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# PHYTOCHEMICAL STUDY OF CASSIA DIDYMOBOTRYA FRES. CULTIVATED IN EGYPT

## PART 1- STUDY OF QUINONES AND URACIL.

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## ABSTRACT

Phytochemical study of the contents of the different organs of C.didumobotrya Fres. resulted in the isolation and characterization of chrysophanol, physcion, aloe-emodin, 2-methoxystupandron, gallacinol, and uracil.

Physcion, emodin, gallacinol and uracil were isolated for the first time from this plant.

The identification was based on, physical, chemical and spectral studies including UV, IR , $^{1}H$, $^{13}C$-NMR and MS analysis. The $^{13}C$-NMR assignments of aloe-emodin are reported here for the first time.

## INTRODUCTION

The genus Cassia (Family Caesalpiniaceae) comprises several species which are commonly used in the folklore medicine as purgative and also for treatment of joints, spleen, liver disorders and leprosy[1,2]. Some species of Cassia have been used as dyes, others have an effect against malaria, black fever, blood poisoning anthrax and dysenteries[3].

One of the species of this genus is C.didymobotrya Fres. which is an ever-green shrub cultivated in public and private gardens for its showy yellow flowers. The leaves of C.didymobotrya Fres. are very poisonous; ingestion results in an intense inflammation of the intestinal canal. Nigerean Masai tribe used the plant as intestinal purgative and antimalarial[3].

The interest in the phytochemical study of C.didymobotrya Fres. arises on the basis of recorded biological effects and folkloric uses of the plant in addition to the lack of a comprehensive study of different constituents of the plant organs.

## EXPERIMENTAL

Plant Material :

The plant material used in this work consists of the dried leaves stems, flowers and fruits of C.didymobotrya Fres. Each organ was powdered to a No. 40 powder. The plant was collected from public gardens in Assiut. The plant materials were collected during April - May (1985,86 and 1987) and identified by Prof. Dr.N.A.El-Keltawy. Prof. of Horticulture, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Extraction :

The powder of each of the different organs of C.didymobotrya Fres. (leaves 2.5 kg, stems 2.5 kg, flowers 2 kg and fruits 1 kg.) was defatted with petroleum ether (b.r.40-60°C) followed by extraction of the marc with ethanol (70%) and concentration of the ethanolic extracts under reduced pressure. TLC of the extracts revealed the presence of anthraquinones, flavonoids, steroids and a nitrogen-containing substance.

Fractionation :

The concentrated ethanolic extracts were fractionated as follows :

a- They were extracted with diethyl ether to exhaustion then dried under reduced pressure.

b- Half of the aqueous mother liquors was alkalinized with dilute ammonium hydroxide solution (pH=ca. 10) and then extracted with chloroform to exhaustion.

c- The second half of the aqueous liquors was extracted successively with ethyl acetate and n-butanol to exhaustion and concentrated under reduced pressure.

The ether fraction was subjected to column chromatography using silica gel and elution was carried out by petroleum ether in increasing polarities with ethyl acetate.

Six compounds were isolated, their physical and chromatographic characters are given in Table 1. $^{1}$H-NMR spectral analyses are shown in Table 2. $^{13}$C-NMR spectral analyses are shown in Table 3.

The butanol extract was fractionated on column chromatography using silica gel, ethyl acetate and ethyl acetate-ethanol in increasing polarities. Compound $S_3$ was isolated.

General Experimental Procedures :

Melting points were uncorrected. $^{1}$H-NMR spectra were carried out in $CDCl_3$ and DMSO-$d_6$ at 300 and 90 MHz. $^{13}$C-NMR spectra were carried out in DMSO-$d_6$ at 300 MHz. Coloumn chromatography : silica gel E.Merck, TLC:silica gel 60F 254 E.Merck.

Apparatus :

UV-VIS. spectrometer 550 S (Perkin-Elmer). (West Germany)

IR. spectrometer 298 (Perkin-Elmer). (West Germany).

$^{1}$H-NMR spectrometer WH-90 (Bruker Physics); (U.S.A.).

$^{1}$H-and $^{13}$C-NMR spectrometer XL-300 (Varian).

Mass spectrometer MS-50 (Kratos), 70 eV.

Melting point apparatus (Gallenkamp).

Solvent Systems :

The following solvent systems were used :

Solvent 1 : Petroleum ether-ethyl acetate (9:1).

Solvent 11: Petroleum ether-ethyl acetate-acetic acid (75:24:1).

Solvent 111: Petroleum ether-ethyl acetate (1:9).

