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CYCLOTRITERPENES FROM THE HEARTWOOD OF *PTEROSPERMUM HEYNEANUM*

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Key Word Index—*Pterospermum heyneanum*; Sterculiaceae; heartwood; 9,19-cyclotriterpenes; 30-norcyclopterospermone; cyclopterospermol; 30-norcyclopterospermol.

Abstract—The new triterpenes, 30-norcyclopterospermone, cyclopterospermol and 30-norcyclopterospermol isolated from the heartwood of *Pterospermum heyneanum* and their structures were established as 30-nor-22-methylene-9,19-cyclolanostan-3-one, 22-methylene-9,19-cyclolanostan-3β-ol and 30-nor-22-methylene-9,19-cyclolanostan-3β-ol, respectively. In addition *n*-octacosanol, 3-hydroxy-5-methoxy-2-methylbenzoquinone, sitosterol and its glucoside were also isolated.

INTRODUCTION

In continuation of our work on the plants of the Sterculiaceae family [1] we have studied *Pterospermum heyneanum* Wall. (Syn: *Pterospermum xylocarpum*). Its leaves have medicinal use in the treatment of leucorrhoea [2]. Kaempferol, its 3-*O*-glucoside, kaempferide-7-*O*-glucoside and sitosterol have been isolated from the stem cuttings of *P. heyneanum*. [3,4]. We now report the isolation and structure elucidation of three new cyclotriterpenes, 30-norcyclopterospermone, cyclopterospermol and 30-norcyclopterospermol.

RESULTS AND DISCUSSION

The dry powdered heartwood of *P. heyneanum* collected locally was extracted successively with *n*-hexane, chloroform and methanol. The residue from the brown hexane extract was chromatographed over silica gel to give three new triterpenes together with *n*-octacosanol and sitosterol. A preliminary spectroscopic study recognised them as new 9,19-cyclotriterpenes and are so designated as 30-norcyclopterospermone (**1**), cyclopterospermol (**2**) and 30-norcyclopterospermol (**3**). The residue obtained after the evaporation of the brown chloroform extract on chromatography over silica gel yielded a rare quinone, 3-hydroxy-5-methoxy-2-methylbenzoquinone (**4**) and sitosterol glucoside. The methanol extract gave further quantities of sitosterol glucoside.

30-Norcyclopterospermol (**3**) $C_{30}H_{50}O$, $[M]^+$ at *m/z* 426, mp 144–145°, $[\alpha]_D + 62.0°$, was obtained in largest quantity and thus formed the key substance for a detailed chemical and spectroscopic study. It gave a pink colour in the Liebermann–Burchard test for a triterpene and a yellow colour with the TNM test for unsaturation. The presence of a cyclopropane ring was evident from its IR peak at 3040 cm^{-1} [5] and from the cyclopropyl protons in its ^1H NMR spectrum (δ0.34, 0.61 AB_q, J = 5 Hz, 2H) (Table 1) [6]. An exo-methylene group was revealed as a doublet at 4.63 but there was no resonance for a methyl on double bond and this ruled out the presence of an isopropenyl group. It yielded a monoacetate (**5**), $C_{32}H_{52}O_2$, $[M]^+$ at *m/z* 468, mp 121–122°, $[\alpha]_D + 136.0°$. From the characteristic mass fragmentation [7] of the compound and its acetate, with the ion at *m/z*, 300 in both (Table 2) (Scheme 1), it was readily recognised as a 9,19-cyclotriterpene with a nine carbon side chain and only one methyl at C-4 as in cycloeucalenol (**10**). With CrO_3–pyridine it formed a 3-ketoderivative (**1**), $C_{30}H_{48}O$, $[M]^+$ at *m/z* 424 which gave a 2,4-DNP derivative (**6**) (Scheme 2) as bright orange yellow needles. The compound (**3**) on treatment with MeOH–HCl gave an isomeric product, $C_{30}H_{50}O$ (**7**) crystallized from MeOH as shining plates, mp 104–105°, $[\alpha]_D + 20.0°$. The isomeric compound showed a weak absorption for a tetrasubstituted double bond (1645 cm^{-1}) in its IR spectrum. Its ^1H NMR spectrum revealed the presence of two methyls at δ1.51 and 1.58 on a double bond, both of which are formed during isomerisation, and its cyclopropane ring was intact with the methylene protons appearing as an AB_q at 0.33 (1H) and 0.61 (1H), (J = 4.5 Hz).

