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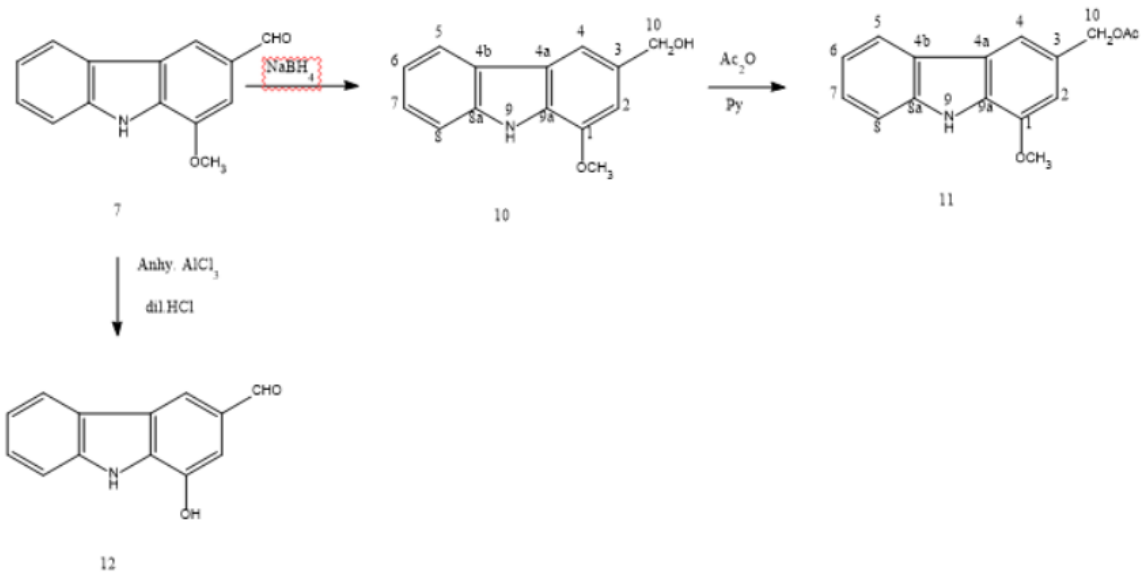
Anti-asthma Activity of Naturally Occurring Carbazole Alkaloids and Their Derivatives

Mumu Chakraborty

Government Girls' General Degree College,7, Mayurbhanj Road, Kolkata – 700023, West Bengal, India

Abstract:

Murrayakoenigii and *Glycosmispentaphylla* are rich sources of carbazole alkaloids. Some of these carbazole alkaloids have shown potent biological activities. In the present work, three different derivatives of the naturally occurring carbazole alkaloid murrayanine **7** were prepared in search of bioactive organic substances. One of the carbazole alkaloids among these have shown potent anti-asthma activitycomparable to the known PDE4 inhibitor, rolipram. This paper is on isolation, characterisation, derivative preparation and determination of biological activities of some carbazole alkaloids obtained from these two plants.



Keywords: *Murrayakoenigii*, *Glycosmispentaphylla*, Carbazole alkaloids, anti-asthma activity

1. Introduction:

Asthma is a non-infectious chronic inflammatory disease of the respiratory system characterized by a reversible airways obstruction. Despite the increase in the prescribed anti-asthmatic treatments, the current trends indicate asthma is set to be the most chronic disease in industrialized countries, affecting mostly the children than the adults.

Chronic obstructive pulmonary disease (COPD) is the most common of all the respiratory disorders in the world. The WHO predicts COPD will become the third most common cause of death world over by

2020 accounting 8.4 million lives [1]. Although asthma for the last 25 years has been managed therapeutically, with a combined bronchodilator and anti-inflammatory therapies, in contrast to this COPD have no effective treatments currently, while the efficacy of the corticosteroids is controversial. Hence, there is an urgent need to develop novel anti-inflammatory drugs having both the bronchodilatory and anti-inflammatory activity, having applicability to treat both COPD as well as asthma. Development of novel PDE-4 inhibitors [2,3] in therapeutic applications has gained importance from the early 1990s. PDE-4 is the selective phosphodiesterase enzyme that metabolises the cAMP. Hence, PDE-4 inhibitors prevent the inactivation of cAMP. The role of cAMP as a second messenger is well established and it modulates the response of immune cells to a variety of stimuli.

