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PLANT METABOLITES. NEW SESQUITERPENE GLYCOSIDES FROM *ERIOBOTRYA JAPONICA*

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ABSTRACT.—Four sesquiterpene glycosides were isolated from the leaves of *Eriobotrya japonica*. The structures were established as **1–4** through spectral studies (fabms and nmr) and chemical methods.

The leaves of *Eriobotrya japonica* Lindl. (Rosaceae), a small tree commonly known as loquat, are documented for use as a folk medicine for the treatment of various skin diseases (1) and diabetes mellitus (2).

Winter *et al.* (3) found that the alcoholic extract of the leaves exhibited an anti-inflammatory effect on carrageenan-induced foot edema in rats. More recently Shimizu *et al.* (1) reported the isolation of some known triterpenes from the Et_2O-soluble fraction of an EtOH extract of the leaves and among these, maslinic acid was found to have strong anti-inflammatory activity. In a recent communication Noreen *et al.* (4) have reported a significant hypoglycemic effect of the alcoholic extract in rabbits.

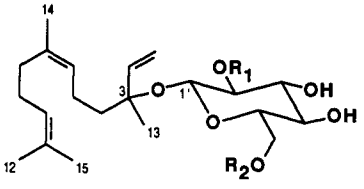
In view of the pharmaceutical interest of this species, we have been investigating the constituents of the $CHCl_3$, MeOH, and H_2O extracts, isolating a new polyhydroxylated terpenoid together with other known triterpenes from the $CHCl_3$ extract (5). The present paper deals with the further isolation and characterization of four new sesquiterpene glycosides **1–4** from the MeOH extract; this is the first report of the occurrence of glycosides of nerolidol in nature.

RESULTS AND DISCUSSION

The glycosidic mixture obtained from the MeOH extract of the leaves of *E. japonica* was purified by Sephadex LH-20 cc and dccc and then fractionated by hplc to yield the new glycosides **1–4**.

On acid methanolysis and glc analysis of the persilylated methylsugars, methylglucoside and methylrhamnoside were obtained in the ratio 1:1 from compound **1**, 1:2 from **2** and **3**, and 1:3 from **4**.

The molecular formulae $C_{27}H_{46}O_{10}$ for **1**, $C_{33}H_{56}O_{14}$ for **2** and **3**, $C_{39}H_{66}O_{18}$ for **4**, and the aglycone formula $C_{15}H_{26}O$ were determined by DEPT ^{13}C nmr, ^{13}C nmr,



1 R_1=Rha, R_2=H
2 R_1=Rha(1↦4)Rha, R_2=H
3 R_1=H, R_2=Rha(1↦4)Rha
4 R_1=Rha(1↦4)Rha, R_2=Rha
5 R_1=R_2=Rha
Rha=α-L-rhamnopyranosyl

and fabms analysis in the negative ion mode (Table 1). The fabms of **1** showed a quasi-molecular anion at *m/z* 529 $[M-H]^-$, prominent fragments at *m/z* 383 and 367 (cleavage of a rhamnose unit with or without the glycosidic oxygen), and a peak at *m/z* 221 (aglycone mass) due to the subsequent loss of a glucose unit (162 units from the 383 peak (Table 1). The above data clearly indicate that **1** possesses a disaccharide chain with glucose directly linked to the aglycone moiety and rhamnose as a terminal unit. The fabms spectra of both **2** and **3** show the $[M-H]^-$ ion at *m/z* 675, shifted 146 mass units relative to **1**. The fragmentation pattern is identical to that of **1** starting from *m/z* 529 $[(M-H)-146]^-$, suggesting that **2** and **3** differ from **1** by the presence of an extra rhamnose unit. In the fabms spectrum of **4**, we observed a quasi molecular anion at *m/z* 821 $[M-H]^-$, which was 146 mass units greater than that of **2** and **3** and suggested the presence of an extra rhamnose unit in **4**. The fragmentation pattern of **4** was identical to that of **2** and **3** starting from *m/z* 675 $[(M-H)-146]^-$.

