

## δ-PYRONE DERIVATIVES FROM CROTALARIA THEBAICA

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من الأجزاء العلوية لنبات كروتالاريا ثيبايقا التابع للعائلة البقلية، تم فصل ثلاثة مركبات من نوع دلتا بيرون بالإضافة إلى مركب ايزوفلافوني (تيكتوريجينين). وقد تم التعرف على التركيب الكيميائي للمركبات المفصلة باستخدام الأشعة تحت الحمراء والأشعة فوق البنفسجية والرنين النووي المغناطيسي بنوعية الهيدروجيني والكربوني بالإضافة إلى مطياف الكتلة.

From the aerial parts of *Crotalaria thebaica* (Del) DC (Fabaceae) three  $\delta$  pyrones (**1-3**) together with an isoflavone (teceroginin) (**4**) were isolated. The structures of the isolated compounds were characterized by using different methods of spectral analysis including, IR, UV, NMR and MS.

### Introduction

*Crotalaria thebaica* (Del) DC. is a small villous shrub grows in Egyptian desert (Known in Arabic as Natash) (1). The genus *Crotalaria* (F. Fabaceae), comprises 550 species distributed mainly in the temperate or tropical regions (2,3).

The genus *Crotalaria* has long been used as medicinal folkloric remedy, especially in India for treatment of different ailments (4-7). This genus is characterized by biological activities of its pyrrolizidine alkaloids (8-10). Some *Crotalaria* species have wide use as diuretic, in treatment of sore throat and inflammation of mouth and to produce cooling sensation, as an expectorant, anti-inflammatory, and for treatment of sore eyes and boils (11-15). Beside pyrrolizidine alkaloids, flavonoids of different skeletons and other constituents (16, 17) were isolated from the genus *Crotalaria* (18-24). In the earlier study of this plant, pyrrolizidine alkaloids, flavonoids, saponins and other constituents were isolated (25-30).

### Experimental

#### Plant material:

*Crotalaria thebaica* (Del) DC. aerial parts were

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collected from the Eastern desert near Aswan in April 2000 and dried in the shade. The plant was kindly identified by Prof. Dr. Nabil El-Hadidy professor of Taxonomy, Faculty of Science, Cairo University, Cairo, Egypt.

The plant material was powdered and kept in dark glass container till used.

#### General:

IR spectra (KBr) were measured on JASCO FT/IR-5300 spectrophotometer.  $[\alpha]_D$  was carried out using DIP-4 polarimeter (JASCO, Japan). UV-analyses were carried out using UV-VIS spectrophotometer, Hitachi 550, double beam spectrophotometer (Japan). The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra were obtained on Bruker (400 MHz for  $^1\text{H}$  and 100 MHz for  $^{13}\text{C}$ -NMR) using TMS as internal standard. EIMS data was obtained with a JEOL JMS- 500 T mass spectrometer. TLC analyses were performed on precoated plates of silica gel 60 F<sub>254</sub> and RP-18 F<sub>254</sub> S (E. Merck, Darmstadt), detection was done by spraying with 10% H<sub>2</sub>SO<sub>4</sub> followed by heating at 110°. Silica gel 60 (60-120 mesh) was utilized for open column chromatography (CC). For final purification, medium pressure liquid chromatography was used [MPLC, CIG column system (22 mm i.d. x 30 cm, Kusano Scientific Co., Tokyo, Japan) packed with RP-18 column (RP 18-37, Fuji Gel, Hanbai Co. LTD, Tokyo, Japan)] LiChroprep C-18 (Merck) material was used.

*Solvent systems:*

- 1- CHCl<sub>3</sub>-EtOAc (4:1).    2- CHCl<sub>3</sub>-EtOAc (9:1).  
 3- CHCl<sub>3</sub>- EtOH (95:5).    4- CHCl<sub>3</sub>- EtOH (9:1).

