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FERULIC ACID ESTERS OF UNSATURATED HIGHER ALCOHOLS FROM *LUPINUS LUTEUS* ROOTS

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Key Word Index—*Lupinus luteus*; Leguminosae; roots; ferulic acid esters; unsaturated higher alcohols.

Abstract—Unsaturated long chain alcohol esters of ferulic acid were isolated and identified in the roots of *Lupinus luteus*. The higher alcohols obtained by hydrolysis of the ferulates were identified as (*Z*)-9-octadecen-1-ol, (*Z*)-11-octadecen-1-ol, (*Z*)-13-octadecen-1-ol, (*Z*)-14-octadecen-1-ol and 11-icosen-1-ol. Octadecyl, icosyl, docosyl and tetracosyl ferulates were also detected from the same source. The content of unsaturated alcohols amounted to 9% of the total long chain alcohols from the ferulates in seven-week-old *L. luteus* roots. © 1997 Elsevier Science Ltd

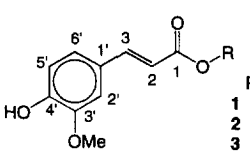
INTRODUCTION

The roots of yellow lupin (*Lupinus luteus*) are known to contain many species of phenylpropanoids, principally isoflavones [1] and flavones [2]. In a previous paper, we described the presence of prenylated flavanones together with ferulates of higher alcohols [3]. Naturally occurring ferulic acid esters of saturated alcohols (C_{16-32}) have been reviewed [4, 5] and occur in the Pinaceae [4, 6], Podocarpaceae, Salicaceae and Leguminosae [4], Linaceae and Bignoniaceae [5], Myristicaceae [7], Crassulaceae [8], Rubiaceae [9], Euphorbiaceae [10] and Aristolochiaceae [11]. In the Leguminosae, ferulates have been isolated from the genera, *Erythrina* [4, 12], *Euchresta* [13] and *Bauhina* [14].

The present study, which was focused on the constitution of alcoholic components in the ferulates of yellow lupin, revealed that they have saturated and unsaturated long-chain alcoholic residues (C_{18-24}) with some of the unsaturated ones possessing a double-bond at unusual positions. Together with the fact that higher alcohol esters of ferulic acid are accumulated in association with suberin biosynthesis in wounded potato tubers [15], our finding of ferulates of unsaturated fatty alcohols may be indicative of their physiological functions in plants.

RESULTS AND DISCUSSION

The less-polar column fraction of a methanol extract of yellow lupin roots [3] was rechromatographed over silica gel to give eluates with chloroform and 1% methanol in chloroform. A part of the latter eluate was concentrated and kept at −20° yielding a fine precipitate (Fr-IIp) which exhibited a blue fluorescent spot under $UV_{365\,nm}$ light on silica gel 60 F_{254} plates (R_f 0.45, hexane–EtOAc, 4:1) and in the ^1H NMR spectrum, a group of proton signals assignable to a feruloyl moiety [δ 8.13, *br s*, (1H. OH-4′), δ 7.59 *d* (*J* = 15.9 Hz, 1H, H-3), δ 7.34 *d* (*J* = 2.0 Hz, 1H, H-2′), δ 7.14 *dd* (*J* = 8.2, 2.0 Hz, 1H, H-6′), δ 6.87 *d* (*J* = 8.2 Hz, 1H, H-5′), δ 6.40 *d* (*J* = 15.9 Hz, 1H, H-2) and δ 3.93 *s* (3H, OCH_3-3′)]. The coupling constant between H-2 and H-3 (*J* = 15.9 Hz) clearly suggested its *trans*-geometry [16] (cf. *cis*-ferulate, $J_{2,3}$ = *ca* 13 Hz [7, 16]), which was unambiguously confirmed by NOEs from H-2 (δ 6.40) to H-2′ and H-6′ protons (9.7 and 5.7%), and from 3′-OCH_3 (δ 3.93) to the H-2′ proton (4.4%). The signals for aliphatic methylene groups (δ 1.26, *ca* 32–34 H), a terminal methyl [0.88 *t* (*J* = 6.9 Hz, 3H)], carbinyl methylene



