ORGANIC LETTERS

2004
Vol. 6, No. 4
633−636

A New Cucurbitacin D Related 16,23-Epoxy Derivative and Its Isomerization Products

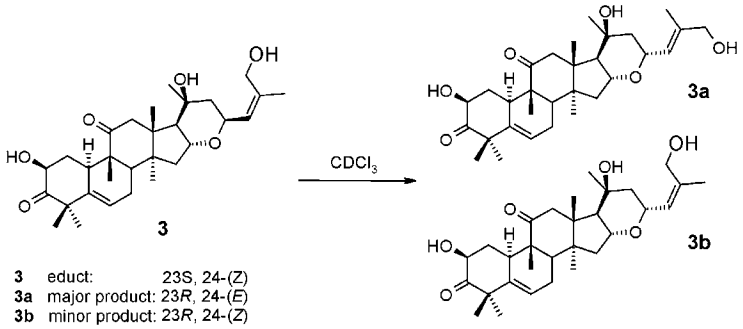
Christoph Seger,*,†,‡ Sonja Sturm,† Ernst Haslinger,‡ and Hermann Stuppner†

Department of Pharmacognosy, Institute of Pharmacy, University of Innsbruck, Innrain 52, A-6020 Innsbruck, Austria, and Institute of Pharmaceutical Chemistry and Pharmaceutical Technology, University of Graz, Universitätsplatz 1, A-8010 Graz, Austria

christoph.seger@uibk.ac.at

Received December 19, 2003

ABSTRACT



A new (23*S*,24*Z*)-16,23-epoxy cucurbitacin derivative was isolated from *Ecballium elaterium* L. (Cucurbitaceae) fruit juice along with known cucurbitacin derivatives. Structure elucidation of these derivatives was accomplished by NMR spectroscopy and mass spectrometry from HPLC-MS data. Isomerization of the epoxy derivative was monitored by 1D and 2D NMR experiments. The configuration of the reaction products was elucidated as (23*R*,24*E*) and (23*R*,24*Z*), and a mechanism for the acid-catalyzed rearrangement process is proposed.

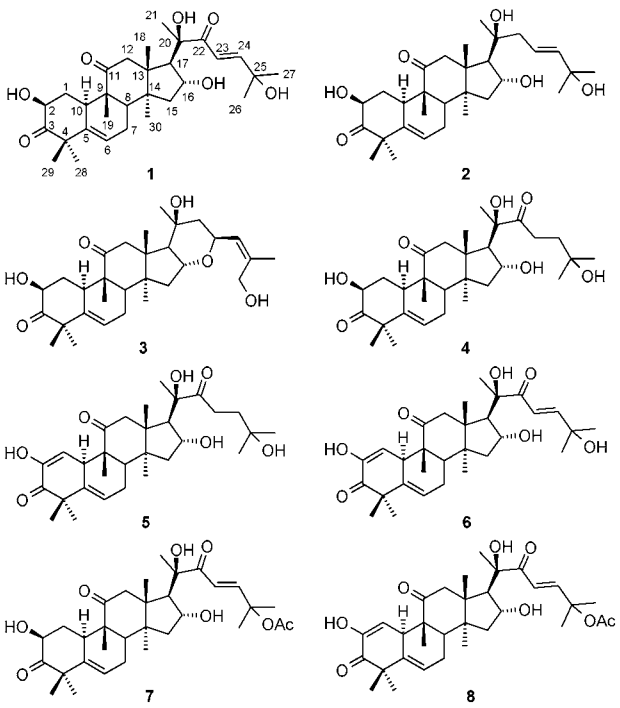
The squirting cucumber *Ecballium elaterium* L. (Cucurbitaceae) is a rich source of highly oxygenated tetracyclic cucurbitacine type triterpenes. This compound class, based on the cucurbitane skeleton (19-(10→9β)abeo-10α-lanost-5-ene) shows a rich variety of side chain derivatives and different ring A substitution patterns. It has been mainly reported from the Cucurbitaceae but is also known from alternative plant families.[1] Cucurbitacins taste bitter and are known for their broad range of bioactivities. Their cytotoxicity, hepatoprotective, antitumor, antiinflammatory, antimicrobial, antihelminthic, and cardiovascular activities have been proven.[2] Furthermore, different derivatives are known as phagostimulants for diabroticite beetles, ecdysteroid antagonists, and active principles against herbivore spidermites.[3] In the case of *E. elaterium* both antihepatotoxic and antiinflammatory properties of fruit juice ("elaterium") have been associated with cucurbitacin B (**7**).[2e,j]

† University of Innsbruck.
‡ University of Graz.

