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THE STRUCTURE AND STEREOCHEMISTRY OF PHLOGANTHOLIDE-A, A DITERPENE FROM *PHLOGACANTHUS THYRSIFLORUS*

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Key Word Index—*Phlogacanthus thyrsiflorus*; Acanthaceae; diterpene lactone; phloganthulide-A.

Abstract—The structure and stereochemistry of phloganthulide-A, a new diterpene lactone isolated from the leaves of *Phlogacanthus thyrsiflorus* has been determined as 2β,15,18-trihydroxy-*ent*-labd-8(17),13-dien-16-oic lactone by chemical and spectroscopic means.

INTRODUCTION

The shrub *Phlogacanthus thyrsiflorus* Nees grows in the sub-tropical regions of the Himalayas. It is used in India for the treatment of bronchial trouble [1]. The isolation of sitosterol, lupeol and betulin from the leaves of *P. thyrsiflorus* has been reported earlier from this laboratory [2]. The present paper reports the structure of a new diterpene lactone called phloganthulide-A (**1a**) from the ethanol extract of the leaves.† The structure **1a** for phloganthulide-A was proposed in an earlier communication [3].

RESULTS AND DISCUSSION

Phloganthulide-A (**1a**), $C_{20}H_{30}O_4$, gave a positive Legal test indicating the presence of an α,β-unsaturated-γ-lactone moiety like that found in 14-deoxyandrographolide [4] and neoandrographolide [5, 6]. Its UV spectrum (EtOH) showed a maximum at 208 nm (ε 9000) and its IR spectrum ($CHCl_3$) exhibited bands at 3440 (OH), 1751 (an α,β-unsaturated γ-lactone) and 902 (exocyclic methylene group) cm^{-1}.

The ^1H NMR spectrum (270 MHz, $CDCl_3$) of phloganthulide-A (**1a**) showed singlets at δ0.71 (3H) and 1.04 (3H) for two tertiary methyl groups. Signals for two hydroxymethylene protons (CH_2OH) appeared as a pair of doublets centred at δ3.37 (1H, J = 11 Hz) and 3.66 (1H, J = 11 Hz). A C-2 axial carbinyl proton resonance appeared as a triple triplet centred at δ3.88 (1H, $W_{1/2}$ = 24 Hz). The large $W_{1/2}$ value and the coupling pattern was in agreement with the presence of the secondary hydroxyl at C-2 in an equatorial orientation. The two exomethylene protons appeared at δ4.63 (1H, *s*) and 4.90 (1H, *s*). The signals for the protons at C-15 appeared as a doublet centred at δ4.79 (2H, J = 1.5 Hz) and these were mutually coupled to a signal for a C-14 proton which appeared at δ7.13 (1H, *t*, J = 1.5 Hz). The small coupling constant (J = 1.5 Hz) suggested that the double bond was endocyclic [4, 7] rather than exocyclic as in andrographolide [8] and related compounds where the olefinic proton on the exocyclic double bond appears as a triplet with $J \approx 6$ Hz.

The mass spectrum of phloganthulide-A was in agreement with the structure **1a**. It contained a very weak molecular ion peak at m/z 334 corresponding to the molecular formula $C_{20}H_{30}O_4$. There were also peaks at m/z 316 and 285 for the ion species $[M - H_2O]^+$ and $[M - H_2O - CH_2OH]^+$, respectively. The loss of the entire side chain ($C_6H_7O_2$) from the $[M - H_2O]^+$ ion by allylic cleavage of the 9,11-bond gave rise to a peak at m/z 205. There was also a peak at m/z 237 due to the loss of 97 mass units ($C_5H_5O_2$) by cleavage of the 11,12-allylic bond.

Phloganthulide-A (**1a**) formed a diacetate (amorphous) (**1b**), $C_{24}H_{34}O_6$, with pyridine and acetic anhydride at 0° indicating the equatorial orientation of the secondary hydroxyl group. Its IR spectrum ($CHCl_3$) showed bands at 1759 (α,β-unsaturated-γ-lactone), 1730–1740 (acetoxy group) and 900 (exocyclic methylene group) cm^{-1}.

