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THREE SECOIRIDOID GLUCOSIDES FROM *LIGUSTRUM JAPONICUM**

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Key Word Index—*Ligustrum japonicum*; Oleaceae; structure elucidation; biogenesis; secoiridoid glucosides; ligustaloside A; ligustaloside B; 10-hydroxyoleuropein.

Abstract—Three new secoiridoid glucosides, ligustaloside A, ligustaloside B and 10-hydroxyoleuropein, along with two known glucosides, oleuropein and ligstroside, were isolated from the leaves of *Ligustrum japonicum* and their structures elucidated. The biogenesis of these secoiridoid glucosides is discussed.

INTRODUCTION

In the course of our studies on the iridoid glucosides of oleaceous plants [1], we elucidated the structure of nüzhenide (**1**), oleuropein (**2**) and the non-iridoid glucoside salidroside, isolated from the ripe fruits of *Ligustrum lucidum* Ait. (Japanese name, Tonezumimochi) and *L. japonicum* Thunb. (Japanese name, Nezumimochi)[2]. It had been reported that the alcoholic extract of the leaves of *L. japonicum*, which contain ursolic acid[3–5], showed a positive inotropic action[6]. The present paper describes the structure elucidation of three new secoiridoid glucosides isolated together with two known glucosides from the leaves of *L. japonicum*. The biogenesis of these glucosides is also discussed. A part of this work was published in the review article [7].

RESULTS AND DISCUSSION

The methanolic extract of the fresh leaves of *L. japonicum* was fractionated by sequential droplet countercurrent chromatography, medium pressure CC and prep. TLC to give three new glucosides, ligustaloside A(**3**), ligustaloside B (**4**) and 10-hydroxyoleuropein (**5**) along with two known glucosides, oleuropein (**2**) [8] and ligstroside (**6**)[9].

Ligustaloside A (**3**), was obtained as a white powder, $C_{25}H_{32}O_{14}$. 1/2 H_2O, $[\alpha]_D$ − 120.1° (MeOH). It showed UV maxima at 231 and 282 nm (log ε 4.21 and 3.50) and IR bands at 3375, 1710, 1630 and 1520 cm^{-1}. These spectral data closely resembled those of oleuropein (**2**), suggesting that ligustaloside A (**3**) had –OC(=O)–C=CH–O– and phenylethyl moieties as chromophores. The ^1H NMR spectrum (in CD_3OD) of **3** exhibited two triplets at δ 2.78 (2H, J = 7.0 Hz) and 4.21 (2H, J = 7.0 Hz) and a multiplet at δ 6.42–6.85 (3H), besides a singlet at δ 3.65 due to a carbomethoxy group and a broad singlet at δ 7.48 due to a proton of the carboxyenolic chromophore, as well as a signal at δ 9.63 integrating for less than one proton, indicating a partly hydrated aldehyde function (see below). The former three signals were attributed to the 3,4-dihydroxyphenylethyl moiety. Acetylation of **3** with acetic anhydride–pyridine gave two products **7**, $C_{37}H_{44}O_{20}$, and **8**, $C_{39}H_{46}O_{21}$, mp 128.5–130.5°. The ^1H NMR spectrum of **7** was similar to that of oleuropein hexa-acetate (**9**) except for the absence of signals due to an olefinic proton and a methyl group of the ethylidene group and the appearance of a new triplet-like signal due to an aldehydic proton at δ 9.69. This observation suggested that **7** differed from **9** only in the moiety consisting of C-8–C-10 and possessed a C-10 aldehyde group. On the other hand, the ^1H NMR spectrum of **8** lacked a signal of an aldehydic proton but showed two one-proton signals at δ 7.0–7.3 and 4.4–4.5 as well as two singlets at δ 1.91 and 1.93 together integrating to one acetoxy group, besides signals of four alcoholic acetoxy groups at δ2.01–2.12. These data suggested that **8** was a mixture of both geometric isomers of the enol acetate corresponding to **7**. This was corroborated through chemical correlation of **7** with **8**, i.e. sodium borohydride-reduction of **7** in carbon dioxide-saturated dioxane gave alcohol **10**, which, on acetylation, afforded the hepta-acetate **11**, mp 124.5–126°. The latter was also obtained on catalytic hydrogenation (Pd–C) of **8**.

Reduction of **3** with sodium borohydride afforded the alcohol **12**, which on alkaline hydrolysis gave 3,4-dihydroxyphenylethyl alcohol (**13**) and a secoiridoid glucoside which was then acetylated and methylated (diazomethane) sequentially to give the dimethyl ester penta-acetate **14**. Its ^1H NMR spectrum exhibited, in addition to all signals of the secoiridoid glucoside moiety of **11**, a signal due to a new carbomethoxy group. Since it seemed rather difficult to distinguish the signals of the two carbomethoxy groups of **14** only from the comparison of their chemical shifts, further information about the two groups was obtained in the following way. Glucoside

*Part 46 in the series "Studies on Monoterpene Glucosides and Related Natural Products". For Part 45, see Uesato, S., Kobayashi, K. and Inouye, H. (1982) *Chem. Pharm. Bull. (Tokyo)* **30**, 927.

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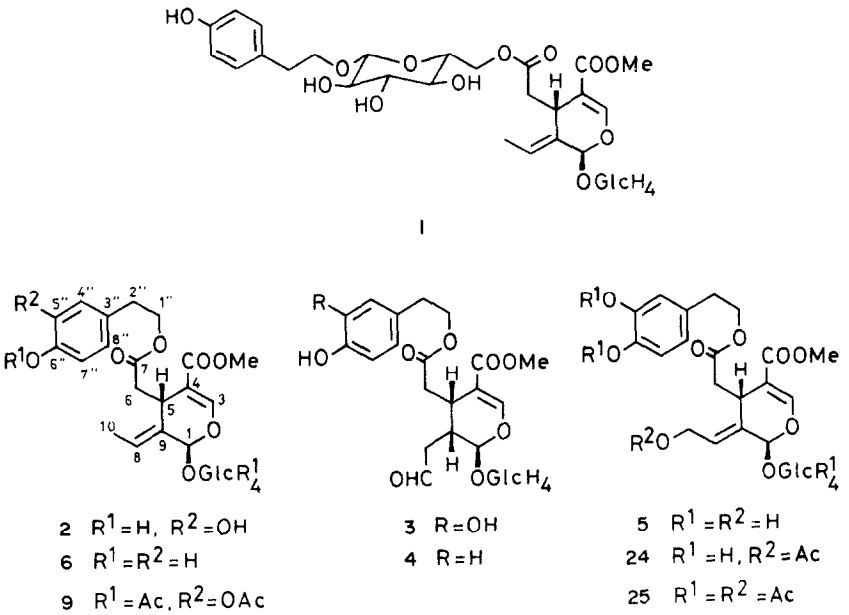

I

2 R^1=H, R^2=OH
6 R^1=R^2=H
9 R^1=Ac, R^2=OAc

3 R=OH
4 R=H

5 R^1=R^2=H
24 R^1=H, R^2=Ac
25 R^1=R^2=Ac

Fig. 1.

