Bull. Pharm. Sci., Assiut University
Vol. 12, Part 2, PP. 325 - 338 (1989)

PHYTOCHEMICAL STUDY OF CASSIA SPECTABILIS DC. CULTIVATED IN EGYPT.

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ABSTRACT

Two aliphatic hydrocarbons, anthraquinone(physcion). B- sitosterol, four flavonoids (apigenin, luteolin, apigenin-7-O-glucoside and 5,7,8, 4-pentahydroxy flavone-6-O-8-O-diglucoside) and the piperidine alkaloids [2-methyl-3-hydroxy-6-dodecyl acetyl piperidine, its isomer and 2-methyl-3-hydroxy-6-(13-tetradecyl acetyl) piperidine] were isolated and identified from the leaves of Cassia spectabilis DC. The identity was accomplished through physical, chemical and spectral studies.

Cassia spectabilis DC. (Leguminosae; Caesalpiniaceae) is an ornamental large tree indigenous to India and the tropicals and recently cultivated in Egypt[1-4]. Many plants of the genus Cassia have been used as purgatives, antimalerials, anthelmintics and as a remedy for dysentry, cough, ophthalmia and skin diseases[5].

Earlier investigations resulted in the isolation of piperidine alkaloids, anthraquinones and B-sitosterol from the leaves of C. spectabilis DC.[6-8]

In previous papers[9,10], the macro-and micromorphology of the different organs of this plant were presented. This work deals with the isolation and identification of hydrocarbons, anthraquinone, steroids, flavonoids and alkaloids from the leaves of C. spectabilis DC. cultivated in Egypt.

EXPERIMENTAL

Plant Material :

The leaves of C. spectabils DC. were collected in April 1986 from flowering plants cultivated in Aswan Botanic Island, Aswan, Egypt. Identification of the plant was confermed by Agricultural Engineer Ali Mousa, Director of the Island. A voucher specimen is on deposit at the Dept. of Pharmacognosy Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Instruments Used :

UV spectra were taken on a Perkin-Elmer model 550 spectrophotometer (W. Germany) and IR spectra (KBr disc or nujol) were determined on a Perkin-Elmer infra-red spectrophotometer 720 (W.Germany). ^{1}H-NMR spectra were recorded on Brucker spectrometer (90 MHz) using TMS as an internal standard and chemical shifts were recorded in δ (ppm) units. Mass spectra were measured with a Kratos, MS-50, A.E.I. 70 eV mass spectrometer. Melting points were determined in a koffler hot stage microscope, type (ESP, Boetius M.).

Authentic Samples :

B-sitosterol, apigenin, luteolin and apigenin-7-O-glucoside were obtained from Carl Roth Chemie GmbH,Karlsruhe , W. Germany. Anthraquinone (physcion) was obtained from Research Dept., Sandoz LTD, Basel, Switzerland. Sugars including glucose, rhamnose, fructose, galactose and arabinose were obtained from E. Merck, Darmstadt, W.Germany.

The solvent systems :

I- Pet. ether-EtOAC (9:1)

II- $CHCl_3$-MeOH-H_2O (65:33:2).

III- EtOAC-HCOOH-H_2O(10:2:3).

IV- $CHCl_3$-MeOH-NH_4OH(90:10:0.5).

were used and all solvents were distilled under reduced pressure at 40°C.

Phytochemical Study of Cassia Spectabilis DC. Cultivated in Egypt.

Extraction and Isolation :

The air-dried powdered leaves (2 kg) were defatted by maceration with pet. ether (60-80°C) (2 x 6 liters) and the extract on evaporation afforded a brown viscous residue (100 g). About (20 g) of this residue was chromatographed over silica gel column (800 g) and eluted, with pet.ether, then with pet,ether-benzene gradient, benzene and benzene-ethyl acetate gradient. (100 ml fractions were collected.) Four compounds (1-4) could be isolated.

