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THREE DIHYDROANTHRACENONES FROM *GASTERIA BICOLOR*

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Key Word Index—*Gasteria bicolor*; Aloaceae; pre-anthraquinones; dihydroanthracenones; chemotaxonomy; cytotoxicity; gasteriacenones A–C.

Abstract—The stem and leaves of *Gasteria bicolor* afforded three new dihydroanthracenones namely 3,4-dihydro-2,6,9-trihydroxy-8-methyl-1(2*H*)-anthracenone (gasteriacenone A), 3,4-dihydro-2,4,9-trihydroxy-6-methoxy-8-methyl-1(2*H*)-anthracenone (gasteriacenone B) and 3,4-dihydro-4,6,9-trihydroxy-7-carbomethoxy-8-methyl-1(2*H*)-anthracenone (gasteriacenone C). Their structures were determined by spectroscopic methods including 2D NMR techniques.

INTRODUCTION

The popular succulent genus *Gasteria*, which comprises 16 species, is endemic to South Africa and has its main centres of distribution in savanna regions of the eastern Cape [1]. *Gasteria* is distinguished from *Aloe*, to which it bears a close resemblance, by examination of its leaves and inflorescence. Many members of both genera adapt well to indoor conditions and are popular in botanical gardens and with collectors in Europe. The genus *Gasteria* belongs to the family Asphodelaceae, subfamily Alooideae according to the classification of Dahlgren *et al.* [2] or the family Aloaceae according to Cronquist [3]. This genus was previously classified in the rather large and heterogeneous family namely the Liliaceae. When the Liliaceae was subdivided into several other families, *Gasteria* and *Aloe* were classified in the Asphodelaceae. However, the current trend is to split *Aloe* and *Gasteria* from the Asphodelaceae and place them in the new family Aloaceae [4].

As there is no work previously on the constituents of any member of the genus *Gasteria*, we set out to examine *Gasteria bicolor* Haw. which belongs to the series *Gasteria* of the section *Gasteria* [5]. Rauwald [6] has recently pointed out that aloesaponol I–IV type dihydroanthracenone compounds, first isolated from *Aloe saponaria* [7, 8] and aloechrysone from *Aloe berhana* [9] may serve as markers of the subterranean anthranoid metabolism in *Aloe* plants. Furthermore, a chemotaxonomic survey of the lipophilic anthranoid aglycones in *Aloe* roots [10] has shown the presence of the 1-methyl-8-hydroxyanthraquinone pathway in 129 out of 172 species. The same survey revealed the presence of a series of unknown stem and root metabolites in *Gasteria* species. This report which reveals the isolation and characterization of three novel dihydroanthracenones, gasteriacenone A, B and C, from the stem and leaves of *G. bicolor*, indicates the close affinity between *Aloe* and *Gasteria*.

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RESULTS AND DISCUSSION

Gasteriacenone A (**1**) was assigned the molecular formula $C_{15}H_{14}O_4$ based on the HRMS which gave a molecular ion at *m/z* 258.0902. The ^{13}C NMR spectrum gave rise to 15 carbon signals assignable to a carbonyl (δ204.9) and from the DEPT experiment to two methylenes (δ27.0, 31.6), a methyl (δ24.7), one aliphatic and three aromatic methines and seven other quaternary carbons. The IR spectrum exhibited absorptions due to a hydroxyl at 3316–3429 cm^{-1}, a chelated carbonyl at 1627 cm^{-1} and aromatic (C–C) stretching at 1602 cm^{-1}. The ^1H NMR spectrum (Table 1) showed a singlet at δ14.86 confirming presence of a chelated hydroxyl proton, two *meta*-coupled aromatic protons, H-5 (δ6.76, *br d*, $J = 2.4$ Hz) and H-7 (δ6.74, *br d*), and an isolated aromatic proton H-10 (δ6.84, *br s*).

