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# STYPANDRONE: A TOXIC NAPHTHALENE-1,4-QUINONE FROM *STYPANDRA IMBRICATA* AND *DIANELLA REVOLUTA*

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**Key Word Index**—*Stypandra imbricata*; *Dianella revoluta*; Liliaceae; blindgrass; blue-flax lily; toxicity; naphthoquinone; stypandrone.

**Abstract**—A compound, previously not isolated from dried, milled samples of *Stypandra imbricata* and *Dianella revoluta*, has now been obtained from fresh samples of these plants. The structure was shown, by spectroscopic techniques, to be identical to that of stypandrone. This quinone was found to be toxic to laboratory mice. However, it produces a different toxic effect to that observed when livestock ingest fresh *Stypandra imbricata* or when stypandrol is administered to laboratory mice.

## INTRODUCTION

The naphthalene-1,4-quinone, stypandrone (**1**), previously isolated from *Stypandra grandis* [1] and *Dianella nigra* [2], has not been isolated from any other source including, specifically, *Dianella revoluta* [1, 3]. It is one of a group of oxidatively related compounds from species of the Liliaceae. The other compounds are the putative precursor, dianellidin (**2**) [4–6] and the products of oxidative coupling, stypandrol (**3**) [5, 6], dianellinone (**4**) [1, 6] and trianellinone (**5**) [3]. Of these, only stypandrol has any reported biological activity. Stypandrol, isolated from toxic samples of *Stypandra imbricata* (blindgrass) [5] has been shown to induce the same toxic effects as those caused by ingestion of the plant by livestock [5, 7, 8]. It has also been recently isolated in small yields from the roots of *Dianella revoluta* (blue-flax lily) [6].

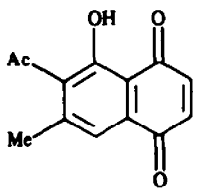
## RESULTS AND DISCUSSION

As a result of a seasonal and growth-site survey of fresh *Stypandra imbricata* for the presence of **3** and related compounds, a previously unobserved TLC spot with a higher $R_f$ (0.6) than **3** ($R_f$ 0.1) and **4** ($R_f$ 0.3) and a lower $R_f$ than **2** ($R_f$ 0.7) was observed. This orange TLC spot turned purple when exposed to ammonia as does dianellinone (**4**) thus indicating the presence of a similar hydroxy-quinone entity. This TLC component was not observed when the fresh plant material or chloroform extracts were fully dried at room temperature for several days or in an oven for several hours at 50°.

The compound was isolated from the chloroform extracts of fresh leaves of *Stypandra imbricata* and roots of *Dianella revoluta* by radial chromatography. The orange solid was then readily purified by vacuum sublimation. It is moderately unstable on silica gel and thus chromatography should not be prolonged. The compound is also unstable in methanolic solution producing a black, insoluble solid. Analysis of the mass, infrared, $^{13}C$ NMR and $^{1}H$ NMR spectroscopic data indicates that the compound is 6-acetyl-5-hydroxy-7-methylnaphtho-1,4-quinone previously isolated from *Stypandra grandis* [1] and named stypandrone (**1**). This structural assignment was confirmed by oxidizing the naphthalene-diol, dianellidin (**2**), with Frémy's salt, to yield **1** identical in all respects to the natural product. A minor product of the Frémy's salt oxidation was shown, by NMR and mass spectroscopy, to be 3-acetyl-5-hydroxy-2-methyl-1,4-naphthoquinone (**6**) as previously described by Cooke *et al.* [1, 4]. The structure of stypandrone (**1**) assigned by Cooke *et al.* was confirmed by synthesis [9, 10]. The stability of stypandrone, but not its isomer (**6**), was in marked contrast to that reported by Briggs *et al.* [2]. The yield of stypandrone (**1**) from the plants was dependent upon the extent of drying of the plants prior to extraction. Fresh plants provided the greatest yield whilst thoroughly dried plants did not afford any stypandrone.

### *Biological activity of stypandrone*

As part of a current investigation into the molecular mechanism of action of the 'blindgrass' toxin, stypandrol (**3**), stypandrone (**1**) was tested for biological activity. Intraperitoneal administration of 1–2 mg to laboratory mice (30 g) resulted in death within 3 hr. There was no clinical or morphological evidence to suggest a similar toxic effect to that observed with stypandrol (**3**) [5, 7, 8]. The other compounds tested, dianellidin (**2**) and dianellinone (**4**), had no clinical effect. **1** also showed no clinical effect when administered intragastrically. A detailed examination of the toxic effect of **1** is currently in progress. However, initial investigations demonstrate that intraperitoneal injection with stypandrone in physiological saline/cremaphor carrier will produce an ascites, where the carrier alone has little or no effect. The loss of this ascites fluid from the vascular circulation results in the elevation of the blood's packed cell volume, thus making the blood more viscous. Stypandrone causes a methaemoglobinaemia in poisoned animals, thus reducing the oxygen-carrying capacity of the blood. It also promotes the conversion of haemoglobin to methaemoglobin *in vitro*. These factors may all contribute to the death of affected animals.

