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QUERSPICATINS A AND B, TWO PENTACYCLIC TRITERPENES FROM *QUERCUS SPICATA*†

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Key Word Index—*Quercus spicata*; Fagaceae; pentacyclic triterpenes; querspicatin A; querspicatin B; lupeol; hexacosanic acid; ^{1}H NMR; ^{13}C NMR; MS.

Abstract—Two new triterpenes designated querspicatin A and querspicatin B have been isolated from the bark of *Quercus spicata*. They have been shown to be 3β-(3′,4′-dihydroxycinnamoyloxy)-9α-hydroxy-7-oxo-lup-20(30)-ene and 3β,9α-dihydroxy-7-oxo-lup-20(30)-ene on the basis of their ^{1}H NMR, ^{13}C NMR and mass spectral data and chemical evidence.

INTRODUCTION

Earlier we reported [2, 3] the isolation of a variety of triterpenes of different skeletons from *Quercus suber*. The related species *Q. spicata* Smith, (syn. *Lithocarpus spicata* Smith) is a moderately tall tree [4, 5] (~8 m) growing throughout Nepal. The stem bark is used in the hilly areas in the treatment of diarrhoea and dysentery; it has hypothermial and hypoglycemic properties. [6].

This bark was collected from a hilly forest near Kathmandu, Nepal, above 2700 m, and was identified at the Herbarium Centre, Department of Medicinal Plants, Kathmandu, Nepal. The isolation of two new triterpenes from this material and the spectral and chemical evidence leading to the elucidation of their structures and stereochemistry are discussed in this paper.

RESULTS AND DISCUSSION

The chloroform extract of the defatted bark of *Q. spicata* afforded by chromatography, a new pentacyclic triterpene designated querspicatin A (**1**) (0.0026% yield), $C_{39}H_{54}O_6$; positive LB test; violet colouration with ferric chloride (phenolic OH); yellow colouration with TNM (C=C); IR ν_{max}^{KBr} cm^{-1}: 3540–3300 (*br*, OH), 1710 (*sh*) (6-membered ring C=O) and 1690 (conjugated ester C=O) 1370 (*gem* dimethyl), 1190 (ester C–O), 975 (*trans* CH=CH) and 880 (C=CH$_2$); UV λ_{max}^{EtOH} 246 nm (log ε 3.99), shifted to 274 nm (log ε 3.8) in alkali (phenolic OH groups).

The ^{1}H NMR (270 MHz, d_6-Me$_2$CO) spectrum of **1** (Table 1) indicated the presence of (i) six tertiary methyls on saturated carbons, (ii) one isopropylidene group, –C(Me)=CH$_2$, [δ1.7, 3H, *br s*, vinyl Me; δ4.58 and 4.73, each 1H, *br d*, J = 2.5 Hz, two geminal olefinic protons)], (iii) one alcoholic OH (exchangeable with D$_2$O), (iv) one deshielded methine proton on C-3 bearing OCOR (δ4.50–4.58, *m*, partially obscured by H-30, (v) a *trans*-disubstituted double bond (δ6.29, 1H, *d*, H$_A$; δ7.53, 1H, *d*, H$_B$; $J_{A,B}$ = 16 Hz, *trans*-Ar–CH=CH–COO–), (vi) a 1,2,4-trisubstituted benzene moiety (δ7.04, 1H, *dd*, J = 8.1 and 2 Hz, H-6′; δ6.85, 1H, *d*, J = 8.1 Hz, H-5′; δ7.15, 1H, *d*, J = 2 Hz, H-2′) and (vii) two phenolic hydroxyl groups (δ8.22 and 8.50 each 1H, *s*, exchangeable with D$_2$O, 3′-OH and 4′-OH respectively). The multiplets at δ3.14 ($W_{1/2}$ ~ 9 Hz) and 2.2 ($W_{1/2}$ ~ 15 Hz) were assigned to the equatorial and axial protons at C-6 [7] respectively. The presence of two phenolic hydroxyl groups was further confirmed by the formation of the diacetate **2** showing a 6H singlet at δ2.32 (two OAc's). The presence of the 3′,4′-dihydroxycinnamoyl moiety was confirmed by decoupling experiments. Irradiation of H-6′ caused each of H-2′ and H-5′ to appear as a singlet, whereas, irradiation on one vinylic proton led to the collapse of the other vinylic proton to a broad singlet and vice versa.

