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Anthraquinones and Other Constituents from the Stem Bark of *Cassia nodosa*

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Thirteen compounds including four anthraquinones were isolated from the stem bark of *Cassia nodosa*. Their structures were elucidated as palmitic acid, lupenone, lupeol, β-sitosterol, stigmasterol, β-sitosterol-β-D-glucoside, eicosanoic acid, ethyl cerotate, chrysophanol, physcion, 1,5-dihydroxy-3-methoxyanthraquinone, 1,5-dihydroxy-3-methoxy-7-methylanthraquinone and pinitol. All of these, except chrysophanol have been isolated for the first time from the stem bark of this plant.

Key Words: *Cassia nodosa*, Caesalpiniaceae, Triterpenoids, Anthraquinones, Sterols, Pinitol.

INTRODUCTION

The genus *Cassia* commonly called as 'Senna' belonging to the family Caesalpiniaceae comprises *ca.* 400 species of herbs, shrubs and trees with about 24 representatives native to India[1]. *Cassia nodosa* Buch.-Ham. is an annual tree grown in gardens for ornamental purpose. The leaves are purgative, used for poulticing boils and as a refrigerant[2]. The present work was undertaken since only one reference on the chemical investigation on its stem bark is available[3].

The chromatographic separation of the petroleum ether, benzene and ethyl acetate fractions from the stem bark of *Cassia nodosa* led to the isolation of 13 compounds, *viz.*, palmitic acid, eicosanoic acid, ethyl cerotate, lupenone, lupeol, β-sitosterol, stigmasterol, β-sitosterol-β-D-glucoside, chrysophanol, physcion, 1,5-dihydroxy-3-methoxyanthraquinone, 1,5-dihydroxy-3-methoxy-7-methylanthraquinone and pinitol. All of these except chrysophanol have been isolated for the first time from the stem bark of this plant. This paper describes the isolation and characterization of these compounds.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were recorded on Jeol AL 300 MHz instrument using $CDCl_3$ and DMSO-d_6 as solvents and TMS as the internal reference. EIMS spectra were recorded on a Hitachi model RMU 6E and Jeol D-300 mass spectrometer. The IR spectra were recorded as KBr pellets on A 400S Shimadzu FTIR spectrometer. Column chromatography was run using silica gel (60-120 mesh) and TLC on silica gel G in different solvent systems. In general, ceric ammonium sulphate and UV light were used for visualization of TLC spots. However, for anthraquinones 10 % methanolic KOH was used. Melting points were determined on electrothermal melting point apparatus.

The stem bark of *Cassia nodosa* was collected from Rajasthan University campus, Jaipur during the rainy season. A voucher specimen has been deposited in the Herbarium [Sheet No. 20159], Department of Botany, University of Rajasthan, Jaipur and identified by one of the authors (Prof. S.C. Jain).

Extraction and isolation: Shade dried and coarsely powdered roots (2.9 kg) were extracted thrice with hot ethanol (95 %) under reflux. The extract was concentrated under reduced pressure to yield a brown semi-solid mass (99.5 g). This extract was fractionated successively with hot petroleum ether, benzene and ethyl acetate, which on concentration afforded the petroleum ether (2.4 g), benzene (1.9 g) and ethyl acetate (96.0 g) fractions. The petroleum ether and benzene fractions exhibited a similar TLC profile (benzene:ethyl acetate, 1:1), hence they were mixed together and chromatographed over a column of silica gel which afforded 6 compounds. Elution was carried out with solvents of increasing polarity *viz.*, petroleum ether, benzene and ethyl acetate. The eluates were collected in 250 mL. portions using stepwise gradient eluting with gradient of pure petroleum ether, petroleum ether:benzene (4 :1 → 1:4) to give 75 subfractions, subfractions 1-4, 20-25, 34-40, 48-52 and 55-69 were crystallized to give **1** (0.104 g), **2** (0.012 g), **3** (0.289 g), **4** (1.101 g) and **5** (0.093 g), respec-