The above information suggests that the new cyclotriterpene has a nine carbon side chain with no methyl on a double bond but a methylene group which on isomerisation gives two methyls on a double bond. The extra carbon can be located at one of the positions (C-22, C-23 or C-24), the more probable being C-24 or C-22. The methylene group is not at C-24 since compound **3** differed in its physical and chemical properties from cycloeucalenol (**10**), which would have given **10a** on isomerisation. Alternatively it may be at C-22 in which case the side chain may have two possibilities, one having the methylene group at C-20 and the methyl at C-22 and the other having the methylene at C-22 and the methyl at C-20. It also differed in its characteristics from 30-norcycloswietenol, the triterpene with the former structure (**8**), isolated from *Swietenia mahagoni* [8].

30-Norcyclopterospermol might thus possess the structure **3** with a methylene at C-22 and a methyl at C-20 in which case 30-norcyclopterospermol and 30-norcyclo-

Table 1. ^{1}H NMR spectra of new cyclotriterpenes in $CDCl_3$ (90 MHz, TMS as internal standard, Chemical shifts in δ)

Compound	Cyclopropyl methylene protons as AB_q*	Methyls	Methylene protons as d*	3α-H	Other protons
1	0.33 (1H) 0.58 (1H) (5)	0.88, 0.96, 1.02 (18H, 6Me)	4.60 (2H) (7)	—	—
2	0.30 (1H) 0.56 (1H) (5)	0.80, 0.89, 0.96, 1.05 (21H, 7Me)	4.65 (2H) (4)	3.25 (1H, *m*)	—
3	0.34 (1H) 0.61 (1H) (5)	0.88, 0.96, 1.03 (18H, 6Me)	4.63 (2H) (5)	3.12 (1H, *m*)	—
5	0.36 (1H) 0.62 (1H) (5)	0.89, 0.96, 1.04 (18H, 6Me)	4.65 (2H) (5)	4.40 (1H, *m*)	2.02 (3H, *s*, -OCOMe)
6	0.35 (1H) 0.60 (1H) (5)	0.90, 1.00, 1.06 (18H, 6Me)	4.69 (2H) (4)	—	3.0 (1H, *brs*, NH), 7.99 (1H, *d*, J = 9Hz, 6′-H), 8.31 (1H, *d d*, J = 9, 2.5 Hz, 5′-H), 9.13 (1H, *d*, J = 2.5Hz 3′-H)
7	0.33 (1H) 0.61 (1H) (4.5)	0.87, 0.93, 1.00 (15H, 5Me) 1.51, 1.58 (6H, 2Me)	—	3.05 (1H, *m*)	—
9	0.32 (1H) 0.62 (1H) (5)	0.82, 0.88, 0.94, 1.03 (21H, 7Me)	4.62 (2H) (4)	4.40 (1H, *m*)	2.01 (3H, *s*, -OCOCH$_3$)
11	0.29 (1H) 0.53 (1H) (5)	0.77, 0.88, 0.94, 1.01 (18H, 6Me) 1.51, 0.58 (6H, 2Me)	—	3.05 (1H, *m*)	—

