# The Characterization of Two Novel Bufadienolides, Lanceotoxins A and B from *Kalanchoe lanceolata* [Forssk.] Pers.

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The characterization of two novel bufadienolides, lanceotoxins A and B, isolated from *Kalanchoe lanceolata* is reported. The assignment of the structures is based on a detailed study of their high-field $^1H$ and $^{13}C$ n.m.r. spectral data and by correlation with known hellebrigenin derivatives.

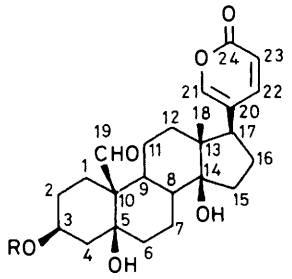
Poisoning of stock by plants of the Crassulaceae, Iridiceae, Liliaceae, and Melianthaceae, which frequently contain bufadienolides as their toxic principles, is of considerable economic importance in Southern Africa.[1] Members of the genera *Cotyledon*, *Tylecodon*, and *Kalanchoe* (Crassulaceae) cause stock losses through 'krimpsiekte' or cotyledonosis, a neurological syndrome which differs from typical cardiac glycoside intoxication.[1,2] These succulent plants grow in the more arid regions of the area and are usually eaten in times of drought or during shortage of feed. We now report the structural elucidation of the toxic principles of *Kalanchoe lanceolata* (Forssk.) Pers.

The isolation of three crystalline toxins from *Kalanchoe lanceolata* by extraction of the fresh plants with ethyl acetate, followed by solvent partition and column chromatography on silica gel, has been reported by Anderson *et al.*[3] All three compounds were identified as bufadienolides by the positive Liebermann colour reactions and their u.v. absorptions at $\lambda_{max.}$ 299 nm (ε 6 100). One compound was identified as 3-*O*-acetylhellebrigenin (**1**) by comparison of its physical and spectroscopic properties with those of an authentic sample.[4]

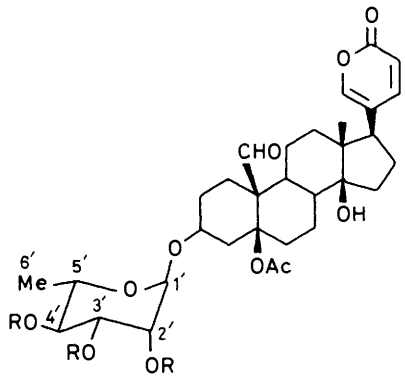
The second toxin, called lanceotoxin B (**3**), gave a green anthrone colour reaction,[5] indicative of a glycoside. Elemental analysis and field desorption (FD) mass spectrometry ($M^+$, 604) established the molecular formula of lanceotoxin B as $C_{32}H_{44}O_{11}$. The i.r. spectrum showed strong carbonyl absorption at 1 710 $cm^{-1}$. The structure of this compound, *viz.* 5-*O*-acetyl-3-*O*-α-L-rhamnosylhellebrigenin (**3**), was deduced mainly from a detailed study of its $^1H$ and $^{13}C$ n.m.r. spectra.

Several structural features were evident from the 500 MHz $^1H$ n.m.r. spectrum of (**3**). A one-proton singlet at $\delta_H$ 10.12 was assigned to an aldehydic proton. Clearly defined signals at δ 7.92 (dd, *J* 9.8 and 2.6 Hz, 22-H), 7.39 (dd, *J* 2.5 and 0.8 Hz, 21-H), and 6.16 (dd, *J* 9.8 and 0.8 Hz, 23-H) are characteristic of the protons constituting the δ-pyrone ring.[6] The nature and stereochemistry of the glycosyl moiety, *viz.* α-rhamnosyl, was defined by the proton resonances at δ 4.68 (d, *J* 1.4 Hz, 1′-H), 3.68 (ddd, *J* 4.3, 2.0, and 1.6 Hz, 2′-H), 3.76 (d, *J* 4.3 Hz, 2′-OH), 2.74 (m, 3′-H), 3.37 (ddd, *J* 9.3, 9.3, and 4.7 Hz, 4′-H), 3.85 (d, *J* 4.8 Hz, 4′-OH), 3.61 (dq, *J* 9.3 and 6.2 Hz, 5′-H), and 1.19 (d, *J* 6.3 Hz, 6′-$H_3$). The linkage of the rhamnosyl moiety to C-3 of the aglycone is evident from the chemical shift of 3-H (δ 3.95, m). A three-proton singlet (δ 1.86) was assigned to the 5-*O*-acetyl group.

