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Isolation and characterization of chemical compounds from flowers of *Cassia alata*

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ABSTRACT

Flowers of cassia alata (commonly known as amaltas or vilayti aghatia) were subjected to column chromatography. The Column was eluted with solvents of different polarities, starting from less polar solvent. It yielded three compounds. On the basis of analytical data, spectral data (UV, IR, ^{1}H NMR) and comparison with authentic samples, these compounds were characterized as stearic acid, alanonal- an anthraquinone and β-sitosterol β-D-glucoside.

Key words: cassia alata, column chromatography, alanonal.

INTRODUCTION

From time immemorial human being is getting food, clothing, shelter and medicines from plants[1].Cassia alata L. (Fabaceae) is an ornamental shrub introduced in India from West Indies. It is a medicinal plant having antinematode[2], antimicrobial[3], antiallergic[4] activities along with purgative effect. Its leaf extract induces cytotoxic effect[5]. According to Telinga and Tamul physicians, cassia alata cures all poisonous bites and veneral outbreakings and also strengthens the body[6]. In the gold coast, its leaves are crushed, mixed with black pepper and are applied to dhoby-itch, craw-craw and ringworm on the head or skin. It is one of the most effective among native medicines[7]. A decoction of the leaves of cassia alata is a good mouth wash[8]. Using column chromatography, chemical compounds from flowers were extracted and characterization was done on the basis of analytical data, spectral data and comparison with authentic samples.

MATERIALS AND METHODS

Chemicals and solvents used were of LR grade and purchased from local vendors. Solvents were purified by standard methods. The absorbents used were silica gel (60-120 mesh) and silica gel-G. Flowers of cassia alata were extracted with hot methanol. Extract was mixed with equal amount of silica gel (60-120 mesh) and dried over hot water bath. The column (silica gel, 70X 10cm) was fed with the dried extract. The successive eluents were petroleum ether(10L), benzene-petroleum ether(1:3, 20L), benzene-petroleum ether(1:1, 20L), benzene(10L), ethyl acetate-benzene(1:19, 10L), ethyl acetate-benzene(1:9, 10L), ethyl acetate-benzene(1:3, 20L), ethyl acetate-benzene(1:1, 30L). The flow rate of column was 15 drops per minute. UV spectral data was taken from HAU, Hisar while IR and ^{1}H NMR data were taken from P. U. Chandigarh.

RESULTS AND DISCUSSION

Column chromatography of the flowers of cassia alata afforded three compounds: First compound in benzene-petroleum ether (1:1), second in ethyl acetate- benzene (1:3) and third in ethyl acetate- benzene (1:1) mixtures.

Compound I: It was crystallized from benzene as shining white crystals, m. pt. 72^0 C (lit.m.pt[9]. 71.5- 72.0^0 C). IR: λ_{max}^{Nuzol} (cm^{-1}): 719, 789, 1073, 1172, 1221, 1377, 1472, 1545, 1685, 1736, 2969, 3470.

^{1}H NMR (δ, $CDCl_3$): 2.03 (t, 2H, J = 5 Hz), 1.33 (s, 30 H, 15 X – CH_2-), 0.88 (br s, 3H, - CH_3).

The IR spectrum of compound I showed the presence of carbonyl (1685 cm^{-1}) and hydroxyl (3470 cm^{-1}) groups. The ^{1}H NMR spectrum showed no peak in aromatic region, which was indicative of its aliphatic nature. A singlet at δ 1.33 for 30 protons could be due to fifteen methylene groups. A broad singlet integrating to three protons observed at δ 0.88 could be due to aliphatic methyl group. A triplet at δ 2.03 (J= 5Hz) for two protons could be due to methylene group vicinal to the carboxylic group. It became evident that compound I had sixteen methylene groups in a straight chain with a methyl group at one end and carboxylic group at other end. The compound could be octadecanoic acid, that is, stearic acid. A direct comparison with the authentic sample of stearic acid confirmed the identity of compound I as stearic acid.

$CH_3(CH_2)_{15}CH_2COOH$

Compound II: It was crystallized from ethyl acetate as orange needles, 100 mg, mp > 300^0C. It gave no colour with alcoholic ferric chloride. It gave red colour with methanolic sodium hydroxide. Its Rf value was 0.27 in acetic acid-benzene (1:49) mixture.

Elemental Analysis: Found: C 63.2, H 2.4, $C_{15}H_8O_6$; Required: C 63.4, H 2.8%.

UV-Visible: λ_{max}^{MeOH} (nm): 230, 260, 430.
$\lambda_{max}^{MeOH-NaOH}$ (nm): $210, 234, 260, 319(sr), 516$.

Methyl ester: The compound (20mg) was mixed with dimethyl sulphate (1.0 ml), anhydrous potassium carbonate (3.0 gm) and dry acetone (20 ml). The mixture was refluxed for four hours under anhydrous conditions. As the reaction completed, mixture was filtered and potassium carbonate was separated. A yellow solid having mp 200^0C was obtained. The Rf value was 0.34 in acetic acid-benzene (1: 49) mixture.

