Inhibition of Oxygen Evolution by Cacalol and Its Derivatives

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The inhibition of ATP synthesis, proton uptake and electron transport (basal, phosphorylating and uncoupled) from water to methylviologen indicates that cacalol and its derivatives act as electron transport inhibitors. Since on one hand photosystem I is not affected and electron transport from DPC to QA is midly affected and on the other hand the electron transport from water to DCIP, and water to silicomolibdate are inhibited, we conclude that the site of inhibition of cacalol is located at the oxygen evolution level. Cacalol derivatives inhibit electron flow between P680 to QA and probably also the Q_B site.

Introduction

Cacalol (9-hydroxy-3,4,5-trimethyl-5,6,7,8-tetrahydronaphtho (2,3-b) furan was isolated from the roots of *Psacalium decompositum* Gray (Syn. *Odontorichum decompositum* Gray Rydb); *Cacalia decompositae* (Gray), a shrub native of northern Mexico, is a furotetralin derivative, a sesquiterpene [1, 2]. Cacalol is a major component of the compounds quantified.

The structure of cacalol was proposed on the basis of chemical and spectroscopic evidences [1, 3–5] and was confirmed by X-ray studies [6] and synthesis [7–9]. It is known that cacalol forms an homodimer with UV light radiation [9]. Cacalol is unstable and oxidized by oxygen and light. During its synthesis cacalol is chemically transformed into methyl cacalol, cacalol acetate or 2-acethylcacalol acetate (Fig. 1).

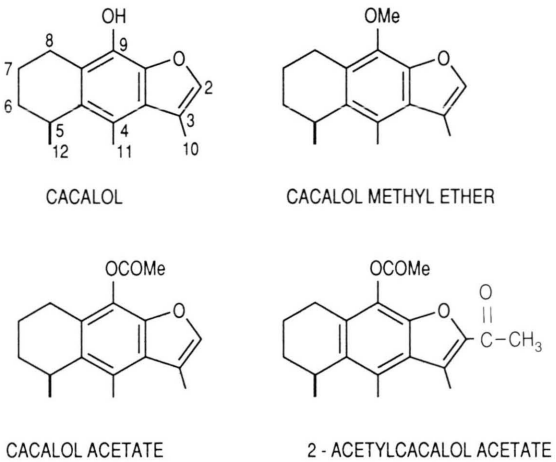

Fig. 1. Structures of cacalol and its derivatives.

The infusion made from the roots of *C. decompositae* Gray is used against rheumatism, colds, back pains, jaundice, colic in babies, diabetes, malaria, fever, to treat snake bites and as a diuretic, tonic or antiseptic wash for wounds ([2], references herein). The effects of the infusion are probably a consequence of cacalol, one of the major components found in it.

The biological role of cacalol in plants is not known, but it probably behaves as an allelochemic agent, interferring with the metabolism of other organisms. The biochemical basis of this action is not well known. Present results, which show that cacalol inhibits oxygen evolution, suggest a way in which they might act as allelochemic agents by interferring with the growth of photosynthetic organisms.

Materials and Methods

Cacalol was obtained as reported [9] and dissolved in acetonitrile. Chloroplast thylakoids were isolated from market spinach leaves (*Spinacea oleracea* L.) as described earlier [10–12] and sus-

