

NEW IONOL GLYCOSIDES FROM *MAERUA CRASSIFOLIA* FORSSK GROWN IN EGYPT

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يعتبر نبات الموريا كراسيفوليا من النباتات ذات الاستعمال الشعبي في علاج الام الأسنان والتهابات الجلد. واستكمالاً لدراسة النبات الكيميائية فقد تم في هذا البحث فصل ثلاث مركبات اينولية جديدة على هيئة جليكوزيدات و تم التعرف على هذه المركبات بدراسة الخواص الكيميائية والفيزيائية واستخدام طرق التحليل الطيفي الحديثة في التعرف على هذه المركبات.

Further investigation of the methanolic extract of the air-dried aerial parts of *Maerua crassifolia* Forssk (F. Capparaceae) resulted in the isolation of three new ionol glycosides. The structure of the isolated compounds had been established by extensive chemical and spectroscopic techniques.

INTRODUCTION

Maerua crassifolia Forssk (F. Capparaceae) is a small tree growing in Egyptian desert.^{1,2} The genus *Maerua* has some folkloric uses³ as ferbifuge, for cephalagia, toothache and infected hairy skin.

As a continuation of the study on *M. crassifolia* Forssk,⁴⁻⁷ we have isolated three new ionol glycosides from the polar fraction of the methanolic extract of the aerial parts of *M. crassifolia* Forssk.

EXPERIMENTAL

General experimental procedures

Melting points were uncorrected, IR were taken in KBr with Perkin-Elmer (Model 457), ¹H- and ¹³C-NMR spectra were recorded in Bruker AM-400 Spectrometer (at 400 MHz for ¹H- and 100 MHz for ¹³C-NMR) using TMS as internal standard. Mass spectra were carried out on Hitachi M-80 spectrometer (Japan). TLC were carried out on silica gel plates (Kieselgel 60 F₂₅₄, E. Merck, Whatman paper No. 1 were used for PC. For isolation, silica gel (230-400) (E. Merck) and irregular reversed phase (RP-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- Ethyl acetate-methanol-water (75:23:2)
- II- Chloroform-methanol-water (70:27:1)

Plant material

The aerial parts of *Maurea crassifolia* Forssk were collected from El-Hafafit in the eastern desert in April 1987. The plant was kindly identified by Prof. Dr. Nabil El-Hadidy, Professor of Taxonomy, Faculty of Science, Cairo University. The aerial parts were air-dried, reduced to No 40 powder and kept in well closed container till used.

Extraction and isolation

2 kg of the air-dried powdered aerial parts were extracted with methanol by maceration. The methanolic extract (132 g) was mixed with 500 ml distilled water, then extracted with hexane (5 x 1.5 L.). The hexane fraction (65 gm) was kept for further investigation. The hydromethanolic mother liquor after evaporation (30 gm) was chromatographed over Amberlite (IR-45) column chromatography (5 x 150 cm),

and eluted with water-methanol gradients. Fractions 500 ml each were collected. The fractions eluted with water-methanol (1:1) were collected, evaporated to dryness (3 gm) and further chromatographed on RP-18 CIG column chromatography (22m x 30 cm). Fractions 25 ml each were collected. The fractions (5-24) eluted with water-methanol (8:2) afforded 10 mg of [1], while fractions (31-50) afforded 49 mg of [2] and fractions (62-70) eluted with water-methanol (7:3) afforded 12 mg of [3].

Acetylation of compounds [1], [2] and [3]

5 mg of each compound was separately mixed with 10 ml of pyridine and 10 ml of acetic anhydride and left for 24 hours at room temperature. The obtained acetates were separately purified using CIG column system using silica gel and hexane-ethyl acetate (55:45) as solvent system.

Enzymatic hydrolysis of compound [2]

7.6 mgs of compound [2] and 7.6 mgs of glycosidase enzyme were mixed at 37°C and left for 20 hours. The aglycone was extracted with ethyl acetate (5 ml x 4), then the aqueous fraction was extracted with n-butanol. The butanol extract was concentrated under reduced pressure and tested for sugars (PC & TLC) using authentic sugars. The ethyl acetate was also evaporated under reduced pressure. The product was purified over silica gel column chromatography using chloroform:Methanol (82:18) as solvent system.

