Electrochemical Properties of the Herbicide Cacalol and Its Derivatives in Protic and Aprotic Solvents by Using Cyclic Voltammetry. Correlation With Hill's Reaction Activities

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The redox behavior of cacalol, a natural product, and its semisynthetic derivatives, which were found acting as electron transport inhibitors in photosystem II, was examined by cyclic voltammetry. The studied compounds were cacalol (**1**), 2-acetylcacalol (**2**), cacalol acetate (**3**), 2-acetylcacalol acetate (**4**), and cacalol methyl ether (**5**). The voltammograms of **1** and **2** in a protic solvent were indicative of an electrochemically irreversible oxidation of the phenolic system. The electrooxidation of compounds **1**−**5** in an aprotic solvent was irreversible and more complex than in the protic solvent but presented similar oxidation patterns; however, compounds containing an unsubstituted phenolic system presented E_{pa}, which is near the potential for the water-splitting enzyme. This suggests that these compounds suffer oxidation and act as inhibitors to the oxygen evolution complex enzyme. Compounds **3**−**5** with higher oxidation potentials could not form a redox pair with the enzymes of photosystem II. Therefore, it is believed that these derivatives acted as inhibitors without any oxidation or reduction reaction.

Keywords: *Redox potentials; cacalol; cacalol derivatives; cyclic voltammetry; alellochemical; Hill's reaction inhibitors; protic and aprotic solvents*

INTRODUCTION

During the past decade there has been increasing research on the isolation, characterization, and evaluation of biologically active natural products. These compounds are secondary metabolites, most of which exhibit allelochemical behavior, a property that makes them of great interest in agriculture (Cutler, 1988).

Cacalol, a secondary metabolite, was isolated from the roots of *Psacalium decompositum* Gray, whose structure was elucidated by chemical (Joseph-Nathan et al., 1966) and spectroscopic (Ruíz et al., 1969) synthetic (Yuste and Walls, 1976) and X-ray (Soriano-García et al., 1987) studies as 9-hydroxy-3,4,5-trimethyl-5,6,7-tetrahydronaphtho[2,3-*b*]furan. The infusion made from the roots of *P. decompositum* Gray is used for the treatment of rheumatism, colds, back pain, jaundice, colic in babies, diabetes, malaria, fever, and snake bites. It is also valuable as a diuretic and antiseptic agent. The effects of the infusion are probably due to the presence of cacalol, one of the major components of this species.

As part of our search for biologically active compounds with medicinal and/or agrochemical importance from plants, we found that cacalol, which is obtained from *P. decompositum* Gray, inhibits Hill's reaction in spinach chloroplasts during photosynthesis, at the level of (oxygen evolution complex (OEC) enzyme (Aguilar-Martínez et al., 1995; Lotina-Hennsen et al., 1991). It was found that cacalol derivatives having a free −OH group inhibited the same target as cacalol (Aguilar-Martínez et al., 1995), and when the −OH group is blocked by acetylation or methylation, the site of inhibition changes to the span of electron transport between P680 and Q_A (Lotina-Hennsen et al., 1991). These results indicated that cacalol may behave as an allelochemic agent by interfering with the growth of photosynthetic organisms, in which the −OH group needs to be free for its action. Anaya et al. (1995) found that cacalol and its derivatives caused inhibition of *Amaranthus hypochondriacus* and to a lesser degree inhibited *Echinochloa Crusgalli* germination. These data suggested that cacalol has phytotoxic activity.

To explain the different target action of cacalol in photosynthesis compared with its other derivatives and their phytotoxic activities, the redox behaviors of these compounds were studied by cyclic voltammetry and their E_{pa} values were correlated with their biochemical properties.

MATERIALS AND METHODS

Chemicals and Solvents. Cacalol (**1**, 9-hydroxy-3,4,5-trimethyl-5,6,7,8-tetrahydronaphtho[2,3-b]furan), a natural product, was isolated from the roots of *P. decompositum* Gray (Romo and Joseph-Nathan, 1964). 2-Acetylcacalol (**2**), cacalol acetate (**3**), 2-acetylcacalol acetate (**4**), and cacalol methyl ether (**5**) were synthesized as reported (Joseph-Nathan et al., 1966; Romo and Joseph-Nathan, 1964). These compounds were purified by crystallization from pentane and petroleum ether before use. Electrochemical measurements were taken in an aqueous solvent containing 100 mM sorbitol, 5 mM $MgCl_2$, and 10 mM KCl buffered with 30 mM sodium tricine at pH 7.6/30% (v/v) acetonitrile (CH_3CN). The measurements made in aprotic environment used tetrabutylammonium tetrafluoroborate (nBu_4NBF_4) as the supporting electrolyte at 0.1 M in CH_3CN. Acetonitrile was dried over anhydrous $CaCl_2$ and distilled before use from phosphorous pentoxide. $LiClO_4$ (0.1 M) in methanol was also used as a third electrolytic medium.

