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IRIDOIDS, IRIDOID–TRITERPENOID CONGENERS AND LIGNANS FROM *DESFONTAINIA SPINOSA*

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Key Word Index—*Desfontainia spinosa*; Loganiaceae; iridoids; seco-iridoids; triterpene-secoiridoids; desfontainic acid; desfontainoside; lignans; 7-*O*-(*p*-coumaroyl)-loganin; secoxyloganin; dimethyl secologanoside; sweroside; (+)-syringaresinol; liriodendrin; (+)-syringaresinol *O*-β-D-glucoside.

Abstract—The leaves of *Desfontainia spinosa* were found to contain the three known seco-iridoids secoxyloganin, dimethyl secologanoside and sweroside in addition to the three iridoids loganin, loganetin and loganic acid previously reported. In addition a novel loganin derivative 7-*O*-(*p*-coumaroyl)-loganin was isolated. The leaves were also found to contain a novel type of compound consisting of ester-linked triterpene and seco-iridoid congeners between 24-hydroxytormentic acid and secoxyloganin. These were named desfontainic acid, a monoglucoside, and desfontainoside, the corresponding diglucoside. Desfontainoside only could be detected in the stems. The stem contained the same iridoids and also the known furofuran lignans (+)-syringaresinol, (+)-syringaresinol *O*-β-D-glucopyranoside and liriodendrin. The iridoids present provide chemotaxonomic evidence for retaining *Desfontainia* in the Loganiaceae as a separate tribe the Desfontaineae. None of the compounds present forms a basis for the reputed hallucinogenic activity.

INTRODUCTION

Desfontainia spinosa Ruiz & Pav., a reputed hallucinogen, has recently been shown to contain the iridoids loganin and loganic acid and also the aglucone of loganin, loganetin [1]. Another recent paper describes the isolation of a cytostatic triterpene, 11-deoxocucurbitacin I [2], and we have recently isolated several other triterpenes [3]. These are the only reported phytochemical investigations into this species.

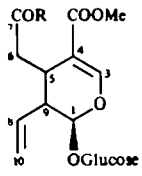
Other iridoids were detected in extracts from *D. spinosa* and this paper describes their characterization. The taxonomic placement of *Desfontainia* is a matter of some controversy. The conflicting views are the assignment of *Desfontainia* to either a separate family, the Desfontainiaceae, or to the tribes Potalieae or Retzieae in the Loganiaceae, some botanists preferring the creation of a separate tribe Desfontaineae [4]. Iridoids have been found useful as chemotaxonomic markers [5] and the knowledge of these compounds would enable clarification of the relationship of *Desfontainia* to the Loganiaceae.

During our fractionation and isolation procedures two compounds giving more complicated spectral features than either the triterpenes or iridoids were obtained. This paper describes their structural elucidation as a new type of compound consisting of a seco-iridoid moiety linked to a triterpene.

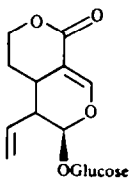
RESULTS AND DISCUSSION

Identification of iridoids and their pharmacological significance

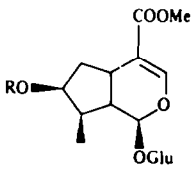
Secoxyloganin (**1**), dimethyl secologanoside (**2**), also known as secoxyloganin methyl ester, and sweroside (**3**) were identified by spectral and chromatographic com-

