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TRITERPENES FROM *ACANTHOSICYOS HORRIDUS*

Mostafa S. M. Magd

Department of Pharmacognosy, Faculty of Pharmacy,
Cairo University, Cairo, 11562 Egypt

ABSTRACT

On continuation of the study on the roots of an unusual member of the Cucurbitaceae family, *Acanthosicyos horridus* Well. ex Hook. f, known as nara or naras; the present work reports the isolation of 24- methylenecycloartanone and (after acetylation) chondrillasteryl glucoside acetate for the first time from this plant. The structures of these compounds, were confirmed by using spectroscopic methods including IR, ^{1}H and ^{13}C NMR and Mass spectra.

INTRODUCTION

Nara occurs in Namibia, South West Africa, particularly in the region of walvis Bay. Leaves are largely absent and the tendrils are reduced to sharp spines. The fruit is a small gourd containing numerous seeds, and both have been used as food by the desert dwellers in the region for over 8000 years.[1] They are still used locally and traded to some extent, particularly the seeds, which are collected for use in the confectionery industry in South Africa[2].

In a previous work[3,4] isolated cucurbitacins, some tetracyclic triterpenes including cycloartanes and sterols[3,5] were isolated from the roots of this plant.

EXPERIMENTAL

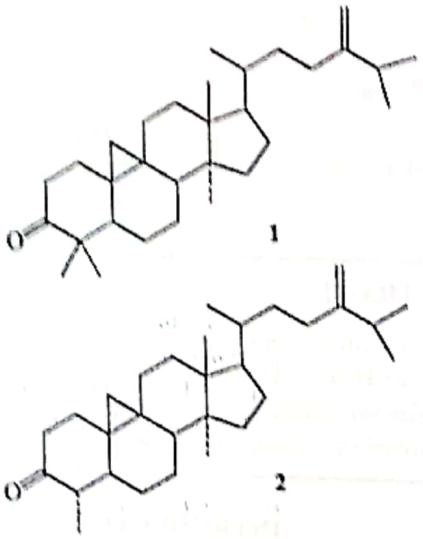
Mps were taken with an electrothermal melting point apparatus and are uncorrected. ^{1}H NMR were recorded at 250 MHz and 400 MHz in $CDCl_3$ using TMS as internal standard. ^{13}C NMR were recorded at 74.88 MHz on a Bruker WH400 NMR spectrometer in $CDCl_3$ using TMS as internal standard. MS were recorded on an AEI MS902 high resolution mass spectrometer having a direct inlet system and operating at 18 and 70 ev. IR spectra were obtained in KBr discs using a Unicam SP200 spectrometer. Analytical TLC was on silica gel 60 PF_{254} (0.5 mm).

Roots of *Acanthosicyos horridus* were collected in 1985 near Walvis Bay, South West Africa[12]. The dried powdered roots (2 kg) were exhaustively extracted successively with petrol (bp 60-80°) and then with MeOH. An oily yellowish brown residue (17 g) and a sticky dark reddish brown residue (210 g) were obtained from the light petroleum and methanol extracts, respectively. These extracts were kept in freezer.

About 16 g of the dried light petroleum extract of the roots was saponified. The unsaponifiable matter (5.2 g) was adsorbed onto a column of silica gel 60 (200 g). The material was chromatographed using the following solvents: petrol (bp 40-60°); petrol-toluene (1:1); toluene; toluene-chloroform (1:1); chloroform; chloroform-methanol (1:1) and methanol; 137 fractions of either 100 ml or 250 ml were collected. Similar fractions were grouped together according to TLC examination. Repeated preparative TLC of fractions 41-49; solvent petrol -toluene 1:1] allowed isolation of 24-methylene-: cycloartanone 1 as white needle crystals from methanol (10 mg), mp 109-111°C [Lit.[13] 111-112°C]; ^{1}H NMR: δ(400 MHz, $CDCl_3$): 0.60 (1H, d, J= 4.2 Hz, C-19), 0.76 (1H, d, J= 4.2 Hz, C-19), 0.81 (3H, S, C-18), 0.88 (3H, S, C-32), 0.91 (3H, d, J= 6 Hz, C-21), 0.96 (6H, S, C-30 & 31), 1.01,(6H, d, J= 6.5 Hz, C-26 & 27), 4.7 (2H,bd, J= 12.5 Hz, C-28); MS : m/z (rel. int.): 438 (4), 424

(19), 423 (31), 395 (9), 354 (4), 340 (9), 339 (17), 313 (13), 300 (11), 285 (10), 216 (11), 201 (28), 187, 175 (41), 163 (32), 160 (36), 138 (5), 125 (11), 123 (41), 109 (44), 108 (21), 95 (41), 84 (14), 83 (26) and 69 (13).