The assignment of the resonances in the $^{13}C$ n.m.r. spectrum of (**3**) (Table) is based on the results obtained from proton-noise-decoupled (p.n.d.) and single-frequency off-resonance proton-decoupled experiments, as well as the reported $^{13}C$ n.m.r. chemical shifts for 3-*O*-β-D-boivinosylstrophantidin[7] and methyl α-L-rhamnoside.[8] The residual splittings observed in a series of off-resonance proton-decoupled $^{13}C$ n.m.r. experiments enabled us to correlate the signals of the proton-bearing carbon atoms with specific proton resonances.[9] The effect of acetylation of the 5-hydroxy-group is evident from the downfield shift of the C-5 resonance (Δδ 10.2 p.p.m.) and upfield shifts of the C-4 (Δδ −2.0 p.p.m.) and C-6 (Δδ −7.2 p.p.m.) resonances.




(1) R = Ac
(2) R = H




(3) R = H
(4) R = Ac

The presence of three secondary hydroxy-groups in (**3**) was confirmed by the formation of the triacetate, $C_{38}H_{50}O_{14}$ (**4**) ($M^+$, 730) upon acetylation. The sterically hindered tertiary hydroxy-group at C-14 remained as the only unesterified hydroxy-function in (**4**).

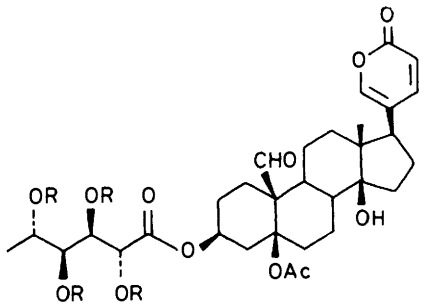
Lanceotoxin B represents the first characterization of a 5-*O*-acetylhellebrigenin glycoside, although the isolation of 3,5-di-*O*-acetylhellebrigenin[10] was reported earlier.

Elementary analysis and fast atom bombardment (FAB) mass spectrometry ($M^+$, 620) established the molecular formula of the third toxic metabolite, lanceotoxin A, as $C_{32}H_{44}O_{12}$. The assignment of its structure as 5-*O*-acetyl-3-*O*-(2,3,4,5-tetra-

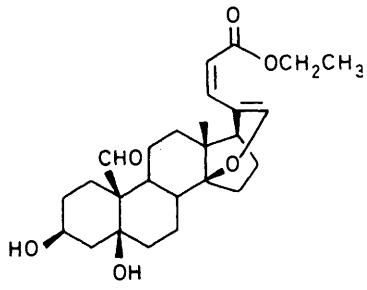
**Table.** $^{13}C$ N.m.r. ($\delta_C$; 20 MHz) data for lanceotoxin A (**5**), tetra-*O*-acetyl-lanceotoxin A (**6**), lanceotoxin B (**3**), and tri-*O*-acetyl-lanceotoxin B (**4**)

