On the basis of its spectral data the structure of *ent*-16-hydroxy-13-*epi*-manoyl oxide (**1**), a diterpenoid isolated for the first time as a natural product, is proposed

EXPERIMENTAL

The ^{1}H NMR spectrum was measured at 200 MHz in $CDCl_3$ soln with TMS as int standard The ^{13}C NMR spectrum was determined at 50 MHz also in $CDCl_3$ soln with TMS added as int reference Plant material was collected in July 1984 in Sierra Javalambre (Teruel) and voucher specimens were deposited at the herbarium of the Faculty of Pharmacy (University of Valencia)

Extraction and isolation of the diterpenoid Dried and powdered plants of *S javalambrensis* (980 g) were extracted with hexane in a Soxhlet The extract (42 g) was chromatographed on a silica gel (Merck, 60) column (1 26 kg) Elution with CH_2Cl_2–EtOAc mixtures of increasing polarity, yielded a diterpenic fraction (2 22 g) which was chromatographed on a 10% $AgNO_3$–silica gel dry column and eluted with CH_2Cl_2–EtOAc (19 1) yielding the compound **1** (55 5 mg)

ent-16-*Hydroxy*-13-epi-*manoyl oxide* (**1**) Treatment of **1** (3 mg) with mesyl chloride (0 2 ml) for 24 hr and later reduction with $LiAlH_4$ (10 mg) for 6 hr gave a substance with $[\alpha]_D^{20}$ −21° ($CHCl_3$, *c* 1 0) ^{1}H NMR δ 5 84 (1H, *dd*, part X of an ABX system, J_{AX} = 11 0 Hz, H-14), 5 03 (2H, part AB of an ABX system, J_{BX} = 18 0 Hz, 2H-15), 3 05 (2H, *q*, *J* = 10 8 Hz, 2H-16) and C-Me singlets at 0 79 (6H), 0 71 (3H) and 0 66 (3H), ^{13}C NMR δ 15 2 (*t*, C-11), 15 9 (*q*, C-20), 18 65 (*t*, C-2), 19 9 (*t*, C-6), 21 3 (*q*, C-19), 24 0 (*q*, C-17), 28 4 (*t*, C-12), 33 3 (*q*, C-18), 33 4 (*s*, C-4), 36 9 (*s*, C-10), 39 3 (*t*, C-1), 42 2 (*t*, C-3), 43 0 (*t*, C-7), 56 5 (*s*, C-5), 58 4 (*s*, C-9), 69 6 (*t*, C-16), 76 7 (*s*, C-8), 77 2 (*s*, C-13), 113 5 (*t*, C-15) and 144 1 (*d*, C-14) MS *m/z* (rel int) M$^+$ absent, 291 [M − Me]$^+$ (0 8), 276 (6), 275 (29), 259 (2), 258 (21), 257 (100), 205 (3), 203 (2), 201 (4), 193 (3), 191 (5), 189 (2), 187 (4), 177 (2), 175 (3), 173 (2), 163 (4), 161 (4), 159 (2), 151 (6), 137 (28), 123 (15), 109 (16), 107 (11), 95 (21), 93 (11)

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CUCURBITACIN GLYCOSIDES FROM *CITRULLUS COLOCYNTHIS*

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Key Word Index—*Citrullus colocynthis*, Cucurbitaceae, cucurbitacin glycosides, hexanorcucurbitacin I glycoside

Abstract—The chloroform extract of *Citrullus colocynthis* yielded four cucurbitacin glycosides which were identified spectroscopically as 2-*O*-*β*-D-glucopyranosyl-cucurbitacin I, 2-*O*-*β*-D-glucopyranosyl-cucurbitacin E, 2-*O*-*β*-D-glucopyranosyl-cucurbitacin L and the novel glycoside, 2-*O*-*β*-D-glucopyranosyl-(22–27)-hexanorcucurbitacin I Detailed ^{1}H and ^{13}C NMR data are provided