The presence of PDE-4 inhibitor in natural product has also been reported earlier. Rolipram, a catechol based compound, is the most effective PDE-4 inhibitor [4] .The plant *Murrayakoenigii*Spreng belonging to the family Rutaceae is native to India and now distributed in most of southern Asia. The leaves of this plant are well-known as curry leaves and have been used as one of the important herbs of south Indian cooking. Various parts of the plant have been used in traditional medicine for the treatment of headache, toothache and stomachaches; influenza, rheumatism, traumatic injury, insect and snake bites and as an antidysentric [5] as well as an astringent. Intake of the leaves can increase digestive secretions and relieve nausea, indigestion and vomiting.

The genus *Glycosmis* belonging to the family Rutaceae consists of nearly 11 species [6]. *Glycosmispentaphylla* (Retz.) is a small (1.5–5 m) tree widely distributed from India, Malaysia and southern China to the Philippine Islands where it occurs in tropical forests at low altitudes. It has been used as a folk medicine in the treatment of fever, liver complaints and certain other diseases.

Both the plants are rich in carbazole alkaloids. A number of monomeric as well as dimericcarbazole alkaloids have been isolated from both the plants [7,8]. Preparation of different derivatives of these naturally occurring carbazole alkaloids have also been reported [9,10,11].

In this present work, a number of naturally occurring carbazole alkaloids and their derivatives were tested for their PDE-4 inhibition activity. One of them showed potent PDE-4 inhibition activity comparable to the known PDE4 inhibitor, rolipram.

2. Results and Discussions:

In the present work, six carbazole alkaloids were isolated from the leaves and stem bark of *Murrayakoenigii*; namely murrayakoeninol**1**, koenidine**2**, koenimbine**3**, girinimbine**4**, mahanimbine**5**, O-methylmurrayamine-A **6** and murrayanine**7**. Investigation on the root bark of *Glycosmispentaphylla* also resulted in the isolation of two carbazole alkaloids viz.glycozoline**8**and glycozolidine**9.**Structures of all the compounds were determined on the basis of 1D and 2D NMR spectral data analysis. Different derivatives of the carbazole alkaloids were also prepared and tested for biological activity.

