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A NEW IONOL GLUCOSIDE FROM *MAERUA CRASSIFOLIA* FORSSK GROWN IN EGYPT

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يعتبر نبات الموريا كراسيفوليا والذى ينمو فى مصر من النباتات ذات الاستعمال الشعبى لعلاج الكثير من الأمراض. لذلك رؤى استكمال إجراء الدراسة الكيميائية المستفيضة للتعرف على مكوناته. وقد تم فصل والتعرف على مركب ايونولى جليكوزيد جديد بالأضافة إلى مركب فلافونى آخر معروف هو الكوارسيتين-٣-أ-جالكتوزيد يفصل لأول مرة من جنس الموريا.

وقد تم التعرف على هذين المركبين بدراسة خواصهما الكيميائية وكذلك استخدام طرق التقنية الحديثة متمثلة فى الاشعة دون الحمراء ومطياف الكتلة والرنين النووى المغناطيسى بنوعيه البروتونى والكربونى.

A new ionol glucoside was isolated from the polar fraction of the methanolic extract of the aerial parts of M. crassifolia Forssk upon repeated column chromatography, together with quercetin 3-O-ß-D-galactopyranoside. The structure of the isolated compounds have been established by extensive spectroscopic study.

INTRODUCTION

Maerua crassifolia Forssk (F. *Capparaceae*) is a small Egyptian desert plant[1,2]. The genus *Maerua* has some folkloric uses[3], the alcoholic extract of the total herb showed neuromuscular blocking and antitumor activities[4,5].

As a continuation to the study on *M. Crassifolia* Forssk[6-8], the present work deals with the isolation and identification of a new ionol glucoside from the polar fraction of the methanol extract together with a known flavonoid galactoside.

EXPERIMENTAL

General experimental procedures

Melting points were uncorrected and determined by electrothermal 9100 digital instrument. IR were taken in KBr pellets with Perkin-Elmer (Model 457). ^{1}H- and ^{13}C-NMR spectra were recorded in Bruker AM-400 spectrometer (at 400 MHz for ^{1}H- and 100 MHz for ^{13}C-NMR) using TMS as internal standard and C_5D_5N and DMSO as solvents. Secondary Impact Mass Spectra (SIMS) and Electron Impact Mass Spectra (EIMS) were carried out on Hitachi M-80 spectrometer. TLC were carried out on silica gel plates (Kieselgel 60 F_{245}, E-Merck) and pre-coated TLC RP-18 F_{254} S, (E.Merck). Whatman paper No. 1 were used for PC. For isolation, silica gel G (230-400) (E.Merck) and irregular reversed phase (R 18-37, Fuji Gel Hanbai Co. LTD; Tokyo, Japan) were used for column chromatography using CIG column system (22 mm i.d. x 30 cm, Kusano Sci. Co., Tokyo, Japan)

The following solvent systems were used:

I Chloroform-methanol (85:15)
II Chloroform-methanol-water (75:24:1)
III Chloroform-methanol-water (30:27:3)
IV Butanol-Acetic acid-water (60:30:10)
V Methanol-water (45:55)

Plant material

The aerial parts of *Maerua crassifolia* Forssk were collected from the Eastern Egyptian desert near Aswan during flowering in April 1992. The plant was kindly identified by Prof. Dr. Nabil El-Hadidy, prof. of Taxonomy,

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Faculty of Science, Cairo University. The aerial parts were air dried, reduced to No. 40 powder and kept in a well closed dark container till used.

Extraction and isolation

1.6 kg of the air-dried powdered aerial parts were extracted with methanol by maceration. The methanolic extract was successively fractionated with n-hexane (fraction A, 36 g), chloroform (fraction B, 28 g), ethyl acetate and n-butanol fractions showed the same spots on TLC (fraction C, 86 g).