In addition to these the presence of an aromatic methyl group (δ2.78, *s*), an aliphatic and phenolic OH at δ5.62 and 10.15 respectively, both of which exchanged on deuteration, and two mutually coupling methylene groups, suggested a dihydroanthracenone system, substituted in ring A by an OH and Me groups, in ring B by an OH at C-9 and right C by an OH at either C-2 or C-4.

Table 1. ^{1}H NMR spectral data for gasteriacenones A (**1**), B (**2**) and C (**3**) (600 MHz, DMSO-d_6)

H	1	2	3
2_{ax}	—	—	2.75 *m*
2_{eq}	—	—	2.80 *m*
2	4.31 *ddd* (11.5, 4.8, 4.2)	4.40 *ddd* (13.0, 5.1, 5.0)	—
OH-2	5.62* *d* (4.2)	5.60* *d* (5.1)	—
3_{ax}	1.85 *m*	1.88 *ddd* (13.0, 12.0, 11.5)	1.98 *m*
3_{eq}	2.20 *m*	2.40 *ddd* (11.5, 5.0, 3.5)	2.18 *m*
4	2.96 *m*	4.80 *ddd* (12.0, 6.8, 3.5)	4.78 *dd* (8.7, 3.3)
OH-4	—	5.70* *d* (6.8)	5.58* *d* (3.6)
5	6.76 *br d* (2.4)	7.12 *d* (2.5)	7.04 *s*
OH-6	10.15* *s*	—	9.90* *s*
7	6.74 *br d*	6.89 *br d* (2.5)	—
Me-8	2.78 *s*	2.82 *s*	2.70 *s*
OH-9	14.86* *s*	14.87* *s*	15.46* *s*
10	6.84 *br s*	7.36 *br s*	7.16 *s*
OMe	—	3.86 *s*	—
CO_2Me	—	—	3.90 *s*

Coupling constants (J in Hz) in parentheses.
*Exchanged after deuteration.

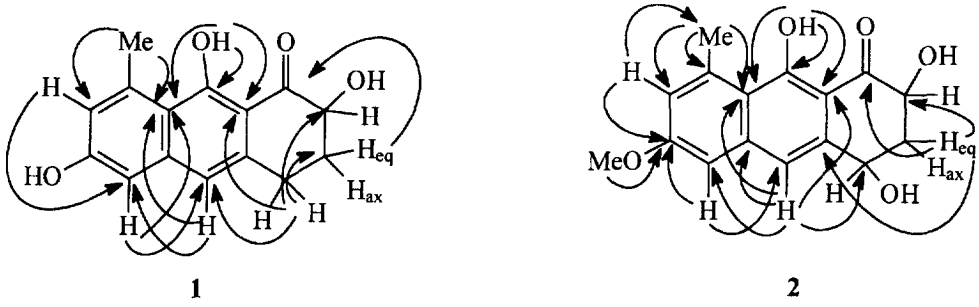


Fig. 1. Longe-range ^{13}C–^{1}H correlations in COLOC spectrum of gasteriacenone A (**1**) and gasteriacenone B (**2**). Arrows indicate the correlation from proton to carbon atom.

Important observations from the ^{1}H-^{1}H COSY and NOSEY experiments were as follows: (i) Long range coupling between the methylene protons (δ2.96) and the aromatic proton (H-10, δ6.84) indicated a methylene group at C-4; (ii) A cross peak of the methylene protons at C-4 (δ2.96) with two protons (δ1.85, 2.20) revealed a remaining methylene group also be placed at C-3; (iii) The methylene protons at C-3 showed further coupling with an oxymethine proton (δ4.31) revealing that the aliphatic OH must be attached to C-2, and (iv) The two aromatic protons H-5 (δ6.76) and H-7 (δ6.74) gave rise to cross peaks with the phenolic OH (δ10.15) at C-6. However, only H-7 showed long range coupling with the signal at δ2.78 confirming that the methyl group is at C-8.