R=
1 -$(CH_2)_{17}CH_3$
2 -$(CH_2)_8CH{=}CH(CH_2)_7CH_3$
3 -$(CH_2)_{10}CH{=}CH(CH_2)_5CH_3$
4 -$(CH_2)_{12}CH{=}CH(CH_2)_3CH_3$
5 -$(CH_2)_{13}CH{=}CH(CH_2)_2CH_3$
6 -$(CH_2)_{18}CH_3$
7 -$(CH_2)_{19}CH_3$
8 -$(CH_2)_{10}CH{=}CH(CH_2)_7CH_3$
9 -$(CH_2)_{21}CH_3$
10 -$(CH_2)_{23}CH_3$

1a ~ **10a** implicate the corresponding alcohols (R-OH), respectively.

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[δ 4.15 *t* (J = 6.7 Hz, 2H)] and the neighbouring methylene [δ 1.68 *qu* (J = 6.8 Hz, 2H)] were assignable to those of a long-chain alcoholic residue. The detection of $[M]^+$ at *m/z* (rel. int) 432 (1.9), 446 (100), 460 (3.0), 474 (51.0), 488 (2.8), 502 (16.7), 516 (1.9) and 530 (2.9) by FD-mass spectrometry revealed IIp to be a mixture of C_{17}–C_{24} alkyl ferulates.

The mother liquor of Fr-II contained further ferulates, which were recovered by preparative TLC to give a fraction (Fr-IIs). Its ^{1}H NMR spectrum exhibited characteristic signals of olefinic (δ 5.36 *m*) and allylic (δ 2.01 *m*) protons, together with aliphatic signals similar to those of the constituents of Fr-IIp. The constituents of Fr-IIs showed $[M]^+$ at *m/z* 444, 446 and 472 in the FD-mass spectrum suggesting that this fraction was a mixture of octadecyl, octadecenyl and icosenyl ferulates.

Alkaline hydrolysis of Fr-IIp and Fr-IIs yielded free higher alcohols and ferulic acid, which corresponded with authentic compounds on TLC (R_f 0.73 and 0.28, respectively, $CHCl_3$–MeOH, 9:1).

The results of a quantitative GC analysis of the higher alcohols in lupin ferulates are summarised in Table 1. The predominant alcohols in both yellow and white lupin ferulates were octadecanol (46 and 58%) and icosanol (27 and 22%), respectively. The unsaturated alcohols, octadecenol and icosenol comprised 9% and a minute amount (<1%), and 12% and a trace amount, in the alcoholic fractions, respectively, from yellow and white lupin ferulates.

Ferulates containing unsaturated alcohols (Fr-IIs) were further purified by medium-pressure liquid chromatography to obtain to major fractions (Fr-IIs-1 and Fr-IIs-2). The former showing a $[M]^+$ molecular ion at *m/z* 444 (FD-mass spectrum) gave a neutral hydrolysate which was subsequently confirmed to be a mixture of positional isomers of octadecenol. Double bond localization was carried out by GC-mass spectrometric analysis of the corresponding *bis*(methylthio)-derivative vicinally substituted at the original double bond, which afforded intense mass fragments arising from cleavage between the two carbons both substituted with a methylthio group [17]. The *bis*(methylthio)alkanols prepared from the alcoholic components of Fr-IIs-1 gave three GC peaks (peaks D1–D3), which showed $[M]^+$ at *m/z* 362 and $[M-47]^+$ at *m/z* 315 formed by loss of a methylthio radical from the $[M]^+$ (Table 2). Peak D1 (R_t = 10.6 min) showed fragment ions at *m/z* 217, 189, 173, and 145. These fragment ions suggested that D1 consisted of two derivatives, namely, 11,12-*bis*(methylthio) octadecanol yielding a pair of mass fragments at *m/z* 217 [fragment A (m = 8)] and 145 [fragment B (n = 4)], and 9,10-*bis*(methylthio)octadecanol yielding another pair of mass fragments at *m/z* 189 [fragment A (m = 6)] and 173 [fragment B (n = 6)] (Table 2). The second GC peak (D2 at R_t = 10.8 min) afforded intense fragment ions at *m/z* 245 [fragment A (m = 10)] and 117 [fragment B (n = 2)] indicating the location of the *bis*(methylthio)-substitution at C-13 and C-14. Peak D3 R_t = 11.1 min, exhibited fragment ions at *m/z* 259 [fragment A (m = 11)] and 103 [fragment B (n = 1)], which indicated that D3 was 14,15-*bis*(methylthio)octadecanol. Thus, the original alcohols were 9-, 11-, 13- and 14-octadecen-1-ols.

