

A Study of the Furocoumarin Derivative of *Ruta Chalepensis* L. (Rutaceae)

Ekbal H.AL-Khateeb, Ali A. AL-Shamma, Bajes A. Nehar

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ABSTRACT

The content of Furocoumarin derivatives (Psoralens) of *Ruta Chalepensis* L. (Whole plant) was studied by simple extraction with petroleum ether (b.p. 60-80°C). The results indicated that the plant contains a total of about (0.015%).

Investigation of these compounds by thin layer chromatography (TLC) revealed the presence of at least four compounds of which methoxsalen (8-methoxypsoralen) was isolated. It was identified and authenticated with a standard by spectral method, IR, NMR, Mass spectra and HPLC. This plant could be considered as a good source for supplying this compound, which is widely used in dermatological preparations.

الخلاصة

ان نبات السذاب من النباتات الطبية الموجودة في العراق والذي يزرع في اماكن متعددة ومعروف لدى متداولي الاعشاب باستعماله الشعبية. ان الدراسات المنشورة عن هذا النبات قليلة ولا توجد اي دراسة عن النبات في العراق، لذا فقد وجد من الضروري دراسة مكونات النبات بصورة كيميائية مفصلة والتعرف عما يحتويه هذا النبات من مواد الفيروكومارين (psoralen) وفصلها بالطرق المتبعة.

ان النتائج الالوية اثبتت لنا بان النبتة تحتوي على الفيروروكومارين (psoralen) لذا تم اولا دراسة شاملة لانتقاء احسن المذيبات العضوية التي يمكن استعمالها لاستخلاص هذه المواد من النبتة ثم تعيين كميتها ونوعيتها في كل من الجذور من جهة وفي الاوراق والسيقان من جهة اخرى. فقد تم جمع كمية من النبات وقد اظهرت النتائج بان البتروليوم ايثر الذي يغلي بدرجة حرارة 60-80°C كان احسن المذيبات العضوية المستعملة لاستخلاص مواد الفيروروكومارين (psoralen). لقد اثبتت لنا نتائج فحص المستخلص بطريقتي كروماتوغرافيا الطبقة الرقيقة عن وجود عدة مركبات في النبات وقد تم فصل اربعة مركبات من هذه المواد سميت كالآتي: BA₁, BA₂, BA₃, BA₄. ان المركب الاول (BA₁) تم فصله على شكل بلورات خفيفة وطويلة بيضاء درجة حرارة انصهاره 147-148°C والتحلل الكيميائي لهذا المركب اثبت بانه مركب الامويدي وهو من مركبات الفيروروكومارين (psoralen). اما المركب (BA₂) فقد تم فصله على شكل بلورات بيضاء اللون درجة انصهاره 76-78°C. لقد تم تعيين الوزن الجزيئي والصيغة الجزيئية لهذا المركب بواسطة تحليل الكتلة (Mass Spectroscopy) وان الوزن الجزيئي للمركب (BA₂) 256 والصيغة الجزيئية C₁₆H₁₆O₃. ثم تم عمل IR و NMR لهذا المركب لتحديد الشكل الكيميائي لهذا المركب، حيث وجد من خلال هذه التحاليل ان المركب (BA₂) يحتوي على نواة الفيروروكومارين (psoralen) وترتبط هذه النواة بسلسلة جانبية. لذا فقد تقرر حفظه في الوقت الحاضر لغرض تحديد تركيبه الكيميائي في المستقبل القريب. اما المركب (BA₃) و (BA₄) كانت كميتهم قليلة وتقرر حفظها في الوقت الحاضر لغرض دراستها بصورة مفصلة اكثر بالمستقبل.

INTRODUCTION

Ruta Chalepensis L. (Rutaceae) is a cultivatec plant^[1] widely grown as garden ornamentals in Baghdad and its vicinity. Ruta, Rue and Sadhab are common names for this plant.