| Carbon atom | (5)† | (6)‡ | (3)† | (4)‡ |
|---|---|---|---|---|
| 1 | 22.9 | 23.4 | 23.5 | 24.1 |
| 2 | 23.6 | 24.1 | 24.5 | 25.0 |
| 3 | 68.2 | 68.1 | 71.2 | 71.0 |
| 4 | 31.7[a] | 31.7[a] | 31.8[a] | 32.2[a] |
| 5 | 83.1 | 83.5[b] | 83.6[b] | 83.6[b] |
| 6 | 28.9[a] | 29.4[a] | 28.8[a] | 28.5[a] |
| 7 | 21.5[b] | 22.0[c] | 21.5[c] | 22.5[c] |
| 8 | 40.7[a] | 41.7[d] | 40.6[d] | 41.7[d] |
| 9 | 38.0[c] | 38.7[d] | 38.0[d] | 38.7[d] |
| 10 | 53.2 | 53.6 | 53.2 | 53.6 |
| 11 | 17.8[b] | 18.2[c] | 17.9[c] | 18.7[c] |
| 12 | 39.5 | 40.1 | 39.6 | 40.4 |
| 13 | 47.6 | 48.1 | 47.6 | 48.2 |
| 14 | 83.1 | 84.3[b] | 83.0[b] | 84.7[b] |
| 15 | 31.0[a] | 32.0[a] | 31.0[a] | 31.7[a] |
| 16 | 28.9[a] | 28.4[a] | 28.1[a] | 28.5[a] |
| 17 | 49.8 | 50.8 | 50.2 | 50.0 |
| 18 | 16.7 | 16.1 | 16.0 | 16.3 |
| 19 | 205.6 | 205.5 | 205.8 | 206.1 |
| 20 | 121.7 | 122.5 | 122.2 | 122.3 |
| 21 | 148.2 | 148.8 | 148.1 | 148.7 |
| 22 | 146.8 | 146.9 | 146.7 | 146.5 |
| 23 | 114.2 | 115.2 | 114.2 | 115.4 |
| 24 | 161.5 | 162.3 | 161.5 | 162.3 |
| 1′ | 172.6 | 166.7 | 99.6 | 96.7 |
| 2′ | 72.2 | 69.7[e] | 71.2[e] | 69.6[e] |
| 3′ | 73.1 | 71.2[e] | 70.4[e] | 69.6[e] |
| 4′ | 70.4 | 69.3[e] | 72.3 | 69.3[e] |
| 5′ | 67.4 | 66.4 | 68.0 | 66.7 |
| 6′ | 19.6 | 22.0 | 17.1 | 17.4 |
| $OCOCH_3$ | 169.7 | 170.0, 169.7, 169.7, 169.6, 169.3 | 169.5 | 170.7, 169.9, 169.9, 169.9 |
| $OCOCH_3$ | 23.1 | 20.3, 20.9, 20.5, 22.3 | 22.0 | 20.8, 20.6, 20.6, 20.6 |

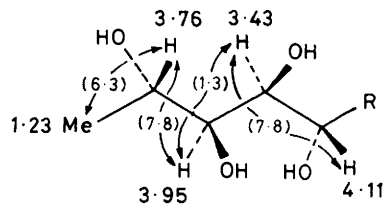
† In $CDCl_3$–[$^2H_6$]DMSO. ‡ In $CDCl_3$. [a–e] Assignments in each column be interchanged.




(5) R = H
(6) R = Ac




(7)




(8)

hydroxyhexanoyl)hellebrigenin (**5**) was based on the analysis of its spectral data and chemical degradation.

A comparison of the $^1H$ and $^{13}C$ n.m.r. data of (**3**) and (**5**) indicated that the two compounds contain the same aglycone. The $^{13}C$ n.m.r. spectrum of (**5**) showed the presence of an ester carbonyl carbon atom ($\delta_C$ 172.6) in addition to the acetate ($\delta_C$ 169.7), the δ-pyrone ($\delta_C$ 161.5) carbonyl carbon atom, and the aldehyde carbonyl carbon atom which resonates characteristically at low field ($\delta_C$ 205.6).