A part of the MeOH extract (about 10.5 g) was adsorbed onto a column of silica gel 60 (400 g). The material was chromatographed using the following solvents: petrol (bp 40-60°); petrol-$CHCl_3$ (1:1); $CHCl_3$- EtOAc (1:1); EtOAc; EtOAc- MeOH(1:1) and MeOH; 425 fractions of either 100 ml or 250 ml were collected. Similar fractions were pooled together according to TLC examination. Fractions 324-352 (2.89 g) collected by elution with EtOAc-MeOH (1:1), were acetylated. The dried acetate (3.10 g) was adsorbed onto another column of silica gel 60 and eluted using the following solvents: cyclohexane - EtOAc (1:); ETOAc and EtOAc- MeOH (1:1). Elution with increasing concentrations of EtOAc in cyclohexane allowed isolation (after prep. TLC, cyclohexane- EtOAc 6:4) of Chondrillasteryl glucoside acetate **3** as white needle crystals from $CHCl_3$- MeOH (1:1) [30 mg]: mp 164-165°C; IR (KBr) cm^{-1}: 1750, 1240 (OAc); ^1H NMR; δ(400 MHz, $CDCl_3$): 0.54 (3H, s, C-18), 0.79 (3H, d, J = 7 Hz, C-26), 0.82 (3H, s, C-19), 0.84 (3H, t, J = 7 Hz, C-29), 0.85 (3H, d, J = 6.5 Hz, C-27), 1.03 (3H, d, J = 6.6 Hz, C-21), 2.01, 2.03, 2.09 (4 acetate singlets, each 3H), 3.63 (1 H, m, 5-H), 3.71 (1H, m, H-3), 4.18 (1H, dd, J = 12 and 2 Hz, 6-H), 4.24 (1H, dd, J = 12 and 4 Hz, 6-H), 4.62 (1H, d, J = 8 Hz, anomeric), 4.96 (1H, t, J = 8 Hz, 2-H), 5.08 (1H, t, J = 8 Hz, 4-H),5.14-5.23 (1H, m, C-7), 5.25 (1H, t, J = 8 Hz, 3-H); ^{13}C NMR: δ (74.88 MHz, $CDCl_3$), C-1 (37.11), C-2 (27.92), C-3 (79.52), C-4 (33.95), C-5 (40.24), C-6 (29.27), C-7 (117.18), C-8 (139.60), C-9 (49.45), C-10 (34.49), C-11 (21.53), C-12 (39.56), C-13 (43.40), C-14 (55.05), C-15 (22.96), C-16 (28.41), C-17 (55.99), C-18 (12.03), C-19 (12.92), C-20 (40.70), C-21 (21.01), C-22 (138.02), C-23 (129.50), C-24 (51.24), C-25 (31.85), C-26 (18.91), C-27 (21.34), C-8 (25.34), C-29 (12.17), C1 (99.63), C2 (77.26), C3 (76.95), C4 (71.69), C5 (76.63), C6 (62.21); M S: m/z (rel. int.): 742 (0.3), 727 (0.1), 603 (1.6), 411 (3), 396 (62), 331 (100), 289 (2), 272 (1), 271 (8), 255 (23), 228 (1), 211 (4), 201 (4), 169 (77), 139 (8), 111 (4), 109 (14) and 43 (16).



RESULTS AND DISCUSSION

I. Petroleum ether extract:

The dried light petroleum extract of the roots *Acanthosicyos horridus* was saponified. Fractionation of the unsaponifiable matter by chromatography on silica gel 60 allowed isolation of a substance, mp 109-111°C. This was assigned structure 1 on the basis of spectral evidence below.

