Arylnaphthalide Lignans from *Cleistanthus collinus*[1)]

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Chemical examination of the aerial parts of *Cleistanthus collinus* afforded the arylanphthalide lignans, cleistanone (1), diphyllin (2), cleistanthins A (3), C (4) and D (5), and 4-*O*-(3″-*O*-methyl-β-D-glucopyranosyl)-diphyllin (6). The first compound is a new member of the rare group of arylnaphthalide lignans containing an alkoxy group on the lactone ring. The structure of the compound was determined from its spectral data, chemical transformations and partial synthesis from diphyllin (2). The new lignan, 1 and its acetyl derivative, 7 were found to exhibit cytotoxicity against MT_2 cell lines.

Key words *Cleistanthus collinus*; arylnaphthalide lignan; cleistanone; cytotoxic activity

Cleistanthus collinus ROXB. (Euphorbiaceae) is a highly poisonous plant.[2)] Its alcoholic extract has been reported[3)] to show anticancer activity against epidermal carcinoma of the nasopharynx in tissue culture. Several lignans of different classes were previously isolated[4—8)] from the plant. During our investigation on the constituents of poisonous species for the discovery of new anticancer and antiviral agents we have examined the aerial parts of the plant. We have isolated a new arylnaphthalide lignan, cleistanone (**1**) along with five known lignans, diphyllin (**2**),[6)] cleistanthins A (**3**),[6)] C (**4**)[5)] and D (**5**)[6)] and 4-*O*-(3″-*O*-methyl-β-D-glucopyranosyl)-diphyllin (**6**).[8)] Here we discuss the structure elucidation of **1** from spectral and chemical evidence. The cytotoxic activity of the compound is also described.

Results and Discussion

Cleistanone (**1**) was analyzed as $C_{22}H_{18}O_8$ from its mass spectrum, elemental analysis and ^{13}C-NMR spectrum. The mass of the compound is 30 amu higher than that of the known constituent diphyllin (**2**) ($C_{21}H_{16}O_7$).[6)] The IR spectrum of **1** indicated the presence of hydroxyl and lactone carbonyl groups as well as an aromatic residue. The ^{1}H-NMR spectrum (400 MHz) showed the signals for five aromatic protons constituted by two singlets [(δ 7.62, 1H and 7.04, 1H)] and an ABX system [(δ 6.96, 1H, d, J=8.0 Hz; 6.84, 1H, dd, J=8.0, 1.5 Hz and 6.80, 1H, d, J=1.5 Hz)], one nonchelated hydroxyl [(δ 6.16, br s)], one methylenedioxy [(δ 6.06 and 6.04, each d, J=1.3 Hz)] and two aromatic methoxy groups [(δ 4.14, 3H, and 3.84, 3H, s)]. This spectrum suggested a close resemblance between the lignans **1** and **2**. Both of these lignans differ only in the structural pattern of the lactone ring. The ^{1}H-NMR spectrum of **1** revealed the presence of an aliphatic methoxy group [(δ 3.68, 3H, s)] and a downfield shifted proton [(δ 6.42, 1H, s)] at C-2a instead of a $-CH_2-$ group present in **2**. The NOESY (Nuclear Overhauser Effect Spectroscopy) spectrum showed the correlations between H-5 [(δ 7.04)] and H-6′ [(δ 6.84)], H-2′ [(δ 6.80)] and MeO-6 [(δ 3.84)], H-8 [(δ 7.62)] and MeO-7 [(δ 4.14)] and H-2a [(δ 6.42)] and MeO-2a [(δ 3.68)]. The structure of **1** was supported from its ^{13}C-NMR spectral data (*vide* Experimental) which were assigned by direct comparison with those of diphyllin (**2**) as well as by comparison with spectral values reported[9)] for the related lignans, phyllamyricins D and E.

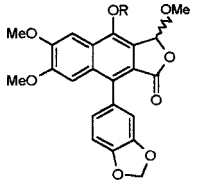
Acetylation of cleistanone (**1**) with acetic anhydride and pyridine afforded a monoacetate (**7**) while the methylation with diazomethane yielded the monomethyl ether (**8**). The spectral properties (^{1}H-NMR and MS) of the latter were found to be similar to those reported for justicidine P.[10)]

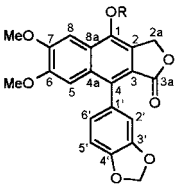
Finally the preparation of cleistanone (**1**) (in racemic form) was achieved from diphyllin (**2**). The latter was treated[10)] with N-bromosuccinimide (NBS) in the presence of azobisisobutyronitrile (AIBN) followed by subsequent treatment with MeOH. The product was identical in spectral properties (^{1}H-NMR and MS) with the natural cleistanone (**1**).