Rutaceae (also termed Rue family) is one of the important families, that comprises many genera and species which have been used as a source of drugs and medicines^[2]. The decoction of this plant has been used in folk remedy as carminative to cure stomach ache in children^[3].

This study was carried in connection with a program of using the chemical constituents of Iraqi Medicinal Plants as a potential for industrial purposes. Literature survey revealed that *Ruta chalepensis* L., contains many important components^[3-9], among which are coumarins and Furocoumarin derivatives. Since

no previous work has been done on this Iraqi plant was found that study of these compounds, is of advantage for its economical value and uses in dermatological preparations, as available imported ones are very expensive.

MATERIALS and METHOD

The plant material was collected during September and October 1997 from the gardens of the vicinity of the city of Baghdad. Four and a half kilograms of powdered plant material (aerial plants and roots) were extracted with 12 liters of petroleum ether (b.p. 60-80°C), under reflux for 72 hours. The petroleum ether extract was filtered and evaporated to dryness, under reduced pressure at a temperature not exceeding 40°C, leaving a dark green residue (80 gm).

Separation and Fractionation of Furocoumarin Derivatives:

Different partition processes together with column chromatography technique were tried but with unsuccessful results. So the separation and fractionation was carried by using preparative thin layer chromatography.

A quantity of 20 gm of the residue was dissolved in a minimum amount of chloroform and applied together with standard psoralen reference compounds (methoxsalen and imperatorin) on a number of preparative TLC plates of silica gel GF254 (0.75 mm thickness) and developed with solvent system benzene-acetone (90:10 v/v).

The developed plates were visualized under U.V light (366 nm wavelength). Four major bands were observed and designated as band I, II, III and IV.

Band II and III were overlapped, and both have very close R_f values to that of the standard reference methoxsalen.

These two bands were separated by multiple development using the same solvent system to give two compounds.

Band I gave compound 1 as yellowish crystals (25 mg) upon recrystallization out of boiling methanol, a fluffy colorless crystals was obtained (20 mg) having a sharp melting point of 147.5°. Out of band II, compound 2 was isolated as yellowish prismatic crystals (125 mg), upon recrystallization out of hot petroleum ether (b.p. 60-80 C°) a colorless long prismatic crystals (120 mg) was obtained having a sharp melting point of 78 C°.

Out of band III, compound 3 was isolated as a yellowish crystals (2.7 mg) upon recrystallization out of boiling ethanol fluffy like crystals (2.5 mg) was obtained.

Out of band IV, compound 4 was isolated as an oily material. The total yields of these compounds isolated from the total extract (80 gm)– were: 80 mg of compound 1; 480 mg of compound 2; 10 mg of compound 3; and oily material designated as compound 4 .

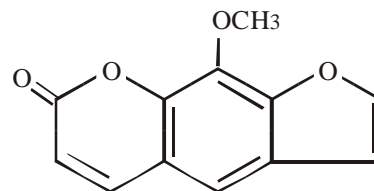
RESULTS and DISCUSSION

Out of these compounds, only compound 1 was identified and confirmed to be methoxsalen (xanthotoxin) as it has similar melting point, CH analysis, and R_f values in different solvent systems with standard reference of methoxsalen.

An assay by HPLC also was done for compound 1, using methoxsalen U.S.P. 1% as a standard reference. The retention time was exactly the same, further identification was carried out by enrichment technique, in which the reference was added individually to compound 1, using three different mobile phases (Fig. 1_{a, b, c}).

