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# FURTHER BENZOFURAN DERIVATIVES AND THE ANTIINFLAMMATORY ACTIVITY OF HELICHRYSUM STOECHAS (L.) GROWN IN LIBYA

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## ABSTRACT

Re-investigation of the aerial parts of H. stoechas (L.) afforded five further benzofuran derivatives and 10 sesquiterpene hydrocarbons in addition to the previously isolated compounds. The structure of these compounds was established on basis of spectral methods and comparison with reference compounds. The different fractions of extract were tested for their antiinflammatory activity to show statistically significant results.

## INTRODUCTION

The plant genus Helichrysum is a large genus belongs to the tribe Inuleae (Compositae), and represented in Libya by two species, H. stoechas (L.) and H. lacteum (Cosson and Durieu) [1].

Many species of this genus have been used in folk medicine [2,3]. H. stoechas is widely grown in Libya and used by the natives as a remedy for kidney troubles. The current literature concerning this species described the isolation of chroman, benzofuran, phloroglucin derivatives as well as triterpenes and sterols, in addition to several known compounds [4-6]. Thus, it was interesting to continue investigation of this substrate, seeking a potential durg or chemical importance. Hence, this represents the isolation of further five benzofuran derivatives together with ten sesquiterpene hydrocarbons. This study is the first report about these constituents form H. stoechas (L.) grown in Libya.

# EXPERIMENTAL

## Material and Methods :

Plant material was collected from Tripoli, in March 1989 and was authenticated by prof. Dr. A. El-Gadi, Botany Department, Faculty of Science, Al-Fateh University. $^1$Hnmr spectra were recorded in $CDCl_3$, $CD_3OD$. with Bruker WM 400.

GC/MS was carried out on a Varian-MAT 445, 70 ev direct inlet, initial temp. 70 °C final temp. 250 °C , injector temp. 250 °C , ion source temp. 200 °C and the detector temp. 280 °C . A column (CP. Sil. 5 (Chrom. pack) containing OV - 17 (0.25 μm film thickness was used) Helium was used as a carrier gas at flow rate of 1-5 ml/min.

## Extraction and Isolation :

The air-dried material (0.9 kg) was extracted at room temperature by maceration with a mixture of methanol-ether-petroleum ether (b.r. 40 - 60 °C) (1 : 1 : 1) affording 18.5 g.

The obtained extract (18.5 g) was fractionated by using column chromatography ($SiO_2$, 5 x 70 cm, 600 g) into nine fractions (Table 5).

The TLC ($SiO_2$, system pet. ether 100%) for fraction **I** and **II**, showed that they were similar. Fraction **II** : by using GC/MS, afforded 10 compounds (Table 1). Fractions **III** and fraction **IV** gave by PTLC ($SiO_2$, system pet. ether, 9 : 1) B-sitosterol and stigmasterol, while fraction **V** gave by PTLC ($SiO_2$, system pet. ether-ether, 7 : 3) compound **1** ($R_f$ 0.018), **2** ($R_f$ 0.063), **3** ($R_f$ 0.28), **4** ($R_f$ 0.48) and **5** ($R_f$ 0.53). The isolated compounds **1-5** were oily.

Fraction **VI** : on crystalization from chloroform-methanal (1:1) gave white crystals (240 mg), which was identified as ursolic acid (m.p. 291-292 °C).

Fraction **VIII** : afforded colourless crystals (79 mg) after crystalization from methanol (m.p. 282 - 283 °C) which was identified as a mixture of B-sitosterol, stigmasterol glucosides, by using COTLC and $^1$Hnmr analysis.

The different fractions of the extract were tested for the anit-inflammatory activity[11].

### Antiinflammatory test of the different fractions :

Mature Albino rats of both sexes, with average weight 200 g. were used. They were divided into eight groups (each of four). Oedema in the rat paw was induced by the S.C. injection of (50 µl) of 1% carrageenan sodium gel. After four hours the volume of the paw was measured using a skin calibre to determine the inflammatory process. The first group was left as control, the second intraperitoneally injected with phenylbutazone 3 mg/100 g body weight.

## RESULTS AND DISCUSSION

Re-investigation of the aerial parts of H. stoechas (L.) by chromatographic methods resulted in the isolation of B-sitosterol, stigmasterol as well as their glucosides and ursolic acid.

Further five benzofuran compounds : 6-methoxy euparin **1**, 6-methoxy - 8 - hydroxy euparin **2**, 6-methoxy - 8 - acetyl euparone **3**, 6-hydroxy termetone **4**, termetone **5**, 5-hydroxy - 6 - (isopenten-2 yl) acetophenone, in addition to ten sesquiterpene hydrocarbons (Table 1) were isolated and identified.

The structure of these compounds could be confirmed unambiguously by the high field $^{1}$Hnmr, MS,IR, M. P. and COTLC. The GC/MS. of fraction II showed ten sesquiterpene hydrocarbons. The identification of these compounds was based on the GC. retention time data and mass spectrometry data, compared with reference samples, in addition to the published data. The obtained results are listed in (Table 1).

