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Formation of a Blue Adduct between 4-*tert*-Butyl-1,2-benzoquinone and 4-Amino-*N*,*N*-diethylaniline

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Abstract—The reaction of 4-*tert*-butyl-1,2-benzoquinone (BQ) with 4-amino-*N*,*N*-diethylaniline (ADA) produces a stable blue adduct with λ_{max} 625 nm (ϵ 11,120 M^{-1} cm^{-1}). This makes the reaction a sensitive analytical tool, suitable for the detection and spectrophotometric determination of this quinone even in the presence of biological compounds. The adduct was identified as 3-*tert*-butyl-4-(4′-diethylamino)-phenylimino-6-hydroxycyclohexa-2,5-dien-1-one.

Introduction

The analytical determination of *o*-quinones is of great practical interest because of the wide occurrence of these compounds in nature[1] and their biological as well as pharmacological importance.[2–4] Nevertheless, several limitations make their quantitative determination often troublesome, especially because of the high reactivity of these electron-deficient structures towards nucleophiles,[5] which seriously affects their stability in any biological-like environment. Therefore the unsubstituted 1,2-benzoquinone is rapidly attacked even by water and *o*-quinones bearing electron-withdrawing substituents such as 4-nitro-1,2-benzoquinone and 1,2-benzoquinone-4-carboxylic acid cannot be isolated. Other *o*-quinones, like 1,2-naphthoquinones, are more stable in the presence of water, although they are still very reactive compounds.

Indeed the high reactivity of *o*-quinones has been exploited in several analytical methods based on their reactions with appropriate nucleophiles to provide coloured and relatively stable adducts suitable for spectrophotometric determination. Among these reactants worthy of mention are dimethylamine,[6] piperidine,[7] cysteine,[8] 3-methylbenzothiazolin-2-one hydrazone[9] and proline.[10] These methods offer possibilities for the determination of *o*-quinones, which are themselves unstable and display only a weak absorption around 400 nm due to an n–π* transition.

We have recently reported[11] that 4-*tert*-butyl-1,2-benzoquinone (BQ) reacts with 4-amino-*N*,*N*-diethylaniline (ADA) to provide a stable, intensely blue adduct. The formation of this adduct is of particular interest because the reaction is rapid and complete and the blue product possesses a high molar extinction coefficient (ϵ 11,120 M^{-1} cm^{-1}) in a region (600–700 nm) free from absorption by the vast majority of biological compounds. So, the detection and the quantitative determination of BQ in biological preparations become reliable and sensitive. Indeed the reaction of BQ with ADA has been successfully employed to reveal polyphenol oxidase activity in polyacrylamide gel electrophoresis, either from purified preparations[11] or from crude extracts,[12] as well as to develop a spectrophotometric assay.[13] Nevertheless, the actual nature of the blue adduct was so far unknown. We report here the result of a detailed investigation aiming to clarify the structure and some properties of the adduct between BQ and ADA and to find analytical applications for the reaction between aromatic amines and *o*-quinones.

Results and Discussion

When ADA is allowed to react with BQ at room temperature, a blue product is quickly formed. This has now been isolated and analysed with NMR, IR, mass spectrometry and UV–Vis spectroscopy. On the basis of the results, the blue compound can be assigned the structure **1** of 3-*tert*-butyl-4-(4′-diethylamino)-phenylimino-6-hydroxycyclohexa-2,5-dien-1-one (the numbering of carbon atoms in **1** is arbitrary and refers to the positions defined for NMR assignments) (see Fig. 1).

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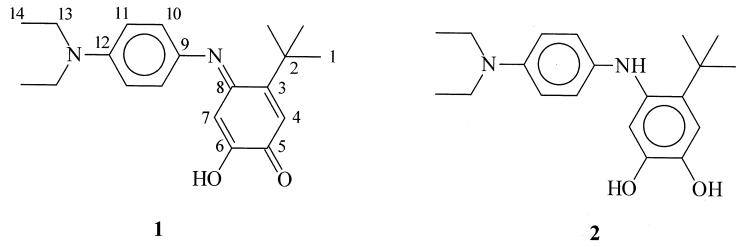

1 **2**

Figure 1.

