## EXPERIMENTAL

*Schefflera octophylla* (Lour.) Harms was identified by Dr. Ph. N. Nguyen, Institute of Biology, National Research Centre of the SRV, Hanoi, and a voucher specimen is kept there.

*Extraction and separation.* Air-dried powdered bark (190 g) of *S octophylla* was extracted with petrol in a Soxhlet for 8 hr. Further extraction with EtOH (24 hr) yielded 10.5 g ethanolic extract. During the ethanolic extraction a yellow precipitate (103 mg) settled down which was separated The precipitate was purified by preparative TLC (petrol–$CHCl_3$, 1:1) yielding **2**.

*Alkaline hydrolysis of* **2**. Compound **2** (100 mg) was boiled with 10 ml 5% KOH in MeOH for 14 hr. The soln was evaporated to dryness and the residue, after addition of $H_2O$, extracted with $CHCl_3$ to remove the triterpene. The triterpene was identified by its MS, $^1$H NMR, IR and TLC data, as well as by direct comparison, as 3α-hydroxy-lup-20(29)-ene-23,28-dioic acid (**1**) [1]. The alkaline soln was acidified with HCl and extracted with EtOAc to remove the fatty acids The soln was evaporated to dryness and esterified with $CH_2N_2$ in $C_6H_6$. The methyl esters were investigated by GC (steel column 2.0 m × 4 mm, 3% SE 30, gaschrom Q 125–150 μm, temperature programming 190–280°, 2°/min, $N_2$ at 21 ml/min) and GC/MS (3% SE 30, temperature programming 205–250°, 2°/min, He at 20 ml/min, 80 eV). The residue from the ethanolic extract was separated by CC on silica gel with $CHCl_3$–EtOAc gradient elution and TLC-monitoring ($CHCl_3$–EtOAc–AcOH, 9:1:0.5). Compound **1** [1] (0.7%) was identified by $^1$H NMR, MS, IR, $[\alpha]_D$ and TLC data, as well as direct comparison with an authentic sample. Using MeOH as eluent a triterpene glycoside mixture (2.9 g) was isolated.

*Acidic hydrolysis of the triterpene glycoside mixture.* The triterpene glycoside mixture (2.9 g) was boiled with 50 ml 1 N HCl in MeOH for 4 hr. The soln was evaporated to dryness and the residue, after addition of $H_2O$, extracted with EtOAc. The EtOAc-phase was dried over $Na_2SO_4$ and concd. The triterpene components were separated by CC on silica gel with gradient elution ($CHCl_3$–EtOAc) and TLC monitoring ($CHCl_3$–EtOAc–AcOH, 9:1:0.5). Oleanolic acid [4], (0.1%) and 3α-hydroxy-lup-20(29)-ene-23,28-dioic acid [1] (0.2%) were identified by MS, $^1$H NMR, IR and TLC data, as well as by comparison with authentic samples.

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# TRITERPENE CONSTITUENTS OF *CALTHA PALUSTRIS**

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**Key Word Index**—*Caltha palustris*; Ranunculaceae; palustrolide; 3β,23-dihydroxylupan-13β→28-lactone; hederagenin; 16,17-dihydroxykauran-19-oic acid; hederagenic acid.

**Abstract**—The structure of a new triterpene lactone, palustrolide, has been elucidated as 3β,23-dihydroxylupan-13β→28 lactone on the basis of physico-chemical studies. In addition, sitosterol, its glucoside, hederagenin, 16,17-dihydroxykauran-19-oic acid and hederagenic acid have been characterized.

## INTRODUCTION

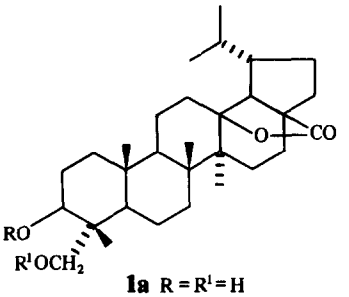
In a preceding paper [1] we described the isolation and structure elucidation of two new 24-norlupane lactones along with the isolation of the chemical constituents from the chloroform-soluble fraction of the alcoholic extract of *Caltha palustris*. The present paper describes the characterization of substance B as sitosterol, substance G as hederagenic acid, substance J as hederagenin, substance K as 16,17-dihydroxykauran-19-oic acid, substance L as sitosterol-β-D-glucoside and the structure elucidation of a new triterpene lactone (substance H), named as palustrolide.

