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CUCURBITACINS FROM THE CRUCIFERAE

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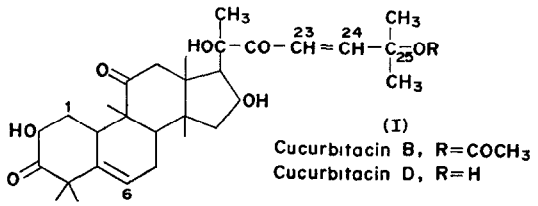
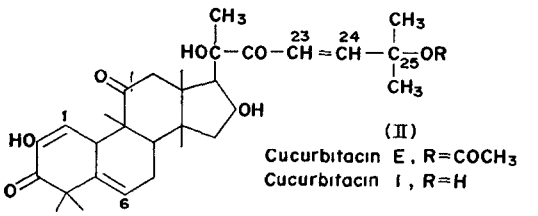
Abstract—The distribution of cucurbitacins in seeds and growing plants of *Iberis umbellata* L. has been investigated. The testas of 'Giant Pink', 'Purple', 'Crimson' and 'Iceberg' (Dutch) strains of *Iberis umbellata* contain Cucurbitacin B with traces of D and E. Seeds of 'Iceberg' (Danish) strain were exceptional in containing cucurbitacins E and I but no B, and this strain was also distinguished by larger redder seeds and by testas which were not readily separable into two layers. Cucurbitacins are rare in other crucifers but they are present in *I. gibraltarica* L., *I. sempervirens* L. and *Lepidium sativum* L.

INTRODUCTION

CUCURBITACINS are a series of tetracyclic triterpenes originally found in members of the cucurbitaceae and later in *Iberis* seeds (Cruciferae) by Bredenberg and Gmelin[1] and Gmelin.[2] Recently three new compounds, cucurbitacins O, P and Q have been found in the crucifer *Brandegea biglovii*.[3] We have found cucurbitacins B, D, E and I (I and II) in I. *umbellata* and cucurbitacins D, E and I in *I. gibraltarica*; this confirms Gmelin's work with the former species, but he did not find any cucurbitacins in the latter.



(I)
Cucurbitacin B, R=$COCH_3$
Cucurbitacin D, R=H



(II)
Cucurbitacin E, R=$COCH_3$
Cucurbitacin I, R=H

RESULTS

Cucurbitacins E and I (II) were isolated from the seeds of *I. gibraltarica* in yields of 2, and 0·2 mg/g seed respectively, and cucurbitacin D (I) was detected by TLC. Cucurbitacin D was detected in growing *I. sempervirens* plants, and cucurbitacin I in the seeds of *Lepidium sativum*.

[1] J. B. BREDENBERG and R. G. MELIN, *Acta Chem. Scand.* **16**, 1802 (1962).
[2] R. GMELIN, *Arzneimittel-Forschg.* **13**, 771 (1963).
[3] S. M. KUPEHAN, R. M. SMITH, Y. AYNEHCHI and M. MARUYAMA, *J. Org. Chem.* **35**, 2891 (1970).

Iberis umbellata 'Giant Pink', 'Crimson', 'Purple' and 'Iceberg' (Dutch) seeds had testas which were readily separable into two layers. The testas contained cucurbitacin B with small amounts of cucurbitacins D and E, and, in the case of 'Giant Pink', traces of cucurbitacin I. In 'Giant Pink' and 'Iceberg' (Dutch) seeds, the two layers of the testa were tested separately, and the cucurbitacins found in the outer layer with traces of cucurbitacin B only in the inner layer.

In 'Giant Pink' cucurbitacin D was detected in the cotyledons and in the first leaves when these formed. One month after germination cucurbitacins D and I were present in the growing point and first leaves, and as growth proceeded the two compounds appeared in new leaves and disappeared from those lower down the plant. At the flowering stage, the leaves were found to contain cucurbitacin I only.

The 'Iceberg' seed from Denmark had testas which were not readily separable into two layers and contained cucurbitacins E and I. Cucurbitacin D was found in the cotyledons and all parts of the plant during growth. This strain was distinguished by having larger redder seeds and completely white flowers. 'Iceberg' (Dutch) seed gave slightly coloured flowers, and had smaller seeds similar to those of the coloured strains. Cucurbitacins D, E and I were present in the flowers of 'Giant Pink' and 'Iceberg' (Danish) strains.