The proton chemical shifts and proton–proton coupling constants of the protons of the side-chain were obtained by first-order analyses of the relevant multiplets in the $^1H$ n.m.r. spectrum. From the values of the coupling constants as corroborated by $^1H$-{$^1H$}homonuclear decoupling experiments and the chemical shifts, a highly oxygenated fragment (**8**) could be constituted. The indicated stereochemistry is, furthermore, based on the proviso that the chain adopts a planar, zig-zag conformation. Previous studies[11] have shown that acyclic polyols adopt an extended, planar, zig-zag conformation if there are no oxygen atoms with parallel 1,3-interactions. The above mentioned structural requirements for lanceotoxin A are satisfied by structure (**5**) in which the 3-hydroxy-group of 5-*O*-acetylhellebrigenin is esterified with 2,3,4,5-tetrahydroxyhexanoic acid. The stereochemistry of the tetrahydroxyhexanoic acid derivative is probably identical to that of rhamnoic acid; this presumption is supported by the co-occurrence of (**3**) and (**5**) in *K. lanceolata*.

The presence of four hydroxy-groups in (**5**) was confirmed by the formation of its tetra-acetate (**6**) ($M^+$, 788) upon acetylation.

Unambiguous proof of the nature of the aglycone and the ester moiety in (**5**) was obtained by alkaline hydrolysis. Treatment of (**5**) with potassium hydroxide in ethanol at 60 °C gave ethyl isohellebrigenate (**7**), identical with the product obtained upon treatment of hellebrigenin (**2**) under the same conditions.

Although the mechanism of bioformation of the hexanoic acid from L-rhamnose by *K. lanceolata* is not evident, it seems likely that oxidation took place prior to the linkage to the 3-hydroxy-group. Acyclic sugar derivatives are rare in nature and this report represents the first example of a bufadienolide glyconate, whereas bufadienolide glycosides are a common occurrence.

Anderson *et al.*[3] recently described the toxicity of lanceotoxins A and B. Typical signs of cardiac glycoside poisoning, involving the gastro-intestinal, neuromuscular, and cardiovascular systems could be induced in sheep by the administration of relatively large doses of lanceotoxin B (**3**). The specific paretic syndrome cotyledonosis, which is characterized by spasmodic contractions of the muscles, occurred only after repeated intravenous administration of smaller doses of lanceotoxin A (**5**) and lanceotoxin B (**3**) to sheep.

## Experimental

M.p.s were determined on a Kofler hot-stage apparatus. U.v. absorptions were measured for solutions in methanol on a Unicam SP-800 spectrophotometer, i.r. spectra on a Perkin-Elmer 237 spectrophotometer, and mass spectra on a Varian MAT spectrometer. $^1H$ and $^{13}C$ N.m.r. spectra were recorded on a Bruker WM-500 (500 MHz) and a Varian CFT-20 (20 MHz) spectrometer, respectively. Merck silica gel (0.063—0.200 mm) was used for column chromatography.

*Isolation of the Metabolites.*—Fresh *Kalanchoe lanceolata* Forssk plants (120 kg) were minced and extracted in a Waring blender with ethyl acetate (3 ×). The solvent was evaporated under reduced pressure and the resultant syrup was partitioned between 95% methanol (3.5 l) and light petroleum (b.p. 40—60 °C). Both extracts were evaporated to dryness and the residues tested for toxicity. Only the residue obtained from the methanol extract (88 g) was toxic.

Repeated chromatography of the toxic residue on silica gel, using chloroform–acetone (7:3 v/v), chloroform–acetone–methanol (60:40:1.5, v/v/v), and benzene–methanol 78:22, v/v) yielded three colourless, crystalline compounds.