Querspicatin A (**1**) on alkaline hydrolysis afforded the ketodiol **3**, [M]$^+$ 456; ^{1}H NMR (100 MHz, CDCl$_3$): δ3.15 (2H, *m*, H-3 and H-6 eq). Acetylation gave the monoacetate **4**, ^{1}H NMR (100 MHz, CDCl$_3$): δ4.48 (1H, *t*, J = 8 Hz, H-3), 3.00 (1H, *m*, H-6 eq) and 2.04 (3H, *s*, –OAc). The other product of hydrolysis of **1** was identified as 3,4-dihydroxycinnamic acid (caffeic acid).

The formation of only the monoacetate, even under refluxing conditions, indicated that one of the two hydroxyl groups (as evident from the mass spectrum) in **3** is attached to an angular position.

The nature of the carbon frame-work of **1** and its derivatives and the location of the oxygen functions in the A/B rings were largely established from their general mass fragmentation behaviour (Scheme 1). The presence of the non-oxygenated fragment at *m/z* 189 (73.7, 64.9 and 100% in **1**, **2** and **3** respectively) with rings D and E intact supported such a conclusion. Although neither **1** nor **2** showed [M]$^+$, other significant and diagnostic ion peaks appeared at *m/z* 189 (characteristic of lupene skeleton [8, 9]), 248, 203 and 163 (247 from **2**), the genesis of which

†Part 29 in the series 'Terpenoids and Related Compounds'. For part 28 see ref. [1].

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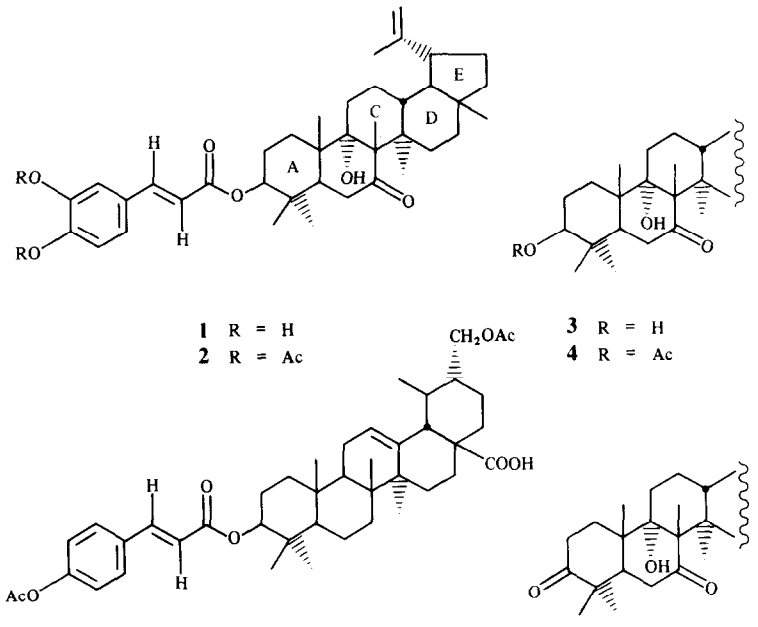