The dried, defatted powdered leaves were extracted by maceration with ethanol (70%) (3 x 5 liters). The alcoholic extract residue (155 g) was divided into two parts, the first (35 g) was used to isolate the flavonoids while the alkaloids were obtained from the second part (120 g) residue as follows :

1- The first part (35 g) was diluted with water and extracted successively with ethyl acetate and n-butanol.

The ethyl acetate extract residue (4 g) was chromatographed over cellulose column (160 g). Elution was started with chloroform, then with chloroform-methanol gradient. Fifty ml fractions were collected and three flavonoidal components (5-7) were obtained.

The n-butanol fraction (residue 12 g) was chromatographed over cellulose column (480 g) eluted with chloroform, then chloroform-methanol gradient. Fifty ml fractions were collected and one flavonoid (8) was obtained.

2- The 2nd part of the ethanolic extract residue (120 g) was stirred with aqueous H_2SO_4 (10%), filtered and the filtrate was extracted with chloroform (1 liter). The aqueous solution was basified with NH_4OH (pH 9-10) and extracted with chloroform (3 x 1 litre) to afford a brown residue(9 g). The residue obtained was chromatographed over silica gel column (360 g) and eluted with chloroform, then chloroform-methanol gradient 100 ml fractions were collected. Three alkaloids (9-11) could be obtained.

Compounds Isolated from Pet.Ether Extract :

Compound I :

Fractions (1-5) eluted with pet.ether showed a single spot (R_f 0.99, Silica gel, Syst. I) were pooled, concentrated, crystallized from pet.ether where white waxy flakes were obtained (150 mg) m.p. 42°C. It gave negative Liebermann-Burchard's and Salkowski's tests[11,12].

IR (nujol) ν (cm^{-1}) : 2970,1470, 1380 and 732. MS, m/z(rel.int.): 464 [M^+(5) $C_{33}H_{68}$] and a base peak at m/z 57[(100), C_4H_9].

Compound 2 :

Fractions (21-25) eluted with pet. ether-benzene (7:3) showed a single spot (R_f 0.84, Silica gel Syst. I were pooled and concentrated to afford white flakes (200 mg), m.p. 84°C. It gave negative tests for unsaturated sterols and/or triterpenes and decolourised-bromine water and potassium permenganate[11,12].

IR (nujol) ν (cm^{-1}): 2970, 1650, 1470, 1380 and 735. MS: m/z (rel,int.) 448 [M^+(8), $C_{32}H_{64}$] and other characteristic peaks at m/z 57 [C_4H_9 (100)], 83(96), 97(94), 111(52) and 125 (27).

Compound 3 :

The pet.ether-benzene (1:4) eluate gave a mixture of three compounds (TLC). Preparative chromatography (silica gel G. system I), provided a band fluorescing under UV (purple to violet). On scratching and elution with MeOH, concentration and then left for crystallization afforded canary yellow needles (20 mg, MeOH) having R_f 0.76 (system I) and melts at 234-36°C. The compound gave rose red colour with 5% KOH solution. The other two spots were faint and hydrocarbon in nature.

UV λ_{max}^{MeOH} (nm) : 258, 272 and 428. ^{1}H-NMR (90 MHz, (δ) $CDCl_3$), (ppm): 2.44(3H,S,C-3,CH_3), 3.95 (3H, S,C-6, OCH_3), 6.67 (1H,d, J=3Hz, H-7), 7.11(1H,d.J=3Hz, H-2), 7.38(1H,d,J=3Hz, H-5), 7.67(1H,d, J=3Hz, H-4), 12.16(1H,S,C-1, OH) and 12.35(1H,S,C-8, OH). MS: m/z(rel. int.): 284 [M^+(100)] and other characteristic peaks m/z 256[M^+-CO, (5.73)] , 228[M^+-2 CO, (1.18)], 241 [M^+-CO-CH_3,(6.46)], 213[M^+-2 CO-CH_3 (4.73)]. 255 [M^+-CHO,(13)] and 226[M^+2 CHO, (5.7)].

Compound 4 :

The benzene-ethyl acetate (95:5) eluate yielded needle-shaped crystals (compound 4) (200 mg), m.p. 135-137°C, (R 0.36,Silica gel G System I).