TABLE 1. Negative fabms Data of Compounds **1–4**.[a]

1 *m/z*	**2** and **3** *m/z*	**4** *m/z*
529 $[M-H]^-$	675 $[M-H]^-$	821 $[M-H]^-$
383 $[(M-H)-146]^-$	529 $[(M-H)-146]^-$	675 $[(M-H)-146]^-$
367 $[(M-H)-162]^-$	513 $[(M-H)-162]^-$	659 $[(M-H)-162]^-$
221 $[(M-H)-(146+162)]^-$	383 $[(M-H)-(2\times146)]^-$	529 $[(M-H)-(2\times146)]^-$
205 $[(M-H)-(162+162)]^-$	367 $[(M-H)-(146+162)]^-$	383 $[(M-H)-(3\times146)]^-$
	221 $[(M-H)-(2\times146+162)]^-$	221 $[(M-H)-(3\times146+162)]^-$
	205	205

[a]The mass units lost correspond to the following fragments: *m/z* 162 glucose or rhamnose with glycosidic oxygen; *m/z* 146 rhamnose without glycosidic oxygen.

Analysis of ^{1}H-, DEPT ^{13}C-, and ^{13}C-nmr data (Table 2), suggested the identity of the aglycone as nerolidol in **1–4**. The ^{1}H-nmr signals were assigned by comparison with values reported for several nerolidol derivatives (6,7), the ^{13}C-nmr signals by comparison with data reported for geraniol and linalool model compounds (8). So we could deduce, for the aglycone of **1–4**, the geraniol partial structure plus one additional isoprene unit consisting of one methylene (δ 42.66), one oxygenated quaternary carbon (δ 82.70), one methyl (δ 23.05), and a vinyl (δ 144.50 and 115.54) group.

The downfield shift (β effect) observed for the C-3 resonance and the upfield shifts (γ effect) experienced by the C-2, C-4, and C-13 resonances in **1–4**, when compared with the corresponding signals in linalool (8), revealed that the sugar chain is attached at C-3. Analogous differences were observed by Miyase *et al.* (9) between the aglycone 3,7,11-trimethyl-1,6-dodecadien-3,10,11-triol and the corresponding glucoside at C-3 (icariside C_3). ^{1}H- and ^{13}C-nmr spectra (Table 2) of **1** indicate that it contains β-D-glucopyranosyl and α-L-rhamnopyranosyl units. The β configuration at the anomeric center of D-glucopyranose was suggested by the chemical shift and the large $J_{1'-2'}$ coupling (7.6 Hz) of the anomeric proton centered at δ 4.48 (1H, d, H-1′ glucose) in the ^{1}H-nmr spectrum, and by the resonances of C-3′ (78.7 ppm) and C-5′ (78.3 ppm) which appear at lower field than those of corresponding α anomers (10). Also, the position of C-1′ (98.1 ppm) is consistent with the linkage of a β-D-glucose to a tertiary alcoholic aglycone carbon (10). H-1′ at δ 4.48 and C-1′ (98.1 ppm) signals were shown to be correlated by direct ^{1}H, ^{13}C chemical shift correlation experiments (HETCOR). The α-configuration at the anomeric center of the α-L-rhamnose was derived by the chemical shift of H-1″ (δ 5.36) in the ^{1}H-nmr spectrum and by the C-3″ (72.7 ppm)

TABLE 2. ^{13}C- and ^{1}H-nmr Data for Compounds **1–4**.[a]

Position	Sugars								Position	Aglycone		
	Compound											
	4		**2**		**3**		**1**					
	δC	δH	δC	δH	δC	δH	δC	δH		δC	DEPT	δH
Glu 1′	98.1	4.48[b]	98.1	4.48[b]	98.1	4.48[b]	98.1	4.48 (1H, d, J = 7.6 Hz)	1	115.54	CH_2	H_t 5.22 (1H, dd, J = 17, 1 Hz)
2′	81.7		81.7		74.2		80.1	submerged by MeOH				H_c 5.25 (1H, dd, J = 10, 1 Hz)
3′	78.2		78.2		78.5		78.7	3.08 (1H, dd, J = 9.5, 9.5 Hz)	2	144.50	CH	5.95 (1H, dd, J = 10, 17 Hz)
4′	72.2		72.3		72.2		72.3	submerged by MeOH	3	81.70	C	
5′	76.4		77.4		76.4		78.3	3.18 (1H, m)	4	42.66	CH_2	1.98–2.12
6′	68.2		62.2		68.2		62.9	3.81 (dd, J = 12.5, 2.5 Hz)	5	25.60	CH_2	—
								3.65 (dd, J = 12.5, 4.5 Hz)	6	125.54	CH	5.24 (submerged)
Rha 1″	103.3	5.20[c]	103.3	5.22[c]	102.2	4.72[c]	102.0	5.36 (1H, d, J = 1.5 Hz)	7	136.17	C	
2″	72.4		73.0		72.2		72.7	3.92 (1H, dd, J = 1.5, 3.7 Hz)	8	40.68	CH_2	1.98–2.12
3″	72.2		72.2		72.3		72.7	3.7 (1H, dd, J = 3, 9 Hz)	9	27.06	CH_2	—
4″	79.8		79.8		79.8		74.0	3.59 (1H, dd, J = 9, 9 Hz)	10	125.72	CH	5.23 (submerged)
5″	68.2		68.2		69.2		69.8	4.11 (1H, dq, J = 6.2, 9 Hz)	11	132.00	C	—
6″	17.8	1.26[d]	17.8	1.26[d]	17.8	1.26[d]	18.0	1.26 (3H, d, J = 6.2 Hz)	12	16.76	Me	1.67 (3H, s)[e]
Rha 1‴	101.4	5.36[c]	101.4	5.36[c]	102.0	5.36[c]			13	23.05	Me	1.40 (3H, bs)
2‴	72.3		72.6		72.2				14	18.74	Me	1.67 (3H, bs)[e]
3‴	72.5		72.6		72.4				15	23.62	Me	1.72 (3H, bs)
4‴	74.2		74.0		74.0							
5‴	69.8		70.4		69.8							
6‴	17.9	1.27[d]	17.8	1.27[d]	17.8	1.26[d]						
Rha 1⁗	102.3	4.72[c]										
2⁗	72.5											
3⁗	72.2											
4⁗	74.0											
5⁗	70.4											
6⁗	17.8	1.26[d]										

