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CUCURBITACINS OF *COLOCYNTHIS VULGARIS**

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Key Word Index—*Colocynthis vulgaris*; *Citrullus colocynthis*, Cucurbitaceae; cucurbitacin I; cucurbitacin J; cucurbitacin T; structure elucidation; biosynthesis.

Abstract—The methanolic extract of the fruits of *Colocynthis vulgaris* on acidic hydrolysis afforded in addition to cucurbitacin I and J a new cucurbitacin designated cucurbitacin T whose structure was elucidated as (24*S*)-16,24-anhydro-25-methoxy-2,16α,20β,24-tetrahydroxy-3,11,22-trioxocucurbita-1,5-diene by spectroscopic methods. The biosynthetic significance of the co-occurrence of cucurbitacins I, J and T in *C. vulgaris* is discussed.

INTRODUCTION

The fruits of *Colocynthis vulgaris*(= *Citrullus colocynthis*; Cucurbitaceae), a plant indigenous to India, Pakistan and Sri Lanka have a variety of ethnomedical claims and finds uses as a purgative [2], powerful cathartic [3], abortifacient [4] and for amenorrhea [5]. Extracts derived from fruits of *C. vulgaris* have been shown to possess cardiac depressant, smooth muscle relaxant, cytotoxic, and antitumour activities [6]. Previous chemical investigations of the fruits of *C. vulgaris* by paper chromatography has revealed the presence of cucurbitacins B,D,E,I,L and the glucosides of cucurbitacins B,E and L [7]. As a continuation of our interest in medicinal and related plants of Sri Lanka we have investigated the fruits of *C. vulgaris* and in this paper we report the isolation of cucurbitacins I, J and a new cucurbitacin (cucurbitacin T) from the acidic hydrolysate of the cold methanol extract.

RESULTS AND DISCUSSION

The cold methanolic extract of the fresh fruits of *C. vulgaris* was subjected to acidic hydrolysis and the hydrolysate was separated by combined CC and prep. TLC into one new and two known cucurbitacins. The structure of the least polar new cucurbitacin (cucurbitacin T) was elucidated as **1** from the evidence presented below.

The UV and IR spectra were characteristic of a cucurbitacin with a ring A diosphenol moiety [8]. The mass spectrum afforded M^+ at *m/z* 528.3120 corresponding to the molecular formula $C_{31}H_{44}O_7$. The presence of a major fragment at *m/z* 496 ($C_{30}H_{40}O_6$) due to the loss of methanol, coupled with the occurrence in the ^1H NMR spectrum of a signal at δ 3.42, indicated the presence of a OMe group in the molecule. The mass spectrum also showed a significant peak at *m/z* 164($C_{10}H_{12}O_2$) arising by a *retro*-Diels–Alder fragmentation of the ring B characteristic of cucurbitacins with a ring A diosphenol system [9]. The loss of the side chain was indicated by the presence of a prominent peak at *m/z* 401 ($C_{24}H_{33}O_5$) (Scheme 1).

The ^1H and ^{13}C NMR spectra were also characteristic of the cucurbitacin structure. These spectra showed close resemblances to those reported for cucurbitacin S (**4**) with a cyclized side chain [8] (see Tables 1 and 2). In the ^1H NMR spectrum ($CDCl_3$, 300 MHz) the eight methyl groups were assigned by direct comparison of their chemical shifts with those of other cucurbitacins reported in the literature [10, 11]. Furthermore, the assignments of H-1, H-10, H-23α, H-23β and H-24 were confirmed by the following INDOR experiments. Irradiation at δ 5.93(H-1) led to the collapse of the broad peak for H-10 at 3.49 into a sharp singlet. Also the irradiation at δ3.49 caused the doublet at 5.93 for H-1 to collapse to a singlet. Irradiation of the double doublet at δ4.12 (J = 8.28, 1.56) assigned to H-24 allowed the location of H-23α and β at δ2.72 and 2.89, respectively. Irradiation at δ2.89 resulted in the double doublet at δ4.12 collapsing to a doublet (J = 8.28 Hz). Many of the coupling constants (Table 1) were calculated from homodecoupling experiments which led to simpler spin coupled patterns. The assignments and spin–spin coupling interactions were confirmed through COSY 45 measurements (Fig. 1) while the multiplicities of the proton signals were determined from the 2D J-resolved spectrum [12].

