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LONG CHAIN ESTERS OF *VIROLA* SPECIES

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Key Word Index – *Virola sebifera*; *V. surinamensis*; *V. melinonii*; Myristicaceae; bark; long chain esters; fatty acid esters of β-sitosterol; β-sitosteryl glucoside; ω-hydroxy fatty acid esters of glucose; feruloylalkanoic acids; 1-monoglycerides of feruloylalkanoic acids.

Abstract—The esters of *n*-fatty acids and ω-hydroxy *n*-fatty acids of β-sitosterol, D-glucose and ferulic acid (*trans* and *cis*) as well as β-sitosterol, fatty acids and β-sitosteryl-β-D-glucoside were isolated from three *Virola* species and identified by optical data and chemical reactions. A novel series of acidic esters derived from C_{22}–C_{29} ω-hydroxy fatty acids and *cis*- and *trans*-ferulic acid is reported for the first time. These compounds also occurred as the corresponding diester 1-monoglycerides whereas the ω-hydroxy acids themselves were also present as the corresponding glucosyl esters.

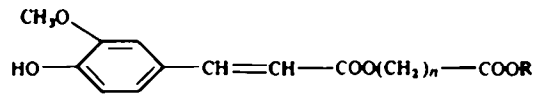
INTRODUCTION

Plants of the myristicaceous genus, *Virola*, have been the subject of ethnobotanical, phytochemical and pharmacological studies because of their uses as hallucinogens and folk medicines in South America [1, 2]. We found that the bark of *V. sebifera*, which is the botanical source of snuffs made by South America Indian tribes [1], contained new alkaloids [3], but the barked wood of *V. sebifera*, barks of *V. surinamensis*, *V. melinonii* and *V. carinata* which are not used for intoxication [1] had no alkaloids. We have reported 11 neolignans in *V. carinata* [4–7], which is used to treat a disease causing blotched discolouration of the skin [1]. In this report, esters of long chain fatty and ω-hydroxy fatty acids were isolated from the barks of *V. sebifera*, *V. surinamensis* and *V. melinonii* but not in the barked wood of *V. sebifera* and bark of *V. carinata* [4–7].

RESULTS AND DISCUSSION

The methanol extract of *V. sebifera* bark was treated with 5% acetic acid to remove basic compounds. The precipitate was dissolved successively in *n*-hexane, benzene and chloroform. The *n*-hexane soluble portion was chromatographed on a silica gel column and three fractions (1–3) which eluted with 30% methanol in chloroform were separated and purified by additional CC. Fraction 1 was β-sitosterol. Fraction 2 was a mixture of long chain fatty acids (IR, ^{1}H NMR and mass spectrometry). Their carbon numbers were determined as 16, 17, 18, 22, 23, 24, 25 and 26 by GC-MS of their corresponding methyl esters. Fraction 3, the last eluted, was a white powder which showed aliphatic, carbonyl and hydroxy groups on IR. ^{1}H NMR suggested that it was composed of three moieties, long chain fatty acids, β-sitosterol and sugar. The ion *m/z* 396 was detected as base peak in the mass spectrum which could be a fragment derived from β-sitosterol. Acid hydrolysis gave three components which were identified as β-sitosterol, a mixture of fatty acids and glucose. Three acetyl protons appeared at δ 2.02, 2.04 and 2.07 ppm in the ^{1}H NMR spectrum of the acetate derivative of fraction 3. It suggested that two of the five hydroxy protons of the glucose moiety were occupied by substitutions of carboxylic acid and β-sitosterol. Alkaline hydrolysis of fraction 3 afforded carboxylic acids and a glycoside. The carbon numbers of the former were 16, 18, 22–26 by GC and GC-MS as methyl esters; the latter was identical with fraction 4 by co-TLC. This fraction was eluted with 40% methanol in chloroform and was obtained as a white powder assumed to be a glycoside of β-sitosterol from its blue-violet colour when heated with 10% sulphuric acid after TLC, and from its IR, ^{1}H NMR and mass spectrum. Acid hydrolysis afforded β-sitosterol and glucose. ^{1}H NMR of fraction 4 and its acetate showed that the anomeric proton of glucose appeared at δ 4.20 (J = 8.0 Hz) and 4.61 (J = 8.0 Hz) ppm, respectively. This suggested that β-sitosterol could be oriented at position 1 of glucose with a β-linkage. β-Sitosteryl-β-D-glucoside is commonly found in nature [8]. The carboxylic acids connected to glucose in the compounds of fraction 3 were at the hydroxy at position 6 of D-glucose because the methylene protons appeared at δ 4.40 and 4.50 (J = 13.0, 2.0 and 13.0, 4.0 Hz, respectively) which were at lower field than those of other protons on carbons bearing hydroxy groups. Thus fraction 3 was a mixture of acyl derivatives with the ester linkage at 6 of glucose of compound 4; 6′-acyl-β-sitosteryl-β-D-glucose carbon numbers 16, 18, 22–26), which have been isolated in nature [8–10].