3-*O*-Acetylhellebrigenin (**1**) (458 mg) crystallized from chloroform–diethyl ether as white needles, m.p. 236—238 °C (lit.,[4] 230—232 °C); $\nu_{max.}$(KBr) 2 860, 2 745, 1 720—1 700br, 1 630, 1 535, 1 240, and 830 $cm^{-1}$; $\lambda_{max.}$ 299 nm (ε 6 000); $\delta_H(CDCl_3)$ 7.85 (dd, *J* 10 and 2.5 Hz, 22-H), 7.28 (dd, *J* 2.5 and 1.0 Hz, 21-H), and 6.28 (dd, 9.5 and 1.0 Hz, 23-H); $M^+$, 485. It gave a blue Liebermann colour reaction.

*Lanceotoxin A* (**5**) (570 mg) had m.p. 206—208 °C (from chloroform–acetone–methanol) (Found: C, 62.2; H, 7.3. $C_{32}H_{44}O_{12}$ requires C, 61.92; H, 7.14%); $[\alpha]_D$ +0.53° (*c* 0.17 in MeOH); $\nu_{max.}$ 3 440, 3 305, 1 714, 1 720, 1 700, 1 630, 1 540 1 240, and 830 $cm^{-1}$; $\lambda_{max.}$ 299 nm (ε 4 800); $\delta_H([^2H_4]MeOH)$ 10.09 (s, 19-H), 7.96 (dd, *J* 9.7 and 2.5 Hz, 22-H), 7.41 (dd, *J* 1.4 and 0.8 Hz, 21-H), 6.26 (dd, *J* 9.7 and 0.8 Hz, 23-H), 5.20 (br s, 3-H), 4.11 (d, *J* 7.8 Hz, 2′-H), 3.95 (dd, *J* 7.8 and 1.3 Hz, 4′-H), 3.76 (qd, *J* 6.3 and 7.8 Hz, 5′-H), 3.43 (dd, *J* 7.8 and 1.3 Hz, 3′-H), 2.00 (s, OAc), 1.23 (d, *J* 6.3 Hz, 6′-$H_3$), and 0.66 (s, 18-$H_3$); *m/z* 620, ($M^+$, FAB) and 458 (100). It gave a blue Liebermann colour reaction and a wine-red anthrone colour reaction.

*Lanceotoxin B* (**3**) (1.28 g) was obtained as white crystals (from chloroform–methanol), m.p. 190—195 °C (Found: C, 62.65; H, 7.4. $C_{32}H_{44}O_{11}$ requires C, 63.56; H, 7.33%); $[\alpha]_D$ −3.56° [*c* 0.27 in MeOH–$H_2O$ (1:1)]; $\nu_{max.}$ 3 430, 1 740, 1 715, 1 705, 1 635, 1 540, 1 225, and 830 $cm^{-1}$; $\lambda_{max.}$ 299 nm (ε 6 200); $\delta_H([^2H_6]acetone)$ 10.12 (s, 19-H), 7.92 (dd, *J* 9.8 and 2.6 Hz, 22-H), 7.39 (dd, *J* 2.5 and 0.8 Hz, 21-H), 6.16 (dd, *J* 9.8 and 0.8 Hz, 23-H), 4.68 (d, *J* 1.4 Hz, 1′-H), 3.95 (br s, 3-H), 3.85 (d, *J* 4.8 Hz, 4′-OH), 3.77 (d, *J* 4.2 Hz, OH), 3.68 (ddd, *J* 4·3, 2·0, and 1·6 Hz, 2′-H), 3.61 (qd, *J* 6.2 and 9.3 Hz, 5′-H), 3.37 (ddd, *J* 9.3, 9·3, and 4·7 Hz, 4′-H), 2·74 (m, 3′-H) 1.86 (s, OAc), 1.19 (d, *J* 6.3 Hz, 6′-$H_3$), and 0.68 (s, 18-$H_3$); $M^+$, 604 (FD). It gave a blue Liebermann colour reaction and a green anthrone colour reaction.

