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A WAX ESTER FROM *PIPER CLARKII*

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Key Word Index—*Piper clarkii*; Piperaceae; wax ester; 3-(4-hydroxyphenyl)propyl tetracosanoate; (+)-crotepoxide; β-sitosterol.

Abstract—A novel type of wax ester has been isolated from a petrol extract of stems and leaves of *Piper clarkii* and its structure established as 3-(4-hydroxyphenyl)propyl tetracosanoate. The structure was verified by synthesis. The oxygenated cyclohexane (+)-crotepoxide and β-sitosterol were also isolated.

INTRODUCTION

Many species of the genus *Piper* have been shown to possess antifungal, insecticidal, anthelmintic and antitumour activities. Presently, we are investigating the constituents of *Piper* species as part of a research programme seeking potent insecticides. We report herein the isolation, structure elucidation and synthesis of a wax ester of novel structure from *P. clarkii*, together with the isolation of the oxygenated cyclohexane (+)-crotepoxide and β-sitosterol.

RESULTS AND DISCUSSION

The petrol extract of stems and leaves of *P. clarkii* yielded three compounds: 3-(4-hydroxyphenyl)propyl tetracosanoate (**1**), (+)-crotepoxide (**2**) and β-sitosterol.

The ^{1}H NMR spectrum of **1** contained signals, which, from a 2D ^{1}H COSY experiment, were found to belong to three isolated spin systems. The aromatic region contained an AA′XX′ system indicating a symmetrically disubstituted ring. The estimated shifts were in agreement with a 4-hydroxyphenyl ring bound to an alkyl group. The aliphatic region contained an AMX system with shifts corresponding to the propyl moiety of a 3-phenyl substituted propyl ester. Furthermore, the aliphatic region contained a complex system giving rise to a broad-end triplet (δ0.88, 3H), a broad intense signal (δ1.26, 40H), a broad quintet (δ1.82, 2H) and a well-resolved triplet (δ2.30, 2H) in agreement with a tetracosanoate moiety. The assignments of the signals are shown in Table 1 together with the correlations obtained from a COSY experiment. The ^{13}C NMR spectrum also corresponded well with the proposed structure. In particular, in the low field region, besides the ester carbonyl at δ174.19, four signals were observed with shifts and intensities that were in excellent agreement with the substitution pattern of the 4-hydroxyphenyl moiety. The high resolution mass spectrum of **1** exhibited a $[M]^+$ which corresponded with the empirical formula $C_{33}H_{58}O_3$ (502.437, calculated 502.439). The mass spectral fragmentation pattern in the low resolution mass spectrum indicated that **1** is an ester with an alcoholic moiety possessing a hydroxyphenylpropyl group. The peak at *m/z* 474 results from loss of CO from the phenol and that at *m/z* 368 is in agreement with the fatty acid ion $[C_{23}H_{47}COOH]^+$ losing OH to form the fragment at *m/z* 351, which again can lose CO to form the fragment at *m/z* 323. The base peak at *m/z* 134 corresponds with [M − carboxylic acid]$^+$ and finally *m/z* 107 could be due to the $[CH_2{-}C_6H_4{-}OH]^+$ ion. The final proof of structure was provided by the synthesis of **1** from the reaction of 3-(4-benzyloxyphenyl)propanol with tetracosanoyl chloride and subsequent removal of the benzyl protection group by hydrogenolysis.