Characters of Isolated Compounds :

Compounds $E_1$-$E_6$ gave red colours with methanolic potassium hydroxide. Crystal form, m.p. and chromatographic behaviour are compiled in Table 1.

UV, IR, MS Spectra of the Isolated Compounds :

Compound $E_1$ : (70 mg, yield=0.0014 %)

UV $\lambda_{max}^{EtOH}$ nm (log $\epsilon$): 222 (4.54), 255 (4.25), 275 (3.93) and 428 (3.96) IR (KBr), $\gamma$ $cm^{-1}$: 1675, 1625 and 1605. MS.$M^+$ at m/z 254 calculated for $C_{15}H_{10}O_4$, other peaks are at m/z = 239, 226, 198 and 169.

Compound $E_2$ : (50 mg. yield=0.001 %).

UV $\lambda_{max}^{EtOH}$ nm (log $\epsilon$ ): 222 (4.52), 250 (4.19), 262 (4.20), 282 (4.17), 430 (3.93). IR (KBr) $\gamma$ $cm^{-1}$: 1672, 1625 and 1610. MS : $M^+$ at 284 calculated for $C_{16}H_{12}O_5$, other peaks are at m/z =256. 241, 227, 226, 213, 198 and 185.

Compound $E_3$: (30 mg, yield=0.0006 %).

UV $\lambda_{max}^{EtOH}$ nm (log $\epsilon$ ): 222 (4.67), 252(4.39), 275(4.07), 282 (4.09) and 428 (4.11). IR (KBr) $\gamma$ $cm^{-1}$ : 3400, 3200, 1675 and 1570. MS:$M^+$ , 270 calculated for $C_{15}H_{10}O_5$, other peaks are at m/z=252, 224, 213, 196 and 185.

Compound $E_4$ : (15 mg, yield=0.0003 %).

UV $\lambda_{max}^{EtOH}$ nm (log $\epsilon$ ): 220 (4.51), 255 (4.22), 262(4.20), 285(4.26) and 435 (4.0). IR (KBr) $\gamma$ $cm^{-1}$ : 3390, 1675 and 1610. NS : $M^+$ 270 calculated for $C_{15}H_{10}O_5$, other peaks are at m/z =242, 214 and 196.

Compound $E_5$ : (5 mg, yield=0.0001 %) :

UV $\lambda_{max}^{EtOH}$ nm (log $\epsilon$ ): 228(4.03), 285 (3.72) 415 (3.26). IR ($CHCl_3$) $\gamma$ $cm^{-1}$ : 2920, 1620, 1680, 1630 and 1600. MS :$M^+$ 260 calculated for $C_{14}H_{12}O_5$, other peaks are at m/z =245, 202, 189 and 161.

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Compound $E_6$ : (70 mg, yield=0.0014 %) :

UV $\lambda^{EtOH}_{max}$ nm (log $\epsilon$ ): 220 (4.58), 250(4.27), 262 (4.28),282(4.23) and 430 (4.09). IR (KBr) $\nu$ $cm^{-1}$ : 3540, 3460, 1680, 1620 and 1565. MS:$M^+$ 300 calculated for $C_{16}H_{12}O_6$, other peaks are at m/z=284,271, 255, 242,226 and 213.

Compound $S_3$: (10 mg, yield=0.0002 %) :

IR (KBr) $\nu$ $cm^{-1}$ : 3100,1710 and 1630. MS:$M^+$ 112 calculated for $C_4H_4N_2O_2$, other diagnostic peaks are at m/z=69,68,42,41 and 40.

## RESULTS AND DISCUSSION

The ether fraction of the ethanolic extract of the leaves, stems, flowers and fruits of C.didymobotrya Fres. gave positive results for the presence of anthraquinones, when chromatographed over silica gel column, six quinones were obtained ($E_1$-$E_6$).

The UV absorption spectra of the isolated anthraquinones show intense benzenoid absorption at ca.250 nm, strong quinonoid absorption band at 275 nm and a weak band at 428 nm. The band at 222 nm is characteristic for hydroxyanthraquinones[4].

The IR spectra of all the isolated compounds show a carbonyl absorption band at 1675 $cm^{-1}$ as well as C=C vibration band at 1605 $cm^{-1}$.

