JOC*Note*

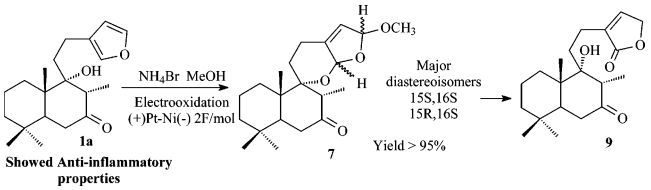
Electro-Oxidation of Hispanolone and Anti-Inflammatory Properties of the Obtained Derivatives

Elizabeth Nieto-Mendoza, Juan A. Guevara-Salazar, María Teresa Ramírez-Apan, Bernardo A. Frontana-Uribe,* Juan A. Cogordan, and Jorge Cárdenas

Instituto de Química de la Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán C.P. 04510, Mexico

bafrontu@servidor.unam.mx

Received February 21, 2005



The electrochemical oxidation ((+)Pt−Ni(−)/NH_4Br/MeOH) of the natural product hispanolone (**1a**) produced, in high yield (>95%), spiro-tetracyclic compounds **7a**−**7d** as a result of the intramolecular addition of the C-9 hydroxyl group into the C-16 position with the simultaneous addition of a CH_3O group at the C-15 position of the hispanolone furan moiety. After the electrochemical oxidation, an acid-catalyzed slow secondary reaction occurred producing the previously undescribed α-butenolide derivative, *iso*-Leopersin G (**9**). An anti-inflammatory study with the electro-synthesized compounds showed that **1a** has higher anti-inflammatory properties with very low cytotoxicity (e.g., the inhibition of TPA-induced ear edema assay $IC_{50} = 1.05$ μM/ear, positive control indomethacin $IC_{50} = 0.27$ μM/ear).

Hispanolone (**1a**) is an abundant natural furanic diterpene with a labdane skeleton.[1] Several research groups have examined its chemical transformations to obtain some important compounds, such as galeopsin (**2**),[2] ambreinolide,[3] drimane derivatives,[4] prehispanolone (**3**), and 14,15-dihydroprehispanolone (**4**) (Figure 1);[5] the last two compounds presented interesting biological activity as specific PAF (platelet activating factor) receptor antagonists which have been implicated with several disease states.[6] Hispanolone and other compounds lacking the tetra-annular spiro skeleton do not exhibit this biological activity.[7] A convenient method for accessing compounds with this structural requirement is through

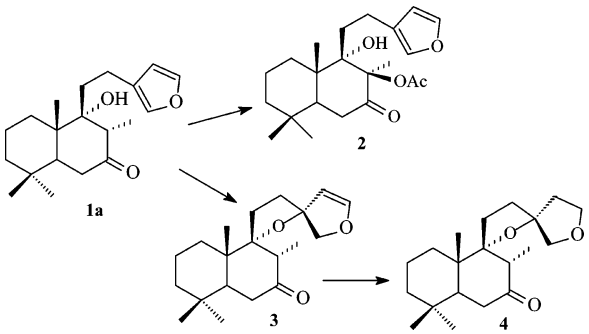


FIGURE 1. Hispanolone and derivatives.

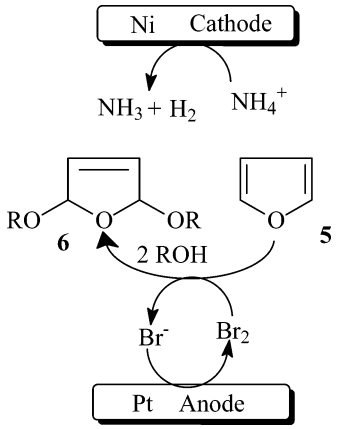


FIGURE 2. Bromine-mediated electro-oxidation of furans.

an electro-oxidation of the furan ring. Furan (**5**) can be electrochemically oxidized under mild conditions in an alcoholic NH_4Br solution to obtain 2,5-dialkoxy-dihydrofurans (**6**) (Figure 2).[8,9]This methodology generates a stoichiometric quantity of bromine[10] which is needed to oxidize the furan ring. In general, the reaction is cleaner and has better yields than the chemical counterpart.

