A new and efficient synthesis of *ortho*- and *para*-benzoquinones of cardanol derivatives by the catalytic system $MeReO_3$–H_2O_2

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A convenient and efficient application of the catalytic system H_2O_2–$MeReO_3$ for the first reported preparation of *ortho*- and *para*-benzoquinones of cardanol derivatives is described.

Introduction

Cardanol, a mixture of 3-*n*-pentadecylphenol, 3-(*n*-pentadec-8-enyl)phenol, 3-(*n*-pentadeca-8,11-dienyl)phenol, and 3-(*n*-pentadeca-8,11,14-trienyl)phenol, is the main component of the roasted 'cashew nut shell liquid' (CNSL), apart from cardol and anacardic acid.[1] CNSL is a side-product from the mechanical processing (hot-bath process) of the cashew nut of *Anacardium occidentale*, a process of importance in view of the edibility of the kernel.[2,3] Therefore, CNSL results as a low-value side-product compared with the valuable edible kernel and a widely available source of distilled cardanol and 3-*n*-pentadecylphenol, obtained by hydrogenation of cardanol, utilizable in fine chemical processes.[2–4]

Cardanol and its derivatives may be used as antioxidants, and in general as stabilizers against light, air, and heat, for several organic materials, *e.g.*, flavours, foods, lubricants, polymers, and rubbers.[5–10] Moreover, CNSL extracts have been shown to have antibacterial,[11,12] antifungal[13] and antitumoral activities.[14]

Based on the above described properties the synthesis of new cardanol derivatives is a straightforward key for the development of convenient industrial applications of CNSL as well as for the design of new drugs. Alkylation,[5,15] acylation,[16] thionation,[17] condensation,[6] nitration, halogenation[18] and a number of other chemical transformations have all been used with different degrees of success for the selective functionalization of cardanol and its isolated components.

In spite of this high number of synthetic transformations, to the best of our knowledge there are no general and efficient catalytic oxidative procedures for the selective synthesis of *ortho*- and *para*-benzoquinones of cardanol derivatives. Benzoquinones are ubiquitous in nature and exhibit biologically important activities.[19] In particular, it has been proposed that the hapten role of some alkyl- and alkenylcardanol derivatives might depend on the *in vivo* oxidative conversion to the corresponding benzoquinones.[20]

A number of oxidants are available in the literature for the preparation of quinones, including Fremy's salt,[21] cerium(IV) ammonium nitrate,[22] lead tetraacetate[23] and other transition metal oxidants.[24] More recently, transition metal complexes such as Co or Mn complexes have been reported[25] to catalyse the oxidation of phenols to quinones with either H_2O_2 or O_2. However, most of the known methods involve reactions either under homogeneous, non-catalytic conditions or show poor selectivity in terms of product distribution.

A novel catalyst useful for the synthesis of benzoquinones is methyltrioxorhenium(VII) ($MeReO_3$, MTO),[26,27] which is stepwise converted by H_2O_2 into the monoperoxorhenium complex $[MeRe(O_2)\cdot O_2\cdot H_2O]$ and the active bis(peroxo)rhenium complex $[MeRe(O_2)_2O\cdot H_2O]$.[28] $MeReO_3$ is a very potent catalytic oxidant and recently it has been used for the synthesis of *para*-benzoquinones by oxidation of 3-methylnaphthalene,[29] naphthols, and *para*-unsubstituted alkylphenols characterized by small alkyl side-chains. In the latter case, *para*-benzoquinones and hydroxy-substituted *para*-benzoquinones were obtained in acceptable yields. The oxidations appeared to be not especially sensitive to steric effects of the alkyl substituents with the sole exception of 3,5-di-*tert*-butylphenol in which case a muconic anhydride was obtained, probably due to overoxidation of an unisolated, reactive 3,5-di-*tert*-butyl-1,2-benzoquinone intermediate.[25c]

We report here a convenient and efficient application of the H_2O_2–$MeReO_3$ system for the first reported catalytic preparation of *ortho*- and *para*-benzoquinones of cardanol derivatives. A fine-tuned substituent effect on the selectivity of the oxidation (*ortho*-benzoquinones *versus para*-benzoquinones) was observed depending either on the steric hindrance of the *meta*-pentadecyl side-chain or that of other alkyl substituents on the phenolic ring. Moreover, the effect of the presence of bromine atoms on the selectivity of the oxidation was also studied.

Due to their biological importance, the cytotoxic effect of selected new prepared derivatives is reported.

