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ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
2011, **8(4),** 1843-1847

Phytochemical Investigations of *Caesalpinia Digyna* Root

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Received 19 December 2010; Accepted 28 February 2011

Abstract: Phytochemical examination of petroleum ether extract of *Caesalpinia digyna* root resulted in the isolation of four compounds namely, friedelin, hexacosanoic acid, *β*-sitosterol and stigmasterol. These compounds have been characterized on basis of physical and spectral data. All the four compounds are being reported for the first time from this plant

Keywords: *Caesalpinia digyna*, Friedelin, Hexacosanoic acid, *β*-Sitosterol , Stigmasterol

Introduction

Caesalpinia digyna (Family: Leguminosae) is a large, scandent, prickly shrub or climber, growing wild in the scrub forests of the eastern himalayas. The plant is one of the ingredients of an indigenous drug preparation, "Geriforte", which has been used for curing senile prurities with excellent results. The drug is also reported to exhibit antifatigue effect in rats[1]. The roots have marked astringent and antipyretic properties. It is given internally in pthisis and scrofulous affections; when sores exist, it is applied externally as well. In some parts of the burma the root, pounded and mixed with water, is drunk as a febrifuge. It is said to have intoxicating effect[2]. The extract of root also exhibited antitubercular, antioxidant, antidiabetic and radioprotective effects[3-5]. Chemical investigations of the plant have shown the presence of caesalpinine A, cellallocinnine, ellagic acid, Gallic acid, pipecolic acid, bergenin and tannins[6-12]. In our earlier studies, the petroleum ether extract of *Caesalpinia digyna* has shown selective *in vitro* cytotoxicity towards cancerous cell lines such as HeLa, HEp-2 and A-549 (unpublished report).This prompted our interest to carry out the phytochemical investigations of petroleum ether extract.

Experimental

Melting points were determined using an electrothermal IA-9200. IR spectrum was obtained on a Perkin Elmer FTIR 1275X type spectrometer with KBr discs. EIMS spectrum was obtained using a Hewlett Packard 5989 B MS spectrometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer and TMS was used as an internal standard. Column chromatography (CC) was performed on silica gel (60-120 mesh and 100-200 mesh, Merck).

Plant material

The root of *Caesalpinia digyna* was purchased from Abirami Botanicals, Tuticorin, Tamilnadu, India and authenticated by Dr. D. Suresh Baburaj, Survey of medicinal plants and collection unit, Ootacamund, India. A voucher specimen (TIFAC 01) has been deposited for further reference at J.S.S College of Pharmacy herbarium, Ootacamund, India.

Extraction and isolation

The root was chopped to small pieces and dried in shade. The dried root was powdered and passed through sieve no. 20 and extracted (750 g) with 2.0 L of petroleum ether (60-80 ^{o}C) in a soxhlet extractor for 18-20 h. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50 ^{o}C). The petroleum ether extract yielded a yellowish green sticky semisolid, weighing 5 g.

The petroleum ether extract (4 g) was chromatographed over silica gel 60-120 mesh and eluted with different mixtures of petroleum ether-dichloromethane, dichloromethane-ethyl acetate and ethyl acetate-methanol gave 17 fractions. The fraction 5 eluted in petroleum ether: dichloromethane (70:30) solvent system, showed one major spot along with minor impurities. Repeated recrystalisation in chloroform yielded a white crystalline solid. Its homogeneity was checked in petroleum ether: dichloromethane (50:50) solvent system. It was designated as Cd-P1 (yield: 200 mg). The fraction 6 eluted in petroleum ether: dichloromethane (60:40) solvent system yielded a white flakes. These white flakes were washed and designated as Cd-P2 (Yield: 150 mg). The fractions 7, 8 and 9, almost showed similar number of spots. All the fractions were mixed together and when rechromatographed over silica gel 100-200 mesh and eluted with different mixtures of petroleum ether-dichloromethane, dichloromethane-ethyl acetate and ethyl acetate-methanol yielded 2 compounds. The separated compounds were recrystallized in absolute alcohol. Two compounds were designated as Cd-P3 (yield 200 mg) and P4 (yield 25 mg), respectively. The remaining fractions were not taken for further purification because of lower yields. These compounds were subjected to physical and spectral studies for characterization.

