrum at 96.9, 100.8 and 101.3 ppm. The first and second signals correspond to those of **3**, and the last one was assigned to C-1 of the second rhamnosyl molecule, which is attached to the C-6 position of glucose, because a lower field shift of the C-6 signal (62.3→67.1 ppm) was observed (Table I-B). Accordingly, **4** is α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→3)-6,7-*trans*-nerolidol. Finally two possibilities remained for the location of the terminal rhamnosyl moiety(z) in **1**, *i.e.*, whether it is attached to the x-rhamnose or y-rhamnose moiety.

Proloquatifoligenin-IV (**5**) was obtained by the alkaline hydrolysis of **1** using *n*-BuOH and NaOH[8] as the reagent. The FD-MS revealed that this compound possesses the same molecular weight, 676, as **4** having a trioside moiety consisting of 1 mol of glucose and 2 mol of rhamnose. The inner rhamnose must be linked to C-2 of glucose, since a lower shift of the C-2 signal was observed. The terminal rhamnose must be located at C-4 of the inner rhamnose, since a lower shift (72.3→79.7 ppm) of that signal was recorded (Table I-B). Consequently **5** is α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-6,7-*trans*-nerolidol.

On the basis of the structures of **4** and **5**, the structure of **1** has been deduced as follows (Chart 2). The ^{1}H-NMR signal of the anomeric proton of D-glucose attached to the C-3 of 6,7-*trans*-nerolidol appeared at 5.0 ppm (J=7.6 Hz), indicating the β-configuration. The ^{1}H-NMR spectral analysis of anomeric protons of rhamnosyl units in the oligoside portion of **1** provided no definite evidence, whereas in the ^{13}C-NMR spectral analysis, the value of $J_{C1,H1}$ is indicative of the anomeric configuration.[9,10] $J_{C1,H1}$ of α-D-glucopyranose is 169.8 Hz and that of β-D-glucopyranose is 161.2 Hz, while $J_{C1,H1}$ of the glucosyl moiety of **1** is 160.6 Hz indicating β-configuration. On the other hand, α-L-rhamnopyranose gives the C-1 signal $J_{C1,H1}$=167 Hz and β-L-rhamnopyranose gives $J_{C1,H1}$=154 Hz, while the rhamnopyranosyl units in **1** show $J_{C1,H1}$=173, 168 and 170 Hz, respectively, revealing that they are linked with α-L-configuration.

The results also showed that z-rhamnose and y-rhamnose are linked with α-L-



loquatifolin A (**1**): R=α-L-Rha(1→4)-α-L-Rha(1→)
(z) (x)
R′=α-L-Rha(1→)
(y)

proloquatifoligenin-I (**4**): R=α-L-Rha(1→)
(x)
R′=α-L-Rha(1→)
(y)

proloquatifoligenin-II (**3**): R=α-L-Rha(1→)
(x)
R′=H

proloquatifoligenin-III (**2**): R=R′=H

proloquatifoligenin-IV (**5**): R=α-L-Rha(1→4)-α-L-Rha(1→)
(z) (x)
R′=H

Chart 2

configuration. All the ^{13}C-NMR signals assigned to the carbon atoms of **1**, and the proloquatifoligenins (**2**—**5**) are shown in Tables I-A and I-B.

Experimental

All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. MS were recorded on a JEOL D-300 mass spectrometer. ^{1}H- and ^{13}C-NMR were measured in C_5D_5N using tetramethylsilane (TMS) as an internal standard on a JEOL GX-400 spectrometer. Optical rotations were measured on a JASCO J-20.

Isolation of 1——The crushed fresh leaves (500 g) of *Eriobotrya japonica* were refluxed with benzene and then with MeOH. The combined MeOH extracts were concentrated *in vacuo*, the residue was dissolved in water, and the solution was extracted with *n*-BuOH saturated with water. The organic layer was evaporated under reduced pressure. The residue was chromatographed on a column of Sephadex LH-20 with MeOH to afford a crude glycoside, which was subjected to HPLC with a preparative PAK-500/C18 column (30 cm × 50 mm i.d.) using MeOH–H_2O (7 : 3) as the solvent to afford crude Lf-A (**1**). The crude Lf-A was purified by HPLC on an Aquasil column (30 cm × 20 mm i.d.) using $CHCl_3$–MeOH–H_2O (40 : 16 : 3) to afford a chromatographically pure major sesquiterpene glycoside, Lf-A (**1**) (0.4 g), mp 128—130 °C, $[\alpha]_D^{22}$ −79.7° (EtOH). *Anal.* Calcd for $C_{39}H_{66}O_{18}\cdot 1.5H_2O$: C, 55.12; H, 8.13. Found: C, 54.89; H, 8.10. FD-MS *m/z*: 845 (M^++Na), 861 (M^++K). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH).

