The Constituents of *Ecballium elaterium* L. Part XXIII.[1] Cucurbitacins and Hexanorcucurbitacins

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In addition to the previously reported cucurbitacins D and I (elatericins A and B) and various phenolics, the ethereal extract of the fruit juice of *Ecballium elaterium* L. has yielded six additional curcubitacins, which have been identified as cucurbitacins B (1b), L (4a), and R (dihydrocucurbitacin D) (2a), and anhydro-22-deoxo-3-*epi*-isocucurbitacin D (5a), hexanorcucurbitacin I (6a), and 16-deoxy-Δ^{16}-hexanorcucurbitacin O (7a). Evidence for the structures of the last three compounds is presented. The diol in ring A of (7a) is assigned the 2β-equatorial, 3β-axial *cis*-configuration.

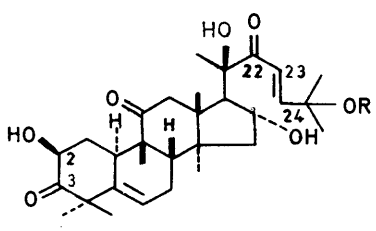
WE have previously reported [1] the isolation of five phenolic compounds from the mother liquors obtained during the crystallisation of cucurbitacins D (1a) and I (3a) (also called elatericins A and B respectively), from the crude ethereal extract of the fruit juice of *Ecballium elaterium* L. Further separation of the mother liquors yielded six other cucurbitacins, characterised as cucurbitacins B (1b),[2] L

[1] Part XXII, M. M. Rao and D. Lavie, *Tetrahedron*, in the press.

[2] W. T. de Kock, P. R. Enslin, K. B. Norton, D. H. R. Barton, B. Sklarz, and A. A. Bothner-By, *J. Chem. Soc.*, 1963, 3828.

(4a),[3] and R † (2a),[4] and anhydro-22-deoxo-3-*epi*-isocucurbitacin D (5a),[5] hexanorcucurbitacin I (6a),[6] and 16-deoxy-Δ^{16}-hexanorcucurbitacin O (7a).

Compounds (1b), (2), and (4) were identified by their spectral properties and confirmed by direct comparison been isolated previously from *Citrullus colocynthis*.[3] Compounds (2a), (5a), (6a), and (7a) were previously unknown in nature. Hexanorcucurbitacin D is the only naturally occurring degraded cucurbitacin reported so far.[8]



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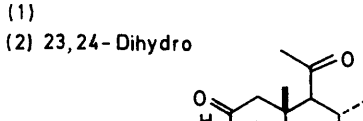
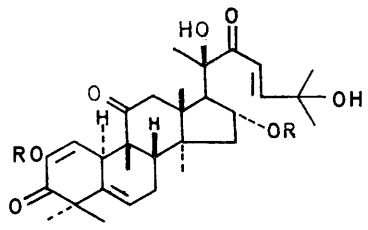


(2) 23,24-Dihydro



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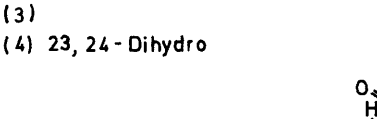
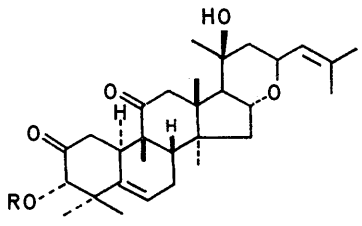


