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Stereostructure, conformation and reactivity of β- and α-gardiol from *Burchellia bubalina*

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Abstract

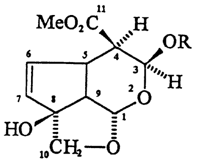
From the leaves of *Burchellia bubalina* (Rubiaceae) pure β- and α-gardiol have been isolated, the latter in crystalline form for the first time. X-ray analysis of β-gardiol establishes its relative configuration. Treatment of the tosyl derivative of β-gardiol with base affords a crystalline elimination product amenable to X-ray analysis. Evidence is presented for the partial conversion of α-gardiol to the epimeric β-gardiol at room temperature. Molecular dynamic studies at 1000 K on the two gardiols provide useful conformational information. In the case of β-gardiol, the analysis shows interesting correlations between conformations adopted at 1000 K and those present in the crystalline state at room temperature. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: *Burchellia bubalina*; Rubiaceae; β-Gardiol; α-Gardiol; Interconversion; Reactivity; X-ray structure; Molecular dynamics

1. Introduction

In 1983, Jensen (1983) isolated β- and α-gardiol from the fresh, unripe fruits of *Rothmannia globosa* (Rubiaceae). Based on NMR spectral evidence, dihedral angles and NOE correlations, he was able to show that β- and α-gardiol have the structures shown in **1a** and **2a**, respectively. In order to assist in establishing the overall structures, Jensen prepared the mono- and dibenzoate derivatives of both gardiols. The final structures indicate that they differ only in being epimeric at the C-4 centre, the point of attachment of the methyl ester moiety.

Subsequent to Jensen's investigations further research on the gardiols has been reported by Bolzani, Izumisawa, Young, Trevisan, Kingston & Gunatilaka (1997). These authors record the isolation of α- and β-gardiol from the leaves of *Tocoyena formosa* (Rubiaceae). In this instance the two gardiols "were obtained as an inseparable mixture", emphasizing our own observations that the separation of the two components presents severe difficulties. Screening tests carried out by Bolzani et al. (1997) on the epimeric mixture of the gardiols showed the existence of antifungal activity using *Cladosporium cladosporiodes* and *C. esphaerospermum* as substrates.



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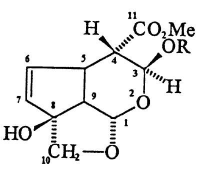
1b R = Ac



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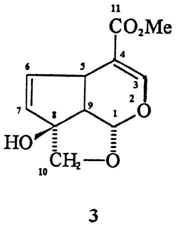

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2. Results and discussion

Leaves from mature plants of *Burchellia bubalina* (Rubiaceae), endemic to South Africa, were collected in

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the National Botanical Garden, Pietermaritzburg, during January 1997. Hutchings (1996) records that the Zulu people use the root of *Burchellia bubalina* in love charm emetics and also in body and nasal spray washes. Roots and bark are also used to bind fractured limbs in animals.

From the dichloromethane extract small quantities of a mixture of α- and β-gardiol were isolated. Repeated purification by column chromatography and centrifugal TLC (chromatotron) finally afforded the pure components. The β-isomer crystallized readily – an observation also reported by Jensen (1983) – but the α-isomer only crystallized, with difficulty, after further separation.

The crystals obtained for β-gardiol were suitable for analysis by X-ray diffraction. The crystal structure shown in Fig. 1 unambiguously establishes the relative configuration of β-gardiol for the first time. The findings fully support the earlier assignment of the relative configuration by Jensen (1983). Indirectly, of course, the relative configuration of α-gardiol is now also beyond doubt. The ^{1}H and ^{13}C data for β-gardiol were recorded at 600 MHz and it was possible to record the multiplicity of *all* the peaks apart from H-5. For this reason this information is given in Section 3.

From the crystal structure accurate information was available with regards to the size of the dihedral angles in the dihydropyran ring. In turn, using the Karplus equation $J_{ab} = 8.5(\cos^2\theta) - 0.28$ for angles $<90°$ and $J_{ab} = 9.5(\cos^2\theta) - 0.28$ for angles $>90°$, exact coupling constants could be calculated (Williams & Fleming, 1995). These J values, together with our observed values for β-gardiol and its 3-acetate derivative (in $CDCl_3$) are shown in Table 1. For comparative purposes the coupling constants recorded by Jensen (1983) for the β-gardiol and the 3-benzoate (in $CDCl_3$) are included. These results show that:

1. the correlation between the two sets of observed J values for the derivatives (3-acetate and 3-benzoate) are very good;
2. the correlation between the two sets of observed J values for β-gardiol itself are of the same order with the greatest difference residing in the $J_{4/5}$ value;
3. the J-values obtained for β-gardiol by calculation from the X-ray data correlate only reasonably well with the observed values based on ^{1}H NMR data.