Other spectral methods were used for further confirmation. The mass spectrum of compound 1 indicates a molecular ion peak at m/e 216. The fragmentation pattern showing significant peaks as shown in (Fig. 2). The NMR spectrum in $CDCl_3$ (Fig. 3) confirmed the presence of eight protons. The proton (H_4) and (H_5) as two doublet at δ 6.8 (H_4) and δ 7.71 (H_5) (shielded (J) is small as expected for the furor ring ($J=1-2$ Cps)). (H_3) proton showed singlet peak at δ 7.82 (aromatic proton) (H_1) and (H_2) protons also appeared as two doublet peaks at δ 7.35, δ 7.26 for (H_1) proton, at δ 6.43 and δ 6.31 for (H_2) proton. Methoxy group protons showed at δ 4.3. The IR spectrum (KBr) (Fig. 4) confirms the structure. C = O stretching band at 1575 cm^{-1} for the α, β -unsaturated lactone. Two bands at 1600 cm^{-1} 1575 cm^{-1} for (C = C) stretching vibration of the aromatic ring. (C = C) olefinic band at 1660 cm^{-1} [10]. Two bands at 1450 cm^{-1} and 1375 cm^{-1} for asymmetric bending vibration of CH_3 group respectively. The stretching (C-O- CH_3) appears at 1280 cm^{-1} for asymmetrical stretching and 1020 cm^{-1} for symmetrical stretching. Two characteristic stretching vibrations for the ester function appear at 1145 cm^{-1} and 1090 cm^{-1} . The most characteristic out of plane C-H bending vibration at 822 cm^{-1} and 760 cm^{-1} . Moreover the C-H out plane bending vibration at 870 cm^{-1} is due to one free (H) in benzene ring. Finally the out of plane olefinic (H) (cisolefin) is present at 755 cm^{-1} in agreement with the structure [10].

All the above mentioned spectral data confirmed that compound 1 is methoxsalen having the following structure:



Compound 2, 3, and 4 left for further identification studies

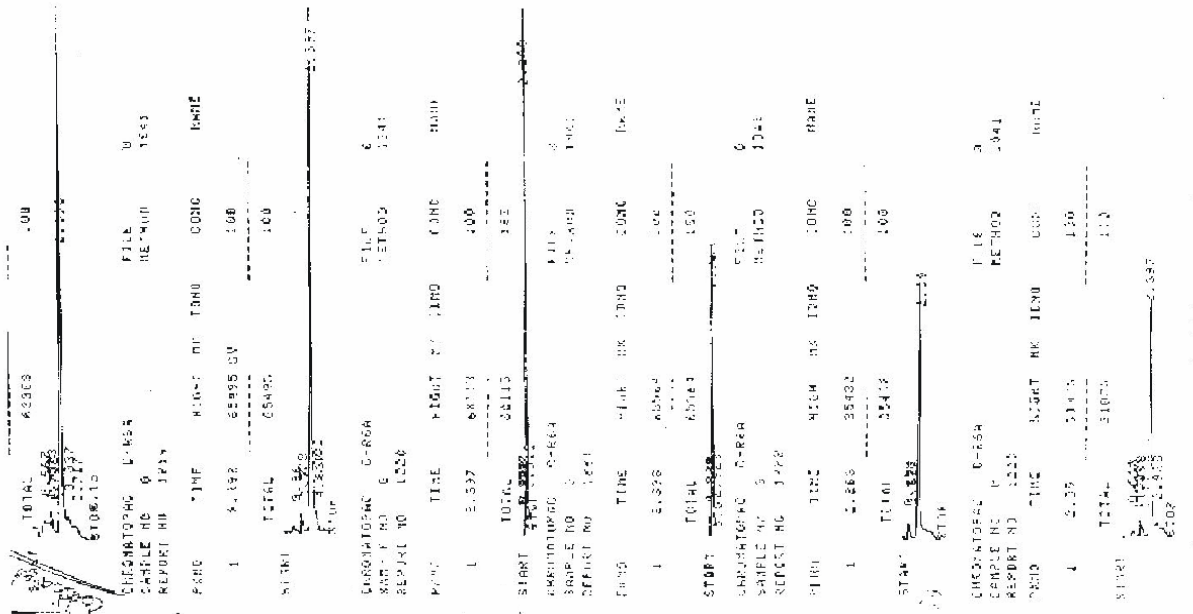


Fig (1a) H.P.L.C for a mixture of equal quantities of the compound (BA₁) and the standard reference Oxorsalen : (Methoxsalen U.S.P 1%) with second mobile phase

