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FURTHER CONSTITUENTS FROM *CROTALARIA THEBAICA* (DEL.) DC.

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نبات الكروتالاريا سبيكا (دل) (النتاش) هو أحد النباتات التابعة للعائلة البقلية والذى ينمو بكثرة فى الصحراء الشرقية. وفى أبحاث سابقة تم فصل بعض الصابونينات واستكمالا للدراسة على هذا النبات فقد أمكن فصل مركبين من مشتقات الأيونون هما ٣-أوكسو-ألفا-ايونيل-بيتا-جلوكوزيد وفوميفوليول بالإضافة إلى سيرنجريزينول-أ-بيتا-د-جلوكوبيرانوزيد ومالتول-٣-أ-بيتا-د-جلوكوبيرانوزيد. وقد أمكن التعرف على هذه المركبات بإستخدام الطرق الطيفية المختلفة. وهذه المركبات تفصل لأول مرة من جنس الكروتالاريا.

Two ionone glycosides namely 3-oxo-α-ionyl-β-glucoside and vomifoliol were isolated from polar fraction of the methanolic extract of the aerial parts of Crotalaria thebaica (Del.) DC upon repeated column chromatography, together with syringresinol-O-β-D-glucopyranoside and maltol-3-O-β-D-glucopyranoside. The structure of these compounds have been established by extensive spectroscopic studies.

INTRODUCTION

The genus *Crotalaria* (Family Leguminoseae) comprises 550 species, and is represented in Egypt by only 5 species.[1] Different *Crotalria* species have long been used as medicinal folkloric remedies especially in India for the treatment of different ailments.[2,3]

As a continuation to the study on *C. thebaica* (Del) DC.,[4-6] the present work deals with the isolation and identification of two ionone glycosides viz 3-oxo-α-ionyl-ß-glucoside and vomifoliol from the polar fraction of the methanolic extract of the aerial parts of the title plant upon repeated column chromatography, together with syringresinol-O-ß-D-glucopyranoside and maltol-3-O-ß-D-glucopyranoside. The structure of these compounds have been established by extensive spectroscopic studies. This is the first report for the isolation of the four compounds from the genus *Crotalaria*.

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EXPERIMENTAL

Mps were uncorrected. ^{1}H, and ^{13}C-NMR were recorded in pyridine-d_5 unless otherwise mentioned at 400 MHz and 100 MHz, respectively on Brucker AM-400 spectrometer. MS spectra on JOEL JMS-SX 102 (JOEL-Japan). For CC: Amberlite IR A-45 (weak anion exchange resin), silica gel (E. Merck), CIG column system (22 mm i.d x 30 cm Kusane Scientific Company, Tokyo, Japan) and RP-18 were used. Precoated silica gel plates (E. Merck) were used for TLC.

The following solvent systems were also used:

I- $CHCl_3$-MeOH-H_2O (75:23:2)
II- $CHCl_3$-MeOH-H_2O (70:27:3)

Plant material

The aerial parts of *Crotalaria thebaica* (Del) DC were collected from El-Hafafit near Aswan in April 1987 while flowering. The plant was kindly authenticated by Prof. Dr. N. El-Hadidy, Professor of Plant Taxonomy, Faculty of Science, Cairo University.

Extraction and isolation

The air dried powdered aerial parts (1.8 kg) of *C. thebaica* was exhaustively extracted with methanol.The methanol was concentrated under reduced pressure (113 g), then mixed with 500 mL water and extracted with hexane. 28 gm of the conc. hydroalcoholic extract was fractionated over Amberlite IR A-45 using water-MeOH gradient. The fraction eluted with water-MeOH (1:1, 4 L) was concentrated and rechromatographed over silica gel CIG columns using $CHCl_3$-CH_3OH-H_2O (80:19:1)and $CHCl_3$-CH_3OH-H_2O (75:23:2) as solvent systems, where four compounds could be isolated in amounts 32 mg [1], 56 mg [2], 26 mg [3] and 46 mg [4].

