Elaeodendroside A: a Novel Cytotoxic Cardiac Glycoside from *Elaeodendron glaucum*[1]

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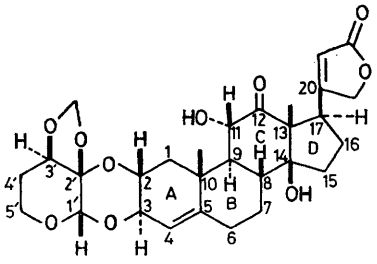
Summary The isolation and structural elucidation of elaeodendroside A (**1**), a novel cytotoxic cardiac glycoside containing a doubly linked sugar substituent, from *Elaeodendron glaucum* Pers. are reported.

IN the course of a continuing search for tumour inhibitors from plant sources, we found that a 95% ethanol extract of seeds of *Elaeodendron glaucum* Pers. (Celastraceae)‡ showed significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB).[2] A chloroform–water partition of the alcohol extract followed by an aqueous methanol (1 : 9)–Skellysolve B partition of the chloroform extract and column chromatography [Silica Gel 60 (E. Merck); methanol–chloroform] of the aqueous methanol extract gave several KB active fractions. The least polar active fraction was rechromatographed (three Silica Gel 60 columns; MeOH–Et_2O, MeOH–$CHCl_3$ and EtOAc–C_6H_6, respectively), and crystallization of one of the resulting KB-active fractions gave elaeodendroside A (**1**, 0·006%): m.p. 299—300 °C (methanol–acetone), $[\alpha]_D^{24}$ + 96° (*c* 0·18, $CHCl_3$). Elemental analysis and high resolution mass spectroscopy indicated a molecular formula of $C_{29}H_{36}O_{10}$, and i.r. [(KBr) 1750, 1720, and 1635 cm^{-1}] and u.v. [λ_{max} (EtOH) 218 nm (log ϵ 4·27)] spectra suggested the presence of a butenolide ring. The ^1H n.m.r. spectrum ($CDCl_3$, Me_4Si) displayed signals at δ 2·50 (1H, dd, J = 14 and 3·2 Hz, 1β-H), 3·60—4·30 (5H, m, 2β-H, 3′ α-H, 5′-CH_2, 17α-H),§ 4·38 (1H, br d, J = 8 Hz, 3α-H), 4·45 (1H, d, J = 12 Hz, 11β-H), 4·67 (1H, s, 1′β-H), 5·16 (2H, ABq, –O–CH_2–O–), and 5·30 (1H, br s, 4-H). The above data and the marked resistance toward acid hydrolysis suggested that (**1**) is a cardiac glycoside containing an unusual sugar linkage. Although similar cardiac glycosides have been reported previously,[3,4] ambiguities have remained concerning the stereochemistry. This is the first complete stereochemical determination of a cardiac glycoside containing the unusual doubly-linked (at C-2 and C-3) sugar unit.



(I)

The chemical structure and molecular stereochemistry of (**1**) were determined by a direct single-crystal *X*-ray analysis. Crystals of (**1**) conform to space group $P2_12_12$, with a = 12·816(4), b = 22·772(4), c = 9·391(2) Å, and Z = 4. Intensity measurements were made using a four-circle diffractometer and scintillation counting. Scattered intensity significantly above background [$I > 3\sigma(I)$] was measured at 1627 of the 2335 independent reciprocal lattice points within a single octant to $2\theta \leqslant 120°$.

The structure was solved by use of the program MULTAN[5] and refined using the block-diagonal least-squares method. Anisotropic thermal parameters were adopted for O and C atoms. All hydrogen atoms, with the exception of that associated with the C(14) hydroxy group, were readily identifiable from difference electron-density maps, and contributions for those atoms in fixed locations and with

† Deceased 19th October, 1976.

‡ Seeds were collected in India in March, 1975. We thank Dr. Robert E. Perdue, Jr. for supplying the plant material in accordance with the programme developed by the National Cancer Institute.

§ The 17α hydrogen is strongly deshielded presumably owing to its co-planarity with and close proximity to the C-12 carbonyl function. *Cf.* L. M. Jackman and S. Sternell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' 2nd edn., Pergamon, New York, 1969, p. 91.

isotropic B values were included in the least-squares calculations. At convergence, the final residual was 0·064. No determination of absolute configuration was made.¶

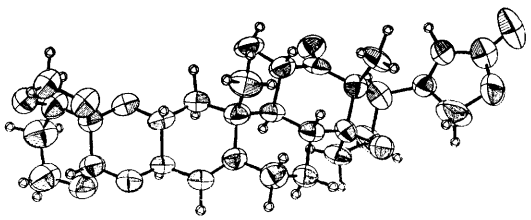


FIGURE. ORTEP drawing of the molecular structure crystal of elaeodendroside A. Thermal ellipsoids for the non-hydrogen atoms are drawn with the 50% probability level as the boundary surface and hydrogen atoms are represented by spheres of arbitrary radius.

A view of the solid-state molecular structure of (**1**) is shown in the Figure. Within the steroidal unit, the cyclohexene ring A adopts a C(2)β sofa conformation. The A/B ring junction is *quasi-trans* and ring B adopts a chair conformation as does ring C with the B/C junction *trans*. The C/D ring junction has the 13β,14β-*cis* configuration noted in the aglycone digitoxigenin[6] and in batrachotoxinin A[7]. Ring D has a distorted half-chair conformation (Δ_{av} 10°, ϕ_{max} 43°)[8] whereas the butenolide ring is planar in a conformation where the ring plane bisects the C(17) endocyclic valence angle. In the glycosidic unit, the six-membered dioxa ring is joined to the steroid nucleus by 2α and 3β bonds to oxygen, and the six-membered oxa and dioxa rings, both with chair conformations, are *cis*-fused. The dioxalan ring adopts a conformation intermediate between the envelope and half-chair forms (Δ_{av} −19°, ϕ_{max} 39°)[8] and is *cis*-fused to the oxa ring.

We thank the National Cancer Institute for financial support.

(*Received*, 17*th January* 1977; *Com.* 035.)

¶ The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

[1] For previous paper in the series Tumor Inhibitors, see S. M. Kupchan, E. J. LaVoie, A. R. Branfman, B. Y. Fei, W. M. Bright, and R. F. Bryan, *J. Amer. Chem. Soc.*, submitted for publication.

[2] Cytotoxicity was assayed under the auspices of the National Cancer Institute, by the procedure described by R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part* 3, 1972, **3**, 1. The cytotoxicity (ED_{50}) against KB cell cultures of (**1**) is *ca.* 10^{-1} μg ml^{-1}.

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