Acid hydrolysis of obtusaside (**1**). Obtusaside (**1**) (39 mg) in 5% H_2SO_4–EtOH (1:1) (15 ml) was refluxed for 2 hr, poured into cold H_2O, neutralised with $BaCO_3$ and filtered. After extraction (×3) with Et_2O and (×3) with EtOAc, the combined extracts were dried over Na_2SO_4 and evapd to dryness. The product mixture was purified by CC (silica gel $CHCl_3$–MeOH 4:1) to give 3-hydroxy-2,6-dimethoxyethylbenzoate (12 mg); R_f 0.8 ($CHCl_3$–MeOH, 4:1, Godin). The aq. layer was filtered and lyophilised to give glucose (8 mg), co-TLC with authentic sample (EtOAc–HOAc–MeOH–H_2O, 13:4:3:3; anisidine phthalate). The glucose was converted to its pentaacetyl glucitol and co-injected with authentic sample (GC). The same retention times were shown.

Enzymatic hydrolysis of **1**. Obtusaside (55 mg) was treated with β-D-glucosidase (110 mg) in NaOAc–HOAc buffer solution (10 ml) (pH 5.5) at 35° with stirring over a weekend, extracted (×3) with *n*-BuOH and evapd. The *n*-BuOH extract was purified by CC (silica gel $CHCl_3$–MeOH 4:1) to give three fractions. Fraction 2 yielded 2,5-dihydroxybenzyl alcohol (22 mg) [R_f 0.5 ($CHCl_3$–MeOH–H_2O, 80:20:3); positive $FeCl_3$ test], directly converted to its triacetate using Ac_2O in the presence of pyridine.

Peracetylation of **1**. Obtusaside (120 mg) in dry pyridine (3 ml) was treated with Ac_2O (10 ml) and stirred at room temp. overnight, poured into cold H_2O, extracted (×3) with Et_2O, dried over Na_2SO_4 and evapd to give the hexaacetate (**1a**) (125 mg). For ^{1}H NMR (in pyridine-d_5) see Table 1.

Permethylation of **1**. Obtusaside (84 mg) was treated with MeI (3 ml) and K_2CO_3 (1.25 g). The mixture was dissolved in Me_2CO (1.5 ml) and refluxed. Usual work-up afforded a product mixture (90 mg) which was purified by CC (silica gel, C_6H_6–EtOAc, 4:1) to give the tetramethyl ether (**1b**) (40 mg) [R_f 0.3 (C_6H_6–EtOAc, 4:1) Godin, $FeCl_3$]; IR (neat): 3400 cm^{-1} (OH). For ^{1}H NMR (in pyridine-d_5) see Table 1.

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PLICATIN A AND B, TWO PHENOLIC CINNAMATES FROM *PSORALEA PLICATA*

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Key Word Index—*Psoralea plicata*; Leguminosae; phenolic cinnamates; 4-hydroxy-3-(2′-hydroxy-3′-methyl-3′-butenyl)-*trans*-methyl cinnamate and 4-hydroxy-3-(3′-methyl-2′-butenyl)-*trans*-methyl cinnamate.

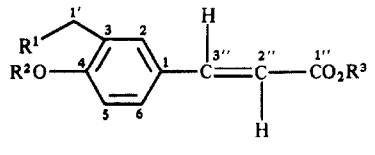
Abstract—Two new phenolic cinnamates, plicatin A and plicatin B, have been isolated from *Psoralea plicata*. Structures have been assigned to these through chemical and spectroscopic studies.

INTRODUCTION

Psoralea plicata Del is a small shrub commonly found in Sindh, Pakistan. It is highly regarded for its medicinal properties in the indigenous system of medicine [1]. Previously we have reported a new triterpenoid from this plant [2]. Following further studies on the fresh and undried plant material, two new phenolic cinnamates have been isolated and named plicatin A and B.

RESULTS AND DISCUSSION

Plicatin A (**1**) crystallized from acetone, mp 182°, $[\alpha]_D$ −59.7°. Its HR mass spectrum gave a molecular ion peak at *m/z* 262.2016 corresponding to the molecular formula $C_{15}H_{18}O_4$ (calcd 262.3050) indicating seven double bond equivalents. Other fragments were observed at *m/z* 247 $[M-CH_3]^+$, 244 $[M-H_2O]^+$, 229 $[M-Me-H_2O]^+$, 192 $[M-C_4H_6O]^+$, 191 $[M-C_4H_7O]^+$ and 160



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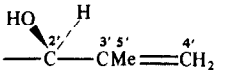
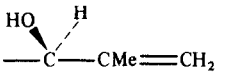
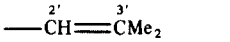
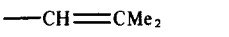
1		H	Me
1a		Me	Me
2		H	Me
2a		H	H

$[M-C_5H_{10}O_2]^+$. The IR spectrum revealed the presence of a hydroxyl group (3240–3450 cm^{-1}), a α,β-unsaturated ester (1710 cm^{-1}) and a 1,3,4-trisubstituted benzene ring (1590, 1525, 860 and 820 cm^{-1}) [3]. The UV spectrum showed maxima at 316, 233 and 215 nm. The maximum absorption at 316 nm corresponded to the reported value for cinnamic ester substituted by oxygen in the *para* position with the alkyl substituent in the *meta* position [3].