Acetylation of the isolated compounds

8 mg of each compound to be acetylated was mixed with suitable volume of pure pyridine and acetic anhydride and left for 24 hours at room temperature.

Acid hydrolysis

8 mg of the glycoside was dissolved in 10 mL of methanol to which an equal volume of 10% H_2SO_4 was added. The mixture was refluxed on a water bath for 3 hours.

Enzymatic hydrolysis

7.5 mg of compounds [2] and [3], was separately subjected to enzymatic hydrolysis using ß-glucosidase (7.5 mg, 37°C for 15 min.). The resulting hydrolysate was extracted with ethyl acetate. The ethyl acetate fraction was concentrated under reduced pressure and purified using CIG column system (silica gel, using ethyl acetate : methanol 96:4 for compound [2] and chloroform-methanol 88:12 for compound [3] where pure aglycones were obtained.

Compound [1]

Obtained as colourless needles (MeOH), mp 189-191°C. hR_f= 32,51 (systems I & II) It is soluble in ethanol, methanol, and sparingly soluble in acetone, ethyl acetate. $[\alpha]_D^{25}$ = -12.3° (MeOH). IR (ν cm^{-1}) 3430 (OH), 1600 (C=C), 1520. ^{1}H-NMR (CD_3OD, δ) 6.71 (2H, s, H-2´ & H-6´), 6.65 (2H, s, H-2´´ & H-6´´), 4.85 (1H, d, J= 7.4 Hz, H-1´´´), 4.71 (2H, d, J= 4.3 Hz, H-2 & H-6), 4.28 (2H, m, H-4 & H-8), 3.92-3.41 (5H, m, sugar protons), 3.85 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.21 (1H, m, H-1 or H-5), 3.12 (1H, m, H-5 or H-1). ^{13}C-NMR (CD_3OD, δ) 55.57 (d, C-1), 55.78 (d, C-5), 72.93 (t, C-4), 73.01 (t, C-8), 87.25 (d, C-6), 87.63 (d, C-2), 135.16 (s, C-1´), 133.21 (s, C-1´´), 105.03 (d, C-2´), 104.74 (d, C-2´´), 154.49 (s, C-3´), 149.46 (s, C-3´´), 139.64 (s, C-4´), 136.40 (s, C-4´´), 154.49 (s, C-5´), 149.46 (s, C-5´´), 105.03 (d, C-6´), 104.74 (d, C-6´´), 56.96 (q-OCH_3), 57.21 (q-OCH_3), 105.5 (d, glu-1), 75.80 (d, glu-2), 77.91 (d, glu-3), 71.46 (d, glu-4), 78.39 (d, glu-5), 62.70 (t, glu-6). SIMS, m/z 603 (M+Na), 581 (M+1) and 418 [(M+1)-hexose].

Acetate of [1]

IR (ν cm^{-1}) 1754 (ester carbonyl), 1602, 1506 (aromatic system). ^{1}H-NMR (CD_3OD, δ) showed five acetate groups at δ 1.95, 1.96, 1.97, 1.98 and 2.72.

Compound [2]

Amorphous powder, soluble in methanol,insoluble in ether and hexane. hR_f= 30, 44 (systems I & II). $[\alpha]_D^{25}$ = +25.6° (MeOH).UV λ_{max} (MeOH), 234, 233 and 204 nm. IR (ν cm^{-1}) 3350 (OH), 2980, 2840, 1642 (C= O), 1594, 1500 and 1225. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2. The assignment of each proton was determined from its coupling constant and by using spin decoupling experiments. SIMS: M+1 at m/z 371 corresponding to the molecular formula $C_{19}H_{30}O_7$, in addition to other fragments for loss of water and hexose.

Acetate of [2]

IR (ν cm^{-1}) 1742 (C=O), 1650 (enone) and 1600 (C=C). ^{1}H-NMR ($CDCl_3$, δ), showed four acetate signals at 2.01, 2.02, 2.04 and 2.07.