Results and discussion

3-*n*-Pentadecylphenol **1**, prepared by hydrogenation of distilled cardanol,[30] was allowed to react with H_2O_2 (4.0 mmol; 35% aq. solution) in ethanol (5 ml) in the presence of catalytic amounts of $MeReO_3$ (2 mol%) at 25 °C to give 4-*n*-pentadecyl-1,2-benzoquinone **2** in 35% yield (Scheme 1), with unchanged substrate.[31] In the absence of $MeReO_3$ less than 2% conversion of **1** takes place under otherwise identical conditions. The oxidation of **1** was further performed both in acetic acid and in the presence of an excess of tetrafluoroboric acid (HBF_4, 8 equiv. mol^{-1}; 54% in diethyl ether) in order to define the best reaction medium for the desired conversion to quinone derivative **2**. Under these experimental conditions, compound **2** was obtained as the only recovered product in 51 and 75% yield,

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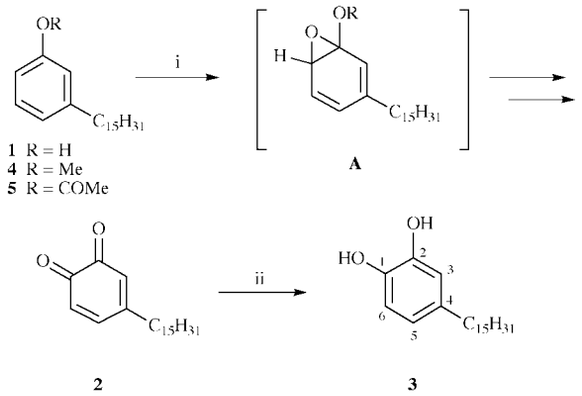
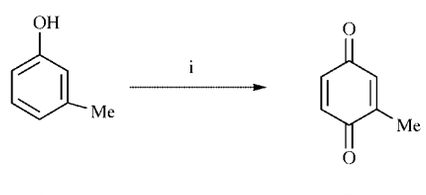


2 3

Scheme 1 *Reagents and conditions*: i, $MeReO_3$, H_2O_2 (35%), EtOH or AcOH, 25 °C; ii, $NaBH_4$, EtOH–THF, 25 °C.

respectively. Thus, the use of the acidic medium was essential for attaining good conversions and complete selectivity in the oxidation of **1**. Due to the ambigous and difficult determination of the oxidation position (*ortho*- or *para*-position of the phenolic ring), the structure of **2** was assigned on the basis of both spectroscopic and chemical evidence. The presence of absorption proton signals in the typical region located between δ 6.3 and 7.3 in the ^{1}H NMR spectrum as well as the presence of two quaternary absorption carbon signals at δ 187.75 and 187.44 in the ^{13}C NMR spectrum of compound **2** were diagnostic for the quinonoid structure.[32] Moreover, the presence of the *ortho*-quinonoid structure was undoubtedly confirmed by the quantitative conversion of **2** to 4-*n*-pentadecylcatechol **3** (side-chain-saturated thitsiol), a phenolic constituent of Burmese lac,[33] by treatment with a small excess of sodium borohydride in a EtOH–THF mixture (4:1 *v*/*v*) at 25 °C (Scheme 1), and comparison with an authentic sample.

It is interesting to note that the reported oxidation of **1** was very selective. In fact, other possible benzoquinone isomers, *e.g.*, 3-*n*-pentadecyl-1,2-benzoquinone and 2-*n*-pentadecyl-1,4-benzoquinone (not shown) or products of benzylic oxyfunctionalization,[34] were not detected in the reaction mixture. The high regioselectivity observed in this transformation may be, in part, attributed to the presence of the 3-*n*-pentadecyl side-chain, as suggested by the oxidation of 3-methylphenol **6** (*m*-cresol), a simple model of **1**, in which case, the expected 2-methyl-1,4-benzoquinone **7** was obtained as the only recovered product in 31% yield (Scheme 2).



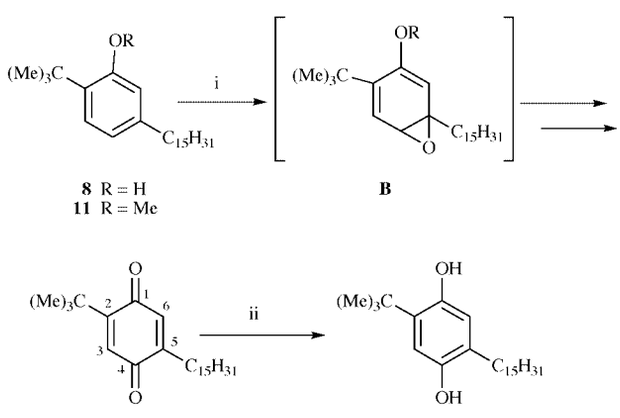
6 7

Scheme 2 *Reagents and conditions*: i, $MeReO_3$, H_2O_2 (35%) EtOH, 25 °C.

In accord with the mechanism previously proposed by Adam *et al.*[25c] it is reasonable to suggest that the oxidation of **1** proceeds through the selective formation of the reactive 1,2-arene oxide intermediate **A** (Scheme 1) and subsequent ring opening, dehydration and further oxidation to **2**. In this case we have no evidence for the formation of other possible epoxide intermediates, probably because of the steric effects due to a spatial disposition of the large 3-*n*-pentadecyl side-chain sterically hindering the adjacent *ortho*-positions of the phenolic ring.