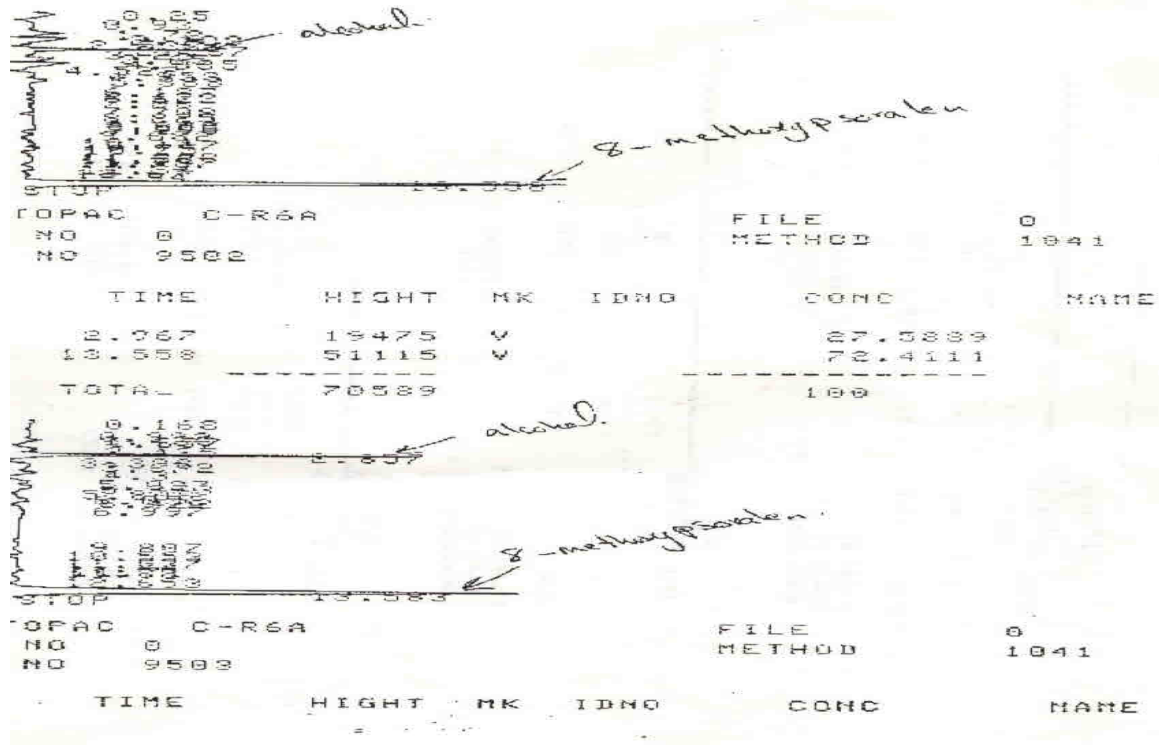


Fig (1b) HPLC for the compound (BA₁) compared to the standard reference oxorsalen ;(Methoxsalen u.s.p 1%)



Fig.(1c) HPLC for a mixture of equal quantities of the compound (BA₁) and the standard reference Oxsoralen ; (Methoxsalen C.S.P 1%) with third mobile phase