3 was reduced with lithium borohydride and then acetylated to give **15**, whose UV and IR spectra still indicated the presence of the chromophore –OC(C=O)–C=CH–O–. Its ^{1}H NMR spectrum showed signals at δ 2.00–2.09 (each *s*) due to six acetoxy groups, at δ 3.71 (*s*) due to a carbomethoxy group and at δ 7.41 (*br s*) for H-3, but did not show any signals attributable to protons of the 3,4-dihydroxyphenylethyl moiety or an aldehydic proton. Since these data meant that the saturated phenylethyl ester and aldehyde groups of **3** were reduced with lithium borohydride, the structure **15** was assigned to the above reduction product. Therefore it was confirmed that the methoxycarbonyl group was located at C-11 in compounds **3** and **11**.

To eliminate the remaining possibility that ligustaloside A (**3**) had the structure with the aldehyde and 3,4-dihydroxyphenylethyl groups at C-10 and C-7, respectively, and to establish its absolute configuration, the following series of reactions were carried out. The alcohol **10** was converted to the tosylate, **16**, which was in turn treated with sodium iodide to give the iodide, **17**, mp 127.5–129°. Deiodination of **17** with tri-*n*-butyl tin hydride[10] gave the hexa-acetate **18**, mp 112–113.5°, along with a small amount of the tetra-acetate **19**. Alkaline hydrolysis of **18** afforded a secoiridoid glucoside, which on acetylation followed by methylation (diazomethane) gave the ester **20**, mp 136–136.5°. This compound was identical with dihydrosecologanoside methyl ester tetra-acetate (**20**) obtained from secologanoside methyl ester tetra-acetate (**21**)[11] through catalytic hydrogenation. Thus, the absolute configuration of ligustaloside A was established as **3**.

In the ^{1}H NMR spectrum of ligustaloside A (**3**), the signal intensity ratio of the aldehydic proton to any other proton was always less than one, though it varied with the solvents used (D_2O, CD_3OD and C_5D_5N). In keeping with this observation, the ^{13}C NMR spectrum (see Table 1) of **3** in CD_3OD showed a very weak signal attributable to an aldehyde carbon. Additionally, no carbon signal other than those due to C-3, C-4 and a phenylethyl moiety appeared in the sp^2 carbon region, and three signals besides that of C-1 appeared at *ca* δ 98. These signals were thought to be attributable to acetal, hemiacetal and hydrate carbons of the compound generated by the solvation of **3** with methanol containing a small amount of water. Accordingly, ligustaloside A (**3**) exists in solution as a mixture of aldehyde, acetal, hemiacetal and hydrate forms.

The second glucoside, ligustaloside B(**4**), was obtained as a white powder, $C_{25}H_{32}O_{13}$, $[\alpha]_D$ – 120.0° (MeOH). It showed UV maxima at 226, 240 (inf), 277 and 284 (sh) nm (log ϵ 4.24, 4.06, 3.25 and 3.18) and IR bands at 3380, 1710, 1630 and 1520 cm^{-1}. Its ^{1}H and ^{13}C NMR spectra were similar to those of ligustaloside A (**3**) except for the signals of aromatic protons and carbons. The ^{1}H NMR spectrum of **4** showed, besides two triplets at δ 2.83 (2H, J = 7.0 Hz) and 4.20 (2H, J = 7.0 Hz), the AA′ BB′ signal pattern centred at δ 6.88, indicative of the presence of a 4-hydroxyphenylethyl moiety. These data implied that the structural relationship between ligustalosides A (**3**) and B (**4**) was the same as that between oleuropein (**2**) and ligstroside (**6**). Compound **4** was, therefore, assumed to possess a 4-hydroxyphenylethyl alcohol (**22**) moiety instead of 3,4-dihydroxyphenylethyl alcohol (**13**) moiety of **3**. This was confirmed in the following way: ligustaloside B (**4**) was reduced with sodium borohydride to the alcohol **23** which, on alkaline hydrolysis, gave a secoiridoid glucoside along with 4-hydroxyphenylethyl alcohol (**22**). Through acetylation followed by methylation, this glucoside was converted to a dimethyl ester penta-acetate, which was identical with compound **14** derived from **3**. The structure of ligustaloside B was therefore established as **4**.

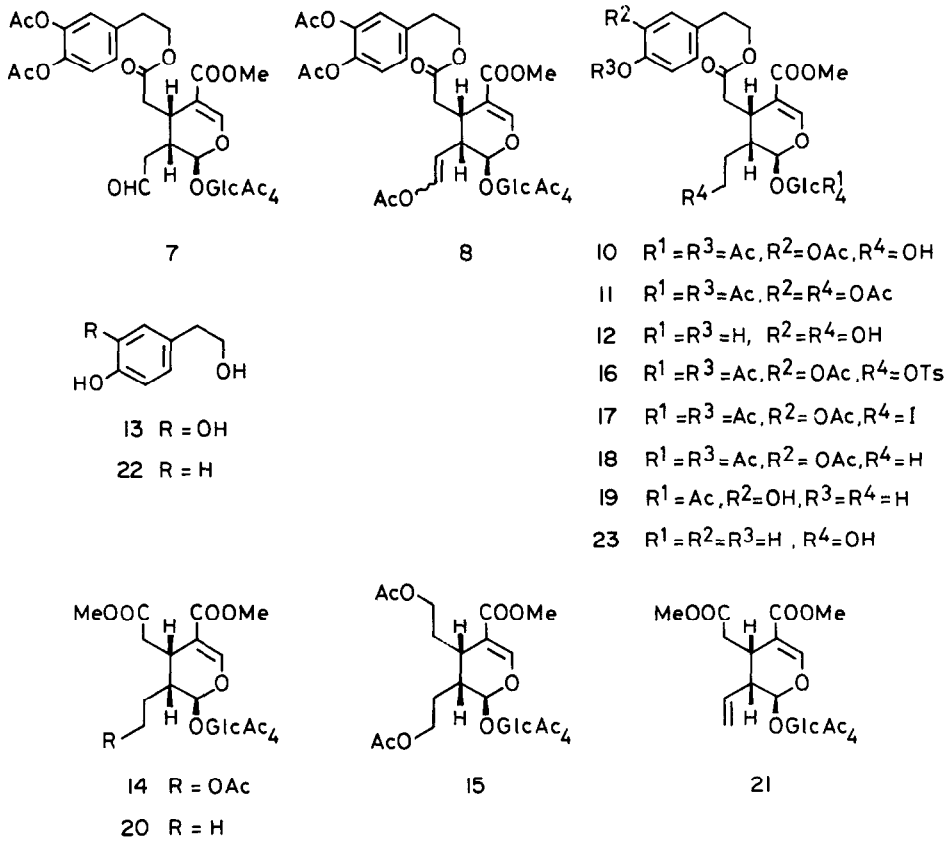

Fig. 2.