Acid hydrolysis

5 mg of [2] was autoclaved in a sealed tube with 1-2 ml 2N trifluoroacetic acid at 120°C/1 bar for 1.5 hour. The aglycone was isolated by addition of distilled water and extracted with CHCl₃, dried over anhydrous magnesium sulphate and crystallised from anhydrous CHCl₃. The remaining aqueous layer was evaporated under reduced pressure and dissolved in the least possible volume of isopropyl alcohol and tested for sugar.

Compound [1]

Compound [1] was obtained as colourless amorphous powder (10 mg). $R_f = 0.23$ and 0.21 in systems I and II, respectively. $[\alpha]_D^{25} = -35.8$ (methanol), IR (KBr cm⁻¹) 3445, 1372, 1377 and 974. SIMS $[M+Na]^+$ at m/z 445, $[M+1]^+$ at m/z 423. ¹H-NMR data are cited in Table 1 and that for ¹³C-NMR in Table 2.

Acetate of compound [1]

IR(KBr) cm⁻¹: 1725 (acetate), ¹H-NMR (CDCl₃) showed 7 acetate signals at δ 1.97, 2.01, 2.02, 2.05, 2.07, 2.10 and 2.19 in addition to other signals in Table 1. ¹³C-NMR (CDCl₃) showed six signals corresponding to 7 CH₃-C=O at δ 169.48, 169.54, 169.42, 170.38 (x2), 170.82 and 171.24 in addition to 5 signals for 7 H₃C-C=O at 20.26, 20.72 (x2), 20.80 (x2), 20.96 and 21.02. Other signals are listed in Table 2.

Compound [2]

This compound was obtained as colourless amorphous powder (49 mg) $R_f = 0.3$ and 0.23 in systems I and II, respectively. $[\alpha]_D^{25} = -46.8$ (methanol), IR (KBr cm⁻¹) 3450, 1372, 1377, 974. SIMS $[M+Na]^+$ at m/z 429 and $[M+1]^+$ at m/z 407. ¹H-NMR data are cited in Table 1 and that for ¹³C-NMR in Table 2.

Aglycone of [2]

Colourless amorphous solid (methanol). $[\alpha]_D^{25} = +91.06$ (methanol), IR (KBr cm⁻¹) 3470, 1379, 1371, 972. ¹H-NMR (C₅D₅N) data are cited in Table 1 and that for ¹³C-NMR (C₅D₅N) in Table 2. MS m/z : 226 $[M^+ - H_2O]$.

Acetate of compound [2]

MS m/z : 658 $[M^+]$. ¹H-NMR (C₅D₅N) showed 5 signals for 6 acetate groups at δ 1.99 (3H), 2.01 (3H), 2.03 (3H), 2.05 (6H), and 2.15 (3H) in addition to other signals listed in Table 1. ¹³C-NMR (C₅D₅N) showed five signals corresponding to 6 CH₃-C=O at δ 169.62, 169.78, 170.28, 170.44 (x2) and 170.52 in addition to 5 signals for 6 H₃C-C=O at 20.43 (x2), 20.55, 20.59, 20.93 and 21.00. Other carbon signals are listed in Table 2.

Table 1: 400 MHz ¹H-NMR data of compounds [1], [2], [3] and their derivatives.