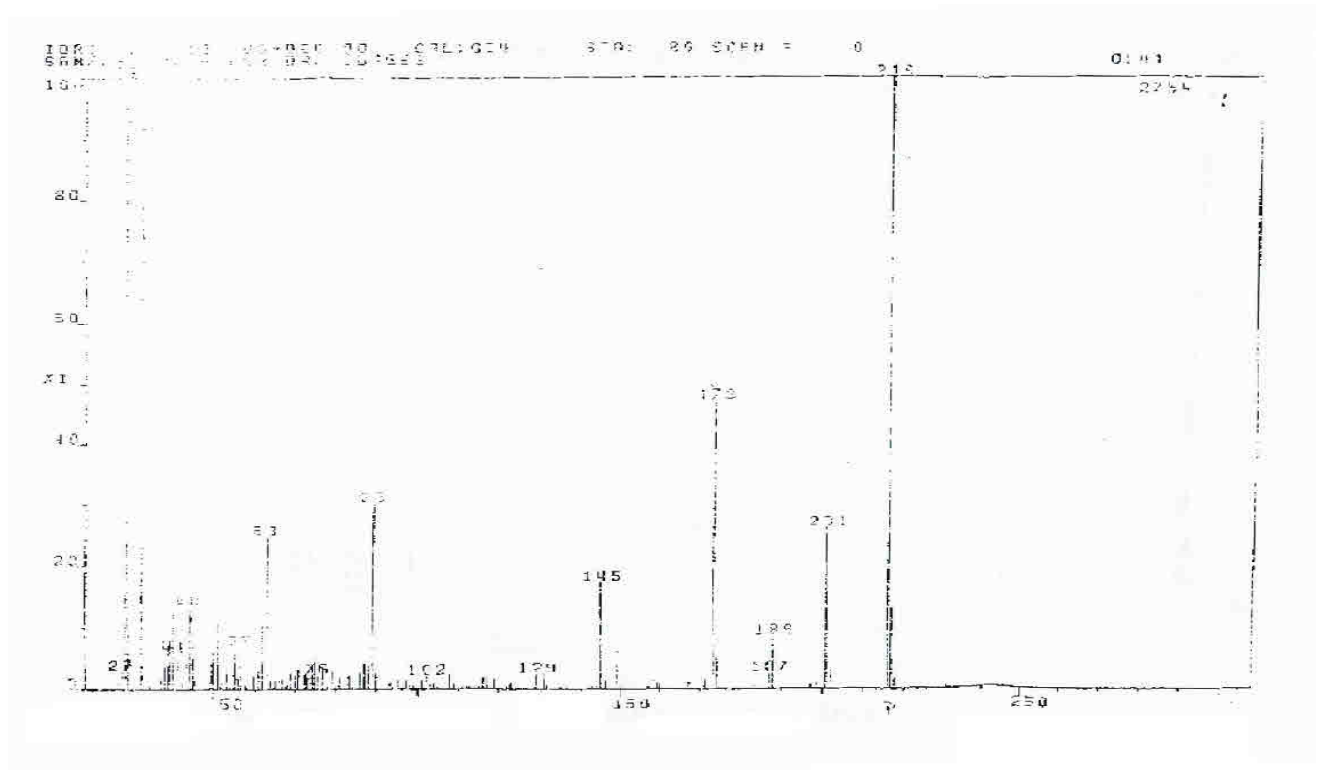


Fig (2) Mass Spectrum of the compound (BA₁)