Hispanolone (**1a**) contains a hydroxyl group on C-9 which can intramolecularly react with the furan ring under the electro-oxidative conditions previously mentioned (Scheme 1) to produce **7a**−**7d**. These tetracyclic compounds could have new and interesting biological activities, as reported for prehispanolone (**3**). With this in mind, we performed the electrolysis of **1a** under conditions which favor the oxidation of the furan ring.

The cyclic voltammetry, under the preparative electrolysis conditions, of hispanolone, its carbonyl-protected derivative **1b**,[11] and furan (**5**) did not show any electrochemical signal before the cathodic and anodic barriers (−0.35 and 1.35 V, respectively). This implies that the

* Corresponding author. Phone: +52 55 56224507. Fax: +52 55 56162203.

(1) Savona, G.; Piozzi, F.; Rodríguez, B. *Heterocycles* **1978**, *9*, 257−261.
(2) García-Alvarez, M. C.; Pérez-Sirvent, L.; Rodríguez, B.; Bruno, M.; Savona, G. *An. Quim., Ser. C* **1981**, *77*, 316−319.
(3) Domínguez, G.; Marco, J. L.; Hueso-Rodríguez, J. A.; Rodríguez, B. *An. Quim., Ser. C* **1988**, *84*, 211−218.
(4) Marco, J. L.; Rodríguez, B. *An. Quim., Ser. C* **1983**, *79*, 56−61.
(5) Wang, E. S.; Choy, Y. M.; Wong, H. N. *Tetrahedron* **1996**, *52*, 12137−12158.
(6) Lee, C. M.; Jiang, L. M.; Shang, H. S.; Hon, P. M.; He, Y.; Wong, H. N. *Br. J. Pharmacol*. **1991**, *108*, 1719−1724.
(7) Hon, P. M.; Wang, E. S.; Lam, S. K. M.; Choy, Y. M.; Lee, C. M.; Wong, H. N. C. *Phytochemistry* **1993**, *33*, 639−641.
(8) Clauson-Kaas, N.; Limborg, F.; Glens, K. *Acta Chem. Scand*. **1952**, *6*, 531−534.
(9) Limborg, F.; Clauson-Kaas, N. *Acta Chem. Scand*. **1953**, *7*, 234−235.
(10) Br_3^- formation cannot be excluded; this anion has almost the same redox value as bromine. See: Dean, J. A., Ed. *Lange's Handbook of Chemistry*, 33rd ed.; McGraw-Hill: New York, 1985; Tables 6-2, pp 6−7.
(11) Cheung, W. S.; Wong, H. N. C. *Tetrahedron* **1999**, *55*, 11001−11016.

10.1021/jo0503308 CCC: $30.25
Published on Web 04/21/2005

SCHEME 1. Bromine-Mediated Electro-Oxidation of Hispanolone and Derivatives and the Observed Follow-Up Reaction

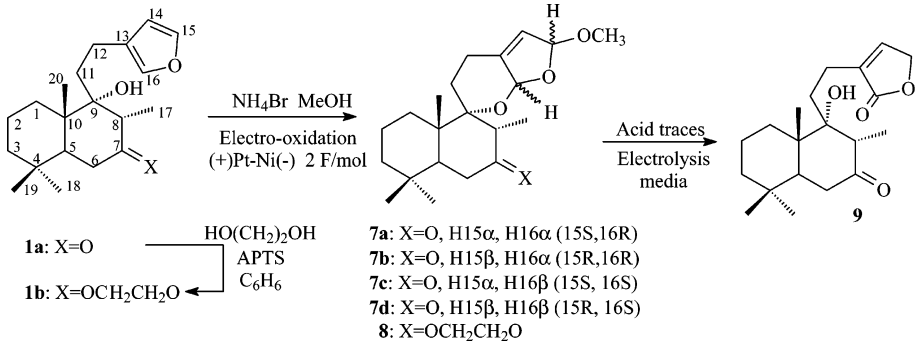


TABLE 1. Electrochemical Oxidation of Furan (5), Hispanolone (1a), and Derivative 1b[a]