*Extraction and Isolation:*

The air-dried powdered of the total herb of *Crotalaria thebaica*. (1.8 Kg) were macerated with ethanol (3X4 L) at room temperature. The combined alcoholic extracts were concentrated under reduced pressure till a semisolid residue was obtained. The solvent free residue was mixed with distilled water and partitioned between hexane (3X1L), chloroform (4X1L) and EtOAc (3X1L). The extract in each case was dried over anhydrous sodium sulphate, and evaporated under reduced pressure till a semisolid residue was obtained to give hexane fraction (Group A), chloroform fraction (Group B) and ethyl acetate fraction (Group C). Group B (20 g) was applied to silica gel column chromatography and eluted with CHCl<sub>3</sub>-EtOAc gradiently. Fractions (100 ml, each) were collected and monitored using silica gel TLC, similar fractions were pooled together. Three subgroups were obtained, subgroup B-1 (8 g) eluted with CHCl<sub>3</sub>-EtOAc (100: 0 to 70:30), subgroup B-2 (1.2 g) eluted with CHCl<sub>3</sub>-EtOAc (60:40 to 40:60) and subgroup B-3 (4.5 g) eluted with CHCl<sub>3</sub>-EtOAc (30-70 to 0:100). Repeated purification of subgroup B-2 by MPLC, [using prepacked Rp-18 column and MeOH-water (97:3)] afforded **compound 1** (28 mg), **compound 2** (23 mg) and **compound 3** (14 mg). Subgroup B-3 upon CC using silica gel CC and CHCl<sub>3</sub>-MeOH gradient, fractions eluted with CHCl<sub>3</sub>-MeOH (93:7) upon repeated purification using MPLC afforded **compound 4** (20 mg).

**Compound 1:** C<sub>16</sub>H<sub>18</sub>O<sub>6</sub>, [α]<sub>D</sub> =+8.4° (EtOH, 1.4). UV (EtOH) λ<sub>max</sub> 222 and 256 nm, IR (film): 3040, 2980, 1735, 1704, 1630, 1602, 1520, 1485, 1450, 1380, 1237, 1105, 1042, 980, 810, 762 and 700 cm<sup>-1</sup>. EIMS m/z (% rel.int.), 306 [M]<sup>+</sup> (5), other peaks at m/z 205 (16), 178 (21), 161 (14), 133 (15), 120 (57) and 90 (100). <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD): δ 7.70 (1H, d, J=16.0 Hz, H-7'), 7.47 (2H, d, J=8.4 Hz, H-2',6'), 6.80 (2H, d, J=8.4 Hz, H-3',5'), 6.35 (1H, d, J=16.0 Hz, H-8'), 5.48 (1H, m, H-5). 4.35 (1H, dd, J=10.2 and 8.0 Hz, H-3), 4.29 (1H, dd, J=13.1 and 6.9 Hz, H-6b), 3.70 (1H, dd, J=13.1 and 3.6 Hz, H-6a), 3.46 (3H, s, OCH<sub>3</sub>), 3.39 (3H, s, OCH<sub>3</sub>), 2.85 (1H, ddd, J=15.0, 10.2 and 7.0 Hz, H-4b), 2.61 (1H, m, H-4a). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD): Table (1).

**Table 1:** <sup>13</sup>CNMR data of compounds 1-2 (100 MHz, CD<sub>3</sub>OD).

Carbon	1	2
2	170.8	171.1
3	73.8	71.4
4	35.8	34.7
5	78.2	76.9
6	71.6	72.6
1'	127.7	130.4
2', 6'	132.2	130.6
3', 5'	117.9	129.6
4'	162.8	134.7
7'	148.8	168.9
8'	114.8	----
9'	169.1	----
Ar-OCH <sub>3</sub>	55.3	
3- OCH <sub>3</sub>	50.3	54.3