The ^1H NMR spectrum of **1** showed signals for two aromatic protons at δ7.35 and 6.90 as doublets, showing *ortho* coupling of 8.2 Hz. The *trans* α,β-unsaturated methyl ester moiety was indicated by the presence of two doublets at δ6.23 and 7.63 ($J=15.9$ Hz) and a sharp singlet at δ3.77 for a methoxyl group, while a terminal methylene group and a methyl group attached to olefinic carbon was revealed by presence of broad singlets at δ4.8, 4.9 and 1.81. In addition, there were signals due to carbinylic proton (multiplet at δ4.27) and non-equivalent protons of a methylene group (doublets at δ2.69 and 2.90, $J_{gem}=13.7$ Hz). The ^{13}C NMR spectrum showed 15 carbon atoms. The multiplicity of each carbon atom was determined by using DEPT experiments [4] which revealed the presence of two methyl, two methylene and six methine carbon atoms.

Compound **1** gave a green colour with $FeCl_3$ solution, indicating the presence of phenolic group. On methylation with diazomethane it gave a monomethyl ether **1a** which still showed the presence of a secondary hydroxyl group in the IR spectrum (3300 cm^{-1}). Based on the above evidence the structure **1** and **1a** have been assigned to plicatin A and its monomethyl ether.

The structure of the side chain at C-3 of **1** was further authenticated by ^1H–^1H correlated spectroscopy (COSY 45°) [5]. It showed connectivity of carbinylic proton with non-equivalent vicinal protons at C-1′ and another cross peak to the olefinic protons due to allylic interaction. Similar allylic interaction was observed between olefinic and methyl protons. The protons at C-1′ showed cross peaks with each other as well as with carbinylic proton at δ4.27. The *R* configuration at C-2′ was finally shown by Horeau method [6]. The reaction of **1a** with racemic-2-phenyl-butanoic anhydride gave 2-phenylbutanoic acid whose positive optical rotation confirmed the *R*-configuration of the hydroxyl group at C-2′ in **1a** and hence in **1**.

Plicatin B (**2**) gave a green colour with $FeCl_3$ solution and showed a molecular ion peak at 246.1256 (HRMS) corresponding to the molecular formula $C_{15}H_{18}O_3$ (calcd 246.3060). Other fragments were observed at *m/z* 231 $[M-Me]^+$, 218 $[M-CO]^+$, 217 $[M-CHO]^+$ and 191 $[M-C_4H_7]^+$. The UV and IR spectrum were very similar to plicatin A. The ^1H NMR spectrum showed two *ortho* coupled aromatic protons at δ7.26 and 6.81 ($J=8.7$ Hz) and an isolated proton at δ7.28. The presence of *trans* methyl cinnamate moiety was shown by pair of doublets for olefinic protons at δ6.23 and 7.63 ($J=15.9$ Hz) and a sharp singlet for a methoxyl group at 3.80. In addition it showed the presence of prenyl group (3H each, singlets at δ1.75 and 1.77, 1H, multiplet at δ5.32 and 2H doublet at δ3.30, $J=7.2$ Hz). The ^{13}C NMR spectrum of **2** showed the presence of 15 carbon atoms; their multiplicity determined by DEPT experiments. It revealed the presence of three methyl, one methylene and six methine carbon atoms.

The key to the structure of **2** was provided by its basic hydrolysis to the corresponding acid **2a**, the physical and spectral data of which coincided with those reported in literature for drupanin [3]. Plicatin B is, therefore, a hitherto unreported ester of drupanin. The structure of **2** was further authenticated by ^1H–^1H correlated spectroscopy and assignments of ^{13}C NMR spectrum was facilitated by ^1H–^{13}C correlated spectrum (hetero-COSY) [5]. Although similar types of phenolic cinnamates have been reported from other plants, the present paper constitutes the first report of their natural occurrence in the genus *Psoralea*.