Cucurbitacins B, D, E and I were not detected in the seeds of the following crucifers: *Alyssum maritimum*, White, *Arabis albida*, *Aubretia deltoidea*, *Brassica alba*, *B. oleracea* var. *capitata*, *B. oleracea* var. *bullata gemnifera*, *Brassica rapa* (turnip) cv. Snowball, *Brassica rapa* (swede) cv. Prizewinner, *Cheiranthus cheiri* Blood red, *Erysimum perofskianum* cv. Deep Orange, *Erysimun perofskianum* cv. Dwarf, compact Golden Gem, *Hesperis matronalis*, *Iberis sempervirens*, *Lunaria annua*, *Matthiola* sp, *Raphanus sativus*.

EXPERIMENTAL

Materials. All the *Iberis* seeds used in this work were supplied by Asmer Seeds Ltd., Leicester. The strains and countries of origin of the *I. umbellata* seeds used were as follows: 'Giant Pink', (England); 'Purple', (England); 'Crimson', (England); 'Iceberg', (Denmark); 'Iceberg', (Holland). The *I gibraltarica* seeds were from Italy.

TABLE 1. NMR OF CUCURBITACINS B, D, E AND I τ ($CHCl_3$)

Assignment	Cucurbitacin B		Cucurbitacin D		Cucurbitacin E*		Curcurbitacin I	
Double bonds								
C_1					4·41	1Hs	4·05	1Hs
C_6	4·20	1Hsb	4·21	sb	4·30	1Hsb	4·26	1Hsb
C_{23}	3·52	1Hd†	3·38	1Hd‡	3·18	2Hs	3·40	1Hd‡
C_{24}	2·93	1Hd	2·84	1Hd‡			2·88	1Hd‡
$COCH_3$								
C_{25}	8·00	3Hs			8·03	3Hs		
CH_3	8·44	6Hs	8·61	3Hs	8·50	6Hs	8·65	15Hs
	8·56	3Hs	8·65	12Hs	8·70	6Hs	8·75	3Hs
	8·63	3Hs	8·70	3Hs	8·78	3Hs	8·97	3Hs
	8·65	3Hs	8·92	3Hs	8·82	3Hs	9·00	3Hs
	8·71	3Hs	9·01	3Hs	9·14	3Hs		
	8·93	3Hs			9·23	3Hs		
	9·02	3Hs						

* DMSO d6.
† $J = 16$
‡ $J = 15$.

Extraction and identification. Iberis seeds were ground in a coffee mill and extracted with EtOH in a column extractor.[4] Fats were separated from the extract by partitioning between aq. EtOH and light petroleum. The fat free aq. EtOH solutions were extracted first with CCl_4 and then with $CHCl_3$. TLC using silica gel GF (Merck) and developing with $CHCl_3$–EtAc–HCOOH (48:48:4) was used where necessary for further purification and identification. Four compounds were isolated from *I. umbellata* and it became evident for the reasons which follow that these were cucurbitacins B, D, E and I previously isolated from this species by Gmelin.[2] The residue from the CCl_4 extract of 'Giant Pink' strain yielded 3 mg/g seed of solid m.p. 178–179°; cucurbitacin B has m.p. 180–182°. From the $CHCl_3$ extract of this strain a minute amount of solid m.p. 146–153° crystallized from EtOH–benzene; cucurbitacin D has m.p. 151–152°. The CCl_4 extract of 'Iceberg' strain of seed from Denmark contained no cucurbitacin B, but gave a solid m.p. 232–234° on crystallization from EtOH; cucurbitacin E has m.p. 234°. The residue from the $CHCl_3$ extract of this batch crystallized from aq. EtOH giving a solid m.p. 146°; cucurbitacin I has m.p. 148–149°. Mass spectrometry did not give the molecular ions of the cucurbitacins. The cucurbitacins have either an acetate or OH group at position 25, and when these are abstracted as H_2O or HOAc leaving an isopropenyl group agreement is obtained with the mass spectrometer results. Hence such a loss probably occurs in the instrument. NMR spectra were consistent with the compounds isolated being the respective cucurbitacins, and τ values are given in Table 1.

[4] P. J. CURTIS, *Chem. & Ind.* 1138 (1956).