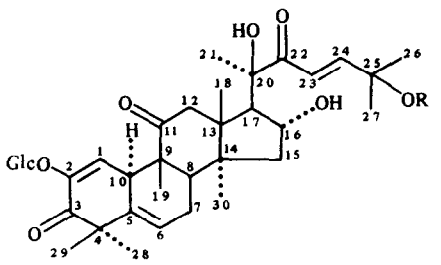
INTRODUCTION

The fruit of *Citrullus colocynthis* L Schrad has been used medicinally since ancient times It has been suggested to possess anti-tumour activity [1 3] Phytochemical investigations of its bitter principles, cucurbitacins, are numerous, but conflicting regarding the type of cucurbitacin and their glycosides present [3–14] In this paper we describe the isolation and structural elucidation of a novel hexanorcucurbitacin glycoside in addition to three other known cucurbitacin glycosides

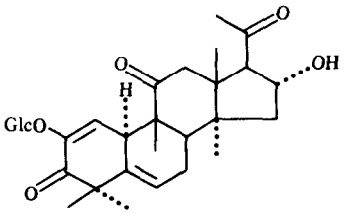
RESULTS AND DISCUSSION

The chloroform extract of the defatted plant was fractionated by preprative TLC (Experimental) to give four glycosides, characterized by spectral analysis as 2-*O*-*β*-D-glucopyranosyl-cucurbitacin I (**1**), 2-*O*-*β*-D-glucopyranosyl-cucurbitacin E (**2**) as the major product, 2-*O*-*β*-D-glucopyranosyl-cucurbitacin L (**3**) and a novel glycoside 2-*O*-*β*-D-glucopyranosyl-(22–27)-hexanorcucurbitacin I (**4**) No free cucurbitacin aglycones were detected in the extract which contradicts previous findings [6, 11, 13, 14], and this is in accordance with the reported presence of cucurbitacins as glycosides only in *Citrullus* due to the absence of the enzyme elaterase, the enzyme capable of hydrolysing the glycosides [15]. Cucurbitacin L glycoside (**3**) has not been previously isolated directly from the plant, although its aglycone was isolated from the enzymatic hydrolysate of the fruit extract [10], so this is the first report of its direct isolation from *Citrullus colocynthis* A following ethanolic extract yielded the glycosides **1**–**3** only, with **1** being the major product

Although ^{1}H and ^{13}C NMR spectra have been presented and used for the structural studies of cucurbitacins



1 R = H
2 R = Ac
3 R = H, 23,24-dihydro



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[17, 18], applications to the field of cucurbitacin glycosides have been very limited [19] Here we report the complete ^1H and ^{13}C NMR data (Tables 1 and 2) for the glycosides isolated. This information should provide important data for further research in this field Assignments of the signals were based on analogy of the different groups, the known effect of substituents and ^1H–^{13}C and ^1H–^1H 2D-correlation spectra. The NMR spectra of **1–3** very closely resemble those of the free aglycones [18]. However, C-1 showed a profound shift in its ^{13}C spectrum to a lower field on glycosidation (δ115–122) The reason for this shift may be due to weaker electron release (+M effect) of glu-O compared to an hydroxy group and therefore less electronic shielding of C-1 in the glycoside, so that it resonates at lower field. The ^{13}C NMR chemical shifts for the sugar moiety were assigned by comparison with those of the glycosides arvenin I and II [19], while their ^1H chemical shifts were determined by ^1H–^{13}C 2D-correlation spectra. The EIMS spectra for **1**, **2** and **3** did not produce observable parent ions. However, FABMS showed observable parent ions either as $[M+1]^+$ or $[M+Na]^+$ ions. The spectra were dominated by the ions $A^+ = [\text{aglycone} - (25\text{-OR})]^+$ and $[A - H_2O]^+$.