(1) (a) Bauer, R.; Wagner, H. *Dtsch. Apoth. Ztg.* **1983**, *123*, 1313−1321. (b) Guha, J.; Sen, S. P. *Plant Biochem. J.* **1975**, *2*, 12−28. (c) Lavie, D.; Glotter, E. *Prog. Chem. Nat. Prod.* **1971**, *29*, 308−362.

(2) (a) Jayaprakasam, B.; Seeram, N. P.; Nair, M. G. *Cancer Lett.* **2003**, *189*, 11−16. (b) Blaskovich, M. A.; Sun, J.; Cantor, A.; Turkson, J.; Jove, R.; Sebti, S. M. *Cancer Res.* **2003**, *63*, 1270−1279. (c) Peters, R. R.; Krebsky, P. B.; Siqueira-Junior, J. M.; Rocha, J. C. S.; Bezerra, M. M.; Ribeiro, R. A.; de Brum-Fernandes, A. J.; Farias, M. R.; Castro da Rocha, F. A.; Ribeiro-do-Valle, R. M. *Life Sci.* **2003**, *73*, 2187−2197. (d) Oh, H.; Mun, Y. J.; Im, S. J.; Seung, Y.; Ho, J.; Lee, H. S.; Woo, W. H. *Planta Med.* **2002**, *68*, 832−833. (e) Agil, A.; Miro, M.; Jimenez, J.; Aneiros, J.; Caracuel, M. D.; Garcia-Granados, A.; Navarro, M. C. *Planta Med.* **1999**, *65*, 673−675. (f) Peters, R. R.; Saleh, T. F.; Lora, M.; Patry, C.; de Brum-Fernandes, A. J.; Farias, M. R.; Ribeiro-do-Valle, R. M. *Life Sci.* **1999**, *64*, 2429−2437. (g) Lazaris, D.; Chinou, I.; Roussis, V.; Vayias, C.; Roussakis, C. *Pharm. Pharmacol. Lett.* **1998**, *8*, 50−51. (h) Peters, R. R.; Farias, M. R.; Ribeiro-do-Valle, R. M. *Planta Med.* **1997**, *63*, 525−528. (i) Duncan, K. L. K.; Duncan, M. D.; Alley, M. C.; Sausville, E. A. *Biochem. Pharmacol.* **1996**, *52*, 1553−1560. (j) Yesilada, E.; Tanaka, S.; Sezik, E.; Tabata, M. *J. Nat. Prod.* **1988**, *51*, 504−508.

10.1021/ol036469r CCC: $27.50
Published on Web 01/28/2004



Our ongoing efforts in the analytics of secondary natural products led us to the isolation of a series of cucurbitacin derivatives from the CH_2Cl_2 extract of *E. elaterium* fruit juice.[4a] A HLPC system was developed to monitor the extraction procedure.[4b] Raw extracts, enriched fractions, and isolated substances were characterized by HPLC-MS.[4c] Three analytes (compounds **1**, **2**, and **3**) were isolated by combining different chromatographic and liquid−liquid separation techniques whereas the others were identified by comparison with appropriate reference material (retention times, UV-spectra, and HPLC-MS single ion traces) as cucurbitacin R (**4**), cucurbitacin L (**5**), cucurbitacin I (**6**), cucurbitacin B (**7**), and cucurbitacin E (**8**) (Figure 1). The isolated substances were characterized by NMR spectroscopy.[4d]