The third glucoside (**5**) was isolated as a white powder, $C_{25}H_{32}O_{14}$. 1/2 H_2O, $[\alpha]_D - 153.7°$ (MeOH). It showed UV absorptions at 231 and 282 nm (log ϵ 4.15 and 3.49) and IR bands at 3370, 1700, 1630 and 1520 cm^{-1}. Its ^1H NMR spectrum was similar to that of 10-acetoxyoleuropein (**24**) except it lacked an acetoxy group. Furthermore, the ^{13}C NMR spectrum of **5** showed a signal of C-10 in the upper field and a frequency of C-8 in the lower field in comparison with the corresponding signals of **24**. Glucoside **5** was therefore assumed to be a deacetyl derivative of **24**, i.e. 10-hydroxyoleuropein. In fact, **5** was converted into the hexa-acetate (**25**) of **24**.

The isolation of the above-mentioned glucosides, especially **3** and **4**, gives important clues with regard to the biogenesis of secoiridoid glucosides of the oleoside-type (**26**) represented by **1** and **6** as well as of the 10-hydroxyoleoside-type (**27**) exemplified by **5** and **24**. All these glucosides, which have so far been found only in the oleaceous plants, are structurally characterized by the esterified C-7 carboxy group and the ethylidene or hydroxyethylidene group at C-9. They are known to be biosynthesized via secologanin (**28**) [12] but the details of the pathway after **28** had not been elucidated. In view of the occurrence of ligustalosides A (**3**), B (**4**) and 10-hydroxyoleuropein (**5**) together with oleuropein (**2**) and ligstroside (**6**) in the same plant, the biogenesis of three series of glucosides could reasonably be explained by postulating the key intermediacy of the epoxide **29**, as follows: (a) cleavage of the epoxide ring of the intermediate **29** accompanied by a hydride shift leads to ligustalosides A (**3**) and B (**4**); (b) cleavage of the epoxide ring of **29** to 8-ol (**30**) followed by dehydration leads to glucosides of the oleoside-type (**26**); and (c) cleavage of the epoxide ring of **29** followed by deprotonation on C-9 leads to glucosides of the 10-hydroxyoleoside-type (**27**) (Fig. 3).

The geometry of the ethylidene group suggests that the C-8 configuration of epoxide **29**, a supposed intermediate, should be *S* on the assumption that the dehydration of **30** would proceed by *trans* elimination. It is noteworthy that eustomoside (**31**), a secoiridoid glucoside with an epoxide ring, was recently isolated from *Eustoma russelianum* [13]. Thus, it seems likely that the epoxide **29** could play a key intermediary role in the biosynthetic pathway of the above-mentioned secoiridoid glucosides. However, an alternative possibility that 10-hydroxy-oleoside-type glucosides could be generated by the hydroxylation of oleoside-type glucosides cannot be ruled out.

In view of the co-occurrence of secoiridoid glucosides of the types **26** and **27** esterified with 3,4-dihydroxyphenylethyl alcohol (**13**) or 4-hydroxyphenylethyl alcohol (**22**), the esterification of the C-7 carboxy group would probably occur after the formation of skeletons of these types via the supposed pathway described above.

EXPERIMENTAL

General procedures. Mps were uncorr. ^1H and ^{13}C NMR spectra were measured at 60 and 50.10 MHz, respectively. TMS was used as int. standard in $CDCl_3$, CD_3OD or C_5D_5N,

Table 1. ^{13}C NMR data of secoiridoid glucosides (50.10 M Hz, CD_3OD, TMS as int. standard)*

Carbon	3†	4†	12	23	2	5	24
1	97.9 (*d*)	97.8 (*d*)	98.4 (*d*)	98.5 (*d*)	95.2 (*d*)	94.9 (*d*)	94.3 (*d*)
3	154.1 (*d*)	154.1 (*d*)	154.0 *d*)	154.1 (*d*)	155.1 (*d*)	155.1 (*d*)	154.9 (*d*)
4	110.6 (*s*)	110.6 (*d*)	11.05 (*s*)	110.7 (*s*)	109.4 (*s*)	109.4 (*s*)	109.1 (*s*)
5	30.8 (*d*)	30.8 (*d*)	30.6 (*d*)	30.7 (*d*)	31.7 (*d*)	32.4 (*d*)	32.4 (*d*)
6	36.6 (*t*)	36.5 (*t*)	36.4 (*t*)	36.5 (*t*)	41.2 (*t*)	41.4 (*t*)	41.2 (*t*)
7	174.3 (*s*)	174.3 (*s*)	174.3 (*s*)	174.4 (*s*)	173.1 (*s*)	173.1 (*s*)	172.8 (*s*)
8	42.0 (*t*)	42.0 (*t*)	30.5 (*t*)	30.6 (*t*)	124.8 (*d*)	129.5 (*d*)	124.4 (*d*)
9	37.3 (*d*)	37.4 (*d*)	37.4 (*d*)	37.6 (*d*)	130.5 (*s*)	131.2 (*s*)	133.9 (*s*)
10	202.8 (*d*)	202.7 (*d*)	60.6 (*t*)	60.7 (*t*)	13.6 (*q*)	59.3 (*t*)	61.8 (*t*)
11	168.9 (*s*)	168.9 (*s*)	168.9 (*s*)	168.9 (*s*)	168.6 (*s*)	168.5 (*s*)	168.3 (*s*)
OMe	51.8 (*q*)	51.8 (*q*)	51.7 (*q*)	51.7 (*q*)	51.9 (*q*)	52.0 (*q*)	52.0 (*q*)
1′	100.8 (*d*)[a]	100.8 (*d*)[c]	100.4 (*d*)	100.6 (*d*)	100.9 (*d*)	100.1 (*d*)	100.9 (*d*)
2′	74.8 (*d*)	74.8 (*d*)	74.7 (*d*)	74.8 (*d*)	74.7 (*d*)	74.8 (*d*)	74.7 (*d*)
3′	78.4 (*d*)	78.4 (*d*)	78.2 (*d*)	78.4 (*d*)	78.3 (*d*)	78.5 (*d*)	78.4 (*d*)
4′	71.7 (*d*)	71.6 (*d*)	71.5 (*d*)	71.7 (*d*)	71.4 (*d*)	71.6 (*d*)	71.4 (*d*)
5′	78.0 (*d*)	78.0 (*d*)	77.9 (*d*)	78.0 (*d*)	77.9 (*d*)	78.0 (*d*)	77.9 (*d*)
6′	62.8 (*t*)	62.8 (*t*)	62.8 (*t*)	62.9 (*t*)	62.7 (*t*)	62.9 (*t*)	62.7 (*t*)
1″	35.4 (*t*)	35.2 (*t*)	35.3 (*t*)	35.2 (*t*)	35.3 (*t*)	35.4 (*t*)	35.4 (*t*)
2″	66.9 (*t*)	66.9 (*t*)	66.8 (*t*)	66.8 (*t*)	66.8 (*t*)	66.9 (*t*)	66.9 (*t*)
3″	130.9 (*s*)	130.1 (*s*)	130.8 (*s*)	130.1 (*s*)	130.7 (*s*)	130.8 (*s*)	130.7 (*s*)
4″	116.5 (*d*)[b]	130.9 (*d*)	116.4 (*d*)[d]	130.9 (*d*)	116.5 (*d*)[e]	116.6 (*d*)[f]	116.4 (*d*)[g]
5″	146.2 (*s*)	116.4 (*d*)	146.1 (*s*)	116.4 (*d*)	146.1 (*s*)	146.3 (*s*)	146.2 (*s*)
6″	144.9 (*s*)	157.0 (*s*)	144.8 (*s*)	157.1 (*s*)	144.8 (*s*)	145.0 (*s*)	146.9 (*s*)
7″	117.1 (*d*)[b]	116.4 (*d*)	117.0 (*d*)[d]	116.4 (*d*)	117.0 (*d*)[e]	117.2 (*d*)[f]	117.1 (*d*)[g]
8″	121.3 (*d*)	130.9 (*d*)	121.2 (*d*)	130.9 (*d*)	121.3 (*d*)	121.4 (*d*)	121.3 (*d*)

