INHIBITION OF MITOCHONDRIAL FUNCTION IN TERMITE QUEEN OVARY BY *RABDOSIA* DITERPENOIDS

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ABSTRACT.—The effect of diterpenoids from plants of the genus *Rabdosia* (Labiatae) on the energy-linked functions in mitochondria from termite queen ovary has been investigated polarographically with an oxygen electrode. Some *Rabdosia* diterpenoids inhibited oxidative phosphorylation and significantly depressed the rate of respiration in state 3 and in 2,4-dinitrophenol uncoupled mitochondria (state 4U) without depressing the respiration rate in state 4.

We have previously reported that the diterpenes from the plants of the genus *Rabdosia*[1] (Labiatae) possess both specific antimicrobial activity against gram-positive bacteria (1) and cytotoxic activity against KB tissue culture (2). These same compounds have been shown to exhibit a weak inhibitory effect on oxidative phosphorylation in mitochondria isolated from rat liver and silkworm midgut (3).

The present work is an investigation of the inhibitory effect of *Rabdosia* diterpenoids on respiratory function in mitochondria isolated from the ovary of a termite queen of *Macrotermes subhyalinus*.

MATERIALS AND METHODS

DITERPENES.—The *Rabdosia* diterpenoids used in this study were all analytically pure samples obtained from our previous studies. The selection of these compounds was based mainly upon the availability of the samples as well as their characteristic structure types.

INSECT.—Termite queens of *M. subhyalinus* were collected from termite mounds located in the Kajiado area near Nairobi, Kenya. Among the 400 species of African termites, *M. subhyalinus* was selected for research material because, although it is not considered an economic pest, it is readily available from the large mounds (up to five meters tall above ground).

PREPARATION OF MITOCHONDRIAL SUSPENSIONS.—The queen ovaries replete with eggs (15 g) were dissected and homogenized. The mitochondrial suspensions were prepared from the homogenate as previously described (4, 5).

MEASUREMENT OF OXIDATIVE PHOSPHORYLATION.—The reaction mixture of mitochondria contained 250 mM sucrose, 30 mM K_2HPO_4, 15 mM KCl, 2 mM EDTA, 5 mM $MgCl_2$, 50 mM Tris-HCl, test compounds, 1 mg of mitochondrial protein and 20 mM succinate as a respiratory substrate at pH 7.4. During the course of the reaction, 0.2 μmole ADP was added to the mixture. A Clark oxygen electrode coupled to a recording system was used to measure oxygen consumption polarographically at 26°. The measurement was repeated five times. Oxidative phosphorylation activity was calculated from the oxygen electrode tracing and expressed as a

[1]This genus was previously known as *Isodon*. (H. Hara, *J. Jap. Bot.*, **47**, 193 (1972))

respiratory control ratio and an ADP/O ratio. The phosphorylation rate was calculated as the ADP/O ratio x the state 3 respiration.

RESULTS AND DISCUSSION

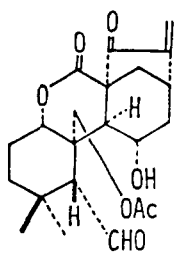
As shown in table 1, the *Rabdosia* diterpenes, isodonal (**1**) (6), enemin (**2**) (7), oridonin (**3**) (8), and nodosin (**4**) (9), exhibited an inhibitory effect on the mitochondrial oxidative phosphorylation. Interestingly, (**2**) and (**4**) lowered the respiratory control ratio and phosphorylation rate but did not affect the ADP/O ratio at 100 and 250 μM, respectively.

TABLE 1. Effect of *Rabdosia* diterpenoids on some energy-linked functions in mitochondria isolated from termite queen ovary.

Compounds	Conc. μM	Respiratory rate[a]			Respiratory control ratio	ADP/O	Phosphorylation rate[c]
		State					
		3	4	4U[b]			
None		148.9±3.2	33.6±1.5	181.4±3.5[d]	4.43	1.72±0.16[d]	256
1	100	117.6±3.1	44.2±1.8	41.2±3.0	2.66	1.03±0.08	121
2	100	114.6±2.6	33.6±1.1	103.6±2.4	3.41	1.62±0.21	186
3	100	117.6±2.9	32.4±0.9	106.0±2.1	3.63	1.33±0.15	156
4	250	94.8±4.0	41.2±1.9	61.8±2.2	2.30	1.79±0.11	170

[a]Expressed as atoms O/mg protein/min. [b]In the presence of 2,4-dinitrophenol at 150 μM. [c]Expressed as nmoles ATP synthesized/mg protein/min. [d]Mean±SE.

These compounds (**1** to **4**) significantly depressed the rate of respiration in state 3 and in state 4U (2,4-dinitrophenol uncoupled mitochondrial), though hardly affecting that in state 4. Thus, they seem to act neither as an uncoupler, *e.g.*, 2,4-dinitrophenol, nor as a phosphorylation inhibitor, *e.g.*, oligomycin, because the former markedly stimulates the respiratory rate in state 3 and in state 4,



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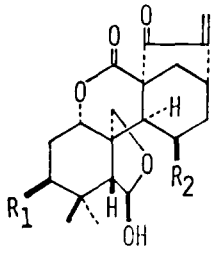
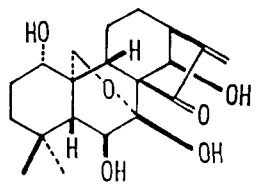


2, R_1=OH, R_2=H



3

4, R_1=H, R_2=OH

while the latter inhibits only the respiratory rate in state 3 but shows no effect in state 4 or state 4U. The *Rabdosia* diterpenoids seem to have an action on the respiratory chain enzymes similar to that of cyanide and antimycin A.

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