entry	compd (mmol)	temp (°C)	current density (mA/cm^2)	time of electrolysis	product (% yield)[d]
1	**5** (20.6)	−22[b]	10	7 h 30 min	**6** (85)
2	**5** (20.6)	−22[b]	20	3 h 50 min	**6** (30)[e]
3	**5** (20.6)	−22[b]	40	2 h	**6** (23)[e]
4	**1a** (0.35)	−45[c]	10	7 min	**7** (86) [**7a**,**7b** (18) **7c**,**7d** (82)][f]
5	**1a** (0.35)	−22[b]	10	7 min	**7** (82) [**7a**,**7b** (52) **7c**,**7d** (48)][f]
6	**1a** (0.35)	−22[c]	10	7 min	**7** (79) [**7a**,**7b** (44) **7c**,**7d** (56)][f]
7	**1a** (0.35)	0[c]	10	7 min	**7** (61) [**7a**,**7b** (15) **7c**,**7d** (85)][f]
8	**1a** (0.35)	20[c]	10	7 min	**7** (60) [**7a**,**7b** (7) **7c**,**7d** (93)][e,f]
9	**1a** (0.35)	40[c]	10	7 min	**7** (45) [**7a**,**7b** (33) **7c**,**7d** (67)][e,f]
10	**1a** (0.35)	60[c]	10	7 min	**7** (55) [**7a**,**7b** (26) **7c**,**7d** (74)][e,f]
11	**1a** (1.60)	20[c]	10	37 min	after 3 days **7c**,**7d** (35), **9** (65)
12	**1b** (0.35)	−22[c]	10	7 min	**8** (75)[g]

[a] 2 F/Mol, (+) Pt−Ni (−)/0.15 M NH_4Br/MeOH in an undivided cell. [b] Anhydrous MeOH. [c] Distilled MeOH. [d] The reported yield corresponds to the purified products: **6** by low-pressure distillation and **7**, **8**, and **9** by medium-pressure chromatography and recrystallization. [e] Secondary products were observed by TLC at the end of electrolysis. [f] Diastereomer ratio determined by the integration of methoxy signals in ^{1}H NMR. [g] Yield of the four diastereoisomers.

compounds are not electroactive under the experimental conditions. These data confirmed that it is possible to work directly with the natural product using bromine as the oxidizing reagent (Figure 2), contrary to a previously reported protocol for the oxidation of the furan ring of hispanolone.[5]

Prior to the preparative electro-oxidation of the natural product, furan behavior was verified as test a compound in an undivided cell (Table 1, entries 1−3). The cleanest reaction (when **6** was the sole product detected by TLC at the end of the electrolysis) and 100% transformation was obtained at current densities lower than 10 mA cm^{-2}. Hispanolone was electrolyzed under these conditions, and the TLC analysis of the solution at the end of the reaction showed one new compound.