tively. Further elution with gradient of benzene:ethyl acetate (4:1 → 1:4) gave 80-130 subfractions in which subfractions 98-108 were crystallized to give **6** (0.056 g). Ethyl acetate soluble fraction was applied over silica gel column using a solvent system of pure petroleum ether, petroleum ether:benzene (3:1 → 1:3) and pure benzene as an eluent to give 68 subfractions. Sub fractions 1-4, 7-9, 16-22, 25-37, 42-59 and 62-68 on crystallization gave **7** (0.051 g), **8** (0.056 g), **9** (0.029 g), **4** (0.561 g) and **5** (0.278 g) and **10** (0.062 g), respectively. Further elution with gradient of benzene:ethyl acetate (3:1 → 1:3) gave 101 subfractions, subfractions 75-78, 108-110, 138-150 and 168-175 were crystallized to give compound **11** (0.035 g), **12** (0.028 g), **6** (0.781 g) and **13** (0.075 g), respectively.

Compound 1 (palmitic acid): White amorphorous powder, m.p. 58-60 °C, Mass (m/z) : 256 [M^+], 239, 227, 213, 211, 199, 185, 171, 157, 143, 60. IR (KBr, ν_{max}, cm^{-1}): 3320-2700 (broad O-H stretching), 1730 (C=O stretching), 730 and 720 [doublet, -$(CH_2)_n$-deformation, n > 4].

Compound 2 (lupenone): White powder, m.p. 166-68 °C, Mass (m/z): 424 [M^+], 409, 381, 218, 205, 204, IR (KBr, ν_{max}, cm^{-1}): 2990, 2850, 1710, 1650, 1380, 1280, 1240, 980, 970 and 830. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 4.69 (1H, s), 4.57 (1H, s), 2.17 (2H, m), 1.68 (3H, s), 1.58-1.13 (23H, m), 1.03 (3H, s), 1.00 (3H, s), 0.96 (3H,s), 0.94 (6H, s) and 0.76 (3H, s).

Compound 3 (lupeol): White powder, m.p. 212-13 °C, Mass (m/z): 426 [M^+], 411, 408, 229, 218, 204, 180, IR (KBr, ν_{max}, cm^{-1}): 3450, 2950, 2890, 1640, 1385, 1360, 1310, 1290, 1270, 1110 and 880. ^{1}H NMR (300 MHz, $CDCl_3$), δ: 4.69 (1H, broad s), 4.56 (1H, broad s), 3.19 (1H, dd, *J* = 6, 4.8), 2.38 (1H, m), 1.92 (3H, s), 1.68-1.20 (2H, m), 0.96 (3H,s), 0.93 (3H, s), 0.87 (3H, s), 0.82 (6H, s) and 0.76 (3H, s).

Compound 4 (β-sitosterol): White needles, m.p. 135-137 °C, Mass (m/z): 414 [M^+], 399, 396, 386 (base peak), 381, 273, 271, 255, 231, 213. IR (KBr, ν_{max}, cm^{-1}): 3400 (-OH), 1640, 1050, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.27 (1H, t), 348 (1H, m) and 2.0-0.70 (47H, m, d, s).

Compound 5 (stigmasterol): White powder, m.p. 135-137 °C, Mass (m/z): 412, 397, 394, 328, 302, 273, 255, 213, 199, 159, 149, 105. IR (KBr, ν_{max}, cm^{-1}): 3400 (OH), 1460, 1380, 1260, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.36 (1H, t), 5.11 (1H, dd), 5.04 (1H, dd), 3.53 (1H, m), 2.29-1.25 (26H, m) and 1.16-0.68 (6×CH_3, s, d).

Compound 6 (β-sitosterol-β-D-glucoside): White granules, m.p. 285-287 °C, FAB Mass (m/z): (NBA) 599 [M+Na]$^+$, 411, 397, 383, 381, 273, 255, 242, 226, 213, 209, 178, 165, 128, 115, 107. IR (KBr, ν_{max}, cm^{-1}): 3400 (broad, OH), 1640 and 1060, ^{1}H NMR (300 MHz, $CDCl_3$ and DMSO-d_6) δ: 5.13 (1H, t), 4.49 (1H, dd), 3.98-3.33 (6H, m), 1.76-0.71 (48H, m).