*Tetra*-O-*acetyl-lanceotoxin A* (**6**).—Treatment of lanceotoxin A (**5**) (100 mg) with acetic anhydride and pyridine at room temperature (12 h) yielded tetra-*O*-acetyl-lanceotoxin A (**6**) as an amorphous solid (90 mg), $\delta_H(CDCl_3)$ 10.06 (s, 19-H), 7.73 (dd, *J* 9.8 and 2.6 Hz, 22-H), 7.20 (d, *J* 2.6 Hz, 21-H), 6.24 (d, *J* 9.8 Hz, 23-H), 5.45 (dd, *J* 9.3 and 2.2 Hz, 3′-H), 5.27 (dd, *J* 8.8 and 2.2 Hz, 4′-H), 5.20 (br s, 3-H), 4.97 (dq, *J* 8.8 and 6.4 Hz, 5′-H), 4.75 (d, *J* 9.4 Hz, 2′-H), 2.11, 2.07, 2.06, 2.06, and 2.05 (5 s, 5 OAc), 1.17 (d, *J* 6.4 Hz, 6′-$H_3$), and 0.65 (s, 18-$H_3$); $M^+$, 788 (FD).

*Tri*-O-*acetyl-lanceotoxin B* (**3**).—Acetylation of lanceotoxin B (**5**) (50 mg) with acetic anhydride–pyridine yielded tri-*O*-acetyl-lanceotoxin B (**4**) (52 mg), m.p. 226—228 °C (from methanol); $[\alpha]_D$ −3.44° (*c* 0.22 in MeOH); $\delta_H(CDCl_3)$ 10.00 (s, 19-H), 7.74 (dd, *J* 9.8 and 2.6 Hz, 22-H), 7.19 (dd, *J* 2.5 and 0.9 Hz, 21-H), 6.22 (dd, *J* 9.8 and 1.0 Hz, 23-H), 5.15 (dd, *J* 10.0 and 3.5 Hz, 3′-H), 5.10 (dd, *J* 3.4 and 1.7 Hz, 2′-H), 5.01 (dd, *J* 10.0 and 9.8 Hz, 4′-H), 4.72 (d, *J* 1.6 Hz, 1′-H), 3.99 (br s, 3-H), 3.82 (dq, *J* 9.7 and 6.3 Hz, 5′-H), 2.10, 2.01, 1.98, and 1.92 (4 s, 4 OAc), 1.17 (d, *J* 6.3 Hz, 6′-$H_3$), and 0.63 (s, 18-$H_3$); $M^+$, 730 (FD).

*Ethyl Isohellebrigenate* (**7**).—Lanceotoxin A (**5**) (60 mg) was treated with a solution of potassium hydroxide (30 mg) in ethanol (2.5 ml) at 60 °C. After 1 h, water was added and the solution acidified with 3M hydrochloric acid. Extraction with $CHCl_3$ and column chromatography (chloroform–acetone) of the residue yielded ethyl isohellebrigenate (**7**) (40 mg), $\delta_H(CDCl_3)$ 10.18 (s, 19-H), 7.20 (d, *J* 9 Hz, 22-H), 6.76 (s, 21-H), 5.86 (d, *J* 9 Hz, 23-H), 4.64 (br s, 3-H), 4.40 (q, *J* 7.1 Hz, $OCH_2CH_3$), 1.78 (t, *J* 7.1 Hz, $OCH_2CH_3$), and 1.47 (s, 18-$H_3$); $\delta_C$ 201.0 (C-19), 167.8 (C-24), 149.4 (C-21), 142.9 (C-22), 120.6 (C-20), 110.1 (C-23), 90.9 (C-14), 74.5 (C-5), 66.8 (C-3), 59.6 ($OCH_2CH_3$), 54.5 (C-10), 43.2, 40.4, 38.8, 38.0, 37.2, 36.3, 31.8, 31.5, 30.7, 27.2, 23.4, 20.9, and 17.6 (skeletal C), 15.2 (C-18), and 14.3 p.p.m. ($OCH_2CH_3$); $M^+$, 444. This product was identical with that obtained from hellebrigenin (**2**) upon treatment with alkaline ethanol.

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