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PHENOLIC FATTY ACID ESTERS FROM *BUDDLEJA GLOBOSA* STEMBARK

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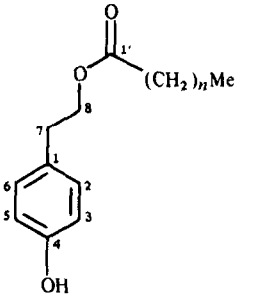
Key Word Index—*Buddleja globosa*; Loganiaceae; phenolic esters, *p*-hydroxycinnamyl docosanoate; *p*-hydroxycinnamyl lignocerate, 2-(4-hydroxyphenyl)ethyl docosanoate, 2-(4-hydroxyphenyl)ethyl lignocerate.

Abstract—A chloroform extract of the stem bark of *Buddleja globosa* yielded a mixture of crystalline compounds which were found to be esters of long chain fatty acids and 4-hydroxycinnamyl alcohol or 2-(4-hydroxyphenyl) ethanol. This type of ester has been proposed as forming the units of the complex polymer suberin but no individual monomers have been reported until now.

INTRODUCTION

The root-bark of *Buddleja davidii* Franch. has been shown to contain caryophyllenoid sesquiterpenes with piscicidal activity [1, 2] The major constituent of this type, buddledin A, has also been extracted from *B. japonica* [3]. The stem- and root-barks of several species of *Buddleja* were screened as part of a programme to investigate the presence of related compounds in hitherto-uninvestigated species. Chloroform extracts of the stem bark of *B globosa* Lam. gave crystals on standing after concentration. The identity of the crystalline matter is discussed in this paper.



1 $n = 22$
2 $n = 20$

RESULTS AND DISCUSSION

Identity of the compounds

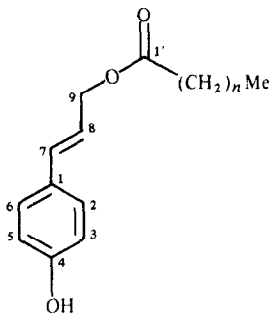
The crystalline material was shown to be a mixture of closely related substances upon consideration of the NMR and mass spectra obtained. The ^{1}H NMR spectra clearly showed the presence of an aromatic portion and a large hydrocarbon moiety. The only acetate signal in the acetylated product was in the aromatic region at δ2.36 and this fact, together with the bathochromic shift observed in the UV spectrum of the parent compounds, indicates that the aromatic portion of the molecule is phenolic. The hydrocarbon part of the compounds was shown to consist of aliphatic fatty acids by GC analysis of the methylated hydrolysis products. The chromatogram obtained by GC showed the presence of two major fatty acid residues corresponding to lignoceric and docosanoic acids.

Separation of the individual constituents of the crystalline mixture proved somewhat difficult but was eventually achieved by reverse-phase impregnated silica gel TLC. Four major compounds were isolated and their identity characterised as described below.

GS13A was identified as the 2-(4-hydroxyphenyl)ethanol ester of lignoceric acid (**1**). The mass spectrum showed a small molecular ion at *m/z* 488 with major peaks at *m/z* 120 and 368 respectively. These correspond to the alcohol and acid portions of the ester. The peak at 1740 cm^{-1} in the IR spectrum showed the presence of an ester group. The signals for the isolated 7,8-methylene groups are clearly seen at δ2.85 and 4.25 respectively in the ^{1}H NMR spectrum. This spectrum also shows signals at δ7.07 and 6.76 typical of a 1,4-disubstituted aromatic ring. Mild alkaline hydrolysis of GS13A produced a compound identical with 2-(4-hydroxyphenyl)ethanol when examined by TLC and also a compound which when methylated had GC behaviour identical with the methyl ester of authentic lignoceric acid.

GS13B was identified as the ester of 2-(4-hydroxyphenyl) ethanol ester of docosanoic acid (**2**). It gave spectral features almost identical with GS13A but the mass spectrum showed a molecular ion peak at *m/z* 460 and a peak corresponding to the fatty acid portion of the molecule at *m/z* 340. Investigation of the hydrolysis products of GS13B showed that the phenolic portion of the molecule was identical to that of GS13A but the fatty acid moiety corresponded to the next member of the descending homologous series, i.e. docosanoic acid.

