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LIGNANS AND AN ALKALOID FROM *HAPLOPHYLLUM TUBERCULATUM*

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فى هذا البحث تم فصل ثلاثة من الليجنانات وهى كوزونوكينين، كاروفيللين ، جستيسيدين أ وذلك بالاضافة الى قلوانى من مجموعة مشتقات الفيوروكينولين وهو ٧-أ-إيزوبنتينيل أوكسى-٨-فاجارين والذى يفصل لأول مرة من النبات موضوع البحث. وكذلك مركب كاروفيللين والذى يفصل لأول مرة من جنس الهابلوفيللم. وقد تم تعيين التركيب الكيميائى للمركبات المفصولة باستخدام الطرق الطيفية والتى شملت الرنين النووى المغناطيسى أحادى وثنائى الاحداثيات.

The aerial parts of Haplophyllum tuberculatum (Forssk) A. Juss grown in Egypt yielded three lignans named as kusunokinin [I], chaerophyllin [II], justicidin A [IV] as well as an alkaloid, 7-isopentenyloxy-8-fagarine [III] which is reported here for the first time from H. tuberculatum., while compound [II] is reported for the first time from the genus haplophyllum. The structure elucidation of the isolated compounds are based on spectroscopic methods including 1D-and 2D-NMR spectra.

INTRODUCTION

The genus *Haplophyllum* (Rutaceae) is represented by about 70 species spread out from the Mediterranean to Eastern Siberia.[1] It is represented in Egypt by *H. tuberculatum* (Forssk) A. Juss, *H. obovatum* (Hochst. ex. Boiss) and *H. longifolium* Boiss.[2]

Studies on *H. tuberculatum* (Forssk) A. Juss resulted in the isolation of several alkaloids as γ-fagarine, evoxine, skimmianine, 3-(1´, 1´-dimethylallyl)-3-(3´´,3´´-dimethylallyl)-1,2,3,4 tetrahydro-2,4-quinoldione,[3-5,9] lignans as polygamain, kusunokinin, justicidin A, justicidin B, dyphyllin and tuberculatin,[5-9] and flavonoids as 5,7,4´-trihydroxy-6-methoxy-3-O-glucosylflavone.[6]

H. tuberculatum (Forssk) A. Juss is used in folk medicine in Egypt for the treatment of nausea, constipation, malaria and gasrtic pain.[10]

This work on the chemical composition of this annual herb has resulted in the isolation of an alkaloid, 7-isopentenyloxy-8-fagarine **[III]** and three lignans, viz, kusunokinin **[I]**, chaerophyllin **[II]** and justicidin A **[IV]**. Compound **[III]** is reported here for the first time from *H. tuberculatum*, while compound **[II]** is reported for the first time from the genus *Haplophyllum*.

The structure of the isolated compounds was established using different spectroscopic techniques including IR, MS, ^{1}H-and ^{13}C-NMR, and different types of 2D-NMR (^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, HMBC and 2D-NOESY).

EXPERIMENTAL

General experimental procedures

Mps were uncorrected. ^{1}H, and ^{13}C-NMR spectra were recorded in $CDCl_3$ unless otherwise mentioned at 300 MHz and 75 MHz, respectively on Varian Unity 300 "Uni 300" spectrometer using TMS as internal standard. MS spectra on Finnigan MAT-312, 70 eV. IR were taken in KBr with Shimadzu, IR-470 (Shimadzu corporation, Japan). Optical rotation was carried out using polarimeter 241, Perkin-Elmer. For CC, silica gel (E. Merck), and pre-

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packed columns [Lobar-Kieselgel columns (40-63 μm) (Merck) size B (310x25 mm)] were used. Precoated silica gel 60 F_{254} plates (E. Merck) were used for TLC.

The following solvent systems were also used:-

System I: Benzene-Ethyl acetate (90:10)
System II: $CHCl_3$-MeOH (90:10)

Plant material

The plant material was collected during flowering stage in May 1997 from El-Kharga, the New Valley. The plant material was air-dried in the shade. The samples were authenticated by Prof. Dr. A. Fayed, Professor of plant Taxonomy, Faculty of Science, Assiut University. A voucher specimen is deposited in herbarium of the Dept. of Pharmacognosy, Faculty of Pharmacy, Assiut University.