Proton No.	[1] ppm mult (J Hz)	[1] Ac. ppm mult (J Hz)	[2] ppm mult (J Hz)	[2] Ag. ppm mult (J Hz)	[2] Ac. ppm mult (J Hz)	[3] ppm mult (J Hz)	[3] Ac. ppm mult (J Hz)
CH ₃ -10	1.41, d (6.4)	1.30, d (6.4)	1.39, d, (6.4)	1.50, d (6.4)	1.36, d (6.4)	1.39, d (6.4)	1.20, d (6.4)
9	4.89, dq (6.9, 6.4)	4.34, dq (7.8, 6.4)	4.89, dq (6.7, 6.4)	4.74, dq (5.8, 6.4)	4.67, dq (7.5, 6.4)	4.87, dq (7.2, 4.4)	4.33, dq (7.3, 6.4)
8	6.21, dd (6.9, 15.4)	5.71, dd (7.8, 15.4)	6.13, dd (6.7, 15.7)	6.35, dd (5.8, 15.6)	6.03, dd (7.5, 15.6)	6.13, dd (7.2, 15.7)	5.52, dd (7.3, 15.6)
7	5.99, d (15.4)	5.38, d (15.4)	6.07, d (15.7)	5.98, dd (15.6, 1.1)	5.84, d (15.6)	6.03, d (15.7)	5.33, d (15.6)
CH ₃ -13	1.55, d (6.5)	0.95, d (6.5)	1.52, d (6.6)	1.51, d (6.7)	1.20, d (6.6)	1.10, d (6.0)	0.80, d (6.6)
5	2.25, dq (10.4, 6.5)	*	2.24, dq (10.4, 6.6)	2.27, dq (10.4, 6.7)	2.25, dq (10.4, 6.6)	2.09, ddq (10.4, 2.9, 6.0)	1.66, ddq (10.4, 2.8, 6.6)
4a	4.24, dd (10.4, 11.6)	5.28, dd (10.4, 11.5)	4.09, dd (10.4, 9.1)	4.13, dd (10.4, 9.1)	5.73 dd, (10.4, 10.5)	2.06, ddq (10.4, 12.8, 10.5)	1.53, ddq (10.4, 12.8, 10.5)
4b	2.08, ddd (4.7, 12.8, 2.8)	1.56, ddd (3.3, 12.8, 2.8)
3	4.08, dd (11.6, 7.4)	5.25, dd (11.5, 7.4)	4.25, ddd (12.3, 4.7, 9.1)	4.26, ddd (12.3, 4.7, 9.1)	5.52, ddd (12.4, 5.0, 10.5)	4.24, dddd (10.5, 11.6, 4.5, 4.7)	5.11, dddd (10.5, 12.3, 3.3, 4.8)
2a	4.27, d (7.4)	5.23, d (7.4)	1.88, dd (12.4, 4.7)	1.92, dd (12.4, 4.7)	1.76, dd (12.4, 5.0)	1.84, dd (12.3, 4.5)	1.58, dd (12.2, 4.8)
2b	2.53, dd (12.3, 12.4)	2.56, dd (12.3, 12.4)	2.32, dd (12.3, 12.4)	2.36, dd (12.3, 11.6)	2.00, dd (12.3, 12.2)
CH ₃ -12	1.15, s	0.91, s	1.15, s	1.16, s	1.07, s	1.13, s	0.83, s
CH ₃ -11	1.39, s	1.17, s	1.19, s	1.19, s	1.15, s	1.16, s	0.85, s
glu. 1'	5.12, d (7.7)	4.52, d (7.9)	5.09, d (7.7)	5.06, d (7.9)	5.08, d (7.7)	4.63, d (7.9)
2'	4.10, dd (7.7, 8.8)	5.01, dd (7.9, 9.2)	4.06, dd (7.7, 8.9)	5.48, dd (7.9, 9.4)	4.09, dd (7.7, 9.0)	5.32, dd (7.9, 9.3)
3'	4.26, dd (8.8, 9.1)	5.14, dd (9.2, 9.3)	4.21, dd (8.9, 9.4)	5.66, dd (9.4, 9.5)	4.19, dd (9.0, 9.3)	5.47, dd (9.4, 9.3)

