A New Abietane Diterpenoid from *Plectranthus bishopianus* BENTH.

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Chemical examination of the MeOH extract of *Plectranthus bishopianus* BENTH., a rare and endangered plant, resulted in isolation of a new abietane diterpene, 6β-hydroxy-7α-methoxyroyleanone (**1**), along with two more diterpenoids, 6,7-dehydroroyleanone, and $6\beta,7\alpha$-dihydroxyroyleanone, a triterpene oleanolic acid, and two sterols, β-sistosterol and stigmasterol. The structures of the isolated compounds were elucidated on the basis of spectroscopic data and also by comparison with authentic samples.

Introduction. – The Lamiaceae are a large family that occur worldwide and have species that are adapted to almost all habitats and altitudes. They belong to the angiosperms and represent a valuable pool of species that contain biologically active compounds [1]. *Plectranthus*, an important genus of Lamiaceae, is used all around the world as ornamental plants and as medicinal herb with economic interest, along with a rich diversity of ethnobotanical uses. The *Plectranthus* species possess antiseptic, vermicidal, and purgative activities, and they are used for the treatment of infections, toothache, stomach ache, and allergies [2]. The chemistry of the genus *Plectranthus* remained relatively unknown; however, several plants have been studied chemically, diterpenoids being found as the most common secondary metabolites; and majority of them are highly modified abietanoids, in addition to entkauranes and phyllocladanes. Essential oils, triterpenoids, flavonoids, and long-chain alkyl catechols were also isolated [3]. Mostly occurring as 'oil-rich' perennial semi-succulent, sub-shrubs, *Plectranthus* is a large and widespread genus with *ca.* 350 species worldwide [4]. In India, *ca.* 30 *Plectranthus* species are known [5], of which *P. amboinicus*, *P. vettiveroides*, *P. barbatus*, *P. mollis*, *P. coetsa*, and *P. incanus* are the most common species used in the traditional Indian Ayurvedic medicine since ancient times to treat many disorders and diseases [6][7]. However, very little is known about the chemical constituents and biological activities of *Plectranthus* species growing in India [8]. *P. bishopianus* is a rare and endangered species available in the Western Ghats of India [9]. The species is included in the possibly extinct category in the *Red Data Book of Indian Plants* [10]. No chemical examination on this plant is reported.

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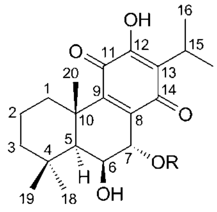
As a part of our phytochemical investigation on medicinal plants and to discover new bioactive natural products, we report the isolation and structure determination of a new abietane diterpene, 6β-hydroxy-7α-methoxyroyleanone (**1**), along with two further diterpenes, 6,7-dehydroroyleanone (**2**) and 6β,7α-dihydroxyroyleanone (**3**), and two sterols, β-sitosterol (**4**) and stigmasterol (**5**), and oleanolic acid (**6**), from the MeOH extract of *P. bishopianus*. Their structures were elucidated on the basis of UV, IR, ^{1}H-NMR, ^{13}C-NMR, and MS data, and by comparison with those of authentic samples.



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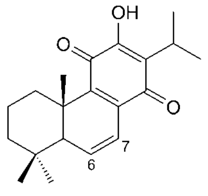

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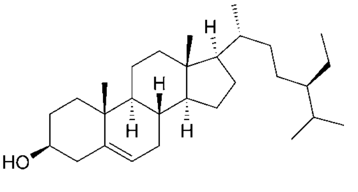

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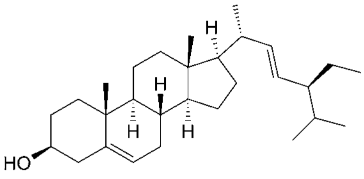

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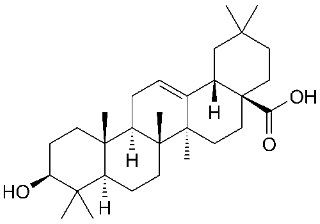

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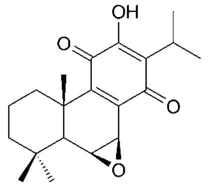

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Results and Discussion. – The hexane-soluble fraction from the MeOH extract of the aerial parts of *P. bishopianus* on purification by repeated column chromatography on SiO$_2$ afforded three diterpenes. The AcOEt-soluble portion gave two further steroids and one triterpenoid compound.