It is probably a reasonable assumption to make that β-gardiol in solution adopts a conformation which differs from that found in the crystal. In order to examine more closely the possible preferred conformation of the two epimers, a molecular dynamics study was carried out, using the HyperChem 5.0 program by Hypercube, Inc.

2.1. Molecular dynamics and conformational analysis of β-gardiol

The principal objectives of this computational study were to (1) determine the accessible conformations for the dihydro-pyran ring of α- and β-gardiol and establish which ring conformation is the most stable, (2) examine the effect of the orientation of the C-4 ester substituent on the total energy of conformations of α- and β-gardiol, and (3) to accurately model the crystallographic conformation of β-gardiol.

Plots of the H-4–C-4–C-5–H-5 dihedral angle (as defined in the X-ray structure of β-gardiol) for α- and β-gardiol against molecular dynamics simulation time at

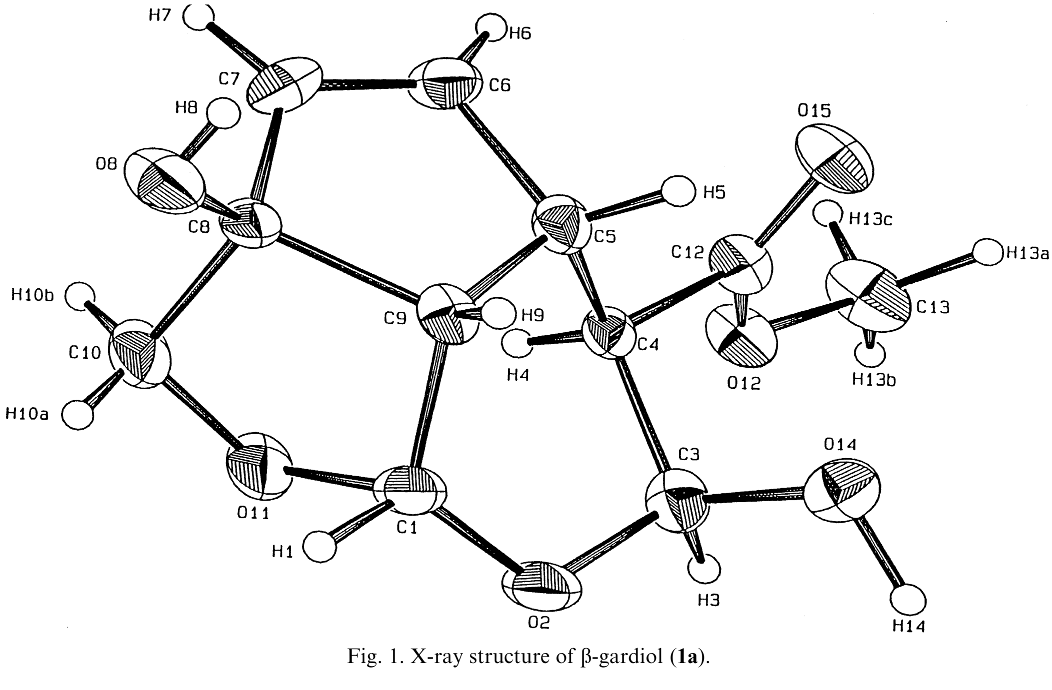


Fig. 1. X-ray structure of β-gardiol (**1a**).

Table 1
Correlation between J values and dihedral angles

	Dihedral angle (θ, deg) from X-ray data	$J_{calc.}$ (Hz)	$J_{obs.}$ (Hz)			
			this paper		Jensen	
			β-gardiol[a]	3-acetate	β-gardiol	3-benzoate
H-3–H-4	65.99	1.12	2.50	2.66	2.1	2.5
H-4–H-5	171.31	9.00	9.16	10.35	6.4	10.5
H-5–H-9	9.28	7.99	9.24	9.27	8.9	9.2
H-9–H-1	46.54	3.87	5.82	5.47	6.3	5.5

[a] Spectra run at 600 MHz in CD_3OD.

1000 K are shown in Fig. 2. For both isomers, two discrete conformational regions are evident. Region A for β-gardiol is characterized by configurations in which the hydropyran ring has a twisted boat conformation and an average H-4–C-4–C-5–H-5 dihedral angle of $\sim -80^\circ$. Region B comprises configurations having a twisted half-chair conformation for the hydropyran ring and an average H-4–C-4–C-5–H-5 dihedral angle of $\sim -160^\circ$. Snapshots taken from both these regions were structurally refined. The minimum energy conformation from region A is shown in Fig. 3 (conformer A); H-4 is clearly equatorial and the hydropyran ring has a twisted boat conformation. The minimum energy conformation from region B (conformer B) has H-4 axial (H-4–C-4–C-5–H-5 $= -167.5^\circ$) and a twisted half-chair hydropyran ring conformation. A local minimum conformation (conformer B′) was also found in region B. Conformer B′ has a similar H-4–C-4–C-5–H-5 dihedral angle to conformer B (-167.0°). However, the ester carbonyl group is oriented oppositely (176.7°) to that of conformer B as a result of rotation about the C-5–C-4–C-12–O-15 dihedral angle. Conformer B′ is slightly higher in energy than conformer B (by 0.11 kcal/mol) and closely matches the crystallographic conformation of β-gardiol.