Since such octadecenols are uncommon as natural products, an alternative method was employed in order to confirm the unusual localization of the double bonds in lupin ferulates. Oxidative cleavage of the olefinic bond according to Lemieux and von Rudloff [18] resulted in the corresponding ω-hydroxyalkanoic acids, which were converted into the corresponding methyl ω-methoxyalkanoates and analysed by GC-mass spectrometry, giving four peaks (K1–K4). When analysed by EI-GC-mass spectrometry, methyl ω-methoxyalkanoates afford not $[M]^+$, but $[M-15]^+$, $[M-31]^+$, $[M-47]^+$ and $[M-64]^+$ fragments together with intense *m/z* 45 and 74 fragments, respectively, characteristic of the primary methyl ether and methoxycarbonylmethylene part structures [19]. Peaks K1–K4 all exhibited $[M-15]^+$ and $[M-31]^+$ fragments, those for K1 at *m/z* 187 and 171, suggesting the M_r 202, K2 at *m/z* 215 and 199, M_r 230, K3 at *m/z* 243 and 227, M_r 258, and K4 at *m/z* 257 and 241, M_r 272 (Table 3). These corresponded with dimethylated C_9, C_{11}, C_{13} and C_{14} ω-hydroxyalkanoic acids, respectively, arising from 9-, 11-, 13- and 14-octadecen-1-ols by oxidative cleavage of their double bonds. The location of a double bond, not only at C-9 but also C-11, C-13 and C-14, in the octadecen-1-ols composing

Table 1. Composition of constituent alcohols in lupin ferulates

Constituent	GC-MS fragment *m/z* (%)		GC content (% FID)	
	$[M-18]^+$	$[M-46]^+$	*L. luteus*	*L. albus.*
Octadecan-1-ol (**1a**)	252 (5)	224 (4)	46	58
Octadecen-1-ol (**2a–5a**)	250 (7)	222 (1)	9	12
Nonadecan-1-ol (**6a**)	266 (4)	238 (4)	n.d.*	1
Icosan-1-ol (**7a**)	280 (3)	252 (3)	27	22
Icosen-1-ol (**8a**)	278 (4)	250 (1)	<1	<1
Docosan-1-ol (**9a**)	308 (2)	280 (2)	13	4
Tetracosan-1-ol (**10a**)	336 (2)	308 (2)	5	4

* n.d.: not detected.

Table 2. GC-MS data of *bis*(methylthio)alkanols prepared from octadecenols in Fr-IIs-1

Peak	R_t(min)	$[M]^-$ *m/z*	$[M-47]^-$ *m/z*	Fragment A *m/z*	Fragment B *m/z*	*m*	*n*
D1* (**2b**)	10.6	362 (26)†	315 (6)	189 (21)	173 (28)	6	6
(**3b**)				217 (100)	145 (90)	8	4
D2 (**4b**)	10.8	362 (19)	315 (5)	245 (100)	117 (51)	10	2
D3 (**5b**)	11.1	362 (17)	315 (4)	259 (100)	103 (40)	11	1

* A mixture of two derivatives (**2a** + **3b**) indistinguishable on a gas chromatogram, but distinguishable in a mass spectrum.
† Relative abundance (%).