**Compound 2:** C<sub>13</sub>H<sub>14</sub>O<sub>5</sub>, [α]<sub>D</sub> =+6.6° (EtOH, 2.3). UV (EtOH) λ<sub>max</sub> 220, 255 nm, IR (film): 3033, 2981, 1743, 1702, 1630, 1601, 1522, 1482, 1455, 1382, 1335, 1104 and 1041cm<sup>-1</sup>. EIMS m/z (% rel.int.), 250 [M]<sup>+</sup> (6), 120 (17) and 90 (100). <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD): δ 8.05 (2H, d, J=8.4 Hz, H-2',6'), 7.63 (1H, m, H-4'), 7.50 (2H, d, J=8.4 Hz, H-3',5'), 5.63 (1H, m, H-5). 4.44 (1H, dd, J =10.2 and 8.0 Hz, H-3), 4.36 (1H, dd, J =13.1 and 6.9 Hz, H-6b), 3.83 (1H, dd, J =13.1 and 3.6 Hz, H-6a), 3.46 (3H, s, OCH<sub>3</sub>), 2.94 (1H, ddd, J =15.0, 10.2 and 7.0 Hz, H-4b), 2.45 (1H, m, H-4a). <sup>13</sup>C-NMR (100 MHz, CD<sub>3</sub>OD): Table (1).

**Compound 3:** C<sub>7</sub>H<sub>12</sub>O<sub>4</sub>, [α]<sub>D</sub> -86° (EtOH, 1.5). IR λ<sub>max</sub>(film) 2990, 1730, 1488, 1375, 1330, 1103 and 1050 cm<sup>-1</sup>. EIMS, m/z (% rel.int): 160 (13), 144 (10), 122(15), 116 (24), 100 (53), 90 (100), 77 (17) and 72 (20). <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD): δ 4.63 (1H, m, H-5). 4.33 (1H, dd, J =10.2 and 7.0 Hz, H-3), 4.05 (1H, dd, J =12.5 and 6.4 Hz, H-6b), 3.47 (1H, dd, J =12.5 and 3.5 Hz, H-6a), 3.36 (3H, s, OCH<sub>3</sub>), 3.28 (3H, s, OCH<sub>3</sub>), 2.70 (1H, ddd, J =15.0, 10.2 and 7.6 Hz, H-4b), 2.37 (1H, m, H-4a).

**Compound 4:** C<sub>16</sub>H<sub>12</sub>O<sub>6</sub>, UV (MeOH) $\lambda_{\max}$  267, 330; + NaOMe; 278, 328 (dec); + AlCl<sub>3</sub>; 278, 378; + AlCl<sub>3</sub> /HCl; 277, 366; + NaOAc; 273, 339; + NaOAc/H<sub>3</sub>BO<sub>3</sub>; 268, 335 nm. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  8.1 (1H, s, H-2), 7.3 (2H, d, *J* = 8.6, H-2',6'), 6.8 (2H, d, *J* = 8.6, H-3', 5'), 6.4 (1H, s, H-8), 3.9 (3H, s, OCH<sub>3</sub>). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>): 181.1 (C-4), 158.1 (C-7), 157.4 (C-4'), 153.7 (C-2), 153.4 (C-5), 153.2 (C-9), 131.7 (C-6), 130.6 (C-2',6'), 122.8 (C-3), 121.8 (C-1'), 115.2 (C-3',5'), 104.9 (C-10), 102.5 (C-8), 60.3 (OCH<sub>3</sub>).

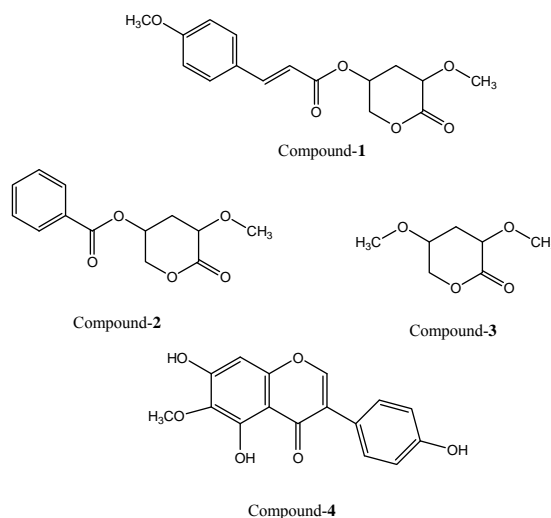
### Results and discussion

The chloroform soluble fraction of the alcoholic extract derived from the air-dried aerial parts of *Crotalaria thebaica* was subjected to silica gel CC. The fractions obtained were rechromatographed over ODS column chromatography followed by reversed phase MPLC to gave three  $\delta$ -pyrones (**1-3**) and an isoflavone (**4**).