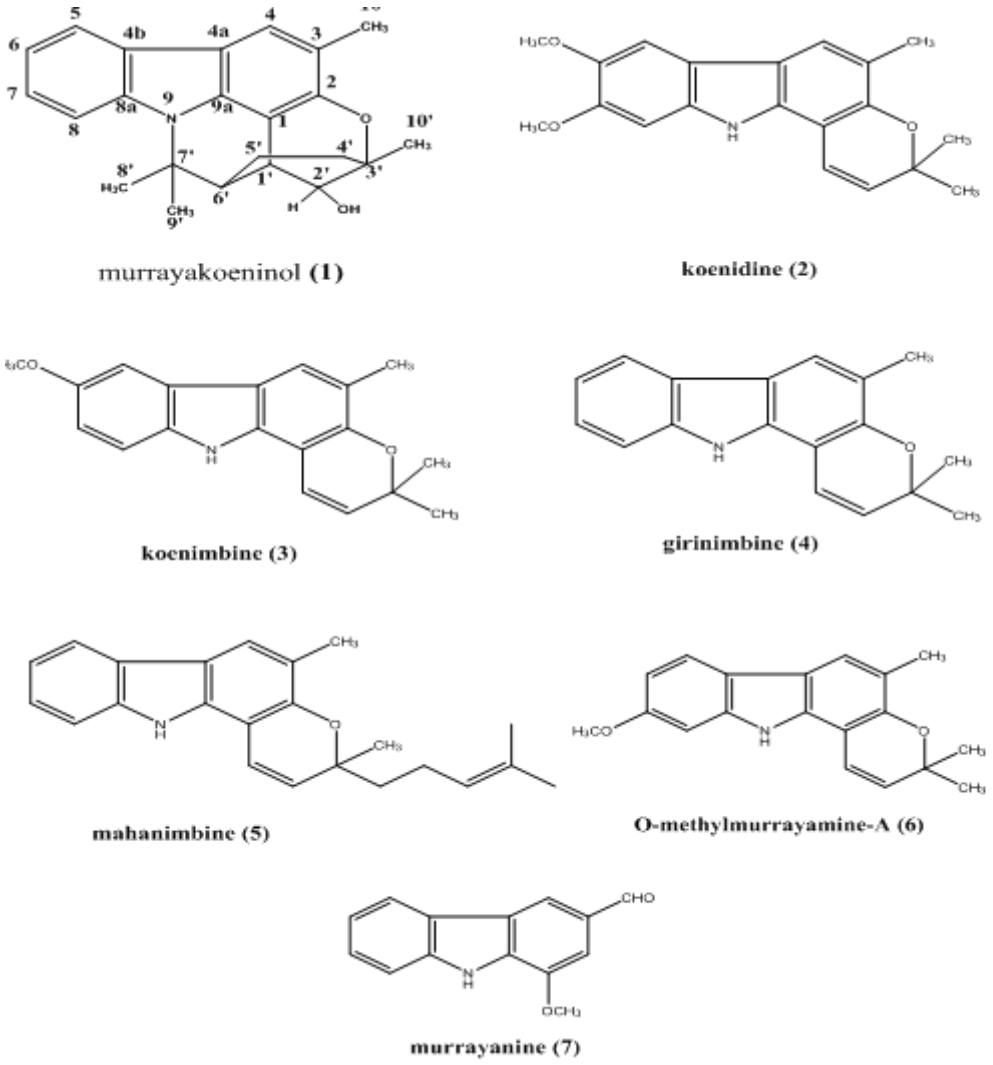


Fig 1: Structure of the compounds isolated from *Murrayakoenigii*

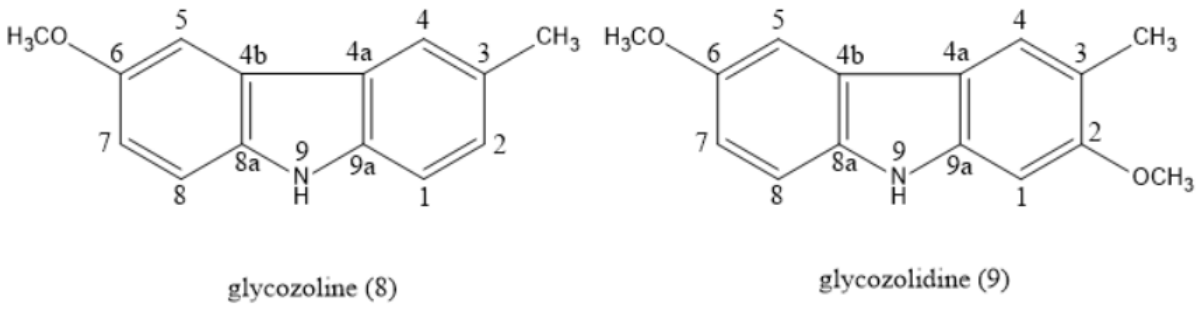
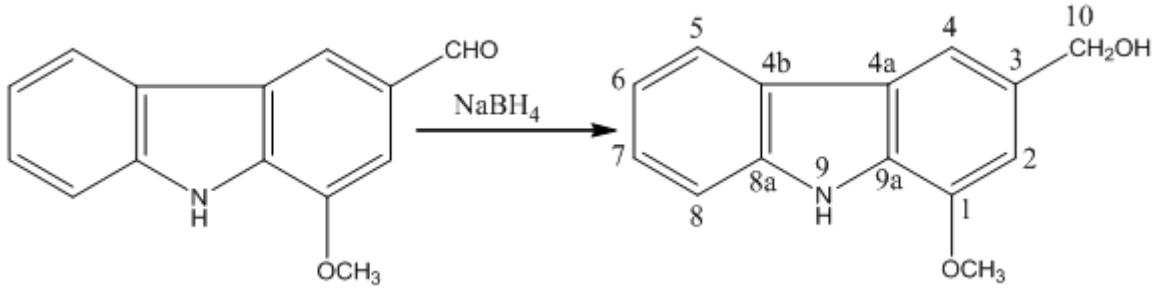