The ^1H NMR spectrum (90 MHz, $CDCl_3$) of the diacetate (**1b**) showed sharp singlets at δ2.03 (3H) and 2.05 (3H) for the two acetoxy groups. The two protons of the acetoxy-methylene group ($-CH_2-OAc$) appeared as a pair of doublets centred at δ3.86 (1H, J = 11 Hz) and 4.18 (1H, J = 11 Hz). The spectrum also showed a signal for a C-2 axial proton at δ4.91 (1H, *m*, *br*) which was partly overlapped with one of the signals at δ4.90 for the exocyclic methylene group. The methyl resonances appeared at δ0.78 (3H, *s*) and 1.02 (3H, *s*). The ^1H NMR spectrum was very similar to that of 14-deoxyandrographolide diacetate (**2b**) [4]. The difference between the two spectra lie in the signals for (i) the methylene protons of the acetoxy-methylene group, (ii) the protons attached to the carbon bearing secondary acetoxy group and (iii) one of the tertiary methyl groups. Thus the ^1H NMR spectra and the other data presented above showed a close similarity in the structure of phloganthulide-A and 14-deoxyandrographolide (**2a**). The above conclusion was also supported by the mass spectral fragmentation pattern of phloganthulide-A and its diacetate.

The mass spectrum of the diacetate (**1b**) showed a weak

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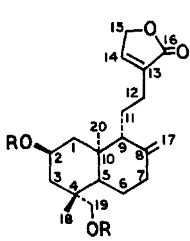
† The report of this work was presented at the symposium on "Advances in the Chemistry of Natural Products and their Biogenesis" held at Nainital, India (1981) *Abstracts*, p. 11.



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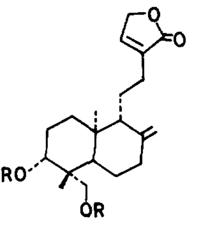

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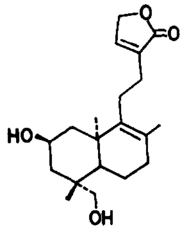

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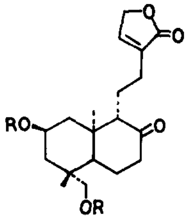
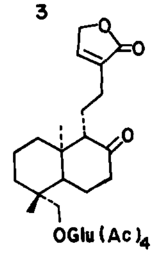


4 R=Ac



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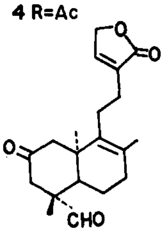

6

molecular ion peak at m/z 418. There were also peaks at m/z 358, 298 and 285 for the ion species $[M-HOAc]^+$, $[M-2HOAc]^+$ and $[M-HOAc-CH_2OAc]^+$, respectively. Loss of 97 mass units ($C_5H_5O_2$) or 111 mass units ($C_6H_7O_2$) from the ion species m/z 298 by appropriate allylic cleavage across the 11,12 and 9,11 bonds gave rise to peaks at m/z 201 and 187 respectively.

On refluxing with ethanolic hydrochloric acid, phlogantholide-A (**1a**) yielded an amorphous isophlogantholide-A (**3**), $C_{20}H_{30}O_4$, formed by migration of the 8,17-double bond to the 8,9 position. The mass spectrum of isophlogantholide-A did not show the molecular ion peak at m/z 334 but showed an $[M-H_2O]^+$ ion at m/z 316. The ^1H NMR spectrum (90 MHz, $CDCl_3$) of **3** showed, as expected, a signal for an olefinic methyl at δ1.67 (3H, s) and there was no signal for the protons of the exocyclic methylene group. In this respect phlogantholide-A behaved like neoandrographolide [5, 6].

