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CLEISTANTHOSIDE B, A DIPHYLLIN GLYCOSIDE FROM *CLEISTANTHUS PATULUS* HEARTWOOD

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Key Word Index—*Cleistanthus patulus*; Euphorbiaceae; heartwood; diphyllin; glycoside; cleistanthoside B.

Abstract—From the heartwood of *Cleistanthus patulus*, a new diphyllin glycoside, cleistanthoside B, has been isolated and its structure elucidated as diphyllin-4-yl 4-*O*-methyl-β-D-xylopyranoside from its chemical properties and spectral data.

INTRODUCTION

Cleistanthus collinus is a rich source of lignans of which cleistanthin A (**1**) [1–3] is reported to be cytotoxic [4]. As part of our study on lignans we earlier examined the heartwood of *C. patulus* Muell. Arg. and reported the isolation and characterization of cleistanthoside A, besides the known lignans (+)-sesamin, taiwanin C, paulownin, diphyllin and cleistanthin A [5]. We have found cleistanthoside A, cleistanthin A and diphyllin to be cytotoxic in 9KB cells (K. V. Sastry, E. Venkata Rao and W. Litcher, unpublished work). We report here the isolation and structure of another new lignan glycoside designated cleistanthoside B (**2**).

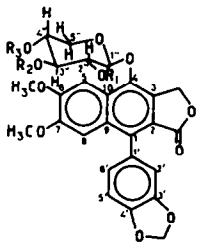
RESULTS AND DISCUSSION

The benzene-soluble residue of the methanol extract of *C. patulus* heartwood when chromatographed over silica gel afforded cleistanthoside B (**2**), mp 178–179°, $[\alpha]_D$ −62.2°, molecular formula $C_{27}H_{26}O_{11}$. Colour tests and UV and IR spectra indicated it to be an arylnaphthalide lignan glycoside.

Hydrolysis of **2** with acid yielded diphyllin as the aglycone, identified by comparison with authentic diphyllin, its acetate and methyl ether, and one sugar that gave a pink colour with aniline oxalate spray, suggesting a methylated pentose. The ^{1}H NMR spectrum of the glycoside diacetate (**3**), mp 138–140°, $[\alpha]_D$ − 10.8°, contained signals due to three methoxyl groups, two derived from the diphyllin portion (δ3.79 and 4.05) and one from an *O*-methylated sugar (δ3.45). As expected for an *O*-glycoside, the mass spectrum of **2** did not show a molecular ion [6]. An ion at *m/z* 380 (relative intensity 100) corresponded to the M_r of the aglycone, diphyllin and ions at *m/z* 147 (7), 129 (3), 115 (5) and 97 (3) arose from the *O*-methylpentose moiety. PC of the sugar along with authentic *O*-methylpentoses in several solvent systems showed it to be 4-*O*-methyl xylose.

GC of the alditol acetate of the sugar gave a peak (R_t 2.15) which corresponded to those of 2-*O*-methyl-, 3-*O*-methyl- as well as 4-*O*-methyl-D-xylose [7]. The mass spectrum of the alditol acetate corresponded with that of 1,3,4,5-tetra-*O*-acetyl-2-*O*-methylpentitol, which can arise from either 2-*O*-methyl- or 4-*O*-methylxylose. Periodate oxidation of the glycoside followed by Smith degradation [8] did not afford the unoxidized sugar or ethylene glycol expected from 3-*O*-methyl- and 2-*O*-methylxylopyranosides, respectively. As expected for a 4-*O*-methylxyloside, the ^{1}H NMR spectrum of **3** showed high coupling constants for the sugar protons because of their diaxial relationship ($J_{1,2}$ = 7.3 Hz, $J_{2,3}$ = 9.2 Hz, $J_{3,4}$ = 9.0 Hz, $J_{4,5ax}$ = 9.65 Hz). The high value of $J_{1,2}$ shows that **2** is a β-xyloside; since the specific rotations of **2** and **3** are both negative, the sugar probably belongs to the *D*-series [9]. The ^{1}H NMR spectrum of **2** itself was less well resolved but showed clearly the presence of a mono-*O*-methyl sugar.

The ^{13}C NMR spectrum of **2** was consistent with the 4-*O*-methylxylopyranoside structure. In methyl- β-D-xylopyranoside, carbons 2, 3 and 4 resonate at δ74.0, 76.9 and 70.4, respectively [10]. *O*-Methylation causes a downfield shift of *ca* 10 ppm [11] so it is reasonable to assume that the signals at δ73.42, 75.27 and 78.34 in the spectrum



1 R_1 = H, R_2 = R_3 = CH_3 CLEISTANTHIN A
2 R_1 = R_2 = H, R_3 = CH_3 CLEISTANTHOSIDE B
3 R_1 = R_2 = $COCH_3$, R_3 = CH_3 CLEISTANTHOSIDE B DIACETATE
4 R_1 = R_2 = R_3 = CH_3 CLEISTANTHIN A METHYL ETHER

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of **2** are due to carbons 2″–4″; *O*-methylation has caused a downfield shift of 8 ppm.

