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EFFECT OF UGANDENSIDIAL ON MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION

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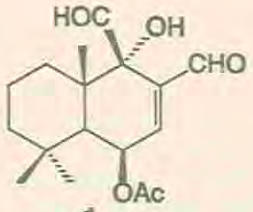
ABSTRACT. Toxicological studies with ugandensidial, a representative of a range of biologically active sesquiterpene dialdehydes, was carried out. Addition of ugandensidial at 10 μg/ml after the respiratory substrates in a reaction medium inhibited both the mitochondrial oxygen consumption and subsequent phosphorylation of ADP. The uncoupler carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) did not relieve the inhibition of oxygen consumption due to ugandensidial hence, this sesquiterpene dialdehyde may have inhibited electron transport in intact mitochondria. Present observations may explain the possible mechanism of the cytotoxicity of ugandensidial as well as closely related sesquiterpene dialdehydes.

INTRODUCTION

Ugandensidial (**1**) [1,2] is a sesquiterpene dialdehyde isolated from the bark of *Warbugia stuhlmanii* Engl. and *W. ugandensis* Sprague species of the genus *Warbugia* (Canellaceae) distributed in East Africa. The two species are widely used in local folk medicine to alleviate toothache, rheumatism, general body pains, diarrhoea and malaria [3]. The bark of *W. ugandensis* is commonly known by several different names in Kenya, depending on the local tribe, such as "Apacha" (Kakamega), "Muthiga" (Kikuyu), "Olosogoni" (Masai), "Soget" (Kipsigis), "Soke" (Tugen) and "Sogomaitha" (Luo).

The distribution of *W. stuhlmanii* is limited to the coastal areas of Kenya and Tanzania, and is known as "Mkaa" (Swahili). Ugandensidial has been reported to exhibit strong insect antifeedant activity against the African-army worm (*Spodoptera exempta*) [4] and tastes very hot to humans [5]. In addition, some closely related dialdehydes, polygodial (**2**), warbuganal (**3**) and muzigadial (**4**) also isolated from *W. ugandensis* and *W. stuhlmanii*, exhibit a number of interesting biological activities including a marked molluscicidal activity against the schistosoma transmitting snail *Biomphelaria glabaratus*, very hot taste to humans, [5,6] and toxicity to *Dendrodoris limbata* (Nudibranchia, Doridacea) when injected directly into the hepatopancreas [7]. Recent studies on the evaluation of polygodial (**2**) and its congeners as antifungal potentiators [8] revealed that when cells of *Saccharomyces cerevisiae* are treated *in vitro* with polygodial for 10 min., the cell membrane becomes severely damaged and many vesicles, possibly formed from fragmented cell membrane, were observed within the cytoplasm. This observation, led Kubo *et al.* [8] to investigate the use of polygodial to facilitate the transmembrane transport of exogenous chemicals into cells. The proposed mode of action of sesquiterpene dialdehydes on cell membranes is not clear,

however, it is thought to involve the interaction of the enal aldehyde which acts as a nucleophilic acceptor for amino and or thiol groups on cell membranes. The resultant adduct then cyclizes with the 9ß-aldehyde system located at a critical distance from the enal aldehyde to form a pyrrolidine ring, thus bearing a close similarity to that proposed for the chemoreceptor membranes of insects [9]. Then there exists a strong possibility that sesquiterpene dialdehydes bearing the enedial moiety with two aldehydes in the correct spatial arrangement as those in ugandensidial (**1**), polygodial (**2**), warbuganal (**3**) and muzigadial (**4**) may interfere with the function of a natural membrane as in



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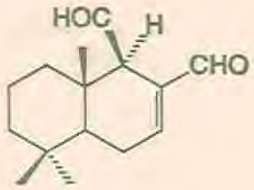

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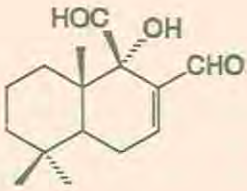

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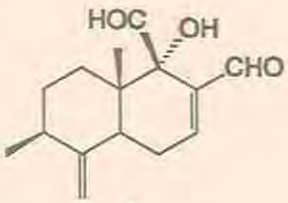

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mitochondria.

To test this hypothesis, the dialdehyde ugandensidial (**1**) which is a representative of a range of biologically active sesquiterpene dialdehydes, was isolated, characterized, and the effect of its interactions with rat liver mitochondrial oxidative phosphorylation characteristics investigated.

EXPERIMENTAL

Plant Material. The bark of *W. ugandensis* was collected in Eldoret, Uasin Gishu District, Kenya, in March 1990. The plant was identified by comparison with a preserved standard specimen at the National Museum Herbarium in Nairobi, Kenya. *Isolation and Identification. W. ugandensis* bark (2.0 kg) was air dried, powdered and allowed to stand in MeOH at room temperature for 1 week. The extract was decanted and the residual pulp similarly extracted a second time. The combined extracts were evaporated under vacuum and the residue partitioned between $CHCl_3$ and H_2O. After drying over Na_2SO_4, the $CHCl_3$ layer was evaporated under vacuum to yield 28 g of a

brown oil. A portion of the oil (10 g) was carefully chromatographed on silica gel (70-130 mesh, Merck) using a CH_2Cl_2/EtOAc gradient. This led to the isolation of ugandensidial (240 mg) as a pale yellow solid. The crude product was recrystallized from EtOAc/hexane to give 200 mg of white needles (mp 138-140°) which exhibited IR, UV, MS, ^1H and ^{13}C NMR spectra essentially identical with those reported in the literature for ugandensidial (**1**) [1,2].