[a] ^{1}H and ^{13}C correlations established by HETCOR experiments: s = singlet, d = doublet, dd = double doublet, b = broad.
[b] 1H, d, J = 7.5 Hz.
[c] 1H, d, J = 1.5 Hz.
[d] 3H, d, J = 6.2 Hz.
[e] Assignments which may be interchanged.

and C-5″ (69.8 ppm) resonances in the ^{13}C-nmr spectrum. H-1 is reported to appear at lower fields, while C-3 and C-5 resonate at higher fields in α-L-rhamnosides with respect to the corresponding β anomers (10).

Unfortunately, the small coupling constant of $J_{H1''\text{-}H2''}$ (1.5 Hz) is of no use in distinguishing between an α-L and a β-L-rhamnopyranoside. However, application of Klyne's rule (11) (Table 3) confirmed the configurations at the two anomeric centers, because the three alternative combinations, namely β-L-rhamnopyranosyl-β-D-glucopyranosyl, α-L-rhamnopyranosyl-α-D-glucopyranosyl, and β-L-rhamnopyranosyl-α-D-glucopyranosyl chains, imply contributions (+104°, +198°, or +478°, respectively) to the [M]D of **1** very different from the observed value (−170°).

TABLE 3. Calculated and Observed Contribution of the Sugar Chain to the [M]D of Compounds **1–4**.

Chain	[M]D
Methyl-α-D-glucopyranoside	+309° [a]
Methyl-β-D-glucopyranoside	− 66° [a]
Methyl-α-L-rhamnopyranoside	−111° [a]
Methyl-β-L-rhamnopyranoside	+170° [a]
[M]D glycoside **1**-[M]D nerolidol	−170°
α-L-rha + β-D-glu	−177°
[M]D glycoside **2**-[M]D nerolidol	−243°
[M]D glycoside **3**-[M]D nerolidol	−236°
α-L-rha + α-L-rha + β-D-glu	−288°
[M]D glycoside **4**-[M]D nerolidol	−377°
α-L-rha + α-L-rha + α-L-rha + β-D-glu	−399°
[M]D nerolidol	+ 34° [b]

[a]Values from Otsuka *et al.* (15).
[b]Value from "Dictionary of Organic Compounds" (16).

The interglycosidic linkage between the terminal rhamnose unit and the inner glucose unit must be at C-2′, which was shifted downfield (β effect) by 6 ppm while the other sugar carbon signals were virtually unchanged, as compared with a methylglycoside model (12). It follows that **1** is nerolidol-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

In compounds **2**, **3**, and **4** the β configuration of the D-glucopyranosyl unit and the α-L configuration of each L-rhamnopyranosyl unit are indicated by analogous considerations of the nmr data and molecular rotation values in light of Klyne's rule (11) (Table 3).