Extraction and Isolation

3 kg of the air-dried powdered aerial parts was extracted with 90% methanol by maceration. The methanolic extract (150 g) was diluted with water (150 ml) and successively extracted with hexane (5x1 L), chloroform (5x750 ml), ethyl acetate (3x500 ml) and finally with butanol (4x500 ml). The thin layer chromatography of the chloroform and ethyl acetate extracts using silica gel G as adsorbent and systems I and II as solvent systems revealed at least 6 similar Dragendorff's positive spots, but the spots are more concentrated in the chloroform extract. The chloroform and ethyl acetate extracts were combined together, concentrated under reduced pressure (20 g) and chromatographed over silica gel column chromatography (3x120 cm) and eluted with hexane-ethyl acetate gradients.

Fractions eluted with hexane-ethyl acetate (95:5) afforded compound **[I]** and further fractions eluted with hexane-ethyl acetate (9:1) afforded a mixture of **[II]** and **[III]**. This mixture was subsequently separated by further chromatography on an efficient Lobar silica gel prepacked column, eluted with Benzene-ethyl acetate (90:10), resulted in the isolation of **[II]** and **[III]**.

Elution with hexane-ethyl acetate (80:20) afforded a mixture of flavonoids which are still under investigation. Elution with hexane-ethyl acetate (70:30) afforded **[IV]**.

Compound [I]

Colourless gummy substance (30 mg), $[\alpha]_D^{20}$ =-30.7° ($CHCl_3$, c= 0.812). MS m/z (rel. Int.%) 370.1 (36.27) [M^+] corresponding to molecular formula $C_{21}H_{22}O_6$, 235 (3.85), 219 (3.03), 192 (8.54), 135 (100), 151 (84.3), 178 (13.5).

IR ν_{max}^{KBr} cm^{-1}, 1762, 1509, 1485, 1458, 925. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2, respectively.

Compound [II]

Colourless needles (35 mg), mp 150-152°C (MeOH), $[\alpha]_D^{20}$ =-65.7° ($CHCl_3$, c= 0.7). MS m/z (rel. Int. %) 368 (12) [M^+] correspoding to molecular formula $C_{21}H_{20}O_6$, 253 (6), 178 (12), 177 (25), 135 (100), 151 (80), 107 (20), 77 (26).

IR ν_{max}^{KBr} cm^{-1}, 1742, 1650, 1600, 1545, 1495, 1480, 932, 885, 850. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2, respectively.

Compound [III]

Colourless needles (50 mg), mp 106-108°C (Acetone), MS m/z (rel. Int. %) 313 (5) [M^+] corresponding to molecular formula $C_{18}H_{19}NO_4$, 245 (60), 227 (100), 230 (27), 216 (25), 202 (65), 187 (37),159 (20).

IR ν_{max}^{KBr} cm^{-1}, 3120, 2915, 1575, 1482, 1007, 991, 728. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2, respectively.

Compound [IV]

Colourless needles (32 mg), mp 270-272°C (Acetone). IR ν_{max}^{KBr} cm^{-1}, 1746, 1603, 1495, 1428, 1258, 1228, 1167, 1083, 1048, 921. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2, respectively.

