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PHLOGANTHOSIDE—A DITERPENE LACTONE GLUCOSIDE FROM *PHLOGACANTHUS THYRSIFLORUS*

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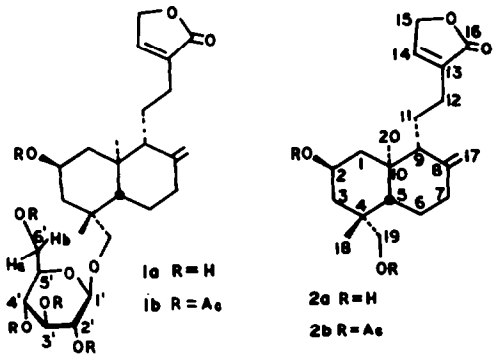
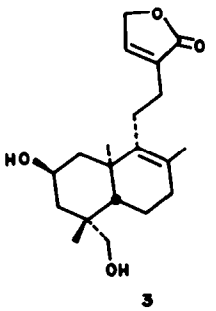
Key Word Index—*Phlogacanthus thyrsiflorus*; Acanthaceae; diterpene lactone glucoside; phloganthoside.

Abstract—A new diterpene glucoside, phloganthoside, has been isolated from *Phlogacanthus thyrsiflorus* and its structure has been established as phlogantholide-A-19-*O*-β-D-glucopyranoside.

INTRODUCTION

The isolation of a new diterpene lactone, phlogantholide-A‡ from the leaves of the plant *Phlogacanthus thyrsiflorus* Nees was reported recently by us [1]. The present communication reports the isolation from the leaves of the same plant of a new glucoside of phlogantholide-A, the structure of which has been established as phlogantholide-A-19-*O*-β-D-glucopyranoside (**1a**).





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‡The structure of phlogantholide-A was inadvertently printed in the abstract of our earlier paper [1] as 2β,15,18-trihydroxy-*ent*-labd-8(17),13-dien-16-oic acid lactone. It should have been 2β,15,19-trihydroxy-*ent*-labd-8(17),13-dien-16-oic lactone.

RESULTS AND DISCUSSION

Phloganthoside (**1a**) formed a pentaacetate (**1b**), $C_{36}H_{50}O_{14}$, M^+ *m/z* 706, which gave a pale yellow colour with tetranitromethane, had a UV absorption maximum (EtOH) at 209 nm (ε 9800) and gave a positive Legal test (*cf.* phlogantholide-A (**2a**) [1]).

The ^{1}H NMR spectrum (200 MHz, $CDCl_3$) of phloganthoside acetate (**1b**) showed singlets at δ2.02 (3H), 2.04 (9H) and 2.11 (3H) for five $-OCOC\underline{H}_3$ groups. There were mutually coupled signals at δ7.15 (1H, *t*, J = 1.5 Hz) and 4.81 (2H, *d*, J = 1.5 Hz) indicative of the presence of an endocyclic α,β-unsaturated γ-lactone moiety [*cf.* the ^{1}H NMR of phlogantholide-A diacetate (**2b**)]. There were also broad singlets at δ4.65 and 4.92 (1H each) for an exomethylene group besides singlets at δ0.78 (3H) and 1.02 (3H) for two tertiary methyl groups. The ^{1}H NMR spectrum of **1b** also displayed a resonance at δ5.02 [1H, *t* (*br*), $J \approx 10$ Hz] analogous to the H-2 resonance (δ4.91) in phlogantholide-A diacetate (**2b**). The non-equivalent methylene proton resonances in the spectrum of **1b** at δ3.31 and 3.90 (each 1H, *d*, $J \approx 10$ Hz) were closer to those of phlogantholide-A (**2a**) (δ3.37 and 3.66, each 1H, *d*, J = 11 Hz) than with those of phlogantholide-A diacetate (**2b**) (δ3.86 and 4.18, each 1H, *d*, J = 11 Hz). Thus phloganthoside is a mono-glycoside of phlogantholide-A linked through a C-19 oxygen function.

Phloganthoside (**1a**) underwent smooth enzymatic hydrolysis with β-glucosidase to yield phlogantholide-A (**2a**) and glucose. The identity of the latter was established by paper chromatography as well as by GC. The experiment indicated the sugar linkage to be β. Upon refluxing with ethanolic hydrochloric acid, phloganthoside (**1a**) afforded, however, isophlogantholide-A (**3**) [1].

The presence of a β-glucoside moiety was further indicated from the ^{1}H NMR spectrum of **1b** which displayed signals at δ4.43 (1H, *d*, $J \approx 8.0$ Hz, H-1'), 5.02 [1H, *t* (*br*), $J \approx 9.0$ Hz, H-2'], 5.10 (1H, *t*, $J \approx 9.5$ Hz, H-3'), 5.21 (1H, *t*, $J \approx 9.5$ Hz, H-4'), 3.67 (1H, *m*, H-5'), 4.14 (1H, *dd*, $J \approx 12.0$ and 2.0 Hz, H_a-6') and 4.28 (1H, *dd*, $J \approx 12.0$ and 4.5 Hz, H_b-6').

The mass spectral data of **1b** also supported the monoglucoside structure. Thus the spectrum was very similar to that of neoandrographolide tetraacetate [2] and

showed, besides the molecular ion peak at *m/z* 706, peaks at *m/z* 405, 359, 345, 331 and 285.