Fraction 5 was eluted by 40% methanol in chloroform and was obtained as a white powder, the ^{1}H NMR of which showed glycosyl and ω-hydroxy fatty acid moieties. An anomeric proton [δ 4.30 (J = 8.0 Hz)] and five acetyl protons (2.00, 2.02, 2.04, 2.07 and 2.09), three methine and two methylene protons on a carbon bearing acetyl group and an anomeric proton for its acetate on ^{1}H NMR suggested that fraction 5 was a mixture of glucosyl esters, of which the ω-hydroxy acyl group was attached to position 1 of D-glucose through a β-linkage. It was hydrolysed to give glucose and ω-hydroxy fatty acids (C_{22}–C_{27}). Such compounds have not previously been isolated from the plant kingdom.



Fraction 6: R = H n = 21–27
Fraction 7: R = OCH_2—CH(OH)—CH_2OH
n = 21, 23–28

The benzene-soluble portion of the extract was chromatographed to obtain two fractions (6 and 7) eluting with 20% methanol in chloroform. Fraction 6 eluted with cyclohexane–ethyl acetate (1:1) on a second CC and was a white powder that showed aliphatic, aromatic, hydroxy and carbonyl groups on IR. ^{1}H NMR showed that it was composed of two moieties. One was *trans*- and *cis*-3(4)-hydroxy-4(3)-methoxy cinnamic acids (*trans*; major); aromatic protons [*trans*: δ6.92 (*d*, J = 9.0 Hz), 7.09 (*d*, J = 2.0 Hz) and 7.10 (*dd*, J = 2.0 and 9.0 Hz) and *cis*: 6.89 (*d*, J = 8.5 Hz), 7.14 (*dd*, J = 2.0 and 8.5 Hz) and 7.58 (*d*, J = 2.0 Hz), methoxy (δ3.96) and ethylene *trans*: δ6.32 and 7.65 (J = 16.0 Hz) and *cis*: 5.83 and 6.84 (J = 13.0 Hz)], the other was for an ω-carboxy fatty alcohol group showing two distinctive methylenes connected to an oxycarbonyl [δ4.22 (*trans*) and 4.14 (*cis*)] and to carbonyloxy (δ2.32, triplet, *trans* and *cis*), two methylenes connected to the methylenes described above (δ1.63, triplet) with the rest of the methylenes as a long aliphatic chain (δ1.27, *s*). Furthermore the alcohol moiety was determined to be a mixture of ω-carboxy fatty alcohols, since [M]$^+$ ions, m/z 616, 602, 588, 574, 560 and 532, were detected on EI-mass spectrometry, which corresponded to carbon numbers 28, 27, 26, 25, 24 and 22, respectively. The phenolic hydroxy and the carboxylic groups of fraction 6 were confirmed by obtaining its methyl derivatives, of which three methyl protons for ethers and ester appeared at δ3.68 and 3.94 on ^{1}H NMR, respectively, and for which [M]$^+$ ions (m/z 616, 588 and 560) corresponded to the addition of 28 mu to the [M]$^+$ ions (m/z 588, 560 and 532) in the mass spectrum. The hydroxy monomethoxycinnamic acid moiety was ferulic acid (commercially available) but not isoferulic acid prepared from caffeic acid [11] on co-TLC and ^{1}H NMR. It was suggested from all these spectra that fraction 6 would be composed by esters of *trans* and *cis* ferulic acids and ω-hydroxy fatty acids. Fraction 6 was hydrolysed with barium hydroxide to obtain a mixture of ferulic acids and a mixture of ω-hydroxy acids. The *trans* form of the acids was confirmed by comparison of TLC and ^{1}H NMR with an authentic sample. The *cis* form was identical with an isomer obtained from an authentic *trans* sample by irradiation with UV (254 nm) in methanol [12]. The hydroxy acid fraction was acetylated and methylated and acetyl and methyl protons were detected by ^{1}H NMR. Their [M]$^+$ ions were detected at m/z 413, 427, 441, 455 and 469 using GC-CI-mass spectrometry, corresponding to C_{22}, C_{23}, C_{24}, C_{25} and C_{26}, respectively. The major compounds were C_{24} and C_{26}. Thus fraction 6 was composed of a mixture of C_{22-28} ω-hydroxy acid esters of *cis*- and *trans*-ferulic acid, which was also found in barks of *V. sebifera* and *V. melinonii*.