(4) 23, 24-Dihydro



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a; R = H
b; R = Ac

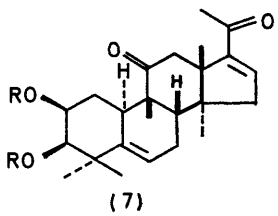

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TABLE 1

N.m.r. signals (δ values; J in Hz) of relevant protons

Compound	6-H	16-H	MeCO	OAc	CMe	Others
Cucurbitacin R (2a)	5·90 (m)	4·37 (m)			0·98, 1·08, 1·23 (2), 1·28, 1·35 (2), 1·43	
Cucurbitacin L (4a)	5·78 (m)	4·33 (m)			1·00, 1·02, 1·23 (3), 1·35—1·41 (3)	1-H 5·93 (d, J 2·5)
Cucurbitacin L diacetate (4b	5·92 (m)	5·81 (m)		1·94, 2·20	1·04 (2), 1·25—1·30 (3), 1·43—1·45 (3)	1-H 6·38 (d, J 2·5)
Anhydro-22-deoxo-3-*epi*-isocucurbitacin D (5a)	5·93 (m)	4·43 (m)			0·77, 0·95, 1·17 (2), 1·28, 1·30, 1·68 (2)	3-H, 3·52 (s) 24-H 5·14 (m)
Anhydro-22-deoxo-3-*epi*-isocucurbitacin D acetate (5b)	5·94 (m)	4·43 (m)		2·17	0·93 (2), 1·17 (2), 1·25, 1·30, 1·70 (2)	3-H 4·92 (s) 24-H, 5·12 (m)
Hexanorcucurbitacin I (6a)	5·80 (m)	4·96 (m)	2·17		0·68, 1·02, 1·25, 1·36, 1·50	1-H 5·90 (d, J 2·5)
Hexanorcucurbitacin I diacetate (6b)	5·87 (m)	5·70 (m)	2·20	2·04, 2·23	0·76, 1·07, 1·34 (2), 1·38	1-H 6·38 (d, J 2·5)
(6a) + TAI *	5·98 (m)	5·83 (m)	2·15		1·05, 1·25, 1·32 (2), 1·43	1-H 6·51; NH 8·38 (s) and 8·79 (s)
16-Deoxy-Δ^{16}-hexanorcucurbitacin O (7a)	5·73 (m)	6·74 (m)	2·29		0·94, 1·00, 1·08, 1·20, 1·23	2-H 3·96 (m); 3-H 3·50 (d, J 2·0); 12-H 3·04 (2H, s)
16-Deoxy-Δ^{16}-hexanorcucurbitacin O diacetate (7b)	5·72 (m)	6·73 (m)	2·29	1·95, 2·07	0·95, 1·07—1·08 (3), 1·20	2-H 5·65 (m) 3-H 5·02 (d, J 2·0) 12-H 3·05 (2H, s)
(7a) + TAI	5·73 (m)	6·70 (m)	2·29		0·96, 1·09, 1·16 (2), 1·27	2-H 5·27 (m); 3-H 5·14 (d) 12-H 3·04 (2H, s) NH 8·29 (s) and 8·36 (s)

* Trichloroacetyl isocyanate.

with authentic samples. We now present evidence leading to the assignment of structures (5), (6), and (7).

Though cucurbitacin B (1b) is found in most species of Cucurbitaceae and almost invariably in association with cucurbitacin D (1a), it is only now that its occurrence in *E. elaterium* L. is demonstrated.[7] Cucurbitacin L (4a) has

Anhydro-22-deoxo-3-epi-*isocucurbitacin D* (5a).—This compound [$C_{30}H_{44}O_5$ (M^+ 484)] has two ketonic carbonyl functions (i.r.) and forms a monoacetate (5b). However, the presence of two hydroxy-groups in (5a) was revealed by the n.m.r. spectrum measured after the addition of trichloroacetyl isocyanate (TAI).[9] The fifth oxygen atom

† We have previously described [4] this compound as dihydrocucurbitacin D, but since this is the first report of it in nature, we have designated it with a separate letter in this series.

[3] D. Lavie, D. Willner, and Z. Merenlender, *Phytochemistry*, 1964, **3**, 51.

[4] D. Lavie, Y. Shvo, O. R. Gottlieb, and E. Glotter, *J. Org. Chem.*, 1963, **28**, 1970.

[5] P. R. Enslin, C. W. Holzappel, K. B. Norton, and S. Rehm, *J. Chem. Soc.* (*C*), 1967, 964.

[6] D. Lavie and Y. Shvo, *J. Amer. Chem. Soc.*, 1960, **82**, 966.

[7] D. Lavie and E. Glotter, *Fortschr. Chem. org. Naturstoffe*, 1971, **29**, 307.

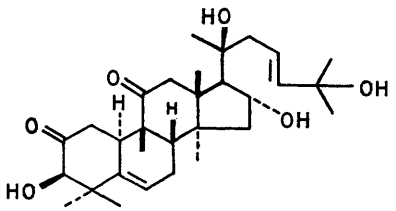
[8] R. W. Doskotch and C. D. Hufford, *Canad. J. Chem.*, 1970, **48**, 1787.