Aglycone [2]

3.1 mg. ^{1}H - and ^{13}C-NMR data are listed in Tables 1 and 2.

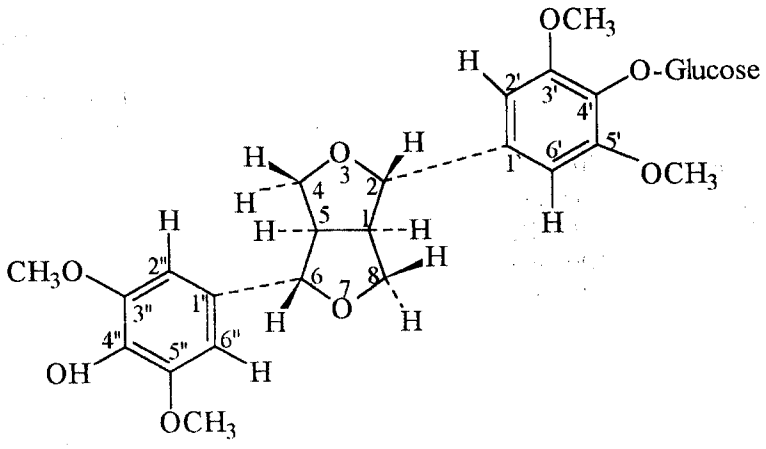
Table 1: 400 MHz ^{1}H-NMR data of compounds [2] and [3], their aglycones and acetate of [2].

Proton No.	[2] (a) δ mult. (J Hz)	[2] acetate (b) δ mult. (J Hz)	[2] aglycone (b) δ mult. (J Hz)	[3] (c) δ mult. (J Hz)	[3] aglycone (a) δ mult. (J Hz)
2u	2.04, d, (16.8)	*	2.07, d (16.7)	2.38, d, (16.6)	2.24, d (17.1)
2d	2.42, d, (16.8)	2.33, d, (16.8)	2.32, d (16.7)	2.66, d (16.6)	2.44, dd (17.1, 1.0)
4	5.88, br.s	5.90, br.s	5.90, br.s	6.07, br.s	5.90, br.s
6	2.67, d (9.1)	2.52, d (9.0)	2.52, d (9.0)	-----	-----
7	5.64, dd, (15.4, 9.1)	5.55, dd, (15.5, 9.0)	5.55, ddd, (1.0, 15.5, 9.0)	6.12, d (15.8)	5.78, dd (15.6, 0.6)
8	5.77, dd, (6.4, 15.4)	5.67, dd, (6.3, 15.5)	5.67, dd, (6.0, 15.5)	6.32, dd (15.8, 6.9)	5.85, dd (15.6, 5.2)
9	4.38, dq, (6.3, 6.4)	4.22, dq, (6.3, 6.4)	4.35, dq, (6.2, 6.0)	4.72, m	4.41, m
10	1.28, d (6.4)	1.23, d (6.4)	1.28, d (6.4)	1.37, d (6.4)	1.37, d (6.4)
11	1.00, s	0.99, s	0.97, s	1.15, s	1.00, s
12	1.02, s	1.03, s	1.07, s	1.25, s	1.02, s
13	1.93, d (1.1)	1.88, d (1.2)	1.89, d (1.1)	2.00, d (1.2)	1.89, d (1.4)
1`	4.35, d (7.8)	4.56, d (8.0)	---	4.91, d (7.7)	---
2`-5`	3.15-3.40	---	---	3.8-4.6	---
6`a	3.81, dd (2.6, 11.7)	---	---	---	---
6`b	3.66, dd (5.0, 11.7)	---	---	---	---

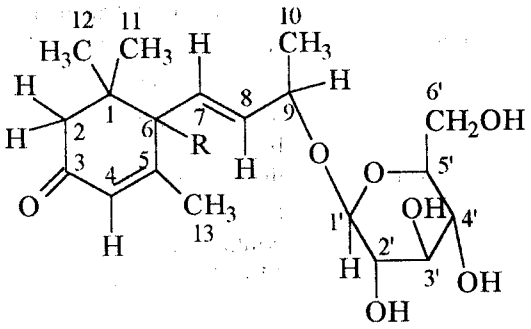
(a)= CD_3OD (b)= $CDCl_3$ (c)= C_5D_5N
u= upfield d = downfield * = under acetate signals.

