A STUDY OF THE COUMARIN CONTENTS OF CITRUS GRANDIS FRUITS GROWING IN EGYPT

Hamida H. El-Gohary, Shadia M. Azzam, Kadria S. El-Deeb and Ibrahim A. Shehata

Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

ABSTRACT:

5-geranoxy-7-methoxy-coumarin, isoimperatorin, bergapten and 5-geranyloxy psoralen (bergamottin) were isolated for the first time from the fruit of Citrus grandis Osbeck (= Citrus decumana Murr.) Their identity was confirmed by spectral methods as well as comparison with authentic samples.

INTRODUCTION

Coumarins have been found to be physiological active in animals as well as other living organisms[1]. Many studies have been carried out to investigate the biological activities of coumarins on different body organs and systems[2]. Several species which contain coumarins have been reported to exhibit anticoagulant[3], estrogenic[4], vasodilatory[5], molluscicidal, anthelmintic[6], diuretic[7] and antibacterial activities as well as molluscicidal and respiratory stimulant effect[8,9].

Five coumarins were isolated from Citrus aurantifolia and Citrus limonia by Iman G.H.[10], while McHale et al[11] isolated meranzin, meranzin hydrate, isoleranzin and B-O-B-D-glucopyranoside from the juice of Citrus grandis .

EXPERIMENTAL

Plant material:

Fruits of Citrus grandis Osbeck were obtained in November 1991 from the trees cultivated in the Experimental Station of Vegetables, Fruits and Medicinal Plants, Qanater, Qalyobieh, Ministry of Agriculture, Egypt Identity of the plant was kindly authenticated by Dr. N. El-Hadidy, Professor of Plant Taxonomy, Faculty of Science, Cairo University. Also, by comparison with herbarium specimens at El-Orman Garden, Guiza. The fruits were peeled. The isolated rinds were air-dried, reduced to no 36 powder and kept in a well-closed container for phytochemical study.

Authentic coumarins:

Bergapten, bergaptol, coumarin, isoimperatorin and bergamottin were obtained from Natural Products Department, NRC, Dokki, Giza Egypt.

Reagents:

1. Iodine-Potassium Iodide Spray reagent[12] : 0.2 g iodine and 4 g potassium iodide were dissolved in 100 ml water.
2. Naturstoff - Polyethylene glycol-reagent[13] : The plates were sprayed first with 1% methanolic solution of Naturstoff reagent (diphenyl boric acid ß-ethyl amino ester) followed by 5% ethanolic polyethylene glycol.
3. Potassium hydroxide spray reagent[13] : 10% ethanolic potassium hydroxide (Borntrager Reaction).

Apparatus:

MS: by a single focussing spectrometer, Hitachi, Perkin-Elmer, model PMU-6D connecticut, USA.

IR: Perkin Elmer 781 Infrared spectrophotometer, all the substances were IR studied using KBr disc technique.

NMR spectra: Joel QT 90, Japan; Melting point: A Reichert melting point microscope (all mp were uncorrected).

Extraction and isolation:

The dried powdered rind of the fruits (1 kg) of Citrus grandis Osbeck, was exhaustively extracted (Soxhlet) with ethyl alcohol 95%. The extract was concentrated to about 500 ml under reduced pressure. It was treated with an equal volume of 10% potassium hydroxide solution at room temperature for one hour with continuous shaking. The alkaline alcoholic extract was diluted with water and extracted with ether (3 X 300 ml). The aqueous mother liquor was acidified with dilute hydrochloric acid and extracted with ether (3 X 300 ml). Ethereal extract was washed with water till free from acidity, dried over anhydrous sodium sulphate and evaporated to dryness to afford crude coumarin mixture (25 g)[10].

This crude coumarin mixture was studied by TLC (silica gel G plates) using benzene-ethyl acetate (4:1) as solvent system.

Visualization was carried out by examination under UV at 365 nm before spraying with Iodine-Potassium iodide[12], Naturstoff-polyethylene glycol or potassium hydroxide spray reagents[13]. This revealed the presence of four spots with R_f 0.62, 0.7, 0.84 and 0.9, respectively. Isolation of these four coumarins was achieved by preparative layer chromatography (silica gel G plates 20 X 20 cm and 3 mm thick) alongside with available authentic samples. Band corresponding to each of the four coumarins was eluted with methanol. The solvent was removed under reduced pressure. The four coumarins isolated were identified by co-chromatography, m.p., m. m.p., UV, IR and NMR data. Compound III was also identified, in addition by mass spectral analysis.