After workup, a colorless crystalline product was isolated. The occurrence of the expected reaction (Scheme 1, **1a** → **7**) was confirmed by IR, high-resolution mass spectrometry (FAB$^+$), and NMR spectroscopy. ^{1}H NMR showed two signals for the CH_3O group with a different integral value: an expansion showed that each signal was actually a pair of singlets.[12] These four methoxy signals (δ 3.271, 3.276, 3.242, and 3.245) were attributed to the four possible diastereoisomers (**7a**−**7d**) where each pair of signals corresponds with two similarly structured diastereoisomers. ^{13}C NMR spectra clearly showed four methoxy carbons at δ 53.7, 53.4, 53.3, and 53.2 which are in agreement with the proposed diastereoisomers. NOESY NMR showed that H-1$_{\beta ec}$ had a strong interaction with the multiplet located at 1.91 ppm which was assigned to C-11 protons. That fact agreed with the spiro feature of C-9. Even though H-16 and H-12 are in 1,3-disposition on the pyran ring, no interaction signal was observed between them in the NOESY NMR experiments; nevertheless, H-16, in both pairs of diastereoisomers, showed a strong interaction with H-11 protons which suggests that all of the diastereoisomers have a boat conformation. This compound feature is supported by theoretical calculations in which the global geometry optimizations and energy for the electrogenerated diastereoisomers **7a**−**7d** were calculated (see section 3 of the Supporting Information). In the NOESY experiment, an interaction between the H-15 and H-16 proton signals, for each pair of diastereoisomers, can also be observed. Furthermore, these protons also interacted with a different methoxyl signal, indicating that each pair of diastereoisomers observed in the ^{1}H NMR methoxy singlets is composed of a diastereoisomer in which protons H-15 and H-16 are on the same side of the dihydrofuran plane (**7a** or **7d**) and a diastereoisomer in which they are on opposite sides (**7b** or **7c**) (Scheme 1). Also in the NOESY experiment, in one molecule, H-16 interacted with the CH_3-17; this evidence corresponds to distereoisomers with a 16*S* configuration in which both groups are on the same side of the molecule. Several attempts to separate the diastereoisomers by fractional crystallization, preparative HPLC, and growing single crystals were unsuccessful. When the carbonyl-protected hispanolone (**1b**) was used in the electro-oxidative reaction, the corresponding four tetracyclic spiro compounds, **8a**−**8d**, were also obtained in good yield (Table 1, entry

(12) See Table 1 of the Supporting Information for the ^{1}H and ^{13}C NMR data and the discussion for this compound.

TABLE 2. Inhibition of TPA-Induced Ear Edema by Hispanolone and Its Derivatives[a]

compd	μmol/ear	% inhibition ± SEM	IC_{50} (μmol/ear)
1a	0.316	21.44 ± 4.8*	1.05
	1.000	40.76 ± 6.3*	
	3.160	83.26 ± 3.6*	
7	0.316	29.89 ± 7.2*	2.26
	1.000	35.65 ± 5.7*	
	3.160	56.35 ± 2.4*	
8	0.316	12.87 ± 4.1*	>3.16
	1.000	24.88 ± 6.7*	
	3.160	56.35 ± 2.4*	
9	0.316	5.10 ± 2.8*	>3.16
	1.000	21.09 ± 4.6*	
	3.160	31.96 ± 7.0*	
	0.13	35.15 ± 6.4*	
	0.24	48.18 ± 2.0*	
indomethacin	0.42	56.29 ± 8.2*	0.27
	0.75	69.42 ± 9.4*	
	1.30	90.35 ± 2.6*	

[a] *$p < 0.05$.

12).[13] The main spectroscopic signals for the spiro moiety are equivalent to those described for **7a**−**7d**.

Experiments at different temperatures (Table 1) showed that at temperatures lower than 20 °C, the reaction was very clean and the sole observed products in TLC were compounds **7a**−**7d**. At higher temperatures, other byproducts appeared on the TLC decreasing the chemical yield of the reaction. This can be explained as a result of a faster but less selective oxidation of the electrogenerated bromine with the starting compound. The reactions at low temperature neither favored a specific diastereoisomeric pair nor affected the ratio of the pairs of diastereoisomers in a predictable way. Nevertheless, it was clearly observed that one pair is almost always present in a higher yield; it has the highest selectivity (93:7) at room temperature. It is possible that this pair of diastereoisomers is more stable than the other, and thus, its formation is thermodynamically favored (vide infra).[14] The electrochemical method was compared with the typical furan oxidation methods. Compound **1a** was allowed to react with NBS[15] (2 mol in 1,4-dioxane/water at room temperature). At the end of the reaction, TLC showed a complex mixture of products that was not separated. GC−MS of the mixture demonstrated the presence of bromated products. The TLC analysis of the bromine oxidation of **1a** in MeOH at room temperature showed the spot for products **7a**−**7d**, but other byproducts were also observed. These experiments showed the high selectivity of the electrochemical method in the furan oxidation. The use of distilled MeOH or anhydrous MeOH at −22 °C (Table 1, entries 5 and 6) had practically the same results.[16]