Compounds **1** and **2** were identified as cucurbitacin D[5] and 22-deoxocucurbitacin-D,[6] respectively, whereas compound **3** proved to be a hitherto unknown derivative. ^1H and ^{13}C NMR spectra of this compound with the molecular formula $C_{30}O_6H_{44}$ showed high similarities with cucurbitacin D related analytes. Especially, ^{13}C NMR signals of the cucurbitane backbone matched well, and the analysis of ROESY cross-peaks allowed assignment of the relative stereochemistry of all chiral positions, which proved to be identical to cucurbitacin D derivatives. Analysis of the remaining signals made the presence of an unusual side chain substitution pattern evident. The signals of the isolated double bond C23−C24 neighbored by the carbonyl function at C-22 were missing. Instead, a prenyl-like double bond structure element in combination with a hydroxymethyl moiety was detectable. Furthermore, an additional ring closure in the side chain was expected from the number of double bond equivalents. The position and size of this ring moiety were derived from the analysis of H−H and H−C coupling networks leading to the identification of a six-membered ring. It connects C-16 in ring D with the side chain C-23 by an epoxy function, thus forming a pyranoid structure element. The mentioned double bond is located at C-24/C-25 and one of the terminal methyl groups is hydroxylated. The (*Z*) configuration of the double bond and the configuration of

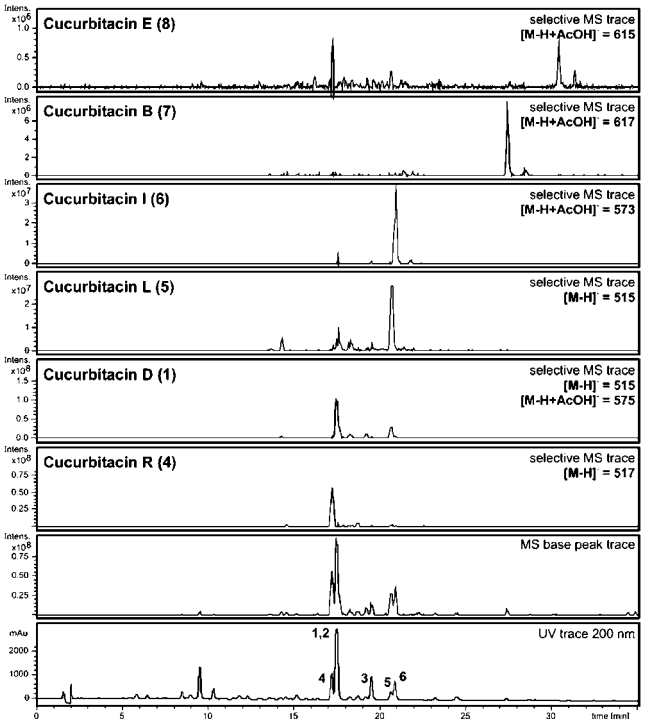


Figure 1. HPLC-UV and HPLC-MS ion trace chromatograms of the *E. elaterium* CH_2Cl_2 extract. Bottom rows: UV trace (200 nm) and MS base peak trace. Above: MS ion traces for masses associated with cucurbitacin R (**4**), cucubrbitacin D (**1**), cucurbitacin L (**5**), cucurbitacin I (**6**), cucurbitacin B (**7**), and cucurbitacin E (**8**).

(3) (a) Balkema-Boomstra, A. G.; Zijlstra, S.; Verstappen, F. W. A.; Inggamer, H.; Mercke, P. E.; Jongsma, M. A.; Bouwmeester, H. J. *J. Chem. Ecol.* **2003**, *29*, 225−235. (b) Dinan, L.; Bourne, P.; Whiting, P.; Dhadialla, T. S.; Hutchinson, T. H. *Environ. Tox. Chem.* **2001**, *20*, 2038−2046. (c) Schroder, R. F. W.; Martin, P. A. W.; Athanas, M. M. *J. Econ. Entomol.* **2001**, *94*, 892−897. (d) Metcalf, R. L.; Metcalf, R. A.; Rhodes, A. M. *PNAS* **1980**, *17*, 3769−3772.