Cyclic Voltammetry. Electrochemical measurements were taken by using a Princeton Applied Research (PAR) Model 173

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potentiostat/galvanostat coupled to a universal programmer (PAR) Model 175. Voltammograms, current−voltage (i−E) diagrams, were registered on a Hewlett-Packard 7004B recorder. A three-electrode, glass electrochemical cell was used with a stationary platinum or a glassy carbon working electrode (area 2 mm^2), a platinum wire auxiliary, and a saturated calomel (SCE) reference electrode. A Luggin tube was used to prevent humidity from entering the cell when the aprotic solvent was used. All potentials are reported as E(V) vs SCE and vs NHE and compared with the Hill's reaction potential values in the biological medium. Solutions in the electrochemical cell were deaerated with purified nitrogen. The measurements were taken in unstirred solutions at room temperature. All of the solutions of cacalol and its derivatives were freshly prepared prior to each experiment and had concentrations of 3 mM in protic and aprotic electrolytic solvents. The scan rate was 100 mV/s. The scans were initiated at −0.5 or 0.0 V in the positive direction.

Electrolysis. *Anodic Oxidation of Cacalol (**1**) in Protic Medium.* A solution of **1** (200 mg) in 100 mM sorbitol, 5 mM $MgCl_2$, and 10 mM KCl buffered with 30 mM sodium tricine (pH 7.6)/30% (v/v) CH_3CN (100 mL) was electrolyzed at a controlled potential (0.52 V vs SCE) by using two Pt plates (10 cm^2) as anode and cathode. The anodic solution was contained within a porous vase for separation, and the electrolysis was quenched at 2 F/mol. During the reaction it was necessary to wash the anode with acetone, because a gray-powdered material was deposited on the electrode, preventing the flow of current. The acetone solution was evaporated under reduced pressure to give a grayish powder (**7**, 45 mg), which decomposed at 250 °C: IR ($CHCl_3$) 3580, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32 (br, H-2), 3.2 (s, br, H-5), 2.8 (s, br, H-8), 1.7 (s, br, C-4 Me), 1.18 (s, br, C-5 Me). The anodic solution was partitioned between AcOEt and H_2O. The AcOEt extract was dried over anhydrous Na_2SO_4 and then filtered. The filtrate was concentrated under reduced pressure and separated by preparative TLC [Kieselgel PF_{254}; hexane−AcOEt (7:3)] to produce cacalone (**6**), a previously known compound (Yuste et al., 1976), as a yellow oil (124 mg) which did not crystallize: IR ($CHCl_3$) 3550 and 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (d, J = 7 Hz, 3H), 1.66 (s, 3H), 1.80 (m, 5H), 2.21 (d, J = 1 Hz, 3H), 2.40 (m, 2H), 3.15 (m, 1 H), and 7.29 (q, J = 1 Hz, 1H).

*Anodic Oxidation of Cacalol (**1**) in Methanol.* A solution of **1** (200 mg) in MeOH (100 mL) containing $LiClO_4$ (2.06 g) was electrolyzed at a constant potential at 1.2 V vs SCE by using a graphite plate (10 cm^2) as an anode and a platinum plate as a cathode. The cathodic solution was contained within a porous vase for separation. The electrolysis was quenched at 2 F/mol. The reaction solution was concentrated under reduced pressure and partitioned between AcOEt and H_2O. The AcOEt extract was dried over anhydrous Na_2SO_4 and then filtered. The filtrate was concentrated under reduced pressure and separated by preparative TLC (Kieselgel PF_{254}; petroleum ether) to produce an isomeric mixture of compounds **7** and **8** (Yuste et al., 1976) as an almost colorless oil (91%): IR ($CHCl_3$) 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 and 1.29 (d, 3H, J = 7Hz, C-5 Me), 1.65 (s, 3H , C-4 Me), 1.70 (m, 4H, C-6 and C-7 protons), 2.19 (d, 3H, J = 1 Hz, C-3 Me), 2.55 (m, 3H, C-5 and C-8 protons), 2.90 and 2.93 (s, 3H, OMe), and 7.40 (q, 1H, J = 1Hz, C-2 proton).