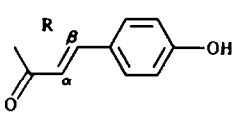


		R
1	Secoxyloganin	OH
2	Secoxyloganin methyl ester (dimethyl secoloqanoside)	OMe



3 Sweroside



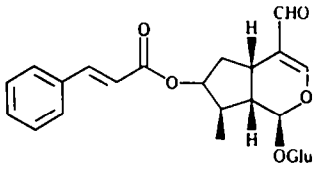


4 7-*O*-(*p*-coumaryl)-Loganin

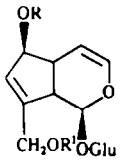
5 Loganin acetate — OAc

parison with authentic samples and literature values [6–9]. 7-*O*-(*p*-Coumaryl)-loganin (**4**) was identified by examination of its spectral data and those of its pentaacetate. In addition to the 239 nm peak typical of an iridoid enol-ether system conjugated with a C-4 carbonyl group the UV spectrum showed a peak at 281 nm indicating the presence of a *p*-disubstituted benzene ring. The IR spectrum showed the presence of a conjugated carbonyl at 1717 cm^{-1}. The ^1H NMR spectrum showed characteristic iridoid signals at δ7.34 (H-3), 5.20 (H-1) and 4.57 (H-1'). In addition there were two pairs of doublets integrating for four and two protons respectively. Their coupling was shown by the COSY spectrum of the compound. One pair (δ7.30, 6.62) is indicative of a *p*-disubstituted aromatic ring and the other pair (δ7.45, 6.14) indicates a *trans*-substituted double bond conjugated with an aromatic ring. On this evidence, and that of the conjugated ester peak in the IR, it therefore appears that the aromatic part of the molecule consists of a *p*-substituted cinnamoyl group. Since the ^1H NMR spectrum of the acetylation product showed an aromatic acetate peak at δ2.32 it seemed likely that the aromatic substituent was a hydroxyl group. The rest of the spectrum was identical to that of loganin apart from the coarse triplet at δ5.16 which was attributed to H-7. In loganin this gives a signal at δ4.03. Since a similar shift is observed for that signal in loganin pentaacetate (**5**) [10] and campenoside (**6**) [11], i.e. where there is an acyloxy substituent at C-7, it seemed reasonable to suppose that C-7 was the point of attachment of the aromatic moiety and that the compound consisted of an ester of *p*-coumaric acid with loganin through the 7-hydroxyl of the loganin. This compound has not previously been reported although other *p*-coumaroyl iridoids are known, e.g. eurostoside (**7**) and odontoside (**8**) [12].

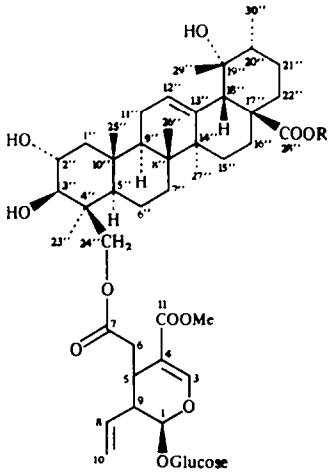
These iridoids were found in both the stem and the leaves and because of their solubility properties are likely to be found in the infusion of the leaves reputed to be hallucinogenic. However none of the iridoids found in *Desfontainia* has been shown to have any CNS activity



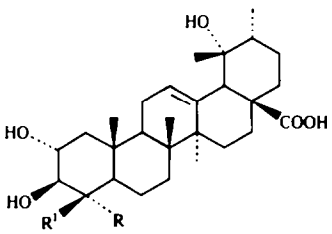
6 Campenoside



7 Eurostoside R = H, R^1 = *p*-coumaryl
8 Odontoside R = *p*-coumaryl, R^1 = H



9 Desfontainic acid R = H
12 Desfontainoside R = D-Glucose



10 23-Hydroxytormentic acid R = CH_2OH, R^1 = Me
11 24-Hydroxytormentic acid R = Me, R^1 = CH_2OH

although investigation has been limited. Some iridoids, e.g. nepetalactone and the valepotriates, are known to affect the CNS but they differ in structure from the iridoids reported here.

Identification of the secoiridoid–triterpenoid congeners

Desfontainic acid (**9**) showed a high $[M]^+$ at *m/z* 890. The UV, IR and particularly the ^1H NMR spectra showed features seen in both triterpenoids and iridoids. The possibility that it was a mixture was ruled out by the MS and the fact that it consistently gave a single spot in various TLC systems. Spin decoupling experiments and the two-dimensional COSY spectrum confirmed that both an urs-12-ene triterpene and a seco-iridoid structure were present.

The features ascribable to the triterpene moiety showed many similarities to 23- or 24-hydroxytormentic acid (**10**, **11**) [3], i.e. the coarse triplet at δ5.22 for H-12, the five tertiary methyl and one secondary methyl signals between δ1.20 and 0.74, the signals at δ4.24 and 3.05 (the carbinol methine protons at C-2 and C-3) and the AB quartet at δ3.82 and 3.28. Alkaline hydrolysis of **9** yielded a compound identical in chromatographic and ^1H NMR spectral data with 24-hydroxytormentic acid (**11**). The triterpene portion of the molecule was therefore reckoned to be this compound.