Table 1: ^{1}H-NMR Data and NOE-correlations of the isolated compounds

H. Atom	Compound [I]		Compound [II]		Compound [III]		Compound [IV]	
	δ, ppm (J, Hz)	NOE with H	δ, ppm (J, Hz)	NOE with H	δ, ppm (J, Hz)	NOE with H	δ, ppm (J, Hz)	NOE with H
2	2.5, m	2´´, 2´, 6´	--------	-------	7.55, d, (3.5)	3	5.6, s	OMe-3
3	2.54, m	---------	3.83, m	-------	7.00, d (3.5)	OMe-4, 2	-------	-----
4	----------	----------	--------	--------	--------	-------	7.49, s	OMe-5
4a	4.15, dd, (9.3, 6.6)	4b	4.24, dd, (9.5, 2.4)	4b	--------	-----	------	-----
4b	3.88, dd, (9.3, 7.1)	4a	4.32, dd, (9.5, 7.1)	4a	--------	-----	-------	----
5a	2.62, dd, (11.5, 5.6)	2´´, 6´´	3.02, dd, (14.2, 9.8)	2´´, 6´´ 5b	7.95, d, (10.5)	6	-------	----
5b	2.49, dd, (11.5, 8.2)	2´´, 6´´	2.64, dd, (14.2, 4.3)	2´´, 6´´ 5a	------	------	-------	-----
6a	2.84, dd, (14.2, 5.1)	2´, 6´	7.52, d, (1.9)	2´	7.20, d, (10.5)	5, 1´	------	------
6b	2.96, dd, (14.2, 6.6)	2´, 6´	--------	------	--------	------	-----	-----
7	----------	--------	-------	-------	--------		6.92, s	OMe-6
1´	----------	--------	-------	-------	4.73, d (6.5)	6, 2´	------	------

Table 1 :Continued

H. Atom	Compound [I]		Compound [II]		Compound [III]		Compound [IV]	
	δ, ppm (J, Hz)	NOE with H	δ, ppm (J, Hz)	NOE with H	δ, ppm (J, Hz)	NOE with H	δ, ppm (J, Hz)	NOE with H
2´	6.60, d, (2.1)	6´ OMe-3´	7.08, d (1.7)	6, OMe-3´	5.55, t (6.5)	1´	6.78, d (1.7)	-------
4´	---------	-------	---------	-------	1.73, 3H, s	-------	---------	-------
5´	6.71, d, (7.5)	6´, OMe-4´	6.86, d, (7.1)	OMe-4´	1.76 3H, s	-------	6.95, d (8.1)	-------
6´	6.58, dd, (7.5, 2.1)	5´	7.10, dd (7.1, 1.7)	-------	-------	-------	6.71, dd (8.1,1.7)	-------
2´´	6.48, d, (2)	---------	6.70, d, (1.7)	-------	-------	-------	---------	-------
5´´	6.77, d, (8)	---------	6.76, d, (7.9)	-------	-------	-------	---------	-------
6´´	6.56, dd, (8, 2)	---------	6.69, dd, (7.9, 1.7)	-------	---------	---------	---------	-------
OCH_2O	5.95, 5.93, dd, (1.5)	---------	6.03, 2H, s	-------	-------	-------	6.04, dd (1.2)	-------
OCH_3	3.86, s [OMe-4´]	-------	3.85	-------	4.38, s [OMe-4]	-------	4.1, s	-------
OCH_3	3.83, s [OMe-3´]	-------	3.87	-------	4.08, s [OMe-8]	---------	3.98, s	-------
OCH_3	---------	---------	---------	------	-------	-------	3.65, s	------

Table 2: ^{13}C-NMR Data and HMBC correlations of the isolated compounds

C. Atom	Compound [I]		Compound [II]		Compound [III]		Compound [IV]	
	δ, (ppm)	HMBC	δ, (ppm)	HMBC	δ, (ppm)	HMBC	δ, (ppm)	HMBC
1	178.44	6a, 6b	172.57	4, 6	------	------	------	-------
2	46.46	3, 6a, 6b	126.34	5, 6	142.89	3	66.67	-------
2a	------	------	------	------	------	------	119.12	2
3	41.42	2, 6a, 6b,4a, 5a, 5b	40.07	5, 6	104.56	--------	147.49	4, 2
3a	------	------	------	------	102.03	3, 2	129.48	4, 2
4	71.22	5a, 5b	69.77	5	157.17	5, OMe-4	100.81	2
4a	------	------	------	------	114.93	6	-------	-------
5	38.28	------	37.67	2, 3, 4, 6´´	117.81	------	151.52	4
6	34.79	------	137.17	3, 2´, 6´	114.39	------	150.25	------
7	------	------	------	------	151.38	5, 6, CH_2-	105.95	------
7a	------	------	------	------	------	------	128.70	7
7b	------	------	------	-------	------	------	125.31	------
8	------	------	------	-------	142.23	6, OMe-8	169.09	2
8a	------	------	------	-------	141.41	5	124.25	------
9a	-------	-------	-------	-------	164.19	2, 3	-------	-------