When the quantity of hispanolone was increased to 1.6 mmol (500 mg) in the same electrolytic cell (Table 1, entry 11), the TLC control also showed only one spot corresponding to products. However, if the reaction was not treated immediately, a new product was obtained after 3 days. After separation, product **9**[17] was obtained in a 65% yield and only the **7c**,**7d** pair of diastereoisomers was obtained in a 35% yield. These diastereoisomers corresponded to the major pair obtained during the electrolysis and, in NMR, to those that have the *S* configuration on C-16. Because of their stability, and the disappearance of the other diastereoisomeric pair, the proposal of a reversible cyclization reaction seems to be supported. From the results obtained from the semiempirical calculations (see section 3 of the Supporting Information), the two diastereoisomers generated by the attack over the *re* face (**7c** and **7d**) corresponded to those with the lowest total energy values. This observation and the experimental results are in agreement with the thermodynamic control of the reaction producing, as the major compounds, the pair of diastereoisomers with lowest energy (configuration 16*S*). The proposed stereochemistry for the compounds observed in high yield at the different temperatures is as follows: **7c** 15*S*, 16*S* and **7d** 15*R*, 16*S* (CH_3O signals at 3.24 ppm in ^1H NMR; 53.20 and 53.36 ppm in ^{13}C NMR).

All the spectroscopic data of compound **9** point to the presence of a butenolide ring, and the α- or β-substitution was proposed by comparison with the typical ^1H and ^{13}C NMR signals for both substitutions in natural products.[18] The observed signals corresponded with an α-substitution, and the proposal was confirmed by a NOESY NMR experiment in which the interactions between the methylene of the butenolide ring at 4.79 ppm and the vinylic proton at 7.1 ppm were clearly observed. In the literature, this α-butenolide has not been described as the only product reported is the product with a β-substitution, Leopersin G;[19] thus, product **9** was named *iso*-Leopersin G. By taking into account that β-butenolide substitution is most common in the natural products isolated from plants of the Lamiaceae family,[18] we can easily reach the alternative substitution from the terminal furan by means of this electrochemical reaction. Another possible application of this methodology is to transform the furan ring of furanic diterpenoids to 1,5-disubstituted pyrrolidin-2-ones; this is a transformation in which the 2,5-dialkoxy-2,5-dihydrofuran could be the key intermediate.[20] Product **9** could be obtained by acidic hydrolysis with subsequent rearrangements from **7a**−**7d** catalyzed by HBrO or HBr, generated from water and traces of the halogen (see Scheme 1 in the Supporting Information for a mechanism proposal). An analogous reaction between phthalaldehyde and amines to obtain unsaturated γ-lactams has been reported.[21,22] To check this proposal, we allowed the mixture of diastereoisomers **7a**−**7d** to react for 3 days with a catalytic quantity of *p*-TSA at room

(13) See Table 2 of the Supporting Information for the ^1H and ^{13}C NMR data for this compound.

(14) Atkinson, R. S. *Stereoselective Synthesis*; John Wiley & Sons: New York, 1995; pp 12−15.

(15) Ferland, J. M.; Lefevre, Y.; Deghenghi, R.; Wiesner, K. *Tetrahedron Lett.* **1966**, 3617−3620.