Fraction 7 was a white powder which was eluted with cyclohexane–ethylacetate (1:3) by CC and consisted of similar components to those of fraction 6 but contained in addition a glycerol moiety on ^{1}H NMR [δ 3.68 (2H), 3.90 (1H) and 4.22 (2H)]. They were mono substituted on position 1 of glycerol, since only one methylene proton of glycerol (δ4.22) shifted downfield. Its acetate derivative showed three acetyl groups; one for phenol (δ2.36) and two for alcohols (δ2.10 and 2.11) on ^{1}H NMR. Several [M]$^+$ ions were detected for fraction 7 (m/z 704, 690, 676, 662, 648, 634 and 606) and its acetate (m/z 774, 746, 732 and 718) confirming that they were monoglycerides of ω-feruloyl fatty acids. Only two phenolic methoxy protons (δ3.94) were detected for the methyl derivatives of fraction 7 by ^{1}H NMR. The methyl derivative was further acetylated to give an acetate, the ^{1}H NMR of which indicated the presence of two aliphatic acetyl protons (δ2.08 and 2.09). Hydrolysis of fraction 7 with HCl followed by methylation gave a product which was identical with the methyl derivatives of fraction 6 on TLC and from their optical spectra. Fraction 7 was then hydrolysed with barium hydroxide followed by methylation to give 3,4-dimethoxy cinnamic acid methyl esters (*trans* and *cis*) and ω-hydroxy fatty acid methyl esters. GC and GC-MS of these fatty acids showed their carbon numbers to be only 24, 26 and 28. Thus fraction 7 was a mixture of 1-(ω-feruloylalkanoyl) glycerols of carbon numbers 22, 24, 25, 26, 27, 28 and 29. Fraction 7 was also detected in bark of *V. surinamensis*. There have been previous reports of esters of hydroxy cinnamic acids such as caffeic acid, ferulic acid, and fatty alcohols (C_{18} to C_{26}), fatty acids (1,26-diol) and ω-hydroxy fatty acids (C_{26} and C_{28}) and a few glycerides in nature [13].