EXPERIMENTAL

General. UV spectra were recorded in MeOH, IR spectra in $CHCl_3$, DEPT experiments were carried out with last pulse angle θ 45, 90 and 135°. The quaternary carbons were determined by substraction of these spectra from the broad band ^{13}C NMR spectrum. The 2D COSY-45° experiment was acquired at 300 MHz with a sweep width of 4000 Hz (2K data points in ω_2) and 2000 Hz (256 t_1 values zero-filled to 1K) in ω_1. The heteronuclear 2D ^1H–^{13}C chemical shift correlation experiments were carried out at 300 MHz with a sweep width of 1280 Hz (2K data point in ω_2) and 1024 Hz (256 t_1 values zero filled to 2K) in ω_1. In both 2D experiments a 2 sec relaxation delay was used and 16 transients were performed for each t_1 value.

Plant material. The plant material was collected from the Karachi region and identified by the Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited.

Extraction and isolation. The freshly collected plant material (20 kg) was extracted with alcohol at room temperature. The gummy residue obtained after removal of solvent was partitioned between $CHCl_3$–H_2O. The hexane soluble portion of $CHCl_3$ fraction was subjected to CC over silica gel, eluting with the solvent gradient of increasing polarity. The eluate obtained with a mixture of hexane–$CHCl_3$ (3:2) was evapd and the gummy residue thereby obtained was rechromatographed over silica gel column, eluting with the same solvent gradient.

Plicatin A. The eluate obtained from the column with hexane–$CHCl_3$ (1:4) was further purified through prep. TLC using hexane–$CHCl_3$–MeOH (35:14:1) as mobile phase. After crystallization with Me_2CO, it melted at 182° and showed $[\alpha]_D$ −59.7° ($CHCl_3$; *c* 0.067); yield (55 mg). UV (MeOH) λ_{max}: 316, 233 and 215 nm. IR ($CHCl_3$) ν_{max} cm^{-1}: 3450–3230 (OH), 1700 (α,β-unsaturated ester conjugated with aromatic ring), 1590, 1525, 860 and 820 (1,3,4-trisubstituted benzene ring); HRMS: *m/z* 262.20163 ($[M]^+$, $C_{15}H_{18}O_4$), 247 $[M-Me]^+$, 244 $[M-H_2O]^+$,

229 $[M-Me-H_2O]^+$, 192 $[M-C_4H_6O]^+$, 191 $[M-C_4H_7O]^+$ and 160 $[M-C_5H_{10}O_2]^+$. ^{1}H NMR: ($CDCl_3$) δ1.81 (3H, *br s*, H-5′), 2.69 (1H, *dd*, J = 13.7, 8.2 Hz, H_a-1'), 2.90 (1H, *dd*, J = 13.7, 4.9 Hz, H_b-1'), 3.77 (*s*, OMe), 4.27 (1H, *m*, H-2′), 4.8, 4.9 (1H each, *br s*, H-4′), 6.23 (1H, *d*, J = 15.9 Hz, H-3″), 7.63 (1H, *d*, J = 15.9 Hz, H-2″), 6.9 (1H, *d*, J = 8.2 Hz, H-5), 7.35, (1H, *d*, J = 8.2 Hz, H-6) and 7.2 (1H, *s*, H-2). ^{13}C NMR ($CDCl_3$ 75.4 MHz), δ126.74 (C-1), 128.63 (C-2), 125.67 (C-3), 159.18 (C-4), 117.9 (C-5), 131.73 (C-6), 32.11 (C-1′), 78.09 (C-2′), 146.14 (C-3′), 111.67 (C-4′), 18.23 (C-5′), 167.85 (C-1″), 144.76 (C-2″), 114.83 (C-3″), 51.55 (OMe).

Plicatin A methyl ether. Prepd via CH_2N_2–Et_2O, the product was purified through prep. TLC using hexane–Et_2O (1:4) and crystallized from EtOH to yield **1a** mp. 167°; $[\alpha]_D$ −57.64 ($CHCl_3$; *c* 0.0721); UV λ_{max}^{MeOH} 316, 234 and 218 nm. IR $\nu_{max}^{CHCl_3}$ 3300 (OH), 1700 (α,β-unsaturated ester conjugated with aromatic ring), 1595, 1520, 865 and 825 (1,3,4-trisubstituted benzene ring); HRMS: *m/z* 276.1361 ($[M]^+$, $C_{16}H_{20}O_4$), 261 $[M-Me]^+$, 258 $[M-H_2O]^+$, 246 $[M-CH_2O]^+$, 243 $[M-Me-H_2O]^+$ and 206 $[M-C_4H_6O]^+$. ^{1}H NMR ($CDCl_3$) δ:1.80 (3H, *br s*, H-5′), 2.70 (1H, *dd*, J = 13.7, 8.2 Hz, H_a-1′), 2.87 (1H, *dd*, J = 13.7, 5.0 Hz, H_b-1′), 4.23 (1H, *m*, H-2′), 3.68 (*s*, OMe), 3.86 (*s*, OMe), 4.82, 4.89 (1H, each, *br s*, H-4′) 6.25 (1H, *d*, J = 15.9 Hz, H-3″), 7.73 (1H, *d*, J = 15.9 Hz, H-2″), 7.0 (1H, *d*, J = 8.2 Hz, H-5), 7.30 (1H, *d*, J = 8.0 Hz, H-6) and 7.20 (1H, *s*, H-2).

