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Note

Isolation of chalkones from the seeds of *Psoralea corylifolia* Linn.

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Isolation and characterization of a new chalkone namely 4,2′-dihydroxy-2″-(1‴-methyl ethyl)-2″-3″-dihydro-(4″,5″,3′,4′) furano chalkone **1** along with a known chalkone 4,2′-dihydroxy-4′-methoxy-5′-(3‴, 3‴-dimethyl allyl)-chalkone **2** have been carried out from the seeds of the desert variety of *Psoralea corylifolia* Linn on the basis of spectral data analysis *i.e.* IR, ^{1}H NMR, mass and chemical reactions.

Keywords: *Psoralea corylifolia*, seeds, chalkones

IPC Code: Int. Cl.[8] A61K

Psoralea corylifolia Linn (Family Leguminosae), is an erect annual herb, found as a weed in the semi-arid regions of Rajasthan and the eastern districts of Punjab adjoining Uttar Pradesh. The plant has immense medicinal importance. It has been widely exploited since ages for its magical effect against several skin diseases like psoriasis, leucoderma and leprosy[1,2]. Cytotoxicity[3], antimutagenicity[4], antiinflammatory[5], repellant[6], antifeedant[7] *etc*. are some of the biological activities reported.

Many bioactive compounds *i.e.* bavachin–a flavonoid, chalkones like bavachromanol, bavachalkone, a phenol derivative-bakuchiol, sterols like β-sitosterol, stigmasterol, fatty acids *etc*. have been isolated from its seeds.

The present paper reports the identification of two chalkones. Compound **1** (**Figure 1**) being reported for the first time while compound **2** (**Figure 2**) has been isolated earlier and characterized by IR and NMR only[8].

Results and Discussion

Compound **1** gave intense yellow colour in NaOH, orange-red colouration in conc. H_2SO_4 which turned yellow on the addition of little quantity of conc. HNO_3 while red on adding few drops of acetic anhydride. A reddish-violet coloured precipitate was obtained with antimony chloride in CCl_4. Reddish-violet colouration with neutral $FeCl_3$ and a negative Gibbs test indicated the presence of a phenolic OH and also that the *para* position of the phenolic group was blocked. The colour reactions indicated the chalkonic nature of the compound. Paper chromatogram sprayed with isonicotinic hydrazide solution gave yellow fluorescence further confirming it to be a chalkone[9,10].

In the UV spectra band at 375 nm (Band I) was the most intense corresponding to Ring B while at 231 nm (Band II) was of ring A of the chalkone nucleus.

A low intensity band appeared at 308 nm while a bathochromic shift of 60 nm in Band I occurred on addition of a few drops of 1 *N* NaOH solution. The band appearing at 308 also shifted to 317 nm. These observations indicated the presence of a *para* hydroxy phenyl group while a bathochromic shift of 53 nm on addition of $AlCl_3$ –HCl solution indicated the presence of a chelating OH group.

The IR spectra gave an intense peak at 1640 cm^{-1}, the characteristic absorption due to the chalkone >C=O group. Peaks at 1360 and 1378 cm^{-1} were due to the C-H deformation vibration of the gem-dimethyl groups while at 1165 and 1120 cm^{-1} were of the CH_3-C-CH_3 skeletal vibrations. Absorption at 1055 was due to the alkyl-O-stretching in a 5-membered

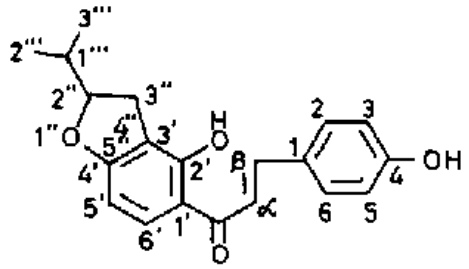

Figure 1 — Compound **1**

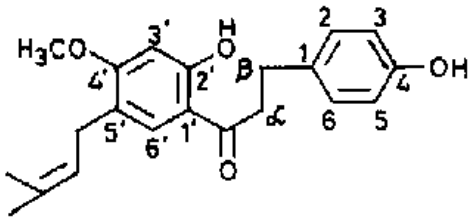

Figure 2 — Compound **2**

cyclic ether ring and 2 bands of almost equal intensity at 1215 and 1240 cm^{-1} were accounted for the aryl-O-stretching vibrations. Other vibrations visible were at 1470, 1510, 1540 and 1570 cm^{-1} of the phenyl ring and at 1610 of the C=C stretching. Peaks at 975 and 1290 of CH=CH out-of-plane and in-plane vibrations and a strong peak at 838 cm^{-1} due to the para substituted benzene ring was also observed. Lastly, bands at 3220 and 3382 cm^{-1} indicated the chelated and non-chelated OH group. The IR spectra hence supported the presence of the chalkone nucleus with a *para* substituted OH group, a chelating OH group along with the presence of an ether linkage and a gem dimethyl group[11,12].