Fraction 8 was isolated from the bark of *V. surinamensis* and *V. melinonii* but not from barks of *V. sebifera* and *V. carinata* [4]. The hexane soluble portion was chromatographed with cyclohexane–chloroform on silica gel to elute fraction 8 as a white powder. The components were esters of β-sitosterol and fatty acids; the methylene protons likaged to the carbonyl group of the acid moiety appeared at δ2.28 (triplet) on ^{1}H NMR. However, [M]$^+$ ions could not be detected by EI-mass spectrometry. Fraction 8 was then compared with authentic palmityl-β-sitosteryl ester [14] on TLC, GC and GC-MS, which suggested that it was a mixture of fatty acid β-sitosteryl esters with carbon numbers of 15 and 16. The latter only has been found before in *Epilobium* species [15].

EXPERIMENTAL

Barks of *V. sebifera* (4.3 kg), *V. surinamensis* (4.2 kg) and *V. melinonii* (1.7 kg) and barked wood of *V. sebifera* (4 kg) were extracted with MeOH. Each extract was treated with 5% HOAc to separate filtrate and ppt. The former was tested for alkaloids with Dragendorff's reagent. The latter was dissolved in *n*-hexane to obtain soluble and insoluble portions. The insoluble portion was treated with C_6H_6 to obtain again soluble and insoluble portions. The latter was treated with $CHCl_3$ to produce soluble and insoluble portions. The latter was treated with EtOAc to yield again soluble and insoluble portions. Each soluble portion from each material was subjected to CC. All spots were detected on TLC by fluorescence (254 nm) and/or with 10% H_2SO_4 followed by heating. Fractions 1–5 were obtained from the hexane-soluble portion by CC elution with a gradient system of $CHCl_3$ and MeOH. Fractions 6 and 7 were obtained from the C_6H_6 soluble portion by CC with $CHCl_3$ and MeOH. Fraction 8

was sep from the hexane soluble portion of *V. surinamensis* and *V. melinonii* barks by CC.

Fractions 1 (yield 0.01%) and 2 (yield 0.007%) were identified as β-sitosterol and fatty acids, respectively, by comparison with authentic compounds mmp, TLC, GC and GC-MS (as Me esters). *Fraction* 3 (yield 0.001%), a white powder, was acyl β-sitosteryl glucoside by IR, ^{1}H NMR and MS. Acetylation (Ac_2O/pyridine with heating) gave the acetate, which was determined by ^{1}H NMR. Acidic hydrolysis: fraction 3 (10 mg) in dioxan (1 ml) and H_2O (0.5 ml) was refluxed in dil. HCl (1 ml) for 3 hr, dried, dissolved in $CHCl_3$ and extracted with 5% NaOH to remove β-sitosterol in the $CHCl_3$ phase, which was confirmed by comparison with authentic compound (TLC). The H_2O phase was acidified and extracted with Et_2O. The Et_2O phase was methylated with CH_2N_2. GC-MS *m/z* [M]$^+$ 270, 298, 354, 368, 382, 396 and 410. *R,s* on GC of the H_2O phase after silylation gave the same *R,* as authentic silyl D-glucose. Alkaline hydrolysis: fraction 3 (10 mg) was refluxed in 5% $NaHCO_3$ in 50% MeOH for 1 hr, the MeOH removed and extracted with Et_2O to give the hydrolysis product, which was confirmed to be β-sitosteryl glucoside by TLC. The H_2O phase was acidified and extracted with Et_2O, from which fatty acids were obtained.

Fraction 4 (yield 0.005%), a white powder, identified as β-sitosteryl glucoside by comparison of optical data [8] and hydrolysis products β-sitosterol and D-glucose.

