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Chemical Analysis of the Roots of *Cassia tora*

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Nine compounds were isolated from the roots of *Cassia tora*. Their structures were elucidated as α-amyrin octacosanoate, palmitic acid, chrysophanol, β-sitosterol, physcion, chrysophanol-8-methyl ether, betulone, β-sitosterol-β-D-glucoside and ononitol. All of these, except β-sitosterol were isolated for the first time from the roots of this plant and betulone is being reported for the first time from this genus.

Key Words: *Cassia tora*, Caesalpiniaceae, α-Amyrin octacosanoate, Chrysophanol, Physcion, Chrysophanol-8-methyl ether, Betulone, Ononitol.

INTRODUCTION

The genus *Cassia* belonging to the family Caesalpiniaceae comprises approximately 400 species of herbs, shrubs and trees with about 24 representatives native to India[1]. *Cassia tora* Linn. is an annual xerophytic shrub which crops up in the arid zones after rainy season. The leaf juice is used in the treatment of conjunctivitis, decoction of leaves is laxative, root is used in snake bite and seeds are useful in treating skin diseases like ringworm, itch and psoriasis[2].

Since the plant is medicinally important and hardly any chemical investigations on its roots[3,4] have been carried out we investigated the chemical constituents of the roots of this plant.

The chromatographic separation of the pet. ether, dichloromethane and ethyl acetate fractions from the roots of *C. tora* led to the isolation of 9 compounds, *viz*., α-amyrin octacosanoate, palmitic acid, chrysophanol, β-sitosterol, physcion, chrysophanol-8-methyl ether, betulone, β-sitosterol-β-D-glucoside and ononitol. All of these except β-sitosterol have been isolated for the first time from the roots of this plant and betulone is being reported for the first time from the genus *Cassia*. This paper describes the isolation and structural determination of these compounds.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded on Jeol AL 300 MHz instrument using $CDCl_3$ and DMSO-d_6 as solvent and TMS as the internal reference. EIMS spectra

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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, for anthraquinones 10 % methanolic KOH was used where the original yellow and orange colour changed to red, violet, green or purple. Melting points were determined on electrothermal melting point apparatus.

The roots of *C. tora* were collected from Gopalpura area located in Central Jaipur during the rainy season. A voucher specimen has been deposited in the Herbarium [Sheet No. 140], 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 (2.5 kg) were extracted thrice with hot ethanol (95 %) under reflux. The extract was concentrated under reduced pressure to yield a brown semi-solid mass (90.5 g). This extract was fractionated successively with hot pet. ether, dichloromethane and ethyl acetate, which on concentration afforded the pet. ether (14.4 g), dichloromethane (6.7 g) and ethyl acetate (65 g) fractions. The pet. ether and dichloromethane 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 8 compounds. Elution was carried out with solvents of increasing polarity *viz*., pet. ether, benzene and ethyl acetate. The eluates were collected in 250 mL portions using stepwise gradient eluting with gradient of pet. ether:benzene (4:1 → 1:4) to give 180 subfractions, subfractions 10-15, 29-38, 39-68, 79-84 and 85-90 (pet. ether:C_6H_6, 4:1) were crystallized to give **1** (0.104 g), **2** (0.056 g), **3** (0.599 g), **4** (1.101 g) and **5** (0.093 g), respectively. Subfractions 110-123 (pet. ether:C_6H_6, 3:2) were crystallized to yield **6** (0.041 g), subfractions 143-156 (pet. ether:C_6H_6, 2:3) on crystallization gave **7** (0.053 g). Further elution with gradient of benzene:ethyl acetate (4:1 → 1:4) gave 181-250 subfractions in which subfractions 218-232 (C_6H_6:EtOAc, 2:3) were crystallized to give **8** (0.056 g). Ethyl acetate soluble fraction was applied over silica gel column using a solvent system of C_6H_6:EtOAc (4:1 → 1:4) as an eluent to give 168 subfractions. Subfractions 3-12 and 22-72 (C_6H_6) on crystallization gave **3** (0.051 g) and **4** (0.019), respectively, subfractions 95-108 (C_6H_6:EtOAc; 2:3) afforded **8** (0.063) and subfractions 125-160 (C_6H_6:EtOAc, 1:4) furnished **9** (0.633 g).