The position of the methylene protons at C-4 was further confirmed by the ^{1}H–^{13}C long-range correlation (COLOC) spectrum, which showed cross-peaks between H-4 (δ2.96) and C-2 (δ71.9), C-3 (δ31.6), C-9a (δ108.8) and C-10 (δ115.9) as depicted diagrammatically in Fig. 1. The chemical shift assignments of quaternary carbons in compound **1** (Table 2) were also supported by the COLOC spectrum. Thus all of the above data are consistent with structure **1** (3,4-dihydro-2,6,9-trihydroxy-8-methyl-1(2*H*)-anthracenone), for which we suggest the trivial name gasteriacenone A, a compound isomeric with aloesaponol II (**5**) [7] and the recently reported antitumour antibiotic, okicenone (**6**) isolated from *Streptomyces* sp. [11] and also synthesized by Snider and Zhang [12]. The relative conformation of the methine proton at C-2 in **1** appears to be in the axial position (see (Fig. 3) as can be deduced from the coupling constant between H-2 and the methylene protons at C-3 (H-2, J_{aa} = 11.5 Hz; J_{ae} = 4.8 Hz). Compound **1** showed only weak cytotoxic activity (LD_{50} 5 μg/ml) towards HL60 (ATCC CCL 240) [13] and against brine shrimp or *Artemia salina* (30% lethality at 100 μg/ml).

Gasteriacenone B (**2**) was assigned the molecular formula, $C_{16}H_{16}O_5$ (HRMS, $[M]^+ = m/z$ 288.1037). The IR spectrum suggested the presence of a hydroxyl and a chelated carbonyl. The ^{13}C NMR spectrum including DEPT indicated a methyl (δ24.5), methylene (δ42.1), a methoxyl (δ55.5), two aliphatic and three aromatic methines, and seven quaternary carbons besides

a carbonyl (δ205.3). The ^{1}H NMR spectrum showed a singlet at δ14.87 confirming a chelated hydroxyl group, singlets at δ2.82 and 3.86 which indicated a methyl and methoxyl groups respectively, two aliphatic OH groups at δ5.60 and 5.70, both of which exchanged on deuteration, a pair of *meta*-coupled aromatic protons H-7 (δ6.89, *br d*, J = 2.5 Hz) and H-5 (δ7.12, *d*, J = 2.5 Hz) and an isolated aromatic proton H-10 (δ7.36, *br s*).

Table 2. ^{13}C NMR spectral data for gasteriacenones A (**1**), B (**2**) and C (**3**) (150 MHz, DMSO-d_6)

C	1	2	3
1	204.9	205.3	204.5
2	71.9	70.2	34.9
3	31.6	42.1	31.3
4	27.0	65.3	66.4
4a	138.5	143.4	142.8*
5	107.6	105.5	108.6
6	159.1	160.4	155.5
7	120.0	119.9	126.0
8	140.7	140.3	136.9
8a	116.1	117.2	116.0
9	166.4	165.7	166.6
9a	108.8	107.7	109.0
10	115.9	114.5	114.7
10a	141.7	141.5	141.0*
Me-8	24.7	24.5	20.8
$CO_2\underline{Me}$	—	—	52.4
$\underline{CO_2}Me$	—	—	168.4
OCH_3	—	55.5	—

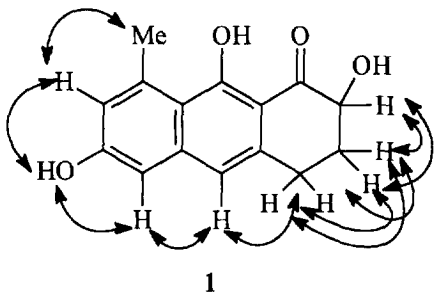
Signal assignments are based on ^{1}H–^{13}C COSY and COLOC spectra.

*Assignments may be interchanged in the column.