[9] I. R. Trehan, C. Monder, and A. K. Bose, *Tetrahedron Letters*, 1968, 67.

is therefore inert and hence may be involved in an ether linkage.

The n.m.r. spectrum of (5a) (Table 1) showed six tertiary and two vinylic methyl groups and two olefinic proton signals. Double resonance measurements indicated that the two olefinic protons are not mutually coupled and that one of them, a multiplet centred at δ 5·14, is coupled to the vinylic methyl groups, thus leading to a tetracarbocyclic five-ring terpenoid skeleton, with a side chain terminating in an isopropylidene unit. A cucurbitacin skeleton for this compound is assumed from its occurrence, as well as from the appearance and location of the second olefinic proton signal which is similar to the 6-H signal of the cucurbitacins. The presence of an α-ketol system in (5a) was detected by a positive triphenyltetrazolium chloride test, while the 1H singlet in the n.m.r. spectrum at δ 3·52 (shifted to δ 4·92 upon acetylation) indicated a 2-oxo-3-hydroxy-ring A. The absence of any other acetylatable hydroxy-group led to the conclusion that the ubiquitous 16-OH group of the cucurbitacins was involved in a cyclic ether linkage accounting for the fifth ring.[7]

Assuming the presence of an 11-oxo-function and a tertiary hydroxy-group (revealed by TAI) at C-20, this compound could then be formulated as anhydro-22-deoxoisocucurbitacin D, a compound earlier obtained by treating 22-deoxoisocucurbitacin D (8) with acid; subsequent treatment with methanolic potassium hydroxide



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afforded the 3-epimer which shows a significant difference in its n.m.r. spectrum.[5] The physical constants and spectroscopic properties of our compound were similar with those of the 3-*epi*-compound and their identity was finally confirmed by a direct comparison of the i.r. and n.m.r. spectra of (5b) with those of an authentic sample.

Hexanorcucurbitacin I (6a).—This compound [$C_{24}H_{32}O_5$ (M^+ 400)] is a diosphenol, as indicated by a positive iron(III) chloride colour test, and confirmed by u.v. and i.r. spectra. Its n.m.r. spectrum (Table 1) showed the presence of five *C*-methyl groups, one methyl ketone, one C*H*OH, and two vinylic protons in positions corresponding to 1-H and 6-H of the diosphenol-containing cucurbitacin I (3a). The presence of two hydroxy-groups, revealed by the addition of TAI, was confirmed by the formation of a diacetate (6b). In both (6a) and (6b) 1-H appears as a characteristic doublet (J 2·5 Hz) indicating the presence of a proton at C-10, and hence a cucurbitane skeleton. While the five *C*-methyl groups can be accommodated on such a skeleton, the methyl ketone represents the degraded side chain. The structure was finally confirmed by direct comparison with an authentic sample of the diacetate prepared by periodate oxidation of (3b).[6] The fragmentation of (6a) under electron impact is in good agreement with the proposed structure. While the base peak is at m/e 43 ($CH_3\overset{+}{C}O$), the second most intense one is at m/e 164 corresponding to $[C_{10}H_{12}O_2]^{+\cdot}$ formed by retro-Diels–Alder cleavage of ring B.[10]

16-*Deoxy*-Δ^{16}-*hexanorcucurbitacin O* (7a).—This compound ($C_{24}H_{34}O_4$) has an unsaturated carbonyl chromophore ($\lambda_{max.}$ 240 nm and $\nu_{max.}$ 1669 cm^{-1}) and a saturated six-membered ring ketone ($\nu_{max.}$ 1690 cm^{-1}). The n.m.r. spectrum (Table 1) measured after the addition of TAI revealed the presence of two hydroxy-groups, confirmed by the formation of a diacetate (7b), thus accounting for all four oxygen atoms. The n.m.r. spectra of both (7a) and (7b) also showed the presence of five tertiary methyl groups, one methyl ketone, and two olefinic protons. While one of the olefinic proton signals is similar to that of 6-H of cucurbitacins, the other (δ 6·74) is assigned to the β-proton of the conjugated chromophore. The molecular formula and the various functional groups discussed above, led to the formulation of the compound as a hexanorcucurbitacin, the methyl ketone being the side chain and forming part of the chromophore.