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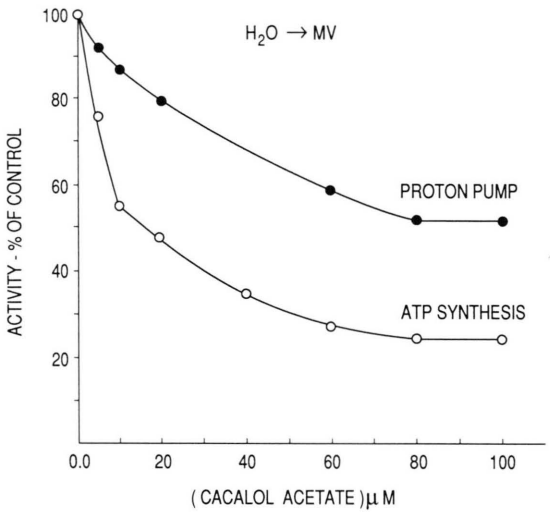
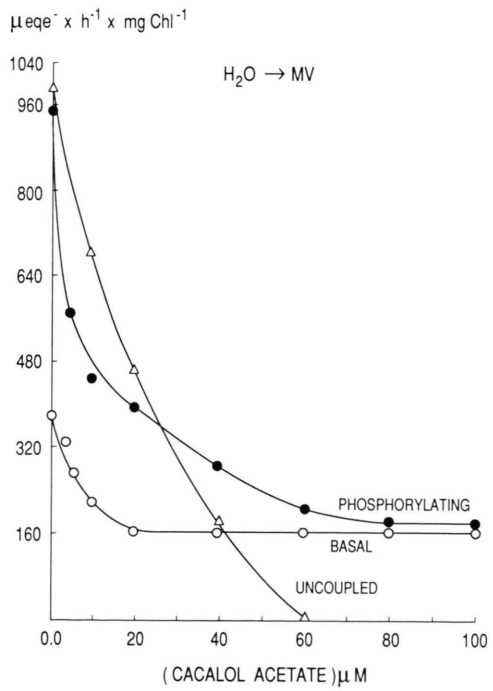
pended, unless indicated, in 100 mM sorbitol, 5 mM $MgCl_2$, 40 mM KCl and buffered with 30 mM Na^+-tricine at pH 7.6; 0.05 mM methylviologen was added as electron acceptor for Hill reaction from water to methylviologen. KCN (1 mM) was added to inhibit catalase activity. Chlorophyll [13], noncyclic electron transport from water to methylviologen [10–12], proton pump [10] and ATP synthesis [10, 11] were determined as described in the literature. All reaction mixtures were illuminated with actinic light of a projector lamp (Gaf 2660) and were passed through a filter of 5 cm of a 1% $CuSO_4$ solution [10–12]. Photosystem I and photosystem II [11, 12, 14], electron transport from water to silicomolibdate [15] and diphenylcarbazide (DCP) to DCIP or DCIP/ferricyanide [16] were measured as reported.





Results and Discussion

Photosynthetic phosphorylation (from water to methylviologen) in spinach thylakoid was inhibited by cacalol acetate (Fig. 2A), the I_{50} (concentration producing 50% inhibition) was about 16 μM. The light-dependent proton uptake was less inhibited (Fig. 2A). The light-dependent synthesis of ATP by illuminated thylakoids may be inhibited in a number of ways: a) by blocking the electron transport, b) by uncoupling ATP synthesis from the electron transport, and c) by blocking the phosphorylation reaction itself. Reagents that block electron transport also inhibit ATP synthesis due to the fact that the generation of the transmembranes electrochemical gradient, the driving

Fig. 2. A) Photophosphorylation and proton uptake from water to methylviologen as a function of cacalol acetate concentrations. Proton uptake was measured at a pH rise between pH 8.0 and 8.1 as described by Dilley [22] using a combination microelectrode connected to a Corning potentiometer with expanded scale. The pH changes were recorded (Gilson recorded). After the illumination period, H^+-produced were quantified by titration with a standard solution 0.01 N HCl. Photophosphorylation was measured as in proton uptake condition, in the presence of 1 mM ADP and 3 mM K^+-phosphate. Each cuvete contained 20 μg chlorophyll per mL in 100 mM KCl, 5 mM $MgCl_2$ and 1 mM Na-tricine pH 8, 0.05 mM methylviologen was added as electron acceptor; reaction time: one minute, aerobic conditions; saturating white light. B) Noncyclic electron transport from water to methylviologen (basal electron transport) as a function of cacalol acetate concentrations, was determined with an oxygraph YSI Model 5300. Each cuvete contained 20 μg chlorophyll per mL in the reaction medium as in A), except that Na-tricine concentration was changed to 15 mM; reaction time 1 min, aerobic conditions, saturating white light. Other conditions as described at Materials and Methods. Phosphorylating electron transport was measured as basal electron transport and 1 mM ADP and 3 mM K_2HPO_4 were added. Uncoupled electron transport was measured as basal electron transport and 3 mM NH_4Cl was added.

force for ATP synthesis, is dependent upon electron flow. Chemicals that increase the proton permeability of thylakoid membranes uncouple phosphorylation from electron flow. Uncoupling agents inhibit ATP synthesis by decreasing the proton gradient but allow electron transport to occur at high rates. In contrast, direct inhibitors of photophosphorylation block phosphorylation and the portion of electron transport that is a consequence of proton efflux linked to phosphorylation [10]. The inhibition of photophosphorylation produced by cacalol acetate can be explained by its effect on either the electron transport or the energy transfer reactions.

In order to obtain further information we studied the effect of cacalol acetate on the photosynthetic electron transport (basal, phosphorylating and uncoupled). Fig. 2 B shows that basal, phosphorylating and uncoupled electron transports from water to methylviologen were inhibited by the addition of cacalol acetate at concentrations similar to those affecting ATP synthesis, and proton uptake (compare Fig. 2 A with 2 B). These results show that cacalol acetate behaves as a typical electron transport inhibitor, since it inhibited uncoupled electron transport and photophosphorylation as well as the proton pump.