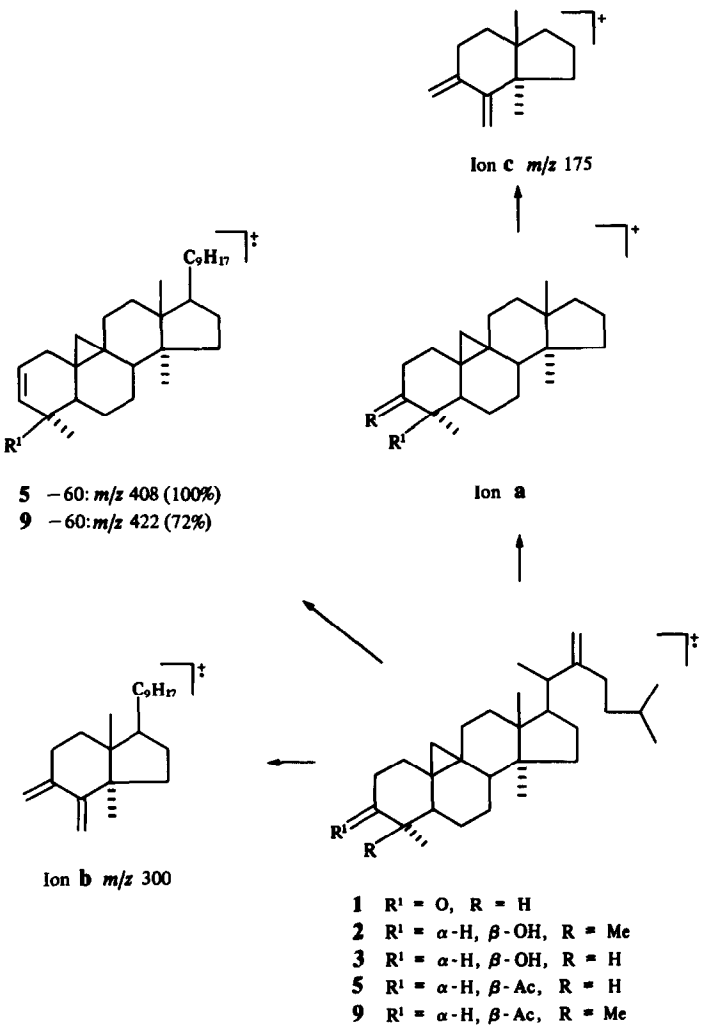
*J values in Hz are in parenthesis.

Table 2. Characteristic mass spectral fragments of the new triterpenes

Compound	M^+	Ion a	Ion b	Ion c
1	424(62)*	299(53)	300(27)	175(50)
2	440(36)	315(10)	300(95)	175(70)
3	426(9)	301(9)	300(15)	175(21)
5	468(24)	343(17)	300(61)	175(100)
9	482(6)	357(9)	300(25)	175(50)

*Rel. int. are given in parenthesis.

swietenol must give the same isomeric product upon treatment with MeOH–HCl. In fact this was found to be the case. Cyclopterospermol (**2**), $[M]^+$ at m/z 440, mp 124–125°, $[\alpha]_D$ + 72°, monoacetate (**9**), mp 98–99°, $[\alpha]_D$ + 84°, was recognised from its ^{1}H NMR spectrum (Table 1) and the prominent mass peak at m/z 300 (95%) as a homologue of 30-norcyclopterospermol and isomeric with cycloswietenol (**12**) another triterpene isolated from *S. mahagoni* [9,10]. Cyclopteropterospermol, like the nor-compound isomerised with MeOH–HCl to give the isomeric product **11** identical in every respect with isocycloswietenol obtained from **12** by similar isomerisation. The structures of 30-norcycloswietenol and cycloswietenol, as well as their isomeric products, were established unequivocally by spectral and extensive degradative evidence [8–10] where the 17-acetyl derivative obtained by ozonolysis of isocycloswietenylacetate was found ultimately to be identical with that obtained from cycloartenylacetate by side chain degradation [11]. The structures of 30-norcyclopterospermol (**3**) and



MS fragmentation of new triterpenes

Scheme 1.