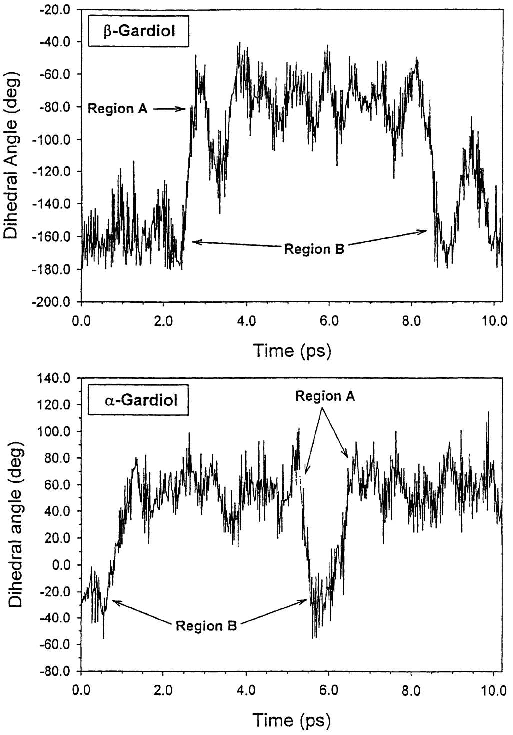


Fig. 2. Molecular dynamics simulations of α- and β-gardiol at 1000 K.

Regions A and B of Fig. 2 for α-gardiol are characterized by configurations having twisted boat and twisted chair dihydropyran ring conformations with average H-4–C-4–C-5–H-5 dihedral angles of $\sim 60^\circ$ and $\sim -20^\circ$, respectively Fig. 3. These conformations are summarized schematically in Fig. 4 along with those for β-gardiol.

Table 2 compares the calculated and observed (X-ray) H-4–C-4–C-5–H-5 dihedral angles for the A, B, and B′ conformations of β-gardiol. The calculated structure of conformer B′ has the same ester carbonyl group orientation as the X-ray structure; the dihedral angles H-3–C-3–C-4–H-4, H-4–C-4–C-5–H-5, and H-5–C-5–C-9–H-9 show particularly good agreement with those measured experimentally (deviations $<4^\circ$). H-4 is *trans* to H-5 (167.0°) in conformer B′, placing H-4 in an axial position and the methyl ester substituent in an equatorial position. Since conformer B′ is calculated to be higher in energy than conformers A and B, the X-ray structure of β-gardiol appears to be a local minimum conformation. Conformational isomers B and B′ are, in fact, conformational isomers differing in the orientation of the methyl ester substituent attached to C-4. The conformation observed in the X-ray structure (H-4 *trans* to carbonyl oxygen O-15) is overall 0.11 kcal/mol higher in energy than the conformation in which H-4 and O-15 are eclipsed, due mainly to a slight increase in van der Waals and bond stretching energy.

2.2. *Epimerization of α-gardiol to β-gardiol*

The two epimeric gardiols are difficult to separate as already observed by Bolzani et al. (1997). It was our