RESULTS AND DISCUSSION

It has been reported (Lotina-Hennsen et al., 1991) that cacalol and its derivatives act as electron transport inhibitors. This property allows them to behave as allelochemical compounds and potential herbicide agents. In this paper, the redox potentials of these compounds were examined by cyclic voltammetry (Heinze, 1984). The chemical structures of these compounds are shown in Figure 1. They have in common the basic structure of tetrahydronaphtho[2-3,b]furan and differ in that the −OH group in cacalol is sometimes methylated or acetylated and in some compounds the furan ring is

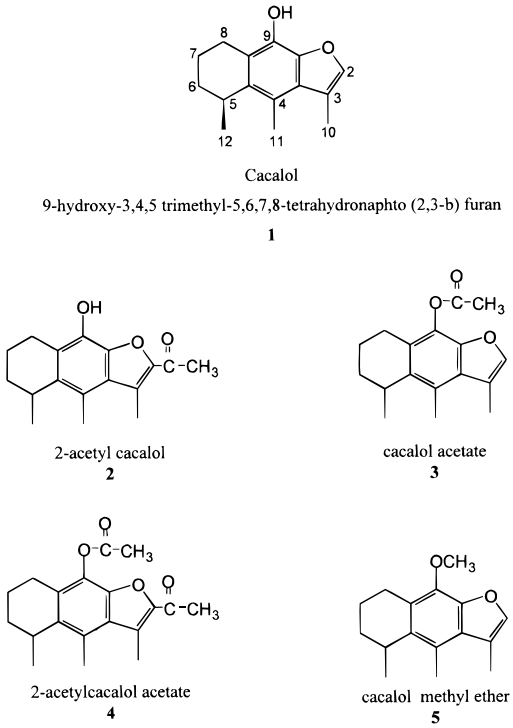


Figure 1. Chemical structures of cacalol and its derivatives.

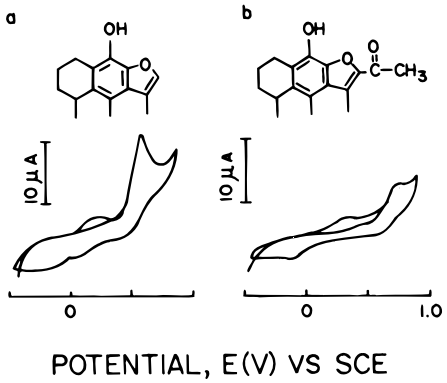


Figure 2. Cyclic voltammograms of 3 mM (a) cacalol and (b) acetylcacalol in 100 mL of sorbitol, 5 mM $MgCl_2$, and 10 mM KCl buffered with 30 mM sodium tricine (pH 7.6)/30% (v/v) CH_3CN. Scans were initiated at 0.5 V in the positive direction at 100 mV/s.

acetylated in the 2-position. It was thought that knowing the redox behavior of these compounds was important to understand their electron transport inhibition effect on photosynthesis. Thus, the voltammetric properties of these compounds were examined in both protic and aprotic solvents to see if their electrooxidation potentials could explain their inhibiting action in the chloroplast membrane and if the compounds became bound to the enzyme target on the hydrophilic or lipophilic side of the membrane.

Protic Medium. The cyclic voltammograms of cacalol (**1**) and 2-acetylcacalol (**2**) in 100 mM sorbitol, 5 mM $MgCl_2$, and 10 mM KCl buffered with 30 mM sodium tricine (pH 7.6)/30% (v/v) CH_3CN are presented in Figure 2. The voltammograms were obtained by measuring the current i at the working electrode as a function of the potential E(V) vs SCE. A scan of the potential was initiated at −0.5 V in the positive direction at 100 mV/s. The shapes of the curves for both compounds were similar. One anodic peak was observed during the initial forward scan, and a small single cathodic peak was seen during the reverse scan.

Table 1. Voltammetric Data for Cacalol and 2-Acetylcacalol in 100 mL of Sorbitol, 5 mM $MgCl_2$, and 10 mM KCl Buffered with 30 mM Sodium Tricine (pH 7.0)/30% (v/v) CH_3CN

compd	E_{pa} (V vs SCE)	E_{pa} (V vs NHE)	E_{pc} (V vs SCE)	E_{pc} (V vs NHE)
cacalol	0.60	0.824	−0.20	−0.044
2-acetylcacalol	0.65	0.894	−0.17	−0.074

[a]Working electrode Pt. Sweep rate 100 mV/s.

At the second cycle a new anodic peak was observed for both compounds. These voltammograms are indicative of an electrochemically irreversible electrode reaction (Bard and Faulkner, 1980).

