Two New Terpenoids from *Pluchea arguta* Boiss.

Viqar Uddin Ahmad*, Azra Sultana, and Kaniz Fizza

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

Z. Naturforsch. 45b, 385–388 (1990); received July 7 / October 23, 1989

Pluchidione, Pluchidinol, Terpenoids, Compositae, *Pluchea arguta* Boiss.

Two new terpenoids pluchidione 1 and pluchidinol 2 were isolated from the ethyl acetate soluble portions of the whole plant of *Pluchea arguta* Boiss. The structures of these compounds were established by using modern spectroscopic techniques.

**Introduction**

In the course of our research on new sesquiterpenes from the fresh whole plant of *Pluchea arguta* (Syn. *Conyza odentophylla* Boiss.) belonging to the family Compositae, we have already reported three new sesquiterpenes [1–3] together with some known compounds. In the present communication we wish to describe the isolation of a new dimeric sesquiterpene pluchidione 1 and a new dimeric sesquiterpene, pluchidinol 2 from the ethyl acetate soluble portion of *P. arguta*. Their structures have been established by ^1^H, COSY-45, NOESY, 2D-J-resolved, ^1^C NMR and heterocosy spectra. Taraxasterol was also isolated from the ethyl acetate soluble portion and was identified by spectroscopic means, as well as by direct comparison with an authentic sample.

**Results and Discussion**

Pluchidione (1): was isolated as light yellow gum from the ethyl acetate soluble portion of the alcoholic extract of fresh whole plant of *P. arguta*. The UV spectrum showed an absorption at 236 nm indicating the presence of a,/?-unsaturation in the molecule [4]. The IR spectrum showed absorptions at 3400 (OH), 1710 (C -O ), 1650 and 1680 cm⁻¹ indicating the presence of four methyl singlets in the ^1^H NMR spectrum, each integrating for 3H at δ 1.09, 1.10, 1.80 and 2.28. These are due to protons at C-10, C-11, C-12 and C-13 respectively. The chemical shift of the last methyl indicated the presence of a COCH group. The methyl group which resonated at δ 1.80 (J12,4 = 0.9 Hz) was attached to an unsaturated carbon. A double doublet at δ 2.34 (Jgem = 17 Hz, J3,4 = 1.14 Hz) is due to H-3/? and another double doublet ascribed to H-3a was observed at δ 2.46 (Jgem = 17 Hz, J3a,4 = 1.04). The doublets at δ 6.40 and δ 6.80 (J7,8 = 14.7 Hz) are due to protons at C-8 and C-7 respectively coupled to each other, their trans olefinic coupling constant also clearly indicated the presence of the double bond outside the ring.

The multiplicities of the proton signals were determined through a 2D-J-resolved spectrum and vicinal coupling was confirmed by COSY-45. A strong cross peak was observed at δ 2.34 and δ 5.90, beside this another cross peak at δ 2.46 and δ 5.90 showing the coupling interaction of H-3a, H-3/? protons with that of C-4 proton. A corresponding cross peak of H-3a and H-3/? protons with each other was also observed in the COSY-45 spectrum, which also showed a cross peak at δ 6.40 and δ 6.80. They are due to vicinal protons in the skeleton of 1. The NOESY spectrum also confirmed structure 1 showing spatial connectivities of H-11 with H-7, H-3/?; CH3-13 with H-8; CH3-12 with H-4.

The terpenoidal nature of 1 was indicated by the presence of four methyl singlets in the ^1^H NMR spectrum, each integrating for 3H at δ 1.09, 1.10, 1.80 and 2.28. These are due to protons at C-10, C-11, C-12 and C-13 respectively. The chemical shift of the last methyl indicated the presence of a COCH group. The methyl group which resonated at δ 1.80 (J12,4 = 0.9 Hz) was attached to an unsaturated carbon. A double doublet at δ 2.34 (Jgem = 17 Hz, J3,4 = 1.14 Hz) is due to H-3/? and another double doublet ascribed to H-3a was observed at δ 2.46 (Jgem = 17 Hz, J3a,4 = 1.04). The doublets at δ 6.40 and δ 6.80 (J7,8 = 14.7 Hz) are due to protons at C-8 and C-7 respectively coupled to each other, their trans olefinic coupling constant also clearly indicated the presence of the double bond outside the ring.