The ^{13}C NMR data for **1**, cleistanthin A methyl ether (**4**) and cleistanthin D (2,3,5-tri-*O*-methylxylofuranosyldiphyllin) have been reported [3]. The sugar carbon signals in **1** and **4** are as expected, but we do not believe that the corresponding signals in cleistanthin D are consistent with a methylated xylofuranoside structure [11].

EXPERIMENTAL

The heartwood of *Cleistanthus patulus* Muell. Arg. was collected from the Bastar forests of central India identified by Dr. P. Narasimha Rao, Department of Botany, Nagarjuna University, Nagarjuna Nagar, India. A voucher specimen has been deposited at the Department of Botany, Andhra University. The following TLC systems (0.25 mm silica gel G) were used: system A, $CHCl_3$–MeOH (9:1); system B, $CHCl_3$–MeOH (99:1). Compounds were detected by observing fluorescence under UV light or by spraying with 10% alcoholic H_2SO_4 followed by heating at 100° for 2–3 min. PC of the sugars was performed on Whatman No. 1 paper using the following systems: system C, *n*-BuOH–C_5H_5N–H_2O (10:3:3); system D, *n*-BuOH–EtOH–H_2O (40:11:19) and system E, EtOAc–C_5H_5N–H_2O (10:4:3). Aniline oxalate spray was used for detecting sugars. GC of the alditol acetates of sugars was carried out on 3% OV 225 (1.52 m × 6.3 mm.) at 175° and 25 ml N_2/min.

Isolation of cleistanthoside B (**2**). The powdered heartwood (2 kg) was extracted with MeOH in a Soxhlet apparatus and concentrated *in vacuo* to a syrup. The syrup was absorbed over the exhausted marc (250 g), air-dried overnight until all the solvent was completely removed, and the absorbed material successively extracted with petrol, C_6H_6 and EtOAc. Evaporation of the C_6H_6 extract *in vacuo* gave a pale brown residue (20 g), which was subjected to CC over silica gel.

The $CHCl_3$–MeOH (49:1) eluate gave a yellowish-brown residue (1.6 g). Crystallization and recrystallization from MeOH afforded cleistanthoside B (**2**) as colourless feathery needles (600 mg), mp 178–179°, $[\alpha]_D$ −62.2° (dioxane; *c* 1), R_f 0.64 in system A. Positive Molisch test for sugars and Labat test for methylenedioxy group. UV λ_{max}^{MeOH} nm (log ε): 348 (3.80), 314 (4.46), 293 (4.12), 274 (4.08), 214 (3.64); IR ν_{max}^{KBr} cm^{-1}: 3345 (*br*) (OH), 1730 (γ-lactone), 1600 (aromatic), 945 (methylenedioxy); MS *m/z* (rel. int.): 380 [M of aglycone]$^+$ (100), 379 (3), 351 (2), 321 (10), 307 (5), 294 (4), 293 (20), 265 (3), 235 (2), 176 (4), 147 (7), 129 (3), 115 (5) and 97 (3); ^1H NMR [200 MHz, $(CD_3)_2SO$–$CDCl_3$]: δ2.77–2.87 (*m*, 1H, H-5″ ax), 3.01–3.12 (*m*, 1H, H-4″), 3.19 (*s*, 3H, 4″-OMe), 3.19–3.26 (*m*, 1H), 3.37–3.43 (*m*, 1H), 3.47 (*s*, 3H, aromatic OMe), 3.73 (*s*, 3H, aromatic OMe), 3.74–3.83 (*m*, 1H, H-5″ eq), 4.44 (*d*, 1H, $J_{1'',2''}$ = 7.4 Hz, H-1″), 4.55 [*s* (*br*), 1H, OH], 5.05–5.26 (*m*, 3H, incl. CH_2OCO), 5.76 and 5.77 (2*s*, 2H, OCH_2O), 6.44–6.50 (*m*, 2H, H-2′,6′), 6.63 (*d*, 1H, $J_{5',6'}$ = ~8 Hz, H-5′), 6.72 (*s*, 3H, H-5 or 8), 7.81 (*s*, 3H, H-8 or 5); ^{13}C NMR [50 MHz $(CD_3)_2SO$–$CDCl_3$]: δ55.33 and 55.95 (aromatic OMe), 58.17 (4″-OMe), 63.00 and 66.69 (Ar$\underline{C}H_2O$ and C-5″), 73.42 and 75.27 (C-2″ and C-3″), 78.34 (C-4″), 100.77, 101.13, 105.17, 105.57, 107.65, 110.28, 118.57, 123.12, 126.87, 128.03, 130.13, 130.21, 135.64, 144.30, 146.98 (×2), 149.74, 151.41, 169.44. (Calc. for $C_{27}H_{26}O_{11}$: C, 61.59; H, 4.98. Found: C, 61.78; H, 5.15%.)

