Anodic C16–C21 fragmentation of catharanthine in methanol. Synthesis of 16-methoxycleavamine

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Two-electron controlled potential oxidation of catharanthine (Cath) affords 2 in 65% yield. Compound 2 is reduced to 16-methoxycleavamine in 95% yield. Cyclic voltammetry experiments show that catharanthine hydrochloride ($CathH^+$) in methanol solution in the presence of 2,6-lutidine exists in acid–base equilibrium with Cath as a free base which is oxidized at 0.7 V *vs.* SCE. Oxidation of Cath to $Cath^{\cdot+}$ and fragmentation of the C16–C21 bond is assumed to occur *via* a concerted mechanism.

Introduction

The dimeric catharanthus alkaloids vinblastine and vincristine are efficacious, clinically useful anticancer reagents which are used routinely for the treatment of a number of human cancers.[1] Unfortunately the isolation and purification of these compounds is a difficult process and consequently these drugs are among the most expensive on the pharmaceutical market. Although much effort has gone into their synthetic development, vinblastine and vincristine are still produced by isolation from the plant.[2]

Synthetic studies have focused on the biomimetic coupling[3] of the catharanthus roseus alkaloids catharanthine (**Cath**) and vindoline (**V**) leading to the pivotal intermediate, a dihydropyridinium ion which can be transformed through further chemical steps into the natural vinca alkaloids anhydrovinblastine (**AVBL**) and vinblastine.[4] The crucial step in the required structural transformation of **Cath** to **AVBL** is fragmentation of the C16–C21 bond to yield a nine-membered cyclic immonium ion $\mathbf{1a}^{2+}$ (Scheme 1). This process was carried out either using the Polanovski reaction[5] or by using electron-transfer reagents.[6] While there have been significant advances following other approaches,[7] the oxidative fragmentation/coupling route to vinblastine remains a topic of interest.[8] We have already reported that an electrochemical methodology can be used to synthesize anhydrovinblastine in good yield and stereoselectivity by coupling of catharanthine and vindoline, even at room temperature.[9]

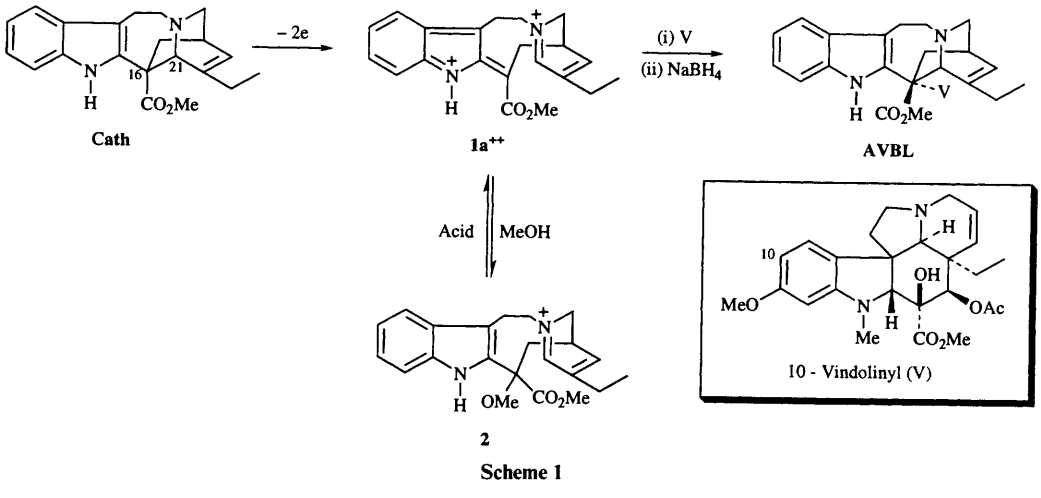
Anodic oxidation is a good alternative to oxidative fragmentation of **Cath** *via* an electron-transfer reaction. It was expected that **Cath** would be transformed through transfer of two electrons and fragmentation of the C16–C21 bond to the dication $\mathbf{1a}^{2+}$ which should be trapped by methanol, leading to 16-methoxy compound **2**. The dication may be recovered by reaction of a Lewis or Brønsted acid on **2** and in the presence of vindoline (**V**) to give **AVBL** (Scheme 1). In this paper we report the basic features of the electrode reaction as well as the preparative results on the anodic oxidation of catharanthine in methanol solution.