The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of cucurbitacin T compared reasonably well with those reported for cucurbitacin S (**4**) [8] and other related cucurbitacins [10], and was consistent with the proposed structure (**1**). The multiplicity assignments were made on the basis of polarization transfer experiments (DEPT) [12]. These revealed the presence of eight methyls, four methylenes and seven methine carbons. The signal at δ83.8 was assigned to C-25 bearing an OMe group. Signals for the oxymethine carbon atoms (C-16 and C-24) appeared at δ71.2 and 81.4. The signals at δ114.8, 144.7 and 198.1 are consistent with the presence of a ring A diosphenol

*Part 17 in the series 'Studies on Medicinal and Related Plants of Sri Lanka'. For Part 16, see ref. [1].

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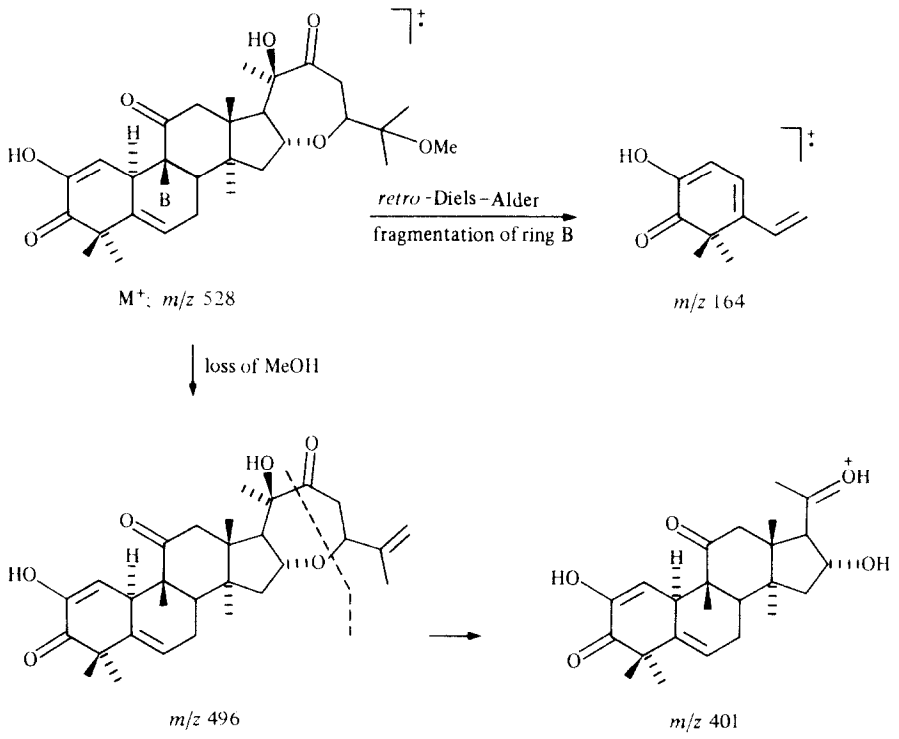


Scheme 1.