Cacalol (**1**) showed during the first cycle the potentials of an anodic E_{pa} peak and a cathodic E_{pc} peak at 0.6 and −0.20 V, respectively. During the second cycle a new anodic peak at 0.20 V was observed (Table 1). The large separation between the anodic and cathodic processes (peak potential separations $\Delta E_p = E_{pa} - E_{pc} > 0.58$ V) and the differences in the magnitudes of the ratio of anodic i_{pa} to cathodic i_{pc} peak currents ($i_{pa}/i_{pc} \gg 1$) are a clear indication of the chemical instability of the primarily formed cation radical **II** (Scheme 1) which deprotonated rapidly to the neutral radical **III,** which, owing to its lower oxidation potential, can be further oxidized to the corresponding phenoxonium ion **IV**. It has been reported (Oyama et al., 1987; Ohnuki et al., 1983) that the electropolymerization of phenols leads to electroinactive polymeric films and in some cases to electroactive oligomeric products, depending on the electrolysis conditions. In the case of cacalol, a conductive compound was formed during the first cycles by continuous cycling within the potential limits of −0.5 and 0.85 V. It was observed that during the first cycles the i_{pa} and i_{pc} of the peaks in the range between 0.5 and −0.5 V tended to increase. However, on electrolysis at a controlled potential at 0.52 V, cacalol was readily converted to cacalone (**6**) (Scheme 2) (Yuste et al., 1976) and a non conductive gray compound on the Pt electrode which gradually stopped the current from flowing. The spectral and electrochemical evidence suggested that cacalone (**6**) was initially formed which then could react with other cacalone molecules, giving rise to an oligomer. The absence of the signal at 7.32 ppm in this compound corresponding to the proton at the 2-position indicates that the coupling of these molecules occurred at the furan ring. This compound may provide valuable information to be studied by polymer researchers.

2-Acetylcacalol (**2**), a cacalol bearing a substituent acetyl at the furan ring in the 2-position (Figure 1) showed by cyclic voltammetry an anodic peak at 0.65 V (Table 1), 50 mV toward more anodic potential than cacalol (see Figure 2b and compare with Figure 2a). This is due to an electron-withdrawing effect of the acetyl group, which made it more difficult for 2-acetylcacalol to lose its electrons. The cathodic peak at −0.17 V and the anodic peak at 0.30 V did not increase by continuous cycling within the potential limits of −0.5 and 0.85 V; however, an electroinactive powder was deposited on and strongly adhered to the electrode, it probably being a dimerization product.

The anodic limit of the protic medium at 0.85 V did not allow the reaching of the potential where the furan ring oxidation must occur for compounds **1** and **2**.

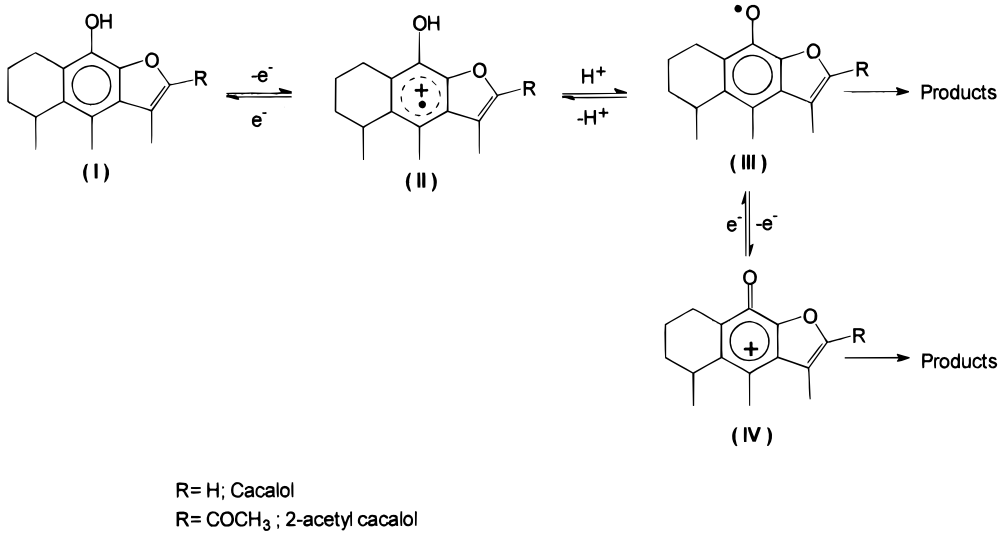
Cyclic voltammograms of cacalol derivatives lacking a free phenolic group showed no anodic peak, since these compounds could not be oxidized at the potential values within this anodic limit in the protic medium. It was observed that when cycling to negative direction, neither cacalol nor the cacalol derivatives were reduced in this solvent, since they were electrochemically unreactive at the cathodic potential limit of −1.8 V of the solvent.

