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Anthraquinones and Arnidiol from *Barleria Longiflora* Linn F.

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The chemical examination of *Barleria longiflora* Linn. F. is described for the first time. Extraction of stems and roots with solvents and column chromatography led to the isolation of four anthraquinones of which two are new. The known anthraquinones were identified as tectoquinone and 1,3,5-trihydroxy-4-methoxy-2-methylanthraquinone and the new anthraquinones characterised as 3,8-dihydroxy-4-methoxy-2-methylanthraquinone and 1,3,4-trihydroxy-5 (or 8)-methoxy-2-methylanthraquinone by chemical and spectral studies. Arnidiol, a pentacyclic triterpene, was isolated without admixture with its isomer faradiol which usually is the case with many plants. A sterol mixture was also isolated, the minor components of which were identified as campesterol, stigmasterol and β-sitosterol by GCMS of the acetate mixture.

Barleria longiflora Linn. F. (Acanthaceae) is a shrub distributed in Southern Deccan Peninsula and Tinnevally of India. Decoction of the root was reported to be used in stricture, dropsy and stone[1]. Of the chemically investigated *Barleria* species, *B. prionitis*[2,8], *B. cristata*[9,10], *B. lupulina*[11,12] and *B. Strigosa*[13] were reported to contain iridoids, flavonoids, sterols and triterpenoids and only *B. buxifolia* was reported to contain anthraquinones, which are 1-hydroxy-7-methylanthraquinone[14] (barleriaquinone), 1-hydroxy-7-carbomethoxyanthraquinone, 1-hydroxy-2-carbomethoxy-7-methylanthraquinone and 1-hydroxy-5-carbomethoxy-7-methylanthraquinone[15]. *B. longiflora* has apprarently not been examined so far and our chemical examination led to the isolation of four anthraquinones of which two are new compounds. Arnidiol and a sterol mixture were also isolated. The structure elucidation of new compounds and identification of known compounds are described herein. The anthraquinones isolated from *B. longiflora* were not reported from *B. buxifolia*.

EXPERIMENTAL

Plant material: The plant material of *B. longiflora* Linn. F. was collected in October 1996 from the Kailasagiri hillytracts of Visakhapatnam. The botanical identity of the plant was established in the Department of Botany, Andhra University, Visakhapatnam. A voucher specimen has been deposited in the herbarium of the Department of Botany, Andhra University.

Extraction and isolation of compounds: The air-dried stems (1.6 kg) were coarsely powdered and successively extracted with hexane, chloroform and methanol. The three extracts when concentrated separately yielded residues of 4.3 g, 6 g and 9 g respectively.

The air-dried roots (0.35 kg) were powdered and successively extracted with chloroform and methanol which on concentration yielded 2 g and 3 g of residues respectively.

The hexane extract of stems and chloroform extract of stems and roots gave positive tests for steroids/terpenoids and anthraquinones. The methanol extracts did not give positive tests for flavonoids, anthraquinones and steroids/terpenoids and the TLC and PC pictures showed only streaks. Column chromatography did not yield any compound. Isolation of compounds from the other extracts by column chromatography over silica gel (ACME, 100-200#) is given in Table-1.

Substance BI-4 (100 mg) was found to be a mixture of anthraquinones, which on rechromatography over silica

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gel (ACME, finer than 200#) and fractional elution with hexane-chloroform (9:1) yielded three anthraquinones **BI-4a** (8 mg), **BI-4b** (10 mg) and **BI-4c** (9 mg) in pure state.

Identification and characterisation of compounds:

BI-1 (tectoquinone) crystallised from petroleum ether as light orange coloured flakes, m.p. 175-76° and showed a single spot in silica gel TLC. It gave pink colour with aq. NaOH and a positive Borntrager reaction. Elemental analysis: Found C, 81.05; H, 4.4%; Calcd. for $C_{15}H_{10}O_2$: C, 81.08; H, 4.5%

BI-2 (Sterol mixture) was obtained as colourless crystals, m.p.146-48°. It showed a single spot in silica gel TLC and gave a positive LB test for sterols. The sterol mixture (20 mg) was acetylated by treating with pyridine (2 ml) and acetic anhydride (3 ml) and keeping aside at room temperature for 24 h. It wan then heated for 3 h at 95°. The acetate derivative crystallised from methanol and showed a single spot in silica gel TLC. It was subjected to GCMS analysis.