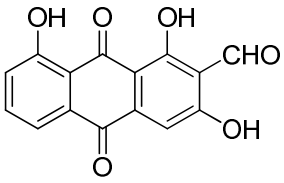
Elemental Analysis: Found: C 65.2, H 3.9, $C_{18}H_{14}O_6$; Required: C 65.8, H 4.2%.; λ_{max}^{Nuzol} (cm^{-1}): 744, 979, 1075, 1182, 1233, 1283, 1343, 1415, 1444, 1462, 1585, 1674, 1731, 2913, 2928.; ^{1}H NMR (δ, $CDCl_3$): 8.45 (s, 1H, CHO-2), 7.97 (s, 1H, H-4), 7.94 (dd, 1H, J= 7.5 Hz and 2.5 Hz, H-5), 7.69 (t, 1H, J= 7.5Hz, H-6), 7.33 (dd, 1H, J= 7.5 Hz and 2.5 Hz, H-7), 4.0 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3).

Colour reactions with alcoholic magnesium acetate and alcoholic sodium hydroxide hinted towards an anthraquinone skeleton. The absorption in the UV and visible regions at 260 nm and 432 nm confirmed anthraquinone nucleus. In the presence of sodium hydroxide, the band in the visible region appeared at 516 nm and this confirmed 1,8- dihydroxylation. The elemental analysis of the compound suggested its molecular formula $C_{15}H_8O_6$.

Its methyl ester was prepared with dimethyl sulphate under anhydrous conditions. ^{1}H NMR of methyl ether in $CDCl_3$ showed three absorption at δ 4.0, δ 3.98, δ 3.96, each integrating to three protons. These were assigned to three aromatic methoxyls. In the aromatic region there was double doublet integrating to one proton at δ 7.33 with J= 2.5Hz and 7.5 Hz. There was a triplet at δ 7.69 with J= 7.5 Hz and it integrated to one proton. These three signals were assignable to three protons of monosubstituted aromatic ring of the anthraquinone. There was a singlet at δ 7.97 integrated to one proton. This represented a peri-proton of another trisubstituted ring of anthraquinone. There was a singlet at δ 8.45 integrated to one proton. This was assignable to an aldehydic proton. This suggested that the derived methyl ether had one formyl group and three methoxy groups. On this basis the molecular formula of the methyl ether could be calculated to be C_{18} H_{14} O_6. This formula of methyl ether was confirmed by elemental analysis. A comparison of molecular formula of original compound (C_{15} H_8 O_6) and that of methyl ether (C_{18} H_{14} O_6)

confirmed that the former was a trihydroxy compound. Thus the original compound had one formyl group and three hydroxyl groups.

In the IR of original compound, there were carbonyl peaks at 1692, 1678 and 1629 cm^{-1}. The peak at 1692 cm^{-1} was due to CO of aldehydic group. The two peaks of the anthraquinone carbonyls confirmed that one CO was chelated and another was non chelated. The original compound did not respond to colour reaction with ferric chloride. This ruled out the presence of vicinal hydroxyls. It has been observed that anthraquinones like chrysophanol, physcion, emodin and alatinone do not respond to colour reaction with alcoholic ferric chloride[10]. On the other hand when two hydroxyls are vicinal as in 6-O-methyl-alaternine, a colour reaction is obtained with ferric chloride[11]. The structure of original compound consistent with its properties was settled as 2-formyl 1, 3, 8-trihydroxyanthraquinone.



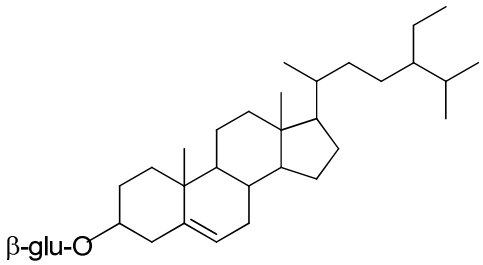
Alanonal

Compound III: It is crystallized from ethyl acetate as brownish-white crystalline powder, 40 mg, mp 280^0C (lit. mp[8], 283-286^0C). It responded to Liebermann-Burchard reaction giving green colour. It gave positive Molisch test. The R_f value in ethyl acetate- methanol- water (40:6.4:5.4) was 0.40.

Acetate: The compound (10mg), pyridine (0.3 ml), and acetic anhydride (0.8 ml) were heated on a water bath for two hours. On keeping overnight and adding ice, it was filtered, yielding a white solid having mp 175^0C. Its R_f value in ethyl acetate was 0.18.

^{1}H NMR (δ, $CDCl_3$): 5.40-4.16 (m, 8H, H-6 and sugar protons), 2.0 (br s, 12H, 4X OAc), 1.20 (s, 6H, 2X Me), 0.94 (s, 6H, 2X Me), 0.80 (s, 3H, Me), 0.60 (s, 3H, Me), 1.33-1.70 (m, 29H, 7X =C-H- and 11X –CH_2).

The ^{1}H NMR of the acetate in $CDCl_3$ showed signals in the range δ 5.40-4.16 for eight protons which could be seven protons of sugar and one olefinic proton of the sterol. At δ 2.0 there was a broad singlet for twelve protons assignable to four acetoxyl groups of sugar. The region δ 1.33-1.70 was characterized by a multiplet for twenty nine protons which could be methine and methylene protons. A singlet at δ 0.94 for six protons could be due to two methyl groups. Three singlets, each for three protons at δ 1.20, δ 0.80 and δ 0.60 were indicative of three methyl groups. Killiani hydrolysis of compound III yielded a sugar and an aglycone. A direct comparison with an authentic sample, the sugar was confirmed as glucose and the aglycone as β- sitosterol. An authentic sample[10] was directly compared and it confirmed identity of compound III as β-sitosterol β-D-glucoside.



β-Sitosterol-β-D-Glucoside

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