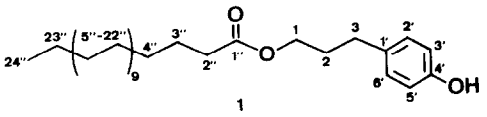
Generally, waxes contain hydrocarbons and esters, but free alcohols, aldehydes, ketones, fatty acids and triterpenes also occur. Wax alcohols occurring free or as esters are usually straight chain carbon compounds with almost exclusively an even number of carbon atoms [1]. Although the hydroxylated cinnamic acids, *p*-coumaric, caffeic, ferulic and sinapic have been detected in vascular plants in a bound form (probably as esters [2]), **1** to the best of our knowledge is the first example of a wax ester possessing an aromatic alcoholic moiety originating from shikimic acid. Polyhydroxy cyclohexanes are rare plant constituents and are reported to have antitumour, antileukaemic and antibiotic activity [3]. They occur in *P. hookeri* [4], *P. cubeb* and *P. clarkii* [5], but the present isolation of (+)-crotepoxide (**2**) in *P. clarkii* has not been reported previously.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were recorded at 250 and 62.9 MHz, respectively. Prep. TLC was performed on silica gel G layers. Silica gel (60–120 mesh) was used for CC. The solvent system for TLC was C_6H_6–EtOAc (9:1).

Plant material. Leaves and stems of the plant *P. clarkii* C. DC. were collected from the Mawsmai forest, Khasi Hills, Meghalaya, India in April 1990. The plant species was identified by Dr B. M. Wadhwa (Deputy Director, Botanical Survey of India, Eastern Circle Office, Shillong) and a voucher specimen (No. T-3, dated 14.4.1990) is deposited in the herbarium of his office.

Extraction and isolation. Crushed and dried plant material



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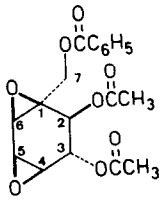

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Table 1. ^{1}H NMR (250 MHz, $CDCl_3$) and ^{13}C NMR (62.9 MHz, $CDCl_3$) spectral data of 3-(4-hydroxyphenyl)propyl tetracosanoate (with respect to TMS ± 0.01 ppm)

C	^{13}C	^{1}H	Multiplicity and coupling constants in Hz (±0.4 Hz)	Relative intensity	COSY Correlations with
1	63.66	4.08	*t* (6.6)	2H	H-2
2	31.28[a]	1.91	*tt* (6.6, 7.6)	2H	H-1, H-3
3	34.44	2.61	*t* (7.6)	2H	H-2
1′	133.30	—	—	—	—
2′, 6′	129.47	7.03	*	2H	H-3′, H-5′
3′, 5′	115.31	6.75	*	2H	H-2′, H-6′
4′	153.95	—	—	—	—
1″	174.19	—	—	—	—
2″	31.95[a]	2.30	*t* (7.5)	2H	H-3″
3″	25.06	1.62	*br quin*	2H	H-2″†
4″	30.49[a]				
5″–22″	29.21–29.73	1.26	*br*	40H	H-3″, H-24″
23″	22.71				
24″	14.11	0.88	*t* (6.6)	3H	†
OH	—	5.05	*br*	1H	—

[a]Assignments may be interchanged.
*Intense doublets from an unresolved AA′XX′ system.
†Both these signals also correlate with the broad signal at 1.26 ppm.

(332 g) was extracted with petrol (bp 60–80°) for 60 hr in a Soxhlet apparatus. The petrol extract was concd and chromatographed over silica gel using petrol, petrol–EtOAc, EtOAc–MeOH in increasing proportions. Three compounds were isolated from these frs in sufficient amounts for identification.

β-Sitosterol. The petrol–EtOAc (33:1) eluate furnished a solid compound. Recrystallization from MeOH gave needles of β-sitosterol, mp 133–134° (lit. [6] 140°). All spectroscopic data were completely in agreement with published data.

3-(4-hydroxyphenyl)Propyl tetracosanoate (**1**). Early frs eluted with petrol–EtOAc (9:1) furnished a mixt. of three compounds. The mixt. was subjected to prep. TLC to obtain the title compound (R_f 0.4) as a thick yellow oil which gave a faint red fluorescence in UV light and soon crystallized. Mp 69–70°. IR ν_{max}^{KBr} cm^{-1}: 3431, 1737, 1615. HRMS *m/z*: $[M]^+$ 502.437 ($C_{33}H_{58}O_3$ requires: 502.439). EIMS *m/z* (rel. int.): 502 $[M]^+$ (<1), 474 $[M-CO]^+$ (<1), 368 $[CH_3(CH_2)_{22}COOH]^+$ (<1), 351 $[CH_3(CH_2)_{22}CO]^+$ (<1), 323 $[351-CO]^+$ (<1), 134 [M −acid]$^+$ (100), 107 $[CH_2{-}C_6H_4{-}OH]^+$ (15). ^{1}H and ^{13}C NMR see Table 1. UV λ_{max}^{MeOH} nm: 208, 236, 286, 314.