Aprotic Medium. Cyclic voltammograms for compounds **1**−**5** are shown in Figures 3 and 4, and their potentials are collected in Table 2. The supporting electrolyte was 0.1 M nBu_4NBF_4 in acetonitrile (Mann and Barnes, 1970). Scans were initiated at 0.0 V in the positive direction at 100 mV/s. These reactions were chemically irreversible (Bard and Faulkner, 1980).

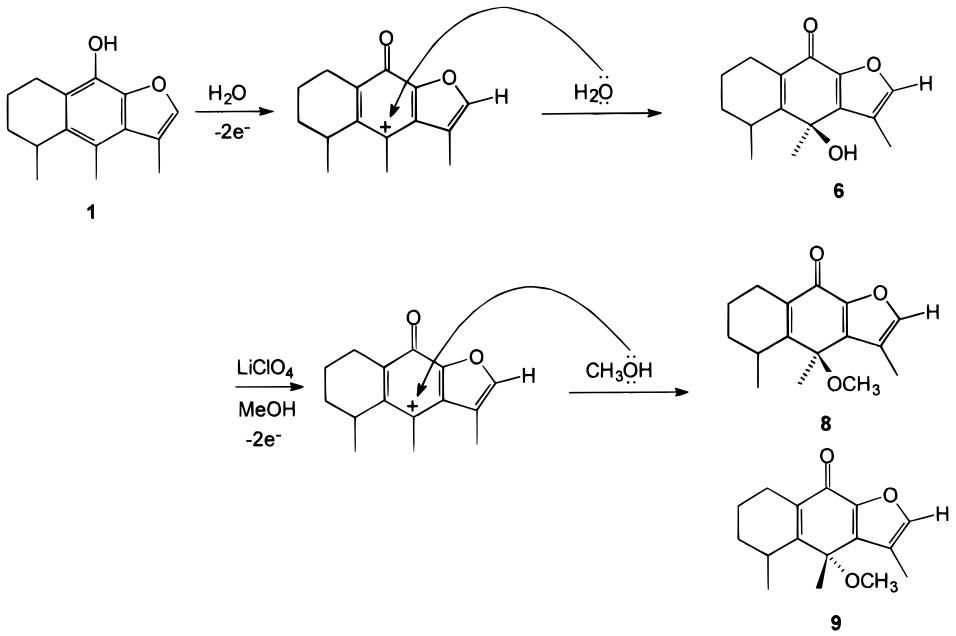
Cacalol (***1***)**.** Two switching potentials E_λ ($E_{\lambda 1}$= 2.25 V and $E_{\lambda 2}$= 1.60 V) (Michielli and Elving, 1968) were used in the cyclic voltammetry experiments with this compound (Figures 3a and 4a, respectively). During the first cycle, when $E_{\lambda 1}$ was used, three anodic peaks at 0.95, 1.13, and 2.1 V and a cathodic peak at −1.27 V were observed as was an anodic peak at −0.18 V. Peaks at 0.95 and 1.13 V were due to the electrooxidation of the phenolic−OH, and the peak at 2.1 V was caused by the oxidation of the furan ring. The oxidation products were chemically unstable on the time scale of the experiment. A second scan having switching potential $E_{\lambda 2}$ = 1.6 V was performed in an attempt to isolate the peaks due to the phenolic system and correlate them with the peaks located in the range from 0.0 to −1.5 V. A new cathodic peak at −0.35 V, which had associated with it an anodic peak at −0.18 V, was observed. Actually, during the first cycles from −0.5 to 1.75 V these peaks tended to grow, perhaps due to the formation of a oligomeric compound from the cacalol radical **III** (see Scheme 1). The products from the cacalol phenoxonium ion followup reactions were reduced at −1.27 V. To corroborate which system was oxidized first in the cacalol molecule, cyclic voltammetry of phenol and furan was performed by separate tests under the same experimental conditions. Cyclic voltammetry of pure phenol showed during the first forward scan an irreversible anodic peak at 1.64 V; one anodic irreversible peak at 1.98 V was also observed for the electrooxidation of the pure furan. These results showed that the phenolic system was oxidized at lower potentials than the furan ring, and it seems that the electrooxidation of phenol and furan is easier if they belong to the tetrahydronaphthofuran system than if they are present as isolated systems. This is due to the major stability of their oxidation intermediaries for resonance and releasing electron effects in the cacalol and its derivative systems.