Compounds Isolated from Ethyl Acetate Extract :

Compound 5 :

The fraction eluted with $CHCl_3$ -MeOH (96:4)(500 ml) on evaporation and crystallization from MeOH afforded yellow needles (10 mg), R_f 0.88 (cellulose, system II), m.p. 343-45°C.

UV λ_{max}^{MeOH} (nm.):-268,328;+NaOMe: 274(+6), 382 (+54); + $AlCl_3$ and $AlCl_3$/HCl: 278(+10), 344(+16), 384(+56); +NaOAc: 276 (+8),328; +NaOAc/ H_3BO_3: 268, 328.

Compound 6:

The $CHCl_3$-MeOH(92:8) eluate (500 ml) on evaopration and crystallization from MeOH gave yellow needles (10 mg.),m.p. 331-33°C, R_f0.75 (cellulose, system II).

UV λ_{max}^{MeOH} (nm.): 252,236; + NaOMe: 274(+22), 398(+62);+$AlCl_3$: 274 (+22), 416(+80); +$AlCl_3$/HCl: 276 (+24), 356(+20); + NaOAc: 262(+10) , 372(+36); + NaOAc/H_3BO_3 : 260(+8), 376(+40).

Compound 7 :

The $CHCl_3$-MeOH(88:12) eluate (500 ml) on evaporation and crystallization from MeOH yielded yellowish-white needles (30 mg), m.p. 178-180°C., R_f0.65 (cellulose, system II).

UV λ_{max}^{MeOH} (nm): 268, 328; + NaOMe: 268, 384(+56); + $AlCl_3$ and $AlCl_3$/HCl: 276(+8), 344(+16), 382 (+54); +NaOAc.268, 332(+4);+ NaOAc/ H_3BO_3 : 268, 328.

Partial Acid Hydrolysis : Compound 7(5 mg)was partially hydrolysed with 2% HCl to its aglycone on one step. indicating that it is a monoside.

Complete Acid Hydroslysis : Compound 7 (10 mg), was subjected to complete acid hydrolysis using N/2 H_2SO_4. The sugar was identified as

glucose by direct comparison with authentic sample and the aglycone was found to be identical with compound 5 in all aspects.

Compound Isolated from n-Butanol Extract :

Compound 8 :

The $CHCl_3$-MeOH (7:3) eluate (500 ml) on evaporation gave a dark yellow powder (50 mg) (MeOH), m.p. 184-86°C (decomp.), R_f 0.15 (cellulose, system II).

UV λ_{max}^{MeOH} (nm): 270, 330; + NaOMe: 278 (+8), 394 (+64); +$AlCl_3$ and $AlCl_3$/HCl: 278 (+4), 382(+52); +NaOAc: 280(+10), 380 (+50); + NaOAc/H_3BO_3: 278(+8), 330. ^{1}H-NMR (90 MHz , (CD_3OD) δ (ppm) 7.91(2H,d, J=8.8Hz, H-2` and H-6`) ,6.89 (2H,d, J=8.8Hz, H-3 and H-5`), 6.44(1H,S, H-3), 3.32-3.85 (12H,m, sugar protons). On partial acid hydrolysis , it was hydrolysed on two steps indicating that it is a bioside. Complete acid hydrolysis gave a sugar identified as glucose. UV of compound 8-aglycone : λ_{max}^{MeOH} (nm.) 280,336, + NaMe: 280, 356(+20,decomp.); + $AlCl_3$: 280, 290 (+10), 404(+68); + $AlCl_3$/HCl:290(+10), 354(+18); +NaOAc : 280(decomp.); + NaOAc/H_3BO_3 : 280, 346 (+10).

Alkaloidal Fraction :

Compound 9 :

Fractions (7-9) eluted with $CHCl_3$-MeOH (95:5) showed single alkaloidal spot (+Ve Dragendorff's and Lassaigne's tests for nitrogen). Crystallization from methanol yielded needle-shaped crystals(100 mg), m.p. 163-65°C, R_f0.65 (silica gel G, system IV).