Acetylation of **2** using Ac_2O–C_5H_5N gave compound **3**, which crystallized from $CHCl_3$–hexane as colourless needles, mp 138–140°, $[\alpha]_D$ −10.8° ($CHCl_3$; *c* 1.04); R_f 0.42 in system B. IR ν_{max}^{KBr} cm^{-1}: 1740, 1605, 925, 830; ^1H NMR (200 MHz, $CDCl_3$): δ2.10 (*s*, 3H, OCOMe), 2.12 (*s*, 3H, OCOMe), 3.32 (*dd*, 1H, $J_{5'' ax/5'' eq}$ = 11.7, $J_{5'' ax/4''}$ = 9.65 Hz, H-5″ ax), 3.45 (*s*, 3H, 4″-OMe), 3.58 (*m*, 1H, H-4″), 3.79 (*s*, 3H, aromatic OMe), 4.05 (*s*, 3H, aromatic OMe), 4.23 (*dd*, 1H, $J_{5'' eq/4''}$ = 5.0 Hz, H-5″ eq.), 5.07 (*d*, 1H, $J_{1'',2''}$ = 7.3 Hz, H-1″), 5.18 (*t*, 1H, $J_{3'',2''} = J_{3'',4''}$ = ~9 Hz, H-3″), 5.37 (*dd*, 1H, $J_{2'',3''}$ = 9.2 Hz, H-2″), 5.42 (*s*, 2H, CH_2OCO), 6.03 and 6.08 (2*d*, 2H, *J* = 1.6 Hz, OCH_2O), 6.74–6.81 (*m*, 2H, H-2′,6′), 6.94 (*d*, 1H, $J_{5',6'}$ = ~8 Hz, H-5′), 7.05 (*s*, 1H, H-5 or 8), 7.49 (*s*, 1H, H-8 or 5). (Calc. for $C_{31}H_{30}O_{13}$: C, 60.98; H, 4.95. Found: C, 61.17; H, 5.18%.)

Hydrolysis of **2**. Compound **2** (300 mg) was hydrolysed with 2 M aq. alcoholic HCl (15 ml) at 100° for 3 hr. The solvent was removed *in vacuo* while adding some H_2O and the product left overnight in a refrigerator. The aglycone that separated was filtered, washed free from sugar, and recrystallized from MeOH to give pale yellow flakes, mp and mmp 289–290° (lit. [12] mp 289–291°). The aglycone formed an acetate, mp and mmp 233–235° (lit. [12] mp 234–235°) and a methyl ether (justicidin A), mp and mmp 262–263° (lit. [13] mp 261–262°).

The filtrate of the hydrolysate was passed through a column of Dowex 2 (CO_3^{2-}) resin. Lyophilization of the eluate yielded a colourless syrup (85 mg), $[\alpha]_D$ +9° (H_2O; *c* 1.01) with the following $R_{rhamnose}$ values in PC: 1.10 in system C, 1.25 in system D and 2.15 in system E. It gave a pink colour with aniline oxalate spray and co-chromatographed with 4-*O*-methyl-D-xylose, obtained by acid hydrolysis of its β-benzyl glycoside obtained through the courtesy of Professor P. J. Garegg. The alditol acetate of the sugar was prepared by reducing it (20 mg) with $NaBH_4$ (80 mg) in H_2O. After decomposition of the excess BH_4^- by the addition of HOAc, the Na^+ ions were removed by ion-exchange chromatography [Dowex 50 (H^+)] and the borate was removed by evaporating *in vacuo* repeatedly while adding a little MeOH each time. The reduced sugar was acetylated with Ac_2O–C_5H_5N [14]; MS *m/z*: 261, 256, 145, 139, 128 and 117. Periodate oxidation of **2** (10 mg) in EtOH (2 ml) with 0.1 M $NaIO_4$ (2 ml) followed by Smith degradation [8] and PC did not yield any sugar or ethylene glycol among the products.

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