Peaks at 0.95 and 1.13 V in cacalol resulted in a two-electron oxidation in an aprotic solvent, giving rise to the corresponding phenoxonium ion **IV** (Scheme 1). The evidence of this intermediary was confirmed by the obtainment of its methoxy derivative when the electrooxidation was performed by using 0.1 M $LiClO_4$ in methanol as a protic electrolytic medium, when the polymer formation was minimized. Cyclic voltammetry of 3 mM cacalol in the above medium with a glassy carbon working electrode at 100 mV/s presented during the initial forward scan two irreversible anodic peaks at 0.68 and 1.15 V corresponding to the formation of cacalol cation radical **II** and cacalol phenoxonium ion **IV,** respectively (Scheme 1; Figure 5). On electrolysis at a controlled potential (1.2 V vs SCE; 2 F/mol) by using a carbon electrode in 0.2 M $LiClO_4$/methanol, cacalol was converted into two −2e oxidation products consisting of a mixture of compounds which were separated

Scheme 1



Scheme 2



by preparative silica chromatoplate in petroleum ether to obtain a racemic mixture of methylcacalone isomers **7** and **8** (Scheme 2) (Yuste et al., 1976) in 91% yield and other compounds not were identified. The structures of **7** and **8** were determined on the basis of their spectral IR and ^{1}H NMR data being similar to those produced by synthesis (Yuste et al., 1976).

2-Acetylcacalol (***2***). Two switching potentials E_λ ($E_{\lambda 1}$ = 2.5 V and $E_{\lambda 2}$ = 2.0 V) (Figures 3b and 4b, respectively) were used in the cyclic voltammetry experiment with **2**. In the forward scan when $E_{\lambda 1}$ was used, **2** showed that the shape of this curve was similar to the cacalol voltammogram (compare Figure 3b with Figure 3a) since both compounds had in common a free phenolic −OH group; consequently, their electrochemical behaviors were similar. However, the anodic 2-acetylcacalol peaks appeared at higher positive potentials than they did in cacalol due to the fact that cation radical **II** and phenoxonium ion **IV** of compound **2**, as well as the intermediaries obtained from the furan oxidation, are destabilized by a 2-acetyl group, which caused a strong electron-withdrawing effect at the furan ring. The irreversible peaks at 1.2 and 1.45 V corresponded to the −2e oxidation of the phenolic group, and the peak at 2.3 V was caused by the furan ring oxidation in the products obtained from phenoxonium ion **IV** followup reactions. On the reverse scan no peak was observed in the region from 0.0 to −0.5 V, presumably caused by an overoxidation of an oligomer or by the presence of electroinactive products obtained from furan oxidation followup reactions. The cathodic peak at −1.18 V resulted from the reduction of the products obtained from the oxidation of the −OH group. This was proved since the peak at −1.05 V newly appeared when $E_{\lambda 2}$ = 2.0 V was used (Figure 4b). Two other cathodic peaks at −0.25 and −0.55 V and an anodic peak at −0.05 V were also observed. This last peak was caused by the oxidation of the products obtained from the followup reactions of the irreversible reduction at −1.05 V; however, it was not coupled to the cathodic peak at −0.25 V since it could not be observed when the scan was reversed at −0.5 V.

Cacalol Acetate (***3***), *2-Acetylcacalol Acetate* (***4***), *and Cacalol Methyl Ether* (***5***). Cyclic voltammetry of compounds **3**−**5** was performed by using two switching potentials E_λ ($E_{\lambda 1}$ = 2.5 V and $E_{\lambda 2}$ = 2.0 V) (see Figures