*CDRI Communication No. 3447.

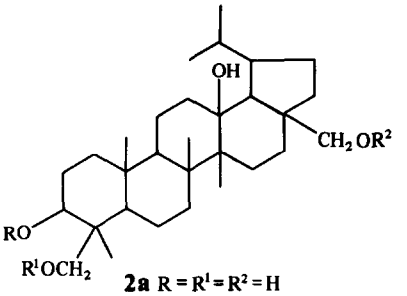
## RESULTS AND DISCUSSION

Palustrolide, $C_{30}H_{48}O_4$, gave positive colour tests for a triterpenoid. The IR absorptions at 3300 and 1760 $cm^{-1}$ suggested it to be a hydroxy-γ-lactone. The acetylation of palustrolide afforded a diacetate whose $^1$H NMR spectrum displayed signals for six methyls in the region δ0.78–1.20, two acetoxymethyls at 1.95 and 1.98 and the corresponding carbinolic protons as an AB*q* ($J$ = 12 Hz) at 3.72 for a primary acetoxymethylene group and the signal for a methine geminal to an acetoxy group appeared as a *dd* ($J$ = 10, 6 Hz) at δ4.7 which confirmed one primary and one secondary hydroxyl group in the molecule.

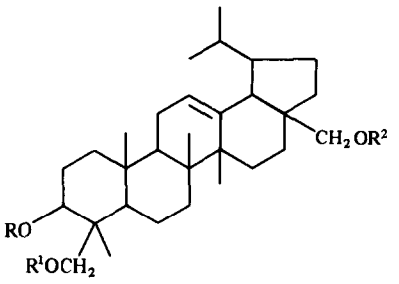
Its mass spectrum showed major fragments at *m/z* 236, 223, 218, 205 (corresponding ions at *m/z* 320, 307, 260 and



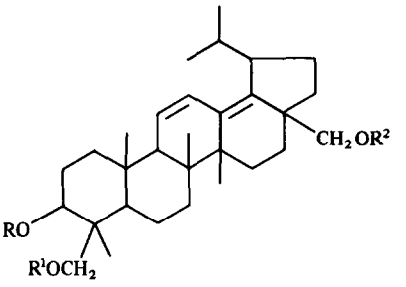
**1a** R = R¹ = H
**1b** R = R¹ = Ac
**1c** R = R¹ = C(Me₂)



**2a** R = R¹ = R² = H
**2b** R = R¹ = R² = Ac



**3** R = R¹ = R² = Ac



**4** R = R¹ = R² = Ac

247 in its diacetate) comprising of rings A and B, characteristic of lupane skeleton [2]. The fragmentation pattern established that the primary and secondary hydroxyl groups were located in rings A and B and also restricted the location of the lactone function to rings C, D and E.

In the $^1$H NMR spectrum of palustrolide diacetate, the most downfield signal in the methyl region at $\delta$ 1.20 was assigned to the C-26 methyl [3] due to the strong diamagnetic anisotropic effect of the lactone group at C-28 while the C-27 methyl appeared as a singlet at $\delta$ 1.12. The isopropyl group methyls, borne on an asymmetric carbon atom do not give a doublet but appeared as a singlet. The stereochemistry of the hydroxyl at C-3 was deduced as $\beta$ (equatorial) [4] from the $^1$H NMR spectrum of the diacetate since the carbinolic proton appeared as a double doublet ($J_{aa}$ = 10 Hz, $J_{ae}$ = 6 Hz) at $\delta$ 4.7.

Palustrolide furnished a monoacetonide which confirmed the presence of a primary hydroxyl group at the C-23 position. Palustrolide diacetate was reduced with lithium aluminium hydride to a tetrol **2a** whose IR spectrum was devoid of carbonyl absorption. Its triacetyl derivative **2b** still showed absorption at 3480 cm$^{-1}$ in the IR spectrum which indicated the presence of a tertiary hydroxyl group. Its $^1$H NMR spectrum exhibited signals for acetoxymethyls at $\delta$ 2.0 (6H) and at 2.04 (3H) and the corresponding carbinolic methylenes as a 2H quartet at 3.74 ($J$ = 12 Hz), an AB $q$ at 4.13, 4.7 (each $d$, $J$ = 12 Hz) and a methine triplet at 4.74.