Results and discussion

Cyclic voltammetry

A typical cyclic voltammogram of the catharanthine hydrochloride salt ($CathH^+$) presented in Fig. 1(*a*) shows two anodic peaks at 1.10 and 1.22 V *vs.* SCE, respectively. When cyclic voltammetry was performed in the presence of 2,6-lutidine (**Lut**) as a base, the peak at 1.10 V corresponding to the oxidation of $CathH^+$ decreased and a new peak at 0.7 V *vs.* SCE appeared due to the oxidation of catharanthine, **Cath**, as a free base [Fig. 1(*b*) and 1(*c*)]. Further addition of 2,6-lutidine results in an increase in the peak at 0.7 V *vs.* SCE, which also shows characteristics of a saturation curve as a function of 2,6-lutidine concentration.

The nature of the peak at 0.7 V *vs.* SCE was studied in some detail. No evidence of reversibility was noticed for sweep rates ranging from 0.02 to 40 V s^{-1}, implying that a one-electron oxidation product, the radical cation, $\mathbf{Cath}^{\cdot+}$, was not observed. Under the experimental conditions employed this wave is



Scheme 1

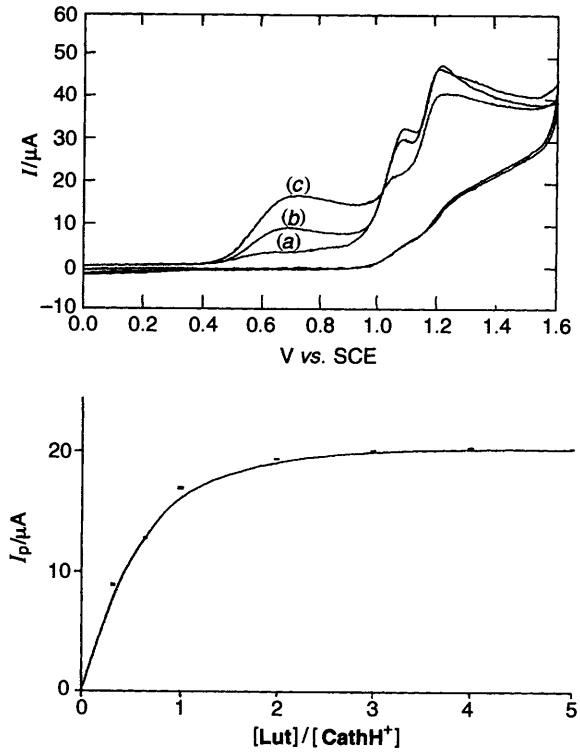


Fig. 1 Top: (*a*) Cyclic voltammogram of **CathH**$^+$ (1 mmol dm^{-3}) in MeOH–0.1 mol dm^{-3} $LiClO_4$ at GCE; $v = 0.1$ V s^{-1}, (*b*) plus **Lut** (0.33 mmol dm^{-3}), (*c*) plus **Lut** (1 mmol dm^{-3}); bottom: dependence of the peak current on concentration of **Lut**

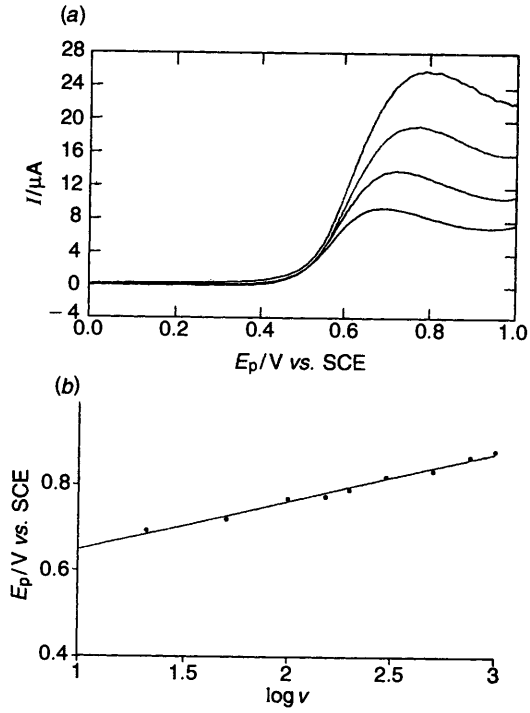


Fig. 2 (*a*) Voltammograms of **CathH**$^+$ (1 mmol dm^{-3}) in MeOH–0.1 mol dm^{-3} $LiClO_4$ at 0.02, 0.05, 0.1 and 0.2 V s^{-1}; GCE; (*b*) E_p–log v plot