Proton No.	[1] ppm mult (J Hz)	[1] Ac. ppm mult (J Hz)	[2] ppm mult (J Hz)	[2] Ag. ppm mult (J Hz)	[2] Ac. ppm mult (J Hz)	[3] ppm mult (J Hz)	[3] Ac. ppm mult (J Hz)
4'	4.29, dd (9.1, 9.3)	5.08, dd (9.3, 10.2)	4.23, dd (9.1, 9.3)	4.54, dd (9.1, 9.5)	4.22, dd (9.3, 9.4)	5.31, dd (9.4, 9.5)
5'	3.98, m	3.93, m	3.95, m	4.13, m	3.97, m	4.49, m
6'a	4.42, dd (11.8, 5.3)	4.26, dd (12.3, 4.7)	4.39, dd (11.8, 5.4)	4.61, dd (12.2, 4.9)	4.41, dd (11.6, 5.4)	4.16, dd (12.3, 4.6)
6'b	4.59, dd (11.8, 2.3)	4.14, dd (12.3, 2.5)	4.57, dd (11.8, 2.3)	4.45, dd (12.2, 2.1)	4.60, dd (11.6, 2.3)	4.31, dd (12.3, 2.5)

Table 2: 100 MHz ¹³C-NMR of compounds [1], [2], [3] and their derivatives.

C No.	[1]	[1] Ac.	[2]	[2] red	[2] Ac.	[2] Ag.	[3]	[3] Ac.
10	22.26 q	22.29 q	22.88	22.23	22.61	25.08	22.90	22.66
9	73.60 d	73.19 d	73.65	77.18	73.70	69.02	73.68	73.72
8	132.43 d	132.88 d	131.48	32.04	132.04	132.87	131.69	131.59
7	135.44 d	133.52 d	137.29	33.29	136.13	136.14	137.61	137.04
6	80.64 s		79.54	77.44	78.98	79.45	77.47	77.04
5	42.23 d	40.61 d	41.66	42.34	39.82	41.99	34.76	34.52
4	74.05 d	71.55 d	78.02	78.04	76.53	78.23	46.43	41.26
3	81.98 d	79.03 d	72.44	72.77	72.36	72.52	66.31	70.41
2	73.68 d	70.90 d	44.05	45.34	40.09	44.22	40.52	35.49
1	41.73 s	41.78 s	39.59	40.62	39.66	39.67	39.98	40.00
13-CH ₃	12.71 q	11.73 q	12.72	12.16	12.34	11.72	16.97	16.67
12-CH ₃	22.98 q	22.28 q	25.16	25.17	24.32	25.20	25.08	24.29
11-CH ₃	23.85 q	23.83 q	25.73	26.13	25.15	25.80	26.13	25.72
glu. 1'	101.56 d	98.59 d	101.21	104.27	98.27	101.18	98.88
2'	75.33 d	71.66 d	75.18	75.40	72.21	75.26	72.24
3'	78.75 d	75.43 d	78.63	78.72	75.72	78.75	75.82
4'	71.90 d	68.82 d	71.82	71.85	69.29	71.94	69.32
5'	78.61 d	71.98 d	78.55	78.31	72.55	78.66	72.36
6'	62.94 t	62.28 t	62.85	62.96	62.55	62.98	62.58

Compound [3]

This compound was obtained as colourless amorphous powder (12 mg) $R_f = 0.26$ and 0.36 in systems I and II, respectively. $[\alpha]_D^{25} = -20.3$ (methanol), IR (KBr cm^{-1}) 3450, 1372, 1377, 974. SIMS $[M+Na]^+$ at m/z 413 and $[M+1]^+$ at m/z 391. $^1\text{H-NMR}$ data are cited in Table 1 and that for $^{13}\text{C-NMR}$ in Table 2.

Acetate of compound [3]

5 mg of [3] was acetylated as mentioned. The obtained acetate was purified using CIG column system (adsorbent RP-18, system methanol-water 7:3) The obtained acetate was subjected to the following: $^1\text{H-NMR}$ (C_6D_6), it showed the presence of 4 signals at δ 2.01 (3H), 2.02 (3H), 2.03 (3H) and 2.06 (6H). Other signals are listed in Table 1. $^{13}\text{C-NMR}$ ($\text{C}_5\text{D}_5\text{N}$), it revealed 5 singlet signals for $\text{H}_3\text{C-C=O}$ at δ 169.55, 169.80, 170.27, 170.38, 170.49, and three quartet signals for five $\text{H}_3\text{C-C=O}$ at δ 20.44 (x2), 20.58 (x2) and 21.32.