IR (KBr) ν (cm^{-1}) : 3350-3425(bd), 2950,2860,1705,1530 and 730. ^{1}H-NMR (90 MHz, $CDCl_3$) δ (ppm): 3.78(1H,bs,H-3 carbinolic proton), 2.8-3.1 (2H,bm,H-6 and H-2), 2.33(2H,t,-CH_2-CO-), 2.04(3H,S,-CO-CH_3), 1.44(3H,d,J=6.9 Hz, C-2 Me) and 1.11-1.37 (intense peak, aliphatic methylene groups), High resolution MS:showed a m/z 325 for[$C_{20}H_{39}NO_2$. (5.76%)] and other characteristic peaks at m/z 326 [M^+ +1, (1.6%)] 310 [M^+-CH_3 (7.7%)], 282[M^+-CO-CH_3,(3.77%)], 268[M^+-CH_2-CO-CH_3,

(4.19%)], 254[M^+ -$(CH_2)_2$-CO-CH_3), (1.6%)], 240[M^+ -$CH_2)_3$-CO-CH_3, (16.26%)] a base peak at m/z 114[M^+ -$cH_2)_{12}$-CO-CH_3, (100%)], and 96[M^+-H_2O-$(CH_2)_{12}$-CO-CH_3, (12.42%)].

Compound 10 :

Fractions (12-14) eluted with $CHCl_3$-MeOH (92:8) gave a single spot. On crystallization from methanol gave needle shaped crystals (20 mg) , R_f 0.50 (silica gel G, system IV). ^{1}H-NMR (90 MHz, $CDCl_3$) δ (ppm): 3.63-3. 85 (1H,H-3), 3.17-3.72(2H, bm,H-6 and H-2), 2.37(2H,t,-CH_2-CO-), 2.06(3H,S,-CO-CH_3), 1.08-1.78(intense peak, aliphatic methylene groups) and 0.87 (2H,d,J=7.1,C-2 Me). MS: showed M^+ at m/z(rel. int.): 325($C_{20}H_{39}NO_2$, 5.85), 326 [M^+ + 1(1.6)] 310 (15.58), 282(3.77), 268 (67.53), 254(13.11), 240 (6.49) and a base peak at m/z 114(100%).

Compound 11 :

The fractions (24-26) eluted with $CHCl_3$-MeOH (82:18) gave a single alkaloid, R_f 0.14 (silica gel G, system IV), on crystallization from methanol yielded rosette-shaped crystals (15 mg) melted at 158-160°C.

^{1}H-NMR (90 MHz, $CDCl_3$ and CD_3OD mixture) δ (ppm): 3.63-3.85 (1H,m,H-3), 2.7-3.16(3H,bm,H-6,H-2 and proton of the hydroxylic carbon of the side chain), 2.13(2H,m,-$\overset{*}{C}H_2$-$\underset{OH}{C}H$ - CH_3), 1.67 (3H,d,J=6.9Hz,-$\underset{OH}{C}H$-$\overset{*}{C}H_3$) 1.19-1.56(intense peak, aliphatic methylene groups and C-2 Me). MS : showed M^+ at m/z (rel.int.), 327($C_{20}H_{41}NO_2$ (2.08) and other characteristic fragments at 326[M^+ -1, (4.1)], 312 [M^+ -CH_3, (5.2)], 268 [M^+-CH_2-CHOH-CH_3, (17.7)], 240(18.8), 212(3.1), 184 (5.7), 156 (8.3) and a base peak at m/z 114(100).

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the different extracts of Cassia spectabilis DC. leaves proved the presence of sterols, anthraquinones, flavonoids and alkaloids and/or basic nitrogenous substances.