The triacetate **2b** yielded a dehydrated product **3** with $BF_3$-etherate which gave a yellow colour with TNM and showed an IR band for a trisubstituted double bond which was confirmed by the appearance of a broad singlet in the $^1$H NMR spectrum at $\delta$5.13. Its mass spectrum showed $[M]^+$ at $m/z$ 584 and the base peak at $m/z$ 203 arising from retro-Diels–Alder cleavage further confirmed the presence of a tertiary hydroxyl group generated by reductive cleavage of the lactone ring at the C-13 position. The dehydrated product **3** on selenium dioxide oxidation yielded a heteroannular diene **4** whose UV spectrum showed maxima at 243, 252 and 262 nm which confirmed the presence of double bond at C-12(13). Thus, the structure of palustrolide was elucidated as 3$\beta$,23-dihydroxy-lupan-13$\beta$ → 28 lactone (**1**).

## EXPERIMENTAL

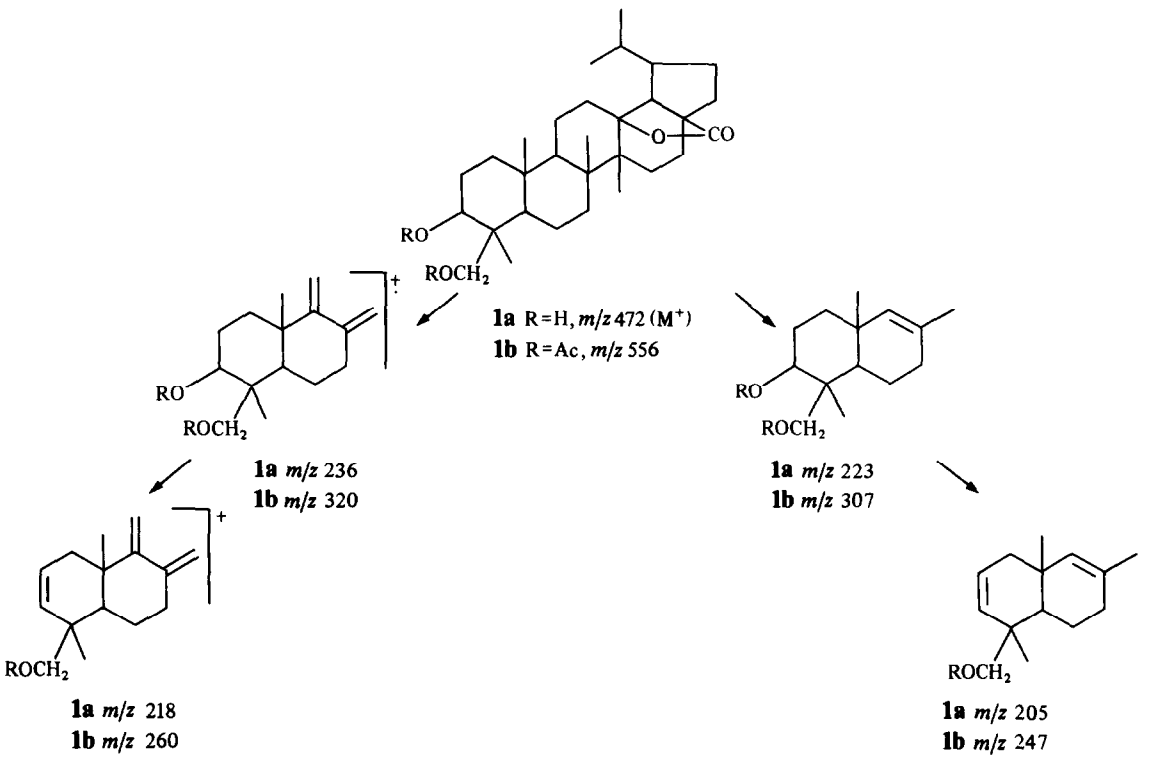
Mps are uncorr. The $^1$H NMR spectra were recorded in $CDCl_3$, unless otherwise stated, with TMS as an internal standard.

*Substance H (palustrolide).* Colourless needles from MeOH, mp 310° dec. $\nu_{max}^{KBr}$ cm$^{-1}$: 3300 (OH), 2920, 1760 ($\gamma$-lactone), 1470, 1450, 1396, 1370, 1230 and 1062. MS $m/z$: 472 $[M]^+$, 454 $[M-18]^+$, 436, 424, 409, 395, 381, 356, 236, 223, 218 (base peak), 205, 203, 191, 189, 187, 175, 173, 147, 145 and 133.