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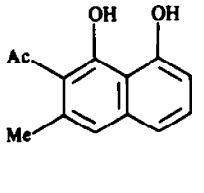


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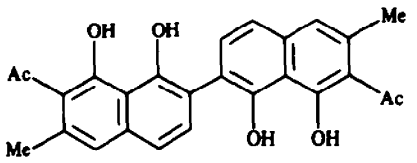


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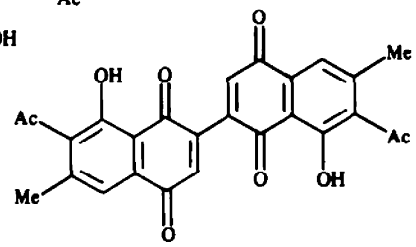


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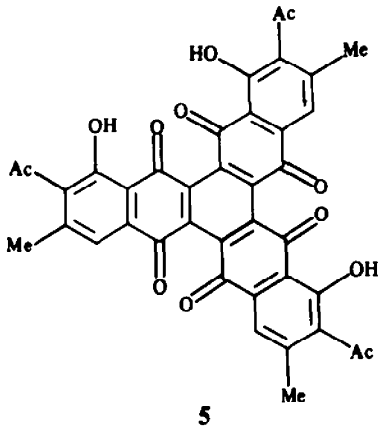


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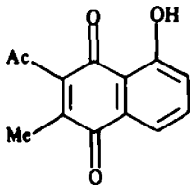


6

## EXPERIMENTAL

Mps are uncorr. The 80 MHz $^1$H and 20.1 MHz $^{13}$C NMR and MS were recorded at the Chemistry Department of the University of Western Australia. Kieselgel 60 $PF_{254}$ gipshaltig (Merck) and Kieselgel 60G (Merck) were used as the adsorbents for radial and thin-layer chromatography. Light petrol refers to the fraction with b.r. 60–80°. Samples of *Stypandra imbricata* and *Dianella revoluta* were collected from various locations in the south-west of Western Australia, and the biological activity of isolated compounds was tested by the intragastric and intraperitoneal administration of suspensions in 10% cremaphor (polyethoxylated castor oil) in physiological saline to laboratory mice and rats.

*Extraction of the plants.* Fresh samples of the leaves from *Stypandra imbricata* or of the roots of *Dianella revoluta* were extracted at room temp. with $CHCl_3$. The crude extracts thus obtained were chromatographed on a rotating disc of silica gel using petrol and petrol–$CHCl_3$ mixtures as the eluents. The first band obtained was yellow and consisted mainly of dianellidin (2), identified as previously described [5], mixed with long chain alcohols and aldehydes. Elution of the first band was closely followed by elution of an orange band. Evaporation of the solvent afforded an orange solid comprised of a mixture of fats and stypandrone (1). The orange TLC spot due to stypandrone rapidly turned purple on exposure to ammonia vapour.

Vacuum sublimation (90–100°/10 mm) afforded stypandrone from the mixture as an orange solid, mp 129–130° (dec.) (lit. [1] mp 135.5–136°). The yield of stypandrone varied from 0–0.01% fr. wt. $^{13}$C NMR ($CDCl_3$) $\delta$202.7, *s*, acetyl carbonyl; 189.9, *s*, C4 carbonyl; 183.9, *s*, C1 carbonyl; 158.5, *s*, C5–OH; 144.9, *s*, C6–$COCH_3$; 139.5, *d*, C2; 138.8, *d*, C3; 136.1, *s*, C9; 131.3, *s*, C7–$CH_3$; 121.4, *d*, C8; 113.3, *s*, C10; 31.7, *q*, acetyl $CH_3$; 20.1, *q*, aryl $CH_3$. $^1$H NMR ($CDCl_3$) $\delta$12.04, 1H, *s*, C5–OH; 7.39, 1H, *s*, H8; 6.87, 2H, *s*, H3 and H2; 2.58, 3H, *s*, acetyl $CH_3$; 2.37, 3H, *s*, aryl $CH_3$. EIMS, *m/e* 230 (M, 61%), 215 (100), 202 (1), 187 (3), 159(4), 131 (14). IR ($CHCl_2$) 1709 (*s*), 1676 (*s*), 1646 (*s*), 1600 (*s*), 1282 (*m*), 1242 (*m*), 1110 (*m*), 1060 (*m*), 851 (*s*).

The next band contained the binaphtho-1,4-quinone, dianellinone (4) as previously reported [6]. $^{13}$C NMR ($CDCl_3$/TFA) $\delta$211.1, *s*, acetyl carbonyl; 187.7, *s*, C1 carbonyl; 186.5, *s*, C4 carbonyl; 160.3, *s*, C8–OH; 147.2, *s*, C2; 145.0, *s*, C7–$COCH_3$; 139.6, *d*, C3; 136.9, *s*, C10; 132.7, *s*, C6–$CH_3$; 123.1, *d*, C5; 114.0, *s*, C9; 32.3, *q*, acetyl $CH_3$; 20.5, *q*, aryl $CH_3$.

*Determination of methaemoglobin in blood.* The blood obtained from a laboratory rat, acutely intoxicated by 1, was assayed for methaemoglobin content as described by Sunshine [11].

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