The structure of **1** was thus established as 2a-methoxydiphyllin, that is, 1-hydroxy-2a-methoxy-2-(-hydroxymethyl)-6,7-dimethoxy-4-(3,4-methylenedioxy-phenyl)-3-naphthoic acid-γ-lactone. The chirality of the molecule could not be determined with all its spectral data.[9)]

It is worth mentioning that the ^{1}H- and ^{13}C-NMR spectra (200 MHz) of cleistanone (**1**) indicated the existence of two inseparable rotamers due to the restricted rotation about the C-4–C-1′ bond. The ^{1}H-NMR signals appearing for H-2′, H-5′ and H-6′ were slightly broad and some of the signals in the ^{13}C-NMR spectrum were split. In the 400 MHz ^{1}H-NMR spectrum the doubled signals for MeO-2a, H-2a and for the protons of the lower ring were clearly observed (Experimental). The existence of such rotamers was also previously noted for the structurally similar lignans, justicidin P[10)] and phyllamyricins D and E.[9)]

Cleistanone (**1**) is a new member of the rare group of aryl-





1 R= H
7 R= Ac
8 R= Me

2 R=H
3 R=3,4-di-*O*-methyl-D-xylose
4 R=β-2,3-di-*O*-methyl-D-xylopyranosyl-(1→4)-β-D-glucopyranose
5 R=4-*O*-3″-*O*-methyl-β-D-glucopyranoside
6 R=2,3,5-tri-*O*-methyl-D-xylose

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naphthalide lignans containing an alkoxy group on the lactone ring.[10] Previously only a few compounds of this group like justicidine P[10] and phyllamyricins D and E[9] were reported.

The known constituents, diphyllin (**2**),[6] cleistanthins A (**3**),[6] C (**4**)[5] and D (**5**)[6] and 4-*O*-(3″-*O*-methyl-*β*-D-glucopyranosyl)-diphyllin (**6**)[8] were characterized by comparison of their physical and spectral properties with those reported in the literature.

The cytotoxicity of cleistanone (**1**) and its acetyl derivative, **7** was tested against MT_2 cell lines using etoposide as standard. The LD_{50} values for the first two compounds were found to be 38.1 μM and 27.2 μM respectively while the value for etoposide was 22.1 μM.

Experimental

General Melting points were measured in a Buchi-510 instrument and are uncorrected. Spectra were recorded with the following instruments: IR, Perkin Elmer spectrophotometer; ^{1}H- and ^{13}C-NMR, Varian Gemini 200 MHz and Varian INOVA 400 MHz; LSI-MS (liquid secondary ionization mass spectroscopy), Micromass Quattro and EI-MS (electron ionization mass spectroscopy), Micromass VG 7070H (70 eV). Optical rotation was determined with a Jasco DIP 360 digital polarimeter. Column chromatography was performed over silica gel (BDH 100—200 mesh) and TLC with silica gel GF 254. The spots were detected in an iodine chamber and under UV lamp. The spots were also visualized by spraying the plates with 10% methanolic H_2SO_4 and subsequently heating on a hot plate.

Plant Materials The aerial parts of *C. collinus* were collected from the forest of Kaleshwaram in Karimnagar district, Andhra Pradesh in the month of February, 2000 and were botanically identified by professor T. Rajugopal, Department of Botany, Osmania University. A voucher specimen (NO IIC-1482) was preserved in the herbarium of our institution.

Extraction and Isolation The shade dried plant materials (5 kg) were powdered and extracted thrice with CH_2Cl_2–MeOH (1 : 1, 6 l) at room temperature. Each extraction was continued for 6 d. The total extract was concentrated under reduced pressure to afford a brown gummy residue (78 g). A part of the residue (3 g) was preserved and the remaining (75 g) was subjected to column chromatography. The column was eluted with solvents of increasing polarity using a mixture of $CHCl_3$ and MeOH. The eluates were collected in fractions of 100 ml each and concentrated. Following the TLC analysis, eluates of similar profiles were combined to give four fractions which were rechromatographed and eluted with a mixture of $CHCl_3$ and MeOH. From the first fraction diphyllin (**2**, 80 mg) and cleistanone (**1**, 21 mg), from the second fraction cleistanthins A (**3**, 1.2 g) and D (**5**, 918 mg), from the third fraction 4-*O*-(3″-*O*-methyl-*β*-D-glucopyranosyl)-diphyllin (**6**, 24 mg) and from the last fraction cleistanthin C (**4**, 1.6 g) were obtained.