GS14A was characterized as the ester of lignoceric acid and 4-hydroxycinnamyl alcohol (**3**). The presence of the lignoceric acid was shown by the same features as observed for GS13A. The ^{13}C NMR spectrum showed the



3 $n = 22$
4 $n = 20$

presence of an extra methylene carbon atom at δ64 9 and the ^{1}H NMR spectrum exhibited signals at δ6.58 and 6 14 for two protons attached to a *trans* double bond The splitting pattern observed for the protons at δ6.58, 6 14 and 4.70 in the ^{1}H NMR spectrum is typical of aromatic propen-2-yl alcohols The phenyl ring is 1,4-disubstituted as signals very similar to those shown by 13A and 13B are seen The acetylated compound gives a single aromatic acetate signal The identity of the alcohol as 4-hydroxycinnamyl was confirmed by TLC examination of the hydrolysis product of GS14A

GS14B was characterised as the ester of 4-hydroxycinnamyl alcohol and docosanoic acid **4**. The nature of these two portions of the molecule was deduced by comparing the signals observed with those given in the spectra for GS13B and GS14A respectively

Biological significance of the esters isolated

Esters of long chain fatty acids and phenols have not been isolated previously from plant material There is, however, considerable evidence for the existence of such compounds as units in the complex polymeric material suberin [4, 5] Suberin is the major substance which impregnates the cell walls of cork tissue in plants and has a considerable passive defence role The existence of such compounds has been determined by chemical degradative studies and this is the first report of the isolation of the intact esters which may form the 'building blocks' of suberin. It is significant that these compounds were isolated from the bark of the plant material examined TLC examination of the bark of other species has shown the presence of similar compounds and work is in progress to isolate and characterise these. It is unlikely that such compounds would contribute to any extent to the biological activity of preparations made from *Buddleja* [6]

EXPERIMENTAL

Source of plant material, extraction and isolation procedures Stem bark was collected by peeling strips from mature bushes growing in the Chelsea Physic Garden The material was authenticated at source and a specimen voucher is deposited in the herbarium of the Chelsea Department of Pharmacy, King's College, London

Concentrated $CHCl_3$ extracts of small amounts of the stem bark of *Buddleja globosa* had yielded soft crystalline matter on standing The shredded stem bark of *Buddleja globosa* was dried, powdered and 200 g of the powder was extracted for 48 hr at 25° with $CHCl_3$ The $CHCl_3$ was filtered off and the extraction repeated with three further portions of $CHCl_3$ The combined $CHCl_3$ extracts were concd under red pres to give 3 2 g of a brown residue

TLC examination of the extract showed the presence of zones which quenched UV light (254 nm) but did not give a strong reaction with anisaldehyde spray These zones were identical to those given by the crystalline matter originally observed 2 0 g of the residue was applied to a silica gel column and eluted with petrol bp range 40–60° with increasing proportions of $(Me)_2CO$ Fractions collected were monitored by TLC The fraction corresponding to a 4 1 mixture of the two solvents showed the presence of the desired compounds and on concentration yielded 250 mg of a soft white crystalline mass Prep TLC using silica gel plates impregnated with liquid paraffin and subsequent recrystallization from $(Me)_2CO$ resulted in the isolation of four compounds GS13A (75 mg), GS13B (30 mg), GS14A (84 mg) and GS14B (36 mg)

Characterization of compounds UV spectra were run in MeOH and MeOH containing 5% NaOH, IR spectra were run in CCl_4 Mass spectra were obtained by using direct insertion probes and electron impact at 35 eV on an AEI MS 902 spectrometer, NMR spectra were obtained in $CDCl_3$ and assignments were made with the help of decoupling experiments