*Signal multiplicities in parentheses were obtained by off-resonance decoupling experiments or INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) experiments.

†**3** and **4** had additional signals arising from acetal, hemiacetal and hydrate forms: 3; C-1, 98.1 (*d*)[h]; C-8, 35.1 (*t*) and 34.9 (*t*); C-10, 98.6 (*d*)[h], 98.5 (*d*)[h] and 98.2 (*d*)[h]; C-1′, 100.7 (*d*)[a]. 4; C-1, 98.0 (*d*)[i]; C-8, 35.1 (*t*) and 34.9 (*t*); C-10, 98.6 (*d*)[i], 98.5 (*d*)[i] and 98.1 (*d*)[i]; C-1′, 100.6 (*d*)[c].

Values with the same superscript may be interchanged.

whereas DSS was employed in D_2O. Si gel AR-100 (Mallinckrodt) was used for CC and Si gel PF_{254} for medium pressure CC. Si gel GF_{254} was used for TLC and spots were visualized by irradiation under UV (245 nm), by exposure to I_2 vapour or by spraying with anisaldehyde–H_2SO_4 reagent followed by heating. Si gel PF_{254} was employed for prep. TLC and bands were detected under UV. Droplet counter current chromatography (DCCC) [using Pyrex glass tubes (120 cm × 2.4 mm) connected to each other by Teflon tubing (140 cm × 1.35 mm)] was carried out by the ascending method with the solvent system, *n*-BuOH–EtOH–H_2O (4 : 1 : 5).

Plant material. Ligustrum japonicum Thunb. was collected at Kyoto Botanical Garden in October. Plant material was identified by Mr. G. Murata of Faculty of Science, Kyoto University. The voucher specimen (H. Inouye No. 3) is deposited in the Herbarium of the Institute of Botany, Faculty of Science, Kyoto University, Kitashirakawa-oiwake-cho, Sakyo-ku, Kyoto 606, Japan.

Isolation of glucosides from L. japonicum. Fr. leaves (2.3 kg) were extracted with hot MeOH (18 l. × 3). After concn of the combined extracts *in vacuo*, H_2O (3 l.) was added and the insoluble material was filtered off through a Celite layer, which was washed with H_2O (2 l.). The combined filtrate and washings were concd *in vacuo* to 1 l. The resulting soln was extracted successively with $CHCl_3$ (0.3 l × 3) and *n*-BuOH (0.7 l × 3). The *n*-BuOH layer* was concd *in vacuo* to give a foamy residue (113.96 g). An aliquot (3.01 g) of the residue was subjected to DCCC and 7 g fractions of the eluted mobile phase were collected. Fractions 20–34, 35–65 and 71–105 were concd *in vacuo* to afford residues R-1 (474 mg), R-2 (761 mg) and R-3 (207 mg), respectively. R-1 was submitted to medium pressure CC on Si gel (15 g) with $CHCl_3$–MeOH of increasing MeOH content. The fractions eluted with $CHCl_3$–MeOH (9 : 1) were combined, concd *in vacuo* and lyophilized to give a white powder (42 mg). This substance was identical with an authentic sample of oleuropein (**2**) (TLC and ^1H NMR). Likewise, the eluate with $CHCl_3$–MeOH (22 : 3) afforded ligustaloside B (**4**) (61 mg) as a white powder and the eluate with $CHCl_3$–MeOH (17 : 3) gave ligustaloside A (**3**) (55 mg) as a white powder. R-2 was submitted to medium pressure CC on Si gel (30 g) in the same way as above, yielding ligustaloside A (**3**) (404 mg). R-3 was subjected to prep. TLC ($CHCl_3$–MeOH, 3 : 1) to give 10-hydroxyoleuropein (**5**) (35 mg) as a white powder.

Ligustaloside A (**3**). $[\alpha]_D^{18} - 120.1°$ (MeOH; c 1.00). ^1H NMR (CD_3OD): δ 2.78 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–), 3.65 (3H, *s*, COOMe). 4.21 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–). 6.42–6.85 (3H, *m*, arom. H), 7.48 (1H, *br s*, H-3), 9.63 (0.2 H, *br s*, CHO). (Found: C, 53.00; H, 5.88. $C_{25}H_{32}O_{14}$. 1/2 H_2O requires: C, 53.10; H, 5.88%.)