Figure 2: Structure of the compounds isolated from *Glycosmispentaphylla*

Preparation of Different Derivatives of Murrayanine (7):

i) Reduction of Murrayanine (7):

Treatment of murrayanine with $NaBH_4$ resulted in reduction of its aldehyde group.Structure ofthe product **10** was established by comparison of its spectral data with that of murrayanine**7**. The singlet at δ10.06 (for –CHO) in the ^{1}H-NMR spectrum of murrayanine was disappeared in the product. Two new signals at δ4.82 and 3.79(-CH_2OH) appeared in the ^{1}H-NMR spectrum of the reduced product. Detailed spectral data has been summarized in Table-1.

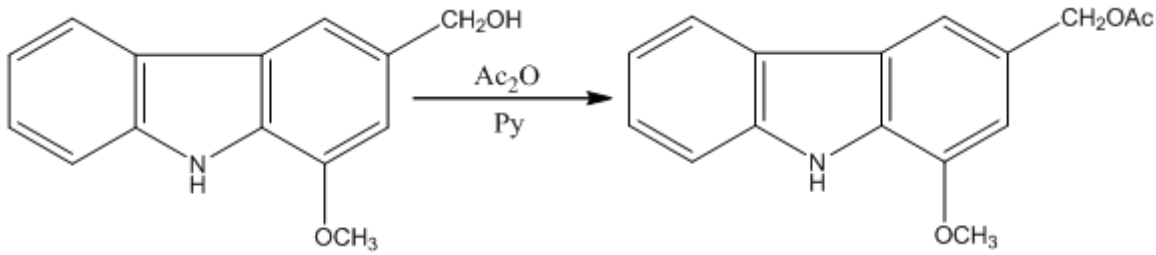


7 **10**

Scheme 1: Reduction of murrayanine (7)

ii) Acetylation of the reduced product (10) of murrayanine :

Compound **10**, on treatment with acetic anhydride and pyridine furnished **11**. A 3H singlet at δ2.12 appeared for the –$COCH_3$ group in the ^{1}H-NMR spectrum of the acetylated product. Detailed spectral data has been summarized in Table-1.

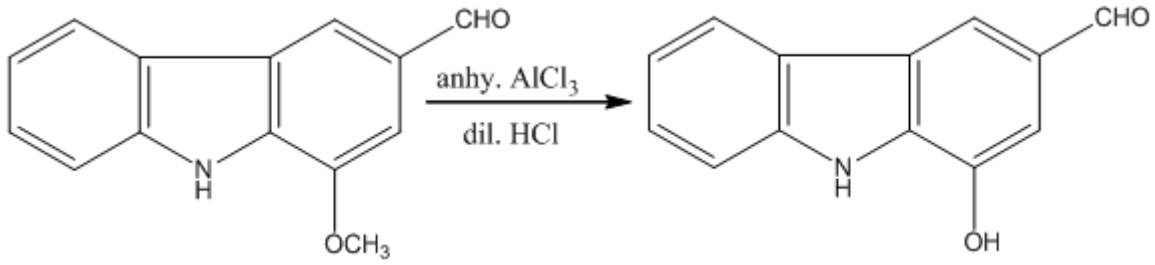


10 11

Scheme 2: Acetylation of the reduced product of murrayanine**(10)**

iii) Demethylation of murrayanine (7) :