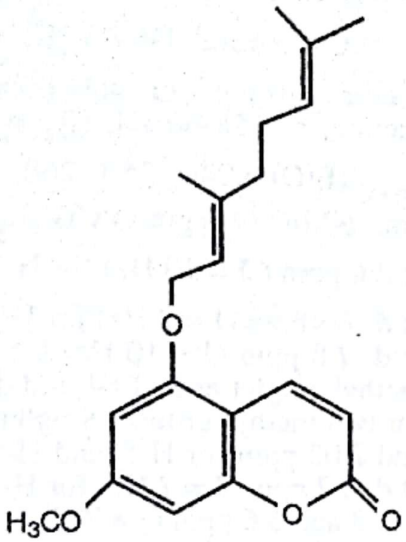
RESULTS AND DISCUSSION

Preparative layer chromatography of the crude coumarins of C. grandis Osbeck resulted in the isolation of four pure compounds. The structure of these compounds were elucidated by comparing their UV, IR, NMR data with those reported in the literature[1,15,16] as follows:

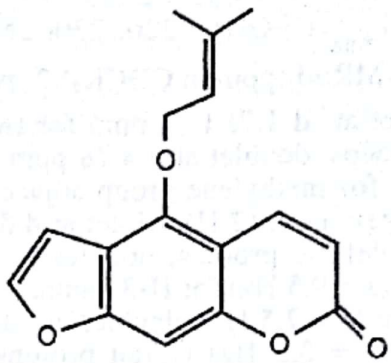
Compound I :

Compound I, ($C_{20}H_{24}O_4$) occurs as fine colourless needles [pet. ether-ethyl acetate (75:25)]; m.p. 85-87°C. (25 mg)], R_f 0.62; UV λ_{max} (EtOH): 220, 260, 330 nm[16] ; NMR : a singlet at d 3.8 ppm characteristics for one methoxy group on C-7[14]. Two doublets d 6 and 8.1 ppm (1 H, d,J = 9.5 Hz) characteristic of coumarin nucleus with oxygen at C-5[14], two doublets at d 6.1 and 6.5 ppm (J = 2.5 Hz) for H_6 and H_8 characteristic for 5,7 substituted coumarin[14], 3 methyl singlets at d 1.5, 1.7 and 1.8 ppm for 3 methyl groups attached to C-7' and C-3'. A singal d 2.1 ppm for 4 H due to CH_2 CH_2 at C-4' and C-5'. A doublet d 4.4. ppm (J = 7 Hz) for CH_2 in C-1'. two triplets at d 5.1 and 5.5 ppm indicating two olefinic protons at C-6' and C-2', respectively.

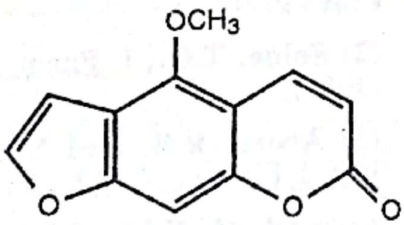
From the m.p., mixed m.p. and co-chromatography with authentic sample UV, superimposability in the finger print region in the IR, NMR and comparison with the reported data[15], this compound was identified as 5-geranyloxy-7-methoxy coumarin.



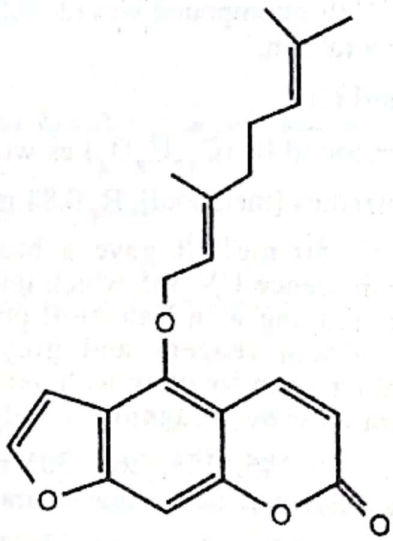
Compound I



Compound II



Compound III



Compound IV

Compound II :

Compound II ($C_{16}H_{14}O_4$) occurs as white needles [ether], m.p. 108-110°C, 40 mg)]; R_f 0.7. It gave a bluish green fluorescence UV 365 which turned to green fluorescence after spraying with potassium hydroxide reagent and intensified after spraying with naturstoff-polyethylene glycol reagent.