The spectral evidence lead to the structural assignment of 2-*O*-β-D-glucopyranosyl-(22–27)-hexanorcucurbitacin I (**4**), which is the only degraded cucurbitacin glycoside reported so far, although, its aglycone has been isolated from *Ecballium elaterium* [16]. This compound was obtained as an amorphous powder, analysed for $C_{30}H_{42}O_{10}$, showed a molecular ion peak at *m/z* 585 $[M(C_{30}H_{42}O_{10})+Na]^+$ in the FAB mass spectrum, and exhibited a ketonic carbonyl absorption (1690 cm^{-1}) in the IR spectrum. A cucurbitane skeleton for this compound was deduced from its co-occurrence with cucurbitacins as well as from its ^1H NMR spectrum (Table 1) which showed the main features of the cucurbitacins. In particular it showed two vinylic protons in position and character corresponding to 1-H, at δ 6.08 (doublet, J = 2 Hz) and 6-H at δ 5.84 (triplet, J = 2 Hz) and 10-H at δ 3.75 (broad singlet). The NMR spectrum also indicated the presence of five C-Me groups, which can be accommodated on the cucurbitane skeleton, and one methyl ketone, for C-21 at δ 2 16, which represents the degraded side chain. The rest of the spectrum was very similar to the other cucurbitacins except for the 16-H and 17-H which appeared at lower field at δ 4 87 (t, J = 7 Hz) and 3.17 (d, J = 7 Hz), respectively, due to deshielding by the carbonyl group. The structure and its resemblance to cucurbitacin glycosides was further confirmed by the ^{13}C NMR spectrum (Table 2) which differed from that of the parent structure **1** by the absence of the side chain signals due to C-22 to C-27 The rest of the spectrum was closely related except for the signals corresponding to the carbons 12, 17, 20 and 21 (Table 2) which are influenced by the nature of the C-20 carbonyl group Both 21-Me

Table 1. ^1H NMR chemical shifts of cucurbitacins **1–4***

H	1	2	3	4	H	1	2	3	4
1	6.11 *d* (2)	6.10	6.11	6.08	18	0.96 *s*	0 94	0.97	0.69
6	5 83 *t* (2)	5.84	5 83	5 84	19	1.47 *s*	1 47	1 48	1.48
7a	2 39 *dd* (8, 20)	2 40	2.40	2.43	21	1 39 *s*	1.42	1.41	2 16
7b	2 11†	2 11	2.11	2.11	26	1 31 *s*	1 55	1.17	—
8	2.05†	2 05	2.05	2.05	27	1 31 *s*	1 52	1 16	—
10	3.75 *br s*	3 71	3 73	3 75	28	1.27 *s*	1 26	1 27	1.27
12a	3 45 *d* (15)	3 41	3 45	3.46	29	1 27 *s*	1.26	1 27	1.27
12b	2 64 *d* (15)	2 59	2 64	2 47	30	0 98 *s*	0 98	0 98	0 98
15a	1.87 *dd* (7, 20)	1 87	1.86	1.97 *dd* (7, 14)	1′	4.66 *d* (8)	4 66	4 68	4.67
15b	1 47†	1 45	1 47	1.58 *d* (14)	2′	3.30 *t* (8)	3.30	3 31	3.29
16	4.47 *t* (7)	4 51	4 39	4 87	3′, 4′	3 48 *m*	3.47	3 46	3.46
17	2 67 *d* (7)	2 65	2 68	3 17	5′	3 37 *m*	3.38	3 38	3 35
23	6.85 *d* (16)	6.84	2.85 *m*	—	6′a	3 98 *dd* (12, 2)	4 01	3 97	3 95
24	7 00 *d* (16)	6.98	1 70 *m*	—	6′b	3.83 *dd* (12, 3)	3 83	3.82	3.82

*Coupling pattern and coupling constants (value in Hz in parentheses) are not repeated if identical with the proceeding column

†Signal is obscured.