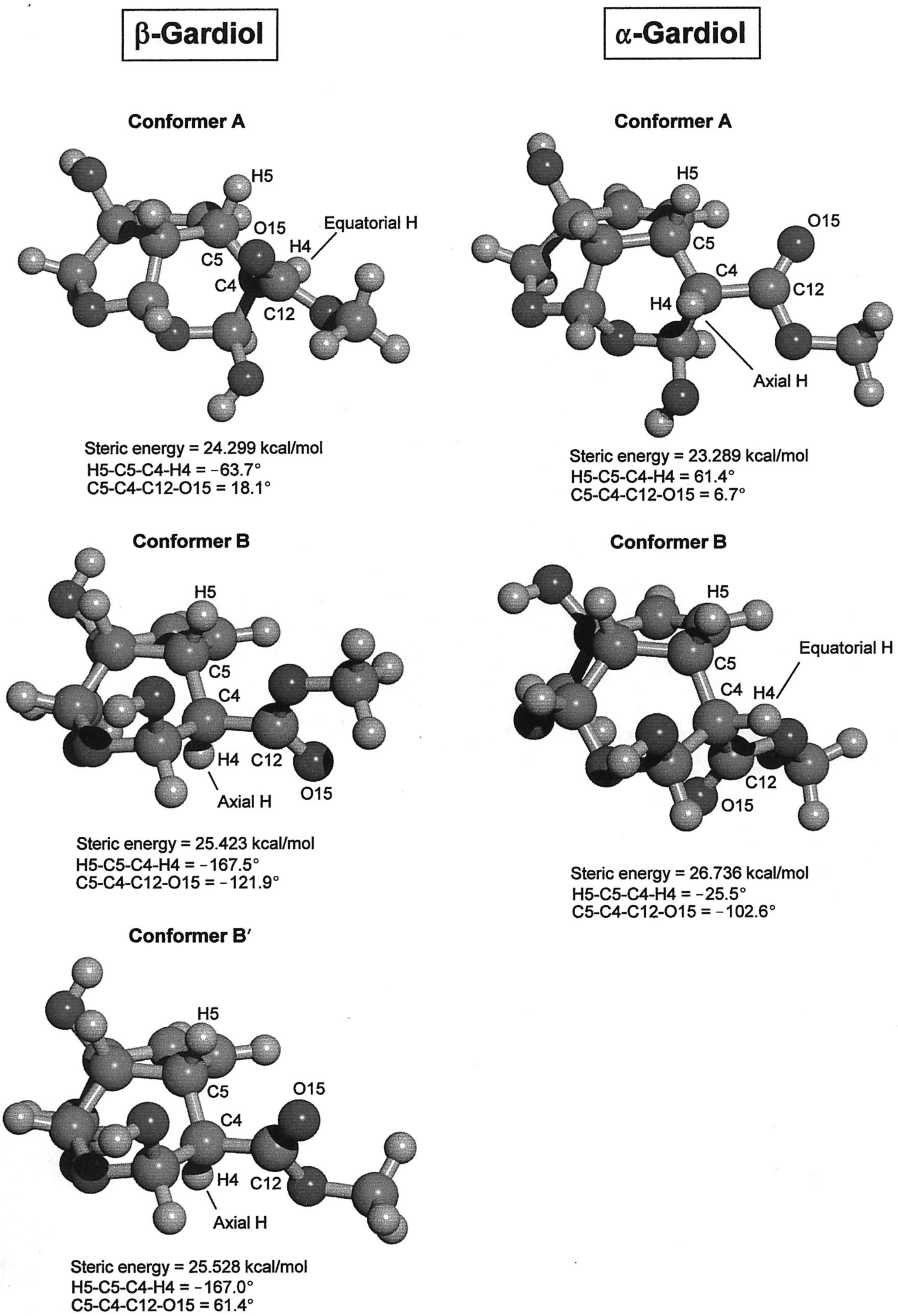


Fig. 3. Minimum energy conformations calculated for α- and β-gardiol.