Compound 7 (eicosanoic acid): White granules, m.p. 70-72 °C, Mass (m/z): 312, 295, 267, 253, 239, 225, ... 60. IR (KBr, ν_{max}, cm^{-1}): 3300-2650 (broad O-H stretching), 1745 (C=O stretching), 1300 (O-C=O stretching), 730 and 720 [doublet, -$(CH_2)_n$-deformation, n > 4].

Compound 8 (ethyl cerotate): White plates, m.p. 68-70 °C, Mass (m/z): 424 [M^+], 410, 396, 368, 340, 325, 297, 241, 185. IR (KBr, ν_{max}, cm^{-1}): 2960, 2890, 1740, 1470, 1180, 730 and 720.

Compound 9 (chrysophanol): Dark orange needles, m.p. 187-188 °C, Mass (m/z): 254 [M^+] 239, 237, 226, 225, 198, 197, 152, IR (KBr, ν_{max}, cm^{-1}): 3400 (-OH), 1680, 1625, 1600, ^{1}H NMR (300 MHz, $CDCl_3$), δ: 2.47 (3H, s, -CH_3), 7.11 (1H, br s, H-2), 7.28 (1H, dd, *J* = 1.1, 8.5 Hz, H-7), 7.65 (1H, br s, H-4) 7.68 (1H, d, *J* = 8.5Hz, H-6), 7.84 (1H, dd, *J* = 1.1, 8.5 Hz, H-5), 12.03 (s, -OH) and 12.14 (s, -OH).

Compound 10 (physcion): Orange crystals, m.p. 198-200 °C, Mass (m/z): 284 [M^+], 256, 254, 241, 227, 213, 198, 185, 128. IR (KBr, ν_{max}, cm^{-1}): 3440, 1710, 1670, 1620, 1590, 1260, 1210, 860 and 780, ^{1}H NMR [300 MHz, $CDCl_3$] δ: 2.45 (3H, s, -CH_3), 3.94 (3H, s, -OCH_3), 6.70 (1H, d, *J* = 2.6 Hz, H-7), 7.09 (1H, br s, H-2), 7.37 (1H, d, *J* = 2.6 Hz, H-5), 7.64 (1H, br s, H-4), 12.13 (s, -OH) and 12.33 (s, -OH).

Compound 11 (1,5-dihydroxy-3-methoxyanthraquinone): Orange needles, m.p. 189-190 °C, Mass (m/z) 254 [M^+], 239, 226, 225, 196, 169, 152, 141, 127, 76. IR (KBr, ν_{max}, cm^{-1}): 3250, 2940, 1630, 1590, 1470, 1400, 1260, 1210, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.20, 12.15 (2×OH, s), 8.34 (1H, dd, *J* = 9, H-8), 7.86 (1H, d, *J* = 3, H-4), 7.79 (1H, dd, *J* = 9, H-7), 7.36 (1H, dd, *J* = 9, H-6), 6.75 (1H, d, *J* = 3, H-2), 3.97 (3H, s, -OCH_3).

Compound 12 (1,5-Dihydroxy-3-methoxy-7-methylanthraquinone): Yellow needles, m.p. 190-92 °C, Mass (m/z): 284 [M^+], 256, 228, 197, 182. IR (KBr, ν_{max}, cm^{-1}): 3500, 2890, 1625, 1160,1100, 850 and 840, ^{1}H NMR (300 MHz, $CDCl_3$ and DMSO-d_6) δ: 12.30, 12.10 (2×OH, s), 7.60 (1H, s), 7.45 (1H, s), 7.07 (1H, s), 6.68 (1H, s), 3.94 (3H, s) and 2.59 (3H, s).