The ^{1}H NMR spectrum indicated the presence of two coupled doublets centered at δ 7.43 and 7.83 corresponding to C-α and C-β protons[13]. Further two *ortho* coupled douplets (coupling between C-3 and C-5 protons and C-2 and C-6 protons) centered at δ 7.53 and 7.72 integrated for four protons of an A_2B_2 system of a *para* substituted phenyl ring. Singlets at δ 13.78 and 6.26 were the OH group protons. Another *ortho* coupled doublets centred at δ 7.37 and 5.59 were the C-6′ and C-5′ positioned protons in ring A. This also indicated that these positions were free.

Presence of two doublets centred at δ 1.41 and 1.25 (J=2.5 Hz) corresponded to the methyl groups in the gem-dimethyl linkage. A doublet centered at δ 3.49 showed the presence of two cyclic methylene protons while a multiplet for a methine proton appeared at δ 4.30. These signals supported the presence of a dihydrobenzofuran ring substituted at 2-position of the furan ring.

The bathochromic shift in the longer wave length in UV spectrum on addition of $AlCl_3$ confirmed the presence of C-2′ OH group and ruled out the possibility of a furan ring attachment to ring A at C-2′ and C-3′. Hence the dihydro furan ring was attached to ring A at C-3′ and C-4′ position. The mass spectra showed important mass fragments at 309 (M-CH_3), 323 (M-H), 296 (M-CO), 190, 189 etc **Chart I**. The structure was strongly supported by the fragmentation pattern as is characteristic for those of chalkones and compounds containing a 2-(1-methyl ethyl)-2,3-dihydro benzo furan ring[14,15].

Compound 2 gave an intense yellow colouration with aqueous NaOH and an intense orange colour with a mixture of conc. H_2SO_4 and few drops of conc. HNO_3 which changed to yellow. A reddish violet coloured precipitate with antimony chloride in CCl_4 further supported the chalkonic nature. The compound also gave a negative Gibbs test. In the I R spectra two characteristic bands at 3420 and 3298 cm^{-1} accounted for the two OH groups one being chelated and the other non-chelated. The chalkonic carbonyl group vibration was visible at 1635 cm^{-1}.

In the UV spectra, peak at 237 nm in the Band II region was less intense. A minor reflection at 308 and a strong band at 372 nm indicated the chalkonic nature. Band at 237 corresponded to ring A and at 373 nm to ring B. A strong bathochromic shift with an increase in intensity in Band I absorption of this compound from 372 to 436 (Δλ = 64 nm) on addition of a few drops of aqueous NaOH indicated the presence of C-4 OH group in ring B.

Again a bathochromic shift of 53 nm on Band I absorption was observed on addition of few drops of $AlCl_3$ + HCl. This shift was indicative of the presence of C-2′ chelating OH group in ring A of chalkone[16,17].

^{1}H NMR showed a singlet at δ 3.87 accounting for three protons of the methoxy group. A pair of doublets centered at δ 7.83 and 7.43 (J=17 Hz) due to C-β and C-α protons to the carbonyl carbon was also observed. Two *ortho* coupled doublets (due to C-3 and C-5 protons and C-2 and C-6 protons) centered at δ 6.89 and 7.57 respectively integrated for four protons of an A_2B_2 system of a *para* substituted phenyl ring. The presence of hydroxyl group protons was indicated by the presence of two singlets, one at δ 13.49 for C-2′ OH and other at 6.44 for C-4 OH respectively. Also the presence of a 3, 3-dimethyl allylic side chain was revealed by a doublet centered at δ 1.75 corresponding to the two methyl group protons of the gem dimethyl group present in the prenyl side chain. The two methylenic protons appeared as a doublet centered at δ 3.26, while a triplet at 5.28 due to one proton linked to the doubly bonded carbon atom of the allyl side chain was also observed.