Compound, Cd-P1, was colorless needles, mp 262-263 ºC; IR ν max (KBr) cm^{-1} : 3405, 2927, 2869, 1715, 1463, 1389, 1050; EI-MS for $C_{30}H_{50}O$ *m/z* (% rel. int.): 426 $[M]^{+}$ (26), 341 (24), 302 (22), 273 (62), 246 (66), 231 (84), 123 (80), 109 (100), 95 (98), 81 (79); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.70 (3H, *s*, Me-24), 0.85 (3H, *s*, Me-25), 0.86 (3H, *d*, *J* = 6.0 Hz, Me-23), 0.93 (3H, *s*, Me-29), 0.94 (1H, *m*, H-22a), 0.98 (6H, *s*, Me-26, Me-30), 1.03 (3H, *s*, Me-27), 1.16 (3H, *s*, Me-28), 1.27 (1H, *m*, H-6a), 1.33 (1H, *m*, H-7a), 1.37 (1H, *m*, H-8), 1.45 (1H, *m*, H-7b), 1.48 (1H, *m*, H-22b), 1.50 (2H, *m*, H-10), 1.53 (1H, *m*, H-18), 1.67 (1H, *m*, H-1a), 1.74 (1H, *m*, H-6b), 1.96 (1H, *m*, H-1b), 2.23 (2H, *m*, H-2a, H-4), 2.39 (1H, *m*, H-2b); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 22.28 (C-1), 41.29 (C-2), 213.22 (C-3), 58.20 (C-4), 42.15 (C-5), 41.63 (C-6), 18.23 (C-7), 53.10 (C-8), 37.45 (C-9), 59.45 (C-10), 35.62 (C-11), 32.42 (C-12), 38.30 (C-13), 39.70 (C-14), 30.51 (C-15), 36.00 (C-16), 29.98 (C-17), 42.79 (C-18), 35.34 (C-19), 28.17 (C-20), 32.77 (C-21), 39.25 (C-22), 6.80 (C-23), 14.65 (C-24), 17.94 (C-25), 18.65 (C-26), 20.25 (C-27), 32.08 (C-28), 31.77 (C-29), 35.01 (C-30)

Compound, Cd-P2, was colorless solid; mp 82-83 °C; IR ν max (KBr) cm^{-1} : 3290, 2917, 2849, 1708, 1472, 1463, 1305, 920, 725; EI-MS for $C_{26}H_{52}O_2$ *m/z* (% rel. int.): 396 $[M]^+$ (7.4), 381 (12.9), 367 (100), 353 (40.1), 339 (28.5), 325 (11.5), 311 (7.4), 297 (7.7), 283 (6.7), 269 (9.1), 255 (6.3), 241 (9.1), 227 (7.1), 213 (6.3), 199 (5.5), 185 (15.5), 171 (9.0), 157 (4.7), 143 (6.0), 129 (33.6), 115 (9.5), 111 (12.2), 99 (11.6), 85 (21.8), 73 (38.3), 71 (31.7), 59 (24.3), 57 (48.5); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.35 (1H, d, J = 7.5 Hz, H_2 – 2a), 2.32 (1H, d, J = 7.5 Hz, H_2 – 2b), 1.63 (2H, m, H2 – 3), 1.25 (44 H, br s, 22 x CH_2), 0.89 (3H, t, J = 7.0 Hz, Me – 26); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 178.00 (C–1), 34.23 (C – 3), 32.15 (C – 4), 29.93 (10 x CH_2)*, 29.87 (4 x CH_2)*, 29.82 $(CH_2)^*$, 29.66 $(CH_2)^*$, 29.59 $(CH_2)^*$, 29.46 $(CH_2)^*$, 29.29 $(CH_2)^*$, 24.91 $(CH_2)_*$, 22.91 (C - 25), 14.32 (C - 2); (*values may be interchanged)