Treatment of murrayanine**(7)** with anhy. $AlCl_3$ afforded the demethylated product **12**. The signal at δ4.08 (3H, s) in murrayanine**(7)** for aromatic methoxy group disappeared in the ^{1}H-NMR spectrum of **12**. ^{13}C-NMR spectrum also showed no quartet. Thus structure of the product was confirmed to be **12**. Detailed spectral data has been summarized in Table-1 and 2.



7 **12**

Scheme 3: Demethylation of murrayanine (7)

Anti-Asthma Activity

In the present work, the naturally occurring carbazole alkaloids and their derivatives were tested for their biological activities.

Among all the compounds, one compound (coded as: ICB/11/D-8) showed very significant biological activity. So detailed biological investigation on this compound was done. As shown in Figure 3, with increasing dose of ICB/11/D-8 the PDE-4 activity was inhibited significantly. The extent of inhibition of PDE4 activity by ICB/11/D-8 is comparable to known PDE4 inhibitor, rolipram. The IC_{50} value of ICB/11/D-8 is 4 ng/ml.

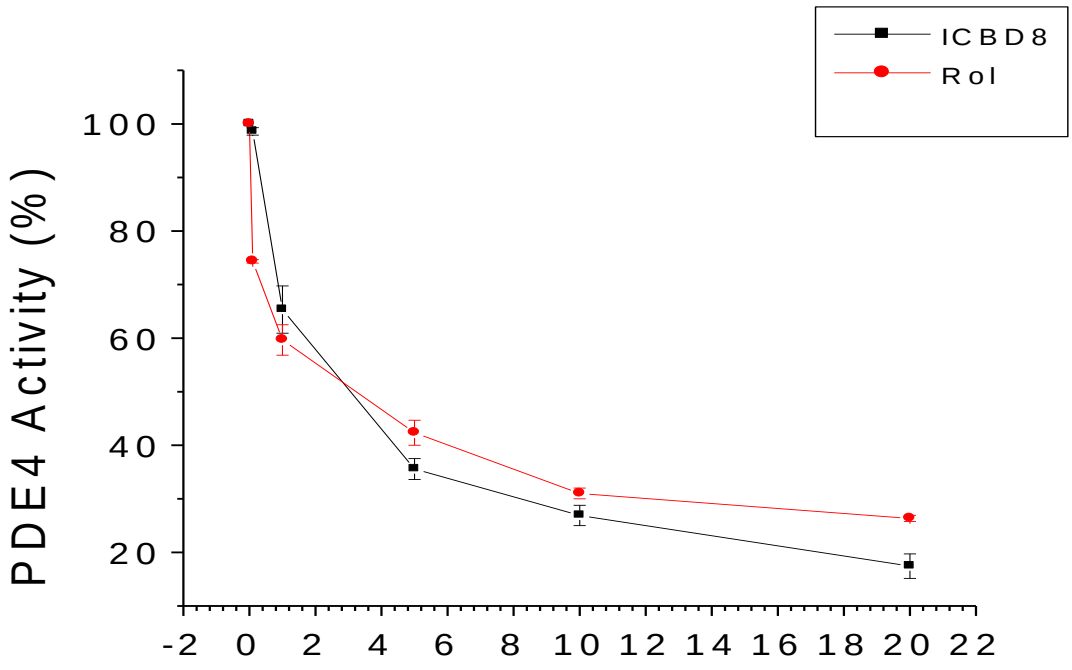


Figure 3. PDE4 inhibition activity of ICB/11/D-8isolated from *Murrayakoenigii*

Table 1.:^{1}H NMR Chemical shifts (δ, $CDCl_3$, 600 MHz) of compounds 7, 10, 11, 12