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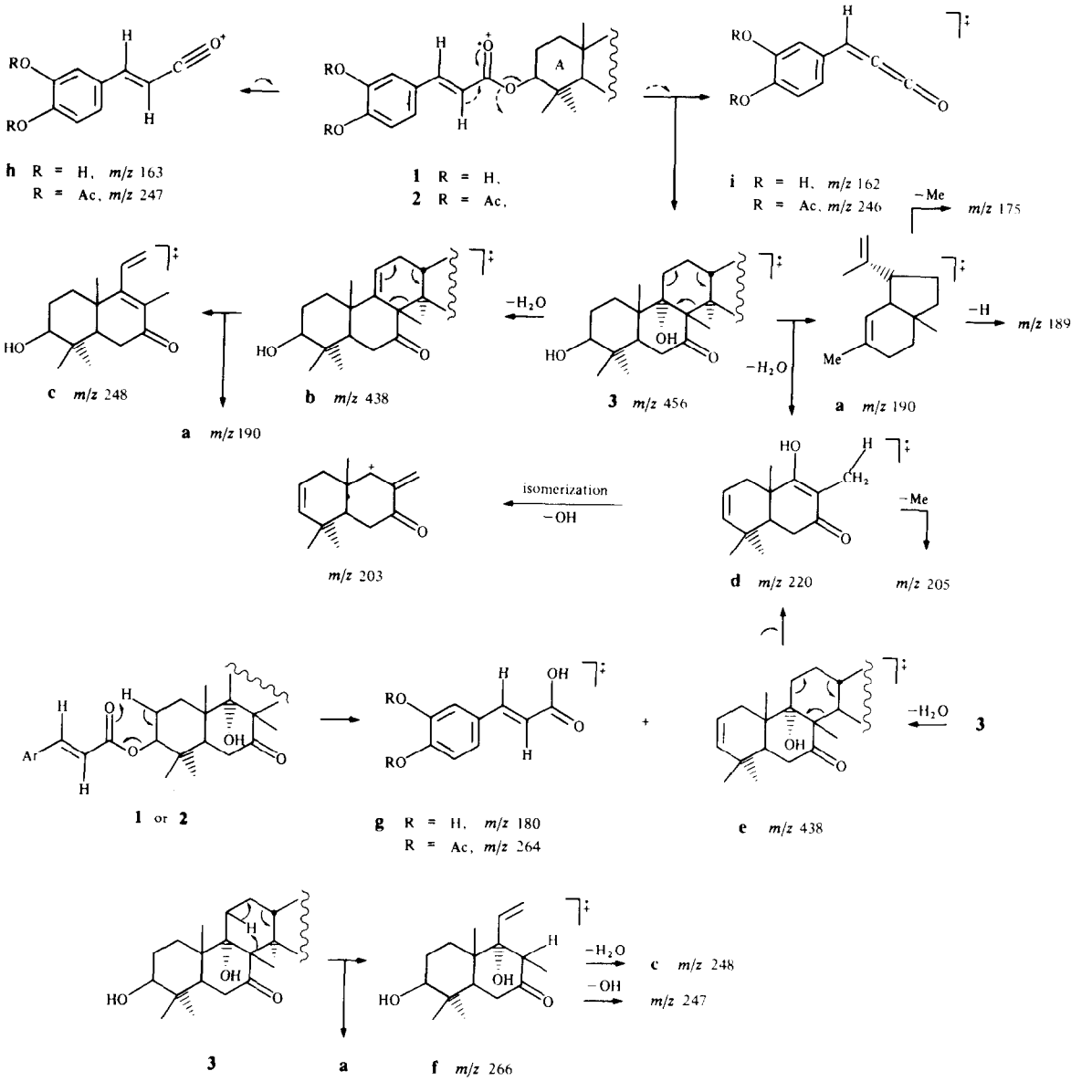
Table 1. ^{1}H NMR chemical shifts (δ, $CDCl_3$) of querspicatin A (**1**), querspicatin B (**3**) and their derivatives