Compound, Cd-P3, was colorless crystal, mp:132-133 °C; IR ν max (KBr) cm^{-1} : 3431, 2935, 2867, 1640, 1464, 1381, 1053, 1022, 958; EI-MS for $C_{29}H_{50}O$ *m/z* (%rel. int.) 414 $[M^+]$ (100%), 396 (54%), 381 (21%); ^{1}H NMR (500 MHz, $CDCl_3$): *δH* 3.52 (1H, m, H-3), 5.35 (1H, m, H-6), 0.69 (3H, s, Me-18), 1.01 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.4 Hz, Me-21), 0.83 (3H, d, *J* = 6.8 Hz, Me-26), 0.81 (3H, d, *J* = 6.9 Hz, Me-27), 0.85 (3H, t, *J* = 7.8 Hz, Me-29). ^{13}C-NMR (125 MHz, $CDCl_3$) *δ:* 37.33 (C-1), 31.63 (C-2), 71.73 (C-3), 42.20 (C-4), 140.71 (C-5), 121.63 (C-6), 31.96 (C-7), 31.81 (C-8), 51.13 (C-9), 36.43 (C-10), 21.09 (C-11), 39.79 (C-12), 42.37 (C-13), 56.75 (C-14), 24.15 (C-15), 28.25 (C-16), 56.02 (C-17), 11.84 (C-18), 19.46 (C-19), 36.07 (C- 20), 18.68 (C-21), 33.95 (C-22), 26.10 (C-23), 45.82 (C-24), 29.15 (C-25), 19.77 (C-26), 19.21 (C-27), 23.13 (C-28), 11.04 (C-29)

Compound, Cd-P4, was colorless crystals, mp:162-164 °C; IR ν max (KBr) cm^{-1} : 3381, 2935, 2862, 1657, 1456, 1375, 1101,1022, 920, EI-MS for $C_{29}H_{48}O$ *m/z* (rel. int.): 412 $[M^+]$ (39.7%), 351 (13.5%), 314 (7.0%), 300 (25.5%), 271 (38.4%), 229 (8.6%), 213 (1.6%), 55 (100%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.40 (1H, m, H-3*α*), 5.30 (1H, t, *J* = 5 Hz, H-6), 0.71 (3H, s, Me-18), 1.01 (3H, s, Me-19), 0.95 (3H, d, *J* = 7 Hz, Me-21), 5.00 (1H, dd, *J* = 8, 14 Hz, H-22), 5.21 (1H, dd, *J* = 8, 14 Hz, H-23), 0.82 (3H, d, *J* = 7 Hz, Me-26), 0.83 (3H, d, *J* = 7 Hz, Me-27), 0.97 (3H, t, *J* = 7 Hz, Me-29) ^{13}C NMR ($CDCl_3$, 125 MHz): δ 140.9 (C-5), 138.5 (C-22), 129.5 (C-23), 121.9 (C-6), 72.0 (C-3), 57.0 (C-14), 56.1 (C-17), 51.4 (C-24), 50.3 (C-9), 46.0 (C-25), 42.4 (C-13), 40.7 (C-20), 39.8 (C-12), 37.5(C-4), 37.4 (C-1), 36.7 (C-10), 32.1 (C-8), 31.9 (C-7), 29.2 (C-16), 28.4 (C-2), 25.6 (C-28), 24.5 (C-15), 21.4 (C-21), 21.3 (C-11), 20.0 (C-27), 19.6 (C-26), 19.1 (C-19), 12.2 (C-29), 12.1 (C-18).

Results and Discussion

The concentrated petroleum ether extract of the roots of *Caesalpinia digyna* was repeatedly fractionated using silica gel column chromatography and compounds, Cd-P1 to Cd-P4, were eluted in the order of increasing polarity. The ^{1}H and ^{13}C NMR spectral data for these compounds revealed that Cd-P1 belongs to the pentacyclic triterpene, Cd-P2 belongs to fatty acid and Cd-P3 and Cd-P4 belongs to sterols.

Compound Cd-P1 was identified as Friedelin (Figure 1). Its EI-MS molecular ion peak at *m/z* 426 corresponded to the molecular formula of $C_{30}H_{50}O$. The fragment peak at *m/z* 273, together with those at *m/z* 302 and 341, was suggestive of a Friedelane derivative with 3-keto substituent[13-14]. Its IR spectrum displayed the carbonyl absorption at 1715 cm-1. One secondary and seven tertiary methyls of the Friedelane skeleton were observed in the ^{1}H-NMR spectrum as a doublet at δ 0.86 (*J* = 6.0 Hz, Me-23) and singlets at δ 0.70 (Me-24), 0.85 (Me-25), 0.93 (Me-29), 0.98 (Me-26, Me-30), 1.03 (Me-27), 1.16 (Me-28), respectively. The most downfield carbon signal at δ 213.2 represented the 3-keto group of Friedelin.