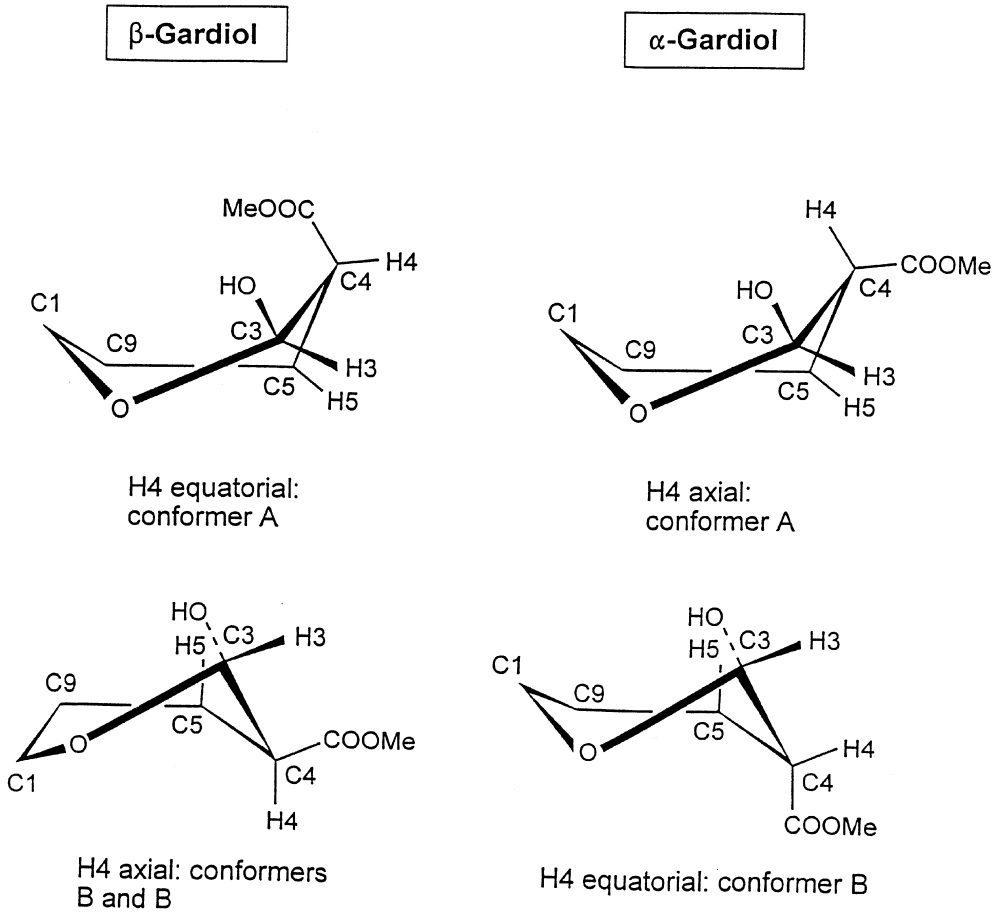


Fig. 4. Schematic representations of the preferred conformations for the 6-membered rings of α- and β-gardiol.

Table 2
Selected calculated and observed H–C–C–H dihedral angles (deg) for conformations of β-cardiol

Dihedral angle	Conformer A	Conformer B	Conformer B′	X-ray
H-3–C-3–C-4–H-4	−23.97	67.53	70.21	65.99
H-4–C-4–C-5–H-5	63.73	167.54	167.00	171.31
H-5–C-5–C-9–H-9	−21.90	8.39	6.95	9.28
H-9–C-9–C-1–H-1	−33.45	33.61	33.21	46.54

experience that pure α-gardiol in $CDCl_3$ is partly converted overnight in the NMR tube to β-gardiol. The clearest evidence for this was the formation of some crystalline material in the NMR tube (α-gardiol is normally an oil whereas the β-epimer gives rise to needle-like crystals). Under the same 'reaction conditions' the 3-acetyl derivative of α-gardiol was perfectly stable.

In order to check further that the α-gardiol isolated in the present investigation was identical to the α-gardiol isolated from *Rothmannia globosa* by Jensen (1983), we prepared the 3-benzoate and the 3,8-dibenzoate derivatives of both gardiols as did Jensen (1983). Not only are these derivatives quite stable, but the allocation of NMR peaks is much simpler, since derivatization has the obvious effect of shifting certain peaks downfield thus alleviating the overlap of peaks. These four derivatives could be obtained very pure (three of them are crystalline) so that identification was unambiguous. The NMR spectra (^{1}H and ^{13}C) are identical to those reported by Jensen (1983) but we find one difference in proton allocation in that the chemical shift values for H-6 and H-7 in the dibenzoate of α-gardiol should be transposed.

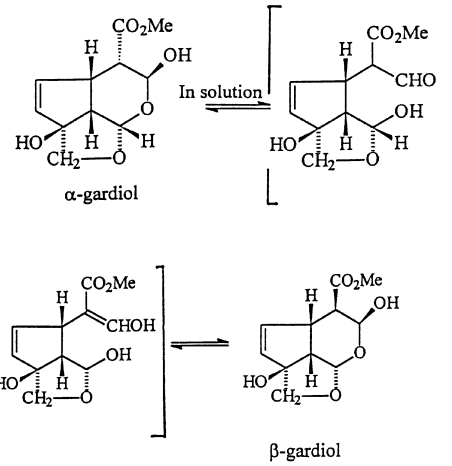
In pursuing the observation that α-gardiol epimerizes rapidly in solution ($CDCl_3$) to β-gardiol the following situations arose:

(1) Pure α-gardiol when left overnight in the NMR tube

in ($CDCl_3$) was converted into an equilibrium mixture of the two epimers. The ^1H NMR spectrum clearly shows the presence of both epimers. Characteristic peaks occur for the CH_2 doublet of doublets at H-10 (shifted upfield in β-gardiol) and the characteristic (but different) peak patterns for H-1 and H-3.

(2) The mixture of gardiol epimers was extracted with CH_2Cl_2 from *Burchellia bubalina* for a very short time (5 h) and purified immediately. Using the usual separation procedures β-gardiol soon crystallized. The α-gardiol fraction was re-fractionated on the chromatotron and the resultant product was shown by ^1H NMR and ^{13}C NMR to be virtually 100% monocomponent. To our surprise this material rapidly crystallized after dissolving it in $CDCl_3$. Previously α-gardiol had only been obtained as an oil. The crystals had mp 105°C (softening), melting 110–112°C, $[\alpha]_D^{25} + 108.3°$, and on a TLC plate (run four times in ether) appeared as a discrete spot clearly separated from β-gardiol and having a lower R_f. The crystals remain reasonably stable but slow conversion to β-gardiol in chloroform solution occurs. In turn pure β-gardiol shows some epimerization to α-gardiol.

The six-membered ring in the gardiol epimers – bearing an acetal and hemi-acetal moiety – represents a very labile system. We suggest that the epimerization from α-gardiol to β-gardiol proceeds by the mechanism shown in Scheme 1.