Table 1. ^{1}H NMR spectral data of cucurbitacins **1**, **3** and **4** (δ values in ppm)*

H	1	3	4†
1	5.93(*d*, 2.6)	5.92(*br d*, 2.9)	5.96(*d*, 2.5)
6	5.76(*m*)	5.75(*m*)	5.77(*br m*)
7	2.02(*br d*, 8.1)	2.02(*br d*, 8.2)	—
8	2.38(*m*)	2.34(*m*)	—
10	3.49(unresolved *d*)	3.53(*br s*)	3.47(*m*)
12α	3.22(*d*, 14.6)	3.21(*d*, 14.4)	2.44(*d*, 12)
12β	2.69(*d*, 14.6)	2.78(*d*, 14.4)	3.05(*d*, 12)
15α	1.84(*dd*, 12.9, 9.0)	1.90(*dd*, 13.1, 9.1)	1.50(*dd*, 11, 4.5)
15β			1.86(*dd*, 11, 4.5)
16	4.32(*dd*, 9.0, 7.0)	4.47(*t*, 7.6)	3.80(*sextuplet*, 9.5, 9.5, 4.5)
17	2.45(*d*, 7.0)	2.61(*m*)	2.14(*dd*, 12, 9.5)
23α	2.72(*dd*, 17.3, 8.3)	2.97(*dd*, 16.5, 9.6)	1.88(*d*, 12)
23β	2.89(*dd*, 17.3, 1.6)	2.61(*m*)	2.26(*dd*, 12.5)
24	4.12(*dd*, 8.3, 1.6)	3.97(*dd*, 9.4, 1.0)	4.00(*d*, 5)
21	1.40(*s*)	1.39(*s*)	1.04(*d*, 7)
18	0.99(*s*)	0.99(*s*)	
19	1.42(*s*)	1.43(*s*)	0.82(*s*), 1.30(*s*)
26	1.40(*s*)	1.18(*s*)	1.32(*s*), 1.34(*s*)
27	1.52(*s*)	1.24(*s*)	1.36(*s*), 1.38(*s*)
28	1.35(*s*)	1.34(*s*)	1.38(*s*)
29	1.24(*s*)	1.24(*s*)	
30	1.03(*s*)	1.02(*s*)	
OMe	3.44(*s*)	—	—

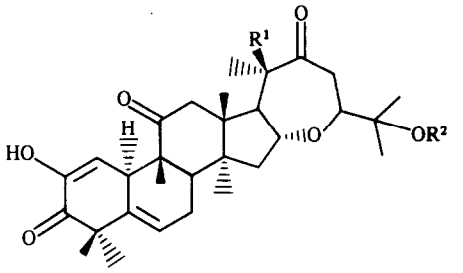
*The multiplicities and *J* values (in Hz) are given in parentheses.
†See ref. [8].

Table 2. ^{13}C NMR spectral data of cucurbitacins **1**, **3** and **4** (δ values in ppm)

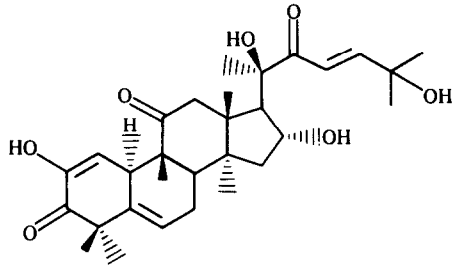
C	1	3	4†
1	114.8	114.7	114.9
2	144.7	144.7	144.6
3	198.1	198.6	198.6
4	47.1	47.6	46.9
5	137.1	137.1	137.0
6	120.7	120.7	120.6
7	23.7	23.7	23.8
8	41.9	41.7	34.6
9	48.4	48.8	43.2
10	34.8	34.8	42.3
11	213.9	212.5	212.9
12	48.9	48.8	47.9
13	45.6	47.8	48.9
14	50.6	50.9	49.3
15	45.9	45.7	41.8
16	71.2	71.8	82.1
17	57.6	56.2	49.6
18	19.9	19.7	11.0*
19	18.3	18.4	18.0*
20	79.2	79.3	32.1
21	24.3	23.7	20.2*
22	213.9	214.1	212.6, 105.5
23	38.3	39.3	47.5
24	81.4	74.5	85.6
25	83.8	72.2	71.1
26	22.7*	25.8	27.9*
27	22.5*	24.9	29.7*
28	20.1	20.1	20.7*
29	28.0	28.0	29.9*
30	20.1	20.2	23.8*
OMe	61.0	—	—

*Assignments within each vertical group may be interchanged.