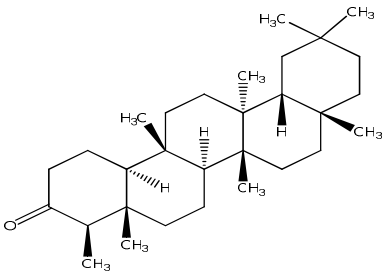


Figure 1. Structure of friedelin

Compound Cd-P2 was identified as hexacosanoic acid (Figure 2). Its IR spectrum exhibits a broad band at 3000-3500 cm^{-1} (-COOH), a carbonyl band at 1708 cm^{-1} and bands at 1472, 1463 and 729 cm^{-1} indicating a long chain fatty acid. Its high resolution ^{1}H NMR spectrum shows a three proton triplet at δ 0.89 (J=7.0Hz) for a terminal methyl group, a 44 proton broad singlet for 22 methylene units and a two proton multiplet at δ 1.63 for the *β*- methylene protons. The ^{13}C NMR spectrum shows a peak at δ 178 for the carbonyl group, the methyl and methylene signals between δ 14 to 30. Its EI-MS molecular ion peak at *m/z* 396 indicates a molecular formula $C_{26}H_{52}O_2$.The structure was confirmed by mass spectrum, which exhibits several ion peaks at an interval of 14 mass units[15].

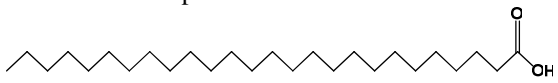


Figure 2. Structure of hexacosanoic acid

Compound Cd-P3 and P4 were given positive test for sterols and alcohols. The melting point of Cd-P3 and P4 were in agreement with the melting point given for *β*-sitosterol (Figure 3) and stigmasterol (Figure 4) in the literature[16]. In ^{1}H NMR spectrum of Cd-P3 displays signals for two tertiary methyl groups at δ 0.69 (s, 3H, C-18) and δ 1.01 (s, 3H, C-19), three secondary methyl groups at δ 0.92 (d, 3H, J=6.4 Hz Me-21), 0.83 (d, 3H, J=6.8Hz Me-26), 0.81 (d, 3H, J=6.9 Hz Me-27) and a primary methyl group at δ 0.85 (t, 3H, J=7.8 Hz Me-29). The broad multiplet signal at δ 5.35 (1H, m,) is due to H-6 and the hydroxy methine proton signal at δ 3.52 (1H, m) is attributed to H-3 for biogenetic considerations. The above data and comparison of ^{13}C NMR data with those of the known sterols[17] suggests that the compound is *β*-sitosterol.

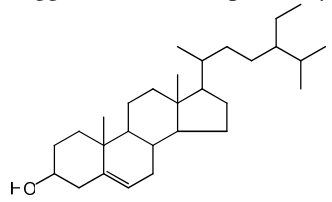


Figure 3. Structure of *β*-sitosterol

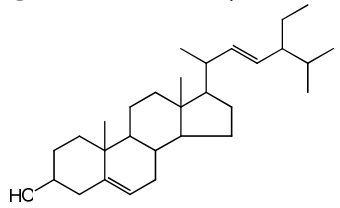


Figure 4. Structure of stigmasterol

Similarly the ^{1}H NMR spectra of Cd-P4 showed the presence of six methyls that appeared at δ 0.71, 0.82, 0.83, 0.95, 0.97 and 1.01. The proton of H-3 appeared as a multiplet at δ 3.40. It also showed olefinic protons at δ 5.30, 5.21 and 5.00. Further, it is supported by its ^{13}C NMR data that shows the presence of a signal at δ 72.00 for a carbinol carbon atom and signals at δ 140.90, 138.50, 129.50 and 121.90 indicating the presence of two double bonds. The above data and a comparison of its ^{13}C NMR signals with known phytosterols[18] suggest that the compound is stigmasterol.

Conclusion

The isolation and identification of compounds friedelin, hexacosanoic acid, *β*-sitosterol and stigmasterol from the roots of *Caesalpinia digyna* was the first ever to be reported from this plant.

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