**Compound 1:** obtained as viscous substance. The molecular formula was deduced as C<sub>16</sub>H<sub>18</sub>O<sub>6</sub> as determined by EIMS, <sup>1</sup>H- and <sup>13</sup>CNMR including DEPT data. EIMS showed fragments at *m/z* 178 [OCH<sub>3</sub>-ph-CH=CH-COO]<sup>+</sup>, 161 [OCH<sub>3</sub>-ph-CH=CH-CO]<sup>+</sup> and at 133 [OCH<sub>3</sub>-ph-CH=CH]<sup>+</sup>, It exhibited UV absorption maximum at 256 nm indicated the presence of conjugated double bond system. The IR spectrum showed the presence of lactone ring (1735 cm<sup>-1</sup>), ester carbonyl (1704 cm<sup>-1</sup>), and aromatic ring (1630, 1602 and 1520 cm<sup>-1</sup>). <sup>1</sup>H-NMR spectrum of compound **1** exhibits signals due to two methoxyl groups at  $\delta$  3.46 and 3.39, *p*-substituted aromatic ring protons at  $\delta$  7.47 (2H, d, *J* = 8.4 Hz) and 6.80 (2H, d, *J* = 8.4 Hz). It also revealed the presence of *trans* olefinic protons at  $\delta$  7.70 (1H, d, *J* = 16.0 Hz) and  $\delta$  6.35 (1H, d, *J* = 16.0 Hz). Furthermore, the <sup>1</sup>H- and <sup>13</sup>C-NMR spectra showed two oxygen bonded methine proton signals at  $\delta$ <sub>H</sub> 5.48 (1H, m, H-5)  $\delta$ <sub>C</sub> 78.2 and  $\delta$ <sub>H</sub> 4.35 (1H, dd, *J* = 10.2 and 8.0 Hz, H-3)  $\delta$ <sub>C</sub> 71.6, methylene protons at  $\delta$ <sub>H</sub> 4.29 (1H, dd, *J* = 13.1, 6.9 Hz H-6b), 3.70 (1H, dd, *J* = 13.1, 3.6 Hz H-6a) ( $\delta$ <sub>C</sub> 73.8) and  $\delta$ <sub>H</sub> 2.85, 2.61 (ddd, H-4b and m, H-4a) ( $\delta$ <sub>C</sub> 35.8) and a carbon signal of a lactone carbonyl at  $\delta$ <sub>C</sub> 170.8. This assignment depends on decoupling experiment, since irradiation of signal at  $\delta$ <sub>H</sub> 5.48 (H-5) resulted in simplification of H-6a and H-6b to be doublet (each, *J* = 12.5) and H-4a to double doublet, while

irradiation of H-4b at  $\delta$ <sub>H</sub> 2.85 simplify H-5 to ddd, H-3 to d and H4a to dd. Irradiation of signal at  $\delta$ <sub>H</sub> 3.70 for H-6a simplify H-5 and H-6b, while no effect was obtained on other signals. Based on these data, part of the structure of **1** was established as  $\delta$ -pyrone with oxygen substituted at C-3 and C-5. Since C-5 is attached to acyl-function, the signal is expected to be downfield compared to its respective position if attached to oxygen function without being acylated (31,32), while H-3 is not affected indicating the presence of oxygen function, not acyl group. The positions of both the acyl group and the methoxyl group were also confirmed by comparing the carbon chemical shifts of both C-3 and C-5 with those calculated (33) (through the <sup>13</sup>C-NMR computer programme). The methine proton signal at C-3 ( $\delta$ <sub>H</sub> 4.35) was coupled with one of the methylene protons at C-4 ( $\delta$ <sub>H</sub> 2.61, m, H-4a) with large coupling constant (*J* = 10.2 Hz), indicating *diaxial* configuration. One of the methylene protons of H-6 was coupled with the methine proton at C-5 with a small coupling constant (*J* = 3.6 Hz). The small coupling constant supported a *diequatorial* configuration between H-5 and H-6a (at  $\delta$ <sub>H</sub> 3.70). The coupling constant between H-6b and H-5 (*J* = 6.9 Hz) still small which may indicate an *axial-equatorial* configuration between H-6a and H-5 (34).