Phlogantholide-A diacetate (**1b**) on osmylation, followed by sodium periodate oxidation gave an amorphous nor-ketone (**4**), $C_{23}H_{32}O_7$ ($[M]^+$ 420). The IR spectrum ($CHCl_3$) of this nor-ketone (**4**) showed bands at 1704 (six-membered ring ketone), 1755 (α,β-unsaturated γ-lactone) and 1740 and 1240 cm^{-1} for the acetate groups (cf. 17-nor-ketone obtained by a similar method from neoandrographolide [6]). The stereochemistry of the bicyclic system in phlogantholide-A was established by the CD data of the 17-norketone **4**: maximum at 294 nm ($\Delta\varepsilon$ + 1.93) and inflections at 302 nm ($\Delta\varepsilon$ + 1.61) and 315 nm ($\Delta\varepsilon$ + 0.56). The curve was very similar to that of neoandrographolide derivative **5** [6] which showed a positive Cotton effect at 293 nm with inflections at 300 and 310 nm and this indicated the relative and the absolute configurations of the decalin systems in **4** and **5**.

Oxidation of isophlogantholide-A (**3**) with chromium trioxide–pyridine complex yielded an amorphous product **6**, $C_{20}H_{26}O_4$. The ^1H NMR spectrum (90 MHz, $CDCl_3$) of **6** displayed the resonance for an aldehyde proton at δ9.75 indicating its axial orientation. The position of this formyl resonance was quite comparable to the axial formyl proton resonances (δ9.64–9.77) in many andrographolide derivatives [9, 10]. Conversely, the equatorial aldehyde groups at C-4 in diterpene and triterpene series resonate at a relatively upfield position (around δ9.3) [11, 12]. Copper–bronze pyrolysis of phlogantholide-A did not afford formaldehyde [13] indicating the absence of a secondary hydroxyl group at C-3.

The equatorial orientation of the secondary hydroxyl and its location at C-2 was established by decoupling experiments. When the carbinyl proton resonance at δ3.88 was irradiated each of the triplets centred at δ0.95 (J = 12.1 Hz) and 1.01 (J = 11.8 Hz) for the two axial protons at C-1 and C-3 collapsed to a doublet. Simultaneously the double doublet at δ2.12 and 2.20 ($J_{gem} \approx 12$ Hz and J_{vic} 2–3 Hz) for the two equatorial protons at C-1 and C-3 were transformed to a doublet each retaining the geminal couplings.

The structures and stereochemistry of phlogantholide-A (**1a**), its acetate (**1b**) and isophlogantholide-A (**3**) received further confirmation from comparative ^{13}C NMR studies of these compounds with those of 14-deoxyandrographolide (**2a**), andrographolide [9] and their derivatives. The signal assignments have been reported earlier by us [3, 14].

EXPERIMENTAL

Mps: uncorr; MS: AEI MS-30 instrument; CC: silica gel (BDH, India) (60–120 mesh); TLC: silica gel G (BDH, India). Deactivated silica gel refers to silica gel treated with 9% H_2O in the usual way. Plant material was collected in November, 1978, from suburbs of Calcutta and identified by the Curator, National Botanic Garden, Shibpur, Howrah 711 103, West Bengal by comparison with a herbarium specimen.

Extraction and isolation of phlogantholide-A (**1a**). Air-dried powdered leaves (4 kg) were first defatted with petrol and then extracted by percolation (× 4) with EtOH (95%) at room temp. The alcoholic extract was concd to a syrup by distillation and then washed with C_6H_6. The residue was extracted (Soxhlet) with $CHCl_3$. The $CHCl_3$ extract was concd and the residue was subjected to CC over deactivated silica gel. Elution of the column with C_6H_6 eventually yielded a crystalline compound which was repeatedly crystallized from $CHCl_3$–petrol to afford phlogantholide-A (**1a**), 810 mg, mp 144–146°, $[\alpha]_D^{26}$ −39.1° ($CHCl_3$); ^{13}C NMR ($CDCl_3$): δ174.4 (s), 146.5 (s), 144.3 (d), 134.3 (s), 107.5 (t), 70.1 (t), 64.6 (t), 64.5 (d), 56.2 (d), 55.3 (d), 47.5 (t), 43.8 (t), 40.6 (s), 40.3 (s), 38.0 (t), 27.2 (q), 24.3 (t), 23.6 (t), 21.7 (t) and 16.1 (q). Found: C, 72.21; H, 9.32. $C_{20}H_{30}O_4$ requires: C, 71.82; H, 9.04%.