Table 2 ^{13}C NMR chemical shifts of cucurbitacins **1–4**

C	1	2	3	4	C	1	2	3	4
1	122 69	123 82	122 69	122 61	18	20 25	20 67	20 24	20 03
2	146 41	146 80	146 42	146 57	19	18 44	18 74	18 46	18 47
3	199 18	198 74	199 18	199 02	20	79 39	79 75	80 33	210 57
4	48 50	49 06	48 59	49 19	21	24 97	25 33	25 11	31 82
5	136 63	137 40	136 64	136 86	22	204 50	203 99	217 13	
6	121 72	122 02	121 74	121 59	23	120 53	122 51	32 62	
7	24 05	24 61	24 05	24 26	24	155 29	151 17	37 57	
8	42 28	42 72	42 24	42 60	25	70 98	80 47	70 24	
9	49 66	50 01	49 66	50 10	26	29 02	26 65	29 02	
10	35 61	36 15	35 61	35 71	27	29 62	27 16	29 11	
11	216 57	215 24	216 60	215 02	28	20 51	20 87	20 55	20 65
12	49 60	49 87	49 51	48 01	29	27 97	28 25	27 97	28 01
13	49 51	49 93	49 66	49 61	30	20 51	21 00	20 31	20 23
14	51 14	51 47	51 05	50 64	1′	100 22	101 19	100 22	100 42
15	46 11	46 77	46 10	45 90	2′	73 43	74 03	73 43	73 57
16	70 93	71 39	70 68	71 81	3′	76 53	77 47	76 53	76 69
17	58 48	59 33	58 51	67 19	4′	69 84	70 77	69 85	70 01
CO Me	-	170 26	-	-	5′	77 13	78 05	77 14	77 31
COMe	—	21.91		-	6′	61 30	62 34	61 31	61 49

and C-17 methine, which are α to the carbonyl group, were shifted to lower field giving rise to signals at δ 31 82 and 67 19, whereas in the parent they occur at δ 24.97 and 58.48, respectively The C-12 was also influenced by the C-20 ketone with an upfield shift of 1 60 ppm which is in accordance with the reported similar effect [18]. The ^{13}C NMR spectrum provided satisfactory information on the sugar moiety by comparison with the spectra of the other glycosides

EXPERIMENTAL

Mps uncorr IR nujol NMR spectra were recorded in $(CD_3)_2CO$ at 400 MHz for ^1H NMR and 100 6 for ^{13}C NMR with TMS as int ref Mass spectra were recorded using the FAB method Analytical TLC was carried out on silica GF_{254} ready made plates (Flucka) For prep TLC a 0 75 mm layer of silica GF_{254} was used, using $CHCl_3$–MeOH (17 3)

Extraction and isolation Air dried and ground fruit of *Citrullus colocynthis* (200 g) collected from southern Iraq, Basrah region, was continuously extracted with petrol (for defatting), $CHCl_3$ and 80% aq EtOH The $CHCl_3$ extract was evapd to dryness to give a brown foam (10 g, 5%) A sample was fractionated by prep TLC using the multiple elution technique (two to three elutions) The various bands were rechromatographed by prep TLC until single spot fractions were obtained The glycosides isolated were as follows according to their decreasing order of R_f values

2-O-β-D-*Glucopyranosyl-cucurbitacin E* (**2**) Obtained as amorphous yellow powder ($CHCl_3$–Et_2O), mp 157–159° (lit [8] 158–160°), IR ν_{max} cm^{-1} 3440 (OH), 1720 (AcO), 1685 (C=O), 1640, 1630 (C=C), FABMS *m/z* (rel int) 741 $[M(C_{38}H_{54}O_{13}) + Na]^+$ (13), 497 [aglycone – AcO]$^+$ (20), 479 [aglycone – AcO $-H_2O]^+$ (7), 396 (23), 203 (28)

2-O-β-D-*Glucopyranosyl-cucurbitacin I* (**1**) Pale yellow needles (EtOH), mp 239–240° (lit [20] 241–242°), IR ν_{max} cm^{-1} 3440 (OH), 1690 (C=O), 1640 (C=C), FABMS *m/z* (rel int) 659 $[M(C_{36}H_{52}O_{12}) - HO]^+$ (11), 497 [aglycone $-HO]^+$ (72), 479 [aglycone – HO – $H_2O]^+$ (33), 401 (34)

2-O-β-D-*Glucopyranosyl-cucurbitacin I* (**3**) Amorphous powder (EtOH-Et_2O), mp 210–215° (lit [21] mp 228–235°), IR ν_{max} cm^{-1} 3400 (OH), 1690 (C=O), 1640 (C=C), FABMS *m/z* (rel int) 701 $[M(C_{36}H_{54}O_{12}) + Na]^+$ (4), 679 $[M + 1]^+$ (7), 661 $[M - OH]^+$ (5), 499 [aglycone – OH]$^+$ (98), 481 (aglycone – OH – $H_2O]^+$ (11)

