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Venusol from *Gunnera perpensa*: structural and activity studies

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Abstract

From the aqueous extract of the dry rhizomes of *Gunnera perpensa* the minor components pyrogallol, succinic acid, lactic acid, and the trimethyl ether of ellagic acid glucoside were isolated. The major constituent was identified as *Z*-venusol, a phenylpropanoid glucoside. Its structure was verified by X-ray diffraction. Tests on isolated uterine smooth muscle from rats showed that the whole extract stimulated a direct contractile response and induced a state of continuous contractility of the uterus once all additives had been removed from the organ bath. By contrast, venusol did not trigger the direct contractile response but induced the state of continuous contractility once the organ bath was flushed.

Keywords: *Z*-venusol; X-ray structure; Lespedezic acid; Uterine contraction; Expulsion of placenta; Spontaneous contractility

1. Introduction

Gunnera perpensa (Gunneraceae), known as river pumpkin, or ''uGhobo'' in isiZulu, is a perennial shrub which grows along stream banks in many parts of South Africa. It is best known in the province of Kwa-Zulu Natal where it has been used traditionally as a ''muthi'' (medicinal) plant by the indigenous Zulu people for a long time. One of the first references to it in the Western literature is by the missionary Father J. Gerstner (1939).

Traditionally the finely ground roots of the plant are boiled in water and administered orally to an animal (cow) after calving in order to expel the retained placenta. The extract operates by inducing vigorous uterine contractions. Human uses of the aqueous decoction are relief of pain in rheumatic fever, to ensure an easy childbirth, and to treat infertility in women (Hutchings, 1996). It is of interest to note that Dr. Tod Collins (1996) a western trained veterinarian from the Underberg district of KwaZulu-Natal, speaks highly of his practical experiences with ''uGhobo'' and endorses the use of the plant by stock farmers.

Some preliminary screening has been done on South African traditional remedies used during labour (Kaido et al. 1997). Masika and Afolayan (2002) have tested other indigenous plants as ethnoveterinary medicines. Despite its widespread use the chemical composition of *Gunnera perpensa* is not known.

2. Results and discussion

2.1. Isolation of components

Gunnera perpensa roots were extracted with boiling water (as is done traditionally) and the dark red solution partitioned against ethyl acetate containing approximately 10% ethanol. Subsequent separation (flash column chromatography with methanol and chloroform) afforded small quantities of succinic acid, lactic acid, pyrogallol and the glucoside of ellagic acid trimethyl ether (**1**). It is known that ellagic acid is a potent antagonist, in particular on histamine liberators (Bhargava and Westfall, 1969). The activity of venusol

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may well be modulated by the presence of the other compounds.

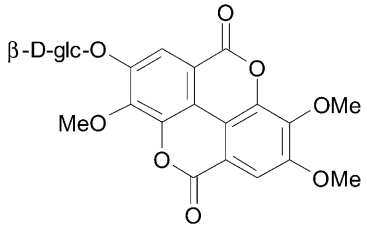
The ethyl acetate extract of aq. *Gunnera* solution yielded buff needles of the compound venusol (0.18% yield based on dry plant material). Recrystallisation afforded orthorhombic needles, suitable for X-ray analysis. Information from this, coupled with the usual ^{1}H- and ^{13}C-NMR and high resolution mass spectral data, enabled us to establish the structure unambiguously as *Z*-venusol, (7,8-dihydroxy-6-(hydroxymethyl)-3-[(*Z*)-(4-hydroxyphenyl)methylidene]tetrahydro-4aH-pyrano[2,3-b][1,4]dioxin-2-one (IUPAC nomenclature). The structure is shown in **2**, but the usual numbering (Proliac et al. 1981) has been retained.

Z-Venusol has been isolated previously by Proliac et al. (1981) and Pagani (1990). Both the *Z* and *E* isomers were obtained recently by Viornery et al. (2000). In all these instances the plant source was *Umbilicus pendulinus* or *Umbilicus ruperstris* (Crassulaceae).