The location and stereochemistry of the two hydroxy-groups was determined by double resonance measurements. The two protons adjacent to the OH groups appeared at δ 3·96 (m) and 3·50 (d, J 2 Hz). By reciprocal irradiation the former signal is converted into a double doublet (J 4·5 and 10 Hz), whereas the latter became a singlet. Hence, the two protons are vicinal and could therefore be assigned to C-2 and C-3 respectively, the C-2 proton exhibiting axial–axial and axial–equatorial coupling with the C-1 methylene protons. The small coupling constant between 2-H and 3-H is therefore an axial–equatorial relationship. Assuming a chair conformation for ring A, the two hydroxy-groups in (7a) are assigned the 2β-*eq*,3β-*ax cis*-configuration.

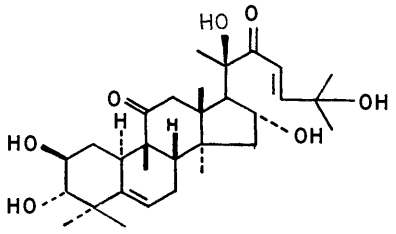
It is interesting that the n.m.r. spectra of ring A ketols in (2a)[4] and the corresponding anhydro-22-deoxo-derivatives[11] both indicated an axial 2-H. However, the equatorial 2-OH group could be either β-oriented when ring A is in a chair conformation or α-oriented when twisted; on the basis of c.d. measurements, the latter conformation has been proposed[11] for these two compounds. It was, nevertheless, noted that the unusual 9β,10α-stereochemistry as well as the combination of the 5,6-double bond and 11-carbonyl group would complicate the interpretation of their Cotton effects. Subsequently, an *X*-ray analysis of the bis-*p*-iodobenzoate of daticoside[12] [(a 16-glycoside of (1a)] revealed that the 2-OH group is β-equatorial (such a stereochemistry had been postulated previously[4,13]). In view of this *X*-ray

[10] H. E. Audier and B. C. Das, *Tetrahedron Letters*, 1966, 2205.
[11] G. Snatzke, P. R. Enslin, G. W. Holzappel, and K. B. Norton, *J. Chem. Soc.* (*C*), 1967, 972.
[12] S. M. Kupchan, C. W. Sigel, J. L. Guttman, R. J. Restivo, and R. F. Bryan, *J. Amer. Chem. Soc.*, 1972, **94**, 1353.
[13] D. Lavie and B. S. Benjaminov, *J. Org. Chem.*, 1965, **30**, 607.

analysis, the 2,3-*cis*-diol system in cucurbitacins O, P, and Q, originally assigned an α-orientation,[14] was revised to the β-configuration.[12]

We conclude therefore that ring A in the hexanorcucurbitacin (7) exists in a chair conformation, which is indeed the preferred conformation, especially in the absence of a trigonal carbon at position 3. This compound represents a degraded cucurbitacin O, P, or Q. It may be added that in view of the presence of a 16-OH group in all the naturally occurring cucurbitacins, the 16,17-double bond in (7) may have been formed during work-up.

The only other natural 2,3-diol in this series is cucurbitacin F (9)[15] which has been assigned a 2α,3β-diequatorial configuration. Since this was based on the



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earlier assumption that the 2-OH group in (1a) was α-oriented, we suggest that this may also be revised to the 2β,3α-diequatorial orientation, and further that the 2-OH group in all known cucurbitacins is β-oriented.

EXPERIMENTAL

M.p.s were taken on a Fisher-Johns apparatus. U.v. spectra were recorded on a Cary 14 instrument (EtOH as solvent). I.r. spectra were taken on a Perkin-Elmer model 237B spectrophotometer and refer to KBr pellets. N.m.r. spectra were recorded on a Varian A-60 spectrometer and refer to 5—10% solutions in $CDCl_3$, using Me_4Si as internal standard. Decoupling experiments were carried out on a Bruker HFX-10 90 MHz spectrometer by Mr. M. Greenberg. Mass spectra were measured on an Atlas CH4 instrument under the direction of Dr. Z. V. I. Zaretskii. Optical rotations were recorded with an automatic Perkin-Elmer 141 polarimeter in $CHCl_3$. The c.d. spectrum was recorded by Mrs. B. Romano with a Cary 60 spectropolarimeter. Analyses were performed in the microanalytical laboratory of the Institute under the direction of Mr. R. Heller. Silica gel 60 (E. Merck) (70—230 mesh) was used for column chromatography, and t.l.c. was carried out on chromatoplates of silica gel F. For preparative layer chromatography (p.l.c.) a 1·0 mm layer of silica gel PF_{254} was used. Acetylations were carried out with acetic anhydride–pyridine at room temperature for 20 h and were worked up by removal of the reagents under reduced pressure and in a hot water-bath.