Compound 13 (pinitol): White crystals, m.p. 185-187 °C, Mass (m/z): 194 [M^+], 158, 144, 73. IR (KBr, ν_{max}, cm^{-1}): 3410, 1510, 1458, 1130, ^{1}H NMR (300 MHz, $CDCl_3$ and DMSO-d_6) δ: 3.92 (2H, q), 3.80-3.76 (2H, ddd), 3.65 (3H, s), 3.62 (1H, t), 3.35 (OH, m), 3.29 (1H, t), ^{13}C NMR (300 MHz, $CDCl_3$ and DMSO-d_6): δ 85.93 (C-1), 74.33 (C-5), 72.02 (C-4), 73.47 (C-6), 72.56 (C-2), 73.76 (C-3), 60.80 (OMe).

RESULTS AND DISCUSSION

In the course of phytochemical study of the alcoholic extract from the stem bark of *Cassia nodosa*, 13 compounds were isolated from the petroleum ether, benzene and ethyl acetate soluble fractions.

Compound **1** was obtained as a white amorphous powder from acetone. The IR showed important peaks at 3320-2700 (broad O-H stretching of COOH), 1730 (C=O stretching), 730 and 720 cm^{-1} (doublet, -$(CH_2)_n$-deformation, n > 4) indicating it to be a long chain fatty acid. In the mass spectrum, the molecular ion peak was observed at m/z 256 corresponding to the molecular formula $C_{16}H_{32}O_2$. The above data led to the identification of compound **1** as palmitic acid[4].

Compound **2** gave positive LB, Noller's test and TNM test indicating it to be an unsaturated triterpenoid. The mass spectrum showed an [M^+] ion at m/z 424. The ^{1}H NMR spectrum showed a pair of doublets at δ 4.57 and 4.69 corresponding to two vinylidene protons of isopropenyl side chain while the singlet at 1.68 could be assigned to the olefinic methyl group in the side chain. A multiplet at δ 2.17 was observed due to C-2 methylene protons in the vicinity of carbonyl function. Six methyl groups located on saturated carbons resonated as singlets at δ 0.76 (3H), 0.94 (6H), 0.96 (3H), 1.00 (3H) and 1.03 (3H). The methylene and methine

protons appeared as multiplet in the region of δ 1.13-1.58. This compound thus appeared to belong to the lupane series and was identified as a lupenone[5].

Compound **3** gave positive LB, Noller's test and TNM test indicating it to be an unsaturated triterpenoid. The mass spectrum showed an [M^+] ion at m/z 426. In ^{1}H NMR spectrum, a pair of doublets at δ 4.57 and 4.69 corresponded to two vinylic protons while the singlet at δ 1.68 could be assigned to the olefinic methyl group in the side chain. A multiplet at δ 2.17 was observed due to H-19 proton and a double doublet was observed at δ 3.19 due to H-3 proton. The six methyl groups located on saturated carbons appeared at singlets at δ 0.76 (3H), 0.82 (6H), 0.87 (3H), 0.93 (3H) and 0.96 (3H). The methylene and methine protons appeared as multiplet in the region of δ 1.20-1.68. Based on these data, the compound was identified as a lupeol[6].

Compound **4** gave positive LB test for sterols. The spectral data resembled those reported for β-sitosterol[7]. The identity was confirmed by preparation of its acetate, m.p. 126-127 °C and benzoate m.p. 143-44 °C and co-TLC with authentic sample.

Compound **5** responded positive LB test for sterols and TNM test for unsaturation. The compound was identified by comparison of its spectral data which resembled those reported for stigmasterol[8]. This was confirmed by the preparation of its acetate, m.p. 143-144 °C and co-TLC with an authentic sample.

Compound **6** responded to LB test for sterols. Further, it gave positive Molisch's test thereby indicating its glycoside nature. The ^{1}H NMR spectrum displayed the presence of an olefinic proton by the appearance of triplet at δ 5.13. The doublet at δ 4.49 corresponded to the anomeric sugar proton indicating it to be axial and thus the linkage of β-nature[9]. The remaining protons of glucose appeared as a multiplet in the region δ 3.33-3.98 and the methyl, methylene and methine protons appeared in high field region *i.e.*, δ 0.71-1.76. These evidences indicated that the compound was a glycoside of sterol. It did not reduce Fehling's solution which indicated that the reducing group of sugar was involved in the glycosidic linkage. It was thus identified as β-sitosterol-β-D-glucoside[10]. Which was confirmed by co-TLC with authentic sample and further by the preparation of its tetra acetate, m.p. 166 °C.