Proton	7	10	11	12
	δ_H(J in Hz)	δ_H(J in Hz)	δ_H(J in Hz)	δ_H(J in Hz)
H-2	7.49 s	8.31 s	6.89 s	7.36 s
H-4	8.21 s	7.64 s	7.69 s	8.21 s
H-5	8.12 d (J=7.8Hz)	8.02 d (J=7.7Hz)	8.03 d (J=7.8Hz)	8.13 d (J=7.8)
H-6	7.32 m	7.42 m	7.45 m	7.25 t (J=7.6Hz)
H-7	7.50 m	7.24 m	7.45 m	7.44 t (J=7.6Hz)
H-8	7.53 (J=7.8Hz)	6.94 m	7.25 m	7.56 d (J=8.1Hz)
H-9	8.59 s	-	8.35 s	-
H-10	10.06 s	4.82 s	5.27 s	9.94 s
CH_3O-1	4.08 s	3.98 s	4.00 s	-
-OH	-	3.79 s	-	-
-$OCOCH_3$	-	-	2.12 s	-
-CHO	10.06			

Table 2.:^{13}C-NMR Chemical shifts (δ, $CDCl_3$, 150 MHz) of compounds 7, 12

Carbon	7 (δ_c in ppm)	12 (δ_c in ppm)
C-1	146.1	141.0
C-2	103.5	107.2
C-3	130.2	130.0
C-4	120.4	119.1
C-4a	120.72	109.0
C-4b	123.6	124.0
C-5	120.72	120.3
C-6	120.71	119.9
C-7	126.6	126.3
C-8	111.5	111.7
C-8a	139.4	141.0
C-9a	134.1	125.0
C-10	191.9	193.1
CH_3O-1	55.8	-

3. Experimental:

General experimental procedure:

TLC was carried out on silica gel 60 F_{254} (Merck) plates and spots were visualized by spraying with Liebermann-Burchard reagent with heating at 120ºC. Column chromatography was performed on silica gel mesh 60-120 (Merck). The mass spectra were recorded on a Q-TOF-Micromass spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded using a BRUKER AVANCE 600 MHz NMR with TLC-cryoprobe using TMS as internal standard. IR spectra were recorded on a JASCO FT-IR Model 140 using sample as KBr pellets. Optical rotation was recorded at 28.9ºC in P-1020 JASCO polarimeter. Data are presented as follows: Chemical shift (in ppm on the δ scale relative to $\delta_{TMS} = 0$), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br.= broad), coupling constant (*J*/Hz). ^{1}H NMR and ^{13}C NMR spectra were recorded at 600 MHz and 150 MHz respectively.

Plant material:

The dried leaves and stem bark of *Murrayakoenigii* were collected from Shantiniketan (India).A voucher specimen has been deposited at IICB, Kolkata, India.

The dried root bark of *Glycosmispentaphylla* was collected from Jhargram (India).A voucher specimen has been deposited at IICB,Kolkata,India.

Extraction and isolation:

The powdered and dried leaves of *Murrayakoenigii* (2 Kg.) were extracted with MeOH at room temperature and concentrated under vacuum at 40ºC to afford 15 g of extract. The MeOH extract was chromatographed on a column of silica gel (mesh size 60-120). Gradient elution was carried out with petroleum ether (bp 60-80ºC)followed by various mixtures of petroleum ether and benzene (3:1, 1:1, 1:3 and 100% benzene) and again various mixtures of benzene and chloroform (200 mL. each). Fractions giving similar spots were combined. Repeated chromatography of the fractions resulted in the isolation of murrayakoeninol **(1)** (with benzene as eluent) together with *O*-methylmurrayamine-A **(6)**, koenidine **(2),** koenimbine **(3)** and mahanimbine **(5)**. The powdered and dried stem bark of *Murrayakoenigi*(1 Kg) was extracted with MeOH at room temperature and concentrated under vacuum at 40ºC. Then the MeOH extract (8 g) was fractionated into three parts: petroleum ether, ethyl acetate and aqueous. The ethyl acetate fraction was kept overnight. A solid was precipitated from it, which was insoluble in chloroform. The solid was filtered, washed with cold chloroform and dried. The compound was characterized to be girinimbine **(4)** by TLC (Benzene) and comparison of the spectroscopic data with authentic sample. The EtOAc extract was chromatographed on a column of silica gel (mesh size 60-120). Gradient elution was carried out with petroleum ether (bp 60-80ºC) followed by various mixtures of petroleum ether and benzene (3:1, 1:1, 1:3 and 100% benzene) and again various mixtures of benzene and chloroform (200 mL. each). Fractions giving similar spots were combined. Repeated chromatography of the fractions resulted in the isolation of rest of girinimbine **(4)** and murrayanine **(7)** (with Petroleum ether: benzene = 1:1 as eluent).

2 kg of powdered and dried root bark of *Glycosmispentaphylla* was extracted with petroleum ether followed by methanol at room temperature. The petroleum ether extract (10 gm) was chromatographed on a column of neutral alumina. Gradient elution was carried out with petroleum ether followed by various mixtures of petroleum ether-$CHCl_3$ (3:1, 1:1 and 1:3) and MeOH in $CHCl_3$ (5%,10%,20% and

30%; 200ml each). Compound **8** and **9** were eluted from this column with a 3:1 mixture of petroleum ether-$CHCl_3$.

Reduction of murrayanine (7)

25 mg of murrayanine**(7)** was dissolved in 10 ml of distilled methanol. 1 pinch of $NaBH_4$ was added to it. The reaction mixture was stirred at room temperature for 24 hours using a magnetic stirrer. Progress of the reaction was monitored by TLC. Methanol was evaporated under reduced pressure. Then the product was extracted with $CHCl_3$ using a separating funnel and washed with water (3 times). Then again it was concentrated under reduced pressure. TLC (Solvent system: Benzene: EtOAc = 6:1) showed the presence of a prominent spot with very little amount of impurity. The major product **10** was purified by preparative TLC using Benzene: EtOAc = 6:1 as solvent system. MS m/z 227$[M]^+$, ($C_{14}H_{13}NO_2$); ^{1}H NMR(Table-1).

Acetylation of reduced product of murrayanine

2 drops of both acetic anhydride and pyridine was added to compound **10** and kept overnight. TLC (Solvent system: Benzene: EtOAc = 6:1) showed the presence of a single product. Ac_2O and Pyridine were removed by repeated distillation under reduced pressure with toluene. Then the product was extracted with $CHCl_3$ using a separating funnel, washed with water (3 times), dried over Na_2SO_4, filtered and concentrated under vacuum. ^{1}H NMR data confirmed the structure of the product as **11.**MS m/z 269$[M]^+$, ($C_{16}H_{15}NO_3$); ^{1}H NMR(Table-1).

Demethylation of murrayanine (7)

25 mg of murrayanine**(20)** was dissolved in 15 ml of dry and distilled benzene. 1 pinch of anhy.$AlCl_3$ was added to it. The reaction mixture was refluxed in a water bath for 6 hours. Progress of the reaction was monitored by TLC. Benzene was evaporated under reduced pressure. The product was acidified with 15%HCl, extracted with $CHCl_3$ using a separating funnel and washed with water (3 times). Then again it was concentrated under reduced pressure. TLC (Solvent system: Benzene: EtOAc = 4:1) showed the presence of a single spot. ^{1}H and ^{13}C NMR data confirmed the structure of the product as **12.** Q-TOF-MS showed (m/z) 211$[M]^+$ ($C_{13}H_9NO_2$); ^{1}H NMR(Table-1) and ^{13}C NMR (Table-2).

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