Isolation Procedure.—Freshly collected fruit juice of *Ecballium elaterium* L. Cucurbitaceae was continuously extracted with ether. From the extract elatericins A and B were separated as previously described,[2] and the resinous residue (125 g) from evaporation of the mother liquor under reduced pressure was dissolved in ethyl acetate (250 ml), mixed with silica gel (200 g), and evaporated to dryness. The dry mass was placed on the top of a dry column of silica gel (2·2 kg) and eluted with n-hexane followed by mixtures with increasing quantities of ether. Fractions (*ca.* 250 ml), were collected and monitored by t.l.c. The fractions from which the various cucurbitacins were obtained are listed in Table 2.

TABLE 2

Chromatographic isolation of cucurbitacins from fruit juice of *Ecballium elaterium* L.

Eluant		Fraction no.	Compound (g)
Ether–hexane	3 : 2	368—379	Anhydro-22-deoxo-3-*epi*-isocucurbitacin D (5a) (0·6)
	3 : 2	380—400	Cucurbitacin E (elaterin) (0·1)
	4 : 1	486—498	Cucurbitacin B (1b) (0·25)
	4 : 1	499—520	Hexanocucurbitacin I (6a) (0·18)
	4 : 1	521—551	Cucurbitacin L (4a) (6)
	4 : 1	573—585	16-Deoxy-Δ^{16}-hexanorcucurbitacin O (7a) (1·5)
	4 : 1	590—620	Cucurbitacin R (dihydrocucurbitacin D) (2a) (1·7)

Anhydro-22-deoxo-3α-isocucurbitacin D (5a).—After evaporation of the solvent from fractions 368—379, the residue was extracted with chloroform to separate the lignan ligballinol,[1] and the extract upon concentration was diluted with ether, when *anhydro-22-deoxo*-3-epi-*isocucurbitacin D* (16α,23-*epoxy*-3α,20-*dihydroxy*-19(10 → 9β)-abeo-10α-*lanosta*-5,24-*diene*-2,11-*dione*) (5a) separated as prisms, m.p. 265—267°, $[\alpha]_D$ +146° (*c* 0·2), $\nu_{max.}$ 3460, 2960, 1711, 1690, 1270, 1102, and 1089 cm^{-1}; *m/e* 484 (M^+) (38·5%), 466 (17·3), 451 (76·9), 319 (9·6), 219 (7·7), 166 (15·4), 133 (16·6), 109 (40·4), 83 (28·8), and 43 (100) (Found: C, 74·1; H, 9·2%; M^+, 484. $C_{30}H_{44}O_5$ requires C, 74·35; H, 9·15%; *M*, 484·65); c.d. (*c* 0·5, dioxan) λ 340 (Δε 0), 320 (+2·68), 307 (+5·44), 298·5 (+5·71), 289 (+4·56), 255 (+0·38), and 246 (+0·17) {lit.,[5,11] m.p. 291—292°, $[\alpha]_D$ +154°; c.d. λ 340 (Δε 0), 320 (+2·34), 307 (+4·97), 298·5 (+5·31), 289 (+4·39), and 255 (+0·42)], acetate (5b), m.p. 240—242°.

Cucurbitacin E.—From fractions 380—400 a crystalline compound was obtained from methanol, m.p. and mixed m.p. with an authentic sample of cucurbitacin E 232—233° (this compound was previously obtained from the sediment of the fruit juice).

Cucurbitacin B (1b).—The residue from fractions 486—498 was purified by p.l.c. (5% MeOH–$CHCl_3$) to give cucurbitacin B (1b), m.p. and mixed m.p. with an authentic sample 182—184°; *m/e* (M^+ 558 not visible) 498 (M^+ −60) (14·3%), 403 (19·6), and 96 (100); *diacetate m/e* (M^+ 640 not visible) 580 (M^+ −60) (3·7%), 164 (15·8), 96 (70), and 43 (100); n.m.r. spectrum in accord with structure (1b).