Fraction 5 (yield 0.0005%), a white powder, ^{1}H NMR (CD_3OD and $CDCl_3$) δ ppm 1.29 [*s*, $(CH_2)\times n$-4], 1.60 (4H, *m*, $CH_2\times 2$), 2.34 (2H, *b*, CH_2 C=O), 3.05–5.0 (9H, *m*, C<u>H</u>$_2$ OH and CH–O–). Acetylation with Ac_2O/pyridine gave an acetate: ^{1}H NMR ($CDCl_3$) ppm 1.26 [*s*, $(CH_2)\times n$], 1.60 (4H, *m*, $CH_2\times 2$), 2.00, 2.02, 2.04, 2.07, 2.09 (3H × 5, *s*, $COCH_3\times 5$), 2.30 (2H, *t*, *J* = 7.0 Hz, CH_2–CO), 3.70 (1H, *br*, H-5'), 4.15 (1H, *br d*, *J* = 12.0 Hz, H-6'), 4.27 (1H, *dd*, *J* = 4.5 and 2.5 Hz, H-6'), 4.29 [2H, overlapped, $(CH_2)_n$ <u>CH_2</u>–O–Ac)], 4.50 (1H, *d*, *J* = 8.0 Hz, H-1'), 4.92 (1H, *t*, *J* = 8.0 Hz, H-2'), 5.08 (1H, *t*, *J* = 8.0 Hz, H-4'), 5.21 (1H, *t*, *J* = 9.0 Hz, H-3'). Hydrolysis: fraction 5 (4 mg) was refluxed in 10% HCl (2 ml) in dioxan for 2 hr and evapd to dryness. H_2O was added and the acids extracted with Et_2O, the alcohols remained in the H_2O phase. Me esters: GC-MS *m/z* [M]$^+$ 370, 384, 398, 412, 426 and 440. The alcohol moiety was D-glucose by GC after prep. of the TMSi derivative in ether.

Fraction 6 (yield 0.001%), a white powder, UV λ_{max}^{MeOH} nm 324, 296 (sh), 234, 216 (sh), 204, IR ν_{max}^{KBr} cm^{-1} 3530, 2930, 2850, 1700, 1620, 1590, 1500, 1445, 1260, 1170. ^{1}H NMR ($CDCl_3$), δ ppm 1.27 [*s*, $CH_2\times n$-4)], 1.63 (4H, *m*, $CH_2\times 2$), 2.32 (2H, *t*, *J* = 7.3 Hz, CH_2–CO), 3.96 (3H, *s*, OCH_3), 4.14 [2H, *t*, *J* = 7.2 Hz, CH_2O–CO (*cis*)], 4.22 [2H, *t*, *J* = 7.2 Hz, CH_2O–CO (*trans*)], 5.83 [1H, *d*, *J* = 13.0 Hz, H-8 (*cis*)], 6.32 [1H, *d*, *J* = 16.0 Hz, H-8 (*trans*)], 6.84 [1H, *d*, *J* = 13.0 Hz, H-7 (*cis*)], 6.89 [1H, *d*, *J* = 8.5 Hz, H-5 (*cis*)], 6.92 [1H, *d*, *J* = 9.0 Hz, H-5 (*trans*)], 7.09 [1H, *d*, *J* = 2.0 Hz, H-2 (*trans*)], 