Table 2: ^{13}C-NMR data of compounds [2] and [3] and their aglycones.

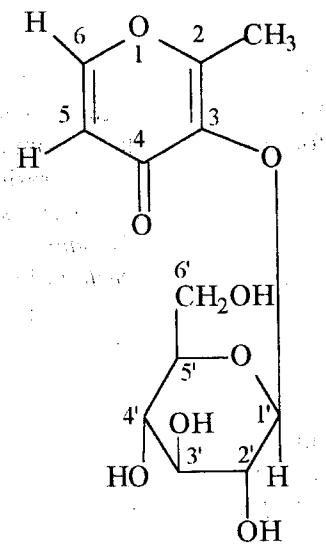
C. No. (multi.)	[2] CD_3OD δ (ppm)	[2] C_5D_5N δ (ppm)	[2] ag. C_5D_5N δ (ppm)	[3] CD_3OD δ (ppm)	[3] C_5D_5N δ (ppm)	[3] ag. $CDCl_3$ δ (ppm)
1 (s)	37.15	36.03	36.90	42.5	41.6	41.98
2 (t)	48.36	47.90	48.01	50.7	50.2	49.95
3 (s)	202.04	198.06	198.12	201.2	197.7	197.98
4 (d)	126.19	126.02	125.49	127.2	126.8	127.20
5 (s)	165.85	161.39	161.84	167.3	164.1	163.46
6 (d)	56.81	55.50	55.56	80.1 (s)	78.9 (s)	78.15 (s)
7 (d)	128.86	127.74	125.94	131.6	131.3	129.26
8 (d)	138.27	137.54	140.71	135.3	134.7	135.98
9 (d)	77.00	75.70	67.48	77.3	76.2	68.26
CH_3 (q)	21.08	21.12	23.18	21.2	21.2	23.11
(q)	23.79	23.16	24.52	23.5	23.5	23.99
(q)	27.90	27.08	26.95	19.6	19.3	19.02
(q)	28.10	27.73	27.76	24.7	24.6	24.26
1′ (d)	102.51	102.66	-----	102.8	102.2	-----
2′ (d)	75.31	75.34	-----	75.3	75.3	-----
3′ (d)	78.00	78.33	-----	78.1	78.3	-----
4′ (d)	71.73	71.69	-----	71.7	71.9	-----
5′ (d)	78.15	78.55	-----	78.2	78.6	-----
6′ (t)	62.75	62.83	-----	62.9	62.6	-----



Compound [1]



	R
Compound [2]	**H**
Compound [3]	**OH**



Compound [4]

Compound [3]

Amorphous powder, soluble in methanol,insoluble in ether and hexane. hR_f 28, 41 (systems I & II) UV λ_{max} (MeOH), 237 and 317 nm (ionone system). IR (ν cm^{-1}) 3350 (OH), 2980, 2840, 1642 (C= O), 1594, 1500 and 1225. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2. The assignment of each proton was determined from its coupling constant and by using spin decoupling experiments. SIMS: M+1 at m/z 387 and M+Na at m/z 409 corresponding to the molecular formula $C_{19}H_{30}O_8$. In addition to other fragment at m/z 225 for loss of hexose moiety.

Aglycone [3]

2.4 mg. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2.