The nature of the iridoid part of the molecule was also determined by examination of the ^1H NMR spectrum. The H-3 signal at δ7.38 indicated a carbonyl group attached to C-4, the H-1 signal (δ5.48) did not give a downfield shift in the acetate thus indicating the attachment of a sugar at this position and the related signals at δ5.70 and 5.16 were typical of a seco-iridoid vinyl substituent at C-9. The presence of a sugar was confirmed by the δ4.61 signal for the anomeric proton. The signals which could be assigned to the secoiridoid were identical to those given by secoxyloganin (**1**) and thus this compound was reckoned to be the iridoid portion of the congener. It could not be detected after hydrolysis but this was possibly due to the small quantities used and more likely due to the fact that the alkaline hydrolysis reaction would hydrolyse the C-11 ester group of the secologanin at the same time as the ester link between the two moieties. The acidification after hydrolysis would also tend to cause disintegration of the iridoid. Since the IR spectrum of **9** showed a peak at 1718 cm^{-1} (ascribed to an ester carbonyl) not observed for **1** or **11**, it was deduced that the two moieties were connected *via* an ester linkage. This must be through the C-7 carboxyl of the secoxyloganin or the C-28 carboxyl of the 24-hydroxytormentic acid. The former was preferred because of the following three reasons: (a) The methylation product of **9** showed an extra –COOMe peak at δ3.48 in the ^1H NMR spectrum. This is characteristic of ursolic acid methyl esters, as can be seen in the data given for the methylation product of **11** [3] rather than the methyl ester of secoxyloganin where the COOMe signal appears at δ3.65 [6]. (b) The ^1H NMR spectrum of **9** compared with **11** showed differences for the H-2″β, H-3″α and H-24″ signals (discussed below) which cannot be explained if attachment was through C-28. (c) The presence of the ester glucoside desfontainoside (**12**; structure discussed below) where a sugar is ester linked through C-28.

The carbonyl group of the secoxyloganin could be linked through the hydroxyl groups at C-2″, C-3″ or C-24″ of **11**. C-2″ can be ruled out because the acetylation product of **9** showed a shift of H-2″ from δ4.24 to 5.66. The remaining two possibilities are through C-3″ or C-24″. The latter is preferred because a carbonyl substituent at C-3″ would mean that the C-3″ carbinol proton would have a shift of about δ4.8 [12] whereas in **9** it is seen at δ3.05. Further evidence for the linkage being through C-24″ is given by the observation that the AB quartet for the C-24″ methylene group is not changed to a 2H singlet upon acetylation as is seen for **11** [3]. The fact that acetylation of the C-3″ OH could not be achieved is probably due to steric hindrance by the bulky seco-iridoid group at C-24″. The highly oxygenated seco-iridoid is spatially close to the H-2″β and has a deshielding effect noted in the difference between the signal for this proton in **9** (δ4.24) and the parent triterpene **11** (δ3.70).

It could be argued that the glucose is attached at some other point than H-1 of the seco-iridoid, this would be an alternative explanation of the difficulty in acetylating the 3″-hydroxyl group. If this were the case the H-1 signal for the seco-iridoid would be seen as a doublet between δ5.50 and 6.00 in the acetate; but no such signal is seen. The possibility that the glucose is attached *via* an ester linkage at C-28″ was discounted for reasons stated above and by noting that in the ^{13}C NMR spectrum the anomeric C-1 of the glucose had a shift of δ98.0 which is typical of the usual iridoid linkage rather than an ester glucoside [13].

Compound **9** was therefore considered to be a novel type of structure consisting of a secoiridoid linked to a triterpene *via* an ester group and was named desfontainic acid (7-*O*-[24″-hydroxytormentic acid (7–24)]-11-*O*-methyl secologanoside).

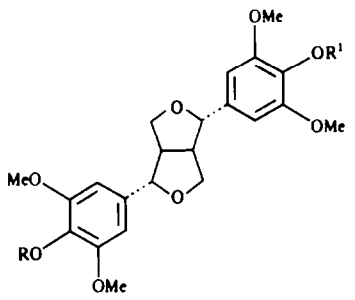
Desfontainoside (**12**) showed many spectral features which were identical to those exhibited by **9**. Additional features noted were extra signals in the ^1H and ^{13}C NMR spectra and its acetate which could be assigned to a second glucose moiety, particularly the signals due to the anomeric C of the glucose and its attached proton. The ^{13}C NMR signal at δ95.8 is characteristic of the anomeric C-1 in an ester-linked glucose and further evidence that the second glucose in **12** is ester linked is provided by the peak at 1698 cm^{-1} in the IR spectrum instead of the acid carbonyl signal seen at 1689 cm^{-1} in **9**. Since the only free acid group in **9** is C-28″ and **12** could not be methylated it is reasonable to suggest that **12** is the ester glucoside of **9**, the second glucose being attached through C-28″. This is confirmed by the formation of **9** as an enzyme hydrolysis product of **12**. Compound **12** was named desfontainoside (7-*O*-[24″-Hydroxytormentic acid ester glycoside (7–24″)]-11-*O*-methyl secologanoside).

Three other types of congeners of secoiridoids are known, namely (1) terpene-conjugated, e.g. menthiafolin [14]; (2) phenolic acid-conjugated, e.g. ligustroside [15] and (3) bis-iridoids and seco-iridoids, e.g. cantleyoside [16]. None of these types has been isolated from the Loganiaceae.