Column chromatography of the different fractions of the extracts resulted in the isolation of 11 components which were studied and identified :

Compound 1 :

The fragmentation pattern in the mass spectrum was characteristic for aliphatic hydrocarbons (subsequent loss of CH_2 group)[16-18], While, the IR spectral data showed bands at 2970 (C-H, stretching), 1470 and 1380 cm^{-1} (C-H bending deformation) were interfering with those of migol[13-15]. So, compound 1 is an aliphatic saturated hydrocarbon having a molecular formula $C_{33}H_{68}$. This represents the first report of its isolation from the plant.

Compound 2 :

The colour reactions suggest the presence of unsaturation which was confirmed by the band at 1650 cm^{-1} (C=C stretching) in IR. A characteristic pattern of aliphatic hydrocarbons is evident in the mass spectrum with the series of peaks (at m/z 83, 97, 111, 125,.. etc.) for olefins at higher masses (i.e. two masses lower than those given by the corresponding paraffin)[13-15] indicating a β-cleavage (allylic cleavage). The latter form carbonium ions which are stabilized by resonance and these are corresponding to the peaks at m/z 83,97,111 , 125.. etc[13,14]. Therefore, it can be suggested that compound 2 is a mono-olefinic hydrocarbon with molecular formula $C_{32}H_{64}$ and it is isolated for the first time from the plant.

Compound 3 :

The UV spectral data showed bands characteristic for benzenoid and quinonoid absorptions as well as those of 1,8 dihydroxyanthraquinones[6,19]. The molecular ion peak M^+ at m/z 284 (100%) is the base peak which is diagnostic for anthraquinones as well as peaks at (M^+-CO) and (M^+-210). Comparison with reported data suggested compound 3 to be physcion[19] (1,8-dihydroxy-3-methyl-6-methoxy-anthraquinone). The identity was confirmed by mmp and co-chromatographic techniques with authentic sample.

Compound 4 :

It was identified as β-sitosterol by direct comparison with authentic sample (mmp, co-chromatography and IR). Chromatography of the acetate derivative over silver nitrate inpregnated plates afforded one single spot identical with β-sitosterol acetate.

Compound 5,6 & 7:

The three compounds were isolated for the first time from the genus and were identified as apigenin, luteolin and apigenin-7-O-glucoside respectively[20-22]. The identity was confirmed by mmp and co-chromatography with authentic samples, together with comparing the UV spectral absorption data and acid hydrolysis of compound 6.

Compound 8 :

The UV spectral data in methanol and with different complexing or ionizing agents suggested that it is a flavone with free OH-groups at C-5, C-7 and C-4'. The presence of free OH-groups at C-6 and C-8 in the aglycone was indicated by the decomposition with both NaOMe and NaOAc (i.e. ring A is polyhydroxylated) and the bathochromic shift with both $AlCl_3$ and NaOAc/boric acid, as well as hypsochromic shift with $AlCl_3$/HCl than that with $AlCl_3$[20-22]. Two doublets at δ 7.91 and 6.89 ppm (J=8.8 Hz) characteristic for ring B protons are noticed in the ^{1}H-NMR. In addition a singlet at δ 6.44 ppm characteristic for H-3 protons and a multiplet at δ 3.22-3.85 ppm integrating for 12 protons of the sugar part confirm that the compound is a bioside[20,21]. The study of hydrolysis products and comparison with the reported data[20,21] suggested that compound 8 is 5,6,7,8,4'-pentahydroxyflavone-6-O-8-O-diglucoside. This represents the first isolation of this compound from the family.

Compound 9,10,& 11 :

The published studies on the stereochemistry of 2,6-dialkyl piperidin-3-ol compounds is relatively comparable with that of compound 9,10,11[6,13,14]. The fragmentation pattern in the mass spectra and the appearance of the base peak at m/z 114 (resulting from the cleavage of the C-6 side chain) are characteristic for 2-methyl-3-hydroxy-6-alkyl-piperidine derivatives[7,8,23]. ^{1}H-NMR spectra of them showed peaks in the range of δ 3.63-3.85 ppm assignable for carbinolic protons and those in the range of δ 1.08-1.78 ppm for aliphatic methylenes of the C-6 side chain. In addition, the methine hydrogens at C-2 and C-6 are represented by peaks in the range of δ 2.7-3.16 ppm and C-2-CH_3 is represented by peaks at δ 0.37-1.44 ppm. The IR spectra showed a broad band at 3350-3425 cm^{-1} attributable to the stretching frequency of a bonded OH-groups with the piperidine nitrogen [6-8,23].