The positive optical rotation of **1** may indicate a L-pentonic lactone (35). So compound **1** was deduced to be 3-methoxy, 5-(*p*-methoxy cinnamate) -  $\delta$  pyrone (Fig 1) and this is the first report for its isolation from nature.



**Fig. 1.** Compounds isolated from *Crotalaria thebaica* (Del) DC.

**Compound 2:** The EIMS spectrum of compound **2** shows  $[M]^+$  at  $m/z$  250  $[M]^+$  consistent with the formula  $C_{13}H_{14}O_5$  which was confirmed by  $^{13}C$ -NMR including DEPT experiment.

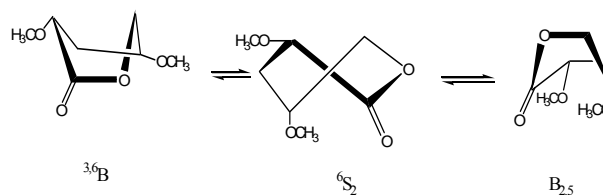
$^1H$ -NMR data showed the presence of signals at  $\delta_H$  3.83 (1H, dd,  $J=13.1$  and  $3.6$  Hz, H-6a) and  $\delta_H$  4.36 (1H, dd,  $J=13.1$  and  $6.9$  Hz, H-6b) assigned for 2H-6, while the two signals at  $\delta_H$  2.94 (1H, ddd,  $J=15.0, 10.2, 7.0$  Hz) and  $2.45$  (1H, m) were assigned for 2 protons of H-4. The signal at  $4.44$  (1H, dd,  $J=10.2$  and  $8.0$  Hz) was assigned for H-3 and the multiplet signal at  $5.63$  was assigned for H-5. It also showed three aromatic signals at  $\delta_H$  8.05 (2H, d,  $J=8.4$  Hz) assignable to H-2',6',  $\delta_H$  7.63 (1H, m) ascribable to H-4' and  $\delta_H$  7.50 (2H, d,  $J=8.4$  Hz) accountable to H-3',H-5' of the mono-substituted benzene ring. This was also deduced from the EIMS spectral data which showed a fragment at  $m/z = 120$  owing to the loss of benzoyl group.  $^{13}C$ -NMR spectra confirmed these suggestions, as it is showing three oxygen bearing carbons at  $\delta_C$  76.9, 72.6 and 71.4, a signal of a lactone carbonyl at  $\delta_C$  171.1, a signal for ester carbonyl at  $\delta_C$  168.9 and six signals for typical mono-substituted benzene ring at  $\delta_C$  130.4, 130.6, 129.6 and 134.7. As in compound **1**, C-5 is downfielded compared to its respective position attached to oxygen function without being acylated. The relative stereochemistry at C-3 and C-5 are similar to those of compound **1**. From the molecular model, it is clear that in a chair form of  $\delta$ -pyrone, the preferred conformation should favor the bulky benzoate into *equatorial* position, and since compound **2** has the same stereochemistry as compound **1**, the bulky benzoate should be *axial*. Consequently compound **2** should have distorted chair form (34). Comparing the carbon chemical shift of C-3 with the published data, the methoxyl group at C-3 should have  $\beta$ -configuration. So compound **2** was deduced to be 3-methoxy, 5-(benzoyl)  $\delta$  pyrone (Fig 1) and this is the first report for its isolation from nature.

**Compound 3:** The EIMS spectrum of compound **3** shows  $[M]^+$  at  $m/z$  160, consistent with the formula  $C_7H_{12}O_4$ ,  $144 [M-O]^+$ ,  $116 [144-CO_2]^+$ . The  $^1H$  and  $^{13}C$ -NMR spectra resembled those of **1** except the absence of the signals of *p*-methoxy cinnamate and the presence of an additional signal for a methoxy group at  $\delta_H$  3.28 (3H, s,  $OCH_3$ ). Because the  $^1H$ -NMR spectral data of H-5 of compound **3** appeared at  $\delta_H$  4.63, which is more