Compound $E_1$

$^1$H-NMR spectrum, Table 2, showed a signal at 2.42 ppm for $CH_3$ group which is confirmed by a signal at 21.46 ppm in the $^{13}$C-NMR spectrum, Table 3. The $^1$H-NMR spectrum showed also two quartets at 7.18 and 7.52 ppm for 2-H and 4-H. two doublets at 7.35 and 7.68 ppm for 7-H and 5-H; a doublet of doublet at 7.78 ppm for 6-H. These signals were confirmed by the presence of five signals in the $^{13}$C-NMR in the C-H region, C-2, 4,5,7 and 6 at 123.89, 120.36,119,12,124.20 and 137.12 ppm respectively. The signal at 11.9 ppm attributable to

the two chelated hydroxyl groups was confirmed by the presence of two signals at 161.45 and 161.14 ppm for C-1 and C-8. The remaining five signals in the $^{13}$C-NMR were corresponding to the quaternary carbon atoms in the molecule.

The fragmentation of $E_1$ as well as the other anthraquinones proceeds as reported in the literature[5], with the expulsion of methyl radical and carbon monoxide. Loss of additional two molecules of carbon monoxide can be also considered.

These data are identical with the previously isolated chrysophanol[4-11] Accordingly, compound $E_1$ was identified as chrysophanol.

Compound $E_2$ :

$^{1}$H-NMR spectrum, Table 2, showed two signals at 2.42 and 3,93 ppm for $CH_3$ and $OCH_3$ groups attached to C-3 and C-6 which were confirmed by two signals at 21.38 and 56.25 ppm in the $^{13}$C-NMR spectrum Table 3 . The spectrum showed also two quartets at 7.09 and 7.67 ppm for 2-H and 4-H, two doublets at 6.69 and 7.4 ppm for 7-H and 5-H which were confirmed by four signals in the $^{13}$C-NMR in the CH region, C-2, 4,5 and 7. Table 3. The two doublets at 21.14 and 12.34 ppm are corresponding to the two hydroxyl groups attached to C-1 and C-8. This was confirmed by the presence of two signals in the $^{13}$C-NMR spectrum, Table 3. The remaining six signals in the $^{13}$C-NMR spectrum are corresponding to the quaternary carbon atoms in the molecule.

These results are identical with those reported for physcion[4-12]. This is the first report on the isolation of physcion from C. didymobotrya Fres.

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Compound $E_3$ :

$^1$H-NMR spectrum showed a doublet at 4.63 ppm for $CH_2$ group confirmed by a signal at 61.97 ppm in the $^{13}$C-NMR spectrum , Table 3. The spectrum showed also a triplet at 5.55 ppm for aliphatic hydroxyl group, two quartets at 7.31 and 7.72 ppm assigned for 2-H and 4-H, two doublet of doublets at 7.38 and 7.74 ppm for 7-H and 5-H and a doublet of doublet at 7.81 ppm assigned for 6-H. These five signals were confirmed by the presence of five signals in the $^{13}$C-NMR spectrum in the CH region, C-4, 2,7,5 and 6, Table 3.

The spectrum showed a signal at 11.9 ppm for the two chelated hydroxyl groups which were confirmed by the two signals in the $^{13}$C-NMR for C-1 and C-8. The remaining five signals in the $^{13}$C-NMR spectrum were assigned for the quaternary carbon atoms in the molecule.

Compound $E_3$ was found to be aloe-emodin on comparison with the data reported for the compound[4-12]. The $^{13}$C-NMR assignments which were reported fcr the first time in addition to MS fragmentation pattern confirmed the structure of the isolated compound.

Compound $E_4$ :

$^1$H-NMR spectrum showed a singlet at 2.42 ppm which was assigned for $CH_3$ group and was confirmed by a signal at 21.43 ppm in the $^{13}$C-NMR spectrum, Table 3. Two doublets at 6.57 and 7.12 ppm, two quartets at 7.18 and 7.51 ppm which were assigned for 7-H, 5-H, 2-H and 4-H respectively and was confirmed by the presence of four signals in the $^{13}$C-NMR spectrum corresponding to CH groups for C-2, 4,5 and 7. The spectrum showed a broad signal at 12.1 ppm for 1,8-dihydroxy groups which was confirmed by two signals in the $^{13}$C-NMR spectrum, a signal at 161.30 ppm was assigned for C-6(-C-OH). The remaining five signals were assigned to the quaternary carbon atoms in the molecule.

Compound $E_4$ was found to be emodin, on comparison with the data reported for the compound[6-11]. The $^{13}$C-NMR assignments are reported for the first time.

It is noteworthy to mention that emodin was isolated for the first time from C.didymobotrya Fres.