(16) This observation was previously reported for furan electrooxidation (see ref 8). The water contained in the distilled MeOH is about 0.01%, and for dry MeOH obtained using Na or Mg as the drying agent, it is about 5×10^{-5}% as reported by Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: Oxford, U.K., 1988; p 217.

(17) See Table 3 of the Supporting Information for the ^1H and ^{13}C NMR data and the discussion for this compound.

(18) Rodríguez-Hahn, L.; Esquivel, B.; Cárdenas, J. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, Ch., Eds.; Springer-Verlag: New York, 1994; Vol. 63, p 145.

(19) Tasdemir, D.; Sticher, O.; Calis, I.; Linden, A. *J. Nat. Prod.* **1997**, *60*, 874−879.

(20) Katritzky, A. R.; Mehta, S.; He, H.-Y.; Cui, X. *J. Org. Chem.* **2000**, *65*, 4364−4369.

TABLE 3. Inhibition of EPP-Induced Ear Edema by Hispanolone and Its Derivatives[a]

compd	μmol/ear	% inhibition ± SEM
1a	1.000	no activity
7	1.000	−73.19 ± 25.5*
8	0.100	22.70 ± 15.8
	0.316	27.11 ± 10.3
	1.000	60.62 ± 8.3*
9	1.000	no activity
dexamethasone	0.010	43.20 ± 10.8*
	0.0316	60.70 ± 12.1*
	0.1000	69.40 ± 10.0*
	0.3160	79.38 ± 4.9*

[a] *$p < 0.05$.

temperature in the electrolysis media; product **9** was obtained in a 70% yield.

To verify the biological activity of the compounds studied, **1a**, **7**, **8**, and **9**, we evaluated them using three anti-inflammation tests in which it is well-known that PAF could be involved.[23] The anti-inflammatory test, using the tetradecanoylphorbol acetate (TPA)-induced ear edema assay as a model (Table 2), showed that hispanolone is the most potent compound tested: the IC_{50} is only 4 times less potent than that the positive control, indomethacin. A clear structure−activity effect was observed with compounds **7** and **8** that contain the spiro skeleton and with compund **9** that does not have the furan moiety. Instead of the abundance of hispanolone as a natural product,[1] the sole report of its positive biological activity corresponds to an antibiotic study.[24] It has been reported that some labdane skeleton compounds have important anti-inflammatory properties that follow the steroid pathway for activity.[25] To gain insight into the possible route for the anti-inflammatory activity of hispanolone, we selected the model using ethylphenyl-propiolate (EPP) because of its specificity toward activation of the glucocorticoid pathway instead of the protein kinase (PKN) pathway in the inflammation process.[26] In this assay (Table 3), hispanolone was inactive; however, compound **8** proved to be the most active. On the other hand, compound **7** favored the inflammatory process showing that bulky compounds close to the steroid structure are required to have the anti-inflammatory activity in this test. These preliminary results might indicate that the anti-inflammatory activity of **1a** is a result of an effect on the eicosanoid pathway, as reported for other nonsteroidal anti-inflammatory compounds,[27] or on the PKN action mechanism,[28] but the PAF route is not affected.[6]

The anti-inflammatory tests were complemented with the inhibition assay of NO_2^- production in live macrophages (mϕ) activated with lipopolysaccharides (LPS) (Table 4). Mature mϕ, when stimulated under inflammation conditions by LPS, produce a large amount of NO which rapidly transforms into NO_2^-. Therefore, the inhibitory effect on NO production is a useful way of evaluating the anti-inflammatory activity of compounds; it also provides information about the capacity of the cells to live in the presence of the compounds tested.[29]

