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Extraction and Identification of Compound Derived from *Ipomoea palmata* Through Various Spectroscopic Techniques

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Extraction and separation of constituents from *Ipomoea palmata* have carried out adopting various chemical routes. Extracted and separated compounds have been identified by various spectroscopic techniques such as UV, and ^{1}HNMR.Thease techniques revel that that two compound can be as 4-hydroxy-2-(3, 7- dimethyl-octa, 2-6, diphanyle) 6-methoxy acetophenones and 4,6-dihydroxy, -2-0-(4 hydroxy butyl) acetophonone, whose melting points are 225^{0}C and 280^{0}C respectively. Elemental analysis and molecular weight determination (M^+ 302) of compound –I (M.P. 225^{0}C) have established that compound has molecular formula as $C_{19}H_{26}O_3$ while compound II (MP. 280^{0}C) depict its molecular formula as $C_{12}H_{16}O_5$. These compound have potential application in various industries like medicine, beverages etc.

Keyword: *Ipomoea palmata*, UV-Vis Spectroscopy, NMR, Mass Spectra, Molecular Weight.

1. Introduction

Ipomoea palmata belongs to a *Ipomoea cairica* (convoluulace) family. It is a climbing herb and is found abundantly in tropical and subtropical region. It has many common name and is also knows as railroad creeper[1]. This vining perennial has palmate leaves and large, showy white to lavender flowers. Each fruit matures at about 1 cm across and contain hairy seeds .Its native range is uncertain , through it is believed to originate from a rather wide area, ranging from Cape Verde to the Arabian Peninsula , including northern Africa. Because of human disperser, it occurs today on most continents as an introduction species and is sometimes a noxious weed . It is a major problem along the coast of New South Wales. In the united State it occurs in Hawaii, California, all the gulf coast states, as well as Arkansas and Missouri[2]. Some plant nurseries sell this plat as an ornamental. It can grow as a separate plant if snapped during attempted removal process[3].

The genus ipomoea has 400 species all over the world from ipomoea palmata forsks or ipomoea caircia L. grow abundantly in Egypt. Ipomoea palmate is used in treatment of various disease[4]. The major bioactive constituents previously isolated from the genus ipomoea were lipoidal matters[5] and Phenolic compounds[6]. The genius ipomoea has been reported to have many biological activities. Pongprayoon et.al[7]. showed a significant analysis effect attributed to the petroleum ether, chloroform and ethanol extract

of ipomoea palmate in albino rats. The structure of compounds have been established although spectroscopic techniques such as NMR, UV etc. These two compounds have been reported for the first time from this plant.

2. Experimental

The air dried and grounded seed (1 Kg) of Ipomoea Palmata were first defatted by extraction with petroleum ether. The defatted plant – material was then extracted with ethanol and concentrated. The extract was then separated into water soluble and insoluble fractions.

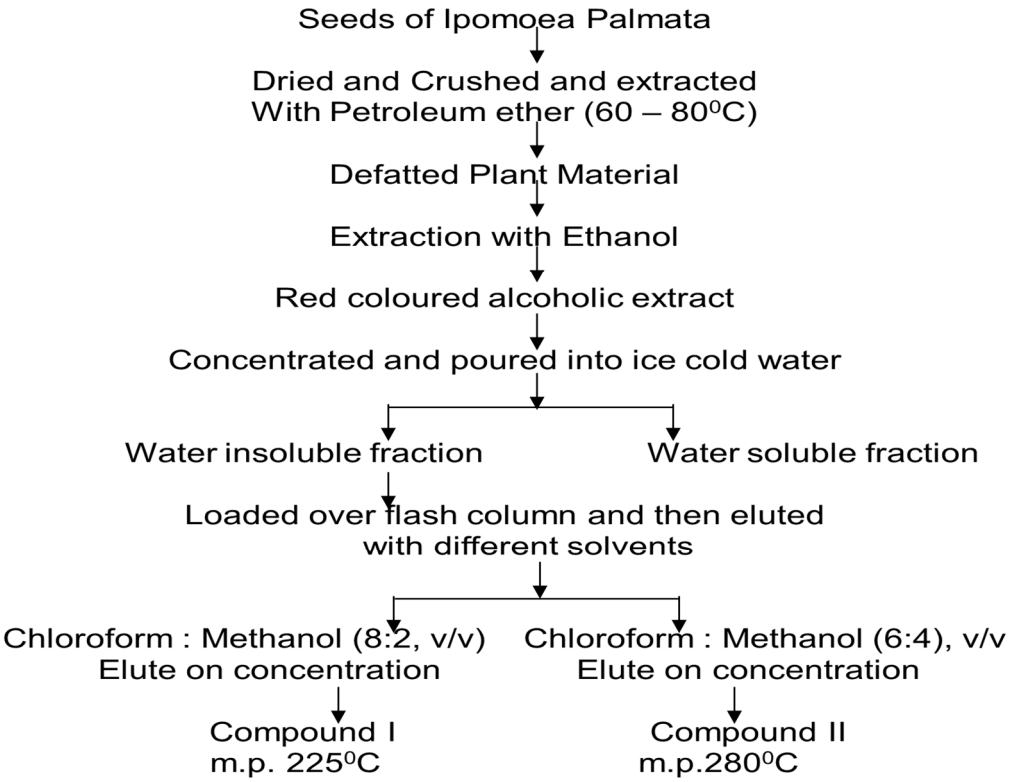
Water soluble portion was loaded over flash column and then eluted with different solvents of increasing polarity. Characterization has been carried out with the help of NMR of Bruker 400 MHz FT-NMR and UV spectrophotometer of Varian UV Visible Spectrophotometer.