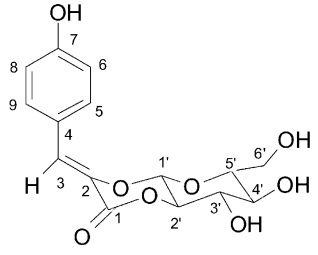
From molecular modeling experiments and NOE data Viornery et al. (2000) was able to distinguish between the *Z*- and *E*-isomers of venusol. This designation of stereochemistry also confirmed the earlier work of Shigemori et al (1990) on lespedezic acid methyl ester (**3**). The chemical relationship between venusol (**2**) and methyl lespedezate (**3**) is a close one. In the aq. *Gunnera* extract we found traces of (**3**) accompanying the major component venusol (**2**). This is likely to be an artefact resulting from the action of methanol (used in the extraction process) on the venusol. Shigemori et al. (1990) were able to show that potassium lespedezate and its geometrical isomer, potassium isolespedezate operate as an "internal clock" in the nyctinastic plant *Cassia mimosoides* and are involved in the leaf-opening and leaf-closing mechanisms.

Recently Ohnuki et al. (1998) have considered the leaf-opening and leaf-closing mechanisms at the molecular level as a result of their findings in nyctinastic plants. These authors were able to isolate both isomers of potassium lespedezate from *Lespedeza cuneata* (leaf-openers). In addition they also isolated the leaf-closing substance potassium D-idarate (**6**). Their detailed studies indicate that leaf movement of nyctinastic plants is controlled by the competitive interaction between leaf-opening [K-lespedezate, (*E* or *Z* isomer)] substances and leaf-closing [K-D-idarate (**6**)] substances. In the evening the enzyme β-glucosidase simply converts K-lespedezate to its deactivated form, potassium 4-hydroxyphenylpyruvate (**7**), i.e. the leaf-opening mechanism is "switched off" by enzymic deactivation of lespedezic acid.

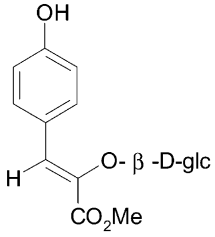
We believe that the above studies are relevant to our work on *Gunnera* as they illustrate the point that all "players" in the activation of the uterine muscles need



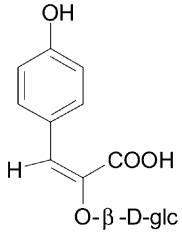
(1)



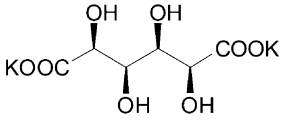
(Z)-Venusol (2)



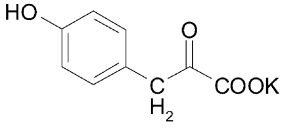
(Z)-Methyl lespedezate (3)
Me = H (Z)-Lespedezic acid (4)



(E)-Lespedezic Acid (5)



(6)



(7)

to be present for the system to function. In the nyctinastic plants all the interacting compounds are present in the plant sap, just as they appear to be present in the crude *Gunnera* extracts. At present our investigations have only yielded one of the compounds involved and this may explain the type of activity observed (see Section 2.3).

While venusol is not a new compound, it has now been isolated from a new plant source (family *Gunneraceae*). From our X-ray data of the compound the Z-stereochemistry around the C-2/C-3 double bond is established unambiguously. The final mechanism remains unclear, but there is good evidence that Z-venusol brings about contraction of uterine muscle (see below).

2.2. Biological activity

The effect of aq. *Gunnera* extract, ethyl acetate extract, ethyl acetate–methanol extract, and pure *Z*-venusol, were tested on uterine and ileal muscle obtained from Sprague–Dawley rats. The procedure has been described previously (Matonhodze et al., 2002).

The aq. *Gunnera* extract directly stimulates a contractile response for both the ileum and uterus muscle. Once the plant extract and the added acetylcholine are rinsed from the organ bath, the tissue enters into a state of continuous spontaneous contractility. Pre-incubation with atropine reduced the contractile response significantly. We speculate from the above that the muscarinic receptor system is involved in the direct contractile response to the whole plant extract.