*In another expt using the leaves of the same plant collected in September, ligstroside (**6**) was isolated from this layer along with the other glucosides, but it was not detected in this expt.

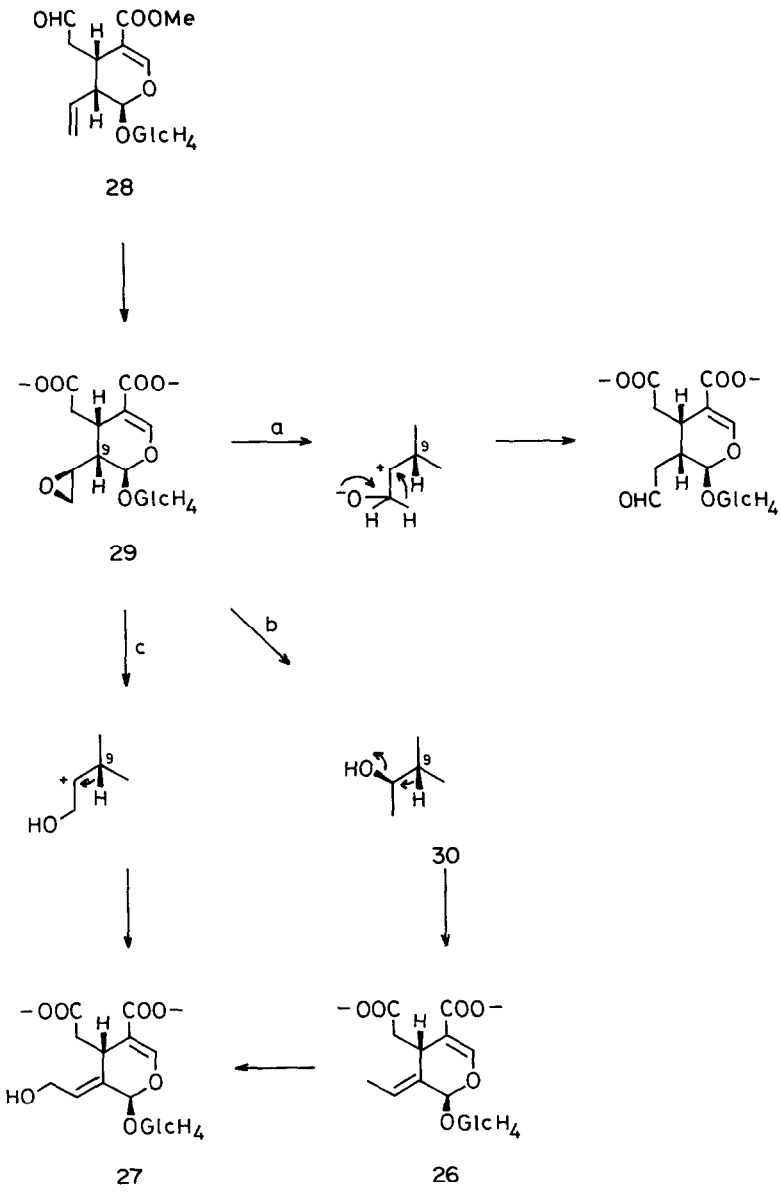

Fig. 3.

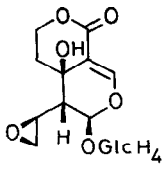

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Fig. 4.

Ligustaloside B (**4**). $[\alpha]_D^{20}$ − 120.0° (MeOH; *c* 0.95), ^{1}H NMR (CD_3OD): δ 2.83 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–), 3.65 (3H, *s*, COOMe), 4.20 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–), 6.88 (4H, AA'BB' pattern, arom. H), 7.45 (1H, *br s*, H-3), 9.58 (0.3H, *br s*, –CHO). (Found: C, 55.25; H, 6.03. $C_{25}H_{32}O_{13}$ requires: C, 55.55; H, 5.97%.)

10-*Hydroxyoleuropein* (**5**). $[\alpha]_D^{20}$ − 153.7° (MeOH; *c*, 0.38); ^{1}H NMR (D_2O): δ 2.78 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–), 3.70 (3H, *s*, COOMe), 4.20 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–) 5.78 (1H, *br s*, H-1), 6.08 (1H, *br t*, *J* = 6.0 Hz, H-8), 6.50–6.95 (3H, *m*, arom. H), 7.49 (1H, *s*, H-3). (Found: C, 53.38; H, 5.96. $C_{25}H_{32}O_{14}.1/2\ H_2O$ requires: C, 53.10; H, 5.88%.)

Acetylation of ligustaloside A (**3**). **3** (1.98 g) was acetylated with Ac_2O–C_5H_5N for 1 hr at room temp. The crude product was submitted to medium pressure CC on Si gel (90 g) with $CHCl_3$–MeOH (99 : 1) as eluent. The first part of the eluate was concd *in vacuo* and the residue recrystallized from Et_2O to give ligustaloside A enol acetates (**8**) (0.42 g) as colourless needles, mp 128.5–130.5°. IR ν_{max}^{KBr} cm^{-1}: 1745, 1695, 1635, 1505; ^{1}H NMR ($CDCl_3$): δ 1.91, 1.93, 2.01, 2.03, 2.10, 2.12 (*s*'s together integrating for 5 × alcoholic OCOMe), 2.28 (*s*, 2 × phenolic OCOMe), 2.93 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–), 4.40–5.40 (altogether 1H, *m*'s, β-enolic H), 6.97–7.30 (4H, *m*, arom. H and)=(H/OAc), 7.40 (1H, *br s*, H-3). (Found: C, 55.32; H, 5.32. $C_{39}H_{46}O_{21}$ requires: C, 55.06;

H, 5.45%.) The last of the eluate furnished ligustaloside A hexa-acetate (**7**) (1.38 g) as a white powder, $[\alpha]_D^{13} - 91.1°$ ($CHCl_3$; *c* 0.99); UV λ_{max}^{MeOH} nm (log ε): 217(4.13), 230 (inf) (4.10), 270 (2.83); IR ν_{max}^{KBr} cm^{-1}: 1755, 1710, 1635, 1510; ^1H NMR ($CDCl_3$): δ 1.98, 2.00, 2.03, 2.08 (each *s*, 4 × alcoholic OCOMe),2.27 (*s*, 2 × phenolic OCOMe), 2.93 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–), 3.68 (3H, *s*, COOMe), 4.28 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–), 7.00–7.18 (3H, *m*, arom. H), 7.39 (1H, *d*, *J* = 1.0 Hz, H-3). 9.69 (1H, triplet-like, H-10). (Found: C, 54.75; H, 5.56. $C_{37}H_{44}O_{20}$ requires: C, 54.95; H, 5.48%.)