2-O-β-D-*Glucopyranosyl-(22–27)-hexanorcucurbitacin I* (**4**) Amorphous powder (EtOH-Et_2O), mp 170–175 (Found C, 63 95, H, 7 55 $C_{30}H_{42}O_{10}$ requires C, 64 06, H, 7 47%) IR ν_{max} cm^{-1} 3400 (OH), 1690 (C=O), 1640 (C=C), FABMS *m/z* (rel int) 585 $[M(C_{30}H_{42}O_{10}) + Na]^+$ (28), 552 $[M + Na - Me - H_2O]^+$ (8), 508 (8), 401 (9)

The EtOH extract was concd and extracted with EtOAc The extract was evapd to give a brown foam Recrystallized from EtOH to give yellow needles which was a mixture of **2** major, and **3** These compounds were separated by prep TLC The mother liquor of the crystallization was fractionated by prep TLC to give further amounts of **1**, **2** and **3**

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(24*R*)- AND (24*S*)-14α-METHYL-5α-ERGOST-9(11)-EN-3β-OLS FROM *GYNOSTEMMA PENTAPHYLLUM*

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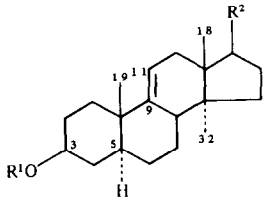
Key Word Index—*Gynostemma pentaphyllum*, Cucurbitaceae, sterol, (24*R*)- and (24*S*)-14α-methyl-5α-ergost-9(11)-en-3β-ol

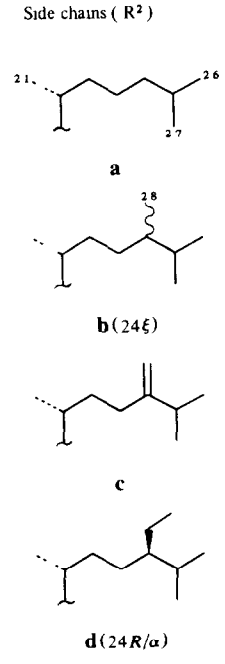
Abstract—Two new sterols were isolated as a mixture from the aerial parts of *Gynostemma pentaphyllum* and shown to be the (24*R*)- and (24*S*)-epimers of 14α-methyl-5α-ergost-9(11)-en-3β-ol.

INTRODUCTION

Gynostemma pentaphyllum Makino (Japanese name, Amachazuru) has been shown by our recent studies to contain several uncommon sterols including a 14α-methylsterol, 14α-methyl-5α-ergosta-9(11),24(28)-dien-3β-ol [or 24-methylene-14α-methyl-5α-cholest-9(11)-en-3β-ol] (**1c**) [1], and four 24,24-dimethylsterols: 24,24-dimethyl-5α-cholest-7-en-3β-ol (24,24-dimethyllathosterol), (22*E*)-24,24-dimethyl-22-dehydrolathosterol and 24,24-dimethyl-25-dehydrolathosterol [2], and 24,24-dimethyl-5α-cholestan-3β-ol [3], in addition to major (22*E*,24*R*/β)-24-ethyl-22-dehydrolathosterol (chondrillasterol) and other sterols [4–6]. Our continuing study on the sterol constituents of *G. pentaphyllum* has led to the isolation as a mixture and identification of two further 14α-methylsterols, (24*R*/α)- and (24*S*/β)-epimers of 14α-methyl-5α-ergost-9(11)-en-3β-ol [or 14α,24-dimethyl-5α-cholest-9(11)-en-3β-ol] (**1b**).



1 R^1 = H
2 R^1 = Ac



RESULTS AND DISCUSSION

Steryl acetate **2b** was isolated from the acetylated sterol fraction of *G. pentaphyllum* by virtue of the procedure described in the Experimental section. The mass spectrum of **2b** showed $[M]^+$ at *m/z* 456, corresponding to