7.10 [1H, *dd*, *J* = 2.0 and 9.0 Hz, H-6 (*trans*)], 7.14 [1H, *dd*, *J* = 2.0 and 8.5 Hz, H-6 (*cis*)], 7.65 [1H, *d*, *J* = 16.0 Hz, H-7 (*trans*)], 7.75 [1H, *d*, *J* = 2.0 Hz, H-2 (*cis*)]. EI-MS *m/z* (rel. %) [M]$^+$ 616 (0.5), 602 (0.9), 588 (3.9), 574 (1.9), 560 (11.6), 532 (1.3), 194 (47.2), 177 (100). HR MS 177.059 ($C_{10}H_9O_3$ calc. 177.017). Methylation of fraction 6 with CH_2N_2 gave a Me ether ester: ^{1}H NMR ($CDCl_3$) δ ppm 1.26 [*s*, $CH_2\times$ (*n*-4)], 1.66 (4H, *m*, $CH_2\times 2$), 2.32 (2H, *t*, *J* = 7.3 Hz, CH_2 CO), 3.68 (3H, *s*, $COOCH_3$), 3.94 (6H, *s*, $OCH_3\times 2$), 4.14 [2H, *t*, *J* = 7.0 Hz, CH_2OCO (*cis*)], 4.21 [2H, *t*, *J* = 7.0 Hz, CH_2–O–CO (*trans*)], 5.86 [1H, *d*, *J* = 13.0 Hz, H-8 (*cis*)], 6.34 [1H, *d*, *J* = 16.0, H-8 (*trans*)], 6.85 [1H, *d*, *J* = 13.0 Hz, H-7 (*cis*)], 6.87 [1H, *d*, *J* = 8.5 Hz, H-5 (*cis*)], 6.89 [1H, *d*, *J* = 8.0, H-5 (*trans*)], 7.09 [1H, *d*, *J* = 2.0 Hz, H-2 (*trans*)], 7.14 (1H, *dd*, *J* = 2.0 and 8.0 Hz, H-6 (*trans*)], 7.23 [1H, *dd*, *J* = 2.0 and 8.5 Hz, H-6 (*trans*)], 7.66 [1H, *d*, *J* = 16.0 Hz, H-7 (*trans*)], 7.82 [1H, *d*, *J* = 2.0 Hz, H-2 (*cis*)]. MS *m/z* (rel. %) [M]$^+$ 616 (15.7), 588 (57.6), 560 (4.5), 208 (27.7), 191 (100). HR MS 191.070 ($C_{11}H_{11}O_3$ calc. 191.070). GC-MS *m/z* [M]$^+$ 616, 588, 560. Hydrolysis: fraction 6 (4 mg) dissolved in DMSO (1 ml) was refluxed in satd. $Ba(OH)_2$ in H_2O (0.5 ml) for 3 hr. The reaction mixture was acidified with HCl and extracted with Et_2O. The Et_2O phase was applied to prep. TLC to isolate alcohol and acid fractions. The former was acetylated followed by methylation for GC and GC-MS. GC-MS (CI isobutane) *m/z* [M]$^+$ + 1 469, 455, 441, 427, 413. The latter was identical with authentic ferulic acid on TLC.