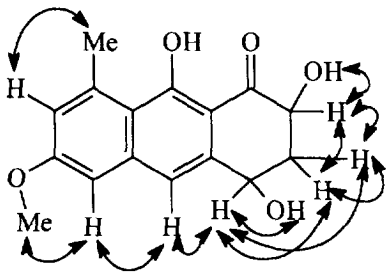
As shown in Fig. 2, the ^{1}H-^{1}H COSY and NOESY experiments revealed: (i) Long range coupling of the aromatic proton (H-10, δ7.36) with the oxymethine proton (δ4.80) at C-4; (ii) Further coupling of the oxymethine proton (δ4.80) with the methylene protons at C-3 (δ1.88, 2.40); (iii) Coupling between the methylene group at C-3 and the remaining oxymethine proton (δ4.40) indicating an OH group at C-2; (iv) Long-range coupling of H-7 (δ6.89) with the methyl group at C-8, and (v) Cross peak between H-10 (δ7.36) and H-5 (δ7.12).

It is interesting to note that in the NOESY spectrum a more intense cross peak resulted between the OMe and H-5 than with H-7, indicating closer proximity of H-5 to the OMe group as shown in Fig. 2.

The COLOC spectrum (Fig. 1) suggested the presence of long-range coupling of H-10 (δ7.36) and H-3$_{eq}$ (δ2.40) with the oxymethine carbon (C-4, δ65.3). All the chemical shift assignments of compound **2** are also in a good agreement with the COLOC spectrum. Consequently, the structure of this compound is determined to be **2** (3,4-dihydro-2,4,9-trihydroxy-6-methoxy-8-methyl-1(2*H*)-anthracenone) or gasteriacenone B. The relative conformation of the methylene protons at C-3 in **2** was assigned to be H-3$_{ax}$ at δ1.88 and H-3$_{eq}$ at δ2.40 based on the coupling constants between H-2 and H-3 (H-2, J_{aa} = 13.0 Hz; J_{ae} = 5.0 Hz), and H-4 and H-3 (H-4, J_{aa} = 12.0 Hz; J_{ae} = 3.5 Hz), and thus placing both methine protons at C-2 and C-4 in axial positions as shown

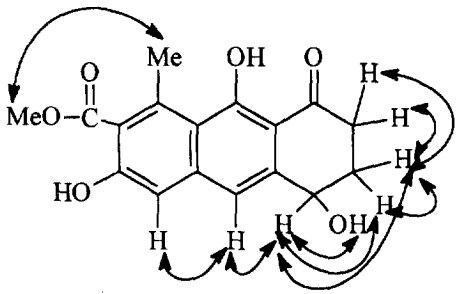


1



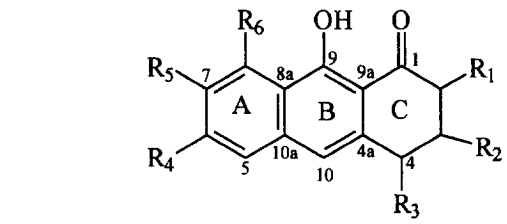
2

Note stronger interaction of OMe with H-5 than with H-7

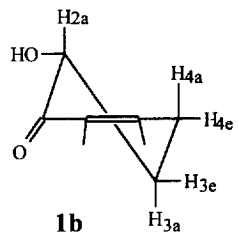


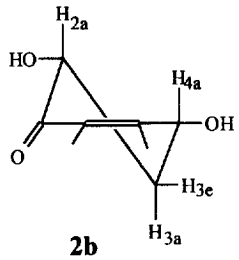
3

Fig. 2. 2D ^{1}H-^{1}H COSY and 2D NOESY correlations for compounds **1**–**3** from *Gasteria bicolor*.