Neither the organic extracts nor the pure Z-venusol (**2**) elicited a direct contractile response. However, the venusol did induce a state of spontaneous contractility once the test substance was flushed from the organ bath. It is possible that the venusol exerts its action in conjunction with substances present in the whole extract. This aspect of the work will be explored in a forthcoming publication.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Varian 500 MHz NMR spectrometer. High resolution mass spectra were measured on a Kratos MS 80 RF double-focussing magnetic sector instrument at 70 eV. The X-ray structure was obtained with an Oxford Xcalibur 2 CCD diffractometer, (M_oK_α) radiation.

3.2. Plant material

Gunnera perpensa roots were collected in May 2002 on a farm in the Underberg district of Kwa-Zulu Natal and allowed to dry out in a shady place. A voucher specimen (leaves and root) was deposited in the Bews Herbarium, University of Natal, Pietermaritzburg (lodged under S.E. Drewes, N.U. 8). Verification of identity was done by Dr. Trevor Edwards, curator of the herbarium.

3.3. Extraction and isolation

The dry root material (910 g) was milled finely and extracted with boiling water (7 l) for 3 h. Preliminary tests had shown that even polar organic solvents, such as ethanol, dissolved almost none of the material. After filtration and autoclaving the red-brown solution was partitioned against ethyl acetate. This was a slow process since an emulsion formed readily which necessitated long periods of waiting between shaking. Emulsion-formation was largely inhibited by resorting to ethyl acetate–methanol (5:1) mixtures and this also resulted in more material being extracted. Concentration of the ethyl acetate–methanol extract to about 2 l, followed by drying (anhyd $MgSO_4$) and subsequent further concentration afforded a brown oil which crystallized on standing (1.67 g, representing 0.18% yield

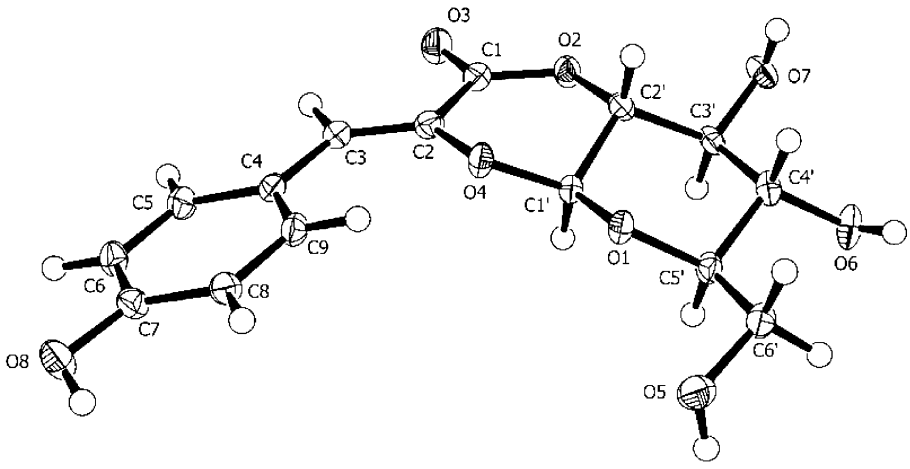


Fig. 1. X-ray structure of (Z) venusol (**2**).

based on dry plant material). The crude crystalline material subsequently proved to be *Z*-venusol which was difficult to get absolutely pure. Final purification required separation by flash chromatography and elution with methanol–chloroform (3:17). The white needles had mp 259 °C (Proliac et al., 1981, quote 259–260 °C) and proved to be suitable for X-ray analysis.

Examination of a tlc plate of the mother liquor of the *Z*-venusol [run in MeOH–$CHCl_3$ (3:17)] and visualized with vanillin–H_2SO_4 dip reagent revealed the presence of at least another seven compounds. Several of these were subsequently identified and proved to be very minor components of known structure. These compounds were: R_F 0.90, 4-*O*-β-D-glucopyranosyl-3,3′,4′-tri-O-methyl-ellagic acid (**1**); R_F 0.81, Blue fluorescent compound (phytosterol); R_F 0.76, Blue fluorescent compound; R_F 0.60, No fluorescence, purple on heating with dip reagent—lactic acid, 15 mg; R_F 0.54, Faint fluorescence, red with dip reagent—pyrogallol, characterized as the triacetate, 37 mg; R_F 0.41, Venusol—strongly fluorescent, buff coloured with dip reagent, 1.67 g; R_F 0.36, Weak fluorescence, green blue with dip reagent—(*Z*) methyl lespedezate, 10 mg; R_F 0.16, Fluorescent—pale purple with dip reagent.