Table (4): The anti-inflammatory activity of the different fractions of H. stoechas (L.) extract.

Mean values ± S.E. n = 4

| | Thickness of Paw skin in mm. after | | |
|---|---|---|---|
| | Zero Time | 3 hours | 6 hours |
| Control | 0.83 ± 0.017 | 0.89 ± 0.03 | 0.90 ± 0.03 |
| Phenylbutazone | 0.90 ± 0.022 | 0.77 ± 0.018$^{++}$ | 0.70 ± 0.02$^{++}$ |
| Fraction IV | 0.84 ± 0.042 | 0.62 ± 0.010$^{+++}$ | 0.59 ± 0.027$^{+++}$ |
| Fraction V | 0.90 ± 0.020 | 0.79 ± 0.028$^{++}$ | 0.71 ± 0.041$^{+++}$ |
| Fraction VI | 0.82 ± 0.044 | 0.67 ± 0.017$^{++}$ | 0.62 ± 0.017$^{++}$ |
| Fraction VII | 0.82 ± 0.028 | 0.78 ± 0.030 | 0.73 ± 0.017 |
| Fraction VIII | 0.90 ± 0.036 | 0.86 ± 0.015 | 0.84 ± 0.017 |
| Fraction IX | 0.80 ± 0.340 | 0.80 ± 0.624 | 0.77 ± 0.020 |

++ P < 0.01 +++ P < 0.001

Table (5): Column chromatography of H. stoechas (L.) extract.

| Fraction No. | Eluting solvent | | Weight (g) |
|---|---|---|---|
| Fraction I | Petroleum ether | 100% | 0.310 |
| Fraction II | Pet. ether-ether | 9:1 | 0.120 |
| Fraction III | Pet. ether-ether | 8:2 | 4.500 |
| Fraction IV | Pet. ether-ether | 1:1 | 5.300 |
| Fraction V | ether | 100% | 1.500 |
| Fraction VI | Ether-methanol | 9:1 | 1.870 |
| Fraction VII | Ether-methanol | 8:2 | 1.400 |
| Fraction VIII | Ether-methanol | 1:1 | 0.840 |
| Fraction IX | Methanol | 100% | 0.520 |

**Table 1 :** GC/MS Analysis of Fraction II.

| Peak No. | RT | % | $M^+$ | Base Peak | Major Peaks " m/z" | Identity | Ref. |
|---|---|---|---|---|---|---|---|
| 1 | 5.17 | 2.40 | 204 | 55 | 175,147, 122, 91 | Unknown | - |
| 2 | 5.26 | 6.00 | 204 | 105 | 161,119,93,55 | Germacrene - D | 7 |
| 3 | 6.55 | 1.60 | 204 | 119 | 161,147,133,105,93,80,55 | Unknown | - |
| 4 | 7.06 | 0.80 | 204 | 69 | 161,133,121,93,55 | Unknown | - |
| 5 | 7.39 | 6.30 | 204 | 55 | 161,133,119,105,91,79 | Alloaromadendrene | 12 |
| 6 | 7.51 | 4.25 | 204 | 55 | 161,147,133,119,105,79 | Unknown | - |
| 7 | 8.07 | 2.00 | 204 | 55 | 189,161,133,105,93 | Isohumulene | 12 |
| 8 | 8.19 | 7.00 | 204 | 105 | 161,147,133,119,93,81 | Guaia-4,6-diene | 12 |
| 9 | 8.44 | 0.70 | 204 | 122 | 161,33,107,91,81,55 | Unknown | - |
| 10 | 8.56 | 8.55 | 204 | 105 | 189,161,134,119,93,55 | δ - cadinene | 10 |

* Compared fragments with the other constituents were slightly different in peak intensities due to different analysis condition.

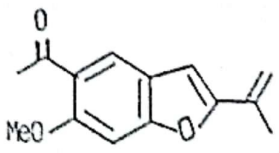
The $^1$Hnmr spectral data of compounds **1-5** (Table 3) showed that these compounds are benzofuran derivatives [5,9]. The $^1$Hnmr data of compound **1** and **2** are nearly similar. In compound **1** the singlet at δ,2.11 indicate the presence of an acetyl group attached to C-5. In compound **2**, the appearance of the signals at δ, 5.14 (br **q** , H-8) and at δ, 1.53 (**d**,H-9) indicated that the carbonyl group in **1** was reduced to a hydroxy group in **2**. The $^1$Hnmr spectrum of compound **3** showed the same signals of compound **2**, except the appearance of a singlet at δ 2.57, which indicate the presence of an acetyl group, attached to C-8 and the disappearance of H-11 and H-11´, in addition to the downfield shift of H-12 (δ 2.11, br **s**), indicate the presence of an acetyl group atteched to C-2. This is the first time to isolate the euparine derivatives from Helichrysum species [5,9]. Concerning compound **4** and **5** the IR spectra of them showed the same signals, except compound **4**, showed a broad band at 3400 $cm^{-1}$ which indicates the presence of a hydroxy group.