†See ref. [8].



1 R^1 = OH, R^2 = Me
4 R^1 = R^2 = H



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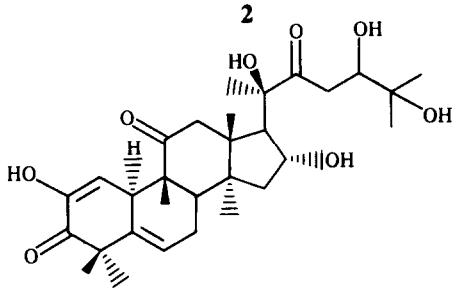

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moiety. The ^{13}C NMR assignments are given in Table 2.

The cucurbitacin of medium polarity was identified as cucurbitacin I (**2**) by comparison of its spectral data (UV, IR, MS, ^1H and ^{13}C NMR) with those reported in the literature [10, 11, 13]. The most polar cucurbitacin was suspected to be cucurbitacin J (**3**) from its UV, IR, MS, ^1H and ^{13}C NMR data. As the ^1H and ^{13}C NMR spectra of cucurbitacin J had not been reported previously these were recorded and found to be in agreement with the structure proposed earlier [14]. The assignments are presented in Tables 1 and 2. This is the first report of the occurrence of cucurbitacin J in *C. vulgaris*.

It is possible that cucurbitacin T (**1**) is an artifact formed from cucurbitacin I (**2**) during the acidic treatment of the methanolic extract (see Experimental), if methanol was still present [8]. However, the artefactual origin of **1** was ruled out as the treatment of **2** with 1 M sulphuric acid in methanol did not afford even a trace amount of **1** (TLC control).

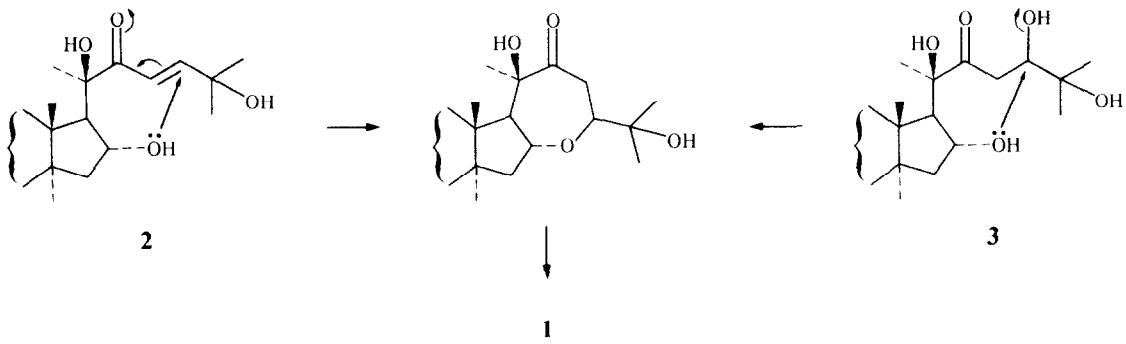
This constitutes the second report of the natural occurrence of a cucurbitacin with a cyclized side chain containing an ether linkage. Biosynthetically cucurbitacin T (**1**) could originate from either cucurbitacin I (**2**) or J (**3**) as shown in Scheme 2. The co-occurrence of these three cucurbitacins in *C. vulgaris* is of biosynthetic significance.

