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SHORT COMMUNICATION

THE ROLE OF CUCURBITACIN Δ^{23}-REDUCTASE IN THE BREAKDOWN PATHWAY OF TOXIC BITTER PRINCIPLES IN *CUCURBITA MAXIMA*

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Abstract—The 23,24-dihydrocucurbitacins were found to be better substrates for *in vitro* breakdown than their precursor cucurbitacins with unsaturated side-chains. A probable breakdown pathway for cucurbitacins and their metabolic interrelationship in *Cucurbita maxima* are proposed.

INTRODUCTION

A CUCURBITACIN Δ^{23}-reductase (NAD(P)H: cucurbitacin Δ^{23}-reductase) which catalyses the reduction of the Δ^{23}- bond in the side chain of cucurbitacin B, D, E, etc, has been found in the Green Hubbard variety of *Cucurbita maxima*.[1] The isolation and purification of the enzyme has been reported as well as some of its major properties.[2]

The constitutional and stereochemical formulae of cucurbitacins A, B, C, D, E, F, G, H, I, J, K and L (toxic[3] bitter principles found in plants[4]) were published recently by Enslin *et al.*[5–10] The structural interrelationship of the different bitter principles are, therefore, well established. The partial formulae of cucurbitacins A to E are presented in Fig. 1.

The primary bitter principles formed in plants of the Cucurbitaceae are cucurbitacin B and E[11]. The other bitter principles are derivatives of B and E produced as a result of metabolic processes which take place during the later stages of development only.[11,12] The important role of cucurbitacin Δ^{23}-reductase in the metabolism and breakdown of these bitter principles is described in this communication.

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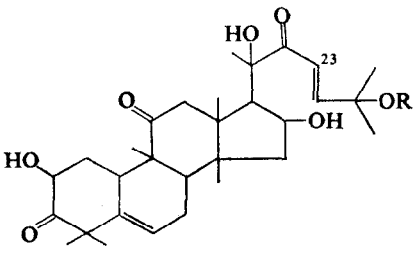
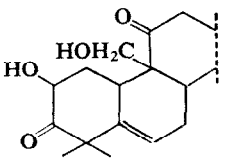
Abbreviations: A_1, B_1, etc. = 23,24-dihydrocucurbitacin A, 23,24-dihydrocucurbitacin B, etc.

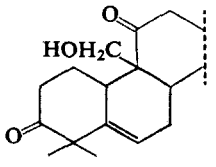
[1] J. C. SCHABORT, D. J. J. POTGIETER and V. DE VILLIERS, *Biochim. Biophys. Acta* **151**, 33 (1968).
[2] J. C. SCHABORT and D. J. J. POTGIETER, *Biochim. Biophys. Acta* **151**, 47 (1968).
[3] D. C. STEYN, *S. African Med. J.* **24**, 713 (1950).
[4] S. REHM, P. R. ENSLIN, J. H. WESSELS and A. D. J. MEEUSE, *J. Sci. Food Agri.* **8**, 679 (1957).
[5] W. T. DE KOCK, P. R. ENSLIN, K. B. NORTON, D. H. R. BARTON, B. SKLARZ and A. A. BOTHNER-BY, *J. Chem. Soc.* 3828 (1963).
[6] P. R. ENSLIN, J. M. HUGO, K. B. NORTON and D. E. A. REVITT, *J. Chem. Soc.* 4779 (1960).
[7] W. T. DE KOCK, P. R. ENSLIN, K. B. NORTON, D. H. R. BARTON, B. SKLARZ and A. A. BOTHNER-BY, *Tetrahedron Letters* 309 (1962).
[8] P. R. ENSLIN and K. B. NORTON, *J. Chem. Soc.* 529 (1964).
[9] K. J. VAN DER MERWE, P. R. ENSLIN and K. PACHLER, *J. Chem. Soc.* 4275 (1963).
[10] C. W. HOLZAPFEL and P. R. ENSLIN, *J. S. African Chem. Inst.* **17**, 142 (1964).
[11] S. REHM and J. H. WESSELS, *J. Sci. Food Agri.* **8**, 687 (1957).
[12] H. L. TEIJEMA, *'n Studie der Cucurbitacine Omzettingen*, Annual Report. Part I and II, South African Department of Agriculture Technical Services, Pretoria (1960–1962).



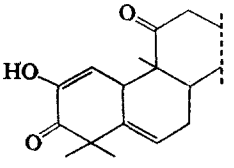
R = OCCH : Cucurbitacin B
R = H : Cucurbitacin D



Cucurbitacin A
(R = $OCCH_3$)



(R = $OCCH_3$)
Cucurbitacin C



(R = $OCCH_3$)
Cucurbitacin E

FIG. 1. PARTIAL FORMULAE OF CUCURBITACINS A, B, C, D AND E.