$NaBH_4$ *reduction of ligustaloside A hexa-acetate* (**7**). A stirred soln of **7** (875 mg) in dioxane (10 ml) was satd with CO_2 by addition of dry ice. $NaBH_4$ (72 mg) was then added to the soln, and the mixture was stirred for 1 hr at room temp. After addition of HOAc, the reaction mixture was diluted with H_2O and extracted with $CHCl_3$ (20 ml × 3). The H_2O washed and dried organic layer was evaporated *in vacuo* to leave a residue (898 mg), which was chromatographed on Si gel (30 g) and eluted with C_6H_6–Me_2CO with an increasing Me_2CO content. Elution with C_6H_6–Me_2CO (9 : 1 and 17 : 3) gave dihydroligustaloside A hexa-acetate (**10**) (803 mg) as a white powder, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 1750, 1705 (sh), 1625, 1500; ^1H NMR ($CDCl_3$): δ 1.98, 2.02, 2.03, 2.08 (each *s*, 4 × alcoholic OCOMe), 1.83–2.13 (1H, *m*, OH, disappeared on addition of D_2O), 2.28 (*s*, 2 × phenolic OCOMe), 2.95 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–), 3.59 (2H, *t*, *J* = 6.5 Hz, H_2-10), 3.68 (3H, *s*, COOMe), 4.30 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–), 6.89–7.28 (3H, *m*, arom. H), 7.38 (1H, *d*, *J* = 1.0 Hz, H-3). (Found: C, 53.47; H, 5.53. $C_{37}H_{46}O_{20}.H_2O$ requires: C, 53.62; H, 5.84%.)

Acetylation of dihydroligustaloside A hexa-acetate (**10**). Compound **10** (28.2 mg) was acetylated (Ac_2O–C_5H_5N) and the product (30.1 mg) was recrystallized from EtOH to give dihydroligustaloside A hepta-acetate (**11**) (17.8 mg) as colourless needles mp 124.5–126°. $[\alpha]_D^{22} - 86.0°$ ($CHCl_3$; *c* 1.00); UV λ_{max}^{EtOH} nm (log ε): end absorption, 269 (sh) (2.80); IR ν_{max}^{KBr} cm^{-1}: 1765, 1745, 1690, 1640, 1510; ^1H NMR ($CDCl_3$): δ 1.98, 2.00, 2.03, 2.08 (*s*'s together integrating for 5 × alcoholic OCOMe), 2.28 (*s*, 2 × phenolic OCOMe), 2.93 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–), 3.67 (3H, *s*, COOMe), 4.12 (2H, *t*, *J* = 6.5 Hz, H_2-10), 4.28 (2H, *t*, *J* = 7.0 Hz, $ArCH_2CH_2O$–), 6.97–7.23 (3H, *m*, arom. H), 7.38 (1H, *d*, *J* = 1.0 Hz, H-3). (Found: C, 55.10; H, 5.93. $C_{37}H_{48}O_{21}$ requires: C, 54.93; H, 5.67%.)

Catalytic hydrogenation of ligustaloside A hepa-acetate (**8**). Compound **8** (80.4 mg) in MeOH (5 ml) was hydrogenated over a Pd–C catalyst [prepared from 5% $PdCl_2$–HCl soln (0.3 ml) and activated charcoal (Darco G-60, 70 mg)]. After an uptake of *ca* 1 mol of H_2, the catalyst was filtered off and the filtrate was concd *in vacuo*. The residue (93.8 mg) was recrystallized from EtOH to give colourless needles (76.6 mg). This substance was identical with a sample of **11** derived from **7** (mmp, ^1H NMR and IR).

$NaBH_4$ *reduction of ligustaloside A* (**3**). To a soln of **3** (300 mg) in EtOH (10 ml) was added $NaBH_4$ (30 mg) under ice cooling and the whole was stirred for 1 hr. After acidification with HOAc, the reaction mixture was concd *in vacuo*. The resulting residue was purified by prep. TLC ($CHCl_3$–MeOH, 7 : 3) to afford **12** (264 mg) as a white powder, $[\alpha]_D^{29} - 177.0°$ (MeOH; *c* 1.03); UV λ_{max}^{MeOH} nm (log ε): 231 (4.13), 282 (3.41); IR ν_{max}^{KBr} cm^{-1}: 3350, 1700, 1630, 1510; ^1H NMR (D_2O): δ 2.80 (2H, *t*, *J* = 6.0 Hz, $ArCH_2CH_2O$–), 3.63 (3H, *s*, COOMe), 3.83 (2H, *d*, *J* = 6.0 Hz, H_2-10), 4.25 (2H, *t*, *J* = 6.0 Hz, $ArCH_2CH_2O$–), 5.37 (1H, *d*, *J* = 8.0 Hz, H-1), 6.55–7.00 (3H, *m*, arom. H), 7.52 (IH, *s*, H-3). (Found: C, 52.20; H, 6.34. $C_{25}H_{34}O_{14}.H_2O$ requires: C, 52.08; H, 6.29%.)

Alkaline hydrolysis of dihydroligustaloside A (**12**). A soln of **12** (323 mg) in 0.5 N NaOH (15 ml) was stirred for 3.5 hr at room temp. The mixture was acidified with Amberlite IR-20 (H^+form) and extracted with EtOAc (20 ml × 4). The dried EtOAc layer was evaporated *in vacuo* to give a syrupy substance (31 mg), which was identical with an authentic sample of 2-(3,4-dihydroxyphenyl)ethyl alcohol (**13**) (^1H NMR and IR). The aq. layer was evaporated *in vacuo* and the residue was subjected to methylation with CH_2N_2–Et_2O followed by conventional acetylation. The product was recrystallized from Et_2O–petrol to give **14** (219 mg) as colourless needles, mp 98.5–99.5°. $[\alpha]_D^{20} - 99.2°$ ($CHCl_3$; *c* 0.40); UV λ_{max}^{MeOH} nm (log ε): 232 (4.08); IR ν_{max}^{KBr} cm^{-1}: 1745, 1690, 1635; ^1H NMR ($CDCl_3$): δ 1.98, 2.01, 2.03, 2.05, 2.10 each *s*, 5 × OCOMe), 3.66, 3.69 (each *s*, 2 × COOMe), 4.15 (2H, *t*, *J* = 6.5 Hz, H_2-10), 7.41 (1H, *d*, *J* = 1.0 Hz, H-3). (Found: C, 51.83; H, 6.20. $C_{28}H_{38}O_{17}$ requires: C, 52.01; H, 5.92%.)