The oxidation of methyl 3-*n*-pentadecylphenyl ether **4** and 3-*n*-pentadecyl acetate **5**, prepared starting from **1** by usual synthetic procedures,[35] was studied with the aim to evaluate the effect of protected phenolic hydroxy groups on the reaction pathway. Treatment of compound **4** with H_2O_2–$MeReO_3$ in AcOH at 25 °C afforded **2** as the only recovered product, in 33% yield (Scheme 1). The fair yield of **2** might be due to ring-opened, water-soluble, products formed by overoxidation, as suggested by the poor mass balance of the reaction (68% substrate conversion). Even in this case, the 3-*n*-pentadecyl side-chain might be expected to exert an important effect on the regioselectivity of the reaction. In fact, as previously reported in the literature,[36] substituted *para*-benzoquinones were the only observed products in the H_2O_2–$MeReO_3$ oxidation of several activated methoxybenzenes characterized by simple alkyl substituents. Moreover, under the conditions of the H_2O_2–$MeReO_3$–AcOH oxidation, compound **5** was quite stable, as confirmed by the low yield of **2** (less than 15% yield; 28% substrate conversion) obtained after 24 h at 80 °C. These data show that the reactivity of **1** toward $MeReO_3$ is decreased by the presence of electron-withdrawing substituent, in accord with the electrophilic character of the activated bis(peroxo)rhenium complex [$MeRe(O_2)_2O{\cdot}H_2O$]. It is interesting to note that the regioselectivity of $MeReO_3$ oxidation of compounds **1**, **4**, and **5** did not depend on the presence of a protective group on the phenolic hydroxy group. Moreover, in the oxidations of compounds **4** and **5**, the use of an acidic medium was essential for attaining acceptable conversions to the *ortho*-benzoquinone **2**.

Attention was then turned to the use of the H_2O_2–$MeReO_3$ system for the oxidation of 2-*tert*-butyl-5-*n*-pentadecylphenol **8**[1,5] and 2-*tert*-amyl-5-*n*-pentadecylphenol **12**,[5] in order to evaluate the effect of a further bulky side-chain alkyl substituent on the phenolic ring. The oxidation of **8** with H_2O_2–$MeReO_3$ in EtOH at 25 °C afforded 2-*tert*-butyl-5-*n*-pentadecyl-1,4-benzoquinone **9** in low yield (less than 20% yield). The same reaction performed both in EtOH–HBF_4 and AcOH systems at 25 °C gives the *para*-benzoquinone **9** in 55 and 71% yield, respectively (Scheme 3). Subsequent treatment of **9** with a small excess of $NaBH_4$ in EtOH–THF mixture (4:1 *v*/*v*) at 25 °C gives 2-*tert*-butyl-5-*n*-pentadecyl-*p*-hydroquinone **10** in quantitative yield (Scheme 3).

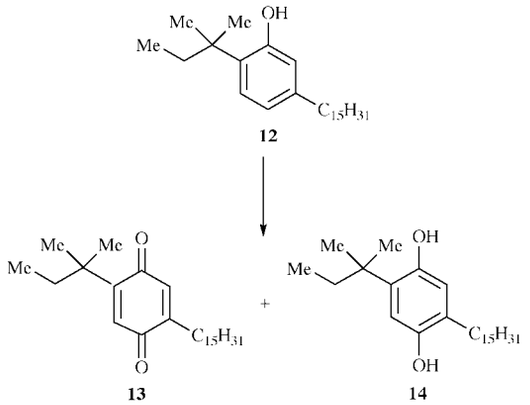


9 10

Scheme 3 *Reagents and conditions*: i, $MeReO_3$, H_2O_2 (35%), EtOH or EtOH–HBF_4 or AcOH, 25 °C; ii, $NaBH_4$, EtOH–THF, 25 °C.

In a similar way, the oxidation of **12** performed both in EtOH–HBF_4 and AcOH systems at 25 °C gives 2-*tert*-amyl-5-*n*-pentadecyl-1,4-benzoquinone **13** in 33 and 72% yield, respectively (Scheme 4). It is worthy of note that in the case of the oxidation of **9** in EtOH–HBF_4 the 2-*tert*-amyl-5-*n*-pentadecyl-*p*-hydroquinone **14** was also obtained, in 23% yield (Scheme 4). Probably, compounds **13** and **14** are present in the reaction mixture as an intramolecularly hydrogen-bonded quinhydrone-type system.

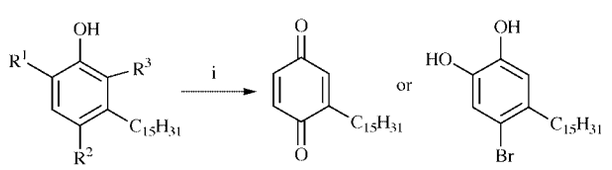
These results indicate that in the oxidation of compounds **8** and **12**, the presence of a bulky alkyl substituent in the C-2 position of the phenolic ring switches the reaction pathway



Scheme 4 *Reagents and conditions*: i, $MeReO_3$, H_2O_2 (35%), EtOH–HBF_4 or AcOH, 25 °C.

toward the selective synthesis of the corresponding *para*-benzoquinones. Probably the reaction proceeds through initial formation of reactive 3,4-arene oxides as intermediates (see, for example, **B** in Scheme 3) because of a finely tuned balance between the steric effects exerted by the alkyl C-2 and C-5 substituents. Moreover, in accord with data previously reported by Adam *et al*.,[25c] the hydroxy group of the phenolic ring may not be attacked first by the bis(peroxo)rhenium complex, as shown by the presence of by-product **14** in the oxidation of **10** performed in EtOH–HBF_4 mixture. In all the cases studied the 6-position was not at all reactive. As previously reported for the oxidation of **4**, the oxidation of the methoxybenzene derivative **11** performed in AcOH at 25 °C gives the *para*-benzoquinone **9** in a yield (49%) smaller than that previously observed for the corresponding phenol **8** (Scheme 3), probably because of ring-opened, water-soluble products formed by overoxidation.