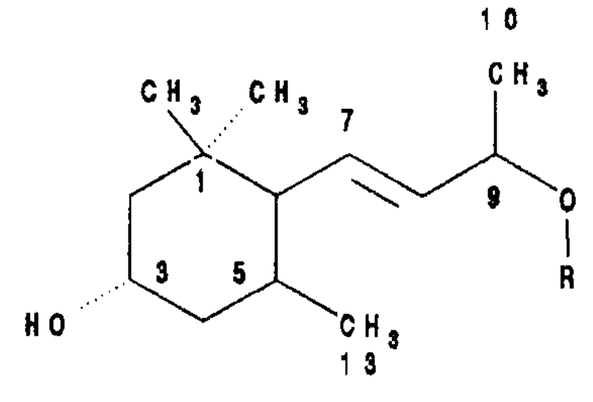
The dried fraction C (10 g) was chromatographed over a column of silica gel (400 g), fractionation was performed with chloroform-methanol mixtures in a manner of increasing polarities. Fractions (100 ml each) were collected and subjected to TLC and inspection of the developed TLC after spraying with 10% H_2SO_4. Similar fractions were collected together. Fractions eluted with $CHCl_3$-MeOH (4:1), (Fr. 86-94) showed the presence of two spots, one of which gave yellow colour with H_2SO_4 (R_F 0.48, system III), and the other gave brownish-violet colour (R_F 0.41, system III). Further purification was carried out using CIG column system using RP-18 column (system V) where compound [1], and [2] were isolated.

Compound [1]

Compound (1) was obtained as a white amorphous powder (28 mg), $[\alpha]_D^{20}$ - 12.3 (methanol), IR (KBr cm^{-1}) 3450 (br. band), 3010, 2970, 2860, 1610, 1377, 1372, 1360, 1250, 1170, 1120, 1050 and 972. SIMS $[M+Na]^+$ at m/z 397, $[M+1]^+$ at m/z 375, other peaks at m/z 213,194, 163 and 145. ^{1}H-NMR data are cited in Table 1 and that for ^{13}C-NMR in Table 2.

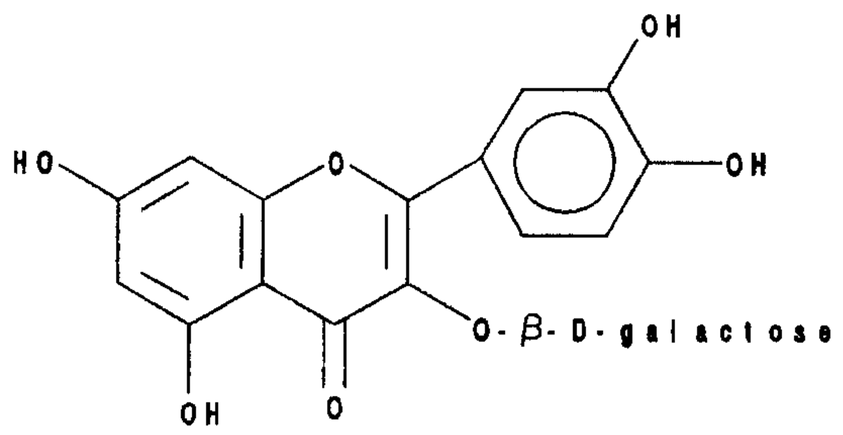
Compound [1A]

Obtained by acid hydrolysis of [1][9] as a viscous gum, IR (KBr cm^{-1}), 3470, 1379, 1371 and 972, EIMS, M^+-H_2O at m/z 194 other peaks at m/z 179 (3%), 176 (13%), 162 (22%), 161 (14%), 149 (7%), 136 (12%) and 123 (90%). ^{1}H-NMR data are cited in Table 1 and ^{13}C-NMR are cited in Table 2.



1) R = glucose

1A) R = H



(2)

Compound [2]

This compound was obtained as yellow amorphous powder (methanol), ^{1}H-NMR (DMSO), δ 7.73 (1H,dd,J= 2.1 and 8.8 Hz, H-6'), 7.59 (1H,d,J= 2.1 Hz, H-2'), 6.82 (1H,d,J= 8.8 Hz, H-5'), 6.44 (1H,d,J= 2.0 Hz, H-8), 6.23 (1H,d,J= 2.0 Hz, H-6), 5.43 (1H,d,J 7.7 Hz, H-1-gal). Other protons at δ 3.2 ~ 5.21. ^{13}C-NMR (DMSO) (aglycone), δ 177.73 (C-4), 164.21 (C-7), 161.18 (C-5), 156.34 (C-2), 156.18 (C-9), 148.64 (C-4'), 145.01 (C-3'), 133.68 (C-3), 121.92 (C-6'), 120.88 (C-1'), 115.82 (C-5'), 115.48 (C-2'), 103.82 (C-10), 98.09 (C-6) and 93.33 (C-8), for galactose δ 101.93 (C-1"), 74.88 (C-5"), 73.23 (C-3"), 71.19 (C-2"), 67.89 (C-4") and 60.32 (C-6"). These data with the UV spectral data with different ionizing and complexing reagents are similar to those of quercetin 3-O-ß-D-galactopyranoside.