The ^{1}H and ^{13}C NMR signals were assigned by comparison with the spectra of authentic samples of ADA and BQ and assignments were confirmed by computer simulation using ACS ChemLab software. FT-IR spectra reveal the presence of an intense and broad band centred at 3390 cm^{-1} (O–H stretching) together with intense signals at 1659 cm^{-1} and 1631 cm^{-1} due to stretching of the C=O and C=N bonds of the quinoneimine system.

The mass spectrum of the purified blue adduct reveals an intense M^{+} peak at *m*/*z* 326, which, together with the observed fragmentation pattern, is in agreement with the suggested structure. The UV–Vis spectrum[13] is also consistent with the highly conjugated structure **1**, since the value of λ_{max} 625 nm and the molar extinction coefficient at this wavelength (ϵ 11,120 M^{-1} cm^{-1}) are indicative of a $\pi-\pi^{*}$ transition in an extended π system conjugated with electron-releasing and -withdrawing groups (C=O and C=N–, $-NEt_2$ and –OH in structure **1**).

When the mass spectrum is recorded on the crude reaction product, a second, much weaker spectrum was observed, which is clearly due to a less volatile compound of slightly higher molecular weight. On the basis of the fragmentation pattern and by comparison with the mass spectra recorded for ADA and *tert*-butylcatechol, this compound could be assigned the structure **2**. Apparently this is an intermediate in the formation of **1** (vide infra).

A possible reaction mechanism leading to the formation of adduct **1** involves, as a first step, a nucleophilic Michael-type addition of ADA on the α-β unsaturated carbonyl system to form an adduct which quickly rearranges to the catechol derivative **2**. This electron-rich structure is then easily oxidised to the final quinoneimine product **1**.

When a water suspension of the adduct **1** reacts with sodium dithionite, ascorbic acid or zinc and dilute acetic acid, the blue colour disappears; it is formed back quantitatively upon standing in air or adding an excess of BQ. This indicates that the quinoneimine adduct **1** can undergo reversible reduction to the catechol adduct **2** and that both molecular oxygen and excess BQ are able to oxidise **2** to **1**, supporting the suggested mechanism for the formation of the blue adduct from ADA and BQ. As **2** is very readily and quickly oxidised to **1**, the overall stoichiometry of the adduct formation is 1:1 with respect to ADA and BQ. Only traces of **2** could be detected after ADA and BQ are mixed in a 1:1 ratio and allowed to react in the presence of air.

An organic solution of the blue product can be held at room temperature in the presence of air for several hours without significant change in UV–Vis spectrum. Moreover, the purified solid compound is indefinitely stable in the dark at 0°C and can be left at room temperature in the dark for several days without significant degradation. Although the blue adduct is not soluble in water, it forms a fine and very stable suspension, which does not precipitate upon centrifugation and does not give light scattering, therefore behaving as a true solution as regards its use for tyrosinase photometric quantitation. It can therefore be directly used for spectrophotometric measurements.[13] The UV–Vis spectral features taken together with the stability of the adduct, make the reaction of BQ with ADA very useful for the spectrophotometric determination of the quinone. This is important especially in the case of biological samples, where several compounds can interfere with absorbance measurements in the range 400–500 nm and in the UV region.