Finally, we studied the oxidation of 4-bromo-3-*n*-pentadecylphenol **15**, 2,4-dibromo-5-*n*-pentadecylphenol **17**, and 2,4,6-tribromo-3-*n*-pentadecylphenol **19**, as representative examples of low-reactivity halogenated derivatives. The oxidation of **15** with H_2O_2–$MeReO_3$ in AcOH at 25 °C afforded a product of oxidative dehalogenation, 2-*n*-pentadecyl-1,4-benzoquinone **16**, as the only recovered product, in 21% yield (Scheme 5) with



15, **17**, **19** **16** **18**

15 $R^1 = R^3 = H$, $R^2 = Br$
17 $R^1 = R^2 = Br$, $R^3 = H$
19 $R^1 = R^2 = R^3 = Br$

Scheme 5 *Reagents and conditions*: i, $MeReO_3$, H_2O_2 (35%), AcOH, 25 or 80 °C.

unchanged substrate. The reaction temperature showed a pronounced effect on this transformation since at 80 °C a higher yield (44%) of **16** was obtained. The oxidation of **17** performed directly at 80 °C in AcOH gave 4-bromo-5-*n*-pentadecylcatechol **18** as the only recovered product, in 31% yield (Scheme 5) with unchanged substrate. In this case, the *ortho*-benzoquinone was not observed in the reaction mixture, probably because of the low reactivity of the substrate toward the electrophilic oxidant. In the latter two reactions no further products were detected in the reaction mixture by TLC analysis. Due to the ambiguous and difficult determination of the attack

Table 1 Cytotoxicity of the phenolic compounds **2**–**5**, **8**, **9**, **12**, **13**, **15**, **17**, and **19** against murine fibroblast cell line (3T3 cells)

Compound	IC_{50} (μg ml^{-1})[a]
2	9
3	9
4	<1
5	<1
8	<1
9	20
12	<1
13	9
15	<1
17	18
19	150

[a] Inhibitory concentration of compound (μg ml^{-1}) required for the 50% inhibition of the cellular growth.

position (2- or 4-position of the phenolic ring), the structure of compound **18** was assigned both on the basis of spectroscopic and chemical evidence. In the first case, the presence of a residual signal at δ_C 114.9 in the ^{13}C NMR spectrum of **18** (characteristic for the C-4 bromine atom as evinced from the ^{13}C NMR spectrum of **15**), as well as the disappearance of the signal at δ_C 107.6 (characteristic for the C-6 bromine atom) were diagnostic for the catechol structure. The presence of the catechol moiety was undoubtedly confirmed by the quantitative conversion of **18** to 5-*n*-pentadecylcatechol by usual reductive treatment,[37] and comparison with an authentic sample. Under the conditions of the H_2O_2–CH_3ReO_3–AcOH oxidation, compound **19** was quite stable, as confirmed by the very low conversion (less than 2%) obtained after 48 h at 80 °C.

It is interesting to note that under our experimental conditions products usually formed by radical pathways, such as diaryl ethers or diaryls,[38] were not observed. A different regioselectivity was observed in the oxidation of the halogenated derivatives **15** and **17** with respect to previously reported oxidations of alkylcardanol derivatives **1**, **8**, and **12**. Thus, the *para*-position in **15** was selectively oxidized in the first step of the reaction independently from the presence of the *n*-pentadecyl side-chain. On the other hand, the *ortho*-position in **17** was selectively oxidized even in the presence of a bulky substituent (the same bromine atom). These latter transformations are noteworthy because of the stringent restriction being imposed by various governing bodies concerning the presence of halogenated phenolic derivatives in the waste water produced by bleaching processes and the consequent necessity to find new and efficient catalytic procedures for the oxidative treatment of these compounds.[39,40]

The cytotoxic effects of compounds **2**–**5**, **8**, **9**, **12**, **13**, **15**, **17**, and **19** were evaluated using murine fibroblast cell line (3T3 cells), plasmocytoma murine cell line (NSO cells), normal human lymphocytes PHA-stimulated and human lymphoblastoid cell line (Daudi cells). Results reported in Table 1 are referred to fibroblast cell line and are representative of all other cell lines studied. Data have been obtained both by tritiated thymidine incorporation analysis and by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test to analyze both proliferation and DNA synthesis in cell cultured in the presence of different compounds. All products studied (with the only exception of **19**) show potent cytotoxic effects reported as IC_{50}-value [inhibitory concentration of compound (μg ml^{-1}) required for the 50% inhibition of cellular growth]. In particular, compounds **4**, **5**, **8**, **12**, and **15**, show an IC_{50}-value < 1.0 μg ml^{-1}, while compounds **2**, **3**, and **13** show an IC_{50} < 10 μg ml^{-1}. Compounds **9** and **17** are relatively less cytotoxic with an IC_{50} < 21 (18 and 20 μg ml^{-1}, respectively) (Table 1). No difference was observed for cytotoxicity between data obtained by using MTT test and DNA synthesis.