$LiBH_4$ *reduction of ligustaloside A* (**3**). To a soln of **3** (218 mg) in dry THF (10 ml) was added $LiBH_4$ (202 mg) and the whole was stirred for 29 hr at room temp. The reaction was stopped with MeOH, the soln was then diluted with H_2O and neutralized with 0.5 M HCl. After removal of THF by distillation *in vacuo*, the aq. soln was chromatographed on active charcoal (1 g), eluting successively with H_2O (200 ml) and MeOH (200 ml). The MeOH eluate was concd *in vacuo* to give a residue (199 mg), which was chromatographed on a Si gel (10 g) column, eluting with $CHCl_3$–MeOH with an increasing MeOH content. $CHCl_3$–MeOH (97 : 3 and 19 : 1) yielded **13** (23.2 mg) as a colourless syrup, and $CHCl_3$–MeOH (23 : 2) gave **12** (60.6 mg) as a white powder. The residue (29.3 mg) of the eluate with $CHCl_3$–MeOH (9 : 1 and 22 : 3) was acetylated (Ac_2O–C_5H_5N) and the product (54.7 mg) was purified by prep. TLC (Et_2O), giving hexa-acetate **15** (24.4 mg) as a white powder, $[\alpha]_D^{17} - 105.4°$ ($CHCl_3$; *c* 0.28); UV λ_{max}^{MeOH} nm (log ε): 232 (4.05); IR ν_{max}^{KBr} cm^{-1}: 1750, 1735, 1700, 1630; ^1H NMR ($CDCl_3$): δ 2.00, 2.03, 2.06, 2.09 (*s*'s together integrating for 6 × OCOMe), 3.71 (3H, *s*, COOMe), 3.93–4.32 (6H, *m*, H_2-6′, H_2-7 and H_2-10), 7.41 (1H, *s*, H-3). (Found: C, 51.08; H, 5.98. $C_{29}H_{40}O_{17}.H_2O$ requires: C, 51.33; H, 6.24%.)

Catalytic hydrogenation of secologanoside methyl ester tetra-acetate (**21**). Compound **21** (200 mg) in MeOH (5 ml) was hydrogenated over a Pd–C catalyst [prepared from 5% $PdCl_2$–HCl soln (0.8 ml) and activated charcoal (Darco G-60, 200 mg)] until H_2 uptake had ceased. The catalyst was filtered off, and the filtrate was concd *in vacuo* to leave a crystalline residue (203 mg), which was recrystallized from EtOH to give dihydrosecologanoside methyl ester tetra-acetate (**20**) (182 mg) as colourless needles, mp 135.5–136°. $[\alpha]_D^{15} - 118.0°$ ($CHCl_3$; *c* 1.02); UV λ_{max}^{MeOH} nm (log ε): 233 (4.07); IR ν_{max}^{KBr} cm^{-1}: 1740, 1700, 1630; ^1H NMR ($CDCl_3$): δ 0.97 (3H, deformed *t*, *J* = 6.0 Hz, H_3-10), 1.98, 2.00, 2.03, 2.08 (each *s*, 4 × OCOMe), 3.23 (1H, *m*, H-5), 3.65, 3.67 (each *s*, 2 × COOMe), 7.36 (1H, *d*, *J* = 1.0 Hz, H-3). (Found: C, 52.81; H, 6.29. $C_{26}H_{36}O_{15}$ requires: C, 53.06; H, 6.17%.)

Tosylation of dihydroligustaloside A hexa-acetate (**10**). Compound **10** (863 mg) was tosylated with *p*-TsCl (305 mg) and C_5H_5N (10 ml) in the usual way. The product (960 mg) was chromatographed on a Si gel (30 g) column, eluting with C_6H_6–Me_2CO with an increasing Me_2CO content. The fractions eluted with C_6H_6–Me_2CO (17 : 1 and 93 : 7) were combine and evaporated *in vacuo* to afford **16** (829 mg) as a white powder, $[\alpha]_D^{30} - 79.5°$ ($CHCl_3$; *c* 1.01); IR ν_{max}^{KBr} cm^{-1}: 1750, 1705, 1635, 1505; ^1H NMR ($CDCl_3$): δ 1.97, 2.00, 2.05

(each *s*, 4×alcoholic OCOMe), 2.26 (*s*, 2×phenolic OCOMe), 2.43(3H, *s*, arom. Me), 2.90 (2H, *t*, J = 7.0 Hz, $ArCH_2CH_2O$–), 3.64 (3H, *s*, COOMe), 4.23 (2H, *t*, J = 7.0 Hz, $ArCH_2CH_2O$–), 6.97–7.20 (3H, *m*, arom. H), 7.33 (1H, *s*, H-3), 7.54 (4H, AA′ BB′ pattern, arom. H). (Found: C, 54.60; H, 5.66; S, 3.02. $C_{44}H_{52}O_{22}S$ requires: C, 54.77; H, 5.43; S, 3.32%.)

Treatment of tosylate (**16**) *with* NaI. The tosylate (**16**) (697 mg) was treated with NaI (163 mg) in dry Me_2CO (7 ml) under stirring for 21 hr at room temp. The reaction was diluted with H_2O and extracted with $CHCl_3$ (40 ml×3). The H_2O washed and dried $CHCl_3$ layer gave a residue (694 mg) which was recrystallized from Me_2CO–Et_2O–petrol to yield the iodide (**17**) (652 mg) as colourless needles, mp 127.5–129°. $[\alpha]_D^{29}$ −78.1° ($CHCl_3$; *c* 1.00); IR ν_{max}^{KBr} cm^{-1}: 1740, 1685, 1630, 1505; ^1H NMR ($CDCl_3$): δ 2.00, 2.07 (each *s*, 4×alcoholic OCOMe), 2.25 (*s*, 2×phenolic OCOMe), 2.94 (2H, *t*, J = 7.0 Hz, $ArCH_2CH_2O$–), 3.18 (2H, *t*, J = 7.0 Hz, H_2-10), 3.66 (3H, *s*, COOMe), 4.28 (2H, *t*, J = 7.0 Hz, $ArCH_2CH_2O$–), 6.97–7.20 (3H, *m*, arom. H), 7.37 (1H, *s*, H-3). (Found: C, 48.55; H, 4.94; I, 13.66. $C_{37}H_{45}O_{19}I$ requires: C, 48.27; H, 4.93; I, 13.66%.)