Hydrolysis of 1 with 2 N H_2SO_4——A solution of **1** (100 mg) in 2 N H_2SO_4 (20 ml)–EtOH (10 ml) was refluxed for 2 h. The aqueous layer was neutralized with ion exchange resin, Amberlite IR 47A (OH^- form), and evaporated *in vacuo*. The residue was proved to be a neutral mixture of glucose (1 mol) and rhamnose (3 mol) by TLC and GLC.

TLC (Silica): Solvent, AcOEt–MeOH–H_2O (6 : 2 : 1); color reagent, 50% H_2SO_4 and aniline hydrogen phthalate.

GLC: Column, Silicone OV-1 3% on Chromosorb W (DMCS) 60—80 mesh; 3.0 m × 2.0 mm glass tube; injector temperature, 250 °C; column temperature, 150—200 °C (2 °C/min); N_2 gas, 1.5 kg/cm^2; detector, hydrogen flame ionization detector (FID); sample, TMS derivative.

Enzymatic Hydrolysis of 1 with Naringinase——A solution of **1** (150 mg) in a buffer solution (20 ml) was treated with naringinase (150 mg) and the whole mixture was incubated under stirring at 25 °C for 48 h. After removal of the residue, the reaction mixture was subjected to chromatography on a column of Sephadex LH-20 with MeOH. The aglycone was identified as 6,7-*trans*-nerolidol from the retention time on GLC in comparison with that of an authentic sample. Beside the aglycone, three partially hydrolyzed glycosides, which were named proloquatifoligenin-I (**4**), -II (**3**) and -III (**2**), were isolated on enzymatic hydrolysis. They were separated by means of HPLC. Buffer solution (pH = 4.0): Citric acid–Na_2HPO_4.

GLC: Column, Apiezone grease L 3% on Gas-chrom Q (2.0 m × 2.0 mm glass tube); injector temperature, 230 °C; column temperature, 180 °C; N_2 gas, 3.0 kg/cm^2; detector, FID.

HPLC: Column, Senshu Pak SSC-Aquasil (8 × 200 mm); solvent, $CHCl_3$–MeOH–H_2O (40 : 16 : 3); pressure, 100 kg/cm^2; detector, refractive index (RI); flow rate, 2.0 ml/min.

Proloquatifoligenin-I (**4**), a white powder, mp 98—100 °C, $[\alpha]_D^{22}$ −43.8° (*c* = 1, EtOH). *Anal.* Calcd for $C_{33}H_{56}O_{14}\cdot 2.5H_2O$: C, 54.91; H, 8.52. Found: C, 55.00; H, 8.50. FD-MS *m/z*: 699 (M^++Na), 715 (M^++K). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH).

Proloquatifoligenin-II (**3**), a white powder, mp 63—65 °C, $[\alpha]_D^{22}$ −31.4° (*c* = 1, EtOH). *Anal.* Calcd for $C_{27}H_{46}O_{10}\cdot 1.5H_2O$: C, 58.15; H, 8.86. Found: C, 58.71; H, 8.92. MS *m/z*: 530 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH).

Alkaline Hydrolysis of 1 with NaOH in *n*-BuOH——A solution of **1** (200 mg) in *n*-BuOH (30 ml) was treated with NaOH (1.5 g), and the mixture was stirred at 80 °C for 4 h. The reaction mixture was chromatographed on a column of Sephadex LH-20 with MeOH to afford **5** besides other proloquatifoligenins.

Proloquatifoligenin-V (**5**), a white powder, FD-MS *m/z*: 699 (M^++Na), 715 (M^++K).

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