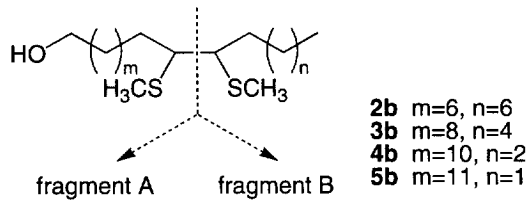
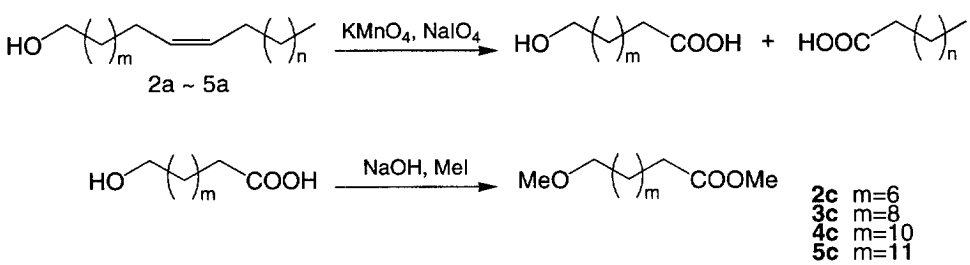


Table 3. GC-MS data of methyl ω-methoxyalkanoates prepared from octadecenols in Fr-IIs-1

			Mass fragment *m/z* (rel. int.)				
Peak	R_t(min)	Content (%)*	$[M-15]^+$	$[M-31]^+$	$[M-47]^-$	$[M-64]^+$	Others
K1 (**2c**)	7.4	13	187 (1)	171 (5)	155 (8)	138 (25)	87 (23), 74 (53), 45 (100)
K2 (**3c**)	9.7	19	215 (2)	199 (4)	183 (13)	166 (7)	87 (39), 74 (65), 45 (100)
K3 (**4c**)	11.8	27	243 (3)	227 (4)	211 (15)	194 (6)	87 (47), 74 (77), 45 (100)
K4 (**5c**)	12.8	41	257 (4)	241 (4)	225 (16)	208 (7)	87 (56), 74 (89), 45 (100)

* Ratio based on FID responses.



lupin ferulates were thus determined unambiguously by duplicate methods.

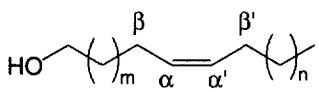
Although another fraction prepared by medium-pressure LC (Fr-IIs-2) gave a single peak on the chromatogram, FD-mass spectrometric analysis revealed that it was a mixture of two components possessing M_rs of 446 (relative intensity of $[M]^+$, 100) and 472 (44%), respectively, corresponding with octadecanol and icosenol, respectively. Hydrolysis of Fr-IIs-2 yielded the expected mixture of octadecanol and icosenol, which was subjected to the oxidative degradation followed by dimethyl derivatization similarly as described above. A notable reaction product (methyl ω-methoxyalkanoate) was observed at R_t = 9.7 min by GC-mass spectrometry and showed mass fragments at *m/z* 215 ($[M-15]^-$, 2%) and 199 ($[M-31]^+$, 4%) together with intense fragments *m/z* 74 (63%) and 45 (100%). The former two fragments indicated the M_r to be 230. The derivative was easily assigned to be methyl 11-methoxyundecanoate and, therefore, the presence of 11-icosenol as an unsaturated alcoholic component in yellow lupin ferulates was thus confirmed.