The mass spectra showed important mass fragments at m/e 337 (M-H), 307 (M-OCH_3), 310 (M-CO), 219 etc. and characterized it as 4,2′-dihydroxy-4′-methoxy-5′-(3‴, 3‴-dimethylallyl)-chalkone **Chart II**.

Experimental Section

The seeds of *Psoralea corylifolia* procured from the local market were sown. The plants which were reproduced, (its identity was established by the Department of Botany, Jai Narain Vyas University, Jodhpur). Seeds of these plants were used for the

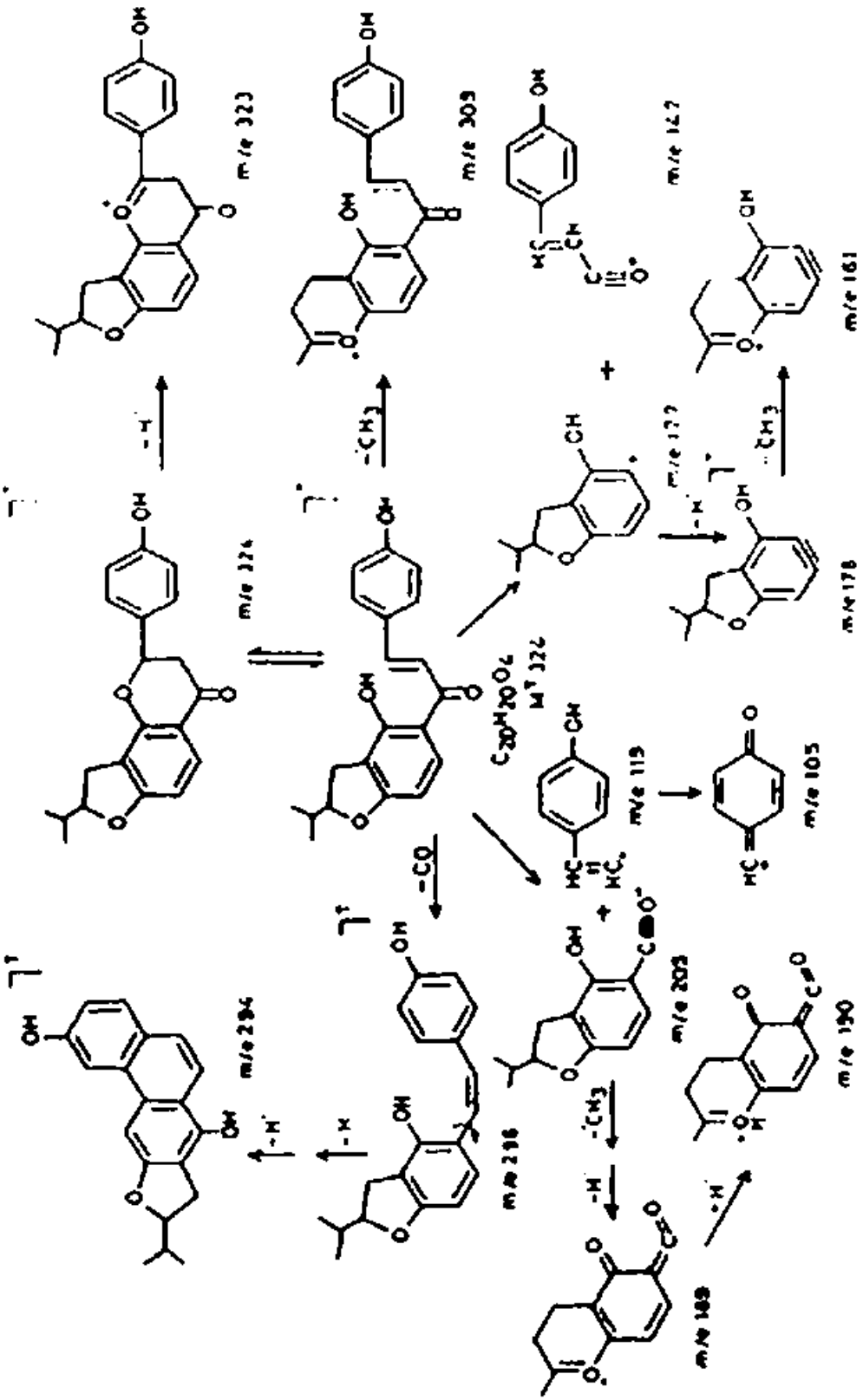

Chart I — The mass fragmentation pattern for compound 1