	R1	R2	R3	R4	R5	R6
1	OH	H	H	OH	H	Me
2	OH	H	OH	OMe	H	Me
3	H	H	OH	OH	COOMe	Me
4	H	OH	H	OH	COOMe	Me
5	H	OH	H	OH	H	Me
6	H	H	OH	OH	H	Me





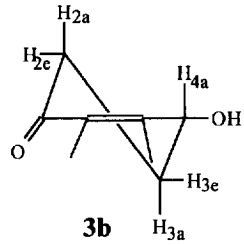


Fig. 3. Gasteriacenones (1–3) and their isomers aloesaponol I (4), aloesaponol II (5) and okicenone (6).

in Fig. 3. Compound **2** proved to be only weakly cytotoxic (LD_{50}, 2 μg/ml) towards HL60 (ATCC CCL 240) [13] and against brine shrimp (40% lethality at 100 μg/ml).

Gasteriacenone C (**3**) gave rise to an $[M]^+$ at 316.0941 in the HR mass spectrum corresponding to the molecular formula $C_{17}H_{16}O_6$. The presence of hydroxyl, an ester and a chelated carbonyl group was suggested from the absorption maxima in the IR spectrum at 3448, 1700 and 1653 cm^{-1}, respectively. The ^{13}C NMR along with a DEPT experiment indicated the presence of two carbonyls (δ204.5, 168.4), a methyl (δ20.8), a methoxyl (δ52.4), two methylenes (δ31.3, 34.9), three CH groups (δ66.4, 108.6, 114.7) and eight other quaternary carbons.

The ^1H NMR spectrum showed signals for a chelated hydroxyl group (δ15.46, *s*), two aromatic protons H-5 (δ7.04, *s*) and H-10 (δ7.16, *s*), an aliphatic OH (δ5.58) and a phenolic OH (δ9.90), both of which exchanged on deuteration, singlets at δ3.90 and 2.70 indicating a methoxyl and a methyl group, respectively. The appearance, in the mass spectra, of the base peak at *m/z* 284 as the result of the loss of MeOH from the molecular ion suggested a hydroxyl group adjacent to an ester functionality [13].

The ^1H–^1H COSY and NOESY experiments revealed: (i) Long range coupling of the aromatic proton (H-10, δ7.16) with the oxymethine proton (δ4.78) indicating an OH group at C-4; (ii) Further coupling of the oxymethine proton at C-4 with the methylene protons at C-3 (δ1.98, 2.18); (iii) Mutual coupling between the methylene groups at C-3 and C-2 (δ2.75, 2.80); (iv) Long range coupling between the methyl group at C-8 (δ2.70) with the OMe (δ3.90) of the carbomethoxyl group, and (v) Cross peak between H-10 (δ7.16) and H-5 (δ7.04).

The relative conformation of methine proton at C-4 in **3** was assigned to be in the axial position by examination of coupling constants between H-4 and methylene protons at C-3 (H-4, J_{aa} = 8.7 Hz; J_{ae} = 3.3 Hz).

These facts are in conformity with structure **3**, for which the trivial name gasteriacenone C is suggested, a compound isomeric with aloesaponol I (**4**) which was first isolated from the subterranean parts of *Aloe saponaria* [7].

The three new compounds are significant for two main reasons. Firstly, they show that the clear-cut distinction between the above-ground and subterranean metabolism (as in most *Aloe* spp.) is absent in *Gasteria*, because the leaves, stem and roots are chemically closely similar. Secondly, some of these new anthranoids have been detected in the related genera *Astroloba*, *Haworthia* and *Poellnitzia* [14] and are therefore of considerable chemotaxonomic interest. Since generic and suprageneric delimitations have not yet reached stability [14], these and structurally related compounds may add a new dimension to our knowledge of phylogenetic relationships in the Aloaceae.

EXPERIMENTAL

General. Mps: uncorr.; Optical rotation: MeOH; UV: MeOH; IR: KBr discs; ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz): DMSO-d_6 with TMS as int. standard. MS: 70 eV; TLC: silica gel PF_{254} (Merck); $CHCl_3$–EtOAc (1:1) (Solvent I).