irreversible and broad. Its peak varies linearly with the logarithm of the sweep rate with a slope of 98 mV (Fig. 2). The values of the transfer coefficient, a, were derived from an E_p–log v slope according to eqn. (1),[10,11] and from the peak

$$E_p = (2.3RT/2aF) \log v + \text{const.} \quad (1)$$

widths[10] using eqn. (2). The value of the transfer coefficient

$$E_p - E_p/2 = 1.857\,(RT/aF) \quad (2)$$

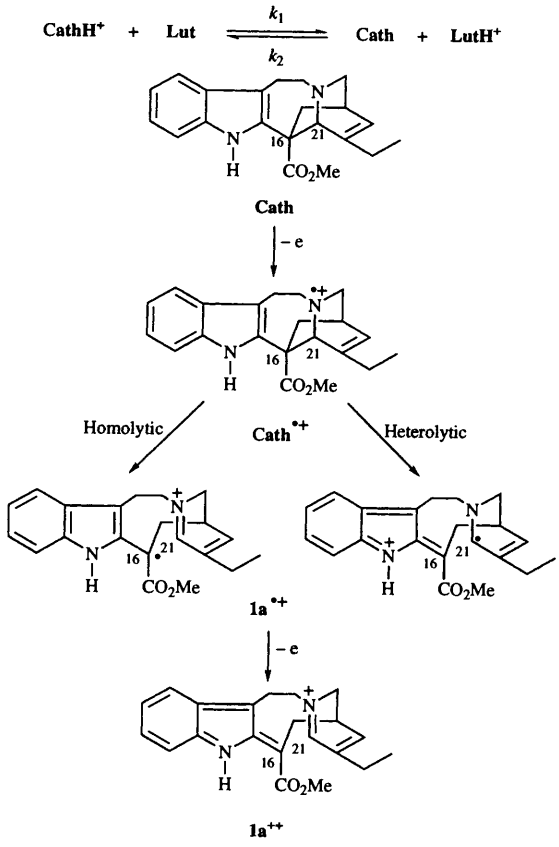
obtained from the E_p–log v slope ($a = 0.30$) was in good agreement with the average value of the transfer coefficient obtained from the peak widths of the voltammograms at five sweep rates ($a = 0.31$). The broadness of the wave and large slope of the E_p–log v plot indicate that the oxidation of **Cath** is governed by the kinetics of an electron-transfer step. Notably, the calculated peak current, i_p, for irreversible charge transfer[12] was higher [i_p (calc.) = 28.10 mA] than the value experimentally determined [i_p (exp.) = 19.22 mA] at 0.1 Vs^{-1} sweep rate. The peak current, i_p, was calculated by assuming the value of the diffusion coefficient for **Cath** ($D = 1.4 \times 10^{-5}$ cm^2 s^{-1}), determined in MeCN where $k_1 \longrightarrow \infty$, according to eqn. (3). The value of the peak

$$i_p = (2.99 \times 10^5)\, na^{1/2}\, ACD^{1/2}v^{1/2} \quad (3)$$

current is presumably determined by the acid–base equilibrium preceding an irreversible electron transfer[10] and it is expected to be proportional to $K(k_1 + k_2)^{1/2}$.

The coulometry at controlled potential ($E = 0.75$ V *vs.* SCE) showed that the overall reaction is a two-electron oxidation of **Cath** leading to the final product **2** (see below). The main features of the electrode reaction are shown in Scheme 2.

The electroactive catharanthine as a free base (**Cath**) is oxidized to the radical cation, **Cath**$^{\bullet+}$, through a slow



Scheme 2

electron-transfer reaction. This process is accompanied by a change in hybridization of the amine moiety from sp^3 in **Cath** to sp^2 in **Cath**$^{\bullet+}$. Strain energy differences resulting from these geometrical preferences distort the preferred planar configuration and thereby increase the free energy for electron transfer.[13]