UV l_{max} (EtOH) : 220, 250, 268, 320 nm, NMR: d (ppm in $CDCL_3$) 2 methyl singlet at d 1.7, 1.62 ppm for two methyl groups, doublet at d 4.78 ppm (J = 7.5 Hz) for methylene group adjacent to an oxygen atom (2 H), triplet at d 5.6 ppm for olefinic protons, doublet at d 6.23 ppm (J = 9.5 Hz) for H-3, doublet at d 6.9 ppm (J = 2.5 Hz), doublet at d = 7.5 ppm (J = 2.5 Hz) (furan protons), singlet at d 7.0 ppm for C-8, doublet at d 8.1 ppm (J = 9.5 Hz) for H-4.

From the m.p., mixed m.p., UV and NMR[14,15] this compound was identified as isoimperatorin.

Compound III :

Compound III ($C_{12}H_8O_4$) as white (50 mg) needles [methanol], R_f 0.84 m.p. 188-190°C. 50 mg]. It gave a bluish green fluorescence UV 365 which intensify after spraying with Naturstoff polyethylene glycol reagent and greyish brown colour upon spraying with iodine-potassium iodide reagent UV l_{max} (EtOH), 220, 245, 255, 265, 305 nm, which are characteristic for linear furanocoumarin[1]. NMR: (d ppm in $CDCL_3$): singlet at d 4.23 ppm for OCH_3, doublet at d 6.2 ppm (J = 9.5 Hz) for C-3, doublet at d 7.0 ppm (J = 2.5 Hz) for C-a furan, singlet at d 7.12 ppm for C-8, doublet at d 7.59 ppm (J = 2.5 Hz) for C-b fuan and doublet d 8.1 ppm (J = 9.5 Hz) for C4. IR: (KBr) cm^{-1} 1720, 1620, 1575, 1510 and 970 cm^{-1}. MS: (rel. inten. %) M^+ at m/z 216 is the base peak, 201, 173, 145, 117 and 89[1]. By combination of UV, NMR, IR, MS, m.p. and mixed m.p., this compound was identified as bergapten.

Compound IV :

Compound IV ($C_{21}H_{22}O_4$) as needle (30 mg) crystals [hexane-ethyl acetate, mp 58-60°C]; (R_f = 0.9); UV l_{max} (EtOH),240, 250, 260, 270, 310. nm; NMR: (d ppm in $CDCL_3$): doublet at d 6 ppm (J = 10 Hz) for H-3_3 doublet at d 7.3 ppm (J = 2 Hz) for H-7; doublet at d 7.8 ppm (J = 10 Hz) for H-4. Two methyl singlet at d 1.64 and d 1.7 ppm for two methyl groups. Singlet at d 2.00 and 2.02 ppm for H-5' and H-4'; doublet at d 4.7 ppm (J = 7 Hz) for H-1 triplet at d 5.3 and 5.6 ppm (J = 7 Hx) or H-2' and H-6', respectively.

From the m.p., mixed m.p., UV, NMR and comparison with the reported data this compound was identified to be bergamottin (5-geranyl-oxy-psoralen).

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دراسة المواد الكومارينية من ثمار نبات سترس جراندس

أوسبك (الشادوك)

حميدة الجوهرى - شادية مظلوم - قدرية الديب و إبراهيم شحاته

قسم العقاقير - كلية الصيدلة - جامعة القاهرة

فى هذا البحث تم التعرف على المكونات الكومارينية بواسطة كروماتوجرافيا الطبقة الرقيقة وتم فصل ٤ مركبات بالطبقة السميكة وهى:

٥-جرانوكسى-٧-ميزوكسى كومارين-أيزوإميراتورين، برجابتين، جيرانيل أوكسى سورالين (برجاجوتين) وقد تم التعرف على التركيب الكيميائى لهذه المواد عن طريق درجة الإنصهار المقارنة بعينة أصلية ، الأشعة دون الحمراء ، الأشعة فوق البنفسجية وطيف الرنتين النووى المغناطيسى .