In summary, $MeReO_3$-catalyzed oxidation of 3-*n*-pentadecylphenol derivatives by 30% aq. H_2O_2 both in EtOH, EtOH–HBF_4 and AcOH affords the corresponding *ortho*- and *para*-benzoquinones in acceptable to good yields. The regioselectivity of the reaction was found to depend on the nature and on the substitution pattern of the substituents. The present catalytic method compares well with the stoichiometric procedures for the synthesis of quinone derivatives from phenols and constitutes a new, convenient, and selective method for the synthesis of *ortho*- and *para*-benzoquinones of cardanol derivatives under environmentally acceptable conditions.

Experimental

Descriptions of analytical instruments and ^{1}H NMR and IR spectrometers have been previously published.[41] Mps were obtained on a Reichert Kofler apparatus and are uncorrected. Mass spectra were recorded on a VG 70/250S spectrometer with an electron beam of 70 eV. Microanalyses were performed with a C. Erba 1106 analyzer. Chromatographic purifications were performed on columns packed with Merck silica gel, 230–400 mesh, for flash technique. TLC was carried out using Merck Kieselgel 60 F254 plates. All reagents and solvents were of highest grade commercially available and were used purified or freshly distilled as required by literature procedures. MTT was purchased from Sigma. It was dissolved at a concentration of 5 mg ml^{-1} in sterile phosphate-buffered saline (PBS) at room temperature and the solution was further sterilized by filtration and stored at 4 °C in a dark bottle. Sodium dodecyl sulfate (SDS) was obtained from Sigma, and DMF was purchased from Fluka. Lysis buffer was prepared as follows: 20% *w*/*v* of SDS was dissolved at 37 °C in a solution of 50% (each) of DMF and demineralized water; pH was adjusted to 4.7 by addition 2.5% of 80% acetic acid and 2.5% 1 M HCl.

Starting materials

3-*n*-Pentadecylphenol **1**, 2-*tert*-butyl-5-*n*-pentadecylphenol **8**, 2-*tert*-amyl-5-*n*-pentadecylphenol **12**, and 4-bromo-3-*n*-pentadecylphenol **15** have been prepared by procedures reported in the literature.[3,30,36]

General procedure for the synthesis of 3-*n*-pentadecyl(methoxy)-benzene derivatives

To a solution of the appropriate substrate (1.0 mmol) and K_2CO_3 (1.5 mmol) in dry DMF (3 ml) was added methyl iodide (3.0 mmol). The reaction mixture was stirred at 25 °C under nitrogen for 12 h. It was then diluted with EtOAc (50 ml) and the excess of MeI was decomposed by addition of a little AcOH. The organic layer was washed with brine (3 × 10 ml) and dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The products were obtained by chromatographic purification and identified by both spectroscopic analyses and mass spectroscopy.

Methyl 3-*n*-pentadecylphenyl ether 4. Oil (Found: C, 82.7; H, 12.1. $C_{22}H_{38}O$ requires C, 82.9; H, 12.0%); ν_{max}/cm^{-1} (KBr) 750, 975, 1000, 1250, 1545, 2850, 2920; δ_H($CDCl_3$) 7.32 (1H, t, *J* 8.2, CH), 6.91 (3H, m, 3 × CH), 3.89 (3H, s, CH_3O), 2.75 (2H, t, *J* 7.4, CH_2Ar), 1.79 (2H, m, CH_2CH$_2$), 1.47 (24H, m, 12 × CH_2CH$_2$), 1.09 (3H, t, *J* 6.7, CH_3CH$_2$); δ_C($CDCl_3$) 159.58 (q), 144.24 (q), 128.96 (t), 120.65 (t), 114.10 (t), 110.59 (t), 54.62 (p), 36.05 (s), 31.97 (s), 31.42 (s), 29.78 (s), 29.68 (s), 29.60 (s), 29.46 (s), 22.73 (s), 14.04 (p); *m*/*z* (EI) 318 (M$^+$).

2-*tert*-Butyl-5-*n*-pentadecylphenyl methyl ether 11. Oil (Found: C, 83.2; H, 12.3. $C_{26}H_{46}O$ requires C, 83.3; H, 12.3%); δ_H($CDCl_3$) 7.20 (1H, d, *J* 8.4, CH), 6.76 (2H, m, 2 × CH), 3.86 (3H, s, CH_3O), 2.60 (2H, t, *J* 7.4, CH_2Ar), 1.61 (2H, m, CH_2CH$_2$), 1.40 (9H, s, Bu^t), 1.30 (24H, m, 12 × CH_2CH$_2$), 0.92 (3H, t, *J* 6.1, CH_3CH$_2$); δ_C($CDCl_3$) 158.39 (q), 141.87 (q), 135.0 (q), 126.28 (t), 120.0 (t), 111.86 (t), 54.90 (p), 35.75 (s), 34.48 (q), 31.95 (s), 31.46 (s), 29.81 (p), 29.73 (s), 29.58 (s), 29.40 (s), 22.72 (s), 14.13 (p); *m*/*z* (EI) 374 (M$^+$).