H	1†	2	3	4	5
H-3	4.50–4.58 *m* 18 Hz* (partially obscured by H-30)	4.58–4.72 *m*	3.15 *m*, 18 Hz*	4.48 *m*, 15 Hz*	—
H-6eq	3.14 *m*, 9 Hz*	3.02 *m*		3.0 *m*, 14 Hz*	3.03 *m*, 8 Hz*
H-6ax	2.2 *m*, 15 Hz*	2.12 *m*	2.24 *m*, 14 Hz*	2.24 *m*, 22 Hz*	2.33 *m*, H-6ax and H-2eq, 18 Hz*
H-19	2.28 *m*	2.3 *m*	2.32 *m*	2.32 *m*	2.22 *m*, H-19 and H-2ax, 16 Hz*
H_A-30	4.58 *d*; 2.5 Hz	4.60 *br s*	4.64 *br s*	4.64 *br s*	4.6 *s*
H_B-30	4.73 *d*; 2.5 Hz	4.70 *br s*	4.74 *br s*	4.75 *br s*	4.6 *s*
CH_2's and CH's	1.2–2.1 *m*	1.30–2.0 *m*	1.20–2.05 *m*	1.26–2.0 *m*	
Me-29	1.70 *s*	1.79 *s*	1.70 *s*	1.70 *s*	1.67 *s*
Tertiary Me's	1.05, 0.98, 0.94, 0.93, 0.92, 0.88	0.84 *br s* (6H) 0.85, 0.88, 1.0 (6H)	0.96 (6H) 0.94 (6H) 0.84, 0.76	0.90, 0.88 0.84 0.82 (9H)	0.90, 0.96 (6H), 0.95, 0.99, 1.04
		Additional ^{1}H NMR chemical shifts			
	1	**2**			**4**
	7.15 *d*, 2 Hz, H-2′	2.32 6H, *s*, OAc			2.08 *s*, 3-OAc
	7.04 1H, *dd*, 8, 2 Hz, H-6′	6.4 *d*, 16 Hz, Hα			
	6.85 1H, *d*, 8 Hz, H-5′	7.65 *d*, 16 Hz, Hβ			
	7.53 *d*, 16 Hz, Hβ	7.29 *d*, 8 Hz, H-5′			
	6.29 *d*, 16 Hz, Hα	7.48 *br s*, H-2′			
	8.22 *s*, C3′-OH	7.41 *d*, 8 Hz, H-6′			
	8.50 *s*, C4′-OH				

*Approximate $W_{1/2}$.
†d_6-Me_2CO.
1: 270 MHz, **2**–**4**: 100 MHz, **5**: 300 MHz.

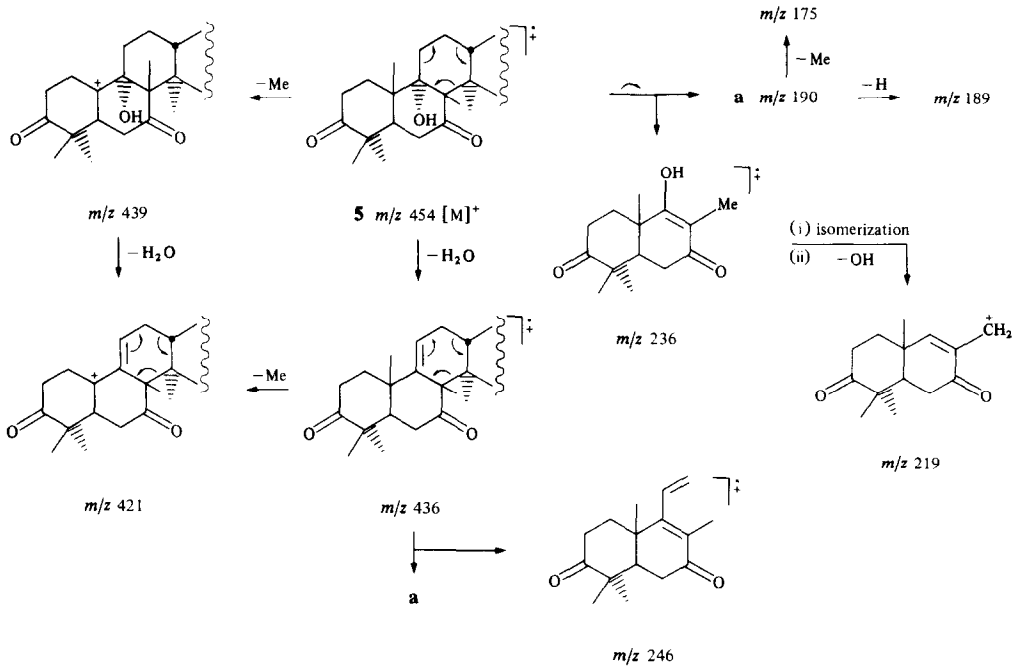
and other ion peaks are rationalized in Scheme 1. The retro-Diels–Alder type fission of ring C of the $[M]^+$ of **3** (*m/z* 456), which is also observed for **1** or **2**, produced the ion peak at *m/z* 189 (after H loss) and subsequent loss of water (*m/z* 220) and methyl from ring A yielded the fragment ion at *m/z* 205 indicating the presence of OH at C-9. Similarly ion *b* at *m/z* 438 also underwent retro-Diels–Alder fission of ring C to produce the fragment ions