Triterpenes linked with acyclic monoterpene acids have been detected in *Gleditsia* (Leguminosae) [17] but there are no reports of similar congeners with iridoids. No evidence is available to suggest that these compounds are responsible for the reputed CNS effects of the plant.

Identification of lignans and their significance

(+)-Syringaresinol (**13**), (+)-syringaresinol *O*-β-D-glucoside (**14**) and liriodendrin (**15**) were identified by



13 (+)-Syringaresinol R, R' = H
14 (+)-Syringaresinol monoglucoside R = H, R' = D-glucose
15 Liriodendrin R, R' = D-glucose

comparison of chromatographic behaviour and spectral characteristics with authentic samples and literature values [18–20]. These furofuran lignans were isolated only from the stem and not the leaves. They are very widespread throughout the higher plants but this is only the second report of lignans being isolated from a member of the Loganiaceae.

It is difficult to assess the pharmacological implications of the presence of these compounds in *Desfontainia* in the light of its reputed CNS effects. Both (+)-syringaresinol and liriodendrin have been shown to inhibit cAMP phosphodiesterase [21], a feature shared by many CNS depressant drugs [22]. (−)-Syringaresinol diglucoside has been shown to be the most active stimulant and anti-stress factor of the components of the adaptogen Eleutherococcus [23] but the (+)-isomer liriodendrin, found in *Desfontainia*, has not been tested. In spite of these possibilities it is unlikely that enough lignan is present in the infusion used to contribute to any CNS activity since the infusion is made mainly from the leaves and not the stems.

Chemotaxonomic considerations

It is the iridoids which are of most use in classifying plants connected with the Loganiaceae. Seco-iridoids are found in related families viz. Menyanthaceae, Gentianaceae and Apocynaceae as well as in the Potalieae in Loganiaceae. Loganin-type iridoids are found in the Strychneae tribe of the same family whilst the Buddlejeae contains aucubin-type iridoids. Since *Desfontainia* has been shown to contain both loganin-type iridoids and seco-iridoids, both found within the Loganiaceae but not reported previously from the same species, it seems reasonable to support the proposition of Leeuwenberg and Leenhouts [24] that *Desfontainia* be retained in the Loganiaceae as a separate tribe the Desfontainieae.

EXPERIMENTAL

Biological material. Fresh leaves and stem of *D. spinosa* were obtained from the Younger Botanic Garden, Argyll, Scotland. The material was authenticated at source and a specimen voucher is deposited in the herbarium of the Chelsea Department of Pharmacy, Kings College London.

Isolation of iridoids. Leaves (100 g) were extracted with 2 l. boiling H_2O. After standing for 30 min, the mixture was filtered and the filtrate mixed with 120 g activated charcoal and filtered on a Kieselguhr bed. The bed was eluted with H_2O (750 ml) until no more free sugars could be detected in the filtrate. The bed was then eluted with 1000 ml MeOH. The eluate was concd under red. pres. to give 8.1 g residue. The residue (3 g) was taken and fractionated by droplet counter-current chromatography (DCCC) using the ascending mode ($CHCl_3$–MeOH–H_2O, 5:5:3). 150 fractions (5 ml) of the mobile phase were collected and then 10 ml fractions of the stationary phase. Fractions were monitored by TLC (systems a–e) and similar fractions combined and evaporated to dryness. Fractions 14–27 (300 mg) and 59–65 (60 mg) showed the presence of iridoids on TLC when sprayed with anisaldehyde reagent. Individual compounds were isolated by prep. TLC [silica gel GF_{254} pre-washed with MeOH, 1 mm thick, $CHCl_3$–MeOH (3:1). Bands detected as quenching zones or by spraying the edge of the plate with the anisaldehyde reagent and heating were eluted with MeOH]. An extract of 150 g stems treated in the same way showed the presence of the same compounds as well as extra spots on TLC.

Secoxyloganin. Obtained as a white amorphous powder (20 mg) from fractions 14–25. Spectral features were identical to those published [6].

Dimethyl secologanoside. Obtained as a white amorphous powder (8 mg) from fractions 26–31. Spectral features were identical to those published [6].

Sweroside. Obtained as a white amorphous powder (15 mg) from fractions 26–31. Spectral features were identical to those published [8].