*Palustrolide acetate.* Acetylation of **1** ($Ac_2O$–$C_5H_5N$) overnight at room temp. afforded a diacetate which crystallized from MeOH, mp 240°. $\nu_{max}^{KBr}$ cm$^{-1}$: 2920, 1760, 1740; $^1$H NMR: $\delta$ 0.78 (6H, $s$, 2Me), 0.84, 0.9, 1.12, 1.20 (3H each, $s$, 4Me), 1.95, 1.98 (3H each, $s$, 2-OCOMe), 3.72 (2H, AB$q$, $J$ = 12 Hz, C-23-$CH_2$OAc), 4.7 (1H, $dd$, $J_{aa}$ = 10 Hz, $J_{ae}$ = 6 Hz, C-3-CHOAc); MS $m/z$: 556 $[M]^+$, 496 $[M-AcOH]^+$, 481, 436 $[M-2AcOH]^+$, 421, 368, 356, 320, 307, 260, 247, 235, 200, 189, 187, 173, 159, 145 and 133.

*Palustrolide acetonide.* Substance H on keeping overnight in dry $Me_2CO$ (10 ml) containing one drop of conc. $H_2SO_4$ at room temp. yielded an acetonide derivative, colourless needles (MeOH), mp 266°. $^1$H NMR: $\delta$ 0.76, 0.83, 0.86, 0.9 (3H each, $s$, 4Me), 1.1 (3H, $s$, Me), 1.2 (6H, $s$, 2Me), 1.3 (3H, $s$, Me), 3.26 (2H, $br\ s$, –$CH_2$OH), 4.0 (1H, $m$, –CHOH).

*Reduction of palustrolide acetate.* Palustrolide acetate was




Scheme 1.

refluxed in dry dioxane containing $LiAlH_4$ for 20 hr. Excess of the reagent was decomposed with EtOAc, diluted with $H_2O$ and then extracted with EtOAc The solvent layer gave **2a** as colourless needles (MeOH), mp 286°. $\nu_{max}^{KBr}$ $cm^{-1}$: 3250 (OH), 1450, 1365, 1054 and 1025.

*Acetylation of* $LiAlH_4$*-reduced product.* Compound **2a** reacted with $Ac_2O$–$C_5H_5N$ at room temp. to afford a triacetyl derivative **2b** as a colourless powder. $\nu_{max}^{KBr}$ $cm^{-1}$. 3480 (OH), 1740; $^1H$ NMR: $\delta$ 0.83 (3H, *s*, Me), 0.90 (9H, *s*, 3Me), 1.08, 1.14 (3H each, *s*, 2Me), 2.0 (6H, *s*, 2-OCOMe), 2.04 (3H, *s*, OCOMe), 3.74 (2H, AB*q*, $J$ = 12 Hz, C-23-$CH_2OAc$), 4.13, 4 7 (1H each *d*, $J$ = 12 Hz, C-28 $CH_2OAc$), 4.74 (1H, *t*, $J$ = 8 Hz, CHOAc).

*Dehydration of* **2b**. Substance **2b** (25 mg) in dry $C_6H_6$ containing $BF_3$-etherate (0.5 ml) was stirred at room temp. for 15 min The reaction mixture was diluted with $H_2O$, extracted with EtOAc which yielded a residue, crystallized from MeOH (**3**) mp 136° $\nu_{max}^{KBr}$ $cm^{-1}$: 2920, 1740, 1365 and 840; $^1H$ NMR: $\delta$ 0.79 (3H, *s*, Me), 0.87–0.96 (9H, *quadruplet*, 3Me), 1.10 (3H, *s*, Me), 1.2 (3H, *s*, Me), 3.55–4.1 (4H, *m*, $2CH_2OAc$), 4.72 (1H, *t*, $J$ = 8 Hz, –CHOAc), 5.13 (1H, *br s*, –C=CH); MS *m/z*: 584 $[M]^+$, 569, 524, 464, 307, 276, 247 and 203 (base peak).