RESULTS AND DISCUSSION

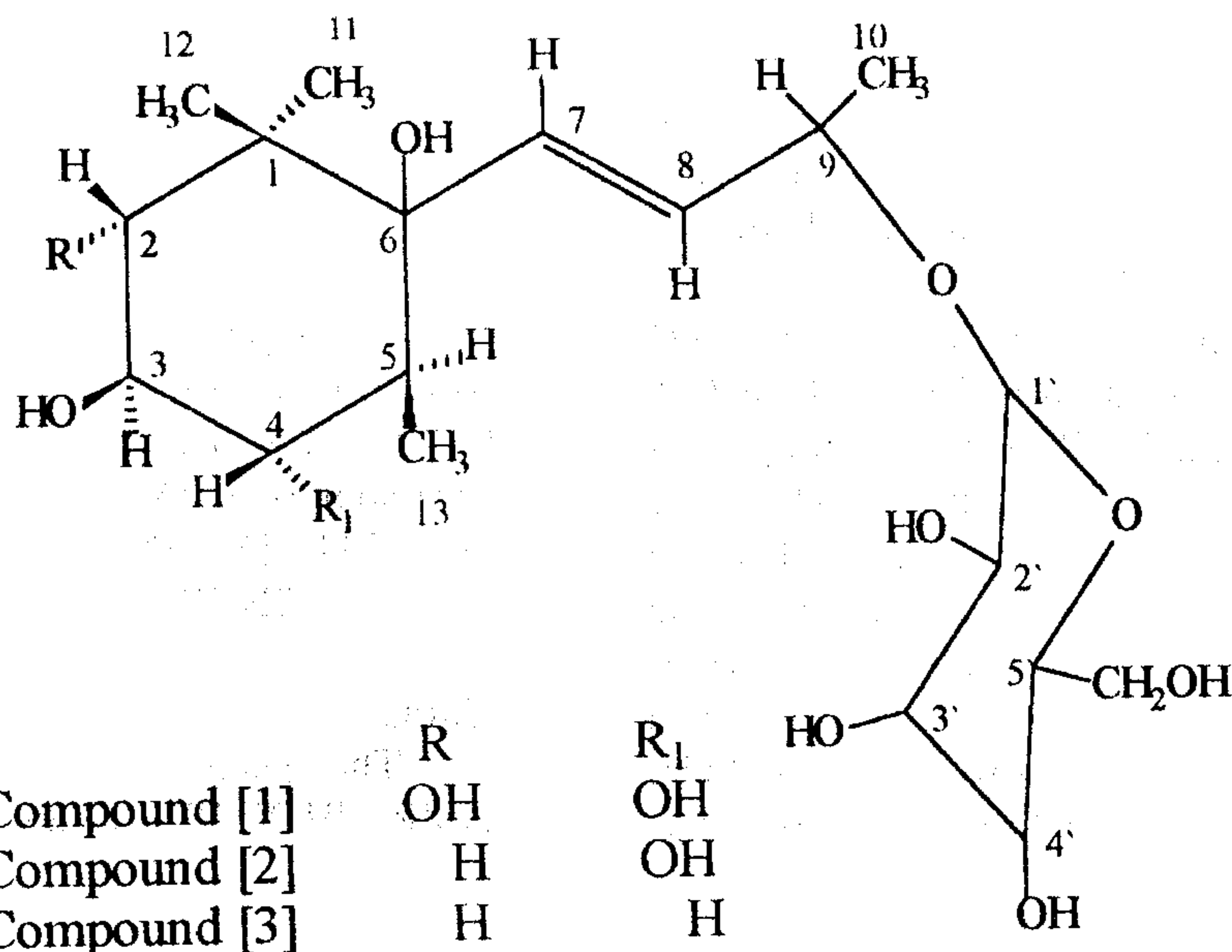
The hydromethanolic mother liquor (30 g) of the air-dried powdered aerial parts of *Maerua crassifolia* Forssk, upon repeated column chromatography afforded three ionol glycosides [1] (10 mg), [2] (49 mg) and [3] (12 mg).