7-O-(p-Coumaroyl)-loganin. Obtained as a white amorphous powder (6 mg) from fractions 54–73. On silica gel it gave a quenching spot under UV (254 nm) and a pink colour with the detecting reagent. UV λ_{max}^{MeOH} (log ε): 239 (3.7), 281 (2.8); IR ν_{max}^{nujol} cm^{-1}: 1717 (conjugated C=O), 1636 (C=C), 1508 (aromatic C=C), 1432, 1375, 1224, 1165, 1039, 838; EIMS (probe) 35 eV, *m/z* (rel. int.): 416 $[M-C_8H_8O]^+$ (4), 389 (16), 308 (43), 312 (23), 311 (82), 297 (16), 272 (14), 196 (100), 181 (58), 180 (53), 105 (74); ^1H NMR (360 MHz, CD_3OD): δ7.45 (1H, *d*, *J* = 16 Hz, H-β), 7.34 (1H, *d*, *J* = 1 Hz, H-3), 7.30 (2H, *d*, *J* = 9 Hz, H-2", 6"), 6.62 (2H, *d*, *J* = 9 Hz, H-3", 5"), 6.14 (1H, *d*, *J* = 16 Hz, H-α), 5.20 (1H, *d*, *J* = 4.5 Hz, H-1), 5.16 (1H, *br t*, H-7), 4.57 (1H, *d*, *J* = 8 Hz), H-1'), 3.81 (1H, *dd*, *J* = 12, 2 Hz, H-6'a), 3.70 (1H, *dd*, *J* = 12, 6 Hz, H-6'b), 3.60 (3H, *s*, OMe), 3.30–3.28 (4H, *m*, H-2' to H-5'), 3.06 (1H, *m*, H-5), 2.24 (1H, *ddd*, *J* = 11, 8, 2 Hz, H-6β), 2.04 (1H, *m*, H-9), 1.89 (1H, *m*, H-8), 1.69 (1H, *m*, H-6α), 1.00 (3H, *d*, *J* = 6.5 Hz, Me-10).

7-O-(p-Coumaroyl)-loganin pentaacetate. EIMS (probe) 18 eV, *m/z* (rel. int.): 746 $[M]^+$ (0.5), 566 $[M-3\times HOAc]^+$ (1), 536 (4), 414 (5), 331 (41), 193 (26), 179 (100), 148 (21); ^1H NMR (360 MHz, $CDCl_3$): δ7.64 (1H, *d*, *J* = 16 Hz, H-β), 7.56 (2H, *d*, *J* = 9 Hz, H-2", 6"), 7.32 (1H, *d*, *J* = 1 Hz, H-3), 7.15 (2H, *d*, *J* = 9 Hz, H-3", H-5"), 6.38 (1H, *d*, *J* = 16 Hz, H-α), 5.31 (1H, *br t*, H-7), 5.28 (1H, *d*, *J* = 3 Hz, H-1), 5.24 (1H, *t*, *J* = 10 Hz, H-3'), 5.15 (1H, *t*, *J* = 10 Hz, H-4'), 5.01 (1H, *t*, *J* = 10 Hz, H-2'), 4.87 (1H, *d*, *J* = 8 Hz, H-1'), 4.32 (1H, *dd*, *J* = 12 Hz, 4 Hz, H-6'b), 4.16 (1H, *dd*, *J* = 12, 2 Hz, H-6'a), 3.77 (1H, *m*, H-5'), 3.72 (3H, *s*, OMe), 3.10 (1H, *m*, H-5), 2.32 (3H, *s* and 2H, *m*, aromatic OAc and H-6β, H-9), 2.11 (3H, *s*, OAc), 2.06 (3H, *s*, OAc), 2.02 (3H, *s*, OAc), 1.92 (3H, *s*, OAc), 1.86 (1H, *m*, H-8), 1.64 (1H, *m*, H-6α), 1.08 (3H, *d*, *J* = 7 Hz, Me-10).

Isolation of iridoid–triterpene congeners. Desfontainic acid (**9**, 30 mg) was isolated as a white amorphous powder from fractions 8–13 obtained from DCCC fractionation (descending mode) of a methanolic extract of the leaves [3]. It gave a deep violet quenching spot under UV (365 nm) and a blue colour with the detection reagent. UV λ_{max}^{MeOH} nm (log ε): 236 (3.15); IR ν_{max}^{KBr} cm^{-1}: 3428 (broad, OH), 1718 (–CO–O–), 1689 (COOH), 1628 (C=C), 1458, 1448, 1441, 1434, 1404, 1381, 1305,