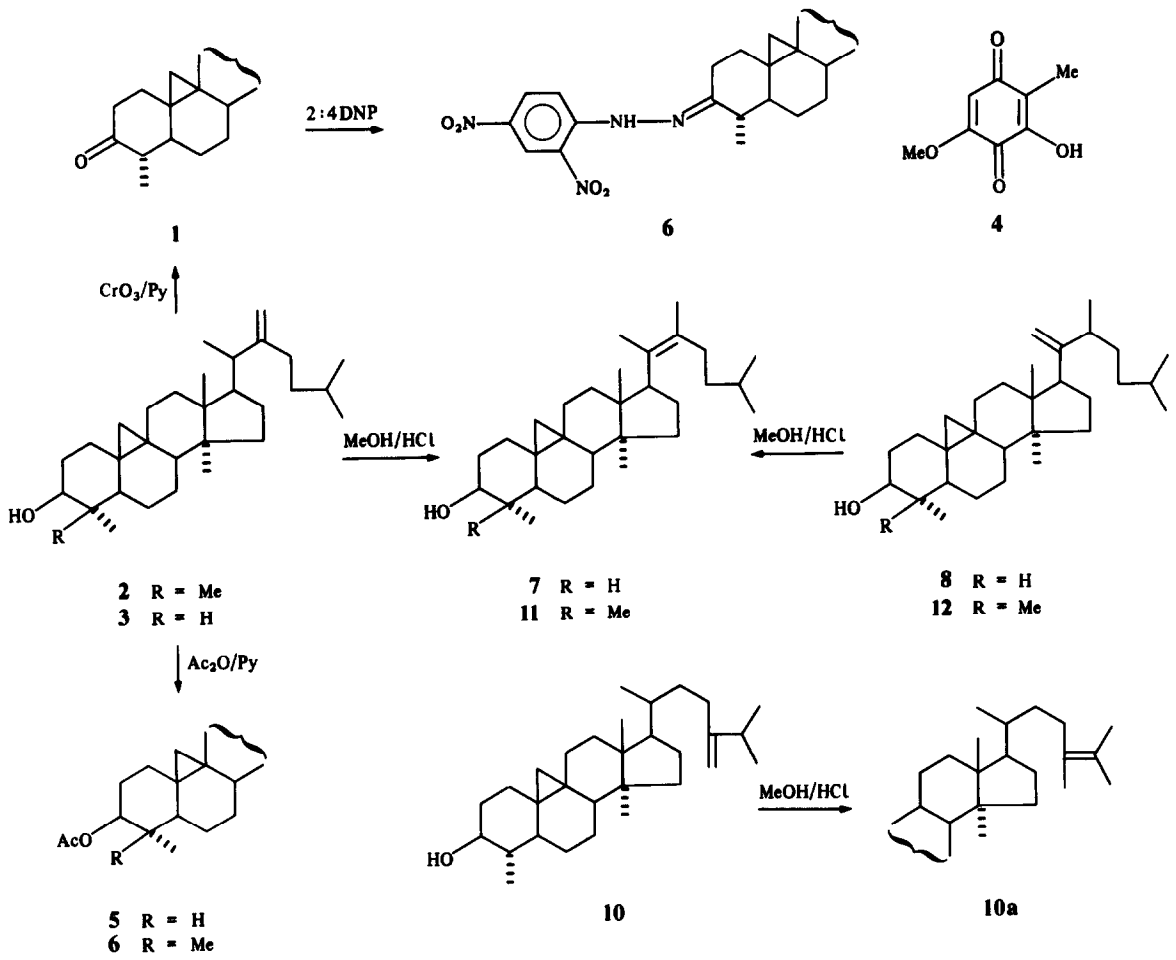
cyclopterospermol (2) were therefore established as 30-nor-22-methylene-9,19-cyclolanostan-3β-ol and 22-methylene-9,19-cyclolanostan-3β-ol, respectively.

30-Norcyclopterospermone (1), $C_{30}H_{48}O$, $[M]^+$ at m/z 424, mp 68–69°, $[\alpha]_D + 35°$ showed the carbonyl absorption (1715 cm^{-1}) in its IR spectrum and yielded a 2,4-DNP derivative, mp 261–162°. It was found to be identical in every respect with the ketone obtained by the oxidation of 3 thereby confirming its structure as 30-nor-22-methylene-9,19-cyclolanostan-3-one.