Compound **7** was obtained as a white amorphorous powder from acetone. The IR spectrum showed important peaks at 3320-2700 (broad O-H stretching of COOH), 1730 (C=O stretching), 730 and 720 cm^{-1} (doublet, -$(CH_2)_n$-deformation, n > 4) indicating it to be a long chain fatty acid. In the mass spectrum, the molecular ion peak was observed at m/z 312. The above data led to the identification of compound **7** as eicosanoic acid[4].

Compound **8** was isolated as white plates from ethyl acetate. The important peaks observed in the infrared spectrum were 1740 (C=O stretching), 1180 (O=C-O stretching), 730 and 720 cm^{-1} [doublet, -$(CH_2)_n$-bending, n > 4]. ^{1}H NMR spectrum exhibited a quartet and a triplet at δ 4.05 and 2.26 due to CH_2O and CH_2CO groups, respectively and one broad singlet at δ 1.25 corresponding to long chain methylene groups and a triplet at δ 0.84 for two methyl groups indicated it to be a long chain saturated aliphatic ester. The molecular ion peak was observed at m/z 424. The base peak appeared at m/z 73 assignable to CH_3CH_2-O-C=O^+ ion characteristic of ethyl esters. Other peaks were observed due to successive loss of 14 mass units (CH_2) confirming the long chain nature of the compound. The above data led to the identification of compound **8** as ethyl cerotate.

Compound **9** showed positive colour reactions with methanolic NaOH and magnesium acetate which indicated its anthraquinone nature. The compound when treated with alkaline formamide gave a dark red colour indicating the presence of 1,8-dihydroxy system in the molecule which was confirmed the appearance of two carbonyl peaks at 1680 and 1625 cm^{-1} in the IR spectrum[11]. Comparison of the data with those reported in literature indicated it to be 1,8-dihydroxy-3-methylanthraquinone (chrysophanol)[12].

Compound **10** gave colour reaction with methanolic NaOH and magnesium acetate characteristic of anthraquinones. The compound gave a dark red colour when treated with alkaline formamide which showed presence of 1,8-dihydroxy system in the molecule. Based on comparison of the spectral data with those reported in the literature[13], it was determined to be physcion which was finally confirmed by co-TLC with authentic sample.

Compound **11** gave the colour reaction of anthraquinones. The appearance of violet colour with conc. H_2SO_4 indicated the presence of 1,5-dihydroxy system in the molecule[14] which was confirmed by the IR spectrum where a single peak at 1625 for carbonyl absorption was observed[11]. The structure was established as 1,5-dihydroxy-3-methoxyanthraquinone on the basis of the comparative spectral data[15]. This is the second report of the isolation of this compound from natural source.

Compound **12** gave colour reaction of anthraquinones. The appearance of violet colour with conc. H_2SO_4 indicated the presence of 1,5-dihydroxy system in the molecule[14] which was confirmed by the IR spectrum where a single peak at 1625 for carbonyl absorption was observed[11]. The structure was determined as 1,5-dihydroxy-3-methoxy-2-methylanthraquinone on the basis of the comparison of the spectral data with those published in the literature[16].

Compound **13** was obtained as white crystals from acetone. Based on the spectral evidences it was identified as the monomethyl ether of inositol, *i.e.* D-pinitol[17]. The identity was finally confirmed by co-TLC with an authentic sample procured from Aldrich and preparation of pentaacetate, m.p. 97 °C.

Palmitic acid (**1**), lupenone (**2**), lupeol (**3**), β-sitosterol (**4**), stigmasterol (**5**), β-sitosterol-β-D-glucoside (**6**), eicosanoic acid (**7**), ethyl cerotate (**8**), physcion (**10**), 1,5-dihydroxy-2-methoxyanthraquinone (**11**), 1,5-dihydroxy-3-methoxy-7-methylanthraquinone (**12**) and pinitol (**13**) have been isolated for the first time from stem bark of *Cassia nodosa*.

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