3. Elution of column

1. Elution with chloroform : Methanol (8:2, v/v) yielded compound I (900 mg)
2. Elution with chloroform: Methanol (6:14, v/v) yielded compound II (100 mg)

Thus two different compounds were isolated from the seeds of Ipomoea Palmata. The process flow charts are as follows:

Fig 1: Flow Diagram of Extraction and Purification of Compounds from Ipomoea Palmata



The seeds of Ipomoea Palmata were collected from F.R.I. Dehradun (a herbarium specimen of the plant on file in Botanical survey of India) and was dried in air circulated oven at 80^0 C and crushed in mixer at 500 rpm. The air dried and crushed seeds of Ipomoea Palmata (1Kg) were extracted with petroleum ether ($60\text{-}80^0$C) in soxhlet extractor till the appearance of colour. The defatted seeds were subsequently extracted under reflux in soxhlet with ethanol. The extract was concentrated under reduced pressure. Now concentrate (100 ml) was poured into ice cold

water and kept for overnight. Then it was filtered and water insoluble and soluble fractions were obtained.

4. Analysis of Water Insoluble Fractions:

Water insoluble portion (40 gm) was then loaded over flash column of silica gel G. then it was eluted with hexane, benzene, chloroform, ethyl acetate, ethanol and methanol and different ratio of solvents in increasing polarity. From the chloroform: Methanol (8:2, v/v), a red coloured compound, m.p. 225^{0}C was separated and marked as I, it was recrystallised from ethanol. The chloroform: Methanol (6:4, v/v) elute, on concentration gave a red coloured compound, m.p. 280^{0}C. It was labeled as II and recrystallised from ethanol. Both these compounds isolated are new and not reported earlier in literatures.

5. Acetylation of Compounds

The compound (40 mg) was dissolved in acetic anhydride (5ml) and pyridine (2ml). It was left for 48 hours at room temperature. The reaction mixture was poured into ice cold distilled water and dried. The residue obtained was crystallized from methanol and was analyzed for one acetyl group.

6. Physical Data

A: Compound I

Compound was crystallized from methanol as red granular compound.

Solubility	:	Soluble in Chloroform, ethylacetate, acetone, methanol and ethanol.	
M.P.	:	225 ºC	
Rf	:	0.71 (Ethyl acetate)	
		0.72 (Methanol: Chloroform) (1: 1, v/v)	
		Found	Calculated for $C_{19}H_{26}O_3$
Elemental Analysis	:	C – 75.49% H – 8.60%	C – 75.50% H – 8.62%
λ_{max}^{EtOH} (UV) nm	:	220, 285; +$AlCl_3$ / HCl: 220, 287; +NaOAc; 230, 29.	
ν_{max}^{KBr} (IR) cm^{-1}	:	3200, 2 900, 2825, 1660, 1600, 1530, 1450, 1380, 1105, 115, 880, 825, 780.	
^{1H}NMR [90MHz, $CDCl_3$]	:	5.70 (d, 2.4Hz), 5.85 (1H, d, J = 2.4 Hz, H-5), 2.45 (s, 3H, H-8), 3.30 (d, 2H, J = 6.2 Hz, H-1), 5.10 – 5.28 (brm, 2H, H – 2, 6'), 2.05 (brm, 4H, H-4' & H-5'), 1.60 (s, 3H), 165 (s, 3H), 1.79 (s, 3H), 7.50 (Ph-OH), 3.59 (s, 3H) ppm.	
Mass Spectra, m/z (%)	:	302 [M^+], 287, 271, 233, 210, 165, 104, 91, 76.	