Hexanorcucurbitacin I (6a).—This compound (2,16α-dihydroxy-19(10 → 9β)-*abeo*-22,23,24,25,26,27-hexanor-10α-lanosta-1,5-diene-3,11,20-trione) was obtained pure after removal of the accompanying cucurbitacin B by p.l.c. (5% MeOH–$CHCl_3$), lower R_F, but could not, however, be crystallized, $\lambda_{max.}$ 270infl and 230 nm; *m/e* 400 (M^+) (26·3%), 385 (6·4), 204 (7·6), 203 (6·1), 189 (11·7), 164 (70·2), 136 (15·2), 122 (19·9), 121 (14·6), 105 (9·9), 93 (9·4), 91 (14·0), 77 (9·4),

[14] S. M. Kupchan, R. M. Smith, Y. Aynehchi, and M. Maruyama, *J. Org. Chem.*, 1970, **35**, 2891.

[15] K. J. van der Merke, P. R. Enslin, and K. Pachler, *J. Chem. Soc.*, 1963, 4275.

and 43 (100); *diacetate* (6b), m.p. 180—182° (chloroform–n-hexane); $\lambda_{max.}$ 230 nm (ε 9700) (Found: C, 69·3; H, 7·65. $C_{28}H_{36}O_7$ requires C, 69·4; H, 7·5%).

Cucurbitacin L (4a).—This was obtained as crystals, m.p. and mixed m.p. with an authentic sample 174—176° (ether–n-hexane), $[\alpha]_D$ −47·6° (*c* 1·3), $\lambda_{max.}$ 270 nm; *m/e* (M^+ 516 not visible) 498 (M^+ −18) (14%), 480 (13), 401 (13·6), 164 (100), 142 (26), and 113 (28).

16-*Deoxy*-Δ^{16}-*hexanorcucurbitacin O* (7a).—The residue from fractions 573—585 (2·5 g) was rechromatographed on Florisin (75 g) and elution with ether–ethyl acetate (2 : 1) afforded cucurbitacin L (0·5 g) followed by 16-*deoxy*-Δ^{16}-*hexanorcucurbitacin O* (2β,3β-*dihydroxy*-22,23,24,25,26,27-*hexanor*-19(10 ⟶ 9β)-abeo-10α-*lanosta*-5,16-*diene*-11,20-*dione*) (7a) (1·5 g); crystallised from benzene as plates, m.p. 230—233°, $[\alpha]_D$ +161·2° (*c* 0·3); $\lambda_{max.}$ 240 nm; $\nu_{max.}$ 3400, 1690, 1660, 1592, 1380, 1040, and 980 cm^{-1}; *m/e* 386 (M^+) (3·5%), 368 (42), 353 (28), 219 (6·1), 191 (10·4), 177 (31·3), 136 (17·8), 119 (9·3), 105 (13·9), 91 (17·0), 79 (8·9), 77 (8·3), 67 (5·9), 55 (11·1), and 43 (100) (Found: C, 74·4; H, 8·75%; M^+, 386. $C_{24}H_{34}O_4$ requires C, 74·55; H, 8·85%; *M*, 386·51); diacetate (7b), m.p. 125—127°.

Cucurbitacin R (2a).—The residue from fractions 590—620 (4·5 g) was rechromatographed on Florisil (125 g). Elution with ether–ethyl acetate (2 : 1) gave (7a) (50 mg), and with ether–ethyl acetate (1 : 1) cucurbitacin R (2β,16α,20,25-tetrahydroxy-19(10 ⟶ 9β)-*abeo*-10α-lanost-5-ene-3,11,22-trione) (2a) (1·5 g), m.p. and mixed m.p. with an authentic sample (prepared by hydrogenation of cucurbitacin D) 140—143°; *m/e* 518 (M^+) (0·5%), 482 (6·4), 403 (35·7) 385 (19), 369 (11·6), 237 (6·6), 219 (6·8), 187 (6·3), 177 (7·6), 166 (9·0), 149 (11·4), 142 (23·8), 113 (54·8), 97 (14·2), 87 (34·5), 69 (24·1), 59 (9·8), 55 (18·5), and 43 (100).

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