The multiplicities of the proton signals were determined through a 2D-J-resolved spectrum and vicinal coupling was confirmed by COSY-45. A strong cross peak was observed at δ 2.34 and δ 5.90, beside this another cross peak at δ 2.46 and δ 5.90 showing the coupling interaction of H-3a, H-3/? protons with that of C-4 proton. A corresponding cross peak of H-3a and H-3/? protons with each other was also observed in the COSY-45 spectrum, which also showed a cross peak at δ 6.40 and δ 6.80. They are due to vicinal protons in the skeleton of 1. The NOESY spectrum also confirmed structure 1 showing spatial connectivities of H-11 with H-7, H-3/?; CH3-13 with H-8; CH3-12 with H-4.
The $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) showed the presence of thirteen carbon atoms in the molecule. The multiplicity assignments were made by DEPT, the pulse sequence with the last polarization pulse angle $\theta = 45^\circ$, $90^\circ$ and $135^\circ$. It showed presence of four methyis at $\delta$ 18.69, 22.97, 24.37 and 28.37 which were assigned to CH$_3$-10, CH$_3$-11, CH$_3$-12 and CH$_3$-13 respectively. There was only one methylene at $\delta$ 49.61 due to C-3. Three methine carbon signals at $\delta$ 41.47, 79.30, 160.31, 196.90 and 197.30 are ascribed to C-1 (a, $\beta$-unsaturated ketone), C-4, C-5, C-2 and C-9. The last two values indicated the presence of a carbonyl function while the value of C-6 indicated an oxygen bearing carbon atom. The assignments of $^{13}$C NMR chemical shifts were also confirmed through a two dimensional $^1$H- $^{13}$C chemical shift correlation experiment (Heterocosy) which showed connectivities of C-11 (6 22.97) with H-11 (6 1.10), C-12 (6 24.37) with H-12 (6 1.80), C-4 (6 127.80) with H-4 (6 5.90), C-3 (6 49.61) with H-3a (6 2.46) and H-3$^\beta$ (6 2.34), C-10 (6 18.69) with H-10 (6 1.09), C-8 (6 130.38) with H-8 (6 6.40), C-7 (6 144.98) with H-7 (6 6.80) and C-13 (6 28.37) with H-13 (6 2.28).

The CD curve in ethanol shows a cotton effect, with a positive maximum around 243 nm and a negative maximum at 210 nm due to the $\pi-\pi^*$ transitions of the a, $\beta$-unsaturated ketone. In addition, there are weaker negative maxima at 318 ascribed to the unconjugated ketone ($n-\pi^*$) and 340 nm due to the enone ($n-\pi^*$). The positive maximum at 243 ($\Delta_{\varepsilon}$ 14.6) corresponds, in the position and intensity to that of $+\alpha$-ionone [5] which has R configuration at C-6. It is therefore concluded that the compound 1 has the same i.e. R configuration at C-6.

Pluchidionol (2): was isolated as a colourless gum and obtained through repeated flash chromatography. Its UV spectrum showed an absorption maximum at 233 nm which indicated the presence of a,$\beta$-unsaturated ketone [4]. The IR spectrum contains peaks at 3200–3600 (OH) and 1674 and 1645 cm$^{-1}$ (a,$\beta$-unsaturated ketone). The fast atom bombardment (FAB) mass spectrum of 2 contains a (M+H)$^+$ peak at $m/z$ 549 corresponding to the molecular formula C$_{31}$H$_{48}$O$_8$. In the EI mass spectrum, the M$^+$ peak was not observed, the peak at $m/z$ 268 which appeared after the breaking of the dimer into the monomeric form. The rest of the important peaks at $m/z$ 235 (base peak) 217, 193, 175, 149 and 123 which are identical to those reported earlier [6].

The $^1$H NMR spectrum (400 MHz) was very similar to that of 4-epiplucheinol [1]. The compound 2 showed methyl singlets, each integrating for 6H, at $\delta$ 0.94 (C-14 and C-14$^\beta$), $\delta$ 1.18 (methyis at C-4 and C-4$^\beta$), $\delta$ 1.42 (C-12 and C-12$^\beta$) and $\delta$ 1.44 (C-13 and C-13$^\beta$). There is a narrow triplet at $\delta$ 3.68 ($J = 2.70$ Hz) characteristic of the protons geminal to hydroxy group at C-3 and C-3$^\beta$. A doublet at 7.03 ($J_{5,6&5,6'} = 2.30$ Hz) is assigned to the olefinic protons at C-6 and C-6$^\beta$. There was an AB quartet centered at $\delta$ 2.30 ppm ($J_{AB} = 15.7$ Hz, $\delta_A - \delta_B = 18.5$ Hz due to H-9). A multiplet at $\delta$ 1.80 was assigned to C-2 and C-2$^\beta$ protons. Besides these signals, there was a multiplet at $\delta$ 1.25 (2H, m, H-16) which was not present in 4-epiplucheinol [1].