Phlogantholide-A diacetate (**1b**). Phlogantholide-A (100 mg) dissolved in C_5H_5N (1 ml) was treated with Ac_2O (2 ml) and kept at 0° overnight. Work-up in the usual way followed by CC over silica gel yielded an amorphous diacetate (**1b**), 90 mg, $C_{24}H_{34}O_6$ ($[M]^+$ 418); ^{13}C NMR ($CDCl_3$): δ174.0 (s), 170.9 (2 × s), 146.0 (s), 144.3 (d), 134.3 (s), 107.9 (t), 70.0 (t), 68.2 (t), 66.2 (d), 56.3 (d), 55.4 (d), 43.8 (t), 40.9 (t), 40.6 (s), 38.4 (s), 37.9 (t), 27.6 (q), 24.3 (t), 23.7 (t), 21.9 (t), 21.2 (q), 20.7 (q) and 15.7 (q).

Isophlogantholide-A (**3**). Phlogantholide-A (100 mg) was refluxed with 50 ml ethanolic HCl (5%) for 6 hr. The reaction mixture was poured on to crushed ice and the precipitate was filtered. The product was purified by CC over deactivated silica gel. The residue from the $CHCl_3$–MeOH (19:1) eluates afforded isophlogantholide-A (85 mg); ^{13}C NMR ($CDCl_3$): δ174.4 (*s*), 144.4 (*d*), 138.1 (*s*), 133.7 (*s*), 126.8 (*s*), 70.1 (*t*), 64.4 (*t*), 64.1 (*d*), 51.5 (*d*), 45.2 (*t*), 43.5 (*t*), 39.8 (2 × *s*), 33.4 (*t*), 26.7 (*q*), 25.8 (2 × *s*), 21.5 (*q*), 19.0 (*q*), and 18.3 (*t*).

Preparation of the 17-*nor-ketone* **4**. To phlogantholide-A diacetate (65 mg) in C_6H_6 (3 ml) was added a soln of OsO_4 (50 mg) in C_6H_6 (1 ml). C_5H_5N (10 drops) was added and left at room temp. for 48 hr. H_2S was then passed through the soln and the black precipitate filtered off. The oily mass obtained after removal of the solvent was dissolved in C_6H_6 and filtered through a short column of silica gel. The oily product thus obtained was dissolved in MeOH (5 ml). A soln of $NaIO_4$ (50 mg) in H_2O (2 ml) was added to the above soln and left for 24 hr. Extraction with Et_2O and usual work-up followed by purification by CC furnished the nor-ketone **4** as an oil (homogeneous on TLC), $[M]^+$ 420.

Oxidation of isophlogantholide (**3**) *to compound* **6**. A soln of isophlogantholide-A (**3**, 35 mg) in C_5H_5N (1 ml) was added to a CrO_3–pyridine complex [prepared by adding CrO_3 (40 mg) to dry C_5H_5N (2 ml) at a temp below $-5°$] and kept overnight at room temp and then worked up in the usual way. The product was chromatographed over silica gel. C_6H_6–$CHCl_3$ (4:1) eluates gave the keto-aldehyde **6**, $[M]^+$ 330; ^1H NMR (90 MHz, $CDCl_3$): δ0.83 (3H, *s*), 1.26 (3H, *s*), 1.70 (3H, *s*), 2.63 (1H, *dd*, $J \approx 14$ Hz and 1.5 Hz), 3.01 (1H, *dd*, $J \approx 15$ Hz and 2 Hz), 4.80 (2H, *d*, $J = 1.5$ Hz) and 7.15 (1H, *t*, $J = 1.5$ Hz).

Copper–bronze pyrolysis of phlogantholide-A (**1a**). Phlogantholide-A (150 mg) was mixed with powdered copper–bronze (450 mg) and heated up to 320° for 1 hr. A slow stream of N_2 was passed through the reaction flask to carry over the evolved gases into a saturated aq. soln of dimedone. But no precipitate of dimedone formaldehyde adduct was obtained.

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