Of the three signals due to anomeric protons in the ^{1}H-nmr spectra of **2** and **3** (Table 2), the one at δ 4.48 (d, J = 7.6 Hz) was assigned to H-1′ of the β-D-glucopyranosyl unit, as it was always correlated by HETCOR experiments to the C-1′ resonance (98.1 ppm). The signals at δ 5.36 (d, J = 1.5 Hz) correlated to the C-1‴ resonance at 102.0 ppm in **3** and at 101.4 ppm in **2** and were assigned to the anomeric protons of the terminal α-L-rhamnopyranosyl units. The signals at δ 5.22 (d, J = 1.5 Hz) in **2** and at δ 4.72 (d, J = 1.5 Hz) in **3**, correlated with the C-1″ signals at 103.3 ppm and 102.2 ppm, respectively, and may be attributed to the anomeric protons of the central α-L-rhamnopyranosyl unit. In the ^{13}C-nmr spectrum of **3** deshielding of C-6′ by 5.3 ppm (from 62.9 in **1** to 68.2 ppm in **2**) and of C-4″ by 5.8 ppm (from 74.0 in **1** to 79.8 in **3**) and shielding of C-5′ by 1.9 ppm (from 78.3 in **1** to 76.4 ppm in **3**) indicates that glucose was substituted at the C-6′ position and the central rhamnose at the C-4″ position.

In the ^{13}C-nmr spectrum of **2**, analogous deshielding β effects experienced by C-2′ and C-4″ (7.5 and 5.8 ppm, respectively) and small shielding γ-effects experienced by C-3′, C-3″, and C-5″ with respect to compound **1** (see Table 2) indicate that glucose was substituted at C-2′ and the central rhamnose at C-4″. The chemical shifts of the carbon signals of the terminal rhamnose unit in both **2** and **3** were virtually unchanged with respect to model compounds (12) and, for the latter, to the analogous unit in **1**. From these data the structure nerolidol-3-*O*-α-L-rhamnopyranosyl-(1↦4)-α-L-rhamnopyranosyl-(1↦6)-β-D-glucopyranoside has been assigned to **3** and nerolidol-3-*O*-α-L-rhamnopyranosyl-(1↦4)-α-L-rhamnopyranosyl-(1↦2)-β-D-glucopyranoside to **2**.

It is noteworthy that in compounds **1**–**4** the anomeric proton of rhamnose linked at a secondary alcoholic carbon (C-6′ of glucose) as in **3** appears at higher field. (δ 4.72) than that of rhamnose linked at a tertiary alcoholic carbon (C-2′ of glucose) (δ 5.2–5.36) as in **1** and **2**. These data are consistent with the literature, which reports anomeric protons shielded in the order primary, secondary, tertiary alcoholic rhamnosides (10).

In comparing compound **4**, the major glycoside isolated from *E. japonica*, with compound **2** proton and carbon resonances of the sugars appeared at essentially the same position except for the presence of signals due to an additional rhamnose unit and for C-6′ and C-5′ resonances. C-6′ was shifted by 6.0 ppm (β effect) while C-5′ was shifted upfield by 1.0 ppm (γ effect) as expected for glycosidation shifts. The carbon signals of the additional terminal rhamnose unit (from C-1‴ to C-6‴) were superimposable with those of analogous units in **1**, **2**, and **3**.

These data indicate that **4** possesses a branched chain sugar with nodal glucose substituted at C-2′ and C-6′ and with rhamnose as one of the branches and rhamnosyl-(1↦4)-rhamnose as the second one.

The above data led to two possible sugar sequences in **4**:

(A) glucose $<^{6\mapsto 1\text{-rhamnose}}_{2\mapsto 1\text{-rhamnose-rhamnose}}$ (B) glucose $<^{6\mapsto 1\text{-rhamnose-rhamnose}}_{2\mapsto 1\text{-rhamnose}}$

Evidence in favor of sequence A was obtained by the results of enzymatic hydrolysis. Partial hydrolysis with pectinase of **4** led to the formation of compound **2** in addition to other glycosides. This established that the branch of the chain linked at C-2′ of glucose consists of two rhamnose units and the branch linked at C-6′ consists of one rhamnose unit. From these data, **4** is nerolidol-3-*O*-{α-L-rhamnopyranosyl-(1↦4)-α-L-rhamnopyranosyl-(1↦2)-[α-L-rhamnopyranosyl-(1↦6)]-β-D-glucopyranoside}.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The following instruments were used: nmr, Bruker WM-250 Spectrospin; ms, Kratos MS 902 spectrometer equipped with Kratos fab source; hplc, Waters Model 6000 A pump equipped with a U6K injector and a 401 refractive index detector; glc, Perkin-Elmer sigma 115 instrument; optical rotation, Perkin-Elmer 241 polarimeter; dccc, 670 Model Buchi apparatus equipped with 300 tubes. The fabms spectra and the DEPT experiments were performed as described earlier (13).