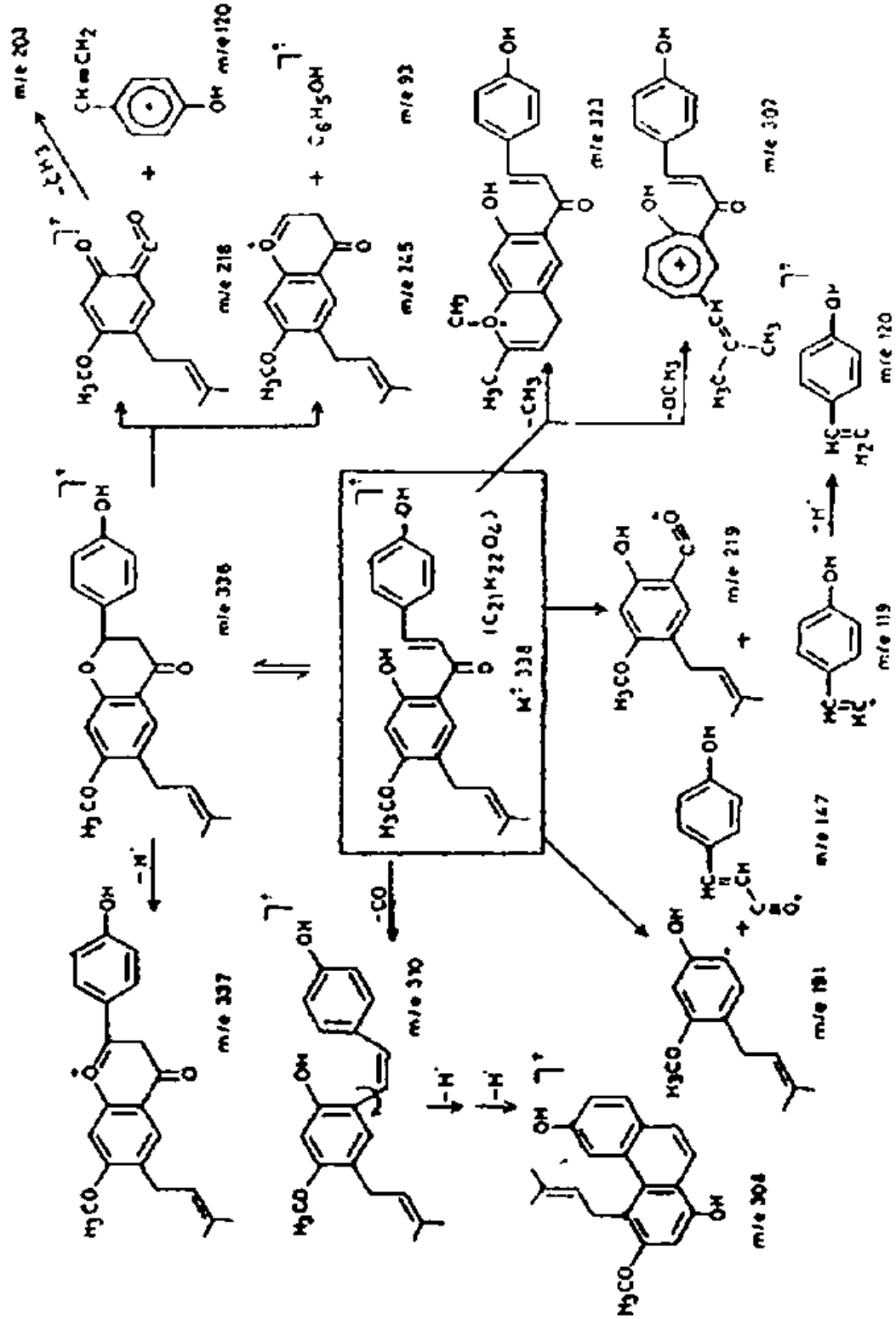

Chart II — The mass fragmentation pattern for compound 2

present chemical investigation. UV spectra were recorded on a Shimadzu-1601 Spectrometer, FTIR on a Shimadzu-8101A spectrometer using KBr pellets, ^{1}H NMR on a Brucker WM 400 model (300 MHz) in $CDCl_3$, and mass spectra were carried out on a JEOL D-300 model.

Extraction and isolation

Two kg of powdered air dried *Psoralea corylifolia* seeds were extracted with chloroform. Excess solvent was distilled off and the chloroform extract was then treated with hexane.