Compound 1 (α-amyrin octacosanoate): White granules, m.p. 63-65 °C, IR (KBr, ν_{max}, cm^{-1}): 1735 (C=O stretching), 1640, 1160 (O=C-O stretching), 730 and 720 (d; $(CH_2)_n$ bonding, n > 4). On hydrolysis gave α-amyrin, m.p. 180-82 °C, mass (m/z): 426 [M^+], IR (KBr, ν_{max}, cm^{-1}): 3350 (O-H stretching), 1640 (C=C stretching) and octacosanoic acid, m.p. 87-89 °C, mass (m/z): 424 [M^+], 407 [M^+ - 17], 379 [M^+ - 45], IR (KBr, ν_{max} cm^{-1}): 3330-2500 (broad O-H stretching), 1715 (C=O stretching), 725 and 710 [doublet, -$(CH_2)_n$-deformation, n > 4].

Compound 2 (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, *etc*., 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 3 (chrysophanol): Dark orange needles, m.p. 187-88 °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, 8.5Hz, H-5), 12.03 (s, -OH) and 12.14 (s, -OH).

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, *etc*. 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 2.00-0.70 (47H, m, d, s).

Compound 5 (physcion): Orange crystals, m.p. 198-200 °C, mass (m/z): 284 [M^+], 256, 254, 241, 227, 213, 198, 185, 128, *etc*. IR (KBr, ν_{max}, cm^{-1}): 3440, 1710, 1670, 1620, 1590, 1260, 1210, 860, 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 6 (chrysophanol-8-methyl ether): Orange needles, m.p. 193-195 °C, mass (m/z): 268 [M^+], 251, 250, 240, 239, 238, 222, *etc*. IR (KBr, ν_{max}, cm^{-1}): 3400 (-OH), 1675, 1630, 1600, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44 (3H, s, $-CH_3$), 4.08 (3H, s, $-OCH_3$), 7.96 (d, 1H, *J* = 7.8 Hz, H-5), 7.33 (d, 1H, *J* = 13.5 Hz, H-7), 7.74 (t, 1H, H-6), 7.60 (br s, 1H, H-4), 7.10 (br s, H-2), 12.93 (s, -OH).

Compound 7 (betulone): White amorphorous powder, m.p. 280-82 °C, mass (m/z): 440 [M^+], 409, 397, 286, 245, 203, 189, 147, 133, 119, 95, 67, 55, IR (KBr, ν_{max}, cm^{-1}): 3455 (-OH), 1705, 1615, ^{1}H NMR (300 MHz, $CDCl_3$ and DMSO-d_6) δ: 4.68 and 4.55 (each 1H, d, H-29), 3.86 and 3.36 (each 1H, d, *J* = 5.3, H-28), 2.58 (2H, m, H-2), 1.88-1.70 (24H, m) 1.67, 0.96, 0.92, 0.91, 0.79, 0.67 (each 3H, s, H-23, 24, 25, 26, 27, 30). ^{13}C NMR (300 MHz, $CDCl_3$ and DMSO-d_6) δ: 60.4 (C-28), 37.3 (C-13), 25.1 (C-12), 215.2 (C-3), 54.7 (C-5), 48.5 (C-18), 49.7 (C-9), 29.2 (C-16), 42.5 (C-14), 40.8 (C-8), 150.3 (C-20), 47.7 (C-19), 47.3 (C-4), 39.5 (C-1), 36.8 (C-10), 33.9 (C-22), 26.6 (C-15), 34.1 (C-7), 27.1 (C-23), 29.7 (C-21), 33.3 (C-2), 21.1 (C-11), 14.6 (C-27), 47.7 (C-17), 19.6 (C-30), 19.0 (C-6), 109.7 (C-29), 21.3 (C-24), 15.9 (C-25), 15.7 (C-26).

Compound 8 (β-sitosterol-β-D-glucoside): White granules, m.p. 285-87 °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), 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 9 (ononitol): White crystals, m.p. 165-67 °C, mass (m/z): 194 [M^+], 158, 144, 73, IR (KBr, ν_{max}, cm^{-1}): 3410, 1510, 1458, 1130, ^1H NMR (300 MHz, $CDCl_3$ and DMSO-d_6) δ: 3.98 (2H, q), 3.69-3.66 (2H, ddd), 3.64 (3H, s), 3.60 (1H, t), 3.35 (OH, m), 3.24 (1H, t), ^{13}C NMR (300 MHz, $CDCl_3$ and DMSO-d_6): δ 81.85 (C-1), 73.36 (C-5), 71.91 (C-4), 71.32 (C-6), 70.66 (C-2), 70.44 (C-3), 58.88 (OMe).