Succinic acid mp 182 °C, in very low concentration was isolated from the mother liquor of venusol. It is not readily detected by tlc.

The ellagic acid derivative, 4-*O*-β-D-glucopyranosyl-3,3′,4′-tri-*O*-methylellagic acid (**1**) was present in low concentration but was readily identified by ^1H and ^{13}C NMR (Khac et al., 1990; Li et al. 1999) as well as by high-resolution mass spectrometry of its aglycone. H-R EI-MS *m/z* 344.05434 M^+ calc. for $C_{17}H_{12}O_8 = 344.05322$.

The methyl *Z*-lespedezate (**3**) was readily identified by comparison with the spectral data provided by Viornery et al. (2000). The NMR features which distiguish it from *Z*-venusol are the sharp methoxy peak at δ_H 3.81 and at δ_C 52.6 (deuteriomethanol). In addition, the anomeric cabon resonates at δ_C 102.8 whereas in venusol it is at δ_C 96.2.

3.4. *Z*-venusol

Fine white needles, mp 259 °C, $[\alpha]_D^{20} = 84.5$ (MeOH, 0.245). ^1H and ^{13}C spectra run in D_2O and CD_3OD were compared with those recorded by Proliac et al. (1981) in DMSO and by Viornery et al. (2000) in C_2D_6CO, and leave no doubt about the identity of the compound. Its stereochemistry about the C-2/C-3 double bond was unambiguously proved to be *Z* by X-ray diffraction (see below) (Fig. 1).

Acetylation of *Z*-venusol afforded an oil, $[\alpha]_D^{20} = 25$ (MeOH, 0.028). ^1H NMR (500 MHz, $CDCl_3$): δ 2.05, 2.09, 2.12 (9H, *s*, 3′, 4′, 6′-OAc), 2.30 (3H, *s*, 7-OAc), 3.95 (1H, *ddd*, *J* 10.1, 4.4, 2.2, H-5′), 4.21 (1H, dd, *J* = 12.7, 2.2, H-6′a), 4.32 (1H, dd, J 12.8, 4.4, H-6′b), 4.32 (1H, dd, *J* 9.9, 7.9, H-2′), 5.09 (1H, *d*, *J* = 7.9, H-1′), 5.13 (1H, *dd*, *J* 10.1, 9.2, H-4′), 5.39 (1H, *dd*, *J* 9.9, 9.2, H-3′), 6.98 (1H, s, H-3), 7.12 (2H, *d*, *J* = 8.7, H-5/9), 7.77 (2H, d, *J* = 8.7, H-6/8); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.5, 20.6, 20.7 (C-3′, 4′, 6′-OAc), 21.1 (C-7-OAc), 61.3 (C-6′), 67.8 (C-4′), 70.9 (C-3′), 73.6 (C-5′), 75.6 (C-2′), 94.6 (C-1′), 120.3 (C-3), 121.9 (C-6/8), 130.0 (C-4), 132.1 (C-5/9), 136.2 (C-2), 151.3 (C-7),159.0 (C-1), 169.1, 169.4, 169.7 (C-3′, 4′, 6′-OAc), 170.5 (C-7-OAc); H-R EIMS m/z 492.126648. M^+ calc for $C_{23}H_{24}O_{12} = 492.126777$. EI-MS *m/z* (rel. int.): 492 (M^+) (77), 450 (10), 408 (3), 289 (22), 194 (48), 176 (88), 169 (100), 163 (20), 134 (60), 127 (35), 109 (95), 43 (58).