Table 2 :Continued

C. Atom	Compound [I]		Compound [II]		Compound [III]		Compound[IV]	
	δ, (ppm)	HMBC	δ, (ppm)	HMBC	δ, (ppm)	HMBC	δ, (ppm)	HMBC
1′	131.37	6a, 6b, 5′	130.43	5′, 6	66.78	--------	132.95	2′, 5′, 6′
2′	109.47	6a, 6b	108.77	6′, 6	120.06	CH_2-1′	110.85	6′
3′	147.89	5′	149.19	-------	137.87	CH2-1′, Me-4′, Me-5′	146.99	2′
4′	146.47	2′	149.19	-------	18.18	-------	147.12	2′
5′	108.17	-------	112.19	-------	25.70	-------	107.87	-------
6′	122.25	6a, 6b, 2′	126.03	6, 2′	-------	-------	123.68	2′
1′′	130.34	5a, 5b, 5′′	128.25	2′′, 5′′	-------	-------	-------	-------
2′′	111.76	5a, 5b	108.48	------	-------	--------	-------	-------
3′′	149.12	5′′	148.11	--------	------	--------	-------	-------
4′′	147.94	2′′	148.36	-------	------	-------	-------	--------
5′′	111.38	-------	111.52	-------	-------	-------	-------	-------
6′′	120.64	2′′	120.91	-------	--------	-------	-------	-------
OCH_2O	101.02	-------	101.42	-------	--------	-------	101.14	------
OCH_3	55.92	--------	55.91	-------	58.92 (OMe-4)	-------	55.19	-------
OCH_3	55.79	--------	--------	-------	64.44 (OMe-8)	-------	55.22	-------
OCH_3	-------	-------	-------	-------	-------	-------	55.60	-------

RESULTS AND DISCUSSION

Compound [I]

This compound was obtained as colourless gummy substance, and exhibited a strong carbonyl absorption at cm^{-1} 1762 in its IR spectrum, suggesting the presence of a butyrolactone system.[11] The 300 MHz ^{1}H-NMR (Table 1) displayed two aromatic methoxyl singlets, a methylenedioxy group as two doublets and six aromatic protons as two ABX systems. The mass spectrum displyed M^+ at m/z 370 corresponding to the molecular formula $C_{21}H_{22}O_6$. The major ions resulting from the facile benzylic cleavages were m/z 135 and 151. These data pointed to a butanolide substituted at C-2 and C-3 with two benzylic moieties bearing two methoxyls in one case and a methylenedioxy substituent in the other. NOE (or 2D-NOESY), one-bond ^{1}H-^{13}C-HETCOR and HMBC experiments, were of equal importance for the precise proton and carbon assignments. The 2D-NOESY was carried out to complement the data. In particular the H-2´ doublet (δ 6.60) exhibited NOEs with the methoxyl signal (δ 3.83) while H-5´ doublet exhibited NOEs with the methoxyl signal (δ 3.86), thus confirming the site of the methoxyl groups. The relative configuration at C-2 and C-3 for compound [I] could be established from the NMR spectrum. The ring fusion is trans because the non-equivalence of the C-4 methylene protons H-4a and H-4b being at δ 4.15 and 3.88.[11-15] The coupling constants $J_{3,4a}$ and $J_{3,4b}$ are 6.6, 7.1 Hz which led to further support to a trans relationship the two benzylic protons.

The assignments of the carbon signals was deduced from one-bond ^{1}H-^{13}C-HETCOR and HMBC spectra (Table 2). For example, the two and three-bond couplings between C-5 and the protons H-3, H-2, H-2´´ and H-6´´ as well as between C-6 and the protons H-2, H-3, H-2´ and H-6´, respectively ascertain the assignments of these signals.

The data obtained were found in good agreement with the data reported for kusunokinin[9], a lignan isolated from *H. tuberculatum* grown in Libya.