The ^1H NMR spectra of compound 1 showed characteristic signals for the hydrogen in a cyclopropane ring. The shifts of these signals agree with those given by Berti et al.[6] The shifts depend upon the substitution at C-3 and C-4 in ring A. Thus, if ring A contains a ketonic group at C-3 and two methyl groups at C-4 the shifts for the two doublets of the cyclopropane hydrogens appear at δ 0.60 and δ 0.76. These two doublets are shifted by δ 0.19 and δ 0.13 upon changing the substitution in ring A to only one methyl group at C-4 in ring A plays a significant role in the determination of the ^1H NMR shift values for the cyclopropane hydrogens which is thus useful in structural elucidation. The other shifts agree with those for cycloeucalenone[3]. The Signals for methyl groups at C-18, C-32, C-30 and C-31 appear as singlets at δ 0.81, 0.88 and 0.96 respectively. Signal for the C-21 methyl group appears as doublet at δ0.91. The doublet at δ1.01 is

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indicative of methyl group at C-26 and C-27. The broad doublets at δ4.7 corresponds with two hydrogens of C-28.

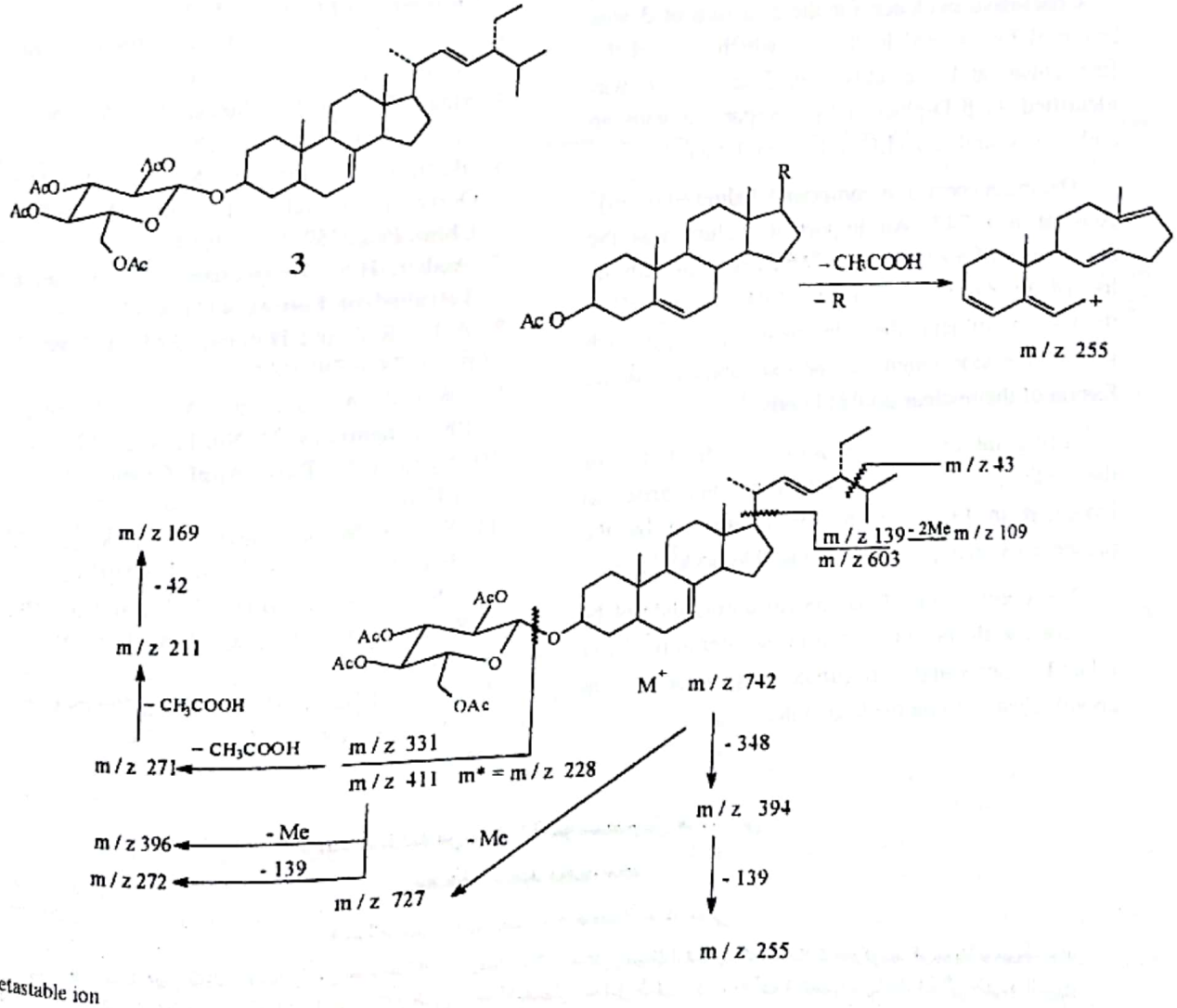
Mass spectroscopy showed the $[M]^+$ peak of compound **1** to be at m/z 438. A characteristic ion fragment at m/z 300 originates probably by rupture of the cyclopropane ring and its formation requires the loss of ring A along with C-6 or C-19[7,3]. The M^+ -84 fragment arises from a Mclafferty rearrangement involving the 24 (28) double bond and H-20[8,3]. The other fragments arise, mostly confirmed by the presence of appropriate metastable peaks[3].