Two dimensional NMR measurements were carried out to verify the $^1$H NMR assignment. The coupling interactions were established through correlated spectroscopy (COSY-45) while the multiplicity of the overlapping proton signals was determined from the 2D-J-resolved spectrum. The assignment for C-2 and C-2$^\beta$ protons at $\delta$ 1.80 could thus be confirmed by its COSY-45 spectrum, which showed a strong cross peak at $\delta$ 1.25 (H-16), 1.80 (H-2, H-2$^\beta$), 3.68 (H-3, H-3$^\beta$). Similarly the assignments of H-5, H-5$^\beta$ at $\delta$ 2.68 and H-6, H-6$^\beta$ at $\delta$ 7.03 were also confirmed by COSY-45 spectrum since they showed interaction with each other.

The broad band and DEPT $^{13}$C NMR spectra of 2 were very useful in elucidating the structure of...
the compound, which showed the presence of eight methyls, seven methylene, six methine and ten quaternary carbon in the structure of 2. The chemical shift and multiplicity of C-2 and C-2' is effected because 2 here they were methines at δ 50.86 (in the case of 4-epiplucheinol it was a methylene) attached to the C-16 methylene which linked the two monomers of same stereochemistry, whereas it was absent in 4-epiplucheinol. This extra methylene i.e. C-16 appeared at δ 29.73.

Heterocosy experiments were carried out to identify the relationship between the carbons and their respective protons. The C-16 signal at δ 29.73 showed a cross peak with protons at δ 1.25. Similarly the C-2 and C-2' at δ 50.86 showed a cross peak with δ 1.80. It also confirmed the assignments of other protons with their respective carbon atoms.

Experimental

UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and IR spectra were recorded on a JASCO A-302 spectrophotometer. HRMS and FDMS were recorded on a Finnigan MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system. The 1H NMR spectra were recorded at 400 MHz on a Bruker AM-400 NMR spectrometer. The 13C NMR spectra were recorded at 100 MHz on the same instrument. The optical rotations were recorded on a Polartronic Universal Australia Standard K-157 digital polarimeter. Flash column chromatography was performed on a Telos Flash Chromatograph model EF-10, using silica gel 60, 230–400 mesh size, (E. Merck) as the stationary phase. The purity of the samples were confirmed by HPTLC silica gel 60 F254 precoated glass plates (nano TLC; E. Merck).

Extraction and isolation of compound 1 and 2

Fresh plant material of P. arguta (only 20 kg) was collected from Karachi and identified by Botany Department, University of Karachi, a voucher specimen has been deposited to the herbarium of Botany Department. The plant was crushed, soaked in hexane and homogenized with an Ultra-Turrax homogenizer. After removal of the hexane extract (twice) from the homogenized material of P. arguta. The residue was soaked in distilled EtOH. The ethanolic extract was taken and the solvent was removed in vacuo. The residue obtained was extracted with ether. The ether insoluble portion was partitioned with ethyl acetate and water. The ethyl acetate layer was separated and aqueous phase was further extracted with ethyl acetate. The ethyl acetate-soluble portion was evaporated in the rotary evaporator. The gummy residue (20 g) was loaded on a large silica gel column and chromatographed in the order hexane, hexane-chloroform mixture, chloroform, chloroform-ethyl acetate mixtures, ethyl acetate, ethyl acetate-methanol mixtures and finally with pure methanol. The fractions eluted from chloroform-ethyl acetate (75:25) contained a mixture of closely moving sesquiterpenes. This sesquiterpenic mixture was subjected to repeated flash column chromatography with chloroform, methanol (96:4) as mobile solvent. The first few fractions afforded compound 1 with some impurities which was further purified through thin layer chromatography on silica plates whereas the more polar fractions contained compound 2. The latter was purified by repeated flash column chromatography.