A dry slurry of the hexane insoluble portion was made in silica gel after dissolving in minimum quantity of chloroform and chromatographed on a silica gel-G (680 g) column. Elution was successively carried out with hexane, hexane-chloroform (95:5, 90:10, 80:20, 50:50) and pure chloroform. The column was eluted at the rate of 150 mL/hr and samples of 50 mL each were collected. Fractions were then grouped together according to the TLC results.

Compound **1** was isolated from fraction (140-158) by eluting with hexane-chloroform (50:50). It was obtained as a yellow coloured compound, crystallized from hexane, yield 1.08 g.

The column was further eluted with pure chloroform. 50 fractions (180-230) were collected, concentrated and treated with hexane. The hexane insoluble portion was subjected to TLC on silica gel. Compound **2** was isolated as an orange coloured, recrystallised with alcohol; yield 0.998 g.

Compound **1**. Elution of the column with hexane-chloroform (50:50) yielded a yellow solid. It was recrystallised from hexane m.p. 195°C; R_f 0.43 (benzene:methanol, 98:2); 0.5 (benzene:ethanol, 95:5); (Found: C, 74.33; H, 6.6; $C_{20}H_{20}O_4$ requires C, 74.07; H, 6.17 %). UV λ_{max}, EtOH + nm (logε): 231.0 (1.16), 280 (0.77), 308 (0.65) and 375 (2.15); UV λ_{max}, EtOH + NaOH nm (logε): 211 (0.94), 229 (0.96), 272 (1.02), 317 (0.58) and 435 (2.29); UV λ_{max}, EtOH + $AlCl_3$ + HCl nm (logε): 230 (1.12), 282 (0.78), 310 (0.58) and 428 (2.18); IR (KBr, cm^{-1}): 3382-3220, 3025, 2970, 2935, 1640, 1635, 1610, 1570, 1540, 1510, 1470, 1378, 1360, 1290, 1285, 1240, 1215, 1165, 1120, 1055, 975, 838, 798 and 670; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 13.78 (s, 1H, C-2′ OH), 7.83 (d, 1H, Cβ-H), 7.72 (d, 2H, C-2 & C-6-H), 7.53 (d, 2H, C-3 & C-5-H), 7.43 (d, 1H, Cα-H), 6.26 (s, 1H, C-4-OH), 7.37 (d, 1H, C-6′), δ 5.59 (d, 1H, C-5′), 4.30 (m, 1H, C-2′′), 3.49 (d, 2H, C-3′′), 2.9 (m, 1H, C-1′′), 1.41 (d, 3H, C-2′′′), 1.25 (d, 3H, C-3′′′); FAB-MS (m/z) (relative intensity): 324 [M]+ $C_{20}H_{20}O_4$, (31.5), 323 (20.15), 311 (4.0), 310 (17.9), 309 (52.5), 296 (15.6), 294 (8.3), 285 (3.8), 205 (6.9), 190 (7.8), 189 (100), 188 (18.6), 177 (12.2), 176 (10.4), 161 (15.5), 147 (11.8), 132 (6.6), 119 (28.3), 105 (8.8), 93 (5.1), 80 (8.9), 79 (8.5), 71 (9.2), 58 (16.9), 46 (22.4), 43 (23.2).

Methylation of 1 (1a): Creamish coloured derivative was obtained by repeated crystallization from ether-alcohol mixture (1:1) m.p. 106-108°C (Found: C, 76.28; H, 6.96. $C_{22}H_{24}O_4$ requires C, 75; H, 6.81%).

Methoxy group estimation of 1a: This was estimated using Cheronis and Ma micro method[18]. Methoxy percentage 18.33 (requires for 2 methoxy groups 17.61%).

Acetylation (1b): Compound **1** was acetylated following the procedure of Verlag[19]. The colourless compound **1b** was isolated as needles by recrystallisation from acetone-petroleum ether mixture (1:1), m.p. 140-42°C (Found: C, 73.83; H, 6.52. $C_{22}H_{22}O_5$: C, 72.13; H, 6.01%).

Acetyl group estimation of 1b: The acetyl group percentage was determined by the known process[20]. Acetyl percentage 10.96 (requires for one acetyl group 11.74%).