Compound [1]

Was obtained as white amorphous powder. It is freely soluble in ethanol, methanol and butanol, insoluble in dichloromethane and hexane. It responded positively to Molish's test indicating its glycosidic nature. The IR spectrum showed the presence of hydroxyl group(s) at 3445 cm^{-1} , geminal dimethyl groups at 1377 and 1372 cm^{-1} and trans double bond at 974 cm^{-1} .

$^1\text{H-NMR}$ ($\text{C}_5\text{D}_5\text{N}$) showed the presence of two singlet methyls at δ 1.15 and 1.39 assigned for the tertiary methyls Me-12 and 11⁸ and two doublet methyls at δ 1.41 (3H, d, $J = 6.4 \text{ Hz}$) and 1.55 (3H, d, $J = 6.5 \text{ Hz}$) assigned for methyl groups 10 and 13,⁸ respectively. The signals centered at δ 6.21 (1H, dd, $J = 6.9, 15.4 \text{ Hz}$) and 5.99 (1H, d, $J = 15.4 \text{ Hz}$) were assigned to the olefinic protons H-8 and H-7 respectively, and their coupling constant ($J = 15.4$) indicates their trans configuration.⁹ The signal at δ 5.12 (1H, d, $J = 7.7 \text{ Hz}$) is assigned for the anomeric sugar proton and the large coupling constant (7.7 Hz) is indicative for its β -configuration.¹⁰ The identification of each proton and its coupling constant was confirmed by using 2-D H-H COSY, 2D-J resolution spectra and acetate formation.

Acid hydrolysis of compound [1] afforded glucose as the sugar moiety (TLC & PC). These data together with $^{13}\text{C-NMR}$ signals of the hexose moiety are in agreement with those reported for β -D-glucopyranoside structure.¹¹⁻¹⁴



The ^{13}C -NMR spectrum showed the presence of 19 carbon signals including six sugar signals. Besides the sugar signals there are additional five oxygen bearing carbons, four of which are doublets at δ 73.60, 73.68, 74.05, and 81.98 assigned to C-9, C-2, C-4 and C-3 respectively, while the fifth is a singlet at 80.64.

SIMS spectrum of compound [1] revealed a diagnostic peak at m/z 423 $[\text{M}+\text{H}]^+$ corresponding to the molecular formula $\text{C}_{19}\text{H}_{34}\text{O}_{10}$. The fragments at m/z 405 $[\text{M}+1-\text{H}_2\text{O}]$ and 387 $[\text{M}+1-2\text{H}_2\text{O}]$ indicate the presence of polyhydroxy compound. The peak at m/z 261 $[\text{M}+1-\text{hexose}]$ supported the presence of hexose as a sugar moiety. The fragments at m/z 243, 225 and 207 indicate the successive loss of water molecules from the aglycone.

Compound [2]

The spectral pattern of [2], ^1H -, ^{13}C -NMR, IR and sugar data were closely related to those of [1] with some conclusive differences. The SIMS of [2] furnished $[\text{M}+\text{H}]$ at m/z 407 decreasing 16 a.m.u. from that of [1]. Also the ^1H -NMR of [2], when compared with [1] (Table 1), showed the same pattern with the exception of an extra two methylene protons at δ 1.88 (1H, dd, $J=4.7, 12.4$ Hz) and 2.53 (1H, dd, $J=12.3, 12.4$ Hz).

Comparatively, the ^{13}C -NMR of [2] showed one less oxygenated carbon than [1]. All these data showed clearly that [2] has one less hydroxyl group than [1] i.e only seven hydroxyl groups are present in [2]. Upon acetylation of [2], it afforded hexacetate which is still showing a tertiary hydroxyl absorption in its IR spectrum indicating the presence of one tertiary hydroxyl function at C-6 as in [1].