The geometry of the double-bonds in the octadecenols prepared from Fr-IIs-1 was determined by ^{13}C NMR (Table 4). The spectrum of the octadecenol mixture exhibited an allylic carbon signal at δ 27.2, which could be reliably assigned to the methylene carbons adjacent to a *cis*-double bond in aliphatic chains [20]; the corresponding allylic methylene carbons adjacent to a *trans*-double bond are usually observed around δ 32.6. The unsaturated fatty alcohols from Fr-IIs-1 showed no signal from δ 30 to 35 except for those assignable to C-2 methylene (δ 32.8) and C-16 methylene of 9-octadecenol (δ 31.9), whilst authentic *trans*-9-octadecenol gave three signals at δ 32.6 (C × 2, allylic carbons, C-8 and C-11), 32.8 (C-2 methylene) and 31.9 (C-16 methylene). These results indicated that the predominant octadecenols in this fraction should have *cis*-geometry. Since the relative

Table 4. ^{13}C NMR data of alcohols in Fr-IIs-1, free alcohols prepared by hydrolysis of Fr-IIs-1, and reference 1-alkenols ($CDCl_3$)

Compound	Carbon*								
	C-1	C-2	C-3	C-β	C-α and C-α'	C-β'	C-18	C-19	C-20
Fr-IIs-1†	64.6	28.7	25.6	27.2	129.6, 130.1§	29.3	13.8¶	—	—
Alcohols of Fr-IIs-1‡	63.1	32.8	25.7	27.2	129.6, 130.1§	29.3	13.8¶	—	—
Reference compounds									
cis-9-Octadecen-1-ol (**2a**)	63.0	32.8	25.7	27.2	129.8, 129.9	27.2	14.1	—	—
trans-9-Octadecen-1-ol	63.1	32.8	25.7	32.6	130.3, 130.4	32.6	14.1	—	—
cis-11-Octadecen-1-ol (**3a**)	63.1	32.8	25.7	27.2	129.9, 129.9	27.2	14.1	—	—
cis-13-Octadecen-1-ol (**4a**)	63.1	32.8	25.7	27.2	129.8, 129.9	26.9	13.9	—	—
cis-14-Octadecen-1-ol (**5a**)	62.9	32.8	25.7	27.2	129.6, 130.1	29.3	13.8	—	—
cis-11-Icosen-1-ol (**8a**)	63.1	32.8	25.7	27.2	129.8, 129.9	27.2	31.9	22.7	14.1

* C-α and -α', olefinic carbons; C-β and -β', allylic carbons close to hydroxy and methyl ends, respectively.
† Mixture of ferulates, **2–5**.
‡ Mixture of **2a–5a**, constituents in neutral hydrolysates of Fr-IIs-1.
§ Accompanied by another signal at δ 129.9 assignable to C-α and -α'.
¶ Accompanied by additional signals at δ 14.0 and 14.1, assignable to C-18.



2a m=6, n=6
3a m=8, n=4
4a m=10, n=2
5a m=11, n=1
8a m=8, n=6

contents of 9-, 11-, 13- and 14-octadecenols are 13, 19, 27 and 41% on a basis of oxidative degradation products, the geometry appeared to be correctly reflected in the ^{13}C NMR spectrum. The octadecen-1-ols in lupin ferulates were thus concluded to be (*Z*)-9-, (*Z*)-11-, (*Z*)-13- and (*Z*)-14-octadecen-1-ols, respectively. The geometry of 11-icosen-1-ol remains to be deduced.

EXPERIMENTAL

General. GCs equipped with single and dual FIDs, were used for qualitative and quantitative analyses. GC columns and analytical conditions were as follows: 1 m × 3 mm glass columns packed with 5% PEG-20M on Chromosorb W AW DMCS (80–100 mesh) for free higher alcohols (carrier gas N_2, 40 ml min^{-1}; inj. temp., 240°; oven temp., prog. from 180° to 220° at 4° min^{-1}) and methyl ω-methoxyalkanoates (N_2, 40 ml min^{-1}; inj. temp., 220°; oven temp., prog. from 100° to 220° at 10° min^{-1}), and a 25 × 0.35 mm fused-silica column with OV-1 as the bonded phase (GL Science Inc., Tokyo) for *bis*(methylthio)alkanols (He, 40 ml min^{-1}; inj. temp., 280°; oven temp., prog. from 200° to 280° at 5° min^{-1}; split ratio, 10:1).