Scheme 1. Possible mechanism for the conversion of α-gardiol to a mixture of epimers.

2.3. Elimination reaction of β-gardiol

Preparation of the tosyl ester of β-gardiol followed by treatment with DABCO afforded the elimination product (**3**) in 63% yield. X-ray analysis of the crystal confirmed that a double bond had formed between C-2 and C-3 (Fig. 5). Numerous naturally occurring iridoids have a double bond in this position and it is likely that such an iridoid, based on the gardiol skeleton, exists in nature. It

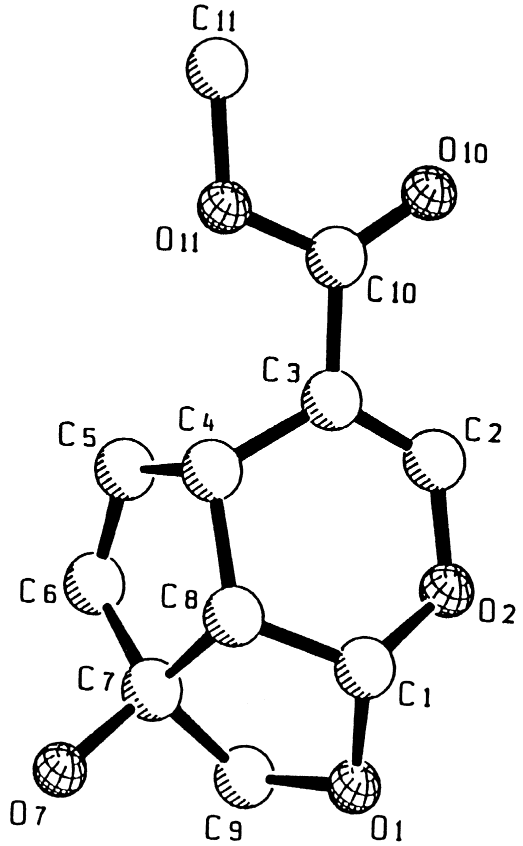


Fig. 5. X-ray structure of compound **3**.

does, however, not occur in the extract of *Burchellia bubalina*.

3. Experimental

3.1. General

NMR: ^1H (200 and 600 MHz) and ^{13}C (50 and 150 MHz); solvents as internal standards: $CDCl_3$ (δ 7.26, 77.01) and CD_3OD (δ 3.30 and 47.58); EI-MS: 70 eV; CD: Jasco J-710 spectropolarimeter; IR: KBr; CC: silica gel 60 (Macherey Nagel); chromatotron: silica gel 60 F_{254}.

Molecular mechanics and molecular dynamics calculations were performed by one of us (OQM) with a HyperChem 5.0 (Hypercube). The MM+ force field was used in all geometry optimizations with a Polak–Ribiere conjugate gradient algorithm, a root mean square gradient termination condition of 0.05 kcal/Å mol), and a dielectric constant of 1.5 D. For molecular dynamics calculations, the system was heated from 100 to 1000 K in 0.2 ps using 50 K steps prior to carrying out a 10 ps constant temperature simulation at 1000 K (0.001 ps time steps). Conformational and energy data were collected at 0.005 ps intervals. Different starting trajectories were employed and selected 'snapshot' conformations structurally refined. Dihedral angle driving studies were performed by driving the ester substituent attached to C-4 of α- and β-gardiol (C-5–C-4–C-12–D-15 dihedral angle) through 360° in 5° steps, calculating single point energies at each point.

3.2. *Plant material*

Leaves (940 g) from mature trees of *Burchellia bubalina* (L.f.) Sims, were collected in the National Botanical Garden, Pietermaritzburg, in January 1996. A voucher specimen (collectors No.: Drewes 3) was deposited in the Bews Herbarium, University of Natal, Pietermaritzburg. Its identity was verified by Mr. Brian Tarr, Curator of the Garden.

Extraction (5 days) at RT with CH_2Cl_2 afforded extract (9.3 g). From EtOAc (7 days) more material (8.2 g) was obtained. Purification of the mixture from EtOAc (1.2 g) by CC (EtOAc–*n*-hexane, 3:2), followed by separation on the chromatotron (Drewes, Horn, Connolly, & Bredenkamp, 1998) using the same eluent, afforded a mixture of the two gardiols (200 mg). Final separation was effected by CC (Et_2O) to give β-gardiol (40 mg) and α-gardiol (25 mg).