The ^{13}C NMR spectrum of 3 has now been studied and the assignments made for all the carbons in comparison with the literature values [12] lends further support to the assigned structure (Table 3). The signal at δ76.651 was assigned to C-3 with the β-OH group and the signals at 43.423 and 29.614 were assigned to C-5 and C-10 of the *trans*-AB junction in 3. The resonance signal at 44.663 was assigned to C-4 to which only one methyl group is attached. The signals at 23.626, 29.614 and 27.294 were assigned to C-9, C-10 and C-19, respectively, of the most strained cyclopropane ring. The cyclopropane ring in 3 resulted in the upfield shift of the C-9 resonance (23.626) with a much lower shift on C-10 (29.614). The signal at 156.970 was characteristic of the end methylene group of the side chain and was assigned to C-22.

The new triterpenes with a 22-methylene and the isomeric derivatives with 20-methylene may be biogenetically conceived to have been formed from a precursor 9,19-cyclolanost-20(22)-ene derivative. Alkylation of the side chain with methionine takes place with the methyl transfer [13] followed by a 1,2-hydride shift from C-22 to C-20 or from C-20 to C-22 leading to the formation of the isomeric triterpenes of the cyclopterospermol or cycloswietenol series, respectively (Scheme 3) [14].

Compound 4, $C_8H_8O_4$, $[M]^+$ at m/z 168, mp 201–202° (dec.) was recognised readily as a hydroxy quinone from its colour reactions, (brown colour with ferric chloride, brownish yellow colour of its alcoholic solution disappeared with the addition of $NaBH_4$ and the same was restored by simple shaking in air) [15] and spectral characteristics (maxima in UV at 288 and 420 nm in ethanol and IR frequencies at 3240 cm^{-1} for OH and



Scheme 2.

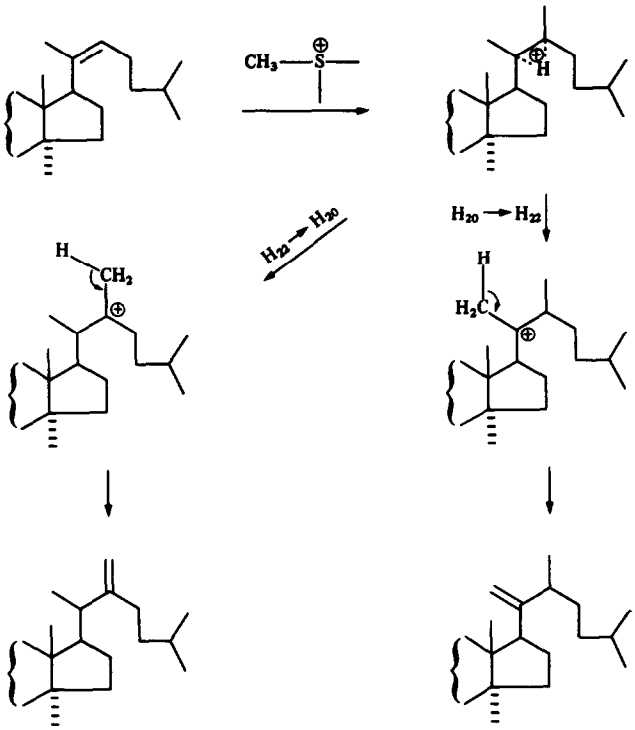
Table 3. ^{13}C NMR spectral data of 30-Norcyclopterospermol (22.63 MHz, TMS as internal standard)

Carbon	Chemical shift in δ	Carbon	Chemical shift in δ
1	30.854	16	27.078
2	34.000	17	52.323
3	76.651	18	17.800
4	44.663	19	27.294
5	43.423	20	36.194
6	24.759	21	18.394
7	28.157	22	156.970
8	46.929	23	33.875
9	23.626	24	35.489
10	29.614	25	31.394
11	25.244	26	21.954
12	55.116	27	22.062
13	45.419	28	19.203
14	48.979	29	14.456
15	32.958	31	106.049