Crotepoxide (**2**). The CC fr. eluted with petrol–EtOAc (9:1) afforded a solid, which recrystallized from hexane–Me_2CO as fine needles, mp 149–150°. $[\alpha]_D^{25}$ +71.2° ($CHCl_3$; *c* 0.34) (lit. [3] 150–151°), +74° ($CHCl_3$; *c* 1.70). The IR and ^{1}H NMR were in agreement with the reported data [3] but the ^{13}C NMR ($CHCl_3$) has not so far been published: 20.40 (2 × Me), 47.90 (C-4), 52.48 (C-5), 53.69 (C-6), 59.27 (C-1), 62.37 (C-7), 69.39 (C-2), 70.28 (C-3), 128.43, 129.08, 129.68, 133.39 (C_6H_5), 165.65, 169.55, 169.87 (C=O).

3-(4-benzyloxyphenyl)Propanol. A soln of 3-(4-hydroxyphenyl)propanol (2 g, 0.013 mol) in EtOH (100 ml) was refluxed with dry K_2CO_3 (2.4 g, 0.017 mol) and benzyl chloride (1.6 ml, 0.014 mol) for 3 hr. On cooling a solid sepd out. This was dissolved in Et_2O and filtered. Et_2O was evapd to give the title product as plates (2.9 g, 94% yield), mp 49–52°. TLC R_f 0.13. HRMS *m/z*: $[M]^+$ 242.135 ($C_{16}H_{18}O_2$ requires 242.131). IR ν_{max}^{KBr} cm^{-1}: 3350, 3060, 3034. ^{1}H NMR ($CDCl_3$) δ1.85 (2H, *m*, H-2), 1.91 (1H, *br s*, OH), 2.64 (2H, *t*, *J* = 7 Hz, H-3), 3.65 (2H, *t*, *J* = 6.4 Hz, H-1), 5.03 (2H, *s*, H-4), 6.89 (2H, *d*, *J* = 8.6 Hz, H-3′, H-5′), 7.09 (2H, *d*, *J* = 8,6 Hz, H-2′, H-6′), 7.36 (5H, *m*, C_6H_5). ^{13}C NMR ($CDCl_3$): 156.98 (C-4′), 134.05 (C-1′), 129.30, 129.20 (C-2′, C-6′), 115.16 (C-3′, C-5′), 62.14 (C-1), 34.24 (C-3), 31.04 (C-2), 137.12, 128.42, 127.76, 127.33, 70.0 (benzyl).

3-(4-benzyloxyphenyl)Propyl tetracosanoate. Tetracosanoic acid (500 mg, 1.3 mmol) was reacted with 1 ml of $SOCl_2$ at 80° for 2.5 hr and excess of $SOCl_2$ dist. off. The resulting acid chloride dissolved in Et_2O was reacted with 3-(4-benzyloxyphenyl)propanol (315 mg, 1.3 mmol) at room temp. for 24 hr. Upon evapn 750 mg of compound was obtained which was purified by flash CC. The product eluted by C_6H_6–$CHCl_3$ (9:1) was re-recrystallized from petrol to give the title compound as