Scheme 1. Significant ions in mass fragmentations of compounds **1**–**3**.

at *m/z* 248 and 190. McLafferty rearrangement of **1** and **2** produced fragment ions at *m/z* 180 or 264 and 438; the latter underwent successive loss of methyl and carbonyl, as expected. Hydrogen transfer from C-11 to C-8 was followed by loss of water forming the fragment ion at *m/z* 248. The fragments containing hydroxyl, carbonyl and a double bond underwent loss of water, carbon monoxide and allylic methyl to give the corresponding ions. The above fragmentations support the presence of two OH's at C-3 and C-9 and an oxo group at C-7 in **3**. The foregoing ^{1}H NMR and mass spectral evidence thus confirmed **3** as 3β,9α-dihydroxy-7-oxo-lup-20(30)-ene and hence querspicatin A as 9α-hydroxy-7-oxo-lup-20(30)-ene-3β-yl caffeate (**1**).

The ketodiol **3**, gave a negative Zimmermann test for 3-oxo triterpenes [10]. However, upon chromic oxide oxidation it yielded the hydroxydiketone **5** which responded to the test, thus suggesting the presence of 3-OH group in **3**. The failure of **5** to give any characteristic colour with ferric chloride excluded the presence of a diosphenol or a β-diketo system and thus confirmed the absence of a keto group in ring-A in **3**. The ^{1}H NMR of **5** gave four signals at δ3.03, (1H, *m*, H-6eq), 2.33 (2H, *m*, H-6ax and H-2eq), 2.21 and 2.38 (1H *m* each, H-2ax and H-19). The mass spectrum of **5** (Scheme 2) fully supported its assigned structure. Thus retro-Diels–Alder collapse of ring C followed by H or Me loss of the fragment retaining rings D and E formed fragment ions at *m/z* 190, 189 or 175. The other fragment, retaining rings A and B, underwent isomerisation and loss of OH to form the ion peak at *m/z* 219, which underwent successive loss of 2CO. In addition, significant ion peaks were observed at *m/z* 439 [M



Scheme 2. Significant ions in mass fragmentation of compound **5**.

$-Me]^+$, 421 [439 $-H_2O$], 393 [421 $-CO$] and 365 [393 $-CO$]. The hydroxydiketone was thus confirmed as 9α-hydroxy-lup-20(30)-ene-3,7-dione (**5**).

The ^{13}C NMR spectrum (67.5 MHz, d_6-Me_2CO) of **1** showed similar characteristic carbon shifts as lupeol acetate (Table 2) for the lupene part of the molecule [11]- the only differences were for a 7-oxo carbon (δ210.00) instead of the methylene carbon at C-7, a 9-oxygenated carbon (δ69.5), a ketomethylene carbon (δ32.77) at C-6 and a quaternary carbon (δ56.72) at C-8, adjacent to carbonyl, as expected. The substituted cinnamic acid part was similar to that of diacetylrubicoumaric acid (**6**) [12] (Table 3). The latter gave rise to signals for α,β-unsaturated carbons (δ116.2, 145.35), an ester carbonyl (δ167.21) and a 3′,4′-dihydroxycinnamoyl moicty (C-3′ at δ146.33, C-4′ at δ146.94).