Table I and Table II show that cacalol, methyl cacalol and 2 acethyl cacalol acetate inhibited uncoupled electron transport from water to methylviologen, ATP synthesis and proton uptake in a very similar way to the way cacalol acetate does; therefore these compounds also act as electron transport inhibitors.

In order to localize the inhibition site of cacalol and its derivatives, photosystem I and II were measured in the presence of those chemicals. The activity of photosystem I was not affected by these compounds (data not shown). However, 60 μM cacalol completely inhibited electron transport from water to DCIP (Table I); methyl cacalol, cacalol acetate an 2-acethyl cacalol acetate only inhibited arround 50% of the activity of photosystem II even at 100 μM of these compounds. Electron transport from water to silicomolibdate was inhibited in the same extent by cacalol derivatives (Table I). Once again cacalol was the only compound which inhibited completely this activity (Table I).

Table I. Uncoupled Hill reaction from water to methylviologen was measured as described in Fig. 2 B. Uncoupled photosystem II from water to DCIP, reaction mixture as described in uncoupled Hill reaction, concentrations of DCIP 100 μM, DBMIB 1 μM, $[Fe(CN)_6]K_3$ 200 mM, NH_4Cl 3 mM. Control value for uncoupled photosystem II electron transport rate expressed in $\mu eqe \overline{\times} ch^{-1} \times mg\ Chl^{-1}$ was 1200. Uncoupled electron transport from water to silicomolibdate, reaction mixture as on photosystem II measurement except that DCIP was changed with 100 μM silicomolibdate and 10 μM DCMU. Control value for electron transport rate from H_2O to SiMo expressed in $\mu eqe \overline{\times} h^{-1} \times mg\ Chl^{-1}$ was 140. DCP to DCIP was measured spectrophotometrically as reported [4] in the resuspension medium, control value for electron transport rate from DCP to DCIP expressed in $\mu eqe \overline{\times} h^{-1} \times mg\ Chl^{-1}$ was 120.

Compounds	Addition μμ	H_2O MV [%]	H_2O DCIP [%]	H_2O SiMo [%]	DPC DCIP [%]
Control	none	0.0	0.0	0.0	0.0
Cacalol	10	44	50	ND	0.0
	20	46	63	25	0.0
	60	100	100	100	0.0
2-Acethyl cacalol acetate	10	22	44	ND	ND
	20	35	44	ND	ND
	100	77	44	50	65
Methyl cacalol	10	32	50	ND	ND
	20	46	53	ND	ND
	100	81.5	53	38	68
Cacalol acetate	10	22	75	ND	ND
	20	54	75	ND	ND
	100	100	75	25	15

ND⁺, not determined.

Table II. ATP synthesis and proton uptake from water to methylviologen were measured as described in Fig. 2A, control value for ATP synthesis was 350 μmol × h^{-1} mg chl^{-1} and for proton uptake was 14.4 μeq × H^+h^{-1} × mg Chl^{-1}.

Compounds	Addition [μM]	Inhibition ATP synthesis [%]	Inhibition Proton uptake [%]
Control	none	0.0	0.0
Cacalol	10	40	32
	20	40	50
	60	31	50
2-Acethyl cacalol acetate	10	45	42
	20	46	43
	100	54	49
Methyl cacalol	10	17	12
	20	22.5	17
	100	44	36
Cacalol acetate	10	45	13
	20	53	22
	100	76	48

To determine the site of electron transport inhibition between water and Q_A of these chemicals, electron flows were measured from DPC to DCIP. The results show that this span of electron transport was not affected by cacalol (Table I), these results indicate that cacalol inhibits electron transport at the level of water splitting enzyme, however 2-acethyl cacalol acetate and methylcacalol inhibit electron transport from water to silicomolibdate in similar percentage to that of DPC to DCIP. These results suggest that these two compounds inhibit electron flow between P680 and Q_A, an cacalol acetate may be acting in two different sites: one at oxygen evolution and the other between P680 and Q_A (Table I). The potency of cacalol, inhibiting 100% of oxygen evolution, suggests that its OH group somehow participates in the inhibition of oxygen evolution. However, the inhibition of electron transport from P680 to Q_A by cacalol derivatives is not clear, because either a releasing electron group (methoxyl) or an attractor electron group (acetate or 2-acethyl) in the structure of cacalol has the same effect of partial inhibition (Table I).

As far as we know there are allelochemicals which act as uncouplers, energy transfer inhibitors and electron transport inhibitors in chloroplasts [20, 21], but the site of inhibition is different to that of the enzyme where oxygen evolution occurs, so this is the first time where it is reported that cacalol inhibits at this target. How significant the effect of cacalol is in the normal growth pattern of the plant has yet to be determined.

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