EXPERIMENTAL

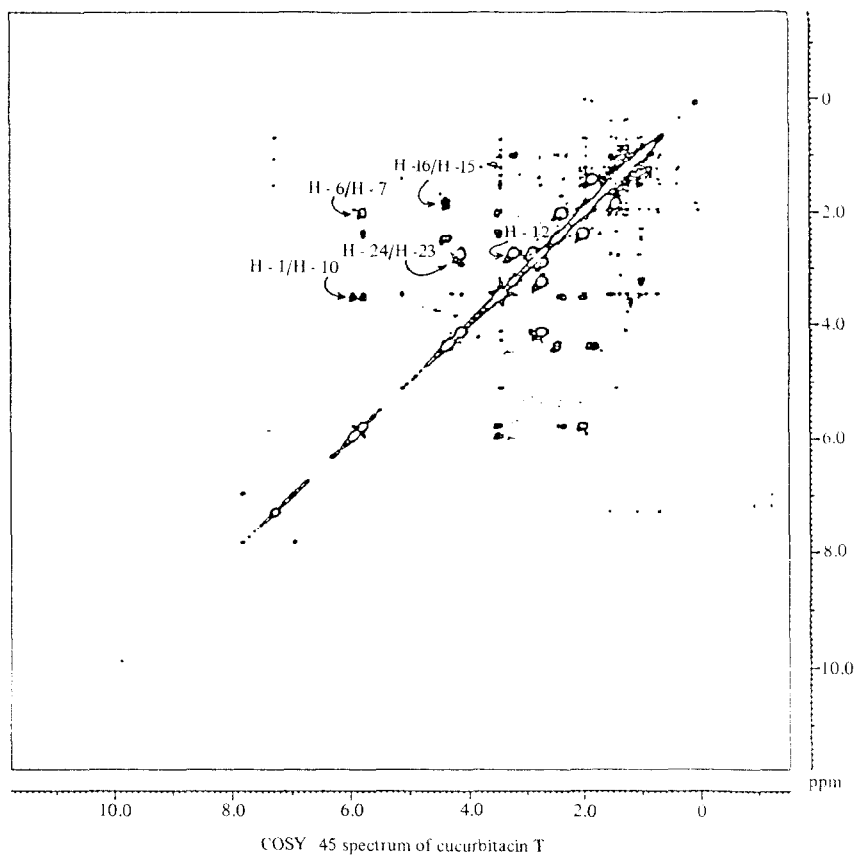
General. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 and 75 MHz, respectively, the multiplicity of carbon signals established by using DEPT measurements.

Isolation and characterization of cucurbitacins. Fresh fruits of *Colocynthis vulgaris* (83 kg) collected in Jaffna, Sri Lanka in Sept. 1983, were extracted with cold MeOH (40 l) for 1 week. Evapn of the solvent *in vacuo* afforded an extract as a brown gum (715 g). A portion (10.3 g) of this material was stirred with 1 M H_2SO_4 (200 ml) overnight and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ extract *in vacuo* afforded a semi-solid (485 mg). Combined CC and prep. TLC over silica gel yielded, in the order of increasing polarity, 14.4 mg (1.15×10^{-3}%) of **1**, 32.5 mg (26×10^{-3}%) of **2** and 24.3 mg (1.94×10^{-3}%) of **3**. Compounds **2** and **3** were identified as cucurbitacins I and J, respectively, from their UV, IR, MS, ^1H and ^{13}C NMR data.

Cucurbitacin T. Obtained as a pale yellow semi-solid which resisted crystallization; $[\alpha]_D^{28} - 53.5°$ ($CHCl_3$; *c* 1.44); UV λ_{max}^{MeOH} nm: 273; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400(OH), 1712(C-22 carbonyl), 1680(C-11 and diosphenol carbonyl); M_r(MS) 528.3120. Calcd for $C_{31}H_{44}O_7$ 528.3087. MS 70 eV *m/z* (rel. int.): 528 [M^+] (6),



Scheme 2.



COSY 45 spectrum of cucurbitacin T

Fig. 1.

496 [$M^+ - MeOH$] (3), 478 [$M^+ - MeOH - H_2O$] (2), 401 [$C_{10}H_{33}O_5$; M^+ – side chain] (4), 386 (3), 341 (3), 333(5), 297(3), 164 [$C_{10}H_{12}O_2$] (45), 143(30), 100(100), 86(99). Some important fragments in the mass spectrum are depicted in Scheme 1. For ^1H and ^{13}C NMR assignments, see Tables 1 and 2, respectively.

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