Synthesis of 3-*n*-pentadecyl acetate 5

Compound **1** (1.0 mmol) was dissolved in a mixture of pyridine (5 ml) and acetic anhydride (5 ml). The reaction mixture was stirred at 25 °C for 4 h. It was then diluted with EtOAc (50 ml) and the excess of pyridine was neutralized by washing with 1 M HCl. The organic layer was further washed successively with aq. $NaHCO_3$ and brine (3 × 10 ml) and dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The product was obtained by chromatographic purification and identified by both spectroscopic analyses and mass spectroscopy. Powder; 36–38 °C (from AcOEt–*n*-hexane) (Found: C, 79.75; H, 11.10. $C_{23}H_{38}O_2$ requires C, 79.7; H, 11.10%); ν_{max}/cm^{-1} (KBr) 750, 1000, 1200, 1540, 1765, 2850, 2920; δ_H($CDCl_3$) 7.28 (1H, m, CH), 7.05 (1H, d, *J* 7.6, CH), 6.91 (2H, m, 2 × CH), 2.62 (2H, t, *J* 7.5, ArCH_2), 2.29 (3H, s, CH_3CO), 1.62 (2H, m, CH$_2$CH_2), 1.28 (24H, m, 12 × CH_2CH$_2$), 0.90 (3H, t, *J* 6.3, CH$_2$CH_3); δ_C($CDCl_3$) 169.40 (q), 150.61 (q), 144.59 (q), 128.99 (t), 125.83 (t), 121.32 (t), 118.62 (t), 35.67 (s), 31.88 (s), 31.13 (s), 29.43 (s), 22.64 (p), 21.03 (p), 14.05 (p); *m*/*z* (EI) 346 (M$^+$).

General procedure for the oxidation of cardanol derivatives

To a solution of the particular cardanol derivative (1.0 mmol) and methyltrioxorhenium(VII) (0.02 mmol) in the appropriate solvent medium (5 ml) was added 35% hydrogen peroxide (2.0–4.0 mmol). The reaction mixture was stirred at the desired temperature (20–80 °C) under nitrogen for 12 h. It was then diluted with both EtOAc (50 ml) and water (3 ml) and the excess of hydrogen peroxide was decomposed by addition of a little MnO_2. After filtration, the organic layer was washed with brine (2 × 10 ml) and dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The products were obtained by chromatographic purification in acceptable to good yields and identified by spectroscopic analyses, mass spectroscopy, and comparison with authentic samples.

4-*n*-Pentadecyl-1,2-benzoquinone 2. Oil (Found: C, 79.0; H, 10.7. $C_{21}H_{34}O_2$ requires C, 79.1; H, 10.7%); ν_{max}/cm^{-1} (KBr) 750, 975, 1000, 1250, 1545, 1655, 2850, 2920; δ_H($CDCl_3$) 6.68 (2H, m, 2 × CH), 6.51 (1H, m, CH), 2.36 (2H, t, *J* 7.4, CH_2C=CH), 1.45 (2H, m, CH_2CH$_2$), 1.20 (24H, m, 12 × CH_2CH$_2$), 0.82 (3H, t, *J* 6.0, CH_3); δ_C($CDCl_3$) 187.75 (q), 187.44 (q), 149.66 (q), 136.73 (t), 136.16 (t), 132.29 (t), 31.87 (s), 29.62 (s), 29.43 (s), 29.30 (s), 29.26 (s), 28.96 (s), 27.73 (s), 22.62 (s), 14.04 (p); *m*/*z* (EI) 318 (M$^+$).

2-Methyl-1,4-benzoquinone 7. Powder; mp 68–69 °C (from AcOEt). Compound **7** was identical to an authentic commercial sample (Aldrich).

2-*tert*-Butyl-5-*n*-pentadecyl-1,4-benzoquinone 9. Powder; mp 51–53 °C (from AcOEt) (Found: C, 80.2; H, 11.3. $C_{25}H_{42}O_2$ requires C, 80.1; H, 11.3%); ν_{max}/cm^{-1} (KBr) 750, 975, 1000, 1245, 1545, 1650, 2850, 2920; δ_H($CDCl_3$) 6.53 (1H, s, CH), 6.42 (1H, t, *J* 1.4, CH), 2.33 (2H, t, *J* 7.5, CH_2C=CH), 1.45 (2H, m, CH_2CH$_2$), 1.24 (9H, s, 3 × CH_3), 1.22 (24H, m, 12 × CH_2), 0.84 (3H, t, *J* 5.8, CH_3); δ_C($CDCl_3$) 188.64 (q), 187.97 (q), 155.63 (q), 147.89 (q), 134.39 (t), 131.69 (t), 34.96 (q), 31.88 (s), 29.63 (s), 29.46 (s), 29.31 (p), 29.14 (s), 28.21 (s), 27.72 (s), 22.65 (s), 14.10 (p); *m*/*z* (EI) 374 (M$^+$).