The petrol extract of the same plant material on chromatography over silica gel afforded another new triterpene designated querspicatin B (0.0005% yield), $[M]^+$ 456 ($C_{30}H_{48}O_3$), positive LB test for triterpenes; it formed a monoacetate. Careful analysis of the IR and ^1H NMR spectra of querspicatin B and its acetate led to their identity with **3** and **4** respectively which was confirmed by direct comparison of the respective samples. Querspicatin B and its monoacetate are, therefore 3β,9α-dihydroxy-7-oxolup-20(30)-ene (**3**) and its 3β-acetate derivative (**4**) respectively. The petrol extract also afforded lupeol and hexacosanic acid.

EXPERIMENTAL

General. Mps: uncorr; IR: KBr; Petrol: 60–80° fraction; CC: silica gel (60–120 mesh); TLC: microscopic slides coated with silica gel G, similar fractions were combined.

Extraction. Dried and powdered stem bark (3 kg) of *Quercus spicata* Smith was extracted exhaustively in a Soxhlet apparatus

Table 2. ^{13}C NMR chemical shifts (δ, $CDCl_3$) of querspicatin A (triterpene part) and lupeol acetate [12]

C	Lupeol acetate (d_6-Me_2CO)	Querspicatin A (**1**) ($CDCl_3$)
1	38.4	38.67
2	23.7	28.36
3	81.0	80.91
4	37.8	38.96
5	55.4	56.18
6	18.2	32.77
7	34.3	210.00
8	40.9	56.72
9	50.4	69.90
10	37.1	37.52
11	21.0	21.71
12	25.1	24.56
13	38.1	37.89
14	42.9	43.21
15	27.5	26.70
16	35.6	35.04
17	43.0	42.00
18	48.0	49.73
19	48.3	47.91
20	150.9	151.54
21	29.9	30.69
22	40.0	39.10
23	28.0	28.97
24	16.5	15.03
25	16.2	16.61
26	16.0	16.61
27	14.5	14.70
28	18.0	17.05
29	19.3	19.50
30	109.4	109.99

Table 3. ^{13}C NMR chemical shifts of the ester moieties of querspicatin A (**1**) and diacetyl rubicoumaric acid (**6**)

	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	C_α	C_β	Ester C=O
Querspicatin A (**1**)	127.53	115.50	146.3	146.9	116.09	122.37	145.35	116.21	167.21
Diacetyl rubicoumaric acid (**6**)	131.8	128.0	121.8	151.9	121.8	128.0	143.0	118.0	166.3

with petrol and $CHCl_3$ successively for 40 hr each. The extracts were evapd *in vacuo* to afford residues B (12 g) and A (10.5 g) respectively which were chromatographed over silica gel (60–120 mesh) using solvents and solvent mixtures of increasing polarity. Fractions of a similar composition, as indicated by TLC, were combined.

Isolation of querspicatin B (**3**). The petrol–EtOAc (9:1) fractions from silica gel CC of residue B afforded a solid which upon repeated chromatography over silica gel yielded **3** (15 mg), crystallized from petrol–EtOAc, mp 280° $[\alpha]_D+2°$ ($CHCl_3$; *c* 0.4). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2910, 2840 (CH and CH_2), 1700 (6-membered ring C=O) 1480, 1450, 1385, 1370, 1185, 1105, 1040, 1030, 980, 940, 880 (C=CH_2); MS: *m/z* (rel. int): 456 $[M]^+$ (51), 438 (18, **b**/**e**), 423 (11, 438 – Me), 395 (10, 423 – CO), 266 (7, **f**), 248 (59, **c**), 247 (9, **c** – H), 233 (15, 248 – Me), 220 (36, **d**), 215 (11, 248 – Me – H_2O), 205 (18, **d** – Me), 203 (39, **d**–OH), 190 (42, **a**), 189 (100, **a** – H), 187 (29, 203 – H – Me), 175 (34, **a** – Me); other strong ions at *m/z* (rel. int.): 396 (18), 234 (32), 221 (18), 208 (24), 207 (68), 201 (20), 191 (30), 177 (22), and > 30 others (rel. int. > 20%) upto 41 (64). (Found: C, 78.68; H, 10.42. $C_{30}H_{48}O_3$ requires C, 78.94; H, 10.52%).