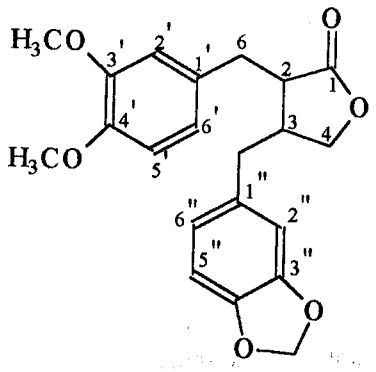
Compound [II]

This compound was obtained as white crystalline powder, melted at 150-152°C. The IR spectrum showed characteristic peaks for an α-ß unsaturated γ-lactone (1742 cm^{-1}), an olefinic double bond (1650 cm^{-1}), an aromatic nucleus (1600 and 1480 cm^{-1}) and a methylene-dioxy (932 cm^{-1}) structure.

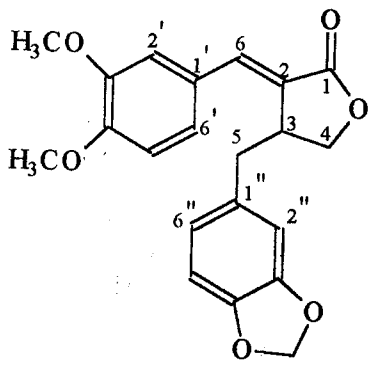
The MS revealed M^+ at m/z 368 corresponding to the molecular formula $C_{21}H_{20}O_6$. The ^{1}H- and ^{13}C-NMR chemical shifts of II were determined using a combination of ^{1}H-^{1}H COSY and ^{1}H-^{13}C-HETCOR technique (Table 1). The spectrum revealed the presence of two methoxyl groups (δ 3.85, 3H,s and 3.87, 3H,s), one methylenedioxy (δ 6.03, 2H, s), two sets of ABX patterns assigned for six aromatic protons (δ 6.7-7.1). The larger chemical shift of the olefinic proton of the the benzylidene moiety (δ 7.52, d, J= 1.9 Hz) is associated with the olefinic proton cis to the carbonyl.[14,15] It is firmly established that in the E-isomers of analogous compounds, the olefinic protons, which is deshielded by the proximal carbonyl group, resonates at ca δ 7.5, whereas the corresponding chemical shift of the Z-counterparts is *ca* δ 6.5.[15-18]

The aliphatic region in the ^{1}H-NMR spectrum accounts for 5 protons, the downfield chemical shift of the geminal couple at δ 4.24, 4.32 allow their assignment as the γ-hydrogens of a γ-butyrolactone ring, the two methylene protons related to the benzylic hydrogen pair appeared at δ 3.02 and 2.64, and a methine proton at δ 3.83. The exact assignments of each proton and carbon were estabished by one-bond ^{1}H-^{13}C-HETCOR, 2D ^{1}H-^{1}H COSY and HMBC experiments (Table 1 and Table 2).

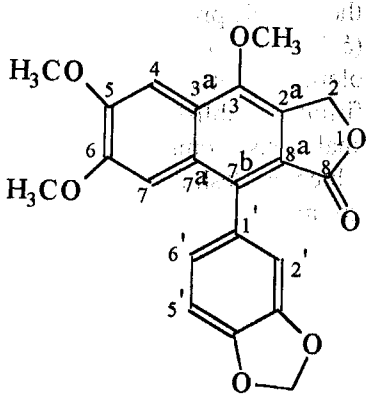
The above mentioned data are coinside with those reported for chaerophyllin isolated from *Chaerophyllum maculatum*[18] and this is the first report of this compound in the genus *Haplophyllum* but the resonances of carbons C-1´ and C-2 must be reversed since they reported these resonances at δ 126.93 and δ 131.51, but in our compound these carbons resonate at δ 130.43 and δ 126.34 respectively. This was confirmed from HMBC experiments. The two- and three bond coupling between C-2 and the protons at H-5a and H-5b and H-6, as well as



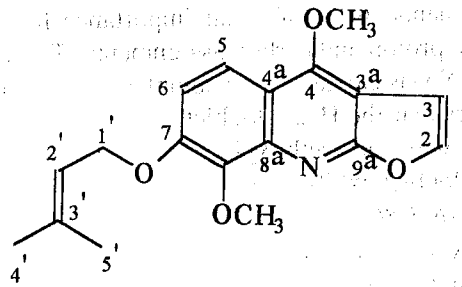
Compound [I]