Compound **1** was obtained as a yellow crystalline compound from MeOH, and has a molecular formula of C$_{21}$H$_{30}$O$_5$ determined by HR-MS (385.1992 (C$_{21}$H$_{30}$NaO$_5^+$, $[M+\mathrm{Na}]^+$; calc. 385.1991), 385.1992; MS: 362 (M^+), 330, 315, 297, 287, 245, 233, 217, 203, 189, 167, 149, 128, 115, 102, 86, 84, 49, 44 (100)). ^{1}H- and ^{13}C-NMR data are compiled in the *Table*. UV Spectra showed maximum absorptions at 200, 271, and 402 nm. IR Spectra exhibited absorptions at 3468, 3373, 1719, 1703, 1225, 1098, 1079, and 952 cm^{-1}, indicating the presence of CO and OH groups. ^{1}H-NMR Spectra displayed five Me signals at δ(H) 1.05, 1.17, 1.21, 1.24, and 1.60, and a MeO signal at δ(H) 3.54. ^{13}C-NMR and DEPT spectra exhibited signals for 21 C-atoms, including one MeO group, five Me, three CH$_2$, and four CH groups, and eight quaternary C-atoms. A *septuplet* at δ(H) 3.20, and the Me *doublet*s at δ(H) 1.17 and 1.24 clearly indicate the i-Pr substitution and revealed the compound as an abietane derivative. Two O-bearing CH-group signals without proper resolution at δ(H) 4.49 and 4.14 in the parent compound and δ(H) 5.57 and 4.08 in the (6-*O*-Ac, 12-*O*-Ac) derivative indicated the *O*-substitution C(6) and C(7) of the abietane. Further, the AcO signals at δ(H) 2.30 and 2.05 in the spectrum of the Ac derivative of **1** indicated one phenolic OH group and one secondary OH group.

Detailed comparison of the ^{1}H- and ^{13}C-NMR data with those of **3** displayed extremely close analogy except for an additional MeO group signal in the spectra of **1** and downfield shift of C(7) signals, suggesting that C(7) of **1** was possibly substituted by a MeO instead of a OH group. Further, remarkably smaller coupling between the CH(6) and CH(7), the single H-atom signal at δ(H) 1.50 (H–C(5)), and the unresolved *multiplet* with $w_{1/2} \approx 2$ Hz indicated that H–C(6) and H–C(7) are in equatorial and axial orientation, respectively. Biogenetically, the Me(19) and Me(20) groups of abietane diterpenoids are β-oriented. Thus, the structure of the compound **1** was established as 6β-hydroxy-7α-methoxyroyleanone (=6β,12-dihydroxy-7α-methoxyabieta-8,12-diene-11,14-dione).

Table. *NMR Spectroscopic Data* (400 MHz, CDCl$_3$) *for Compound* **1** *and Its 6-*O*-Ac,12-*O*-Ac Derivative*

	Compound **1**		6-*O*-Ac,12-*O*-Ac derivative of **1**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH$_2$(1)	1.16–1.17 (*m*), 2.58–2.61 (*m*)	38.23 (*t*)	1.20–1.21 (*m*), 2.49–2.52 (*m*)	38.17 (*t*)
CH$_2$(2)	1.50 (*dt*, *J* = 13.6, 3.5), 1.81 (*qt*, *J* = 13.6, 3.5)	19.01 (*t*)	1.53 (*dt*, *J* = 13.5, 3.5), 1.81 (*qt*, *J* = 13.5, 3.5)	18.85 (*t*)
CH$_2$(3)	1.44 (*dt*, *J* = 13.2, 3.0), 1.27–1.30 (*m*)	42.11 (*t*)	1.45 (*dt*, *J* = 13.2, 3.3), 1.25–1.29 (*m*)	42.19 (*t*)
C(4)		33.73 (*s*)		33.21 (*s*)
H–C(5)	1.50 (*s*)	49.05 (*d*)	1.58 (*s*)	48.05 (*d*)
H–C(6)	4.49 (*s*)	65.95 (*d*)	5.57 (*s*)	67.26 (*d*)
H–C(7)	4.14 (*s*)	75.83 (*d*)	4.07 (*s*)	72.85 (*d*)
C(8)		139.26 (*s*)		139.51 (*s*)
C(9)		147.60 (*s*)		150.29 (*s*)
C(10)		38.53 (*s*)		38.97 (*s*)
C(11)		183.64 (*s*)		180.06 (*s*)
C(12)		150.80 (*s*)		150.29 (*s*)
C(13)		124.71 (*s*)		137.50 (*s*)
C(14)		186.72 (*s*)		186.40 (*s*)
H–C(15)	3.20 (*sept.*, *J* = 7.1)	24.22 (*d*)	3.13 (*sept.*, *J* = 7.1)	25.24 (*d*)
Me(16)	1.24 (*d*, *J* = 7.0)	19.87 (*q*)	1.25 (*d*, *J* = 7.0)	20.29 (*q*)
Me(17)	1.21 (*d*, *J* = 7.0)	21.77 (*q*)	1.22 (*d*, *J* = 7.0)	21.42 (*q*)
Me(18)	1.05 (*s*)	33.44 (*q*)	1.06 (*s*)	21.59 (*q*)
Me(19)	1.17 (*s*)	24.29 (*q*)	1.06 (*s*)	23.41 (*q*)
Me(20)	1.60 (*s*)	19.74 (*q*)	1.58 (*s*)	20.23 (*q*)
MeO–C(7)	3.54 (*s*)	58.58 (*q*)	3.60 (*s*)	58.63 (*q*)
AcO–C(6)			2.30 (*s*)	170.27 (*s*)
AcO–C(12)			2.05 (*s*)	168.12 (*s*)