GS13A Crystals mp 61–62 , R_f values (A) 12, (B) 60, (C) 31, (D) 44 UV λ_{max}^{MeOH} (log ε) 220 (3 6), 272 (3 1) (NaOH) 220 (3 8), 242 (3 6), 292 (3 2), IR ν^{Nujol} cm^{-1} 3450 (OH), 1740 (ester C=O), 1510, 1465, 1380, 1170, MS *m/z* (% base peak) 488 $[M]^+$ (5), 368 (76), 120 (100), ^{1}H NMR, 7 07 (2H, *d*, *J* = 8 4 Hz, 3', 5'-H), 6,76 (2H, *d*, *J* = 8 4 Hz, 2', 6'-H), 4 25 (2H, *t*, *J* = 7 1 Hz, 8-CH_2), 2 85 (2H, *t*, *J* = 7 1 Hz, 7-CH_2), 2 28 (2H, *t*, α-CH_2 of fatty acid), 1 59 (2H, *m*, β-CH_2 of fatty acid), 1 25 (40H, *m*, CH_2) 0 91 (3H, *t*, ω-Me) ^{13}C NMR see Table 1

GS13B Crystals mp 64–66 R_f values (A) 12, (B) 60, (C) 31, (D) 50 UV λ_{max}^{MeOH} (log ε) 220 (3 8), 273 (3 1) (NaOH) 220 (3 8), 242 (3.6), 292 (3 2), IR ν^{Nujol} cm^{-1} 3450 (OH), 1740 (ester C=O), 1510, 1455, 1365, 1165, MS *m/z* (% base peak) 460 M^+ (7), 340 (79), 120 (100), ^{1}H NMR, 7 07 (2H, *d*, *J* = 8 4 Hz, 3', 5'-H), 6 76 (2H, *d*, *J* = 8.4 Hz, 2', 6'-H), 4 25 (2H, *t*, *J* = 7 1 Hz, 8-CH_2), 2.85 (2H, *t*, *J* = 7 1 Hz, 7-CH_2), 2.28 (2H, *t*, α-CH_2 of fatty acid), 1 59 (2H, *m*, β-CH_2 of fatty acid), 1 25 (36H, *m*, CH_2), 0 91 (3H, *t*, ω-Me) ^{13}C NMR see Table 1

Table 1 ^{13}C NMR spectral data of compounds isolated

C	Chemical shift (δppm from TMS) GS13A	GS13B	GS14A	GS14B
1	129 0	129 0	130 0	130 0
2,6	128 1	128 1	130 1	130 1
3,5	115 5	115 5	115 3	115 3
4	155 6	155 6	154 4	154 3
7	31 9	31 9	133 8	133 8
8	65 2	65 2	121 0	121 0
9	- -	–	64 9	64 9
1'	174 0	174 0	174 0	174 0
2'	34 4	34 4	34 4	34 4
3'–22'	29 5	-	29 5	—
3'–20'	–	29 5	-	29 5
23'	22 7	-	22.7	-
21'	–	22 7	-	22 7
24'	14 1	-	14 1	-
22'	- -	14 1		14 1

GS14A. Crystals mp 59–60°: R_f values A 10 B 55 C 28 D 55, UV λ_{max}^{MeOH} (log ε) 232 (3 6), 275 (3 1) 295sh (3 05), (NaOH) 248 (3 5) 345 (3.7) IR ν^{Nujol} cm^{-1} 3450 (OH), 1740, 1675, 1600, 1455, 1385, 1090, 1050, MS *m/z* (% base peak) 500 M^+ (4), 368 (72), 129 (100), ^{1}H NMR, 7.27 (2H, *d*, *J* = 7 7 Hz, 3′, 5′-H), 6 78 (2H, *d*, *J* = 7.7 Hz, 2′, 6′-H), 6.58 (1H, *d*, *J* = 15 9 Hz, 7-H), 6.14 (1H, *dt*, *J* = 15 9 and 6 5 Hz, 8-H), 4 70 (2H, *d*, *J* = 6 5 Hz, 9-CH_2), 2 34 (2H, *t*, α-CH_2 of fatty acid), 1 63 (2H, *m*, β-CH_2 of fatty acid), 1 30 (40H, *m*, CH_2), 0 88 (3H, *t*, ω-Me). ^{13}C NMR. see Table 1