Reduction of iodide (**17**) *with* n-Bu_3SnH. To a soln of the iodide (**17**) (316 mg) in dry C_6H_6 (15 ml) were added *n*-Bu_3SnH (317 mg) and α, α′-azobis-isobutyronitrile (5.7 mg) and the whole was refluxed under Ar for 15 hr. After cooling, the reaction was concd *in vacuo* to give a syrupy residue which was subjected to prep. TLC (C_6H_6–Me_2CO, 17 : 3, two developments). Of the two major bands, the more mobile one gave **18** (134 mg) which was crystallized from Me_2CO–Et_2O–petrol to give colourless needles. The less mobile band afforded **19** (50 mg) as a white powder. **18**, mp 112–113.5°; $[\alpha]_D^{20}$ −101.0° ($CHCl_3$; *c* 0.51); IR ν_{max}^{KBr} cm^{-1} 1750, 1735, 1685, 1630, 1505; ^1H NMR ($CDCl_3$): δ 0.92 (3H, deformed *t*, J = 6.0 Hz, H_3-10), 1.97, 1.99, 2.00, 2.07 (each *s*, 4×alcoholic OCOMe), 2.26 (*s*, 2×phenolic OCOMe), 2.93 (2H, *t*, J = 7.0 Hz, $ArCH_2CH_2O$–), 3.65 (3H, *s*, COOMe), 4.27 (2H, *t*, J = 7.0 Hz, $ArCH_2CH_2O$–), 6.93–7.23 (3H, *m*, arom. H), 7.37 (1H, *br s*, H-3). (Found: C, 55.91; H, 5.75. $C_{37}H_{46}O_{19}$ requires: C, 55.92; H, 5.83%.) **19**, IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 1750, 1700 (sh), 1630; ^1H NMR ($CDCl_3$): δ 0.88 (3H, deformed *t*, J = 6.0 Hz, H_3-10), 1.97, 1.99, 2.02, 2.08 (each *s*, 4×OCOMe), 2.82 (2H, *t*, J = 6.5 Hz, $ArCH_2CH_2O$–), 3.68 (3H, *s*, COOMe), 4.24, (2H, *t*, J = 6.5 Hz, $ArCH_2CH_2O$–), 5.50–6.67 (2H, *m*, 2×OH, disappeared on addition of D_2O), 6.33–7.17 (3H, *m*, arom. H), 7.37 (1H, *s*, H-3). (Found: C, 55.06; H, 5.98. $C_{33}H_{42}O_{17}.1/2\ H_2O$ requires: C, 55.07; H, 6.02%.)

vacuo to give a residue, which was acetylated (Ac_2O–C_5H_5N) (79.2 mg) was heated with 0.5 N NaOH at 80° for 1 hr. The cooled mixture was neutralized with Amberlite IR-120 (H^+-form). The resin was filtered off and the filtrate was concd *in vacuo* to give a residue, which was acetylated (Ac_2O–C_5H_5N and subsequently methylated with CH_2N_2–Et_2O. The product (75.9 mg) was purified by prep. TLC (C_6H_6–Et_2O, 1 : 1) and recrystallized from EtOH, giving rise to colourless needles (43.7 mg), 136–136.5°; $[\alpha]_D^{15}$ −114.0° ($CHCl_3$; *c* 0.80). (Found: C, 53.25; H, 6.21. Calc. for $C_{26}H_{36}O_{15}$: C, 53.06; H, 6.17%.) This substance was identical with dihydrosecologanoside methyl ester tetra-acetate (**20**) (mmp, ^1H NMR and IR).

$NaBH_4$ *reduction of ligustaloside B* (**4**). To a soln of **4** (131 mg) in EtOH (4 ml) was added $NaBH_4$ (10 mg) under ice cooling. After stirring for 1 hr, the mixture was worked-up as for **12** to give dihydroligustaloside B (**23**) (118 mg) as a white powder, $[\alpha]_D^{19}$ −126.7° (MeOH; *c* 1.00); UV λ_{max}^{MeOH} nm (log ε): 229 (4.16), 277 (3.24), 284 (sh) (3.16); IR ν_{max}^{KBr} cm^{-1}: 3350, 1710, 1630, 1520; ^1H NMR (D_2O): δ 2.80 (2*H*, *t*, J = 6.0 Hz, $ArCH_2CH_2O$–), 3.60 (3H, *s*, COOMe), 4.20 (2H, *t*, J = 6.0 Hz, $ArCH_2CH_2O$–), 5.31 (1H, *d*, J = 7.0 Hz, H-1), 6.95 (4H, AA′ BB′ pattern, arom. H), 7.46 (1H, *s*, H-3). (Found: C, 54.44; H, 6.24. $C_{25}H_{34}O_{13}.1/2\ H_2O$ requires: C, 54.44; H, 6.40%.)

Alkaline hydrolysis of dihydroligustaloside B (**23**). A soln of **23** (61.5 mg) in 0.5 N NaOH (3 ml) was stirred for 4 hr at room temp. After acidifying the soln with 1 N HCl, the mixture was extracted with EtOAc (15 ml×3). The H_2O washed and dried EtOAc layer was evaporated *in vacuo* to leave a crystalline residue (17.5 mg), which was recrystallized from Me_2CO–Et_2O to give colourless needles, mp 91–92°. This substance was identical with an authentic sample of 2-(4-hydroxyphenyl)ethyl alcohol (**22**) (mmp, ^1H NMR and IR). The aq. layer was chromatographed on activated charcoal (15 g), eluting successively with H_2O (100 ml) and MeOH (200 ml). Concn of the MeOH eluate gave a residue (46.2 mg) which was subjected to acetylation (Ac_2O–C_5H_5N) followed by methylation with CH_2N_2–Et_2O. The product (69.2 mg) was purified by prep. TLC (C_6H_6–Me_2CO, 4 : 1) and recrystallized from Et_2O–petrol to give colourless needles (45.7 mg), mp 98.5–99.5°. This compound was identical with a sample of **14** derived from dihydroligustaloside A (**12**) (mmp, ^1H NMR and IR).

Acetylation of 10-*hydroxyoleuropein* (**5**). 10-Hydroxyoleuropein (**5**) (29.1 mg) was acetylated (Ac_2O–C_5H_5N) and the product (45.6 mg) was purified by prep. TLC (C_6H_6–MeCO, 4 : 1) to furnish the hepta-acetate (42.5 mg) as a white powder, $[\alpha]_D^{29}$ −120.1° ($CHCl_3$; *c* 0.80) (lit. $[\alpha]_D$ −117.4°)[1]. (Found: C, 55.36; H, 5.69. Calc. for $C_{39}H_{46}O_{21}$: C, 55.06; H, 5.45%.) This substance was identical with an authentic sample of 10-acetoxyoleuropein hexa-acetate (**25**) (^1H NMR and IR).

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