Plant material. The plant material was collected near Port Elizabeth in the eastern Cape, South Africa in April 1994 and vouchers deposited at the Rand Afrikaans University Herbarium under the cipher B.-E. van Wyk 3556b. The plant was identified as *Gasteria bicolor* var *bicolor* by the long stems and paniculate inflorescence. The identity was confirmed by G. F. Smith and E. J. Van Jaarsveld, both from the National Botanical Institute, South Africa. The material was sliced into fragments and air-dried for several days. Preliminary TLC screening

showed no detectable difference between the fresh and dried material, and no apparent qualitative difference between stems, leaves and roots.

Extraction and isolation. Powdered, air-dried stem and leaves (300 g) were extracted with MeOH for 24 hr. The evapd MeOH extract (15 g) was subjected to flash CC over silica gel using EtOAc–$CHCl_3$ (1:1). Eight frs, each 250 ml, were collected. Concn of fr. 3, and recrystallization from MeOH afforded pale yellow crystals of compound **2** (17 mg). The remaining fractions were further purified by prep. TLC over silica gel with EtOAc–$CHCl_3$ (1:1) to afford **1** (10 mg) and **3** (10 mg).

Gasteriacenone A (**1**). Pale yellow amorphous, $[\alpha]_D$ $-43°$ (MeOH; *c* 0.19). R_f 0.6 (Solvent I). UV λ_{max} nm: 226, 273, 319, 331, 383. Appearance under UV lamp, 366 nm: strongly bluish. IR ν_{max} cm^{-1}: 3429, 3316, 2926, 2857, 1627, 1602, 1461, 1381, 1291, 1164, 1086; ^1H NMR: Table 1; ^{13}C NMR: Table 2; MS *m/z* (rel. int.): 258.0902 $[M]^+$ (100) ($C_{15}H_{14}O_4$ requires 258.0892), 241 (13), 240 (82), 229 (12), 214 (14), 212 (31), 211 (15), 197 (12); CD: $\Delta\varepsilon_{245}$ -0.86, $\Delta\varepsilon_{273}$ -2.2, $\Delta\varepsilon_{282}$ -0.31, $\Delta\varepsilon_{291}$ -0.36, $\Delta\varepsilon_{313}$ -0.25, $\Delta\varepsilon_{333}$ -1.41, $\Delta\varepsilon_{381}$ $+0.11$ (MeOH).

Gasteriacenone B (**2**). Pale yellow crystal from MeOH, Mp 185–187°, $[\alpha]_D$ $-66°$ (MeOH; *c* 0.10). R_f 0.3 (Solvent I). UV λ_{max} nm: 226, 272, 314, 326, 373. Appearance under UV lamp, 366 nm: strongly bluish. IR ν_{max} cm^{-1}: 3418, 2922, 1128, 1607, 1461, 1373, 1164, 1043; ^1H NMR: Table 1 ^{13}C NMR: Table 2; MS *m/z* (rel. int.): 288.1037 $[M]^+$ (100) ($C_{16}H_{16}O_5$ requires 288.0998), 270 (39), 242 (30), 241 (26), 216 (33), 215 (17); CD: $\Delta\varepsilon_{249}$ -2.0, $\Delta\varepsilon_{272}$ -5.5, $\Delta\varepsilon_{287}$ -0.34, $\Delta\varepsilon_{312}$ $+0.40$, $\Delta\varepsilon_{332}$ -1.45, $\Delta\varepsilon_{385}$ $+0.52$ (MeOH).

Gasteriacenone C (**3**). Pale yellow amorphous, $[\alpha]_D$ $+8°$ (MeOH; *c* 0.19). R_f 0.26 (Solvent I). UV λ_{max} nm: 224, 274, 318, 330, 376. Appearance under UV lamp, 366 nm: strongly bluish. IR ν_{max} cm^{-1}: 3448, 2933, 1700, 1653, 1628, 1561, 1461, 1394; ^1H NMR: Table 1. ^{13}C NMR: Table 2; MS *m/z* (rel. int.): 316.0941 $[M]^+$ (41) ($C_{17}H_{16}O_6$ requires 316.0946), 284 (100), 266 (9), 256 (19), 228 (10), 210 (14).

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