BI-3 (Arnidiol) was crystallised from hexane-ethylacetate, m.p. 257-59°, $[\alpha]_D^{28}$ + 76.25° (c. 0.13 $CHCl_3$). It gave violet colour in LB reaction and purple colour with conc. H_2SO_4. Elemental analysis: Found: C, 81.12, H, 11.02%; Calcd. for $C_{30}H_{50}O_2$: C, 81.44; H, 11.31%, IR_{vmax} (KBr): 3380, 2940, 1450, 1380 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 3.58 (1H, br d, J=11.3 Hz, 3α-H), 0.89 (3H, s, 23-CH_3), 0.66 (3H, s, 24-CH_3), 0.86 (6H, s, 25&27-CH_3), 0.95 (3H, s, 26-CH_3), 0.73 (3H, s, 28-CH_3), 0.87 (3H, d, J=6.8 Hz, 29-CH_3),4.46 & 4.58 (1H each, *br s*, 30=CH_2). EIMS *m/z* (rel. int.) 442 $[M]^+$ (14), 411 (50) 385 (11), 234 (13) 189 (100).

Preparation of diacetate: Ten milligrams of **BI-3** was acetylated at room temperature with 1 ml of pyridine and 0.5 ml of acetic anhydride and heated in a water bath for 2 h. The product when worked up in the usual way gave diacetate as crystals from hexane-ethyl acetate, m.p. 192-94°.

BI-4a (3,8-Dihydroxy-4-methoxy-2-methylanthraquinone): It was obtained as yellow crystals, m.p. 210-12° from hexane-chloroform. It gave a pink colour with aq. alkali and violet colour with conc. H_2SO_4 which slowly turned into pink. It was soluble in Na_2CO_3 solution. Elemental analysis: Found: C, 67.24; H, 4.03%, Calcd. for $C_{16}H_{12}O_5$: C, 67.6; H, 4.22%. Visible spectrum λ_{max} ($CHCl_3$): 405 nm. IR_{vmax} (KBr): 3434, 2922, 1684, 1630 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.39 (3H, s, 2-CH_3), 4.00 (3H, s, 4-OCH_3), 6.76 (1H, s, D_2O exchangeable, 3-OH), 7.25 (1H, dd, J=8.1 HZ, 1.1 Hz, 7-H), 7.61 (1H, t, J=8.1 Hz,6-H), 7.76 (1H, dd, J=8.1 Hz, 1.1 Hz, 5-H) 7.96 (1H, s, 1-H), 12.82 (1H, s, D_2O exchangeable, 8-OH). EIMS m/z (rel. int.) 284 $[M]^+$ (8), 266 (100), 238 (92), 210 (8) 181 (9).

BI-4b (1,3,5-Trihydroxy-4-methoxy-2-methylanthraquinone): It was obtained as orange red needles from hexane-chloroform, m.p. 209-11°. It gave dark pink colour with aq. alkali and violet colour with conc. H_2SO_4 which turned slowly pinkish violet. It was soluble in aq. Na_2CO_3 and gave light greenish yellow colour with $FeCl_3$.

Elemental analysis: Found: C, 63.84; H, 3.94%; Calcd. for $C_{16}H_{12}O_6$: C, 64.0; H, 4.0%. Visible spectrum λ_{max} ($CHCl_3$): 442 nm. IR_{vmax} (KBr) : 3434, 2922, 1608, cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) : δ 2.28 (3H, s, 2-CH_3), 3.99 (3H, s, 4-OCH_3), 6.99 (1H, s, D_2O exchangeable, 3-0H), 7.28 (1H, dd, J=8.1 Hz, 1.1 Hz, 6-H), 7.65 (1H, t, J=8.1Hz, 7-H), 7.82 (1H, dd, J=8.1 Hz, 1.1 Hz, 8-H), 12.91 (1H, s, D_2O exchangeable, 5-OH), 14.02 (1H, s, D_2O exchangeable, 1-OH). EIMS *m/z* (rel. int.): 300 $[M]^+$ (100), 282 (67), 266 (28), 257 (52), 238 (25), 226 (26), 201 (12),173 (14).

