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Studies on the Isolation of Parahydroxy Benzoic Acid from the Leaves of *Cassia Alata*

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Abstract

Studies were carried out on the leaves of *Cassia alata*. Parahydroxy benzoic acid was isolated from the leaves of *Cassia alata* with the help of column and thin layer chromatography using a gradient of organic solvents with increasing polarity. The compound was characterized on the basis of UV, IR, ^{1}H-NMR and Mass spectrometry.

Introduction

A large and predominantly tropical genus of about 580 species of herbs, shrubs and trees, with about twenty representatives including alata are found in Indo-Pak-Bangladesh subcontinent.[1] The tree is often cultivated by the Indians for the sake of its leaves which are held in high esteem as a local application in skin diseases. A belief in their powers of this character prevails also in the West Indies, Brazil, Mauritania, Java and other tropical countries. Their efficiency, especially in Herpescircinatus, is confirmed by Mekenna[2] and others. The leaves taken internally act as an aperient.[3,4] George[4] remarks that according to the Telinga and Tamil Physicians, the leaves cure all poisonous bites as well as venereal affections and strengthen the body. The fresh leaves are often employed to cure ringworm.[4,5]

Earlier chemical investigations of *Cassia alata* mentioned only some anthraquinones,[7,8,9] rhein and its reduced from with other unidentified anthraquinone derivations.[3]

Despite some works done on the anthraquinones, reports on the isolation of other forms of compound from *Cassia alata* are lacking. So, the present work had, therefore, been taken with a view to carry on a complete investigation for the isolation of compounds other than anthraquinones from the leaves of *Cassia alata* and accordingly Parahydroxy benzoic acid was isolated from the plant. The structure of Parahydroxy benzoic acid was elucidated by using spectroscopic techniques.

Materials and Methods

A Reichart micro melting point apparatus was used for recording the melting point. UV spectra (MeOH) was recorded on a Shimadzu UV-240 spectrophotometer, IR spectra (KBr) on a Shimadzu IR-460 instrument, ^{1}H-NMR spectra (CD_3OD) on a Bruker AM-500 FT NMR spectrometer (500 MHz) using TMS as internal standard and Mass spectra on a Varian-MAT 112S spectrometer. Electron Impact (EI) and Peak Matching experiments were performed on a MAT-312A mass spectrometer.

Fresh leaves were collected from the plants grown in the adjoining areas of BCSIR Laboratories, Rajshahi campus during August-September period. The leaves were washed with water to remove extraneous materials and then dried in shade. Care was taken to avoid exposure to sunlight. The dried material was crushed to powder.

The air-dried *Cassia alata* leaf powder (6.60 Kg) was soaked in 80 % ethanol for a week. The ethanolic extract was then filtered and the solvent was removed under reduced pressure to obtain a viscous residue (487 g). The crude ethanolic extract was then defatted with n-hexane. The n-hexane solvent was then removed under reduced pressure to yield the residue (160 g).

The defatted extract was then treated with water, shaken well to resolve into water soluble and water insoluble parts. The water soluble part was extracted with ethyl acetate. The ethyl acetate soluble part was chromatographed over a silica gel (70-230 mash) column and successively eluted using increasing polarity of ethyl acetate and methanol. Elution of the column with ethyl acetate : methanol (60 : 40 v/v) afforded compound **1** along with minor impurities.

Purification of the compound by Preparative thin layer chromatography (PTLC)

The compound **1** with some impurities was applied to a PTLC card of silica gel $^{60}GF_{254}$ (thickness 0.1mm) and eluted with ethyl acetate : methanol (9 :1 v/v). A distinct single band (R_f 0.69) was observed on the PTLC card. The band was collected and washed out with ethyl acetate to obtain a crystalline solid (compound **1**, 10.9 mg, m.p 215 - 216^{O} C, R_f = 0.69).

Spectroscopic analysis of compound 1

Uvλ_{max} (MeOH)nm : 254, 203, 196

IRυ_{max} (KBr) cm^{-1} :3350 (O - H), 1445, 840 (C - H), 1675 (C = 0), 1601 (C = C)

EIMS $^{m}/_{z}$ (rel. int %) : 138(74), 121(100), 110(1), 93(26), 81(3), 65(32), 53(7).

Peak matching $^{m}/_{z}$ (formula) : 138.02643 ($C_7H_6O_3$).

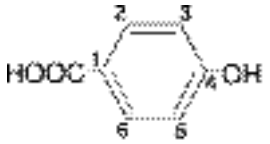
^{1}H-NMR (500MHz) δ_{TMS} : (CD_3OD)

δ7.84 [H-2, H-6, 2H, dd, $J_{(H\text{-}2,\ H\text{-}3)}$ 8.75 Hz, $J_{(H\text{-}2,\ H\text{-}6)}$ 2.19 Hz]

δ6.76 [H-3, H-5, 2H, dd, $J_{(H-3,\ H-2)}$ 8.75 Hz, $J_{(H-3,\ H-5)}$ 2.19 Hz]

Results and Discussion

The ethyl acetate triturate of the ethanolic extract of *Cassia alata* leaves yielded compound **1** as a crystalline solid after purification by preparative TLC. The IR spectrum (KBr) showed a band at 3350 cm^{-1} indicating the presence of hydroxyl group. The presence of carboxylic group was supported by OH stretching at 3000 cm^{-1} and C = O stretching at 1675 cm^{-1}. The absorption band at 1601 cm^{-1} was assigned to C = C stretching of aromatic skeleton vibration. It showed absorption bands at 1445 cm^{-1} and 840 cm^{-1} for C-H bending vibration.



Parahydroxy benzoic acid

The EI mass spectrum showed the molecular ion as well as base peak at $^{m}/_{z}$ 138. The molecular formula was established with the help of ^{1}H-NMR and peak matching experiments as $C_7H_6O_3$ corresponding to the mass $^{m}/_{z}$ (138.03349).

The compound was identified by ^{1}H-NMR spectroscopy (CD_3OD, 500 MHz). The peaks in the region δ6.74 to δ7.86 indicated the presence of benzene ring. The peaks at δ6.74 to δ6.77 and δ7.82 to δ7.86 were observed due to four protons, two protons each forming a magnetically equivalent group exhibiting two double doublets which suggest that two substituted groups are attached at positions 1 and 4. Two protons at 2 and 6 positions gave a double-doublet at δ7.84 due to coupling between H-2, H-3 protons and H-2 and H-6 protons having coupling constants 8.75 and 2.19 Hz respectively. Two protons at 3 and 5 positions gave another double-doublet at δ6.76 due to coupling between H-3, H-2 protons and H-3, H-5 protons having coupling constants 8.75 and 2.19 Hz respectively.

From the exact matching of the UV, IR, ^{1}H-NMR and mass spectral evidences compound **1** was characterized as Parahydroxy benzoic acid.

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