Cleistanone (**1**): Pale yellow crystals, mp 217—218 °C (MeOH), $[\alpha]_D^{25}$ +4° (c=1.5, MeOH); IR (KBr) ν_{max} 3415, 1729, 1618, 1504, 1432 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ: 7.58 (s, H-8), 7.07, 7.06 (each s, H-5), 6.93 (br d, J=8.0 Hz, H-5′), 6.83, 6.79 (each dd, J=8.0, 1.5 Hz, H-6′), 6.81 (br d, J=1.5 Hz, H-2′), 6.42, 6.41 (each s, H-2a), 6.08 (br s) and 6.04 (t, J=1.3 Hz, –OCH_2O–), 4.07 (s, MeO-7), 3.81, 3.801 (each s, MeO-6), and 3.69, 3.68 (each s, MeO-2a); ^{13}C-NMR (50 MHz, $CDCl_3$): δ: 167.3 (C-3a), 151.3 (C-7), 150.8 (C-6), 147.5 (C-3′, C-4′) 145.7 (C-1), 132.4 (C-4), 131.7 (C-4a), 128.3 (C-1′), 123.9, 123.7 (C-6′), 123.5 (C-2), 119.2 (C-3), 110.9, 110.7 (C-2′), 108.2 (C-8), 106.4 (C-5′), 101.2 (–OCH_2O–), 100.8 (C-5), 99.7 (C-2a), 56.1 (MeO-6, MeO-7), 55.8 (MeO-2a); LC/MS: m/z 433 $[M+Na]^+$. EI-MS. m/z: 410 (12), 384 (8), 360 (9), 341 (5), 275 (27), 260 (13), 214 (28), 202 (14), 155 (28); *Anal.* C, 64.28; H, 4.46%, Calcd. for $C_{22}H_{18}O_8$ C, 64.39; H, 4.39%.

Acetylation of Cleistanone (1) Ac_2O (0.2 ml) and pyridine (0.2 ml) were added to cleistanone (**1**, 5 mg) and the mixture was kept overnight. Usual work up afforded a monoacetyl derivative, **7** (5 mg). IR ν_{max} 3415, 1770, 1618, 1505, 1435 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$): δ: 7.20 (1H, s, H-8), 7.11 (1H, s, H-5), 6.94 (1H, d, J=8.0 Hz, H-5′), 6.82 (1H, dd, J=8.0, 1.5 Hz, C-6′), 6.80 (1H, d, J=1.5 Hz, H-2′) (the signals appearing for H-2′, H-5′ and H-6′ were slightly broad), 6.28 (1H, s, H-2a), 6.07 and 6.05 (2H each d, J=1.3 Hz, –OCH_2O–), 4.06 (3H, s, MeO-6), 3.80 (3H, s, MeO-7), 3.52 (3H, s, MeO-2a), 2.51 (3H, s, –OAc); LSI-MS m/z: 452 (M^+) (68), 439 (31), 421 (60), 379 (100), 307 (21).

Methylation of Cleistanone (1) Cleistanone (**1**, 5 mg) was dissolved in MeOH (5 ml) and an excess of fresh CH_2N_2 (in Et_2O) was added. After 2 h the solvents were removed to yield the monomethyl ether **8** (5 mg). The spectral properties (IR, ^{1}H-NMR and MS) of the compound were identical to those reported[10] earlier for justicidine P.

Preparation of (±) Cleistanone [(±) 1] from Diphyllin (2) A mixture of diphyllin (**2**, 20 mg) in CCl_4 (10 ml) containing NBS (20 mg) and AIBN (8 mg) was heated at 80 °C for 3 h. The mixture was cooled and the solvent was removed under reduced pressure. The residue was dissolved in a mixture of MeOH and CH_2Cl_2 (1 : 1, 20 ml) and was stirred for 3 h at room temperature. After concentration the product was purified by column chromatography to afford (±) cleistanone [(±) **1**] (14 mg), $[\alpha]_D^{25}$ +0° (c=1.25, MeOH). The spectral data of the latter were similar to those of the new lignan.

Determination of Cytotoxicity The compounds, **1** and **7**, were tested for their anti-proliferative activity using MTT colorimetric assay.[11] The assay was performed in a flat bottomed 96 well tissue culture plate. The MT_2 cell lines were cultured in RPMI 1640 (Sigma cat # R4130) with 10% FBS (Sigma cat # F2442). Medium (100 μl) containing 5×10^4 cells were cultured with increasing concentration of the compounds and incubated for 24 h at 37 °C under 5% CO_2. Methylthioazotetrazolium (20 μl) (MTT) (Sigma cat # M5655) was added to the culture to achieve a final concentration of 0.5—2 mg/ml and incubation continued for 4 h under similar conditions. A purple-blue formazan precipitate will be formed upon reduction of MTT by mitochondria of proliferating cells. The precipitate was dissolved by incubating with 100 μl of acidic isopropanol for 30 min. The intensity of color developed was measured at 490 nm. Etoposide was used as standard inhibitor. The % viability is calculated based on a control experiment conducted in absence of an inhibitor. The LD_{50} was determined based on the concentration required for inhibiting 50% viability of the given cell lines. The data are presented as the average of three independent determinations.

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References and Notes

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