TABLE 4. Nitrite Production Effect of Hispanolone and Its Derivatives in the INOS Inhibitory Test Using Live Macrophages (mϕ) Activated with Lipopolysaccharides (LPS)[a]

compd	C (μM)	production of NO_2^- [μM] ± SEM
1a	10	83.20 ± 2.0* (a)
	31	71.51 ± 0.6* (a)
	100	52.63 ± 2.1* (a)
7	10	93.70 ± 3.3 (a)
	31	93.93 ± 2.7 (a)
	100	77.74 ± 2.1* (72)
8	10	87.25 ± 2.4* (a)
	31	81.52 ± 2.2* (a)
	100	71.26 ± 1.1* (27)
9	10	95.09 ± 2.0 (a)
	31	87.34 ± 1.9* (a)
	100	51.38 ± 1.1* (a)
aminoguanidin	10	97.82 ± 4.2 (a)
	31	80.01 ± 9.4* (a)
	100	66.16 ± 3.5* (a)

[a] *$p < 0.05$; (% Cellular viability), (a) means 95−100% of the cells lived after the test.

It can be observed that, as in the case of the TPA assay, at all of the concentrations tested, hispanolone was the compound with the highest anti-inflammatory activity. Its response was comparable to that observed for the positive control (in the same order and dose dependency), and more interestingly, it had a very low cytotoxicity. The second highest activity is that of compound **9** which like hispanolone does not have the spiro moiety; this shows that the closing of the ring (compounds **7** and **8**) is not beneficial for the anti-inflammatory properties.

In this work, the current controlled electrochemical oxidation of hispanolone was described. The reaction yielded the tetra-annular spiro compound with current efficiencies of >95% by means of intramolecular addition of the C-9 hydroxyl group into the C-16 position with a high selectivity for furan oxidation. The anti-inflammatory properties of hispanolone and its derivatives were evaluated showing that the parent compound has good anti-inflammatory activity with very low cytotoxicity. The presence of the electrochemically constructed tetra-annular spiro skeleton decreased the activity.

Acknowledgment. We thank Isabel Chávez, Alejandrina Acosta, Javier Pérez, Luis Velasco, Rocío Patiño, Antonio Nieto, and Elizabeth Huerta for technical assistance. Gabriela Salcedo carried out the English style correction. This work was partially supported with CONACyT-México grants (J34873-E and 25305-E).

Supporting Information Available: Complete Experimental Section with procedures for the electrochemical transformations and anti-inflammatory assays, ^1H and ^{13}C NMR spectroscopic data, discussion for compounds **7**, **8**, and **9**, the proposed mechanism for **9**, and theoretical calculations for geometry optimization. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0503308

(21) Grigg, R.; Gunarante, H. Q. N.; Sridharan, V. *J. Chem. Soc., Chem. Commun.* **1985**, 1183−1184.
(22) DoMinh, T.; Johnson, A. L.; Jones, J. E.; Senise, P. P. *J. Org. Chem.* **1977**, *42*, 4217−4221.
(23) Saunders, R. N.; Handley, D. A. *Annu. Rev. Pharmacol. Toxicol.* **1987**, *27*, 237−255.
(24) Citoglu, G.; Tanker, M.; Sever, B.; Englert, J.; Anton, R.; Antalar, N. *Planta Med.* **1998**, *64*, 484−485.
(25) Razmilic, B. I.; Schmeda-Hirschmann, G. *Planta Med.* **2000**, *66*, 86−88.
(26) Cameron, G. S.; Baldwin, J. K.; Klann, R. C.; Patrick, K. E.; Fischer, S. M. *Cancer Res.* **1991**, *51*, 5642−5648.
(27) Rao, T. S.; Currie, J. L.; Shaffer, A. F.; Isakson, P. C. *Inflammation* **1993**, *17*, 723−741.
(28) Huguet, A. I.; Recio, M. C.; Máñez, S.; Giner, R. M.; Rios, J. L. *Eur. J. Pharmacol.* **2000**, *410*, 69−81.
(29) Fushiya, S.; Kishi, Y.; Hattori, K.; Batkhuu, J.; Takano, F.; Singab, A.; Okuyama, T. *Planta Med.* **1999**, *65*, 404−407.