RESULTS AND DISCUSSION

Products obtained after *in vitro* incubation of bitter principles with homogenates of the immature fruits of *Cucurbita maxima* indicated that reactions involving hydrogenation (catalysed by cucurbitacin Δ^{23}-reductase[12]), dehydrogenation, hydroxylation, isomerization as well as other unkown conversions must be of major importance in the metabolism and breakdown of these toxins. The products after incubation were detected by paper chromatography.[12–16] The breakdown of cucurbitacins in plants also involves the deacetylation of certain cucurbitacins by an acetyl esterase.[5, 17]

According to Schabort and Potgieter,[2] a single cucurbitacin Δ^{23}-reductase might be responsible for the reduction of the Δ^{23}-bond. It was found that the primary cucurbitacins and their derivatives tested can act as substrates for this enzyme.[2]

The important role of cucurbitacin Δ^{23}-reductase as well as the 23,24-dihydrocucurbitacins as intermediates in the metabolism and breakdown of bitter principles is clearly illustrated in Fig. 2. All the bitter principles referred to in Fig. 2 were detected after *in vitro* incubations of B, D or E as substrates. Cucurbitacins G and H, and cucurbitacins J and K were found to be intermediates in the breakdown of cucurbitacins D and I respectively.[12] As indicated in Fig. 2, most of the reactions seem to be irreversible except for the interconversions of the primary cucurbitacins $B \rightleftharpoons E$, $B_1 \rightleftharpoons E_1$ etc, which were found to be completely

[13] S. REHM, personal communication.
[14] P. R. ENSLIN, T. G. JOUBERT and S. REHM, *J. Sci. Food Agri.* **8**, 673 (1957).
[15] P. R. ENSLIN, T. G. JOUBERT and S. REHM, *J. Sci. Food Agri.* **7**, 646 (1956).
[16] J. C. SCHABORT and D. J. J. POTGIETER, *J. Chromatog.* **31**, 235 (1967).
[17] H. M. SCHWARTZ, S. I. BIEDRON, M. M. VON HOLDT and S. REHM, *Phytochem.* **3**, 189 (1964).

reversible. It has been postulated that this reaction is catalysed by a cucurbitacin Δ^1-dehydrogenase. Further breakdown of cucurbitacin J and K and probably also G and H may result in the splitting of the side chain between C_{23} and C_{24} to yield ecballic acid and acetoin.[8] The

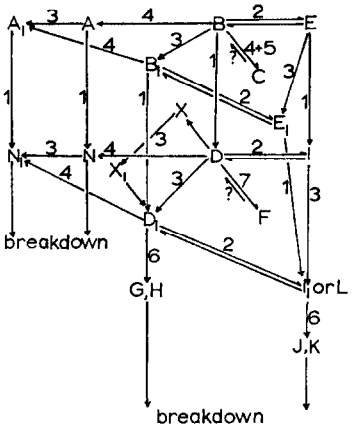


FIG. 2. PROPOSED METABOLIC INTERRELATIONSHIP AND BREAKDOWN PATHWAY OF CUCURBITACINS IN *Cucurbita maxima*.

The enzymes postulated to catalyse the different reactions are indicated by numbers, as follows: 1, cucurbitacin acetyl esterase;[17] 2, cucurbitacin Δ^1-dehydrogenase; 3, cucurbitacin Δ^{23}-reductase;[1,2] 4, cucurbitacin C18-hydroxylase; 5, cucurbitacin C2-hydroxylase; 6, cucurbitacin C24-hydroxylase; 7, cucurbitacin C3-hydrogenase.

important role of cucurbitacin Δ^{23}-reductase in the breakdown pathway is more expressly shown by the fact that the 23,24-dihydrocucurbitacins, viz. A_1, B_1, D_1 and E_1, were found to be better substrates (about two times) for *in vitro* breakdown than their precursor cucurbitacins with unreduced side-chains, namely cucurbitacin A, B, D and E (Table 1). The formation of the 23,24-dihydrocucurbitacins from the cucurbitacins is almost irreversible at pH 6·5 (see Ref. 2).

TABLE 1. A COMPARISON OF THE RATE OF CONVERSION OF CUCURBITACINS AND 23,24-DIHYDROCUCURBITACINS TO OTHER COMPOUNDS AND CUCURBITACINS IN THE PROPOSED CATABOLIC PATHWAY OF BITTER PRINCIPLES

Cucurbitacin	Decrease in concentration (% per 6 hr)
A	25±4
A_1	46±6
B	39±4
B_1	67±7
D	28±6
D_1	52±8
E	37±3
E_1	63±7

EXPERIMENTAL

Materials

Golden Hubbard fruits were supplied by Dr. S. Rehm of the Horticultural Research Institute, Roodeplaat, Pretoria. Green Hubbard fruits were obtained from the local market and Mr. R. Starke of Malelane. Cucurbitacins were supplied by Dr. S. Rehm and Dr. P. R. Enslin of the National Chemical Research Laboratories, C.S.I.R., Pretoria.

Methods

In vitro incubations of bitter principles with homogenates of the immature fruits of Golden and Green Hubbard varieties were performed on 0·05 M Maleic acid–NaOH buffer (pH 6·6) according to methods described by Rehm[18] and Teijema.[12]

In order to compare the respective cucurbitacins and 23,24-dihydrocucurbitacins as substrates for *in vitro* breakdown, the rates of disappearance of substrates or appearance of products in the incubation mixtures were determined. The cucurbitacins were extracted quantitatively with chloroform and their presence determined paper chromatographically[12–16]. Their concentrations were estimated paper chromatographically[12–16] but were also determined spectrophotometrically in chloroform or methanol[12] by using their respective absorbancy indexes at their respective u.v. maxima.[19,20]

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[18] S. REHM, *Ber. Deutch. Bot. Ges.* **69**, 26 (1956).
[19] W. C. EISENHUT and C. R. NOLLER, *J. Org. Chem.* **23**, 1948 (1958).
[20] A. MELERA, W. SCHLEGEL and C. R. NOLLER, *J. Org. Chem.* **24**, 211 (1959).