Table 1: 400 MHz ^{1}H-NMR of compounds 1 and 1A (C_5D_5N).

H No.	1 δ, multi, (J Hz)	1A δ, multi, (J Hz)
2	1.89,dd (12.6, 12.2) 2.18,dd (12.6, 4.4)	1.91,dd (12.3, 12.1) 2.20,dd (12.3, 4.5)
3	4.14,m	4.16,m
4	1.80,ddd (12.8, 3.3, 2.8) 2.02,ddd (12.8, 10.4, 10.5)	1.81,ddd (12.7, 3.4, 2.8) 2.05,ddd (12.7, 10.4, 10.6)
5	1.69,m	1.71,m
6	1.36,dd (8.8,5.8)	1.39,dd (8.8, 5.8)
7	5.51,dd (15.5, 8.8)	5.48,dd (15.6, 8.8)
8	5.59,dd (15.5, 6.5)	5.64,dd (15.6, 6.5)
9	4.72,dq (7.5, 6.3)	4.64,dq (7.1,6.3)
10	1.31,d (6.3)	1.42,d (6.3)
11	1.01,s	1.00,s
12	0.94,s	0.94,s
13	1.39,d (6.5)	1.38,d(6.5)
1'	5.04,d (7.8)	--
2'	4.04,dd (7.8, 9.0)	--
3'	4.24,dd (8.9, 9.0)	--
4'	4.11,dd (8.9, 9.3)	--
5'	3.95,ddd (9.3, 5.5, 2.2)	--
6a	4.38,dd (12.0, 5.5)	--
6b	4.56,dd (12.0, 2.2)	--

Table 2: 100 MHz ^{13}C-NMR of compounds 1 and 1A (C_5D_5N).

C No.	1	1A
1(s)	34.58	34.63
2(t)	47.84	49.21
3(d)	68.41	69.50
4(t)	38.94	39.05
5(d)	38.00	38.03
6(d)	56.40	56.34
7(d)	133.32	133.60
8(d)	134.47	136.22
9(d)	74.05	67.82
10(q)	21.80	24.30
11(q)	23.00	22.18
12(q)	31.59	31.32
13(q)	17.54	17.68
1'(d)	101.31	--
2'(d)	75.40	--
3'(d)	78.68	--
4'(d)	71.60	--
5'(d)	78.26	--
6'(t)	62.82	--

RESULTS AND DISCUSSION

The polar fraction of the methanolic extract of the aerial parts of *M. Crassifolia* afforded a new ionol glucoside [1]. The IR of [1] showed OH groups (3450, 1170 cm^{-1}), bands for glycosidic linkage (1360 ~ 1250 cm^{-1}), geminal dimethyl groups (1377 and 1372 cm^{-1}) and trans double bond (972 cm^{-1})[10]. Secondary Impact Mass Spectrum (SIMS) showed $[M+Na]^+$ at m/z 397 and $[M+1]^+$ at m/z 375 consistent with the molecular formula $C_{19}H_{34}O_7$. The peak at m/z 213 $[(M+H)-Glu]^+$ for the loss of hexose while the peak at m/z 194 $[M-(Glu+H_2O)]^+$ for the loss of hexose and one molecule of water.