Comparison of the ^{13}C -NMR pattern of [2] with that of [1] showed an upfield shift of C-1 (2.1 ppm) and upfield shift of C-3 (9.6 ppm) in [2] as well as the upfield shift of C-2 (29.71 ppm) compared with [1] indicating that the lacked hydroxyl group of [2] is absent from the position number 2 of the cyclohexyl ring. Similar shifts could be observed when comparing the acetate derivatives of [1] and [2]. Also, in ^1H -NMR the signals at δ 4.27 (H-2) has

disappeared and upfield shifted to give two signals at δ 1.88 and 2.53.

The ^{13}C -NMR spectrum of the aglycone showed the presence of three carbon-bearing oxygen, while the glycoside afforded hexacetate (four for sugar residue), this indicates that the compound has two secondary and one tertiary hydroxyl groups in addition to the sugar hydroxyl groups. The position of sugar linkage could be explained as follows:-

- a- Comparing the chemical shift of C-9 in both the original compound and the aglycone, revealed that C-9 was upfield shifted in the aglycone by 5.63 ppm while the other sp^3 carbon bearing oxygen were slightly affected i.e the sugar moiety was linked to C-9 of the aglycone.
- b- The LSPD data showed that the anomeric sugar carbon only affected when the H-9 proton of the aglycone was irradiated indicating that the sugar moiety was linked to C-9 of the aglycone moiety.
- c- Comparing the chemical shift of C-1' and C-9' of the original compound with its reduced product, showed the downfield shifting of both C-1' and C-9' in the reduced product by 3.06 and 3.53 ppm respectively, confirming the glycosylation at C-9.

The acid and enzymatic hydrolysis afforded glucose as the sugar moiety (PC and TLC using authentic sugars). ^{13}C -NMR spectrum of the glucose moiety is in agreement with those obtained for other β -D-glucopyranosides.¹¹⁻¹⁴ The β -D-configuration of the glycosidic linkage was corroborated by the coupling constant of 7.7 Hz of the anomeric proton in ^1H -NMR spectrum.

SIMS gave m/z 429 $[\text{M}+\text{Na}]$, 407 $[\text{M}+1]$ corresponding to the molecular formula $\text{C}_{19}\text{H}_{34}\text{O}_9$ for the glucoside. EIMS showed m/z 388 corresponds to $[\text{M}^+-\text{H}_2\text{O}]$ and other fragments m/z 244 $[\text{M}^+(\text{hexose}+1)]$ and 209 $[\text{M}^+(\text{hexose}+2\text{H}_2\text{O})]$.

Compound [3]

The spectral pattern of compound [3] (^{13}C -NMR, ^1H -NMR, IR and SIMS) showed very

close relation to those of [1] and [2] with only important differences.

- a- The SIMS showed $M+1$ at m/z 391 with one oxygen function less than [2] and two oxygen function less than [1], so the molecular weight of [3] is 390 corresponds to the molecular formula $C_{19}H_{34}O_8$, also other fragments appear in the spectrum at m/z 227 and 209 corresponding to the loss of hexose [$M^+ + 1$ -hexose] and [$M^+ + 1$ - (hexose + H_2O)] respectively.
- b- The ^{13}C -NMR (Table 2) as expected showed one less oxygenated carbon than [2] as it revealed the presence of four quartet signals, two singlet signals (as in [1] and [2]), three triplet signals ([1] showed one triplet carbon while [2] showed two triplet carbon) and ten doublet signals ([1] showed twelve doublet carbons and [2] showed eleven doublets, this means that [3] has less oxygen bearing carbons than [1] (by 2 carbon atoms) and [2] (by one carbon atom). Similarly behaved the 1H -NMR (Table 1) where it showed two pairs of methylene protons (only one pair in [2] and disappearance of the signal at δ 4.09 corresponding to H-4 methine proton.

As in case of [1] and [2], the sugar moiety was identified as glucose (acid hydrolysis, PC and TLC). The ^{13}C -NMR chemical shift of the sugar carbons were in agreement with those obtained for other β -D-glucopyranosides.¹¹⁻¹⁴ The β -D-configuration of the glycosidic linkage was corroborated by the coupling constant of 7.7 Hz of the anomeric proton in 1H -NMR spectrum.

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