Compound $E_5$ :

$^1$H-NMR spectrum showed three singlets in $CH_3$ group region at 2.36,2.61 and 3.94 ppm for 7-$H_3$, 6-$CH_3$=CO and 2-$OCH_3$ respectively. The spectrum showed also two singlets at 6.13 and 7.56 ppm for the quinone and aromatic protons at C-3 and C-8 respectively. The singlet at 12.55 ppm was assigned for the chelated hydroxyl group at C-5.

The fragmentation of compound $E_5$ proceeds through the expulsion of acetyl group and two molecules of carbon monoxide.

The results obtained for compound E5 are identical with those reported for 2-methoxystypondron isolated from the stem bark of Rhamus fallax Boiss.[4,13,14]. However this is the first report on the isolation of this compound from a member of the Family Caesalpiniaceae.

Compound $E_6$ :

$^1$H-NMR spectrum showed a singlet at 3.93 ppm which was assigned for the methoxyl group at C-6, a doublet at 4.61 ppm for $CH_2$ at C-3. These signals were confirmed by the two signals in the $^{13}$C-NMR spectrum for C-16 and C-15, Table 3. The spectrum showed also four doublets at 6.85, 7.66, 7.18 and 7.25 ppm which were assigned for 7-H, 5-H, 2-H and 4-H respectively . These four signals were confirmed by the presence of four signals in the $^{13}$C-NMR spectrum, Table 3, corresponding to C-7,5,2 and 4,

The signal at 12 ppm assigned to the two chelated hydroxyl groups at C-1 and C-8 evidenced by the two signals in the $^{13}$C-NMR spectrum. The triplet at 5.54 ppm was assigned to the aliphatic hydroxyl group at C-3.

Compound $E_6$ was identical with fallacinol which was previously isolated from Lichens Teloschites flavicans (SW.) Norm., T.exilis (Michaux) Winio[4] and this is the first report of this compound in family Leguminosae (Caesalpiniaceae).

Compound $S_3$ :

IR spectrum showed a carbonyl absorption band at 1710 $cm^{-1}$, band at 3400 $cm^{-1}$ for NH stretching, the absorbtion bands at 1630 and 3100 $cm^{-1}$ were assigned to C=C and the aromatic C-H stretching respectively. $^1$H-NMR spectrum showed two doublets at 7.44 and 5.5 ppm assigned to H-3 and H-2 respectively, a broad signal at 10.95 ppm was assigned to two NH groups (exchangeable with $D_2O$).

The mass spectrum was found to be identical with that reported for the pyrimidine base uracil[15] and this is the first report of this compound in family Caesalpiniaceae.

Uracil has been obtained from wheat germ, bovine thymus or spleen and earlier by hydrolysis of herring sperm[16]

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# دراسة كيميائية لنبات الكاسيا ديديموبوتريا فرس المنزرع فى مصـــــر

## ١ ـ المواد الكينونيـــه واليوراســـــيل

سامية محمد الصياد ، عفاف محمد عبد الباقى ، انعام يونس بخـــــــــيت وكارل فرنرجلومـــــــيتزا *

قسم العقاقير ـ كلية الصيدلة ـ جامعة آسيوط و * معهد الصيدلة ـ جامعة بون بالمانيا

تعتبر بعض النباتات من جنس الكاسيا من أغنى المصادر الطبيعية للمـــــواد الانثراكينونية الى جانب العديد من المركبات الاخرى ذات النوعيات المختلفة ٠

وقد ذكر الباحثون أن نبات الكاسيا ديديمويوتريا فرس سام بسبب التهابات القناه الهضمية مصحوبة باسهال وقئ ٠

وبدراسة هذا النبات وجد أنه يحتوى على مواد انثراكينونيه وفلافونيديـــه وكذلك استيرولات غير مشبعه ومواد نيتروجينية ٠

وتشتمل هذة الدراسة على فصل والتعرف على المواد الكينونيه والنيتروجينيـــه

وقد تم التعرف على المركبات المفصوله والمحضره فى صوره نقية من خـــــلال دراستها فيزيقيا وكيميائيا وطيفيا باستعمال الرنين النووى البروتونى والكربونى ومطياف الكتله بنوعيه وكذلك الاشعه دون الحمراء وفوق البنفسحية ٠

والمواد المتعرف عليها هى كالاتى :

١ ـ كينونات ، كريزوفانول ، آلو ـ ايمودين ، ايمودين ،فسيون ، ٢ ـ ميثوكسى استباندرون والفلاسينول ٠

٢ ـ مواد نيتروجينيـــه : يوارســـيل

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