2-*tert*-Amyl-5-*n*-pentadecyl-1,4-benzoquinone 13. Powder; mp 52–54 °C (from AcOEt) (Found: C, 80.2; H, 11.4. $C_{26}H_{44}O_2$ requires C, 80.3; H, 11.4%); ν_{max}/cm^{-1} (KBr) 750, 975, 1000, 1245, 1540, 1650, 2850, 2920; δ_H($CDCl_3$) 6.50 (1H, s, CH), 6.41

(1H, t, *J* 1.4, CH), 2.35 (2H, t, *J* 7.6, CH_2C=CH), 1.71 (2H, q, *J* 8.0, CH_2CH$_3$), 1.45 (2H, m, CH$_2$), 1.22 (24H, m, 12 × CH_2-CH$_2$), 1.18 (6H, s, 2 × CH$_3$C), 0.81 (3H, t, *J* 6.8, CH$_3$), 0.66 (3H, t, *J* 8.0, CH_3CH$_2$); δ_C(CDCl$_3$) 188.36 (q), 188.01 (q), 154.58 (q), 147.97 (q), 134.33 (t), 133.44 (t), 38.71 (q), 33.28 (s), 31.88 (s), 29.63 (s), 29.46 (s), 29.31 (s), 29.14 (s), 28.63 (s), 28.21 (s), 27.67 (s), 27.02 (t), 22.65 (s), 14.07 (p), 9.29 (p); *m*/*z* (EI) 388 (M$^+$).

2-*tert*-Amyl-5-*n*-pentadecyl-*p*-hydroquinone 14. Oil (Found: C, 79.8; H, 11.8. $C_{26}H_{46}O_2$ requires C, 79.9; H, 11.8%); ν_{max} (KBr)/cm^{-1} 1193, 1450, 3275; δ_H(CDCl$_3$) 7.05 (1H, s, CH), 6.49 (1H, m, CH), 4.90 (1H, br s, OH), 4.8 (1H, br s, OH), 2.41 (2H, t, *J* 7.6, CH_2C=CH), 1.75 (2H, q, *J* 8.0, CH_2CH$_3$), 1.45 (2H, m, CH$_2$), 1.20 (24H, m, 12 × CH_2CH$_2$), 1.16 (6 H, s, 2 × CH$_3$C), 0.82 (3H, t, *J* 6.8, CH$_3$), 0.65 (3H, t, *J* 8.0, CH_3CH$_2$); *m*/*z* (EI) 390 (M$^+$).

2-*n*-Pentadecyl-1,4-benzoquinone 16. (Found: C, 79.1; H, 10.7. $C_{21}H_{34}O_2$ requires C, 79.1; H, 10.7%); δ_H(CDCl$_3$) 7.34 (1H, m, CH), 6.94 (1H, m, CH), 6.10 (1H, m, CH), 2.32 (2H, t, *J* 8.0, CH_2C=CH), 1.45 (2H, m, CH_2CH$_2$), 1.20 (24H, m, 12 × CH_2CH$_2$), 0.88 (3H, t, *J* 6.0, CH$_3$); *m*/*z* (EI) 318 (M$^+$).

4-Bromo-5-*n*-pentadecylcatechol 18. Oil (Found: C, 63.21; H, 8.80. $C_{21}H_{35}BrO_2$ requires C, 63.15; H, 8.80%); ν_{max} (KBr)/cm^{-1} 810, 860, 3400; δ_H(CDCl$_3$) 7.64 (1H, s, CH), 7.60 (1H, s, CH), 5.93 (1H, br s, OH), 5.90 (1H, br s, OH), 2.85 (2H, t, *J* 7.1, CH_2Ar), 1.23 (2H, m, *J* 6.8, CH_2CH$_2$), 0.93 (24H, m, 12 × CH_2CH$_2$), 0.59 (3H, t, *J* 5.9, CH_3CH$_2$); δ_C(CDCl$_3$) 152.0 (q), 141.83 (q), 140.65 (q), 134.46 (t), 133.82 (t), 114.9 (q), 37.26 (s), 34.45 (s), 31.85 (s), 30.52 (s), 29.60 (s), 29.51 (s), 27.89 (s), 22.62 (s), 14.0 (p); *m*/*z* 398 (EI) (M$^+$).

General procedure for the reduction of *ortho*- and *para*-benzoquinones of cardanol derivatives

To a solution of the particular benzoquinone cardanol derivative (1.0 mmol) in EtOH–THF (5 ml) mixture (4:1 *v*/*v*) was added a small excess of sodium borohydride (1.5 mol equiv.). The reaction mixture was stirred at 25 °C under nitrogen for 1 h. It was then diluted with EtOAc (50 ml) and the excess of $NaBH_4$ was decomposed by addition of a little amount of aq. HCl (1 M). The organic layer was washed with brine (3 × 10 ml) and dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The products were obtained by chromatographic purification and identified by spectroscopic analyses, mass spectroscopy, and comparison with authentic samples.