The possibility that the compound **1** might be an artefact due to MeOH extraction was considered and ruled out by extracting the plant material with AcOEt and EtOH yielding also compound **1**. Another possibility of obtaining **1** from **3** *via* epoxide **7** by neighboring group participation (elimination of H$_2$O, followed by nucleophillic opening of the resultant epoxide **7**) was also considered and ruled out by refluxing **3** with MeOH for 8 h. No change in compound **3** was observed. The structures of the other compounds were elucidated and confirmed on the basis of their spectroscopic data, and comparison with those of authentic samples.

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Experimental Part

General. Silica gel (SiO_2; *Qualigens*; 100–120 mesh) were used for column chromatography, and percolated silica gel (*Merck 60 F-254*) plates were used for TLC with hexane/AcOEt 8 :2 or 7:3. The spots on TLC were detected by spraying with 5% H_2SO_4 and then heating at 110°. UV Spectra: *Shimadzu 260* UV spectrophotometer. IR Spectra: *FT-IR Perkin Elmer* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker-AM-400* and *-300* spectrometers; at 400 and 300 MHz (^1H), and 100 and 75 MHz (^{13}C), resp.; in CD_3OD; δ in ppm rel. to Me_4Si as internal standard, J in Hz . HR-MS: *Micromass Q-TOF* micro mass spectrometer in electronspray-ionization (ESI) mode.

Plant Material. The plant *P. bishopianus* was collected from the Nandhi Hills (13° 21′ N, 77° 41′ E), and it was further multiplied at CIMAP, RC Bangalore farm (13° 4′ N, 77° 35′ E). The plant was identified by Dr. *R. R. Rao*, CSIR Emeritus Scientist, and the herbarium specimen with No DST-72 was deposited with CIMAP, RC, Bangalore. The leaves of the plant were collected during March 2009 and shade-dried before extraction.

Extraction and Isolation. The shade dried and powdered leaves of *P. bishopianus* (1.0 kg) were extracted with hot MeOH (6 l, three times, 4 h each) and concentrated under reduced pressure. After evaporation of the org. solvent, the extract (0.156 kg) was partitioned between AcOEt and H_2O. The AcOEt soluble material (0.103 kg) was further dissolved in EtOH and kept in the refrigerator. The separated wax was removed by filtration. The process was repeated three times. From the defatted fraction (0.042 kg), 0.020 kg was subjected to CC (SiO_2 (100–200 mesh); hexane/AcOEt 1:0 to 0.7:0.3). *6,7-Dehydroroyleanone* (**2**; hexane/AcOEt 9:1), *6β-hydroxy-7α-methoxyroyleanone* (**1**; hexane/AcOEt 8:2), *6β,7α-dihydroxyroyleanone* (**3**), *β-sistosterol* (**4**), *stigmasterol* (**5**), and *oleanolic acid* (**6**; hexane/AcOEt 7:3) obtained were purified by repeated crystallization.

6β-Hydroxy-7α-methoxyroyleanone (= *(6β,7α)-6,12-Dihydroxy-7-methoxyabieta-8,12-diene-11,14-dione*; **1**). UV (MeOH): 200, 271, 402. IR (KBr): 3468, 3373, 1719, 1703, 1225, 1098, 1079, 952. ^1H- and ^{13}C-NMR: see the *Table.* MS: 362 (M^+), 330, 315, 297, 287, 245, 233, 217, 203, 189, 167, 149, 128, 115, 102, 86, 84, 49, 44 (100). HR-ESI-MS: 385.1992 ($C_{21}H_{30}NaO_5^+$, $[M+Na]^+$; calc. 385.1991).

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