Chemicals. Commercially available *cis*-9- and *cis*-11-octadecenoic acids were reduced with $LiAlH_4$ to yield *cis*-9-octadecen-1-ol (**2a**) and *cis*-11-octadecen-1-ol (**3a**). *trans*-9-Octadecenoic acid was prepared as a *cis–trans* isomerization product [21] and similarly reduced to give *trans*-9-octadecen-1-ol (*trans*-form, 96%, HPLC, $UV_{208\ nm}$). *cis*-13-Octadecen-1-ol (**4a**) and *cis*-14-octadecen-1-ol (**5a**) were acquired as neutral hydrolysates of Wittig reaction products from 13-benzoxytridecanal and triphenylphosphonium *n*-pentylide, and from 14-benzoxytetradecanal and triphenylphosphonium *n*-butylide, respectively. The coupling reactions of ω-benzoxyalkanal prepared from the corresponding ω-benzoxyalkan-1-ols by PCC oxidation [22] with triphenylphosphonium *n*-alkylides [23] were carried out using *t*-BuOK as a base and THF as a solvent [24] (*cis*-form, 94%, HPLC, $UV_{208\ nm}$). *cis*-11-Icosen-1-ol (**8a**) were purchased from Nu-Check-Prep, Inc.

Plant material and fractionation of ferulate mixtures. Yellow lupin (*L. luteus* L.) roots (fr. wt 13 kg) harvested immediately after the flowering stage, were extracted twice with 90% MeOH and the extracts fractionated as described previously [3]. The combined eluates with benzene and 10% EtOAc in benzene from a silica gel column were conc and stood at −20° to yield a large amount of solid consisting mainly of lonchocarpol A [3]. Constituents in the mother liquor (13.55 g) were rechromatographed over silica gel (180 g) and eluted with $CHCl_3$ (1 l) and 1% MeOH in $CHCl_3$ (200 ml × 3). Ferulic esters of long-chain alcohols in the $CHCl_3$ eluate (Fr-I) and in the first MeOH–$CHCl_3$ eluate (Fr-II) were detected as blue fluorescent spots under $UV_{365\ nm}$ light on silica gel 60 F_{254} plates (R_f 0.45 in hexane–EtOAc, 4:1). When stood at −20° Fr-II afforded ppts (Fr-IIp, 177 mg) and a mother liquor containing predominantly unsaturated higher alcohol esters of ferulic acid. The latter was subjected

to prep. TLC (hexane–EtOAc, 3:1) to give a ferulate mixt. (Fr-IIs, 130 mg). Further fractionation of Fr-IIs was performed by medium-pressure LC (pre-packed 310 × 25 mm column, Merck LiChroprep RP-18, solvent; MeOH, detection; $UV_{322\ nm}$) and two major frs (earlier one: Fr-IIs-1, 52 mg and a later one Fr-IIs-2, 44 mg) were obtained. Constituents and chromatographic properties of these frs were checked by FD-MS and HPLC using a Develosil-ODS-N5 (Nomura Chemical) column (250 × 4.6 mm, MeOH, 1 ml min^{-1}, $UV_{322\ nm}$).