Preparation of rat liver mitochondria. Rat liver mitochondria were prepared from Sprague-Dawley strains. Mitochondria were isolated using 0.25 M sucrose in presence of 0.5 mg/ml BSA (fatty acid free). The procedure used was essentially as described by Hogeboom and Schneider [10]. All preparations were carried out at 4°.

Determination of oxygen consumption. The oxygen consumption rate was recorded polarographically by an oxygen electrode operated at 25° in a closed and magnetically stirred glass chamber. The reaction medium contained 210 mM mannitol, 70 mM Tris-HCl buffer (pH 7.4) , 3 mM phosphate and 1 mg/ml mitochondrial protein in a total volume of 1.70 ml. The substrate used was either 5 mM K-malate plus 5 mM K-glutamate or 5 mM succinate, in the latter case 5 μM rotenone was included in the reaction mixture. When used, the concentration of other reagents were as follows: 294 μM ADP, 3 μM CCCP, ugandensidial (in ethanol solution) was added at a concentration of 10 μg/ml.

Determination of mitochondrial protein. Mitochondrial pellet protein was determined using biuret method as described by Jacobs *et al* [11].

Determination of respiration control ratio (RCR). Respiration control ratio (RCR) was calculated as the rate of oxygen consumption during "state 3" respiration to that of "state 4" respiration as described by Chance and Williams [12].

Determination of ADP/O ratio. The ADP/O ratio was calculated as the ratio of the amount of ADP (n mol) used over the amount of oxygen (n atoms) consumed in the presence of ADP.

RESULTS AND DISCUSSION

In the absence of ugandensidial (control), the respiratory control ratio (RCR) was 4.72 and 4.64 using malate plus glutamate and succinate, respectively. The ADP/O ratio using malate plus glutamate as the substrate was 2.83 and 1.87 when succinate was used (Fig. 1 trace B and Fig. 2 trace D). These observations indicate that the mitochondria were actively respiring and could effectively phosphorylate ADP to ATP.

In both Fig. 1 trace A and Fig. 2 trace C, addition of ugandensidial after the substrates (malate + glutamate and succinate, respectively) inhibited both the oxygen consumption and subsequent oxidative phosphorylation of ADP. The observation that further addition of uncoupler CCCP did not relieve the inhibition of oxygen consumption due to ugandensidial points to a possibility that this sesquiterpene dialdehyde may have inhibited the electron transport [3-14]. However, since this inhibition was observed both with malate plus glutamate as well as succinate plus rotenone, it is most likely that ugandensidial inhibits the electron transport at a point beyond the rotenone site.

Muzigadial (**4**) possessing a reactive enedial functionality, has been shown to react with L-cysteine to form a pyrrolidine ring [9]. This ability to chemically interact with an amino acid by this compound may explain in part the mechanism of the cytotoxicity of

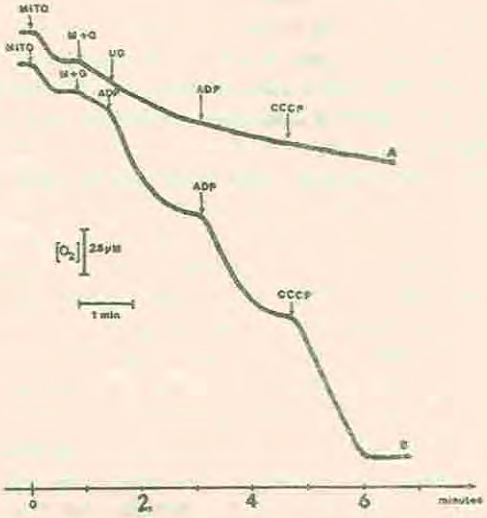

Fig. 1. Oxygen electrode recording of rat liver mitochondria respiring in a medium of 210 mM mannitol, 70 mM sucrose, 20 mM Tris-HCl (pH 7.4), 0.5 mg/ml mitochondrial protein (MITO), 294 µM ADP, 3 µM carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) and 10 µM ugandensidial (UG). Substrate is malate + glutamate (M + G) 5 mM each. Chamber vol.= 1.70 ml at 25°.

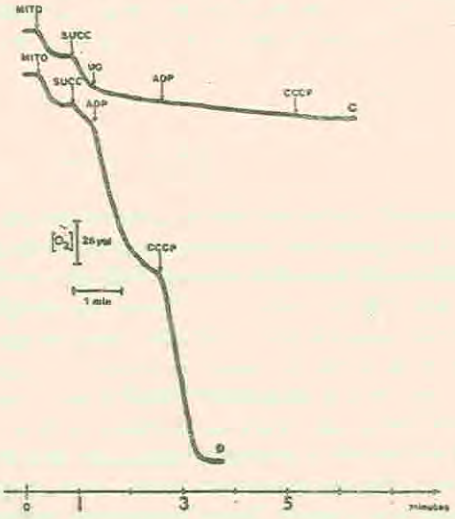

Fig. 2. Oxygen electrode recording of rat liver mitochondria respiring in a medium of 210 mM mannitol, 70 mM sucrose, 20 mM Tris-HCl (pH 7.4), 0.5 mg/ml BSA and 3 mM phosphate (pH 7.4) when indicated, 1 mg/ml mitochondrial protein (MITO), 294 µM carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) and 10 µM ugandensidial (UG). Respiratory substrate is 5 mM succinate (SUCC). Reaction chamber volume was 1.70 ml and temperature 25°.

ugandensidial which also possesses a similar enedial functionality. Mitochondria are essentially lipo-protein [15-19] and ugandensidial may have, interacted with the inner mitochondrial membrane proteins (enzymes) hence, inhibiting the flow of electrons as well as oxidative phosphorylation of ADP to ATP (Fig. 1A and 2C). Further work on the structure-activity relationship of the sesquiterpene dialdehydes is in progress in our laboratory.

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