Compound 9 :

It showed M^+ at m/z 325 and characteristic ion fragment peaks at m/z 268, 254, 240.. etc with a gradient loss of 14 mass units corresponding to the methylene groups of the side chain at C-6 and the base peak at m/z 114 corresponds to $[M^+-(CH_2)_{12}-COCH_3]$. The bonded OH in the IR spectrum is characteristic for all cis-piperidine alkaloids with axial OH group at C-3. The discussed data and comparison with reported data led to the structure of 2-methyl-3-hydroxy-6-dodecyl acetyl-piperidine for compound 9. This compound was previously isolated from the same plant growing in India[6].

Compound 10 :

It has the same molecular weight (MS, m/z 325) as that of 9 and shows the same fragmentation pattern with some differences in the relative intensities. Moreover, the ^{1}H-NMR spectra gave the same data with slight variations in

chemical shifts. However, 9 and 10 differ in their melting points and chromatographic behaviour giving an evidence of being isomers. Subsequently, we suggest that both compounds 9 and 10 are isomers.

Compound 11 :

The molecular ion peak M^+ at m/z 327 is accompanied by a characteristic fragment at m/z 326 (M^+-1) which can be attributed to the elimination of the hydrogen atom to the secondary alcoholic group giving a stable oxonium ion[25]. The fragmentation starts by loss of the terminal methyl group of the side chain giving a peak at m/z 312 then 268 ($M^+ -CH_2-CHOH-CH_3$) which followed by gradual loss of methylene groups of the side chain till the base peak at m/z 114 (2-methyl-3-hydroxy-piperidine)[6-8,23,24]. So, compound 11 is suggested to be : 2-methyl-3-hydroxy-6(13-hydroxy tetradecyl) piperidine.

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دراسة كيميائية لنبات الكاسياسبكتابيليس دى كندول المنزرع فى مصـــــــــر

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نبات الكاسياسبكتابيليس دى كندول ينتمى العائلة البقلية وموطنـــه الاصلى فى الهند أو المناطـــق الاستوائية فى افريقيا أو أمريكا . ولجنـــــس الكاسيا استعمالات عديدة كملينات ، طارد للديدان ، فى علاج الملاريا كذلـــك فى علاج بعض أمـــراض العيون والامراض الجلدية بالاضافة للدوسنتاريا والكحه .

وفى هذة الدراسة تم فصل والتعرف على مركبين من الهيدروكربونـــات ، فيسيون (من الانثراكينونات) ، البيتا سيتوستيرول ، أربعة فلافونيدات منـــها ثلاثة تـــم فصلها لأول مره من جنس الكاسيا وهم : أبيجنين ،لوتيولين وابيجنين ـ ١٧ ـ جلوكوزيد ، أما الفلافونيد الرابع (٥ ، ٦ ، ٧ ، ٨ ، ٤ـ خمـــــــاسى هيدروكسى فلافون ـ ٦ ـ أ ـ ٨ ـ أ ـ داى جلوكوزيد) فقد تم فصله لأول مره مـــن العائلة البقلية .

كذلك تم فصل ثلاثة قلوانيات وهـــى :

١ ـ المادة القلوانية الاولى عباره عن : (٢ـ ميثيل ـ ٣ هيدروكس ـ ٦ ـ دوديسيل اسيتيل-بيبريدين) .

٢ ـ المادة القلوانية الثانية : وهى متجازئ للســـابق .

٣ ـ المادة القلوانية الثالثة :(مركب رقم ١١) وهى : ٢ ـ ميثيل ـ ٣هيدروكسى ـ ٦ ـ هيدروكسى تتراديسيل -بيبريدين .

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received in 5/10/1989 & accepted in 10/12/1989