On the basis of the above accumulated evidence structure **1** was established as 24- methylene-cycloartanone.

II. Alcoholic Extract:

A methanolic extract of the roots of *Acanthosicyos horridus* was chromatographed on silica gel colum. A substance, mp 164-165°, was obtained after acetylation from the polar fractions of the column. This is assigned structure 3 on the basis of the chemical and spectral evidence below.

The ^1H NMR spectra of compound 3 showed the characteristic signal for the anomeric proton as doublet at δ4.62. The signals were assigned by comparing with those given by Wasim et al.[9]. Tertiary methyl groups appear as singlets at δ0.54 and δ0.82 for C-18 and C-19. The triplet at δ0.79 (J = 7 Hz) is due to C-29 methyl signal. The signals of three further 2nd methyl groups were observed as doublets at δ0.79 (J= 7 Hz), 0.85 (J= 6.5 Hz) and 1.03 (J= 6.6 Hz) for C-26, C-27 and C-21



m* = metastable ion

Fig. 1: Mass fragmentation of chondrillasteryl glucoside acetate **3**.

respectively. Multiplets at δ3.71 and 5.14- 5.23 are due to H-3 & H-7. The attachment of the sugar moiety shifted the carbinylic proton resonance to δ3.71.

The ^{13}C NMR spectrum showed the presence of 35 carbon atoms in the molecule. out of these, six carbon signals were in the glycosidic region corresponding to a hexose moiety, the remaining 29 carbon signals were due to the aglycone. The anomeric carbon signal of sugrar moiety appeared at δ99.63. Among the methine resonances the low field ones at δ117.18 and 79.52 were assigned to C-7 and C-3. Concerning the methylene resonances the lowest field quaternary resonance at δ139.60 is attributed to C-8. The lowest field methine resonances at δ138.02 and 129.50 were assinged to C-22 and C- 23.

Conclusive evidence for the structure of **3** was provided by its acid hydrolysis which yielded the free sugar and the aglycone. The former was identified as β-D-glucose by comparison with an authentic smaple on TLC and spectral data[9].

The mass spectra of compound **3** showed th $[m]^+$ peak at m/z 742. An important feature was the appearance of a peak at m/z 255 associated with the loss of sugar moiety (-331) and -OH (-17) as well as the C-17 sustituent (the side chain, - $C_{10}H_{19}$), such loss of the side chain can be explained by allylic fission of the nuclear double bonds[10].

A fragmnt ion appeared at m/z 603 due to loss of the side chain. The other fragments arise as indicated in Figure 1, mostly confirmed by the presence of an appropriate metastable peak[3].

The comparison of the spectroscopic data of he aglycone with those reported in the literature[11] for related compound confirmed its structure as chondrillasteryl glucoside acetate.

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ترديبينات ثلاثية من نبات الاكانثوسيصيوص هوريدوس

مصطفى سعيد محمد مجد

قسم العقاقير - كلية الصيدلة - جامعة القاهرة- مصر

تم فى هذا البحث فصل مادتين لأول مرة من جذور هذا النبات وهما: ٢٤ - ميثلين سيكلوارتينون وكذلك خلات جلوكوزيد كوندريلا ستيرول، وقد تم التعرف على التركيب الكيميائى لهاتين المادتين عن طريق درجة الانصهار، تحليل طيف الكتلة والأشعة الحمراء، بالاضافة الى التردد النووى المغناطيسى.