4-*n*-Pentadecylcatechol 3. Powder; mp 91–92 °C (lit.,[33,40] 91, 91–92 or 92–93 °C) (Found: C, 78.5; H, 11.3. Calc. for $C_{21}H_{36}O_2$: C, 78.6; H, 11.3%); ν_{max} (KBr)/cm^{-1} 810, 860, 3400; δ_H(CDCl$_3$) 6.26 (1H, d, *J* 8.5, CH), 6.22 (1H, d, *J* 2.8, CH), 6.12 (1H, dd, *J* 8.5, *J′* 2.9, CH), 4.51 (2H, br s, OH), 2.18 (2H, t, *J* 7.2, CH_2Ar), 1.23 (2H, m, *J* 6.8, CH_2CH$_2$), 0.93 (24H, m, 12 × CH_2CH$_2$), 0.59 (3H, t, *J* 5.9, CH_3CH$_2$); δ_C(CDCl$_3$) 150.49 (q), 148.65 (q), 131.22 (q), 117.35 (t), 116.41 (t), 113.58 (t), 32.74 (s), 30.99 (s), 30.74 (s), 30.52 (s), 30.40 (s), 30.20 (s), 23.45 (s), 14.41 (p); *m*/*z* (EI) 320 (M$^+$).

2-*tert*-Butyl-5-*n*-pentadecyl-*p*-hydroquinone 10. Oil (Found: C, 79.8; H, 11.7. $C_{25}H_{44}O_2$ requires C, 79.7; H, 11.7%); ν_{max} (KBr)/cm^{-1} 1193, 1456, 3270; δ_H(CDCl$_3$) 6.69 (1H, s, CH), 6.44 (1H, s, CH), 4.81 (1H, br s, OH), 4.72 (1H, br s, OH), 2.48 (2H, t, *J* 7.3, CH_2Ar), 1.55 (2H, m, CH$_2$CH_2), 1.35 (9H, s, Bu^t), 1.26 (24H, m, 12 × CH_2CH$_2$), 0.87 (3H, t, *J* 6.1, CH_3CH$_2$); δ_C(CDCl$_3$) 147.82 (q), 146.66 (q), 134.61 (q), 126.64 (q), 117.89 (t), 114.32 (t), 34.18 (s), 31.90 (q), 29.78 (s), 29.69 (s), 29.55 (s), 29.42 (p), 29.36 (s), 22.68 (s), 21.04 (p), 14.11 (p); *m*/*z* (EI) 376 (M$^+$).

Biological experiments

Cell lines. All cell lines were obtained from ATCC. The cells were cultured in RPMI 1640 supplemented with 5% FCS (foetal calf serum), 0.1 mM glutamine, 1% penicillin and streptomycin. Cells were grown in Nunc clone plastic bottles (TedNunc, Roskilde, Denmark) and split twice weekly at different cell densities according to standard procedures. 3T3 cells were grown as monolayer cultures and were split by using trypsin. Peripheral blood mononuclear cells (MNC) were separated from heparinized whole blood, obtained from healthy donors, on a Ficoll-Hypaque gradient as previously reported.[42,43] MNC thus obtained were washed twice with RPMI 1640 supplemented with 10% FCS, glutamine and antibiotics, suspended at 200 000 viable cells ml^{-1} in medium containing, as mitogen, 5 μg ml^{-1} PHA (phyto haemo agglutinin) and used in toxicity tests.

Toxicity tests. Cells were plated at different concentrations on flat-bottom 96-well microplates (0.1 ml well^{-1}). Normal lymphocytes were plated out at 20 000 cells well^{-1}; 3T3 cells (murine fibroblast line) were plated at 10 000 cells well^{-1}; NSO cells (plasmocytoma murine cell line) were plated out at 3000 cells well^{-1}; Daudi cells (human lymphoblastoid cell line) were plated at 3000 cells well^{-1}. 12 Hours after plating, different concentrations of each compound, diluted in ethanol, were added to each well. After 48 h, MTT assay was performed to analyze cytotoxicity of the different compounds. Some experiments were performed by using confluent cells: compounds were added on 3T3 monolayer 3 days after plating. Tests were then run as described above. Control wells were performed by addition of the same amount of ethanol. DNA-synthesis experiments were performed as previously reported.[44]

MTT–formazan extraction procedure. 20 μl of the 5 mg ml^{-1} stock solution of MTT were added to each well; after 2 h of incubation at 37 °C, 100 μl of the extraction buffer were added. After an overnight incubation at 37 °C, the optical densities at 570 nm were measured using a Titer-Tech 96-well multiscanner, employing the extraction buffer as the blank. Standard deviation (S.D.) never exceded 5% of the mean value. Compounds **2**–**4**, **6**, **8**–**10**, **12**, and **13** induce, at high concentrations, *per se* modulation of MTT absorbance. IC_{50}-Values are reported for 3T3 and are very similiar to those found in other cell lines.

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