3.3. *β-Gardiol (**1a**)*

Fine needles, mp 122°C (CH_2Cl_2–hexane) (lit. 122–124°C, Jensen, 1983), $[\alpha]_D^{25}+209°$ (MeOH, *c* 0.07) (lit. 211°, Jensen, 1983); IR, ν (cm^{-1}): 3418, 2920, 1688 (CO), 1418, 1010, 896; EIMS *m/z* (rel. int.): 242 [M$^+$] (0.8), 224 (18), 211 (18), 193 (9), 179 (8), 119 (18), 110 (78), 103 (100), 82 (58); ^{1}H NMR (CD_3OD, 600 MHz): δ 2.65 (1H, dd, $J=9.2$, 5.8 Hz, H-9), 2.67 (1H, dd, $J=9.2$, 2.5 Hz, H-4), 3.52 (1H, m, H-5), 3.53 and 3.77 (2H, dd, $J=9.5$ Hz, H-10), 3.71 (3H, s, CO$_2$*Me*), 5.36 (1H, d, $J=2.5$ Hz, H-3), 5.49 (1H, d, $J=5.8$ Hz, H-1), 5.73 (1H, dd, $J=5.5$, 2.0 Hz, H-7), 5.90 (1H, dd, 5.5, 2.1 Hz, H-6); ^{13}C NMR (CD_3OD), 150 MHz): δ 40.8 (C-5), 49.2 (C-4), 49.8 (C-9), 52.7 (*CO$_2$*Me), 75.1 (C-10), 90.8 (C-3), 93.8 (C-8), 101.6 (C-1), 135.9 (C-7), 138.3 (C-6), 173.3 (*CO$_2$*Me); CD (EtOH, *c* 0.06): $\Delta\varepsilon_{196}$ -4.1, $\Delta\varepsilon_{202}$ $+7.5$, $\Delta\varepsilon_{204}$ -9.6, $\Delta\varepsilon_{207}$ -3.8.

3.4. *X-ray data for β-gardiol (**1a**)*

The compound has a molecular formula of $C_{11}H_{14}O_6$. Diffraction data were measured on a Nonius CAD-4 diffractometer with graphite-monochromated Mo K radiation. The unit cell parameters were determined by a least squares fit of 25 reflections with $\theta > 12°$. No crystal decay was observed. The structure was solved by direct methods and the final *R* value was 0.0426. Crystals were orthorhombic with cell parameters a (pm) $=601.9(5)$, $b=984.1(4)$, $c=1863.1(6)$, V (pm^3) $=1103.45(96)\times10^6$, space group $P2_12_12_1$, $Z=4$ Fig. 1. Data deposited with the X-ray Crystallographic Centre, Cambridge, UK.

3.5. *α-Gardiol (**2a**)*

The α-gardiol initially isolated by us was an oil, $[\alpha]_D^{25}+100°$ (MeOH, *c* 0.25) (lit. +152°, Jensen, 1983). It subsequently crystallized on further purification on the chromatotron (Et_2O). The crystals soften at 105°C and melt at 110–112°C, $[\alpha]_D^{25}+108.3°$, $[\alpha]_{546}^{25}+128.1°$, $[\alpha]_{365}^{25}+336.4°$ (MeOH, *c* 0.096). Crystalline α-gardiol has limited solubility in $CDCl_3$ (the solvent from which it crystallized) and the NMR (at 200 MHz) spectrum is recorded in CD_3OD: δ 2.63 (1H, dd, $J=8.5$, 5.7 Hz, H-9), 2.71 (1H, dd, $J=8.7$, 5.3 Hz, H-4), 3.56 (1H, m, H-5), 3.73 (3H, s, CO$_2$*Me*), 3.78 and 3.93 (2H, dd, $J=9.2$ Hz, H-10), 5.18 (1H, d, 8.7 Hz, H-3), 5.54 (1H, d, 5.7 Hz, H-1); 5.79 (2H, s, 5.79 H-6, H-7); ^{13}C NMR (CD_3OD, 50 MHz): δ 40.6 (C-5), 48.7 (C-4), 52.3 (CO$_2$*Me*), 52.5 (C-9), 76.6 (C-10), 90.9 (C-3), 93.6 (C-8), 101.8 (C-1), 135.2 (C-7), 137.1 (C-6), 173.9 (*CO$_2$*Me).

3.6. *β- and α-Gardiol-3-acetates (**1b**, **2b**)*

Fresh leaves (650 g) of *Burchellia bubalina*, collected October 1996, were extracted at RT for 7 days with EtOAc (6.5 g). The usual separation by CC gave a mixture of reasonably pure epimers (R_f 0.2 and 0.15 resp. (ether) for β- and α-gardiol, purple stain with anisaldehyde) (370 mg). Acetylation (pyridine/Ac_2O) and work-up after 2 h at RT afforded 420 mg which was separated by chromatotron (Et_2O) to give pure β-gardiol-3-acetate (40 mg), mp 111–112°C, $[\alpha]_D^{25}+135.6°$ ($CHCl_3$, *c* 0.081) and α-gardiol-3-acetate (35 mg) as an oil $[\alpha]_D^{25}+98.4°$ ($CHCl_3$, *c* 0.126).