Spectral data after Acetylation

		Found	Calculated for $C_{21}H_{28}O_4$
Elemental Analysis	:	C = 73% H = 8%	C = 73.25% H = 8.13%

IR (KBr) $_{max}$ cm^{-1} : 1760, 1260 (Acetate), 1655 (C=0), 1600, 1520 (aromatic), 1180, 1010, 880, 820 cm^{-1}

^{1H}NMR [90 MHz, $CDCl_3$] : δ – 6.02 (d, 1H, J = 2.5 Hz, H – 3), 6.20 (d, 1H, J = 2.5 Hz, H – 5), 2.40 (s, 3H), 3.31 (d, 2H, J = 6.2 Hz, H-1), 5.10 – 5.28 (brm, 2H, H – 2', H - 6'), 2.06 (brm, 4H, H-4' & H-5'), 1.62 (s, 3H), 1.65 (s, 3H), 1.80 (s, 3H), 3.16 (s, 3H), 2.16 (s, 3H, 1XOAC) ppm.

Mass Spectra, m/z (%) : 344 [M^+]

B: Compound II

Compound was crystallized from methanol as red granular compound.

Solubility : Chloroform, DMSO, methanol.

M.P. : 280ºC

Rf : 0.25 Meo, $CHCl_3$, (1:1, v/v)
0.72 MeOH : $CHCl_3$ (1 : 1, v/v)

		Found	Calculated for $C_{12}H_{16}O_5$
Elemental Analysis	:	C – 60% H – 6.7%	C – 60% H – 6.66%

UV (EtOH) $_{max}$ nm : 230, 290, +$AlCl_3$ / HCl : 251, 285, +NaOAc- 242, 287.

IR (KBr) $_{max}$ cm^{-1} : 3380, 3200, 2918, 2850, 1650, 1540, 1450, 885, 825, 780.

^{1}HNMR [90MHz, $CDCl_3$] : 5.65 (d, 1H, J = 2.5 Hz, H-3), 5.80 (d, 1H, J=2.5 Hz, H-5), 2.48 (s, 3H), 4.30 (t, 2H, H-1′),1.79(s, 4H, H-2′ and H-3′), 3.65 (dd, 1H, H-1′), 3.58 (dd, 1H, H-4′), 7.90 (ph-OH), 13.10 (1H, ph-OH), 2.62 (brt, J=6.2 Hz, 1H) ppm.

MS m/s : 240 [M^+]

Spectral Data after Acetylation

Elemental Analysis	:	C: 59.2% H: 6.01%	C : 59% H : 6%

IR (KBr) $_{max}$ cm^{-1} : 1760, 1680, 1600, 1580, 1370, 880, 810

^{1}H NMR[90MHz, $CDCl_3$]: 6.00 (d, 1H, 2.5 Hz, H - 3), 6.31 (d, 1H,J = 2.5 Hz, H – 5), 2.35 (s, 3H), 4.31 (t, 2H, H-1'), 1.80 (s, 4H, H-2', H-3') 3.65 (dd, 1H, H-4') 3.59 (dd, 1H, H-4') 2.11 (s, 3H, - $OCOCH_3$), 211(s, 3H, - $OCOCH_3$), 2.02(s, 3H, - $OCHOCH_3$) ppm.

Maas Spectroscopy, m/z : 366 [M^+]

7. Results and Discussion

A. Compound I

The compound I, m.p. 225^{0}C, on elemental analysis and molecular weight determination (M^+300) established its molecular formula as $C_{19}H_{26}O_3$. The compound showed UV spectrum characteristic of acetophenone derivatives. Appearance of peaks at 3200 cm^{-1} in its IR spectrum showed the presence of hydroxyl group. The peak at 1660 cm^{-1} was found due to presence of α-β- unsaturated keto group.

On acetylation with acetic anhydride and pyridine, it formed monoacetato, showing the presence of one hydroxyl group in the molecule. The compound induced bathochromic shift of 10 nm in its UV spectrum, on addition of sodium acetate, to the ethanolic solution of compound and no shift with $AlCl_3$. This showed the presence of free hydroxyl group at para position with respect to keto group.

The ^{1}HNMR spectrum of the compound showed singlet at δ2.45 (3H) and δ3.59 (3H) ppm for CH_3 Co- and – OCH_3 protons respectively. ^{1}HNMR displayed signals at δ 1.60 (s, 3H), 1.65 (s, 3H), 1.79 (s, 3H), 2.05 (br m4h) 3.30 (d, 2H, J=6.2 Hz), 5.10 – 5.28 (2H, br) ppm for the presence of 3, 7 dimethyl octadienyl chain[3] (see compound II). ^{1}HNMR also gave two signals for aromatic protons of acetophenone ring at δ 5.70 (d, ^{1}H, J=2.4 Hz) and 5.85 (d, ^{1}H, J = 2.4 Hz). These protons are meta, coupled as indicated by J value. NMR values are shown in compound II discription.