The ^{13}C NMR spectra of the glycoside acetate **1b** (20 MHz, $CDCl_3$) further established its structure and stereochemistry. The spectrum displayed signals for five non-protonated carbons [δ39.0 (C-4), 40.4 (C-10), 134.1 (C-13), 146.1 (C-8) and 173.9 (C-16)], four methines [δ55.0 (C-9), 56.1 (C-5), 67.9 (C-2) and 143.8 (C-14)], nine methylenes [δ21.7 (C-11), 24.1 (C-6 and C-12), 37.9 (C-7), 41.1 (C-3), 43.7 (C-1), 69.8 (C-15), 72.9 (C-19) and 107.4 (C-17)] and two methyls [δ15.3 (C-20) and 27.4 (C-18)] in addition to signals for an acetate function [δ21.0 (CH_3) and 170.2 (C=O)] and signals for a tetraacetylhexose moiety. The signals for the aglycone part closely resembled those of its congener phlogantholide-A (**2a**) and the corresponding diacetate (**2b**) [3]. A notable difference between the spectra of **2b** and the aglycone part of **1b** was the downfield shift of the C-19 methylene carbon resonance from δ68.2 in **2b** to δ72.9 in **1b**. This showed that the glycoside residue in **1a** had to be attached to C-19. The presence of a β-D-glucosyl moiety in the parent compound (**1a**) was also derived from the ^{13}C NMR spectrum of **1b**. Besides four acetate methyl resonances at δ20.4 and four acetate carbonyl signals at δ168.7, 169.0, 169.8 and 169.9 the spectrum also exhibited signals for a methylene carbon at δ61.7 (C-6'), and five methine carbons at δ68.2 (C-4'), 71.0 and 71.4 (C-2' and C-3'), 72.6 (C-5') and 100.4 (C-1'). The assignments were derived from comparison with the ^{13}C NMR data for methyl-β-D-glucoside and methyl-α-D-glucoside [4] and from a consideration of the small downfield shift of the α-carbon and the small upfield shift of the β-carbon upon acetylation.

Information concerning the pyranose form of the sugar and the configuration of the glucosidic linkage was obtained from the ^1H NMR data discussed earlier. This was further corroborated by calculation of the molecular rotation [5–7]. The molecular rotation of crystalline phloganthoside acetate (**1b**) was observed to be $-161.67°$ showing an acceptable difference of $-12.4°$ from the calculated value $-149.27°$ (based on a reported [8] molecular rotation value $-65.88°$ for tetraacetyl-β-D-glucopyranoside and $-83.39°$ for phloganthoside acetate (**1b**)). This indicated the sugar in phloganthoside to be D-glucose. If the sugar was L-glucose then the calculated [M] value would be $-17.51°$.

On the basis of the data presented above the structure of phloganthoside may be represented as phlogantholide-A-19-*O*-β-D-glucopyranoside (**1a**).

EXPERIMENTAL

Mps: uncorr; MS: AEI MS-30 instrument; CC: silica gel (BDH, India) (60–120 mesh); TLC: silica gel G (BDH, India). Plant material was collected from suburbs of Calcutta in November, 1982, when in full leaf and identified by the Keeper, National Botanic Garden, Shibpur, Howrah 711103, West Bengal, India by comparison with a herbarium specimen.

Isolation of phloganthoside. The ethanolic extract of the defatted air-dried crushed leaves (5 kg) of *P. thyrsiflorus* was concentrated under reduced pressure and the residue successively extracted with $CHCl_3$ and *n*-BuOH. The residue obtained from the *n*-BuOH extract was subjected to CC over deactivated silica gel. The $CHCl_3$–MeOH (93:7) eluates afforded an almost homogeneous (TLC) material. Further purification by preparative TLC furnished phloganthoside (**1a**), amorphous (230 mg).

Phloganthoside pentaacetate (**1b**). Phloganthoside (100 mg), C_5H_5N (1 ml) and Ac_2O (2 ml) were heated on steam bath for 3 hr. Work-up in the usual way followed by CC over silica gel yielded the crystalline pentaacetate **1b**, 90 mg, mp 193–194°, $[\alpha]_D^{26}$ $-22.9°$ ($CHCl_3$). (Found: C, 61.35%; H, 7.45%. M^+ 706. $C_{36}H_{50}O_{14}$ requires C, 61.16%; H, 7.13%. M_r 706.)

Enzymatic hydrolysis of phloganthoside. A solution of phloganthoside (25 mg) in acetate buffer, pH 5 (0.5 M, 15 ml), was incubated with β-glucosidase (25 mg, Sigma) overnight at 37°. The mixture was then diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract gave phlogantholide-A (15 mg).

The sugar obtained after removal of H_2O from the aqueous phase was silylated in C_5H_5N with hexamethyldisilazane and trimethylchlorosilane for 30 sec. On GC analysis [3% SE-30 (silicone polymer) on Diatomite C (dimethylchlorosilane treated, 80–100 mesh) (6' × ¼" coiled stainless steel column), column temp. 160°, N_2 60 ml/min, detector and injector port temp. 250° and 225°, respectively], the derivative had the same R_t as that of the derivative of D-glucose. The sugar was also identified as glucose by PC [Whatman No. 1 filter paper; *n*-BuOH–HOAc–H_2O, 4:1:1 (descending); spray reagent: aniline hydrogen phthalate].

Acid hydrolysis of phloganthoside. Phloganthoside (100 mg) was refluxed with ethanolic HCl (5%; 50 ml) on a steam bath for 6 hr. Dilution with H_2O and extraction with $CHCl_3$ gave, after work-up isophlogantholide-A (**3**), 65 mg, mp 144°, M^+ 334.

The aqueous part was neutralized with Ag_2CO_3 and the sugar identified as glucose by PC.

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