1680 cm^{-1} for the quinone carbonyl). It gave a mono methyl ether $C_9H_{10}O_4$, mp 124–125°. Its ^{1}H NMR spectrum showed a methyl resonance at δ1.91, a methoxyl at 3.82 and a proton at 5.9 (1H, *s*), which suggested that it might be a trisubstituted benzoquinone such as a hydroxy-methoxy-toluquinone. Two isomeric quinones, 5-methoxy-6-hydroxy-2-methyl benzoquinone (fumigatin) and 3-hydroxy-5-methoxy-2-methylbenzoquinone are known with these structural features [16, 17]. A comparison of the physical and spectral characteristics of **4** proved its identity with 3-hydroxy-5-methoxy-2-methylbenzoquinone. A direct comparison with an authentic sample, however, could not be made. Its mass spectrum showed the characteristic peaks of a methoxybenzoquinone [18] at *m/z* 97 [M − 71, 15%]$^+$ and 69 [M − 99, 97%]$^+$ in addition to the usual fragments of a benzoquinone. Incidentally, this is the first report of its isolation from a plant source.

EXPERIMENTAL

Mps are uncorr. IR spectra were recorded on KBr discs and UV spectra were taken in EtOH. ^{1}H NMR and ^{13}C NMR



Biogenesis

Scheme 3.

spectra were taken in $CDCl_3$ and the values were reported in δ downfield to TMS. Optical rotations were taken in $CHCl_3$. For chromatography Achme's silica gel was used.

Isolation of compounds **1, 2, 3, 13** *and* **14**. The *n*-hexane extract from the heartwood powder of *P. heyneanum* (2 kg) was found to contain five spots on TLC (R_f 0.50 *n*-hexane–C_6H_6, 2:8; 0.51, 0.40, 0.30 and 0.26, C_6H_6; spray reagent; methanolic H_2SO_4 at 80°). The crude residue (15 g) obtained by the evaporation of the solvent under vacuum was dissolved in hot MeOH (60 ml) and the waxy material settled at the bottom was separated by decantation. The residue (4.5 g) from the defatted extract was chromatographed on a silica gel column eluting with varying proportions of *n*-hexane–C_6H_6 mixtures collecting 500 ml fractions to give the five compounds **1, 2, 3, 13** and **14**; 9:1 (fr. 1–6, **13**), 8:2 (fr. 7–12, **1**), 7:3 (fr. 13–30, **2**), 6:4 (fr. 31–50, **3**), 5:5 (fr. 51–65, **14**).

Identification. Compound **13**, colourless plates (MeOH, 100 mg) was identified as *n*-octacosanol and **14**, shining needles ($CHCl_3$–MeOH, 300 mg) as sitosterol by direct comparison with the authentic samples.

30-*Norpterospermone* (**1**). Colourless plates, mp 68–69° (MeOH, 60 mg). IR, ^1H NMR and mass spectral data are described in the text, ^1H NMR (Table 1); MS (Table 2). (Found: C, 84.86; H, 11.30. $C_{30}H_{48}O$ requires: C, 84.90; H, 11.32%).

2,4-*Dinitrophenyl hydrazone of* **1**. Compound **1** (20 mg) in MeOH (10 ml) was treated with 2,4-dinitrophenylhydrazine (15 mg) in MeOH (10 ml) and a drop of conc H_2SO_4. The reaction mixture was warmed for 5 min and left for 1 hr. The 2,4-DNP derivative was obtained as bright orange needles (MeOH, 18 mg), mp 261–262°. (Found: C, 71.48; H, 8.59. $C_{36}H_{52}O_4N_2$ requires: c, 71.52; H, 8.62%).

Cyclopterospermol (**2**). Colourless plates, mp 124–125° (MeOH, 100 mg). (Found: C, 84.50; H, 11.81. $C_{31}H_{52}O$ requires: C, 84.54, H, 11.82%). *Cyclopterospermyl acetate* (**9**). Compound **2** (30 mg) gave an acetate (Ac_2O–pyridine) as colourless needles (MeOH, 25 mg), mp 98–99°. (Found C, 82.09; H, 11.22. $C_{33}H_{54}O_2$ requires: C, 82.15, H, 11.20%).

Isocyclopterospermol (**2**) *conversion into isocycloswietenol* (**11**). Compound **2** (30 mg) in MeOH (10 ml) and HCl (5% 10 ml) was refluxed for 1 hr. The solid separated showed a purple spot on TLC, silica gel, C_6H_6, R_f: 0.40 (Spray reagent: 10% methanolic H_2SO_4) and crystallised from MeOH as shining plates (20 mg), mp 117–118°, $[\alpha]_D$ + 16.0°. (Found: C, 84.49; H, 11.81, $C_{31}H_{52}O$ requires: C, 84.54; H, 11.82%).