Table 1
X-ray data for Z-venusol (**2**)

Molecular formula	$C_{15}H_{16}O_8$
Formula mass	324.28
Temperature	120 (2) K
Wavelength	0.71073 ?
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions $b = 9.2990(16)$ Å, $\beta = 90°$ $c = 28.397(5)$ Å, $\gamma = 90°$	$a = 5.113(2)$ Å, $\alpha = 90°$
Volume	1350.1(7) Å
Z	4
F(000)	680
Crystal size	$0.40 \times 0.20 \times 0.10$ mm^3
Theta range for data collection	4.24–31.87°
Reflections collected	13,403
Independent reflections	4251 [R(int) = 0.0335]
Completeness to theta = 25.00°	99.1%
Absorption correction	None
Refinement method	Full-matrix least squares on F^2
Goodness of fit on F^2	1.009
Final R indices [$I > 2$ sigma (I)]	$R1 = 0.0364$, w$R2 = 0.0814$
R indices (all data)	$R1 = 0.0455$, w$R2 = 0.0850$
Absolute structure parameter	−0.7 (7)

The complete set of data (atomic coordinates, equivalent isotropic displacement parameters, bond lengths and bond angles, anisotropic displacement parameters, hydrogen coordinates and torsion angles) will be lodged with the Cambridge Crystallographic center.

3.5. *X-ray crystallography*

The X-ray data is collected in Table 1.

Acknowledgements

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References

Bhargava, U.C., Westfall, B.A., 1969. Antagonistic effects of ellagic acid on histamine liberators. Proceedings of the Society for Experimental Biology and Medicine 131, 1342. –1345.

Collins, T., 1996. "Ghopho"-a traditional herbal enigma. Letter to interested persons, published by the author.

Gerstner, J., 1939. A preliminary checklist of Zulu names of plants with short notes. Bantu Studies, 13, 49–64, 131–149, 307–326.

Hutchings, A., Scott, A. H., Lewis, G., Cunningham, A. B., 1996, Zulu Medicinal Plants, University of Natal Press, Pietermaritzburg.

Khac, D.D., Tran-Van, S., Campos, A.M., Lallemand, J.-Y., Fetizon, M., 1990. Ellagic compounds from *Diplopanax stachyanthus*. Phytochemistry 29, 251–256.

Kaido, T.L., Veale, D.J.H., Havlik, I., Rama, D.B.K., 1997. Preliminary screening of plants used in South Africa as traditional herbal remedies during pregnancy and labour. Journal of Ethnopharmacology 55, 185–191.

Li, X.-C., Elsohly, H.N., Hufford, C.D., Clark, A.M., 1999. NMR assignments of ellagic acid derivatives. Magnetic Resonance in Chemistry 37, 856–859.

Masika, P.J., Afolayan, A.J., 2002. Antimicrobial activity of some plants used for treatment of livestock disease in the Eastern Cape, South Africa. Journal of Ethnopharmacology 83, 129–134.

Matonhodze, B.B., Katsoulis, L.C., Hofmeyr, G.J., 2002. Labour induction and meconium: in vitro effects of oxytocin, dinoprostone and misoprostol on rat ileum relative to myometrium. Journal of Perinatal Medicine 30, 405–410.

Ohnuki, T., Ueda, M., Yamamura, S., 1998. Molecular mechanism of the control of nyctinastic leaf-movement in *Lespedeza cuneata* G. Don. Tetrahedron 54, 12173–12184.

Pagani, F., 1990. Constituents of *Umbilicus ruperstris*. Bollettino Chimico Farmaceutico 129, 279. - 280.

Proliac, A., Desage, M., Favre-Bonvin, J., 1981. Structure of venusol, a novel substance from *Umbilicus pendulinus*. Tetrahedron Letters 22, 3583–3584.

Shigemori, H., Sakai, N., Miyoshi, E., 1990. Bioactive substances from *Lespedeza cuneata* LG. Don and their biological activities. Tetrahedron 46, 383–394.

Viornery, L., Saliba, C., Daskiewicz, J.B., Bayet, C., Comte, G., Fenet, B., Gutierrez, G., Barron, D., 2000. Phenylpropanoids from *Umbilicus pendulinus*. Chemical and Pharmaceutical Bulletin 48, 1768–1770.