Compound 1: Light yellow gum, [α]D30 +23.25° (c = 0.043, CHCl3). - UV (MeOH): λmax nm (log ε) 236 (3.96). - IR (CHCl3): 3400 (OH), 1680 (C = O), 1720 (C=O), 1360 (C-O). - 1HNMR (CDCl3, 400 MHz): δ 1.09 (s, 3-H-10), 1.10 (s, 3-H-11), 1.80 (d, J = 0.9 Hz, 3-H-12), 2.28 (s, 3-H-13), 2.46 (dd, Jgem = 17 Hz, J3,4 = 1.04 Hz, H-3a), 2.34 (dd, Jgem = 17 Hz, J5,6 = 1.14 Hz, H-3β), 5.90 (m, H-6), 6.40 (d, J7,8 = 14.7 Hz, H-7), 6.50 (d, J7,8 = 14.7 Hz, H-7). - 13C NMR (CDCl3, 100 MHz): δ 41.47 (C-1), 197.3 (C-2), 49.6 (C-3), 127.8 (C-4), 160.3 (C-5), 79.3 (C-6), 144.98 (C-7), 130.38 (C-8), 196.9 (C-9), 18.69 (C-10), 22.97 (C-11), 24.37 (C-12), 28.37 (C-13). - FDMS m/z: 222. - HRMS: m/z: 222.1263 [M+], 189.09188 (C11H13O3, calcd.), 189.091549 (M+ - CH3O), 124.0575 (C7H4O3, calcd.), 124.05246 (M+ - CH3O). - CD (EtOH, 0.03 g/l): [θ]340 = -1918, [θ]318 = -2730, [θ]245 = 41874, [θ]225 = 0, [θ]210 = 44400.

Compound 2: Colourless gum, [α]D30 +74.07° (c = 0.0067, CHCl3). - UV (MeOH): λmax nm (log ε) 233 (4.29), 204 (4.31). - IR (CHCl3): 3200–3600 (OH), 1710 (C=O), 1645, 1674 (a,b-unsaturated ketone) cm−1. - 1HNMR (CDCl3, 400 MHz): δ 0.94 (s, 6-H-14, 14'), 1.25 (s, 6-H-12, 12'), 1.44 (s, 6-H-13, 13'), 1.18 (s, 6-H-15, 15'), 2.25 (2H, m, H-16), 2.30 (ABq, J = 15.7 Hz, δ5-H - δ6-H = 18.5 Hz, 2H-9, 2H-9'), 3.68 (2H, t, J = 2.7 Hz, H-3 and H-3'), 2.68 (2H, d, J5,6,8,9 = 2.10 Hz, H-5 and H-5'), 7.03 (2H, d, J5,6,8,9 = 2.30 Hz, H-6 and H-6'). - 13C NMR (CDCl3, 100 MHz): δ 31.98 (C-1, 1'), 50.86 (C-2, 2'), 73.52 (C-3, 3'), 72.1 (C-4,
4'), 18.9 (C-5, 5'), 143.2 (C-6, 6'), 145.0 (C-7, 7'), 201.3 (C-8, 8'), 57.8 (C-9, 9'), 39.25 (C-10, 10'), 71.99 (C-11, 11'), 29.36 (C-12, 12'), 28.90 (C-13, 13'), 17.77 (C-14, 14'), 22.70 (C-15, 15'), 29.73 (C-16). - FAB + ve m/z: 549 [M+H]^+. - EIMS m/z (rel. int., %): 268 (10), 253 (12), 235 (100), 217 (12), 193 (14), 175 (10), 149 (74), 132 (12), 123 (16), 109 (22), 95 (14), 83 (14), 77 (12), 55 (12).

**Acetylation of compound 2:** A solution of 2 (8 mg) in dry CHCl₃ in 3.5 ml solutions was cooled. AcCl (1 ml) and pyridine (0.75 ml) was added to it and the mixture was allowed to stand overnight at 0°. Ice was added and the reaction mixture was worked up in the usual manner. The residue from CHCl₃ extract was collected [7].

EI m/z: 799 (M⁺ - 1) 537.269, 253 [a]D + 76.92 (c = 0.013, CHCl₃). - UV (MeOH): λmax nm (logε) 254 (2.42), 204 (2.19). - IR (CHCl₃): 1735 (C=O), 1300, 1050, - CO - O cm⁻¹.

---