Alkaline hydrolysis of 1: Compound **1** (5.0 mg) was refluxed with 5 mL of 50% aqueous potassium hydroxide for 3 hr. The solution was cooled, acidified with HCl and extracted with 5% aqueous $NaHCO_3$. The sodium bicarbonate extract was neutralized and reextracted with diethyl ether 2 to 3 times. The combined ether extract was distilled and the concentrate on examination by paper chromatography in *n*-butanol and 5% acetic acid (1:1) showed the presence of *para* hydroxy benzoic acid as one of the products on comparison with the authentic sample. (R_f : 0.87)

Ring cyclisation of 1: It was cyclised with ethanolic 10% sulphuric acid by the method of Naik *et al*[21]. A colourless crystalline compound was obtained when recrystallised with petroleum ether. It gave a positive test for flavanone, m.p. 188°C.

Compound 2: Isolated by eluting the column with pure chloroform. It was purified from alcohol as orange coloured needles, m.p. 338°C, R_f 0.3

(benzene: methanol 98:2), 0.8 (benzene : ethyl acetate 4:1), (Found: C, 74.4; H, 7.0. $C_{21}H_{22}O_4$ requires C, 74.5; H, 6.6%). UV_{max}, EtOH, nm (logε): 237 (1.67), 308 (1.30), 372 (3.35); UV_{max}, EtOH + NaOH nm (log ε): 255 (1.65), 297 (1.33), 436 (3.52); UV_{max}, EtOH + $AlCl_3$ nm (logε): 253 (1.69), 310 (1.29), 425 (3.55); IR (KBr, cm^{-1}): 3420, 3298, 3015, 2965, 2910, 2855, 1635, 1560, 1505-1520, 1445, 1370, 1280, 1240, 1200, 1170, 1135, 1000, 975, 838 and 600 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, δ, ppm): 13.49 (s, 1H, C-2′-OH), 7.83 (d, 1H, C-β), 7.57 (d, 2H, C-2 and C-6), 7.43 (d, 1H, C-α), 7.26 (s, 2H, C-3′ and C-6′), 6.89 (d, 2H, C-3 and C-5), 6.44 (s, 1H, C-4, -OH), 5.28 (t, 1H, methine proton of prenyl side chain), 3.87 (s, 3H,-OCH_3), 3.26 (d, 2H, methylene protons of prenyl side chain), 1.75 (d, 6H, gem di-methyl group of prenyl side chain); FAB-MS (m/z) (relative intensity): 340 $[M]^+$ $C_{21}H_{22}O_4$ (5.9), 339 (28.1), 338 (100), 337 (20.0), 323 (15.4), 310 (9.8), 268 (10.4), 247 (6.4), 245 (15.6), 220 (14.6), 219 (87.1), 218 (47.6), 217 (9.8), 204 (9.5), 203 (70.7), 191 (8.5), 190 (7.0), 189 (5.0), 187 (14.5), 177 (6.9), 176 (6.7), 175 (16.9), 169 (5.3), 163 (12.0), 161 (22.0), 160 (14.8), 151 (9.2), 148 (8.5), 147 (31.8), 120 (8.3), 119 (14.5), 105 (12.1), 93 (28.6), 78 (18.2), 69 (20.0), 55 (16.8), 42 (22.0), 41 (21.3).

Methylation of 2 (2a): Light yellow coloured methyl derivative was obtained, m.p. 85-86°C (Found: C, 75.6; H, 7.03. $C_{23}H_{26}O_4$ requires C, 75.4; H, 7.1%).

Methoxy group estimation of 2a: Estimation results indicated the presence of three methoxy groups when these were determined by Cheronis and Ma micromethod[17]. Methoxy percentage 26.12 (requires for 3 methoxy groups 25.40%)

Methoxy group estimation of 2: This was estimated following the Cheronis and Ma′s procedure[17]. Methoxy percentage 9.32 (requires for one methoxy group 9.17%).

Acetylation of 2 2b: A colourless compound was isolated which was recrystallised from chloroform, m.p. 112°C (Found: C, 71.62; H, 6.8. $C_{23}H_{25}O_5$ requires, C, 72.44; H, 6.56%).

Acetyl group estimation of 2b: Acetyl percentage 11.16 (requires for one acetyl group 11.28%), indicating that only one hydroxyl group in compound **2** was free for acetylation.

Alkaline hydrolysis of 2: One of the products identified was similar to that as for compound **1**, i.e. *p*-hydroxy benzoic acid by following the same procedure.

Ring cyclisation of 2: Cyclisation was carried out as given in compound **1**. The isolated compound was purified and crystallized from benzene-ethyl acetate mixture (1:1). It gave positive test for flavanone, m.p. 154-55°C.

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