30-*Norcyclopterospermol* (**3**). Colourless feathery needles (MeOH, 130 mg). (Found: C, 84.45; H, 11.7. $C_{30}H_{50}O$ requires: C, 84.50, H, 11.73%). 30-*Norcyclopterospermyl acetate* (**5**). Compound **3** (40 mg) gave the acetate (Ac_2O–pyridine) as colourless needles, (MeOH, 30 mg). (Found: C, 81.98; H, 11.13. $C_{32}H_{52}O_2$ requires: C, 82.05; H, 11.11%). 30-*Norcyclopterospermone* (**1**). Compound **3** (40 mg) in pyridine (0.5 ml) was added to CrO_3 in pyridine (3 ml containing 40 mg of CrO_3) and left overnight at room temp. MeOH (10 ml) was added to decompose the excess of unreacted CrO_3 and the reaction mixture was poured into H_2O. The product was extracted with $CHCl_3$ and crystallized from MeOH as colourless plates (30 mg), mp 68–69°, $[\alpha]_D$ + 35.0°; (Found: C, 84.62; H, 11.21. $C_{30}H_{48}O$ requires: C, 84.90; H, 11.32%).

30-*Norisocyclopterospermol* (**3**) *conversion into* 30-*Norisocycloswietenol* **7**. Compound **3** (40 mg) in MeOH (20 ml) was refluxed with methanolic HCl (5% 10 ml) for 1 hr on a water bath and poured into ice water. The solid separated, showed a purple

spot on TLC, silica gel, benzene (R_f: 0.30, spray reagent: 10% methanolic H_2SO_4) and crystallized from MeOH to yield the pure isomeric product as shining plates (20 mg). (Found: C, 84.49; H, 11.70. $C_{30}H_{50}O$ requires: C, 84.50, H, 11.73%).

The crude residue (5 g) from the $CHCl_3$ extract of the heartwood of *P. heyneanum* was chromatographed over a silica gel column prepared in C_6H_6 and fractions of 200 ml were collected. The first 14 fractions with the C_6H_6 eluant yielded compound **4**. The next 20 fractions with C_6H_6–Me COOEt (8:2) yielded compound **15**.

3-Hydroxy-5-methoxy-2-methylbenzoquinone (**4**). Brownish orange prisms (MeOH, 60 mg) mp 201–202° (dec.). UV λ_{max}^{EtOH} nm (log ε): 288 (4.20) and 4.20 (2.72). IR ν_{max}^{KBr} cm^{-1}: 3240 (OH), 1680, 1660, 1620, 1610, MS *m/z* (rel. int.): 168 $[M]^+$ (100), 140 (13), 138 (38), 125 (24), 112 (11), 97 (15), 80 (52), 69 (97), 59 (21), 57 (13), 53 (22), 41 (10). (Found: C, 57.02; H, 4. 74. $C_8H_8O_4$ requires: C, 57.14; H, 4.76%).

3,5-Dimethoxy-2-methylbenzoquinone (**4a**). Compound **4** (20 mg) was dissolved in dry Me_2CO (10 ml) and freshly purified and dried $(Me)_2SO_4$ (0.2 ml) was added to it and the mixture was refluxed over dry K_2CO_3 (50 mg) for 12 hr. The filtrate after usual work-up yielded methyl ether **4a** which approved as orange yellow needles (MeOH, 15 mg), mp 124–125°. (Found: C, 59.29; H, 5.43. $C_9H_{10}O_4$ requires: C, 59.34; H, 5.49%).

Sitosterol-3-O-β-D-glucoside (**15**). Colourless plates ($CHCl_3$–MeOH, 100 mg), mp 282–284°, $[\alpha]_D$-40° (*c* 0.6 in pyridine). It gave LB test for steroids and Molische's test for a glycoside. It was identified as sitosterol-3-*O*-β-D-glucoside by comparison with an authentic sample.

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