In order to explore the usefulness for analytical purposes of the reactions of *o*-quinones with aromatic amines, BQ has been reacted with other aniline derivatives. Furthermore, to determine whether the formation of a blue adduct upon reaction with ADA is a general behaviour for quinones or is specific to BQ, a number of quinones have been reacted with ADA under experimental conditions similar to those used for the reaction with BQ. Besides ADA, other aromatic amines are found to react with *o*-quinones to give intensely coloured adducts. However, the adducts obtained show UV–Vis spectra with λ_{max} values falling at shorter wavelengths, and a number of unidentified by-products are found. The reaction products arising from BQ and aniline (3-*tert*-butyl-6-hydroxy-4-phenyliminocyclohexa-2,5-dien-1-one, **3**) and from BQ and 4-methoxyaniline (3-*tert*-butyl-6-hydroxy-4-(4′-methoxyphenyl)-iminocyclohexa-2,5-dien-1-one, **4**) were formed in poor yields (40% for **3** and 15% for **4**). Therefore ADA can be considered the most interesting reactant among the aniline derivatives tested so far. The formation of a blue or blue-violet adduct upon reaction with ADA is not a general behaviour of *o*-benzoquinones but appears to be confined to those in which the 5-position is available (and accessible) for nucleophilic attack. Thus, no colour development was observed when ADA was mixed with 3,5-di-*tert*-butyl-1,2-benzoquinone or with *tert*-butyl-1,4-benzoquinone. In these cases no reaction takes place at all as demonstrated by spectrophotometric observations, showing the patterns of the unchanged quinones 30 min after mixing. When ADA was reacted with other *o*-quinones, susceptible of nucleophilic attack at the 5-position, coloured adducts absorbing in the range

500–650 nm were observed. Nevertheless, in all cases the reaction proceeded slowly and generated a multitude of side products which reduced the yield to less than 10%. Therefore, the adducts are of no analytical interest and no attempts were made to purify and characterise them. 1,2-Naphthoquinones also give blue adducts with ADA and it is worth noting that, in the case of 1,2-naphthoquinone-4-sulfonic acid, the substituent is presumably eliminated as sulfite ion, and the violet adduct obtained is identical to that formed with unsubstituted 1,2-naphthoquinone (λ_{max} 598 nm, ϵ 7470 $M^{-1}\,cm^{-1}$). Preliminary observations suggest that in the case of 1,2-naphthoquinones the coloured adducts arise, as expected, from a nucleophilic attack of ADA at the 4-position.

Experimental

^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 (or Varian Gemini 200) spectrometer using the purified adducts dissolved in $CDCl_3$ (Sigma). FT-IR spectra of the purified adducts were recorded with a Perkin–Elmer FT-IR 1600 from a solution of the sample in chloroform (Aldrich), using a KBr cell with an optical path 0.1 mm. GC–MS analysis of the products after mixing ADA and BQ was performed both on the crude reaction mixture and on the purified adduct using a Hewlett Packard 5890 series II gas chromatograph equipped with an HP 5971 mass detector. Since the blue adduct proved to be too unstable to pass the chromatographic column (HP5, 30 m, 0.025 mm i.d.) with the necessary temperature programming (50–250°C) without massive degradation, mass spectra of the reaction product before and after purification were performed also on a double-analyser spectrometer (Fison), EI+70 EV, with direct probe-introduction of the sample. UV–Vis spectra were recorded on a double beam Varian Cary 2300 spectrophotometer and with a HP diode array.

A pure sample of the blue adduct was prepared by adding a concentrated solution of BQ (2 mmol) in dioxane (Aldrich) to 3 mmol of ADA sulfate (Fluka) dissolved in water. The blue adduct was quantitatively formed (as judged from spectrophotometric analysis) immediately after mixing at room temperature and was extracted with dichloromethane (Aldrich). The organic solution was washed with water, dried over Na_2SO_4 and evaporated in vacuo to yield the blue product which was purified by preparative TLC on silica gel (Merck, 20 cm×30 cm×1 mm) by eluting with ethyl acetate/petroleum ether 15:85. The purified product was extracted from the plate with isopropanol which was evaporated in vacuo at room temperature. The overall yield of purified adduct on the basis of BQ was 87%. Since 4-*tert*-butyl-1,2-benzoquinone (BQ) is not a commercial product, it was prepared from 4-*tert*-butylcatechol (Fluka) by stirring in the dark for 1 h at room temperature a concentrated dioxane solution with an excess of Ag_2O in the presence of an excess of dry Na_2SO_4. The inorganic material was then removed by filtering and the solution was deoxygenated by bubbling with nitrogen and evaporated in vacuo at room temperature. No further purification was necessary.