upfielded than that of compound **1** ( $\delta_H$  5.48), the cinnamoyl moiety at C-5 must be substituted by a methoxy group. The methine proton signal at C-3 ( $\delta_H$  4.33) was coupled with one of the methylene protons at C-4 ( $\delta_H$  2.37, m, H-4a) with large coupling constant ( $J=10.2$  Hz), indicating *diaxial* configuration. One of the methylene protons of C-6 was coupled with the methine proton at C-5 with a small coupling constant ( $J=3.5$  Hz). The small coupling constant supported a *diequatorial* configuration between H-5 and H-6a (at  $\delta_H$  3.47). The coupling constant between H-6b and H-5 ( $J=6.4$  Hz) still small which may indicate an *axial-equatorial* configuration between H-6b and H-5 (34).

From the observed coupling constant for the substituted lactones, it is clear that compound **3** adopts a conformation close to a skew conformation  $1^6S_2$ , intermediate between the two boat conformations  $1^{3,6}B$  and  $1-B_{2,5}$ , which is the conformation of minimal energy but when the lactone is substituted in position 3 or 5, the molecule may force into the half chair conformation (36) (Fig.2) as in compound **1** and **2**.

The negative optical rotation of **3** may indicate a D-pentonic lactone (35). It is interesting to note that D-xylano-1,5-lactone was expected to be levorotatory (37). So compound **3** was deduced to be 3, 5-dimethoxy  $\delta$ -pyrone (Fig.1) and this is the first report for its isolation from natural source, but it is previously synthesized from 2,3,4-tri-*O*-methyl-D-xylano-1,5-lactone and 2,3,4-tri-*O*-methyl-D-arabino-1,5-lactone (36).



**Fig. 2.** The possible conformation of compound **3**.

**Compound 4:** gave blue colour with alcoholic solution of ferric chloride, indicating its phenolic nature. The UV spectrum showed two absorption bands at  $\lambda_{max}$  267 and 330 nm characteristic for isoflavonoids. The presence of 4' hydroxyl group was deduced by the bathochromic shift of band I after the addition of NaOMe, a free hydroxyl group at C-7 was deduced by the bathochromic shift of band II after the addition of NaOAc and the free

hydroxyl group at C-5 and the absence of *ortho*-dihydroxy groups from the bathochromic shift with  $\text{AlCl}_3$  in band I which does not change after the addition of HCl. The  $^1\text{H-NMR}$  spectrum showed a singlet signal at  $\delta_{\text{H}}$  8.1 characteristic for H-2 of isoflavonoids (38). Also it showed signals at  $\delta_{\text{H}}$  7.3 and 6.8 (each 2H, d,  $J=8.6$  Hz), assigned to the aromatic protons H-2',6' and H-3',5' respectively. A singlet signal at  $\delta_{\text{H}}$  6.4 (1H, s) indicated only one hydrogen to be present on the ring A, moreover a signal due to one methoxy at  $\delta_{\text{H}}$  3.9 (3H, s,  $\text{OCH}_3$ ) was observed.  $^{13}\text{C-NMR}$  spectral data confirmed the isoflavonoid skeleton of 4 from the signal at  $\delta_{\text{C}}$  153.7 characterized for C-2 of isoflavonoids (39).  $^{13}\text{C-NMR}$  showed fourteen carbon signals for sixteen carbon atoms. The chemical shift values at  $\delta_{\text{C}}$  130.6 & 115.2 were assigned to carbon atoms 2',6' and H-3',5' respectively, suggesting that compound 4 contained a 4'-hydroxyphenyl group as in genisten, but not a 4'-methoxyphenyl group ( $\delta_{\text{C}}$  131.1 and 114.5). The position of the methoxy group was confirmed to be at C-6 from the chemical shift of methoxy group at 60.3 (39). From all the above data compound 4 was deduced to be 5, 7, 4'-trihydroxy, 6-methoxy isoflavone (tectogenin).

It is worthily to mention that  $\delta$ -lactones play an outstanding role in many dairy products, they were also isolated from depot animal fat and furthermore found in certain products of vegetable origin such as coconut, pine apple, apricot and strawberry (40), many of D-aldono-1,5-actones showed many biological activities (41-45).

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