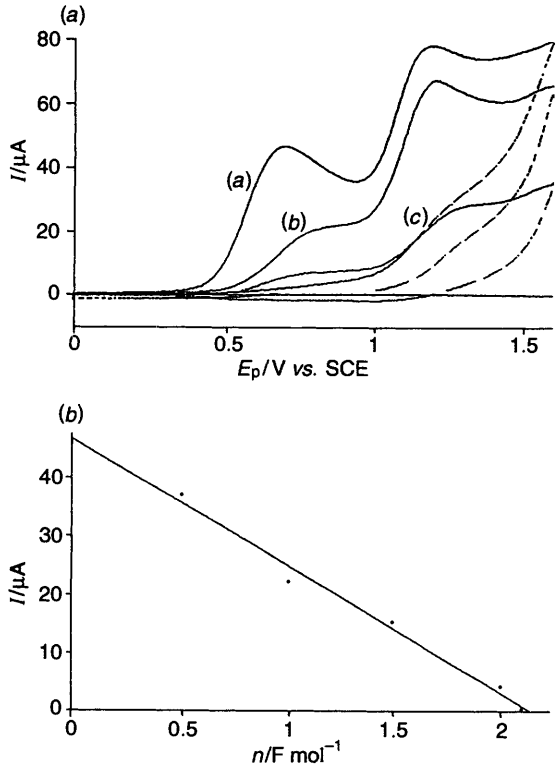


Fig. 3 (*a*) Cyclic voltammograms of **CathH**$^+$ (50 mg, 0.13 mmol) in MeOH–0.1 mol dm^{-3} $LiClO_4$ (50 cm^3) plus **Lut** (55 mm^3); $v = 0.1$ V s^{-1}; GCE; (*a*) 0.0, (*b*) 1.0, (*c*) 2.0 Fmol^{-1}. (*b*) Dependence of the peak current on charge passed.

The flattening at the nitrogen is accompanied by an increase in the p character of the lone-pair orbital. This results in an accumulation of strain in the molecule, so that the C16–C21 fragmentation reaction would be expected to release most of this in the transition state. The alignment of the σ-orbital of the C16–C21 bond with the p orbital on nitrogen would facilitate the fragmentation process leading to a new radical cation **1a**$^{\cdot+}$. In principle, the unimolecular C16–C21 fragmentation of the radical cation **Cath**$^{\cdot+}$ may follow a homolytic or heterolytic mode. This depends mainly upon the relative potentials[14] of the indolyl radical formed ($E_1°$) or the azaallyl radical ($E_2°$). If $E_1°$ is more negative than $E_2°$, the homolytic mode of fragmentation would be favourable and *vice versa*. It is difficult to discriminate between the two modes of fragmentation at the present time since we do not know the redox potentials of the two possible radicals. However, the product obtained by photolysis of **Cath** in the presence of an electron acceptor (product 4 in ref. 15) suggests that the homolytic mode of C16–C21 fragmentation is more feasible.

On the basis of the experimentally determined transfer coefficient, it is possible to address the question concerning the mechanism of anodic fragmentation of the C16–C21 bond in **Cath**, *i.e.* whether electron transfer and C16–C21 bond fragmentation are concerted or successive steps. Dissociative electron-transfer theory[16] predicts that the activation driving force relationship is quadratic and that the symmetry factor, α (the electrochemical transfer coefficient), varies linearly with the driving force and is expected to be smaller than 0.5 for a large driving force.

$$\alpha = 0.5\,(1 + \Delta G°/4\Delta G_0^{\ddagger}) \qquad (4)$$

It follows that the electrochemical transfer coefficient is a useful experimental tool in discrimination between stepwise and concerted mechanisms.[17] Excellent agreement was found between theoretical predictions and experimental data[18] in the electrochemical reductive cleavage of arylmethyl halides. Nitrobenzyl halides with large α (*i.e.* significantly larger than 0.5) are reduced through a stepwise mechanism involving the intermediacy of the radical anion. Substituted benzyl halides having weaker electron-withdrawing groups as well as unsubstituted benzyl halides with a small α (*i.e.* significantly smaller than 0.5) are reduced *via* a concerted mechanism.

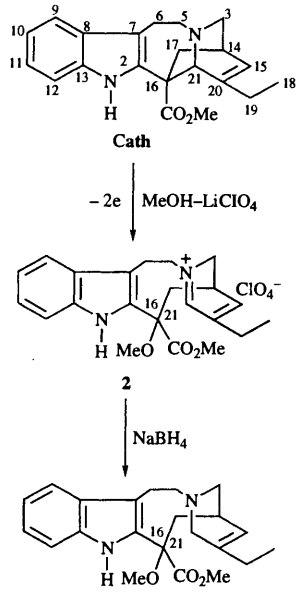
Taking into account the theoretical arguments and the experimental fact that the α value is markedly less than 0.5 (*i.e.* $\alpha = 0.30$ from E_p–log v plot and $\alpha = 0.31$ from the peak width), we may conclude that the anodic C16–C21 fragmentation of **Cath** occurs according to the concerted mechanism.