Determination of absolute configuration at C-2′ *of* **1a**. Compound **1a** (10 mg) (*ca* 00003 mol) was added to a solution of racemic 2-phenylbutanoic anhydride (*ca* 8.02 μl) in dry pyridine (*ca* 0.2 ml). The resulting mixt. was allowed to stand for 10 hr 0.1M NaOH was then added dropwise until the pH was 9 and the soln was then extracted with $CHCl_3$. The aq. layer was acidified to pH 3 using 1 M HCl and the acidic layer extracted with C_6H_6 (10 ml). The benzene extract was evapd to adjust the vol. to 1 ml. The optical rotation of 2-phenylbutanoic acid in solution was found to be positive establishing the *R* configuration of the OH group at C-2′ in **1a** and hence in **1**.

Plicatin B. The fraction obtained with hexane–$CHCl_3$ (5:3) was repeatedly crystallized with Me_2O–MeOH (1:1), yielded light yellow crystals (98 mg) of **2** mp. 72°; $[\alpha]_D$ +17.46° ($CHCl_3$; *c*0.683); UV λ_{max}: 317, 234 and 216 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3230 (phenolic OH), 1695 (α,β-unsaturated ester), 1620, 1595, 860 and 830 (1,3,4-trisubstituted benzene ring); HRMS *m/z* 246.1249 ($[M]^+$, $C_{15}H_{18}O_3$), 231 $[M-Me]^+$, 218 $[M-CO]^+$, 217 $[M-CHO]^+$ and 191 $[M-C_4H_7]^+$; ^{1}H NMR: ($CDCl_3$) δ:1.77 (3H, *br s*, H-4′), 1.75 (3H, *br s*, H-5′), 3.30 (2H, *d*, J = 7.2 Hz, H-1′), 3.8 (*s*, OMe) 5.32 (1H, *m*, H-2′), 6.81 (1H, *d*, J = 8.7 Hz, H-6), 7.26 (1H, *d*, J = 8.7 Hz, H-5), 7.28 (1H, *s*, H-2), 7.63 (1H, *d*, 15.9 Hz, H-2″), 6.23 (1H, *d*, J = 15.9 Hz, H-3″). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ135.01 (C-1), 127.76 (C-2), 127.99 (C-3), 156.64 (C-4), 116.07 (C-5), 130.09 (C-6), 29.32 (C-1′), 121.24 (C-2′), 135.01 (C-3′), 17.91 (C-4′), 25.81 (C-5′), 168.22 (C-1″), 145.23 (C-2″), 114.73 (C-3″) and 51.68 (OMe).

Hydrolysis of plicatin B. Plicatin B (30 mg) hydrolysed with 5% KOH in MeOH at reflux temp. for 45 min. The reaction mixt. was worked-up in the usual manner. The gummy residue thereby obtained was crystallized with C_6H_6–Me_2O (1:1) to provide **2a** (24.6 mg); mp 149°; $[\alpha]_D$ +41.060 ($CHCl_3$; *c* 0.0487). UV λ_{max} nm: 316, 235 and 218; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2800–3450 (broad absorption band. OH), 1660 (carbonyl of an α,β-unsaturated acid), 1595, 1520, 865 and 825 (1,3,4-trisubstituted aromatic ring); HRMS *m/z* 232.9851 ($[M]^+$, $C_{14}H_{16}O_3$); 217 $[M-Me]$, 215 $[M-OH]^+$ and 177 $[M-C_4H_7]^+$; ^{1}H NMR: ($CDCl_3$) δ:1.78 (3H, *br s*, H-5′), 1.77 (3H, *br s*, H-4′), 3.33 (2H, *d*, J = 6.0 Hz, H-1′), 5.3 (1H, *m*, H-2′), 6.28 (1H, *d*, J = 15 Hz, H-3″) 6.78 (1H, *d*, J = 8.6 Hz, H-5), 7.12 (1H, *d*, J = 8.6 Hz, H-6), 7.72 (1H, *d*, 15 Hz, H-2″), 7.25 (1H, *s*, 2-H). The physical and spectral data coincided with those reported in literature for drupanin [3].

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