1297, 1278, 1205, 1074, 932, 812, 786; EIMS (probe) 18 eV *m/z* (rel. int.): 890 $[M]^+$ (0.5), 744 (1), 522 (2), 440 (3), 368 (5), 260 (2), 246 (2), 243 (4), 219 (2), 183 (14), 165 (10), 163 (58), 161 (100), 154 (13), 133 (5), 126 (11), 108 (28); ^{1}H NMR (360 MHz, CD_3OD): δ7.38 (1H, *s*, H-3), 5.70 (1H, *ddd*, $J_{8,9}$ = 16 Hz, $J_{8,10a}$ = 10 Hz, $J_{8,10b}$ = 18 Hz, H-8), 5.48 (1H, *d*, *J* = 7 Hz, H-1), 5.22 (1H, coarse *t*, H-12″), 5.16 (2H, *m*, H-10a, H-10b), 4.61 (1H, *d*, *J* = 8 Hz, H-1′), 4.24 (1H, split *t*, *J* = 10, 4 Hz, H-2″β), 3.82 (1H, *d*, *J* = 11 Hz, H-24″a), 3.79 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.62 (3H, *s*, OMe-12), 3.59 (1H, *dd*, *J* = 12, 4 Hz, H-6′b), 3.28 (1H, *d*, *J* = 11 Hz, H-24″b), 3.20 (4H, *m*, H-2′ to H-5′), 3.05 (1H, *d*, *J* = 10 Hz,, H-3″β), 2.90 (1H, *m*, H-5β), 2.52 (2H, *br s*, overlapping *m*, H-18″β, H-9β), 2.28 (1H, split *t*, *J* = 13, 4.5 Hz, H-5″α), 1.20, 1.18, 1.10, 0.96, 0.74 (3H each, *s*, Me-23″, 25″ to 29″), 0.82 (3H, *s*, *d*, *J* = 6 Hz, Me-30″); ^{13}C NMR ($CDCl_3$, 100.63 Hz): see Table 1.

Desfontainic acid pentaacetate. EIMS (probe) 18 eV, *m/z* (rel. int.): 800 $[M - 5 \times HOAc]^+$ (0.3), 769 $[M - 331]^+$ (0.4), 580 (4), 556 (17), 414 (17), 395 (21), 331 (71), 258 (21), 240 (36), 220 (921), 208 (29), 169 (100); ^{1}H NMR (360 MHz, $CDCl_3$): δ7.38 (1H, *s*, H-3), 5.66 (1H, split *t*, *J* = 10, 4 Hz, H-2″β), 5.59 (1H, *ddd*, $J_{8,9}$ = 16 Hz, $J_{8,10a}$ = 10 Hz, $J_{8,10b}$ = 18 Hz, H-8), 5.35 (1H, coarse *t*, H-12″), 5.16 (2H, *m*, H-10a), 5.29–5.24 (3H, *m*, H-1, H-10a, H-10b), 5.22 (1H, *t*, *J* = 10 Hz, H-3′), 5.10 (1H, *t*, *J* = 10 Hz, H-4′), 4.99 (1H, *t*, *J* = 10 Hz, H-2′), 4.90 (1H, *d*, *J* = 8 Hz, H-1′), 4.29 (1H, *dd*, *J* = 12, 4 Hz, H-6′b), 4.13 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.95 (1H, *d*, *J* = 11 Hz, H-24″a), 3.73 (1H, *m*, H-5′), 3.69 (3H, *s*, OMe-12), 3.48 (1H, *d*, *J* = 11 Hz, H-24″b), 3.36 (1H, *d*, *J* = 10 Hz, H-3″α), 2.88 (1H, *m*, H-5β), 2.76 (1H, *m*, H-9β), 2.55 (2H, *br s* overlapping *m*, H-18″β, H-5″α), 2.11, 2.04, 2.03, 2.00, 2.00, 1.93 (3H each, *s*, OAc) 1.38, 1.25, 1.21, 1.10, 0.74 (3H each, *s*, Me-23″, 25″ to 29″), 0.96 (3H, *s*, *d*, *J* = 6 Hz, Me-30″).

Desfontainic acid methyl ester. ^{1}H NMR (360 MHz, CD_3OD): δ7.38 (1H, *s*, H-3), 5.69 (1H, *ddd*, $J_{8,9}$ = 16 Hz, $J_{8,10a}$ = 10 Hz, $J_{8,10b}$ = 18 Hz, H-8), 5.49 (1H, *d*, *J* = 7 Hz, H-1), 5.23 (1H, coarse *t*, H-12″), 5.18 (2H, *m*, H-10a, H-10b), 4.61 (1H, *d*, *J* = 8 Hz, H-1′), 4.26 (1H, split *t*, *J* = 10, 4 Hz, H-2β), 3.82 (1H, *d*, *J* = 11 Hz, H-24″a), 3.79 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.62 (3H, *s*, OMe-12), 3.59 (1H, *dd*, *J* = 12, 4 Hz, H-6′b), 3.48 (3H, *s*, OMe-31), 3.28 (1H, H-24″b), 3.20 (4H, *m*, H-2′ to H-5′), 3.08 (1H, *d*, *J* = 10 Hz, H-3″α), 2.91 (1H, *m* H-5β), 2.53 (2H, *br s* overlapping *m*, H-5″α, H-9β), 2.43 (1H, *br s*, H-18″β), 1.24, 1.24, 1.10, 0.97, 0.60 (3H each, *s*, Me-23″, 25″ to 29″), 0.82 (3H, *s*, *d*, *J* = 6 Hz, Me-30″).