$$\mathbf{Cath} - e \longrightarrow \mathbf{1a}^{\cdot+} \qquad (5)$$

Preparative anodic oxidation

Anodic oxidation of **Cath** was performed at a platinum gauze electrode in methanol solution containing $LiClO_4$ in a divided cell at a controlled potential ($E = 0.65$–0.75 V *vs.* SCE). The electrolysis was monitored by HPLC and terminated after all starting material was consumed. Cyclic voltammograms under constant conditions were obtained as a function of the charge passed. The results show that under controlled potential electrolysis, the oxidation wave decreases at a rate corresponding to the consumption of 2F mol^{-1} (Fig. 3).

Compound **2** was isolated as the major product in 65% yield. The $NaBH_4$ reduction of **2** gave 16-methoxycleavamine, **3**, in 95% yield (Scheme 3).



Scheme 3

El mass spectra of compound **2** showed a molecular peak at *m/z* 367 ($M^{\cdot+} - ClO_4$) and IR spectra showed characteristic vibrations at 1730 (C=O), 1665 (=N=CH–) and 1051 (ClO_4^-) cm^{-1}, respectively. ^{1}H NMR spectra run in CD_2Cl_2 showed all relevant protons. A noteworthy feature of the spectrum is the signal at 9.3 ppm assigned to the imine proton at C21. 16-Methoxycleavamine, **3**, showed a molecular peak at *m/z* 368 as the parent peak. Using the published assignments of the ^{1}H and ^{13}C spectra of the cleavamine half in anhydrovinblastine as a guide,[19] interpretation of the spectra **3** was undertaken by use of a 2D ^{1}H–^{13}C correlation and ^{13}C–DEPT assignments of the CH_2 type. The assignments made are shown in Table 1.

Cath undergoes a two-electron oxidation to produce **1a**$^{2+}$ which is subsequently attacked by methanol as a nucleophile at

Table 1 Spectral assignments for 16-methoxycleavamine **3**

Position	^{13}C NMR	^{1}H NMR
2	132.5	
3	52.4	2.70, 2.85, m[a]
5	56.1	3.35, m[a]
6	25.7	2.90, 3.40, m[a]
7	113.1	
8	128.6	
9	119.1	7.48, d
10	122.1	7.15, t
11	119.6	7.22, t
12	110.7	7.43, d
13	134.5	
14	33.2	2.25, m[a]
15	124.2	5.53, d
16	51.1	
17	38.3	
18	12.2	1.05, t
19	27.5	2.01, q
20	139.3	
21	51.1	2.21, 2.50, m[a]
C=O	169.4	
CH_3O	51.8	3.70, s
$COOCH_3$	52.1	3.12, s
NH		8.63, s

[a] Overlaps another signal.

position C16 to yield **2** (Scheme 3). Notably, DDQ-oxidation of **Cath** in methanol solution also proceeds with formation of $\mathbf{1a}^{2+}$ which is attacked by two molecules of methanol at positions C16 and C21 yielding 16,21-dimethoxycleavamine in 30–35% yield.[20] The differences in the chemical reactions of $\mathbf{1a}^{2+}$ under conditions of anodic and DDQ oxidation can be explained by the existence of $\mathbf{1a}^{2+}$ as an adduct with DDQ,[20] presumably attached covalently at position 21. We have isolated the product **2** in 65% yield, but the estimated yield of **2** (R_t = 5.06 min) by HPLC at the end of the electrolysis was > 85%. No attempts were made to analyse the by-products. The reduction of **2** with $NaBH_4$ gave 16-methoxycleavamine, **3**, which can be used as a synthon in the presence of vindoline for a new, non-oxidative method for the preparation of anhydrovinblastine.

Experimental

Apparatus and procedures

IR spectra were run on a Perkin-Elmer 1600 FTIR instrument. NMR spectra were recorded on a Bruker AC 250 MHz spectrometer and EI mass spectra were recorded on an AEI MS 902 instrument. HPLC analyses were performed using Varian Vista 5500 equipment with a UV-200 detector and Varian 440 integrator using a MCH-5N CAP micro column (15 cm × 4 mm) at 284 nm with MeOH (0.5 cm^3 min^{-1}) as eluent. Voltammetric measurements were performed in a 25 cm^3 conical cell with a glassy carbon disc electrode [GCE; A = 0.071 cm^2)], platinum as a counter electrode and saturated calomel electrode (SCE). All electrochemical measurements were carried out using a PAR M-270-1 electrochemical analysis system.