Ferulate mixture Fr-IIp and Fr-IIs. Fr-IIp. UV λ_{max}^{MeOH}nm: 294 and 324. ^{1}H NMR δ (acetone-d_6, 500 MHz): δ 8.13 (1H, *br s*, OH-4′), 7.59 (1H, *d*, *J* = 15.9 Hz, H-3), 7.34 (1H, *d*, *J* = 2.0 Hz, H-2′), 7.14 (1H, *d*, *J* = 8.2, 2.0 Hz, H-6′), 6.87 (1H, *dd*, *J* = 8.2 Hz, H-5′), 6.40 (1H, *d*, *J* = 15.9 Hz, H-2), 4.15 (2H, *t*, *J* = 6.7 Hz, H-1″), 3.93 (3H, *s*, CH_3-3′), 1.68 (2H, *m*, *J* = 6.8 Hz, H-2″), 1.24 (*br s*-like *m*), 0.88 (3H, *t*, *J* = 6.9 Hz, CH_3-ω). Fr-IIs. UV λ_{max}^{MeOH}nm: 295 and 325. ^{1}H NMR δ (acetone-d_6, 500 MHz): δ 8.13 (1H, *br s*, OH-4′), 7.59 (1H, *d*, *J* = 15.9 Hz, H-3), 7.34 (1H, *d*, *J* = 2.0 Hz, H-2′), 7.14 (1H, *dd*, *J* = 8.2, 2.0 Hz, H-6′), 6.87 (1H, *d*, *J* = 8.2 Hz, H-5′), 6.40 (1H, *d*, *J* = 15.9 Hz, H-2), 5.36 (*m*, -CH_2-CH=CH-CH_2-), 4.15 (2H, *t*, *J* = 6.7 Hz, H-1″), 3.93 (3H, *s*, CH_3-3′), 2.01 (*m*, -CH_2-CH=CH-CH_2-), 1.68 (2H, *m*, *J* = 6.8 Hz, H-2″), 1.24 (*br s*-like *m*) 0.90-0.88 (3H, *m*, *J* = 6.9 Hz, CH_3-ω).

Composition of ferulates in yellow and white lupin roots. Roots of seven-week-old yellow and white lupins, fr. wt 44 and 49 g, respectively, were extracted × 3 with MeOH. Each extract was evapd *in vacuo* and the residue obtained partitioned between EtOAc (× 3, 60 ml) and H_2O (20 ml). The EtOAc layer was washed with 1M aq. HCl, 5% aq. $NaHCO_3$ and satd aq. NaCl successively, dried (Na_2SO_4) and concd. The concentrate was chromatographed on a Wako gel C-200 (15 g) column. A ferulate mixt. eluted with 90 ml of benzene and 30 ml of benzene–EtOAc (19:1) was collected and subjected to prep. TLC (hexane–EtOAc, 4:1). The yields of ferulate mixts from yellow and white lupin roots were 1.8 and 2.7 mg, respectively. The constitutive alcohols of lupin ferulates thus obtained were analysed by GC after alkaline hydrolysis.

Hydrolysis of ferulates and derivitization of higher alcohols. Hydrolysis. The ferulate mixt. (2–4 mg) was hydrolysed in 1 ml of 90% MeOH containing 0.4 M KOH at 37° for 2 hr. The resulting fatty alcohols were extracted with hexane and cleaned-up by prep. TLC in hexane–EtOAc (4:1, R_f 0.34).

Bis(*methylthio*)-*derivatives.* These were prepd according to refs [17, 25]. To a mixt. of higher alcohols (1–2 mg) dissolved in Me_2S_2 (0.2 ml) was added 50 μl of an I_2 soln in Et_2O (60 mg ml^{-1}) and the reaction mixt. left at room temp. for 24 hr. The reaction mixt. was then subjected to prep. TLC (hexane–$CHCl_3$, 2:1, R_f 0.18–0.30) to give a mixt. of *bis*(methylthio)alkanols, which were analysed by GC-MS.