plates (700 mg, 92% yield). Mp 72–73°. TLC R_f 0.76. HRMS *m/z*: $[M]^+$ 592.485 ($C_{40}H_{64}O_3$ requires 592.486). IR ν_{max}^{KBr} cm^{-1}: 3036, 2953, 1743. ^1H NMR ($CDCl_3$) δ0.88 (3H, *t*, J=6.9 Hz, H-24″), 1.25 (40H, *br s*, H-4″, H-23″), 1.62 (2H, *br quin* H-3″), 1.91 (2H, *tt*, J=6.8, 7.8 Hz, H-2), 2.29 (2H, *t*, J=7.5 Hz, H-2″), 4.07 (2H, *t*, J=6.4 Hz, H-1), 5.04 [2H, *s* (C_6H_5C) H_2], 6.90 (2H, *d*, J=8.4 Hz, H-3′, H-5′), 7.09 (2H, *d*, J=8.4 Hz, H-2′, H-6′), 7.42 (5H, *m*, C_6H_5). ^{13}C NMR ($CDCl_3$) δ173.95 (C-1″), 157.16 (C-4′), 133.59 (C-1′), 129.32 (C-2′, C-6′), 114.82 (C-3′, C-5′), 63.54 (C-1), 34.39 (C-3), (C-2″, C-2, C-4″),(31.95, 31.28, 30.46), 29.72–29.20 (C-4″, C-22″), 25.05 (C-3″), 22.71 (C-23″), 14.13 (C-24″), 137.19, 128.56, 127.90, 127.45, 70.05 (benzyl).

Synthetic 3-(4-*hydroxyphenyl*)*propyl tetracosanoate* (**1**). 3-(4-benzyloxyphenyl) Propyl tetracosanoate (100 mg, 0.17 mmol) dissolved in EtOAc (50 ml) was hydrogenated at 7 atm over 10% Pd-C at 25° for 3.5 hr. The reaction mixt. was filtered and evapd to dryness. The resulting product was recrystallized from petrol to give **1** as shining needles (73 mg, 95% yield), mp 73–74°. All spectral data were identical to those for the natural product.

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6′-*O*-FERULOYL- AND 6′-*O*-SINAPOYL-DEMETHYLALANGISIDES, TETRAHYDROISOQUINOLINE-MONOTERPENE GLUCOSIDES FROM *ALANGIUM PLATANIFOLIUM*

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Key Word Index—*Alangium platanifolium*; Alangiaceae; leaves; tetrahydroisoquinoline-monoterpene glucosides; demethylalangiside; 6′-*O*-feruloyl-demethylalangiside; 6′-*O*-sinapoyl-demethylalangiside.

Abstract—From the leaves of *Alangium platanifolium* var. *trilobum*, two new tetrahydroisoquinoline-monoterpene glucosides, 6′-*O*-feruloyl-demethylalangiside and 6′-*O*-sinapoyl-demethylalangiside, were isolated along with demethylalangiside. Their structures were determined by spectroscopic methods.

INTRODUCTION

Alangium platanifolium Harms var. *trilobum* Ohwi is a deciduous shrub which is distributed throughout northeast Asia, including Japan. Recent phytochemical studies of this plant resulted in the isolation of several flavonoid glycosides, phenolic compounds, iridoid glucosides and xyloglucosides of some alcohols [1–3]. We investigated the constituents of the leaves of *A. platanifolium* var. *trilobum* because of our interest in nitrogenous glycosides, since alangiside (**1**), a tetrahydroisoquinoline-monoterpene glucoside, as well as several ipecac alkaloids had been isolated from an Indian medicinal plant, *A. lamarckii* Thwaites in the same family [4]. We now report the isolation and characterization of two new tetrahydroisoquinoline-monoterpene glucosides.

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RESULTS AND DISCUSSION

Dried leaves of *A. platanifolium* var. *trilobum* were extracted with hot methanol. The extract was separated by a combination of chromatographic procedures to yield three tetrahydroisoquinoline-monoterpene glucosides (**2–4**).

Compound **2** was isolated as needles, mp 180–182°, $[\alpha]_D^{27}$ −76°. It showed an IR band at 1654 cm^{-1} and a ^1H NMR signal at δ7.40 (*d*, J=2.5 Hz), suggesting the presence of the β-alkoxyacrylic amide chromophore. Its ^1H NMR spectrum exhibited signals for two acetal protons at δ4.69 (*d*, J=8.0 Hz) and 5.49 (*d*, J=1.5 Hz), a