BI-4c (1,3,4-Trihydroxy-5(or8)-methoxy-2-methylanthraquinone): This was obtained by crystallisation from hexane-chloroform, m.p. 255-57°. It gave dark pink colour with alkali and blue colour with conc. H_2SO_4. It was soluble in Na_2CO_3 solution.It gave pink colour with methanolic magnesium acetate and green colour with $FeCl_3$. Elemental analysis: Found: C, 63.63; H, 3.86%; Calcd. for $C_{16}H_{12}O_6$; C, 64.0; H, 4.0%. Visible spectrum λ_{max} ($CHCl_3$): 448 nm. IR ν_{max} (KBr): 3434, 2922, 1619 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.26 (3H, s, 2-CH_3), 3.97 (3H, s, 5 or 8-OCH_3), 7.05 (1H, s, D_2O exchangeable, 3-OH), 7.25 (1H, dd, J=8.1 Hz, 1.1 Hz, 6 or 7-H) 7.63 (1H, t, J=8.1Hz, 7 or 6-H), 7.78 (1H, dd, J=8.1 Hz, 1.1 HZ, 8 or 5-H), 12.20 (1H, s, D_2O exchangeable, 4-OH), 13.23 (1H, s, D_2O exchangeable, 1-OH). EIMS *m/z* (rel. int.) : 300 $[M]^+$ (100), 282 (56), 271 (10), 257 (40), 226 (50), 201 (15), 173 (18).

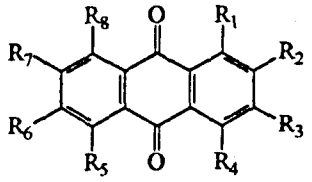
RESULTS AND DISCUSSION

BI-1 gave positive tests for anthraquinones and showed $[M]^+$ ion peak *m/z* 222 and analysed for the formula $C_{15}H_{10}O_2$. Its IR, UV, ^1H NMR and MS suggested it to be 2-methylanthraquinone (tectoquinone) which has been reported to be present in heartwood and sapwood of *Tectona grandis* (teak wood) and in many other plants[16]. The identity was confirmed by direct comparison with an authentic sample.

Table 1 : Isolation of compounds

CC of extract	Eluant composition	Substance isolated	Yield (mg)
Hexane extract of stems	5% EtOAc in hexane	BI-1	25
	10% EtOAc in hexane	BI-2	35
	10% EtOAc in hexane	BI-4c	15
	15% EtOAc in hexane	BI-3	20
Chloroform extract of stems	5% EtOAc in hexane	BI-1	15
	10% EtOAc in hexane	BI-2	40
Chloroform extract of roots	5% EtOAc in hexane	BI-2	20
	10% EtOAc in hexane	BI-4	100

CC : Column chromatography.

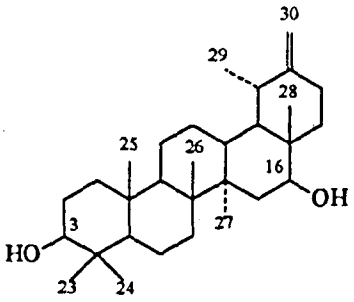


1. $R_2 = CH_3$, others = H

4a. $R_2 = CH_3$, $R_3 = R_8 = OH$, $R_4 = OCH_3$, others = H

4b. $R_1 = R_3 = R_5 = OH$, $R_2 = CH_3$, $R_4 = OCH_3$, others = H

4c. $R_1 = R_3 = R_4 = OH$, $R_2 = CH_3$, R_5 or $R_8 = OCH_3$, others = H



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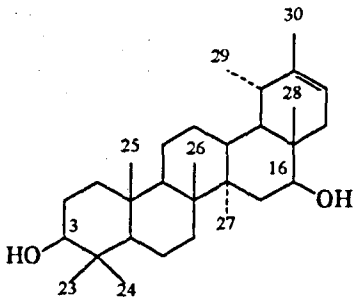

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BI-2 gave positive LB reaction. The ^1H NMR and MS data indicated that it could be sterol mixture. It was acetylated and the acetate was subjected to GCMS analysis. The GCMS showed four peaks with retention times 6.093, 13.067, 13.475 and 14.408 respectively. The major component of the mixture (88.01%) was not identified due to the absence of $[M\text{-}CH_3COOH]^+$ peak. The fragment ions observed were at *m/z* 279, 167, 149, 113, 83, 71, 57 and 43. The minor components were identified as campesterol (2.19%) [*m/z* 382, $[MCH_3COOH]^+$], stigmasterol(6.52%)[*m/z* 394, $[M\text{-}CH_3COOH]^+$]and β-sitosteol (3.28%) [*m/z* 396, $[M\text{-}CH_3COOH]^+$].