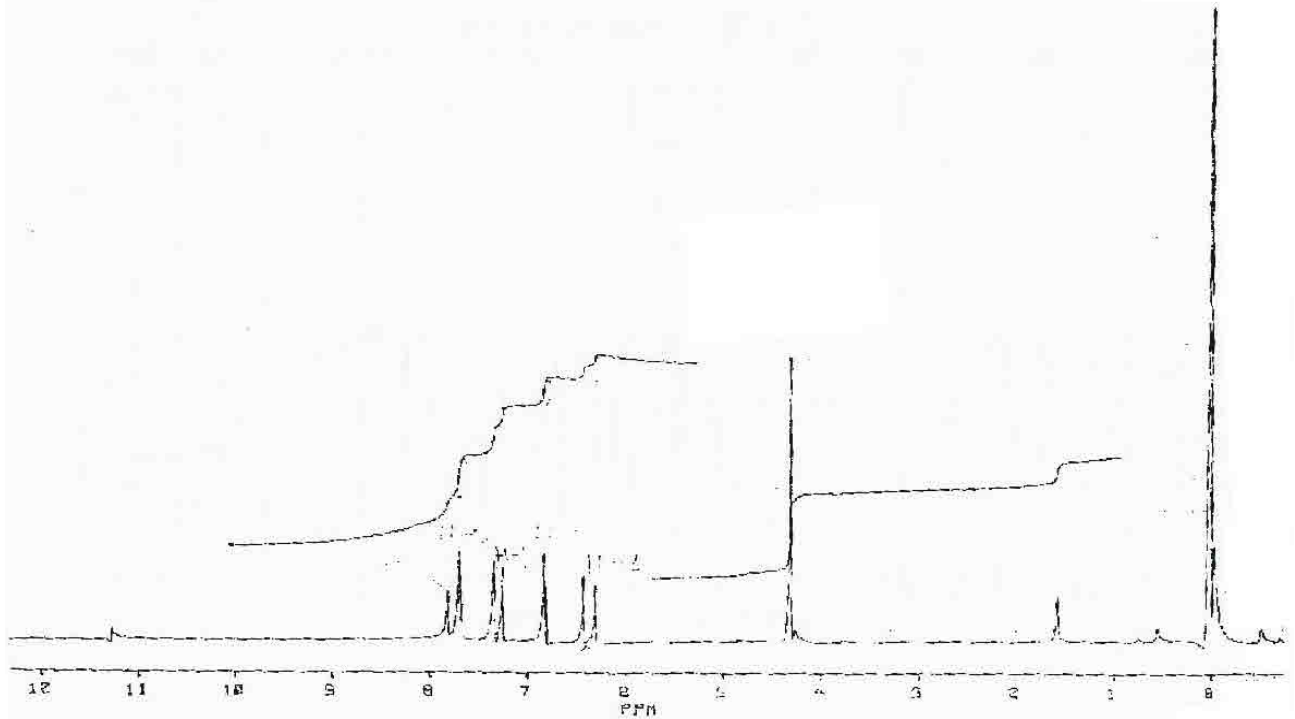


Fig (3): NMR Spectrum of the compound (Ba₁) determined in CDCl₃

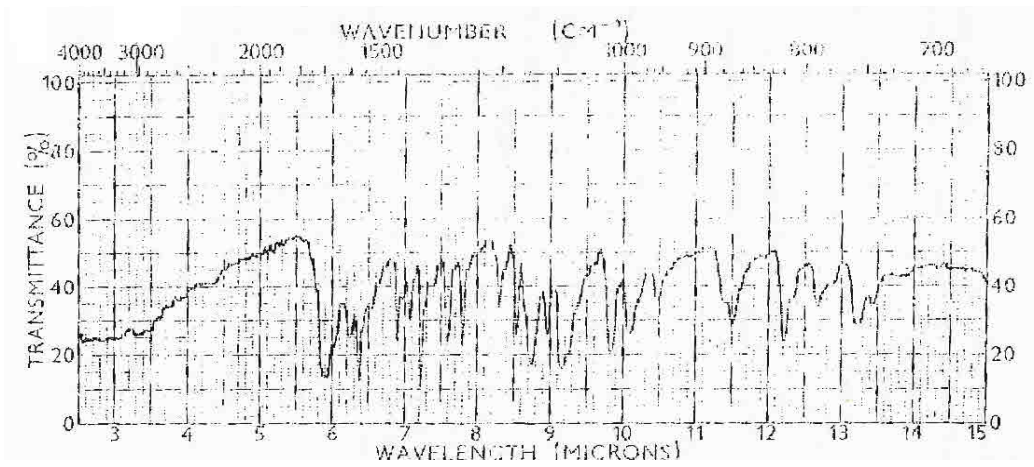


Fig (4) :IR Spectrum of compound (BA1)

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