GC-MS of bis(*methylthio*)-*derivatives.* Peak D1 (**2b**+**3b**). *m/z* (rel. int.): 362 $[M]^+$ (26), 315 $[M-47]^+$ (6), 217 (100), 189 (21), 173 (28), 145 (90), 144 (17), 109 (16), 97 (24), 96 (15), 95 (35), 87 (13), 83 (23), 82 (15), 81 (32), 69 (33), 67 (30), 61 (45), 55 (53), 43 (15), 41 (23). Peak D2 (**4b**). *m/z* (rel. int.): 362 $[M]^+$ (19), 315 $[M-47]^+$ (5), 246 (16), 245 (100), 117 (51), 116 (13), 109 (10), 95 (17), 83 (10), 81 (15), 69 (24), 67 (12), 61 (25), 55 (22), 41 (12). Peak D3 (**5b**). *m/z* (rel. int.): 362 $[M]^+$ (17), 315 $[M-47]^+$ (4), 260 (18), 259 (100), 109 (10), 103 (40), 102 (10), 95 (15), 83 (10), 81 (15), 69 (16), 67 (12), 55 (24), 41 (12).

Methyl ω-methoxyalkanoates. The higher alcohols originated from Fr-IIs-1 and Fr-IIs-2 were subjected to oxidative cleavage of their double bonds followed by a dimethylation reaction. To the alcohols (*ca* 2 mg) in *tert*-BuOH (1.5 ml) and 2 mM aq. Na_2CO_3 (1.5 ml) was added a mixt. of $KMnO_4$ (0.6 mg) and $NaIO_4$ (6.5 mg) in 3 ml of 2 mM aq. Na_2CO_3 at 0°. The reaction mixt. was stirred at 0° for 12 hr, at which point, 5 drops of conc. H_2SO_4, *ca* 5 mg of Na_2SO_3 and 3 ml of H_2O were added successively [18]. The resulting ω-hydroxyalkanoic acids were extracted with Et_2O (× 3, 15 ml) and derivatized by the method of ref. [26] into the corresponding methyl ω-methoxyalkanoates, which were analysed by GC-MS.

GC-MS fragments for methyl ω-methoxyalkanoates. Peak K1 (**2c**). *m/z* (rel. int.): 187 $[M-15]^+$ (1), 171 (5), 170 (2), 155 (8), 138 (25), 127 (5), 110 (13), 97 (17), 96 (13), 87 (23), 84 (11), 74 (53), 69 (19), 68 (12), 55 (33), 45 (100), 43 (11), 41 (24). Peak K2 (**3c**). *m/z* (rel. int.): 215 $[M-15]^+$ (2), 199 (4), 198 (3), 183 (13), 166 (7), 138 (10), 124 (7), 98 (23), 97 (10), 96 (15), 95 (10), 87 (39), 84 (20), 83 (21), 82 (14), 81 (13), 74 (65), 69 (40), 68 (12), 67 (11), 55 (41), 45 (100), 43 (14), 41 (31). Peak K3 (**4c**). *m/z* (rel. int.): 243 $[M-15]^+$ (3), 227 (4), 226 (3), 211 (15), 194 (6), 166 (4), 152 (6), 143 (10), 124 (8), 112 (10), 111 (14), 110 (9), 98 (35), 97 (22), 95 (15), 95 (15), 87 (47), 84 (25), 83 (41), 82 (19), 81 (15), 75 (10), 74 (77), 71 (9), 69 (39), 68 (13), 67 (14), 57 (10), 56 (10), 55 (84), 45 (100), 43 (15), 41 (38). Peak K4 (**5c**). *m/z* (rel. int.): 257 $[M-15]^+$ (4), 241 (4), 240 (5), 225 (16), 208 (7), 180 (5), 166 (6), 143 (12), 112 (14), 111 (14), 109 (11), 98 (44), 97 (27), 96 (19), 95 (16), 87 (56), 84 (27), 83 (34), 82 (19), 81 (17), 75 (13), 74 (89), 71 (11), 69 (50), 68 (15), 67 (17), 59 (10), 57 (13), 56 (12), 45 (100), 42 (10), 41 (43).

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