$SeO_2$ *oxidation of dehydrated product* (**3**) Compound **3** (12 mg) was refluxed in glacial AcOH containing freshly sublimed $SeO_2$ for 2 hr. After removal of AcOH *in vacuo*, the residue was dissolved in $Et_2O$, filtered and the solvent layer yielded an amorphous powder of **4**. $\lambda_{max}^{MeOH}$ nm: 243, 252 and 262

*Substance G* (*hederagenic acid*). Mp 228°; $C_{30}H_{46}O_4$. $\nu_{max}^{KBr}$ $cm^{-1}$: 3410, 2920, 1715 (COOH), 1698 (C=O), 1460, 1370, 1365, 1044, and 830; $^1H$ NMR: $\delta$ 0.80, 0.83, 0.93, 1.06 (3H each, *s*, 4Me), 1.17 (6H, *s*, 2Me), 3.42 (2H, AB*q*, $J$ = 12 Hz, $CH_2OH$), 5.2 (1H, *br s*, –C=CH). Methyl ester· mp 217°. $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1728 (COOMe), 1694; $^1H$ NMR: $\delta$ 0.70, 0.80, 0.86, 0.93 (3H each *s*, 4Me), 1.06 (6H, *s*, 2Me), 3.4 (2H, *m*, $-CH_2OH$), 3.5 (3H, *s*, COOMe), 5.23 (1H, *br s*, –C=CH); MS *m/z*: 484 $[M]^+$, 454, 425, 407, 399, 339, 262, 249, 203 (base peak), 189 and 133. Methyl ester acetate; $^1H$ NMR: $\delta$ 1.96 (3H, *s*, OCOMe), 3.58 (3H, *s*, COOMe), 4.01 (2H, *br s*, $-CH_2OAc$), 5.20 (1H, *br s*, –C=CH)

*Substance J* (*hederagenin*). Mp 315°; $C_{30}H_{48}O_4$; $\nu_{max}^{KBr}$ $cm^{-1}$. 3240 (OH), 2900, 1690 (COOH), 1460, 1384, 1264, 1230, 1188, 1090, 1038, 960 and 920 MS *m/z*. 472 $[M]^+$, 454, 436, 424, 408, 385, 381, 248 (base peak), 223, 203, 189, 175 and 161. Diacetate, mp 170°; $^1H$ NMR: $\delta$ 0 68, 0.78, 0.82, 0.86, 0.9, 1.05 (3H each, *s*, 6Me), 1.94, 1.96 (3H each *s*, 2-OCOMe), 4.68 (1H, *t*, $J$ = 8 Hz, –CHOAc), 5.16 (1H, *br s*, –C=CH).

*Lactonization of hederagenin diacetate.* Accomplished with $CHCl_3$–HCl (gas); mp 238°; $^1H$ NMR: $\delta$ 0 78, 0 83, 0.89, 0.94, 1.0 and 1.11 (3H each *s*, 6Me), 1.95, 1.98 (3H each, *s*, 2OCOMe), 3 72 (2H, AB*q*, $J$ = 12 Hz, $-CH_2OAc$), 4.7 (1H, *dd*, $J$ = 10 Hz, 6 Hz, –CHOAc). MS *m/z*· 496 $[M - AcOH]^+$, 481, 452, 436, 421, 392, 369, 307, 262, 260, 248, 234, 203 and 189.

*Substance K* (16α,17-*dihydroxykauran*-19-*oic acid*). Mp 260°; $C_{28}H_{32}O_4$; $\nu_{max}^{KBr}$ $cm^{-1}$. 3350 (OH), 2900 and 1690 (COOH), Methyl ester; mp 145°; $\nu_{max}^{KBr}$ $cm^{-1}$. 3150, 2800, 1720 (COOMe) $^1H$ NMR: $\delta$ 0.66, 0.82 (3H each, *s*, 2Me), 2.63 (2H, *br s*), 3.58 (3H, *s*, –COOMe). MS *m/z*· 350 $[M]^+$, 332, 319, 300, 273, 259 and 241. Methyl ester acetate; mp 106°; $\nu_{max}^{KBr}$ $cm^{-1}$. 2900, 1740, 1720; $^1H$ NMR. $\delta$ 0.70, 0.84 (3H each, *s*, 2Me), 2.02 (3H, *s*, OCOMe), 3.59 (3H, *s*, COOMe), 4.15 (2H, *s*, $-CH_2OAc$).

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# LATEX EXTRACTABLES OF *CALOTROPIS GIGANTEA*

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**Key Word Index**—*Calotropis gigantea*; Asclepiadaceae; latex; 3′-methylbutanoyl, acetyl and free α-amyrin, β-amyrin, ψ-taraxasterol, taraxasterol and lupeol; alkanes.