The reaction of other quinones with ADA and that of aniline and anisidine (Fluka) with BQ was performed at room temperature by mixing a water solution of the amine with the quinone dissolved in dichloromethane or ethanol or water as appropriate. Whenever a 2 phase system occurred vigorous stirring was necessary.

1,2-Benzoquinone, 4-methyl-1,2-benzoquinone, 1,2-benzoquinone-4-carboxylic acid, 3,5-di-*tert*-butyl-1,2-benzoquinone and 2,3-naphthoquinone were prepared by oxidation of the parent catechols with sodium periodate in 50 mM sodium acetate buffer (pH 5) at room temperature, followed by extraction in dichloromethane. All the other quinones used (1,2-naphthoquinone, 1,2-naphthoquinone-4-sulfonic acid, 3,4,5,6-tetrachloro-1,2-benzoquinone) were commercially available (Aldrich, Fluka).

1. ν_{max}/cm^{-1} 3390, 3013, 1659, 1631. δ_H (300 MHz, $CDCl_3$) 1.47 (1, s, 9H), 6.68 (4, s, 1H), 6.69 (7, s, 1H), 6,97 (10, d, 2H, J=6.0 Hz), 6.73 (11, d, 2H, J=6.0 Hz), 3.44 (13, q, 4H, J=7.5 Hz), 1.22 (14, t, 6H, J=7.5 Hz), 1.57 (OH, broad s, 1H). δ_C (75 MHz, $CDCl_3$) 31.9 (1), 30.4 (2), 150.9 (3), 125.2 (4), 183.6 (5), 154.8 (6), 103.8 (7), 162.8 (8), 139.3 (9), 125.9 (10), 112.4 (11), 147.8 (12), 45.4 (13), 13.4 (14). *m/z* 326 (M^+, 100%); 311 (42%); 283 (37%); 269 (14%); 255 (12%); 190 (76%); 163 (14%); 149 (24%); 134 (40%); 119 (6%). HRMS calcd for $C_{20}H_{26}N_2O_2$ 326.1994 found 326.1991. UV–Vis λ_{max} 625 nm, ϵ 11120 $M^{-1}\,cm^{-1}$. Anal. Calcd for $C_{20}H_{26}N_2O_2$: C, 73.59, H 8.03, N 8.58. Found: C 73.56, H 8.01, N 8.60.

2. *m/z* 328 (M^+, 100%); 313 (63%); 300 (26%); 283 (14%); 226 (35%); 190 (23%); 164 (20%); 149 (49%); 120 (15%); 119 (15%).

3. ν_{max}/cm^{-1} 3446, 3015, 1647, 1623, 1311. δ_H (200 MHz, $CDCl_3$) 1,49 (s, 9H), 6.39 (s,1H), 6.66 (s, 1H), 6.92 (d, 2H, J=8.0 Hz), 7.22 (m, 3H), 1.60 (OH, broad s, 1H). UV–Vis (CH_2Cl_2) λ_{max} 491 nm, ϵ 3596 $M^{-1}\,cm^{-1}$. Anal. Calcd for $C_{16}H_{17}NO_2$: C 75.27, H 6.71, N 5.49. Found: C 75.24, H 6.69, N 5.47.

4. ν_{max}/cm^{-1} 3438, 2961, 1645, 1630, 1321. δ_H (200 MHz, $CDCl_3$) 1.27 (s, 9H), 3.67 (s, 3H), 6.40 (s, 1H), 6.70 (s, 1H), 6.82 (m, 4H), 1.54 (OH, broad s, 1H). UV–Vis (CH_2Cl_2) λ_{max} 459 nm, ϵ 1974 $M^{-1}\,cm^{-1}$. Anal. Calcd for $C_{17}H_{19}NO_3$: C 71.56, H 6.71, N 4.91. Found: C 71.53, H 6.70, N 4.89.

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