Compound [4]

hR_f= 39 (system II), fine needle crystals (methanol-ethyl acetate 1:2), mp 108-110°C. It is soluble in ethanol, methanol, and sparingly soluble in acetone, ethyl acetate. IR (ν cm^{-1}) 3360, 1665, 1620, 1560, 1462. 1260, 1210 and 850. ^{1}H-NMR (CD_3OD, δ) 7.90 (1H, d, J= 5.6 Hz, H-6), 6.35 (1H, d, J= 5.6 Hz, H-5), 4.71 (1H, d, J= 7.2 Hz, H-1´), 3.1-3.3 (3H, m, sugar protons), 3.73 (1H, dd J= 12.8, 2.2 Hz, H-6´a), 3.32 (1H, dd J= 12.8, 5.2 Hz, H-6´b), 2.36 (3H, s, 2-CH_3). ^{13}C-NMR (CD_3OD, δ) 177.21 (s, C-4), 164.57 (s, C-3), 157.13 (d, C-6), 143.70 (s, C-2), 117.39 (d, C-5), 105.55 (d, C-1´), 75.47 (d, C-2´), 78.09 (d, C-3´), 71.20 (d, C-4´), 78.59 (d, C-5´), 62.62 (t, C-6´), 15.83 (q, 2-CH_3). SIMS: M+1 at m/z 289 corresponding to the molecular formula $C_{12}H_{16}O_8$.

Acetate of [4]

Needle crystals, mp 118-120°C.IR (ν cm^{-1}) 1752, 1654, 1624 and 1567. ^{1}H-NMR ($CDCl_3$, δ) 7.62 (1H, d, J= 5.7 Hz, H-6), 6.32 (1H, d, J= 5.7 Hz, H-5), 5.36 (1H, d, J= 7.9 Hz, H-1´), 5.17 (1H, dd, J= 9.6, 7.9 Hz, H-2´), 5.28 (1H, t, J= 9.6 Hz, H-3´), 5.11 (1H, dd, J= 9.6, 9.8 Hz, H-4´), 3.65 (1H, ddd, J= 9.8, 4.5, 4.2 Hz, H-5´), 4.12 (1H, dd J= 12.3, 2.5 Hz, H-6´a), 4.20 (1H, dd J= 12.3, 5.6 Hz, H-6´b), 2.30 (3H, s, 2-CH_3), in addition to four acetate groups at δ 2.12, 2.03, 2.02 and 2.01. ^{13}C-NMR ($CDCl_3$, δ) 173.78 (s, C-4), 161.36 (s, C-3), 153.81 (d, C-6), 141.43 (s, C-2), 117.52 (d, C-5), 99.55 (d, C-1´), 71.60 (d, C-2´), 72.04 (d, C-3´), 68.73 (d, C-4´), 72.78 (d, C-5´), 61.79 (t, C-6´), 15.37 (q, 2-CH_3).

RESULTS AND DISCUSSION

Compound [1]

The IR spectrum showed the presence of hydroxyl group(s), and aromatic system. Both ^{1}H- and ^{13}C-NMR showed signals characteristic for sugar, tetrasubstituted phenolic rings and fused bifuran ring. These spectra showed that the two phenolic rings and the fused furan rings are not equivalent. Most of the signals of the bifuran ring and the phenolic system were duplicated and this indicates that one of the two phenolic rings was glycosylated and the other containing free hydroxyl group. SIMS showed M+Na at m/z 603 and M+1 at m/z 581 corresponding to the molecular formula $C_{28}H_{30}O_{13}$, the peak at m/z 418 indicates the loss of hexose from the molecule. The sugar moiety was identified as glucose and the ß-configuration was determined from the coupling constant of the anomeric proton in the ^{1}H-NMR (J= 7.4 Hz). The presence of phenolic hydroxyl group was confirmed from the acetate. A phenolic acetate group was detected in its ^{1}H-NMR (at δ 2.26) and only four alcoholic acetates were detected indicating the presence of only one sugar moiety. From all the above mentioned data, compound [1] was identified as (+)-Syringaresinol-O-ß-D-glucopyranoside which has been isolated from same plants including *Eucommia ulmoides* (F. Fucommiaceae) and *Desfontainia spinosa* (F. Loganiaceae).[7-9] This is the first report on this compound in the genus *Crotalaria.*

Compound [2]

The IR spectrum showed the presence of hydroxyl group(s), enone and olefinic group(s). The ^{1}H-NMR spectra (in both CD_3OD and

C_5D_5N) showed the presence of four methyls, two singlets and two doublets, one methylene proton, two methine protons and three olefinic protons two of them were in trans configuration (J= 15.4 Hz) in addition to the sugar protons.