Controlled potential oxidation of Cath

Preparation of 2. Into the anodic compartment of a divided cell with a Pt-gauze anode (2 × 9 cm) and a Ni-cathode filled with a 0.1 mol dm^{-3} solution of $LiClO_4$ in MeOH (50 cm^3, Merck HPLC-grade) were added catharanthine hydrochloride (128 mg, 0.345 mmol) and 2,6-lutidine (160 mm^3, 1.45 mmol). The temperature in the cell was maintained at 10 °C during the electrolysis by external cooling with cold water. The anode potential was set at 0.65 V *vs.* SCE at the beginning of electrolysis and was gradually increased during the electrolysis up to 0.75 V *vs.* SCE. The electrolysis was terminated after the initial current (50 mA) had decayed smoothly to 1–2 mA. During the electrolysis an electronic integrator was used to record the quantity of electricity passed. The product **2** precipitated during the electrolysis. The reaction mixture after the electrolysis was evaporated to *ca.* 20 cm^3 and left in a refrigerator for 1 h. The resulting precipitate was filtered and washed with cold MeOH, EtOAc and ether giving an analytically pure product **2**. Yield 65%, mp 168–170 °C (Found: C, 56.61; H, 5.77; N, 6.12. $C_{22}H_{27}N_2ClO_7$ requires: C, 56.59; H, 5.78; N, 6.00%); ν_{max}/cm^{-1} 3375, 2967, 1723, 1665, 1579, 1434, 1238, 1212, 1102, 1051, 1006, 755, 622; ^{1}H NMR $\delta_H(CD_2Cl_2)$ 9.3 (s, 1 H, H-21), 8.2 (s, 1 H, NH), 7.52 (d, 1 H, 9-H), 7.41 (d, 1 H, 12-H), 7.26 (t, 1 H, 10-H), 7.17 (t, 1 H, 11-H), 6.95 (d, 1 H, 15-H), 4.64 (m, 1 H, 5-H), 4.12 (m, 1 H, 5-H), 3.95 (m, 1 H, 3-H), 3.67 (s, 3 H, CH_3O), 3.60 (m, 1 H, 3-H), 3.48 (s, 3 H, CH_3O), 2.8 (m, 2 H, 17-H), 2.48 (q, 2 H, 19-H), 1.20 (t, 3 H, 18-H); EIMS *m/z* (relative intensity) 367 (59), 355 (21), 337 (18), 233 (19), 238 (12), 202 (10), 135 (36), 124 (76), 107 (92), 91 (39), 79 (40), 43 (100).

Reduction of 2. Preparation of 3. Compound **2** (80 mg) was suspended in MeOH (10 cm^3). To the stirred solution mixture was added $NaBH_4$ (15 mg), gradually at room temperature during 30 min. The solution was evaporated to *ca.* 5 cm^3 and 10 cm^3 of water was added. The precipitated product **3** was filtered and recrystallized from 70% aqueous methanol. Yield 95% (60 mg), mp 97–99 °C; R_f 0.75 (toluene–MeOH, 8:2) (Found: C, 71.70; H, 7.50; N, 7.47. $C_{22}H_{28}N_2O_3$ requires: C, 71.74; H, 7.61; N, 7.61%); ν_{max}/cm^{-1} 3454, 2953, 2909, 1741, 1604, 1476, 1453, 1244, 1127, 1012, 756; ^{1}H NMR $\delta_H(CDCl_3)$ 8.63 (s, 1 H), 7.48 (d, *J* 7.3 Hz, 1 H), 7.43 (d, *J* 7.3 Hz, 1 H), 5.53 (d, *J* 6 Hz, 1 H), 3.70 (s, 3 H), 3.5–3.1 (m, 3 H), 3.12 (s, 3 H), 3.1–2.5 (m, 4 H), 2.5 (d, *J* 7 Hz), 2.4–2.05 (m, 3 H), 2.01 (q, *J* 7 Hz, 2 H), 1.05 (t, *J* 7 Hz, 2 H); EIMS *m/z* (relative intensity) 368 (100), 353 (32), 337 (23), 305 (20), 251 (10), 171 (16), 136 (94), 123 (38), 108 (19).

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