Querspicatin B monoacetate (**4**). A soln of querspication B (**3**) (4 mg) in pyridine (0.25 ml) was refluxed with Ac_2O (0.35 ml) on a steam bath (4 hr). Usual work-up and crystallization from petrol–$CHCl_3$ gave pure crystals of **4**, mp 250°. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2920, 2860 (CH_2 and CH), 1735 (OAc), 1690 (C=O), 1640, 1450, 1370, 1245, 1025, 980, 880 (C=CH_2).

Isolation of querspicatin A (**1**). The petrol–EtOAc (4:1) eluate fractions from silica gel CC of the residue A afforded a solid residue, which upon repeated chromatography over silica gel yielded pure querspicatin A (**1**), crystallized from Me_2CO–petrol as white microcrystalline powder (80 mg), mp 260°, $[\alpha]_D+13°$ (EtOH; *c* 0.3); IR ν_{max}^{KBr} cm^{-1}: 3540–3300 (OH), 2940, 2860 (CH_2 and CH), 1710 (*sh*, 6-membered ring C=O), 1690 (conjugated ester), 1640 (C=C), 1605, 1510, 1440 (aromatic system), 1370 (*gem* dimethyl), 1280, 1190, 1110, 1090, 975, 880 (C=CH_2). MS *m/z* (rel. int.): $[M]^+$ (not observed) 438 (44, **b**/**e**), 423 (20, 438 – Me), 395 (52, 423 – CO), 266 (13, **f**), 248 (16, **c**), 220 (13, **d**), 219 (12, 220 – H), 215 (16, **c** – Me – H_2O), 205 (17, **d** – Me), 203 (27, **d** – OH), 190 (34, **a**), 189 (74, **a** – H), 187 (43, 203 – Me – H), 180 (54, **g**), 175 (34, **a** – Me), 163 (100, **h**), 162 (13, **i**); other strong ions at *m/z* (rel. int.): 439 (24), 259 (21), 204 (21), 202 (23), 201 (20), 191 (29), 177 (22), 173 (23), 161 (39) and > 30 others (rel. int. > 20%) upto 41 (47). (Found: C, 75.37; H, 8.51. $C_{39}H_{54}O_6$ requires C, 75.73; H, 8.74%).

Hydrolysis of querspicatin A (**1**). Querspicatin A (**1**) (50 mg) was refluxed with 4% KOH–MeOH soln (10 ml) on a steam bath (8.5 hr) (the progress of the reaction was monitored at 1 hr intervals by TLC). The reaction mixture was diluted with water (50 ml), extracted with EtOAc and evapd. The residue was crystallized from petrol–EtOAc to afford the ketodiol **3** (20 mg), needles, mp 282°, $[\alpha]_D+3°$ ($CHCl_3$; *c* 0.4), $[M]^+ = m/z$ 456; ν_{OH} (3450 cm^{-1}) and $\nu_{C=O}$ (1700 cm^{-1}). It showed mp and rotation similar to those of querspicatin B; its identity was confirmed by mmp determination and spectroscopic (IR, ^1H NMR and MS) data comparison. Its monoacetate, mp 252°, prepared in the usual way was also found to be identical with querspicatin B monoacetate (**4**) by direct comparison (mmp, IR, ^1H NMR).

The aq. part was neutralized with 2 M HCl and evapd. The addition of MeOH and evapn was repeated × 3. The residue was finally crystallized from MeOH–petrol to give caffeic acid (3,4-dihydroxycinnamic acid), mp 194° (lit. 194–198°) [13]. The identity was confirmed by direct comparison with an authentic sample.