Fraction 7 (yield 0.0007%), a white powder, ^{1}H NMR ($CDCl_3$) δ ppm 1.26 [*s*, $CH_2\times$ (*n*-4)], 1.63 (4H, *b*, $CH_2\times 2$), 2.36 (2H, *t*, *J* = 7.5 Hz, CH_2–CO), 3.68 (2H, *b*, CH_2–OH), 3.90 (1H, *b*, C<u>H</u>OH), 3.94 [3H, *s*, OCH_3 (*cis*)], 3.95 [3H, *s*, OCH_3 (*trans*), 4.20 [*m*, CH_2O–CO, CH_2O (*trans*) and (*cis*)], 5.84 [1H, *d*, *J* = 13.0, H-8 (*cis*)], 6.32 [1H, *d*, *J* = 16.0 Hz, H-8 (*trans*)], 6.89 [1H, *d*, *J* = 13.0 Hz, H-7 (*cis*)], 6.93 [1H, *d*, *J* = 8.5 Hz, H-5 (*cis*),], 6.96 [1H, *d*, *J* = 8.0 Hz, H-5 (*trans*)], 7.07 [1H, *d*, *J* = 2.0 Hz, H-2 (*trans*)], 7.11 [1H, *dd*, *J* = 2.0 and 8.0 Hz, H-6 (*trans*)], 7.17 [1H, *dd*, *J* = 2.0 and 8.5 Hz, H-6 (*cis*)], 7.64 [1H, *d*, *J* = 16.0 Hz, H-7 (*trans*)], 7.80 [1H, *d*, *J* = 2.0 Hz, H-2 (*cis*)]. MS *m/z* (rel. int. %) [M]$^+$ 704 (0.9), 690 (2.6), 676 (1.2), 662 (4.3), 648 (1.9), 634 (4.6), 606 (2.4), 194 (33.7), 177 (100). Acetate: ^{1}H NMR ($CDCl_3$) δ ppm 1.26 [*s*, $CH_2\times$ (*n*-4)], 1.60 (4H, *b*, $CH_2\times 2$), 2.10 (3H, *s*, $COCH_3$), 2.11 (3H, *s*, $COCH_3$), 2.34 (2H, *t*, *J* = 7.0 Hz, CH_2CO), 2.36 (3H, *s*, $COCH_3$), 3.89 [3H, *s*, OCH_3 (*cis*)], 3.90 [3H, *s*, OCH_3 (*trans*)], 4.13 [2H, *t*, *J* = 7.0 Hz, CH_2O–CO (*cis*)], 4.18 [1H, *dd*, *J* = 6.0 and 12.0 Hz, C<u>H</u>$_2$O–Ac–(gly)], 4.19 [1H, *dd*, *J* = 6.0 and 12.0 Hz, C<u>H</u>$_2$–OAc–(gly)], 4.23 [2H, *t*, *J* = 7.0 Hz, CH_2O–CO (*trans*)], 4.26 [1H, *dd*, *J* = 4.0 and 12.0 Hz, CH_2O–CO (gly)], 4.27 [1H, *dd*, *J* = 4.0 and 12.0 Hz, CH_2O–CO (gly)], 5.28 [1H, *m*, CH–OAc (gly)], 5.97 [1H, *d*, *J* = 13.0 Hz, H-8 (*cis*)], 6.42 [1H, *d*, *J* = 16.0 Hz, H-8 (*trans*)], 6.87 [1H, *d*, *J* = 8.0 Hz, H-5 (*cis*)], 6.89 [1H, *d*, *J* = 8.0 Hz, H-5 (*trans*)], 6.90 [1H, *d*, *J* = 13.0 Hz, H-7 (*cis*)], 7.08 [1H, *d*, *J* = 2.0 Hz, H-2 (*trans*)], 7.14 [1H, *dd*, *J* = 2.0 and 8.0 Hz, H-6 (*trans*)], 7.22 [1H, *dd*, *J* = 2.0 and 8.0 Hz, H-6 (*cis*)], 7.62 [1H, *d*, *J* = 2.0 Hz, H-2 (*cis*)], 7.68 [1H, *d*, *J* = 16.0 Hz, H-7 (*trans*)]. MS *m/z* (rel. %) [M]$^+$ 774 (0.2), 746 (1.6), 732 (0.3), 718 (2.0), 43 (100). Methylation gave a Me ester: ^{1}H NMR ($CDCl_3$) δ ppm 1.27 [*s*, $CH_2\times$ (*n*-4)], 1.60 (4H, *m*, $CH_2\times 2$), 2.37 (2H, *t*, *J* = 7.0 Hz, CH_2–CO), 3.66 (2H, *m*, CH_2OH), 3.94 (6H, *s*, $OCH_3\times 2$), 4.11 [2H, *t*, *J* = 7.0 Hz, CH_2O–CO (*cis*)], 4.18 [2H, *t*, *J* = 7.0 Hz, CH_2OCO (*trans*)], 4.22 (3H, *m*, O–CH_2–C<u>H</u>(OH)C<u>H</u>$_2$OH), 5.86 [1H, *d*, *J* = 13.0 