Desfontainoside (**12**). Isolated from fractions 54–73 of the DCCC (descending mode) separation of the methanolic leaf extract. It was isolated as a white amorphous powder (15 mg) giving a deep purple quenching spot under UV (365 nm) and a blue colour with the detecting reagent. UV λ_{max}^{MeOH} nm (log ε): 236 (3.10); IR ν_{max}^{KBr} cm^{-1}: 1728 (–CO–O–C–), 1698 (–CO–O–C–), 1635 (C=C), 1575, 1412, 1357, 1075, 1024, 928, 810, 769; EIMS (probe) 18 eV, *m/z* (rel. int.): 728 $[M - 2 \times C_6H_{10}O_5]^+$ (0.5), 412 (2), 317 (6), 279 (4), 261 (4), 246 (3), 240 (4), 236 (3), 223 (4), 218 (7), 146 (17), 133 (17); ^{1}H NMR (360 MHz, CD_3OD): δ7.38 (1H, *s*, H-3), 5.70 (1H, *ddd*, *J* = 16, 10, 18 Hz, H-8), 5.49 (1H, *d*, *J* = 7 Hz, H-1), 5.21 (2H, *d*, *J* = 8 Hz, *m*, H-1‴, H-10b), 5.18 (1H, coarse *t*, H-12″), 5.15 (1H, *dd*, *J* = 10, 1 Hz, H-10a), 4.61 (1H, *d*, *J* = 8 Hz, H-1′), 4.20 (1H, split *t*, *J* = 10 Hz, 4 Hz, H-2″β), 3.83 (1H, *d*, *J* = 11 Hz, H-24″a), 3.80 (1H, *dd*, *J* = 12, 2 Hz, H-6‴a), 3.70 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 3.62 (3H, *s*, OMe-12), 3.58 (2H, *m*, H-6′b, H-6′a), 3.30–3.10 (9H, *m*, H-24″b, H-2′ to H-5′, H-2‴ to H-5‴), 3.05 (1H, *d*, *J* = 10 Hz, H-3″α), 2.91 (1H, *m*, H-5β), 2.52 (2H, *m*, H-5″α, H-9β), 2.42 (1H, *br s*, H-18″β), 1.24, 1.20, 1.11, 0.97, 0.66 (3H each, *s*, Me-23″, 25″ to 29″), 0.83 (3H, *s*, *d*, *J* = 6 Hz, Me-30″); ^{13}C NMR (CD_3OD, 100.63 Hz): see Table 1.

Desfontainoside nonaacetate. ^{1}H NMR (360 MHz, $CDCl_3$): δ7.38 (1H, *s*, H-3), 5.66 (1H, split *t*, *J* = 10, 4 Hz, H-2″β), 5.59 (1H, *ddd*, $J_{8,9}$ = 16 Hz, $J_{8,10a}$ = 10 Hz, $J_{8,10b}$ = 18 Hz, H-8), 5.38 (1H, coarse *t*, H-12″), 5.32–5.10 (7H, *m*, H-10a, H-10b, H-1‴, H-3′, H-3‴, H-4′, H-4‴), 4.99 (2H, *t*, *J* = 10 Hz, H-2′, H-2‴), 4.90 (1H, *d*, *J* = 8 Hz, H-1′), 4.29 (2H, *m*, H-6′b, H-6‴b), 4.13 (1H, *dd*, *J* = 12, 2 Hz, H-6′a), 4.06 (1H, *dd*, *J* = 12, 2 Hz, H-6‴b), 3.96 (1H, *d*, *J* = 11 Hz, H-24″a), 3.79 (1H, *m*, H-5′), 3.73 (1H, *m*, H-5‴), 3.70 (3H,

Table 1. ^{13}C NMR data of desfontainic acid (**1**) and desfontainoside (**4**)