400 MHz ^{1}H-NMR (C_5D_5N) aided with extensive ^{1}H-^{1}H spin-decoupling experiments showed the presence of four methyl signals, where the two singlets at δ 1.01 and 0.94 were assigned to CH_3-11 and CH_3-12 and two doublets at δ 1.31 (d, 6.3 Hz) and 1.39 (d, 6.5 Hz) for CH_3-10 and CH_3-13. A pair of doublet at δ 2.18 (12.6 and 4.4 Hz) and 1.89 (12.6 and 12.2 Hz) were assigned to methylene protons at

C-2, while the two signals at δ 1.80 (1H,ddd, 12.8, 3.3 and 2.8 Hz) and 2.02 (1H,ddd, 12.8, 10.4 and 10.5 Hz) could be assigned to methylene protons at C-4. Two multiplets at δ 4.14 (1H, m) and 1.69 (1H, m) for H-3 and H-5, respectively. Two signals at δ 5.51 (1H,dd,J= 15.6 and 8.8 Hz) and 5.59 (1H,dd,J= 15.6 and 6.5 Hz) were assigned to the trans olefinic protons (H-7 and H-8 respectively). An anomeric sugar proton at δ 5.04 (1H,d,J= 7.8 Hz) and from its coupling constant, it is clear that it is present in ß-configuration[11]. The other protons were cited in Table 1 as shown from their coupling constant and from extensive ^{1}H-^{1}H spin decoupling experiments.

Careful inspection of the broad-band proton decoupling ^{13}C-NMR and Distortionless Enhancement by Polarization Transfer (DEPT) spectra (C_5D_5N) showed the presence of nineteen carbon signals (Table 2), one singlet, eleven doublets, three triplets and four quartets and this confirm the MS. The two doublet signals at δ 133.32 and 134.47 were assigned to the disubstituted double bond carbons C-7 and C-8 while the two oxygenated methane carbons at δ 68.41 and 74.05 were assigned for the free hydroxy group carrying carbon C-3 and the carbon carrying glycosylated one at C-9 respectively and the singlet carbon at δ 34.58 was assigned for C-1. The ^{13}C-NMR spectral data of the glucose moiety are in a good agreement with those reported for D-glucopyranoside[12]. The assignment of each carbon signal and it multiplicity as cited in (Table 2) by comparing these data with those reported for other ionones [13-16].

Acid hydrolysis of compound [1] gave aglycone [1A] and one sugar molecule identified as glucose (PC, system IV, using authentic sugars). The aglycone gave in its EIMS, M^{+}-H_2O at m/z 194 (3%), so the molecular weight of the aglycone consistent with the molecular formula $C_{13}H_{24}O_2$. 400 MHz ^{1}H-NMR (C_5D_5N) of the aglycone (Table 1) showed two singlet and two doublet methyls, two -CH_2-, two >CH, two >CH-O- and two =CH- protons. The assignment of each proton and its multiplicity as cited in Table 1. The ^{13}C-NMR of the aglycone showed 13 carbon signals, one singlet, six doublets, two triplets and four quartets and this support the MS. The two oxygenated methane carbons at δ 69.50 and 67.80 were assigned for the two secondary alcoholic hydroxy group carbons C-3 and C-9 while the two doublet signals at δ 133.60 and 136.22 were assigned for the disubstituted double bond. Since there was only one olefinic double bond, the last unsaturation degree (No. of unsaturation = 2) was accounted for by a cycle. The identification of each carbon and its multiplicity as cited in Table 2 by comparing the chemical shift and its multiplicity with those reported for other ionones with related structures[13-17].

The glycosidic linkage of the sugar with the aglycone part was confirmed to be at C-9 by comparing the ^{13}C-NMR of the glucoside and that of the aglycone (Table 2), since C-9 was upfield shifted (6.23 ppm) but C-8 and C-10 were downfield shifted (1.75 and 2.50 ppm respectively) while the other carbon signals are slightly affected or unchanged. From all the above mentioned data, compound [1] was identified as a new ionol glucoside having the structure [1].

Compound [2], Quercetin 3-O-ß-D-galactoside. The physical, chemical, chromatographic and UV spectral data with different ionizing and complexing reagents of compound [2] proved its flavonoidal glycoside nature[18,19]. ^{1}H-NMR showed the characteristic pattern for quercetin[18] and one anomeric sugar proton at δ 5.43 (1H,d,J= 7.7 Hz) characteristic for ß-glycosidic linkage. ^{13}C-NMR showed characteristic signals for quercetin and other 6 carbon signals for hexose sugar. The sugar signals are assignable for D-galactopyranose[20,21]. Acid hydrolysis yielded sugar identified as galactose (PC and TLC using authentic sugars). The aglycone was identified as quercetin (mp, mmp, co-chromatography and spectral data).

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