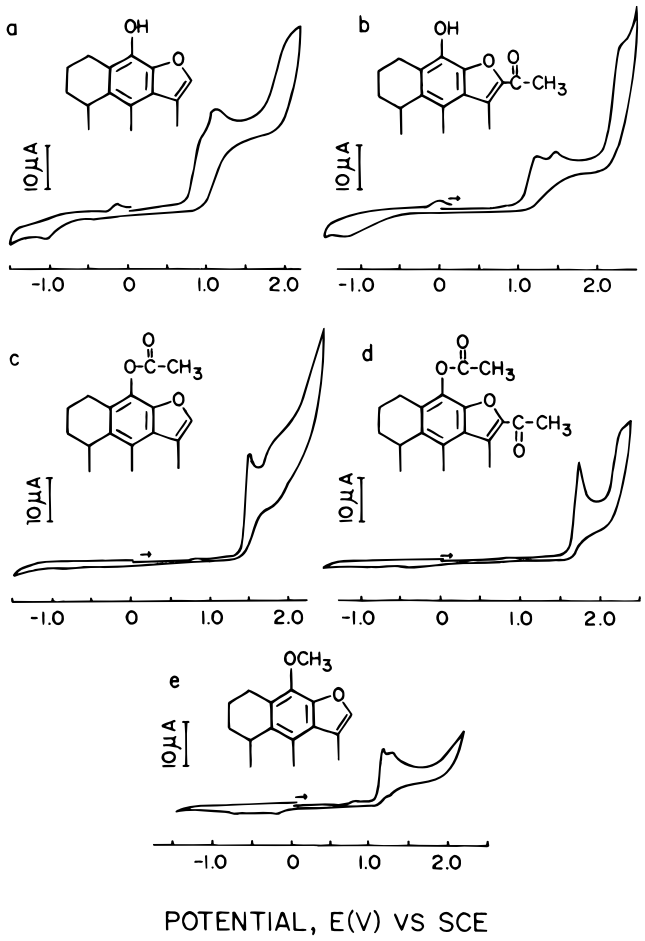


Figure 3. Cyclic voltammograms of 3 mM cacalol and cacalol derivatives in 0.1 M nBu_4NBF_4 in CH_3CN. Scans were initiated at 0.0 V in the positive direction at 100 mV/s, when $E_{\lambda 1} = 2.5$ V.

3c−e and 4c−e) and showed that the oxidation of the substituted phenolic system was carried out probably by following the same mechanism as cacalol. This was suggested on the basis that when $E_{\lambda 2}$ was used, compounds **3**−**5** presented curves similar to those of cacalol and 2-acetylcacalol. It is important to mention that the anodic peaks at 1.5 and 1.8 V of compound **3** and the anodic peak at 1.70 V for compound **4** appeared at higher anodic potentials than those corresponding to cacalol, whereas anodic peaks at 1.15 and 1.30 V for compound **5** appeared at potentials similar to those of cacalol (Table 2). This can be explained in terms of an electron-withdrawing effect of the acetyl group which increased the positive charge on the ring, destabilizing the oxidation intermediaries for compounds **3** and **4**, and an electron-donating effect of the $-OCH_3$ group which stabilized the oxidation intermediaries in compound **5**.

It was found that cacalol (**1**) (Lotina-Hennsen et al., 1991) and 2-acetylcacalol (**2**) (Aguilar-Martínez et al., 1995) inhibit Hill's reaction in chloroplasts at the water splitting enzyme level. However, the target for the other cacalol derivatives [cacalol acetate (**3**), 2-acetylcacalol acetate (**4**), and cacalol methyl ether (**5**)] was one of the redox enzymes in the span of P680 to Q_A electron transport chain (Lotina-Hennsen et al., 1991). These results suggested that the −OH group of cacalol participates in the inhibition of oxygen evolution since by methylating or acetylating this −OH group, the derivative did not show inhibition on the water splitting enzyme. It is well-known that the midpoint potential

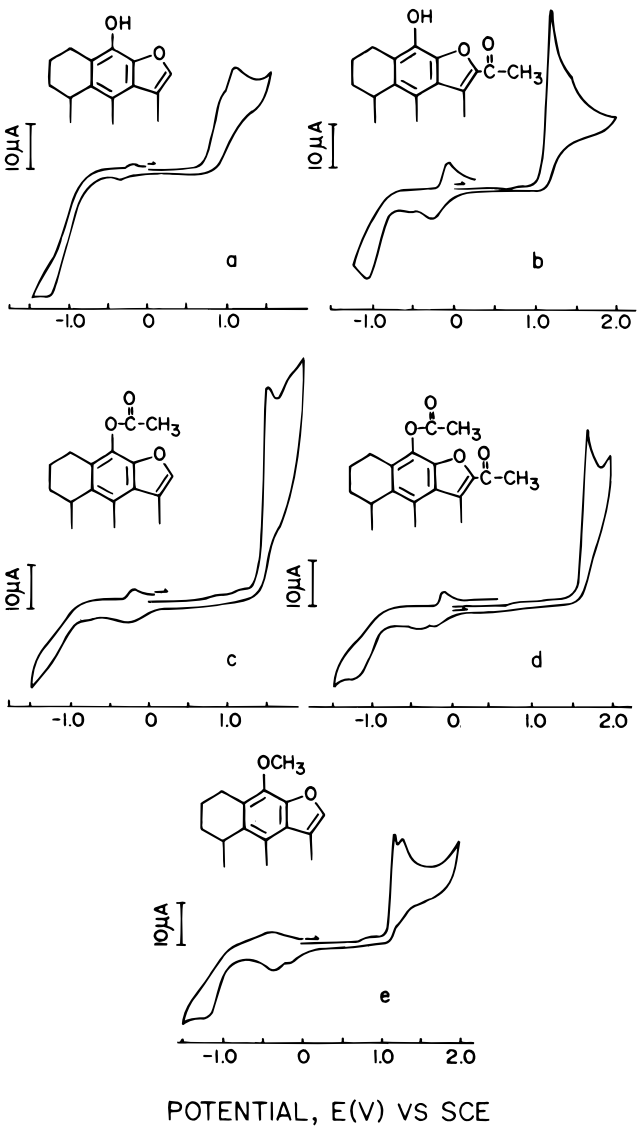


Figure 4. Cyclic voltammograms of 3 mM cacalol and cacalol derivatives in 0.1 M nBu_4NBF_4 in CH_3CN. Scans were initiated at 0.0 V in the positive direction at 100 mV/s, when $E_{\lambda 2} = 2.0$ V.