Querspicatin A diacetate (**2**). A soln of querspicatin A (**1**) (8 mg) in pyridine (0.25 ml) was treated (20 hr, room temp.) with Ac_2O (0.35 ml). The mixture was poured into ice-cold 1 M HCl and extracted with ice-cold $CHCl_3$. The residue obtained after evapn was crystallized from EtOAc–petrol as fine colourless needles (3 mg), mp 220°, IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1775 (OAc), 1710 (OAc), 1700 (C=O), 1640 (C=C), 1370, 880 (C=CH_2). MS: *m/z* (rel. int.): $[M]^+$ (not observed), 438 (32, **b**/**e**), 423 (8, **b**/**e** – Me), 395 (25, 423 – CO), 266 (2, **f**), 264 (3, **g**), 248 (11, **c**), 247 (4, **h**), 246 (4, **i**), 220 (6, **d**), 205 (14, **c** – Me – CO), 203 (**d** – OH), 190 (29, **a**), 189 (65, **a** – H), 175 (26, **a** – Me), 43 (100); other strong ions at *m/z* (rel. int.): 439 (13), 393 (13), 215 (13), 180 (30), 163 (32) and 20 others (rel. int. > 20%) upto 55 (53).

Oxidation of querspicatin B (**3**). To a soln of **3** (8 mg) in dry pyridine (1 ml), CrO_3 (20 mg) in dry pyridine (1 ml) was added at 0° with stirring. After 24 hr the mixture was decomposed with excess 2 M HCl, and then extracted with EtOAc. The organic layer was washed with aq. $NaHCO_3$ and H_2O, dried and evapd. The residue (10 mg) was purified by chromatography over silica gel to give the hydroxydiketone **5** as colourless needles (5 mg), mp 230° (EtOAc–petrol), $[\alpha]_D+25°$ ($CHCl_3$; *c* 0.36). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1705, 1700 (C=O), 1640 (C=C), 1455, 1375, 880 (C=CH_2); MS: *m/z* (rel. int.): 454 $[M]^+$ (33), 439 (20, M – Me)$^+$, 436 (6, M – H_2O)$^+$, 421 (3, 439 – H_2O or, 436 – Me), 411 (10, 439 – CO), 408 (30, 436 – CO), 393 (16, 421 – CO), 383 (2, 411 – CO), 365 (9, 393 – CO), 246 (5, 436 – **a**), 236 (19, arising from RDA type collapse of ring C), 235 (34, 236 – H), 231 (5, 246 – Me), 219 (34, 236 – OH), 218 (21, 246 – CO), 208 (14, 236 – CO), 203 (49, 231 – CO), 191 (11, 219 – CO), 190 (5, **a** or, 218 – CO), 189 (76, **a** – H), 175 (44, **a** – Me), 163 (20, 191 – CO), 55 (100); other strong ions at *m/z* (rel. int.): 410 (18), 248 (52), 205 (58), 143 (63) and 10 others (rel. int. > 20%) upto 67 (89).

Isolation of lupeol and hexacosanic acid. The petrol–EtOAc (19:1) fractions from CC residue B (two spots), upon repeated chromatography afforded lupeol (500 mg), mp 210° ($CHCl_3$–petrol), $[\alpha]_D+26°$ ($CHCl_3$; *c* 0.8); it was found to be identical with an authentic sample [14] by direct comparison (mmp, co-TLC, IR). The acetate (Ac_2O–pyridine, room temp., 24 hr) crystallized from $CHCl_3$–petrol as needles (20 mg), mp 219°, $[\alpha]_D$ +42° ($CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1735, 1370, 1247, 880.

The later petrol–EtOAc (19:1) fractions afforded hexacosanic acid (30 mg), mp 78° ($CHCl_3$–petrol), $[M]^+$ 396. Its identity was established by comparing its physical and spectral data with those reported [15].

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