The ^{13}C-NMR spectrum (in both CD_3OD and C_5D_5N) revealed the presence of nineteen carbon signals including six of the hexose at δ 102.51, 75.31, 78.00, 71.73, 78.15 and 62.75 in CD_3OD. The other 13 carbon signals were assigned for the aglycone moiety indicating that this compound is ionone glycoside. The methylene protons each appeared as doublet (J= 16.8 Hz, geminal coupling) indicating that these two protons were surrounded by quaternary carbon from both sides, i.e, located between the carbonyl and the carbon bearing the two singlet methyls.

The ^{13}C-NMR spectrm showed the presence of four olefinic carbons in addition to the signal at δ 202.04 (CD_3OD) for conjugated carbonyl, while ^{1}H-NMR showed the presence of three olefinic protons. This indicates the presence of cyclohexanone with butenol side chain. Upon acetylation it afforded tetra acetate indicating the presence of hexose and the absence of acetylated hydroxyl group in the aglycone moiety, while both acid and enzymatic hydrolysis gave aglycone and glucose as a sugar moiety. The ß-configuration of the glucose was determined from the coupling constant of the anomeric proton in the ^{1}H-NMR (J= 7.8 Hz), and its ^{13}C-NMR data is in good agreement with those reported for ß-D-glucopyranosides.[10-13] SIMS showed M+1 at m/z 371 corresponding to the molecular formula $C_{19}H_{30}O_7$ and the peak at m/z 208 for the loss of hexose from the glycoside. From all of the above mentioned data, this compound was deduced to be an ionone glucoside[6-(3´-O-ß-D-glucopyranosyl-trans-1´-butenyl) 1,1,5-trimethyl cyclohex-4-en-3-one].[14] This is the first report of the ^{13}C-NMR data of this compound.

Compound [3]

The UV spectrum showed the absorption characteristic for enone system. The IR supported this finding (ν^{-1} 1650). The ^{1}H-NMR showed two tertiary methyls, a secondary methyl and a methyl attached to an olefinic carbon, an isolated methylene group α-to carbonyl group and three olefinic protons. The ^{13}C-NMR in CD_3OD and C_5D_5N showed the presence of nineteen carbon signals indicating the presence of ionone glycoside. The acid hydrolysis gave glucose as a sugar part. The ß-configuration of the glucose was deduced from the coupling constant of the anomeric proton in the ^{1}H-NMR (J= 7.7 Hz), and its ^{13}C-NMR data is in good agreement with those reported for ß-D-glucopyranosides.[10-13] The SIMS gave M+1 at m/z 387 corresponding to the molecular formula $C_{19}H_{30}O_8$. From all the above mentioned data compound **[3]** was identified as Vomifoliol glucoside which had been isolated from *Vinca rosea* (F. Apocyanaceae).[15] and *Vitis vinifera* (F. Vitaceae).[16] This is the first report of this compound in the genus *Crotalaria*.

Compound [4]

The IR spectrum showed the presence of hydroxyl group(s), carbonyl group and unsaturated system. The ^{1}H-NMR showed the presence of methyl group,two olefinic protons and sugar protons. The ^{13}C-NMR showed the presence of three singlet and two doublet aromatic carbons in addition to six sugar carbons and methyl groups. The SIMS showed M+1 at m/z 289 corresponding to the molecular formula $C_{12}H_{16}O_8$. Acid hydrolysis gave glucose as a sugar moiety and acetylation afforded tetraacetate. All the above mentioned data (IR, ^{1}H-NMR, ^{13}C-NMR, SIMS, acid hydrolysis and acetylation) are similar to those reported for maltol-3-O-ß-D-glucoside.[17] This compound is reported for the first time in the genus *Crotalaria*.

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