RESULTS AND DISCUSSION

In the course of phytochemical study of the alcoholic extract from the roots of *C. tora*, 9 compounds were isolated from the pet. ether, dichloromethane and ethyl acetate soluble fractions.

Compound **1** showed positive Liebermann-Burchard and Noller's test characteristic of triterpenoids. The compound on hydrolysis yielded the alcoholic moiety, m.p. 180-82 °C identified as α-amyrin[5] and the acidic part which was a long chain fatty acid, m.p. 87-89 °C characterized as octacosanoic acid. Thus compound **1** was identified as α-amyrin octacosanoate.

Compound **2** was obtained as a white amorphorous powder from acetone. The IR showed important peaks at 3320-2700 (broad O-H stretching), 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 **2** as palmitic acid[6].

Compound **3** showed positive colour reaction 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[7]. Comparison of the data with those reported in literature indicated it to be 1,8-dihydroxy-3-methylanthraquinone (chrysophanol)[8].

Compound **4** gave positive Liebermann-Burchard test for sterols. The spectral data resembled those reported for β-sitosterol[9]. The identity was confirmed by preparation of its acetate, m.p. 126-27 °C and benzoate m.p. 143-44 °C and co-TLC with authentic sample.

Compound **5** gave colour reaction with methanolic NaOH and magnesium acetate characteristic of anthraquinones. The compound when treated with alkaline formamide gave a dark red colour which showed presence of 1,8-dihydroxy system in the molecule. Based on comparison of the spectral data with those reported in the literature[10] it was determined to be physcion which was finally confirmed by co-TLC with authentic sample.

Compound **6** gave colour reaction with methanolic NaOH and magnesium acetate characteristic of anthraquinones. The structure of **6** was determined to be chrysophanol-8-methyl ether on the basis of above data, together with a comparison of the data with those published in the literature[11].

Compound **7** showed positive result in Liebermann-Burchard test. The mass spectra showed an [M^+] ion at m/z 440. The IR spectrum exhibited absorption bands for hydroxy (3455 cm^{-1}) and ketonic (1705) groups. The ^{1}H NMR of spectrum of **7** showed signals for an isopropylene function at δ 4.68 and 4.55, each 1H doublet and 1.67, 3H singlet, a set of geminal protons at δ 3.86 and 3.32 for hydroxy methyl group and five singlets for methyl groups at δ 0.96, 0.92, 0.91, 0.79 and 0.67. The ^{13}C NMR spectrum of **7** exhibited the presence of 30 carbon signals and also showed a carbonyl signal, two olefinic signals at δ 150.3 and 109.7 and a oxygenated carbon signal of C-28 at δ 60.4. From these results, compound **7** was indicated to be a 3-oxo lupane type triterpene. Beside the above evidences and by direct comparison of its spectral data with those of the reported literature[12], the structure of **7** was determined to be lup-20(29)-ene-28-ol-3-one (betulone), a rare triterpenoid which has been isolated from *Betula lenta*[13].

Compound **8** responded to Liebermann-Burchard 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 indicated it to be axial and thus the linkage of β-nature[14]. 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[15]. Which was confirmed by co-TLC with authentic sample and further by the preparation of its tetraacetate, m.p. 166 °C.

Compound **9** was obtained as white crystals from acetone. Based on the spectral evidences it appeared to be a cyclohexitol methyl ether. A comparison of the data with those published in the literature indicated it to be 4-O-methyl-myoinositol *i.e.*, ononitol[16]. The identity was finally confirmed by comparing the R_f value (0.22) in TLC [water:ethyl acetate:2-propanol, 6:11:83, v/v/v][17].

α-Amyrin octacosanoate (**1**), chrysophanol (**3**), physcion (**5**), chrysophanol-8-methyl ether (**6**), betulone (**7**), β-sitosterol-β-D-glucoside (**8**) and ononitol (**9**) were isolated for the first time from roots of *C. tora*.

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