(4) (a) **Isolation**: The CH_2Cl_2 extract was subjected to Sephadex LH-20 chromatography in methanol and subsequent counter-current chromatography (HSCCC; CH_2Cl_2, MeOH, H_2O). Compound **1**, **2**, coeluting in the analytical HPLC, and **4** were separated by HSCCC yielding pure compound **1** (6 mg) and a mixture of compounds **2** and **4** (37 mg, >95% **2**). Compound **3** was further purified by preparative HPLC (3 mg) from another HSCCC fraction. (b) **HPLC**: HP 1100 liquid chromatograph (stationary phase, Zorbax SB-C-18 (150 × 4.6 mm, 3.5 μm); mobile phase, solvent A water, solvent B acetonitril; gradient 0 min 80% A, 22 min 60% A, 40 min 20% A) equipped with a diode array detector (detection at 200, 230, and 267 nm) and operating at room temperature. (c) **HPLC-MS**: coupling of the HPLC system described above to a Bruker Esqire 3000plus (ion source ESI, negative mode; spray voltage 4500 V; nebulizer gas N_2, 40 psi; dry gas N2, 10 L/min, 350 °C). The HPLC setup was equal to the HPLC-DAD method, only solvent A was replaced by 0.15% acetic acid. (d) **NMR**: 1D and 2D NMR spectra were recorded on a Varian Unity Inova 600 in $CDCl_3$ solution at 300 K. All signals were unambiguously assigned by using 2D correlation data (DQF-COSY, HSQC, HMBC, ROESY).

(5) Jacobs, H.; Singh, T.; Reynolds, W. F.; McLean, S. *J. Nat. Prod.* **1990**, *53*, 1600−1605.

(6) (a) Galindo, A.; Villegas, N.; Mansilla, H. *Nat. Prod. Lett.* **1999**, *13*, 285−292. (b) Panosyan, A. G.; Nikishchenko, M. N.; Avetisyan, G. M. *Khim. Prir. Soedin.* **1985**, *5*, 679−687. (c) Enslin, P. R.; Holzapfel, C. W.; Norton, K. B.; Rehm, S. *J. Chem. Soc. C* **1967** *10*, 964−972.

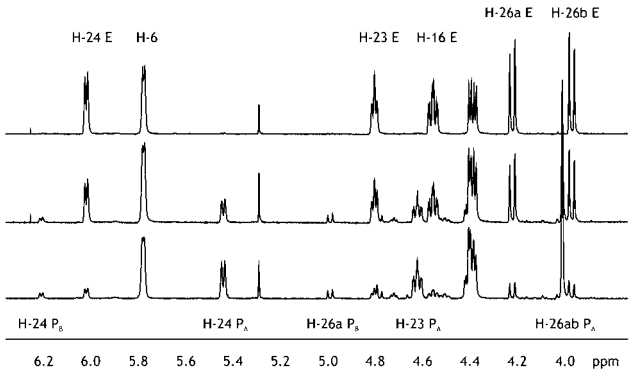


Figure 2. Time series (0, 8, and 21 h) of the low-field region of ^{1}H NMR spectra (600 MHz) showing the increase and decrease of product (index E parent compound **3**) and educt peaks (index P_A main product **3a**, index P_B minor product **3b**), respectively.

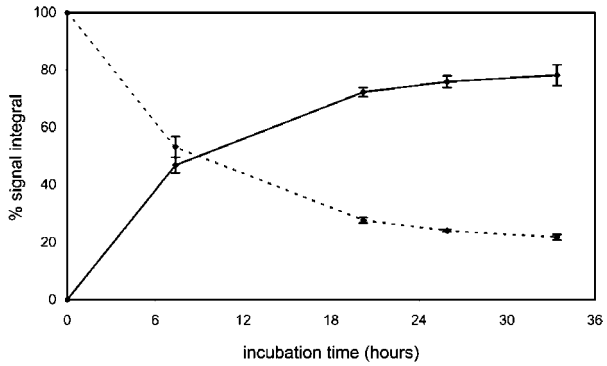


Figure 3. Breakdown curve (full line: sum of products **3a** and **3b**, dashed line educt **3**) derived from the integration of ^{1}H NMR signals H23, H24, and H_2-26.