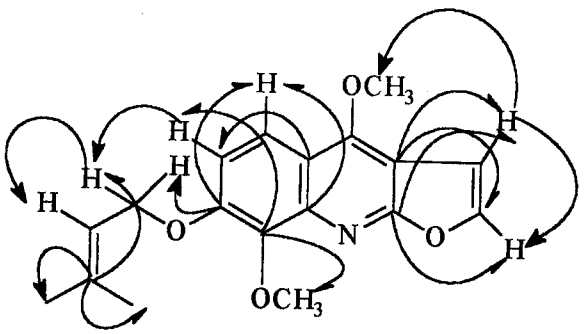
Compound [II]



Compound [IV]



Compound [III]



HMBC
NOE

Fig. 1: HMBC and NOE of compound **III**

between C-1′ and H-5′ ascertain the assignments of these signals.

Compound [III]

The ^{1}H-NMR spectrum showed the presence of two methoxyl groups at δ 4.38 and 4.08 with the ^{13}C-NMR signals at δ 58.92 and 61.44 for OMe located at C-4 and C-8, respectively. The presence of two AB quartet in the aromatic region of the ^{1}H-NMR provided confirmation of the furan protons (δ 7.00 and 7.55, J= 3.5 Hz) and for two adjacent aromatic protons (δ 7.20 and 7.95, J=10.5 Hz). Hence the additional substituents must be located at either C-7 or C-5, the former being preferred on biogenetic grounds.[5]

The assignment of these signals was established by the NO effects between OCH_3-4 and H-3, as well as H-5 and H-6 and also between H-6 and CH_2-1′. The OCH_3-8 showed no NO effects. The ^{1}H-NMR also indicated the presence of two tertiary methyl groups at δ 1.76 and 1.73, C= CH (δ 5.55, t, J= 6.5 Hz) and O-CH_2 group in the molecule (δ 4.73, d, J= 6.5 Hz) which suggested the presence of isopentenyloxy group.

The one- bond ^{1}H-^{13}C-HETCOR and DEPT experiments led to the exact assignment of the protonated carbons, while that of the quaternary carbons was deduced from HMBC spectrum (Table 2 and Fig .1). The protons H-5 and O$\underline{CH_3}$-4 show three-bond couplings to C-4, while H-6 and O$\underline{CH_3}$-8 show three bond coupling to C-8. The signals of C-4a and C-8a were established from the three bond couplings to H-6 and H-5, respectively. The two and three-bond coupling between C-3a and the protons H-3 and H-2, respectively, support the doubtless assignment of these signals, etc. The location of OCH_3 groups was also confirmed by 2D-NOESY.

This compound was identified as 7-isopentenyloxy-8-fagarine,[19,20] which reported for the first time in *H. tuberculatum* and its ^{13}C-NMR data is reported also for the first time.

Compound [IV]

This compound was obtained as fine needles (acetone) melted at 270-272°C. Its IR spectrum exhibited a strong carbonyl absorption at cm^{-1} 1746. The ^{1}H-NMR spectrum showed three methoxyls at δ 4.1, 3.98 and 3.65 and one methylenedioxy group at δ 6.04 (2H, s), and an isolated and deshielded methylene group at δ 5.6. The spectrum also revealed five aromatic protons, two of which occured as singlets at δ 7.49 and δ 6.92 and located in the ring carrying the methoxyl groups at δ 3.98 and 3.65 (2D NOESY), and two protons as an AB system at δ 6.71 and 6.95 showing ortho coupling J= 8.1 Hz while the fifth at δ 6.78 showed the meta coupling to one half of the AB system (that at δ 6.71). These data are in accordance with the data reported for Justicidin A.[3,6,13] The position of the methylene protons at C-2 was ascertained from the 2D-NOESY between methoxyl group at C-3 (δ 4.1) and the methylene protons at δ 5.6. The locations of the other two methoxyls was also established in the same manner (Table 1).

The ^{13}C-NMR spectral data of justicidin A (Table 2) is reported here for the first time and the assignments of each carbon was ascertained by one-bond ^{1}H-^{13}C-HETCOR, and HMBC experiments (Table 2).

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