GS14B Crystals mp 60–61° R_f values A 10 B 55 C 28 D 60, UV λ_{max}^{MeOH} (log ε) 232 (3 6), 275 (3 1) 295sh (3 05), (NaOH) 248 (3 5) 345 (3.7 IR ν^{Nujol} cm^{-1} 3450 (OH), 1740, 1675, 1600, 1455, 1385, 1090, 1050; MS *m/z* (% base peak) 472 M^+ (6), 340 (72), 129 (100), ^{1}H NMR, 7 27 (2H, *d*, *J* = 7.7 Hz, 3′, 5′-H), 6.78 (2H, *d*, *J* = 7.7 Hz, 2′, 6′-H), 6.58 (1H, *d*, *J* = 15 9 Hz, 7-H), 6.14 (1H, *dt*, *J* = 15 9 and 6.5 Hz, 8-H), 4 70 (2H, *d*, *J* = 6 5 Hz, 9-CH_2), 2.34 (2H, *t*, α-CH_2 of fatty acid), 1.63 (2H, *m*, β-CH_2 of fatty acid), 1 30 (36H, *m*, CH_2), 0.88 (3H, *t*, ω-Me) ^{13}C NMR see Table 1

Chromatographic procedures TLC was carried out using silica gel GF254 (Merck) solvent systems used were (A) petrol (40–60 bp range)–$CHCl_3$ (1·1); (B) toluene–EtOAc (6·1); (C) petrol (40–60 b p. range)–MeCOEt 9 1. Zones were detected by examination under UV light 254 nm and spraying with anisaldehyde reagent (0 5% anisaldehyde in H_2SO_4–HOAc–MeOH, 5 10 85) and heating at 105° for 10 min.

Reverse phase TLC was carried out by impregnating the prepared silica gel plates with 5% liquid paraffin in petrol then removing the petrol in a current of air before applying the mixture to be separated The plates were run in $CHCl_3$–MeOH–H_2O (25 75 5) (TLC system D) When this method was used preparatively the bands were eluted with $CHCl_3$, taken to dryness and the residue dissolved in petrol This was put through a small silica gel column until no more liquid paraffin could be detected in the eluate The desired compounds were then eluted with $CHCl_3$–MeOH (4.1), taken to dryness under red. pres and recrystallized from Me_2CO

Gas-liquid chromatography of the methyl esters of the fatty acids was carried out using N_2 20 ml/min and a SGE BP28 column i.d. 0.22 mm, 12 m long, temperature programming 100° rising to 200° at 5°/min An FID detector was used.

Chemical reactions Acetylation was carried out using normal procedures. The acetates formed for each compound gave ^{1}H NMR spectra very similar to the parent compounds apart from some downfield shift of the aromatic ring protons and a 3H singlet at δ2.31. Hydrolysis of the compounds was carried out by refluxing for 30 min at 100° with 5% NaOH in MeOH. The hydrolysis mixture was passed through cation-exchange resin and then concd TLC examination showed the presence of a quenching spot which gave colour reactions and TLC behaviour identical with authentic samples of either 4-hydroxycinnamyl alcohol or 2-(4-hydroxyphenyl)ethanol Methylation of the fatty acids released on hydrolysis was achieved by refluxing with BF_3 in MeOH for 30 min.

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REFERENCES

1 Yoshida, T, Nobuhara, J., Uchida, M and Okuda, T (1978) *Chem. Pharm Bull.* **26**, 2535.
2. Yoshida, T, Nobuhara, J, Uchida, M., Fujii, N and Fujii, O (1978) *Chem. Pharm. Bull.* **26**, 2543
3 Suspuglas, P., Suspuglas, C and Rossi, J C (1978) *Plant Med Phytother* **12**, 148.
4 Riley, G R and Kolattukudy, P E (1975) *Plant Physiol* **56**, 650
5 Kolattukudy, P. E (1984) *Can J Botany* **62**, 2918
6 Houghton, P J (1984) *J. Ethnopharmacol* **11**, 293