the chiral carbon centers within the ring system were deduced from NOE contacts.[7] The stereochemistry at positions C-16, C-17, and C-20 remained identical with that of related cucurbitacin derivatives and the proton at the new stereogenic center C-23 was (*S*) configured. Thus, this analyte, a new cucurbitacin derivative, can be assigned as (2*β*,9*β*,10α,16α,-23*S*)-16,23-epoxy-2,20,26-trihydroxy-9-methyl-19-norlanosta-5,24-(*Z*)-diene-3,11-dione. All other derivatives (**1**, **4**−**8**) besides 22-deoxocucurbitacin D (**2**), which is new for *E. elaterium* but has been described from two other sources,[6] already have been found in this taxon and are widespread. The formation of pyranoid side chain ring structures is an uncommon feature within this compound class and only a few derivatives have been reported to date.[6a,b,8] The ^{1}H and ^{13}C NMR shift value assignments of these derivatives are in good agreement with the data sets established for compound **3**. It is noteworthy that all known derivatives lack the C-22 carbonyl function and mainly C-23 isomer pairs have been reported. Moreover, the in vitro synthesis of these cyclic derivatives by acid catalysis has been proven.[6a] A complex mixture of isomerization products including 16,23-epoxy derivatives isomeric at C-23 were formed from derivative **2** upon incubation in chloroform acidified with HCl gas.

It can be argued that the mentioned paired occurrence of the side chain epoxides from biological sources may be the result of the workup of the plant material under weakly acidic conditions in protic solvents. This hypothesis is supported by the isomerization process, which was observed for compound **3**. In NMR solution about 78% were transformed into two products **3a** and **3b** yielding a ternary educt/product mixture in the ratio of 23:66:11 within 33 h. This process was monitored by ^{1}H NMR (Figure 2) and a breakdown curve (Figure 3) was constructed from the integration of well-separated proton signals. With 2D NMR methods the structures of these rearrangement products were elucidated from the reaction mixture. Both were 16,23-epoxides too, but the configuration at C-23 and C-24 had changed. The analysis of the ROESY spectrum did unequivocally prove that both **3a** and **3b** are C-23 epimers of **3**. The major product **3a** has additionally isomerized at C-24 whereas **3b** retained the (*Z*) configuration of the double bond.[9] This assignment is further supported by characteristic changes in the NMR shift values of the side chain double bond substituents C-26 and C-27 and the collapse of the diastereotopic proton signals of H_2-26 in the sterically hindered (24*Z*) derivatives **3** to a singlet in the (24*E*) isomer **3a**.[8a,c,10] Thus **3a** (major product, 85%) and **3b** (minor product, 15%) can be assigned as (23*R*,-24*E*) and (23*R*,24*Z*) derivatives of **3**, respectively. The observed reaction might have been catalyzed by traces of acid in the NMR solution. In conclusion, a reaction mech-

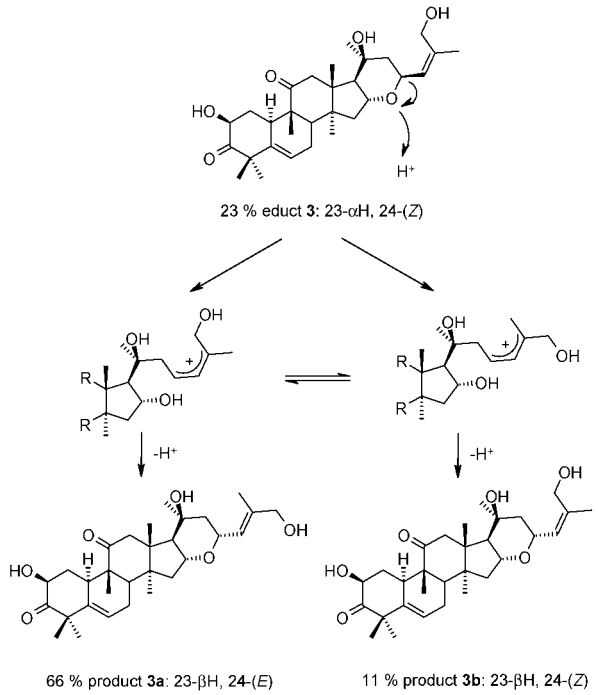


Figure 4. Proposed reaction mechanism for the acid-catalyzed rearrangement of compound **3**.

(7) Cross-peaks H-16 with H-24, H-24 with H_3-27, and H_2-22 with H-23.