**Abstract**—The hexane and methanol soluble extract of the latex coagulum of *Calotropis gigantea* afforded two new triterpene esters, *viz.* 3′-methylbutanoates of α-amyrin and ψ-taraxasterol, besides the known 3′-methylbutanoates of three triterpene alcohols. The compositions of the alkane fraction, total triterpene alcohol fraction, and free, acetyl and 3′-methylbutanoyl triterpene alcohol fractions of the extract were determined.

## INTRODUCTION

The milk-weed family (Asclepiadaceae) is very rich in latex bearing plants and a number of species have been investigated for the constituents of solvent extractables [1]. Although the leaves and root-bark of *Calotropis gigantea* have previously been examined [2–4], no attempt has yet been made to characterize the latex constituents of this plant. We have, therefore, undertaken here the detailed analysis of the latex constituents of *C. gigantea*.

## RESULTS AND DISCUSSION

The coagulum of *C. gigantea* latex was extracted with hexane and methanol. The hexane extract was subjected to chromatography over silica gel which afforded a hydrocarbon fraction (2.5% of the hexane extract). The GLC analysis of the fraction showed the predominance of $C_{14}$, $C_{16}$ and $C_{18}$ alkanes with a consequently higher even : odd ratio (3 : 1). This is interesting since the alkane profile in cuticular lipids usually display a high odd : even ratio with maxima of $C_{29}$, $C_{31}$ and $C_{33}$. The hexane and methanol extracts were combined and a portion of the hydrocarbon free mixture from the combined extracts was hydrolysed and subjected to silica gel TLC which afforded one major band corresponding to 3β-monohydroxy triterpene. The fraction from this band was acetylated and the composition was determined by GLC as shown in Table 1. The following five triterpenes were identified by GLC and GC/MS as the acetates (3β-OAc = **b**): α-amyrin (5α-urs-12-en-3β-ol, **1b**), β-amyrin (5α-olean-12-en-3β-ol, **2b**), ψ-taraxasterol (5α-taraxast-20-en-3β-ol, **3b**), taraxasterol [5α-taraxast-20(29)-en-3β-ol, **4b**], and lupeol [5α-lup-20(29)-en-3β-ol, **5b**].

The remaining portion of the hydrocarbon free triterpene mixture was separated into four major bands (referred to as bands 1–4 in order of polarity, beginning with the least polar) on silica gel TLC. Co-chromatographic studies with authentic triterpene acetate (**4b**) and free triterpene alcohol (**4a**; 3β-OH = **a**) indicated the occurrence of such components in bands 3 ($R_f$ 0.58) and 4 (0.15), respectively. The IR spectra showed that the fractions from bands 1 ($R_f$ 0.79), 2 ($R_f$ 0.72) and 3 were the mixtures of esterified components ($\nu_{max}$ = 1720–1730 $cm^{-1}$). The components from band 2 (*ca* 30%) showed very long retention times on GLC, and GC/MS analysis revealed that the major five components were the esters (3β-$OCOC_4H_9$ = **c**) of $C_5$-saturated fatty acids with five triterpene alcohols (*m/z* 510, $[M]^+$; *m/z* 408, $[M - C_5H_{10}O_2]^+$), (**1c–5c**). The composition of this fraction was determined by GLC and is shown in Table 1. The fraction was further fractionated by silver nitrate–silica gel TLC which afforded four bands (referred to as bands 2-1 to 2-4 in order of polarity, beginning with the least polar). Band 2-1 ($R_f$ 0.89) gave a mixture of the pentanoic acid esters of α-amyrin (**1c**) and β-amyrin (**2c**). The fractions from bands 2-2 ($R_f$ 0.69), 2-3 ($R_f$ 0.57) and 2-4 ($R_f$ 0.50) were the pentanoic acid esters of ψ-taraxasterol (**3c**), taraxasterol (**4c**) and lupeol (**5c**), respectively. The pentanoic acid esters of **3c** and **4c** showed the methylene multiplet at δ2.17 (2H, H-2′), deshielded by the adjacent carbonyl group, and the isopropyl doublet at δ0.96 (6H, *J* = 6 Hz, H-4′, H-5′) in the $^1H$ NMR spectra besides the signals arising from the triterpene moieties which suggested that the pentanoic acid moiety was 3-