NOTIZEN

New Bufadienolides from *Kalanchoe daigremontiana Hamet et Perr. (Crassulaceae)*

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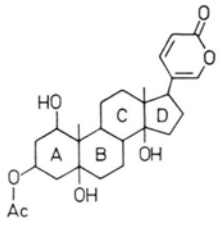
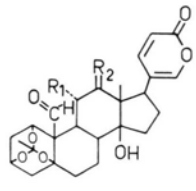
Kalanchoe daigremontiana, Bufadienolides, X-Ray, Daigremontianin, Daigredorigenin, Bersaldegenin Derivatives

Five biosynthetically related bufadienolides were isolated from Kalanchoe daigremontiana Hamet et Perr. and structurally elucidated by X-Ray and NMR techniques. Two compounds were orthoacetates which showed strong CNS activity.

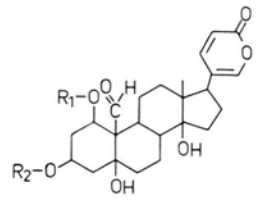
Some *Crassulaceae* belonging to the genera Cotyledon or Kalanchoe are known as toxic and cause Cotyledonosis ("Krimpsiekte") in live-stock [1]. The problem is of economic importance in Southern Africa. Using an animal test system for fractionation, we succeeded in isolating five toxic bufadienolides from the upper parts and roots of *Kalanchoe daigremontiana* [2]. Combined preparative column chromatography (solvent system: ethylacetate/toluene/acetone = 10/3/2), TLC (same system) and HPLC on a RP-18 column with 30% acetonitril, yielded pure **1–5** in amounts of 5–80 mg.



1



2: R_1 = OH, R_2 = O
3: R_1 = H, R_2 = H/H



4: R_1 = H, R_2 = Ac
5: R_1 = Ac, R_2 = H

The structure of **1** ($C_{26}O_7H_{36}$, m.p. = 280–290 °C) was determined by X-ray analysis of a crystal obtained from methanol/acetone = 1/3. Crystal data: Space group orthorhombic $P2_12_12_1$; a = 11.254, b = 14.199, c = 14.361 Å; d_m = 1.33 g cm^{-3}; Z = 4. The intensities of 1632 reflexions (observed $1482 \geq 4\sigma(I)$) were measured (Ni-filtered CuK_α-radiation, Ω-scan) with a Nicolet R 3M diffractometer. Calculations succeeded with direct methods by means of SHELXTL. The refinement converged at an R-factor of 7.9% [3]. **1** is the 3-O-acetate of the novel bufadienolide *Daigredorigenin,* with a hitherto not described 1β-3β-5β-14β-tetrahydroxy substitution pattern. The rings A, B, C and D are connected *cis*/*trans*/*cis;* the lactone ring at C17 is β-oriented.

The structure of **2,** the second novel bufadienolide, *Daigremontianin* ($C_{26}O_9H_{30}$, m.p. = 270–280 °C), was also determined by X-ray analysis, with a crystal obtained from ethanol/heptane = 1/1.5. Crystal data: Space group monoclinic $P2_1$; a = 6.691, b = 18.456, c = 9.257 Å; β = 96.11°, d_m = 1.421 g cm^{-3}; Z = 2. The intensities of 2127 reflexions (observed $2085 \geq 2\sigma(I)$) were measured (MoK_α-radiation, graphite monochromator, scan $\theta/2\theta$) with an Enraf Nonius CAD-4 diffractometer. Calculations in $P2_1$ failed due to a superimposition of the true structure with its mirror image, but succeeded in the most common space group P1 with a doubled set of data by means of SHELXTL. The refinement converged at an R-factor of 7.26% [3]. Daigremontianin has a 11α-hydroxy, 12-oxo substitution pattern in ring C and represents the rare case of a naturally occurring orthoacetate. The main compound **3** ($C_{26}O_7H_{32}$, m.p. = 290–300 °C), which could also be detected in Kalan-

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choe tubiflora Hamet, was determined as *Bersaldegenin-1,3,5-orthoacetate* by 2D-NMR spectroscopy. **3** was, like **4,** first isolated from Bersama abyssinica Fresen. (Melianthaceae) [4]. Apart from a strong positive inotropic activity, which is comparable to g-Strophanthin, both orthoacetates **2** and **3** are strongly sedative after doses of 0.1–0.5 mg/kg and affect the CNS at higher doses, which induce paralysis and muscle contractions in mice. The same symptoms are described for "Krimpsiekte" [5]. The isomeric compounds **4** ($C_{26}O_8H_{34}$, m.p. = 255–260 °C) and **5** ($C_{26}O_8H_{34}$, m.p. = 235–245 °C) were identified by ^{1}H NMR spectroscopy as *Bersaldegenin-3-O-acetate* and *Bersaldegenin-1-O-acetate*. The latter compound has not been described as a naturally occurring bufadienolide. All isolated compounds **1–5** possess the basic structure of a 5β-14β-androstane and obviously follow a clear biosynthetical sequence: **1** – oxidation → **4** (**5**) – cyclisation/-H_2O → **3** – oxidation → **2.** The occurrence of bufadienolides in a Kalanchoe species is of chemotaxonomic interest. After completion of this work, bufadienolides with other substitution patterns were reported to occur in Kalanchoe lanceolata [Forssk.] Pers. [6].

[1] H. Friedrich and E. Krüger, Dtsch. Apoth. Ztg. **108,** 1273 (1968).
[2] Most plants were grown in our own greenhouse; only plants that were collected in Teneriffa (Canary Islands) contained **1.**
[3] The atomic co-ordinates are deposited at the Cambridge Crystallographic Data Centre.
[4] S. Morris Kupchan and J. Ognyanov, Tetrahedron Lett. **21,** 1709 (1969).
[5] J. Watt and M. Breyer-Brandwijk, Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd Edition, E. And S. Livingstone Ltd., Edinburgh and London 1962, p. 311.
[6] L. A. P. Anderson, P. Steyn, and F. van Heerden, J. Chem. Soc., Perkin Trans. I, 1573 (1984).