Table 1. Selected ^{1}H and ^{13}C NMR Data for Compound **3** and the Isomerization Products **3a** and **3b**[a]

	3		**3a**		**3b**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
no.	δ, mult.	δ, mult, (J)	δ, mult.	δ, mult, (J)	δ, mult.	δ, mult, (J)
16	70.0 d	4.56 dt (3.4, 9.7)	76.2 d	4.41 dt (3.9, 9.7)	69.9 d	4.52 dt (3.4, 9.7)
17	55.1 d	2.00 d (9.2)	55.0 d	1.98 d (9.7)	n.d.	n.d.
18	19.8 q	0.92 s	19.9 q	0.96 s	n.d.	n.d.
20	72.3 s		72.5 s		n.d.	
21	29.2 q	1.34 s	29.2 q	1.34 s	n.d.	n.d.
22	45.7 t	1.89 dd (6.8, 14.1)	48.8 t	1.49 dd (13.6, 8.3)	n.d.	n.d.
		1.56 d (14.1)		1.38 dd (13.6, 2.4)		n.d.
23	70.8 d	4.81 t (6.8)	72.5 d	4.63 dt (2.4, 8.3)	70.2 d	4.73 t (6.8)
24	128.7 d	6.03 d (6.8)	124.7 d	5.45 d (8.3)	134.5 d	6.22 d (6.8)
25	141.0 s		138.6 s		n.d.	
26a	62.5 t	4.23 d (13.2)	67.9 t	4.02 s	70.0 t	5.00 d (12.2)
26b		3.98 d (13.2)				4.79 d (12.2)
27	22.3 q	1.83 s	14.2 s	1.72 s	21.1 s	1.84 s

[a] Shift values δ are expressed in ppm, coupling constants J in Hz. Abbreviation: n.d. = not detected.

anism (Figure 4) can be proposed: upon protonation of the epoxide oxygen the subsequent ring opening reaction leads to the formation of an allylic cation. Within this system, free rotation around the C-24/C-25 bond allows the isomerization at the former double bond position. This results in an approximate 5-fold excess of the sterically less hindered (*E*) configuration in the product mixture.

Assuming identical activation energies for the reformation of the 16,23-epoxide ring structure with the new (23*R*) configuration, this product ratio mirrors the equilibrium at the stage of the allylic intermediates. Although this proton catalyzed reaction cascade should be completely reversible, no traces of the (23*S*,24*E*) isomer were found in the reaction mixture. Hence, the reformation of the epoxide function in the (23*S*) configuration of the educt seems not feasible. Indeed, molecular modeling calculations did prove that the educt configuration is associated with an axial orientation of the bulky side chain. Since the HPLC-MS analysis of the *E. elaterium* raw extracts did not show any traces of **3a** or **3b**, the occurrence of (23*R*) derivatives in this plant matrix can be excluded—thus leaving compound **3** as the sole biogenic stereoisomer. However, the demonstrated instability in acidified protic environments leading not to degradation but isomerization may be of biological significance. The high energy diastereomer **3** could act as precursor and the catalyzed isomerization to **3a** and **3b** may be associated with an altered (enhanced) activity profile, directed against unspecialized herbivores.

Acknowledgment. The authors thank Prof. Dulio Arigoni for valuable discussions regarding the reaction mechanism and the Bionorica AG, Neumarkt, Germany for financial support.

Supporting Information Available: Complete ^{1}H and ^{13}C NMR data sets for **3**, **3a**, and **3b**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL036469R

(8) (a) Kanchanapoom, T.; Kasai, R.; Yamasaki, K. *Phytochemistry* **2002**, *59* 215−228. (b) Sekine, T.; Kurihara, H.; Waku, M.; Ikegami, F.; Ruangrungsi, N. *Chem. Pharm. Bull.* **2002**, *50*, 645−648. (c) Kubo, H.; Ohtani, K.; Kasai, R.; Yamasaki, K.; Nie, R. L.; Tanaka, O. *Phytochemistry* **1996**, *41*, 1169−1174. (d) Schenkel, E. P.; Farias, M. R.; Mayer, R.; Breitmaier, E.; Rucker, G. *Phytochemistry* **1992**, *31*, 1329−1333.

(9) C-23 isomerization: in both derivatives the NOE contact H-16/H-24 of **3** is replaced by NOE contacts between H-16 and H-23. C-24 isomerization: **3a**, NOE contact between H23 and H_3-27; **3b**, NOE contact H-24/H_3-27.

(10) Jakupovic, J.; Boeker, R.; Grenz, M.; Paredes, L.; Bohlmann, F.; El-Din, A. S. *Phytochemistry* **1988**, *27*, 1135−1140.