C	1	4
1	95.6	97.6
3	151.8	154.0
4	109.8	111.9
5	28.5	29.6
6	34.9	34.3
7	170.0	170.0
8	133.7	136.1
9	46.4	45.5
10	117.3	119.3
11	167.7	169.7
12	50.0	52.0
1′	98.0	100.2
2′	72.5	74.7
3′	76.9	78.3
4′	69.5	71.6
5′	76.2	78.6
6′	60.7	62.8
1″	44.3	46.5
2″	61.1	63.2
3″	85.3	87.3
4″	39.0	39.0
5″	53.6	54.9
6″	18.0	18.9
7″	32.4	30.8
8″	40.7	41.3
9″	47.7	51.5
10″	36.9	36.9
11″	24.9	25.0
12″	126.3	129:5
13″	138.9	139.8
14″	40.9	42.8
15″	28.6	30.0
16″	25.1	26.5
17″	47.8	48.0
18″	54.7	56.7
19″	72.2	73.6
20″	43.4	43.0
21″	27.8	27.2
22″	37.5	38.3
23″	22.9	26.7
24″	69.0	71.0
25″	16.2	17.8
26″	14.7	16.6
27″	22.3	24.8
28″	183.7	178.0
29″	24.8	27.1
30″	15.6	17.6
1‴		95.8
2‴		73.9
3‴		78.0
4‴		71.1
5‴		78.5
6‴		62.5

s, OMe-12), 3.47 (1H, d, J = 11 Hz, H-24"b), 3.35 (1H, d, J = 10 Hz, H-3"α), 2.89 (1H, m, H-5"β), 2.76 (1H, m, H-9"β), 2.53 (2H, br s overlapping m, H-18"β, H-5"α), 2.11, 2.08, 2.06, 2.04 (9H), 2.03, 2.01, 1.93 (3H each, s, OAc), 1.38, 1.23, 1.21, 1.10, 0.71 (3H, each, s, Me-23", 25" to 29"), 0.94 (3H, s, d, J ≈ 6 Hz, Me-30").

Signals in the ^{1}H NMR spectra were measured from TMS and assigned with the aid of spin-decoupling experiments and the two-dimensional COSY spectrum. Signals in the ^{13}C NMR spectra were also measured from TMS and assigned using DEPT spectra.

Isolation of lignans. Finely divided stem (150 g) were extracted as above and fractionated using the same DCCC system but in the descending mode. TLC examination of the fractions revealed the presence of three compounds not seen in the leaf extract which gave a bright purple colour with the spray reagent. Fractions containing these compounds (19–32, 46–60; stationary phase) were combined and the compounds isolated by prep. TLC as above.

Syringaresinol. Obtained as a cream amorphous powder (30 mg) from fractions 19–32. The compound was identical to that isolated from *Buddleja davidii* [18].

Syringaresinol-O-β-D-glucoside. Obtained as a white amorphous powder (22 mg) from fractions 46–60. Spectral features were identical to those published [19].

Liriodendrin. Obtained as white crystals (10 mg) (MeOH), mp 253°, from the stationary phase. Spectral features were identical to those published [20].

TLC. Silica gel GF_{254}. Solvents: (a) EtOAc–MeOH (3:1); (b) $CHCl_3$–MeOH (3:1); (c) $CHCl_3$–MeOH (10:1); (d) EtOAc–MeOH (6:1); (e) $CHCl_3$–EtOAc (1:1). Detection of zones was by examination under UV light (254 nm), in daylight after spraying with 0.5% anisaldehyde in HOAc–H_2SO_4–MeOH (2:1:17) and heating at 105° for 10 min. Loganin derivatives appear as quenching spots giving a pink colour after spraying and some other iridoids as quenching spots giving a brown or grey–brown colour after spraying. The iridoid–triterpene congeners appear as quenching spots giving a blue colour after spraying. Furofuran lignans appear as quenching spots giving a bright purple colour.

Acetylation of compounds was carried out using standard procedures.

Methylation of compounds was carried out using the Aldrich Mini-Diazald apparatus and reagent.

Enzymatic hydrolysis. A sample (10 mg) was suspended in 5 ml H_2O with 100 mg glucosidase. The mixture was incubated in a water bath at 37° for 3 days. The reaction mixture was then extracted with 2 × 50 ml $CHCl_3$–MeOH (3:2), the $CHCl_3$ layers combined and taken to dryness under red. pres. The concd extract was examined by TLC and where required the products were isolated in a pure form using prep. TLC.

Alkaline hydrolysis of desfontainic acid (**9**). Compound **9** (8 mg) was dissolved in 10 ml 5% KOH in MeOH and refluxed gently for 30 min. The reaction mixture was neutralized with dil. HCl and quickly extracted with 2 × 25 ml $CHCl_3$–MeOH (2:1). The organic layers were combined, dried (Na_2SO_4) and concd under red. pres. The mixture was examined by TLC and products were isolated by prep. TLC. The chromatographic behaviour and ^{1}H NMR spectrum of the major product was identical to that of 24-hydroxytormentic acid (**11**) [3]. Compound **1** could not be detected in the reaction mixture. The aq. part of the reaction mixture was freeze-dried and also investigated by TLC. No spot corresponding to **1** could be detected.

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