For the HETCOR experiments, Bruker commercial microprograms were used; delays were adjusted to an average CH coupling of 135 Hz on a 256–1024 data matrix (Bruker WM-250).

PLANT MATERIAL.—The plant was collected in April 1987, at Suzhou, Chang-Sou province, China, and was taxonomically examined by Dr. Jiang Mi of the Analytic Group of the Department of Traditional Chinese Medicine, Shanghai. A voucher sample of the plant is deposited at the herbarium of this department.

EXTRACTION AND ISOLATION.—The air-dried leaves (4 kg) were defatted with petroleum ether and $CHCl_3$ and extracted with MeOH to give 150 g of residue. Part of the residue (8.8 g) was chromatographed on a Sephadex LH-20 column (100 × 5 cm). Fractions (8 ml) were eluted with MeOH and checked by tlc [Si gel plates, *n*-BuOH–HOAc–H_2O (60:15:25)].

Fractions 18–27 (600 mg) containing the crude glycosidic mixture were further purified by dccc with $CHCl_3$-MeOH-H_2O (7:13:8) in which the stationary phase consisted of the lower phase (ascending mode, flow 12 ml/h). Fractions (4 ml) were collected to yield the more polar glycosides **4** and **2** in fractions 18–20 (250 mg), **2** and **3** in fractions 22–28 (120 mg), and the less polar **1** in the fractions 28–34 (60 mg).

Fractionation of each glycoside was achieved by hplc on a C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.) with MeOH-H_2O (65:35) to yield pure **4** (88 mg), Rt 6 min, $[\alpha]^{25}D$ −50° (*c* = 1, MeOH), [M]D −411°; **2** (44 mg), Rt 8 min, $[\alpha]^{25}D$ −41° (*c* = 1, MeOH), [M]D −277°; **3** (40 mg), Rt 8 min, $[\alpha]^{25}D$ −40° (*c* = 1, MeOH), [M]D −270°; and **1** (10 mg), Rt 12 min, $[\alpha]^{25}D$ −25.4° (*c* = 1, MeOH), [M]D −136°.

The ^1H- and ^{13}C-nmr data of **1**–**4** are in Table 2 and fabms data in Table 1.

Acid methanolysis.—Methanolysis of each glycoside (0.5–1 mg) was achieved as described earlier (12).

Enzymatic hydrolysis of **4**.—A solution of **4** (70 mg) in McIlvain buffer (pH = 4.35 ml) was incubated with crude pectinase from *Aspergillus niger* (Sigma, 35 mg) at 37° for 24 h (14). Progress of hydrolysis was monitored by tlc [Si gel, *n*-BuOH–HOAc–H_2O (60:15:25)] every 4 h, and the reaction was stopped when four main spots were detected. The reaction mixture was extracted with *n*-BuOH and concentrated in vacuo. The residue was purified by hplc [μ-Bondapak C_{18} column, MeOH-H_2O (65:35)] to give **4** (20 mg), **2** (3 mg), **5** (4 mg), and **1** (3 mg). Compounds **1**, **2**, and **4** were identified by R_f, Rt, and ^1H-nmr data.

Nerolidol-3-O- α-L-rhamnopyranosyl-(1↦2)-[α-L-rhamnopyranosyl-(1↦6)]-β-D-glucopyranoside [**5**].—^1H- and ^{13}C-nmr (CD_3OD, 250 MHz) aglycone signals superimposable on those of compounds **1**–**4**; sugar signals, ^1H nmr δ 1.26 (6H, d, *J* = 6.2 Hz), 4.48 (1H, d, *J* = 7.6 Hz), 4.72 (1H, d, *J* = 1.5 Hz), 5.36 (1H, d, *J* = 1.5 Hz); ^{13}C nmr (multiplicities deduced by DEPT) 98.1 (CH, C-1′), 80.8 (CH, C-2′), 78.2 (CH, C-3′), 72.0 (CH, C-4′), 76.4 (CH, C-5′), 68.2 (CH_2, C-6′), 102.2 (CH, C-1″), 72.7 × 2 (CH, C-2″ and C-3″) 74.0 (CH, C-4″), 69.0 (CH, C-5″), 18.0 (Me, C-6″), 102.0 (CH, C-1‴), 72.2 and 72.4 (each CH, C-2‴ and C-3‴), 74.2 (CH, C-4‴), 69.8 (CH, C-5‴), 17.8 (Me, C-6‴).

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