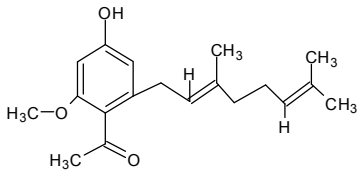
Thus, ^{1}HNMR of the compound is consistent with p-hydroxy acetophenone having methoxy and dimethyl octa dienyl group as substituents.

As two aromatic protons are meta coupled so the two substituents either must be present at position C-2 and C-6 or position C-3 and C-5.

The protons at position C-2 and C-6 are deshielded (ortho to carbonyl group), so they should absorb at higher δ value (δ 7.57 and 7.73 ppm) and proton at C-3 and C-5 are shielded by – OH group, so they should absorb at lower δ value (δ 5.70 and 5.85 ppm) so the substituent must be present at position C-2 and C-6.

Position of substituent at these positions was further confirmed by the ^{1}HNMR data of acetate (J') of compound, which suggested that these two aromatic protons must be present at position C-3 and C-5 because aromatic protons showed a considerable down field of phenolic–OH group.

Compound I was thus established as 4-hydroxy-2-[3', 7'-dimethylocta-2',6' diphanyle] 6-methoxy acetophenone.



The compound is new and not reported earlier from any other plant source, but some natural compound having this type of side chain are reported earlier [8-10].

B. Compound II

The compound IInd, m.p. 280ºC was analyzed for $C_{12}H_{16}O_5$. The compound gave characteristic UV spectrum of acetophenones. It showed IR absorption corresponding to α, β – unsaturated and hydroxyl group (1650, 3200, and 3380 cm^{-1}).

The compound induced shift in UV spectrum on addition of NaOAc (12 nm) as well as on addition of $AlCl_3$ / HCl (21 nm), showing the presence of free hydroxyl group at ortho and para position with respect to – COMe group (see compound III). Acetylation of compound IInd yielded triacetate (IIIrd) showing the presence of three hydroxyl groups.

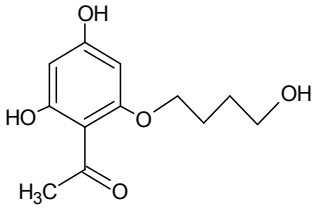
NMR values of IInd and IIIrd compounds showed the presence of –O-hydroxy-butyl side chain. The structure of hydroxyl butyl side chain was established unambiguously by ^{1}H NMR. There are two –OCH_2 groups present in the side chain. One shows up as a triplet of 2H and the other is represented by two diostereotopic protons with different chemical shifts. The letter must be associated with –CH_2OH group, since in very

pure NMR a direct coupling of the two diastereomeric oxymethylene protons with the hydroxyl protons is observed. The other $–OCH_2$ group was therefore directly linked to acetophenone ring with aromatic ether linkage.

The broad singlet for $–CH_2-CH_2-$ group was observed at δ 1.79 (4H, Br, S) so sequence in side chain is given as Ar – OCH_2 CH_2 CH_2 – CH_2 OH.

The ^{1}H NMR also showed signals for two phenolic hydroxyl group, so the ^{1}H NMR of compound was consistent with a tetra substituted aromatic ring with $–COCH_3$ group, one –O- hydroxyl butyl residue and two phenolic hydroxyl groups as substituents.
^{1}H NMR also showed presence of two aromatic protons at δ 5.65 (d, J = 2.5 Hz) and 5.80 (d, J = 2.5 Hz) ppm. The protons are meta coupled as indicated by J value. So -O- hydroxyl butyl residue must be present at meta position to hydroxyl group. Thus substituents must be present at C-2 and C-6.
Position of substituents at these positions was further confirmed by ^{1}H NMR. As aromatic protons at C-3 and C-5 are shielded by –OH group so they should absorb at lower value (δ 5.65 and 5.80).

Thus compound was established as 4, 6 dihydroxy, 2-O-(4' hydroxy butyl) acetophenone.



The compound is not reported earlier from any other plant source.

8. Conclusion

Extracted and separated compounds have been identified as 4-hydroxy-2-(3, 7- dimethyl-octa, 2-6, diphanyle) 6-methoxy acetophenones and 4, 6-dihydroxy, -2-0-(4 hydroxy butyl) acetophonone, whose melting points are 225^{0}C and 280^{0}C respectively. Elemental analysis and molecular weight determination have established that compound I has molecular formula as C_{19} $H_{26}O_3$ while compound II has molecular formula as $C_{12}H_{16}O_5$.

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