3.7. *β-Gardiol-3-acetate (**1b**)*

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.06 (3H, s OCO*Me*), 2.78 (1H, dd, $J=9.3$, 5.5 Hz, H-9), 2.91 (1H, dd, $J=10.4$, 2.7 Hz, H-4), 3.54 (1H, tt, $J=9.3$ Hz, H-5), 3.73 (3H, s, CO$_2$*Me*), 3.65 and 3.92 (2H, dd, $J=9.80$ Hz, H-10), 5.56 (1H, d, $J=5.5$ Hz, H-1), 5.82 (1H, dd, $J=5.5$, 1.8 Hz, H-7), 6.01 (1H, dd, $J=5.5$, 2.3 Hz, H-6), 6.42 (1H, dd, $J=2.70$, 0.4 Hz, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 21.0 (OCO*Me*), 38.7 (C-5), 45.8 (C-4), 49.5 (C-9), 52.3 (CO$_2$*Me*), 74.3 (C-10), 88.7 (C-3), 93.6 (C-8), 99.4 (C-1), 134.6 (C-7), 137.2 (C-6), 169.4 (O*CO$_2$*Me), 170.4 (*CO$_2$*Me); EIMS *m/z* (rel. int.): 254 (10) [M$-$30]$^+$, 225 (35), 194 (86), 162 (36), 135 (41), 103 (100), 85 (75), 77 (98).

3.8. *α-Gardiol-3-acetate (**2b**)*

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.06 (3H, s, OCO*Me*), 2.87 (1H, d, $J=4.8$ Hz, H-9), 2.91 (1H, d, $J=4.9$ Hz, H-4), 3.61 (1H, m, H-5), 3.77 (3H, s, CO$_2$*Me*), 4.07 and 4.18 (2H, dd, $J=9.6$ Hz, H-10), 5.56 (1H, d, $J=4.8$ Hz, H-1), 5.85 (1H, dd, $J=5.6$, 1.7 Hz, H-7), 5.95 (H, dd, $J=5.6$, 2.6 Hz, H-6), 6.32 (1H, d, $J = 8.7$ Hz, H-3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 21.1 (OCO*Me*), 41.6 (C-5), 43.8 (C-4), 52.5 (CO_2*Me*), 54.4 (C-9), 78.0 (C-10), 90.2 (C-3), 93.5 (C-8), 100.0 (C-1), 134.2 (C-7), 136.5 (C-6), 169.5 (O*CO*$_2$Me), 171.0 (CO_2Me); EIMS *m/z* (rel. int.): 224 (13) $[M-60]^+$, 193 (9), 177 (18), 162 (51), 135 (30), 107 (46), 85 (38), 77 (100).

3.9. Elimination product (**3**) from β-gardiol

β-Gardiol (10.0 mg) in CH_2Cl_2 (2 ml) was treated with 4-bromobenzene sulfonyl chloride (31.6 mg) and DABCO (18.5 mg). The mixture was stirred at room temp. for 48 h and then purified by CC (CH_2Cl_2/MeOH, 92:8) to give the crystalline **3** (5.83 mg 63%). Mp 141–143°C, IR, ν (cm^{-1}): 3470, 2960, 1690, 1640, 1300, 1050, 800, $[\alpha]_D^{25} + 171.6°$ (MeOH, *c* 0.239), and starting material (2.36 mg, 24%). For the crystals ^{1}H NMR ($CDCl_3$, 600 MHz): δ 2.89 (1H, dd, $J = 9.5$, 6.2 Hz, H-9), 3.74 (3H, s, CO_2*Me*), 3.84 (1H, bdd, H-5), 3.93 (2H, s, H-10), 5.65 (1H, d, $J = 6.2$ Hz, H-1), 5.74 (1H, dd, $J = 5.5$, 2.4 Hz, H-7), 6.02 (1H, dd, $J = 5.5$, 2.2 Hz, H-6), 7.49 (1H, s, H-3); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 37.7 (C-5), 51.4 (CO_2*Me*), 51.6 (C-9), 75.6 (C-10), 95.2 (C-8), 100.6 (C-1), 107.6 (C-4), 131.4 (C-7), 137.8 (C-6), 151.8 (C-3), 167.3 (*CO*$_2$Me); EIMS *m/z* (rel. int.): 224 (24) $[M]^+$, 193 (22), 177 (33), 174 (32), 162 (100), 134 (64), 106 (62), 85 (74), 77 (70), 65 (34). HRMS calcd. for $C_{11}H_{12}O_5$: 224.0684, found 224.0686.

3.10. X-ray data for **3**

Solution was by direct phase determination and refined by full-matrix LSQ. The final *R* value was 0.060, crystals were orthorhombic with cell parameters a (pm) = 595.4(4), $b = 992.2(1)$, $c = 1736.9(2)$, V (pm^3) = $1026.2(2) \times 10^6$, space group $P2_12_12_1$, $Z = 4$, number of reflections 3533 (Fig. 5).

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