Table (3): $^1$Hnmr spectral data of compounds 1-5 (TMS. as Int. stand., $CDCl_3$)

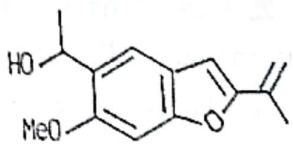
| H-NO | Compound 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| H-2 | - | -- | - | 5.26 t | 5.26 t |
| H-3 | - | - | - | 3.38 dd | 3.38 dd |
| H-3` | 6.54 brs | 6.54 brs | 7.01 s | 3.06 dd | 3.06 dd |
| H-4 | 7.44 brs | 7.47 brs | 7.65 brs | 7.82 brs | 7.82 brs |
| H-6 | - | - | - | - | 7.80 brd |
| H-7 | 7.02 brs | 6.99 brs | 7.44 brs | 6.92 brs | 6.82 d |
| H-8 | - | 5.14 q | 6.25 q | - | - |
| H-9 | 2.11 s | 1.49 d | 1.52 d | 2.54 s | 2.54 s |
| H-11 | 5.69 brs | 5.69 brs | - | 4.99 brs | 4.99 brs |
| H-11` | 5.09 brs | 5.09 brs | - | 4.93 brs | 4.93 brs |
| H-12 | 2.09 brs | 2.09 brs | 2.11 brs | 1.67 brs | 1.67 brs |
| Meo | 3.92 s | 3.91 s | 3.91 s | - | - |
| Aco. | - | - | 2.57 s | | |

(Hz): Compound 1-3: 9,8 = 7

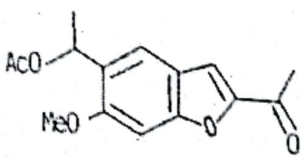
Compound 4-5: 6,7 = 7; 2,3 = 9; 2`,3 = 8; 2,2 = 17.



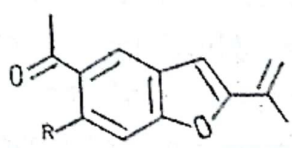

1




2




3




4 R: OH

5 R: H

The MS spectrum of compound **4** suggested a molecular formula of ($C_{13}$ $H_{14}$ $O_3$) ($M^+$ 218) whereas the MS spectrum of compound **5** showed the same fragments but the molecular ion peak appeared at (m/z 202) which suggested a molecular formula of ($C_{13}$ $H_{14}$ $O_2$) (Table 2). These data indicate that the two compounds are similar except that compound **4** has a hydroxy group more than compound **5**. The $^1$Hnmr spectral data for the two compounds confirmed the above assumption, which showed the same signals, except that the $^1$Hnmr spectrum of compound **5** showed another aromatic proton attached to C-6 and this explain the upfield shift of H-7 (δ -6.82, **d**) whereas compound **4** has a hydroxy group attached at C-6 this also explain the downfield shift of H-7 in this compound (δ-6.92, br **s**) (Table 3).

**Table 2 :** Mass Spectral Data of Compound **4** and **5**

| | |
|---|---|
| For compound **4** | m/z (rel. Int., %) $M^+$ 218 ($C_{13}$ $H_{14}$ $O_3$) (16%), 201 (12), 187 ($C_{12}$ $H_{11}$ $O_2$) (100), 159 (50). 144, (34) 115 (26), 57 (38). |
| For compound **5** | m/z (rel. Int., %) $M^+$ 202 ($C_{13}$ $H_{14}$ $O_2$) (98%), 187 ($C_{12}$ $H_{11}$ $O_2$) (100), 159 (50), 144 (34), 131 (24), 115 (21), 91 (26), 57 (38). |

The Antiinflammatory activity test for the different fractions of the extract, showed that, fractions IV, V and VI significantly decrease the thickness of the rat paw skin. ($p < 0,01 - 0,001$)., fraction VII, VIII and IX showed weak effect (Table 4), while fractions I, II, III, are devoid of such effect.

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## مشتقات بنزوفيوران إضافية والنشاط الحيوى كمضاد للإلتهابات من نبات هيلكريزوم ستويكاس (عشبة الأرنب) الذى ينمو فى ليبيا

سميح إبراهيم الدهمى
قسم العقاقير - كلية الصيدلة - جامعة الزقازيق

فى هذا البحث تم دراسة وفحص محتويات الأجزاء الهوائية فى هذا النبات وعن طريق التحليل الكروماتوجرافى أمكن فصل خمسة مشتقات بنزوفيوران وكذلك التعرف على عشرة مركبات هيدروكربونية من نوع السيسكويتربين . وقد تم التعرف على هذه المركبات عن طريق طيف الكتلة والرنين النووى المغناطيسى (٤٠٠ ميجاهيرتز) كذلك بالمقارنة بمواد مفصولة من قبل .
ولقد تم إختبار خلاصات النبات المختلفة كمضادات للإلتهابات وأعطت نتائج إيجابية .