Table 2. Voltammetric Data for Cacalol and Cacalol Derivatives 3 mM in 0.1 M nBu_4NBF_4 in CH_3CN[a]

compd	E_{pa} (V vs SCE)	E_{pa} (V vs NHE)
cacalol	0.95	1.19
	1.13	1.37
	2.10	2.34
2-acetylcacalol	1.20	1.44
	1.45	1.69
	2.30	2.54
cacalol acetate	1.50	1.74
	1.95	2.19
2-acetylcacalol acetate	1.74	1.98
	2.30	2.54
cacalol methyl ether	1.17	1.41
	1.28	1.52

[a] $E_{\lambda 1} = 2.5$ for the oxidation of the phenolic and furan systems. Working electrode Pt. Sweep rate 100mV/s.

of water splitting enzyme is 0.81 V vs NHE (Diner and Joliot, 1977) and for those for $P680^+/P680$, Ph/Ph^-, and Q_A/Q_A^- are ~1.1, −0.16, and 0.0 V vs NHE, respectively (Diner, 1977). From the results reported here (Tables 1 and 2), the inhibition at the water splitting enzyme by cacalol and 2-acetylcacalol may be due to the forma-

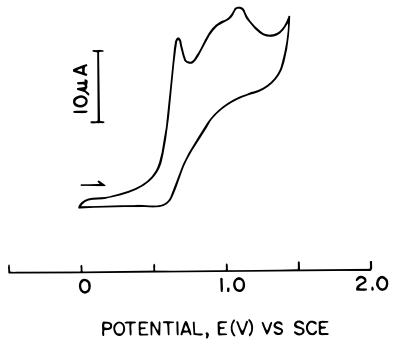


Figure 5. Cyclic voltammogram of 3 mM cacalol in 0.2 M $LiClO_4$ in acetonitrile. Scan was initiated at 0.0 V in the positive direction at 100 mV/s.

tion of a redox pair and the product of the redox reaction inhibits the enzyme. This is due to the fact that the anodic E_{pa} peaks for cacalol and 2-acetylcacalol were 0.824 and 0.894 V vs NHE, respectively, in protic medium. Notice that electrooxidation of cacalol and its derivatives in aprotic medium showed at least two or three anodic E_{pa} peaks (Table 2) with a higher anodic potential value compared to that obtained in protic medium. The first anodic E_{pa1} peak in aprotic medium for cacalol and 2-acetylcacalol may correspond to the anodic E_{pa} found in protic medium determination.

CONCLUSIONS

The results of these studies have shown that cacalol and its derivatives are molecules which are very reactive to electrochemical oxidation, due to the presence of phenol and furan rings, giving irreversible reactions in protic and aprotic solvents. Voltammetric data showed that the phenol oxidation occurred at lower potentials than the furan ring oxidation. The obtainment of cacalone and methylcacalone derivatives by electrolysis is clear evidence of the initial formation of the cacalol phenoxonium ion.

The E_{pa} of the phenol and furan systems in cacalol and its derivatives was very sensitive to the substituent electronic effects. Substituents with an electron-withdrawing effect caused the oxidation of phenol and furan systems to appear at higher potentials than in the unsubstituted systems.

The E_{pa} values determined for cacalol and its derivatives agreed with their previously reported activity for inhibiting Hill's reaction. It was observed that for these compounds to be good Hill's reaction inhibitors, it was necessary to have a potential close to the OEC enzyme and also have the phenolic −OH group free. According to this, it is suggested that cacalol and 2-acetylcacalol were oxidated by the excited photosystem II at the chloroplast and their phenoxonium ion was the species bound to the OEC enzyme, inhibiting, in this way, the electron transport during photosynthesis.

The insolubility of these compounds in protic solvents may suggest that they act at the lipophilic site of the chloroplast membrane in which the OEC enzyme is located. As far as we know, this is the first time that the relationship structure−redox potentials of allelochemical compounds−Hill's reaction inhibiting properties has been studied.

LITERATURE CITED

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