Hz, H-8 (*cis*)], 6.34 [1H, *d*, *J* = 16.0 Hz, H-8 (*trans*)], 6.40 [1H, *d*, *J* = 13.0 Hz, H-7 (*cis*)], 6.60 [1H, *d*, *J* = 8.5 Hz, H-5 (*cis*)], 6.90 [1H, *d*, *J* = 8.5 Hz, H-5 (*trans*)], 7.09 [1H, *d*, *J* = 2.0 Hz, H-2 (*trans*)], 7.14 [1H, *dd*, *J* = 8.5 and 2.0 Hz, H-6 (*trans*)], 7.23 [overlapped with solvent, H-6 (*cis*)], 7.66 [1H, *d*, *J* = 16.0 Hz, H-7 (*trans*)], 7.81 [1H, *d*, *J* = 2.0 Hz, H-2 (*cis*)]. Acetylation of the Me ester of fraction 7 gave a Me ether acetate: ^{1}H NMR ($CDCl_3$) δ ppm, 1.27 [*s*, $CH_2\times$ (*n*-4)], 1.60 (4H, *b*, $CH_2\times 2$), 2.08 (3H, *s*, O–Ac), 2.09 (3H, *s*, O–Ac), 2.37 (2H, *t*, *J* = 7.0 Hz, CH_2CO), 3.92 (6H, *s*, $OCH_3\times 2$), 4.11 [2H, *t*, *J* = 7.0 Hz, CH_2OCO (*cis*)], 4.16 [1H, *dd*, *J* = 6.0 and 12.0 Hz, C<u>H</u>$_2$OAc (gly)], 4.17 [1H, *dd*, *J* = 6.0 and 12.0 Hz, CH_2–O–Ac(gly)], 4.19 [2H, *t*, *J* = 7.0, CH_2OCO (*trans*)], 4.30 [1H, *dd*, *J* = 4.0 and 12.0 Hz, CH_2O(gly)], 4.31 [1H, *dd*, *J* = 4.0 and 12.0 Hz, CH_2O–(gly)], 5.27 [1H, *m*, C<u>H</u>–O–Ac(gly)], 5.86 [1H, *d*, *J* = 13.0 Hz, H-8 (*cis*)], 6.33 [1H, *d*, *J* = 16.0 Hz, H-8 (*trans*)], 6.84 [1H, *d*, *J* = 13.0 Hz, H-7 (*cis*)], 6.87 [1H, *d*, *J* = 8.0 Hz, H-5 (*cis*)], 6.89 [1H, *d*, *J* = 8.0 Hz, H-5 (*trans*)], 7.08 [1H, *d*, *J* = 2.0 Hz, H-2 (*trans*)], 7.14 [1H, *dd*, *J* = 2.0 and 8.0 Hz, H-6 (*trans*)], 7.22 [1H, *dd*, *J* = 2.0 and 8.0 Hz, H-6 (*cis*)], 7.65 [1H, *d*, *J* = 16.0 Hz, H-7 (*trans*)], 7.69 [1H, overlapped with H-7, H-2 (*cis*)]. Hydrolysis and methylation: fraction 7 (8 mg) dissolved in dioxan (0.6 ml) and H_2O (0.6 ml)

was refluxed with dil. HCl (1 ml) for 3 hr, concd to dryness and dissolved in $CHCl_3$. This was methylated with CH_2N_2, purified by prep. TLC and found to be identical to fraction 6 and its Me ether esters on TLC, respectively. Fraction 7 (6 mg) dissolved in DMSO (1 ml) was refluxed with satd. $Ba(OH)_2$ in H_2O (0.5 ml) for 3 hr. The reaction mixture was acidified with HCl and extracted with Et_2O. The Et_2O phase was dried and methylated with CH_2N_2 to obtain two hydrolysed methylated products. These were separated by prep. TLC and shown to be dimethoxy cinnamic acid Me ester and ω-hydroxy fatty acid Me esters on TLC and GC/GC-MS (CI) (m/z $[M]^+ + 1$ 427, 455).

Fraction 8 (yield 0.0002%), a white powder, showed ^1H NMR signals which indicated that it consisted of β-sitosterol and long chain fatty acids moieties. GC-MS $[M]^+$ m/z 638, 652.

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