BI-3 gave a positive LB reaction and showed $[M]^+$ ion at m/z 442, consistent with the formula $C_{30}H_{50}O_2$. The IR spectrum showed broad absorption bands at 3580 to 3240 cm^{-1}. It formed a diacetate with acetic anhydride

and pyridine and did not form a methyl derivative with ethereal diazomethane. This suggested that it contains only two alcoholic hydroxyl groups. The ^{1}H NMR spectrum showed singlets at δ 0.66, 0.73, 0.86, 0.86, 0.89 and 0.95 corresponding to six tertiary methyl groups and a doublet at δ 0.87 (J=6.8 Hz) characteristic of secondary methyl group. A broad doublet at δ 3.58 (3α-H) and two broad singlets at δ 4.46 and 4.58 characteristic of exocyclic methylene protons were also observed. By correlating the m.p., optical rotation and spectral data **BI-3** was identified as arnidol which was first isolated from *Arnica montana* and *Tussilago farfara*[17,18]. Later the structure was revised by Pyrek and Baraboweka[19]. We isolated arnidiol (3) as a single component without admixture with faradiol (5) which was usually the case.

BI-4a showed $[M]^+$ ion peak at *m/z* 284 and analysed for the formula $C_{16}H_{12}O_5$. The IR spectrum showed bands at 3434 cm^{-1}, (OH), 1684 (unchelated carbonyl) and 1630 (carbonyl). The ^{1}H NMR spectrum showed peaks corresponding to a *peri*-OH, a β-OH, a methoxy and a methyl group. Thus it could be a dihydroxy monomethoxy monomethyl anthraquinone. A set of peaks characteristic of aromatic protons in the ^{1}H NMR spectrum revealed that one of the rings of anthraquinone is mono substituted and the other one is trisubsitituted. The presence of aromatic proton singlet at δ 7.96 in ^{1}H NMR, indicates its position adjacent to methyl, because the proton adjacent to hydroxyl usually appears at lower δ value i.e. around 7.0. The compound did not respond to Shibata's test[20] (methanolic magnesium acetate), characteristic of vicinal hydroxyl groups which gave support for placement of methoxyl at 4- position. The remaining hydroxyl may be at 5 or 8 position. The 5-hydroxy compound was already reported as obtusifolin from *Cassia obtusifolia*[21] and its m.p. was given as 237-238° while the m.p. of **BI-4a** was found to be 210-212° and hence it could be in all probability the 8-hydroxy compound i.e. 3,8-dihydroxy-4-methoxy-2-methylanthraquinone which has not been so far reported either as a natural product or by synthesis.

BI-4b displayed $[M]^+$ peak at m/z 300, consistent with the formula $C_{16}H_{12}O_6$. The IR spectrum showed bands at 3434 (OH), 1608 cm^{-1}. (chelated carbonyl). The ^{1}H NMR spectrum showed, two *peri* hydroxyls, a β-OH, a methoxyl and a methyl group indicating it could be a trihydroxy monomethoxy monomethylanthraquinone. A set of peaks characteristic of aromatic protons in ^{1}H NMR spectrum revealed that one of the rings of anthraquinone is monosubstituted and the other one is tetrasubsitututed. It was confirmed by ^{1}H-^{1}H COSY spectrum which showed connectivities between aromatic protons. Based on the IR spectrum, 1,3,8-trihydroxy structure was ruled out, because of the presence of only one carbonyl absorption band (at 1608 cm^{-1}), characteristic of chelated carbonyl. The compound did not respond to Shibata's test and hence 1,3,4-trihydroxy structure was also ruled out leaving 1,3,5-trihydroxy structure. The chemical tests, spectral data and m.p. suggested that it could be 1,3,5-trihydroxy-4-methoxy-2-methylanthraquinone which was reported earlier from *Ventilago calyculata*[22] and also synthesized[23]. The other properties also corresponded with the compound described in literature. This is the second report of its occurrence in nature.

BI-4c also showed $[M]^+$ peak at m/z 300, consistent with the formula $C_{16}H_{12}O_6$. Thus it could be an isomer of compound **BI-4b.** The IR spectrum showed bands at 3434 (OH), 1619 cm^{-1} (chelated carbonyl). The ^{1}H NMR spectrum showed, two *peri* hydroxyls, a β-hydroxyl, a methoxy and a methyl groups. It gave pink colour with methanolic magnesium acetate and hence the two *peri* hydroxyls and the β-hydroxyl were assigned to 1,3 and 4 positions respectively. The methoxyl group may be at 5 or 8 positions. As in **BI-4b** connectivities between aromatic protons in ^{1}H-^{1}H COSY spectrum were also observed. From the colour reactions and spectral data it was characterised as 1,3,4-trihydroxy-5 (or8)-methoxy-2-methylanthraquinone. With the available data it has not been possible to distinguish between these two possible structures. Both are not reported in the literature so far.

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