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Note

Reaction of Gibbs reagent (2,6-dichlorobenzoquinone 4-chloroimine) with the antioxidant BHA (3-*tert.*-butyl 4-hydroxyanisole): isolation and identification of the major product

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Gibbs reagent (2,6-dichlorobenzoquinone-4-chloroimine) is a widely-used analytical reagent for the detection of phenolic compounds[1,2]. The reagent is rapidly hydrolyzed is basic solution, yielding 2,6-dichlorobenzoquinone imine, which reacts at the *para* position of the phenol ring[3]. The product indophenols are intensely colored. Thus, Gibbs reagent may be used as a spray reagent for visualization of phenolic compounds on thin-layer chromatography plates[4].

The reactions of Gibbs reagent with *ortho*- or *meta*-substituted phenols yield the corresponding substituted indophenols. Some *para* substituents (for example, nitro, phenyl, or carboxyl groups) completely block the reaction, and no colored product forms. On the other hand, certain *para* substituents do not prevent the reaction with Gibbs reagent. These "anomalous" Gibbs reactions have been noted frequently (ref. 5 and references therein). We have shown that this phenomenon is due to the cleavage of the *para* substituent (presumably as an anion) resulting in a product identical to that formed from the unsubstituted phenol[6]. Thus, *para*-halogenophenols and *para*-alkoxyphenols react with Gibbs reagent to give 2,6-dichloroindophenol; the yield of product (and hence the intensity of the color formed) varies with the nature of the *para* substituent[6].

In this paper, we extend our investigations on the Gibbs reagent and describe the reaction with 3-*tert.*-butyl-4-hydroxyanisole (BHA). This compound is an important antioxidant and food additive, and appears to offer protection against certain carcinogens[7]. The major product of the reaction of BHA with Gibbs reagent is identified as 2′-*tert.*-butyl-2,6-dichloroindophenol, and the chromatographic and spectroscopic properties of this compound are described.

MATERIALS AND METHODS

3-*tert.*-Butyl-4-hydroxyanisole (BHA, major isomer) was obtained from Fluka, Hauppauge, NY, U.S.A., and re-crystallized from hexane–diethyl ether. 2-*tert.*-Butylphenol (Aldrich, Milwaukee) and Gibbs reagent (Fisher) were used without further purification. All solvents were reagent grade or better. Stock solutions (0.1 *M*) were prepared in acetone.

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BHA (0.1 ml, 0.1 *M*) was added to 10 ml of aqueous 0.1 *M* K_2HPO_4, and Gibbs reagent (0.1 ml, 0.1 *M*) was added with stirring. For comparison, the same reaction was performed using 2-*tert.*-butylphenol in place of BHA. In both cases, a deep purple product formed immediately. The indophenolate anion product was converted to neutral indophenol by addition of concentrated hydrochloric acid, dropwise, until the color change was complete. The product was extracted with diethyl ether, dried, and evaporated. The residue was taken up in 1 ml chloroform for chromatographic analysis.

Thin-layer chromatography (TLC) was carried out using aluminum-backed silica gel sheets with fluorescent indicator (Merck), developed in chloroform (100%). Bands were visualized directly or by fluorescence quenching. Preparative scale TLC was performed in the same solvent, using 1000-μm silica plates with pre-adsorbent zones for sample application (Whatman type PLK5F). High-performance liquid chromatography (HPLC) was performed as follows. Column: Alltech CN polar-bonded phase (5 μm), 250 × 4.6 mm, preceded by a Waters "Guard-Pak" CN pre-column. Solvent: hexane–diethyl ether (60:40). Flow-rate: 2.0 ml/min, supplied by Waters 510 pump. Detector: Waters Model 440 UV detector, 254 nm.

Optical spectra were obtained using a Hewlett-Packard 8451A diode-array spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained using the Brüker 400-MHz instrument of the Southwestern Ontario NMR Facility at the University of Guelph.

RESULTS

Gibbs reagent reacts rapidly with BHA[8,9] yielding a deep purple coloured product, λ_{max} = 586 nm in 0.1 *M* K_2HPO_4 solution. The identical λ_{max} was obtained following the reaction of 2-*tert.*-butylphenol with Gibbs reagent under the same con-

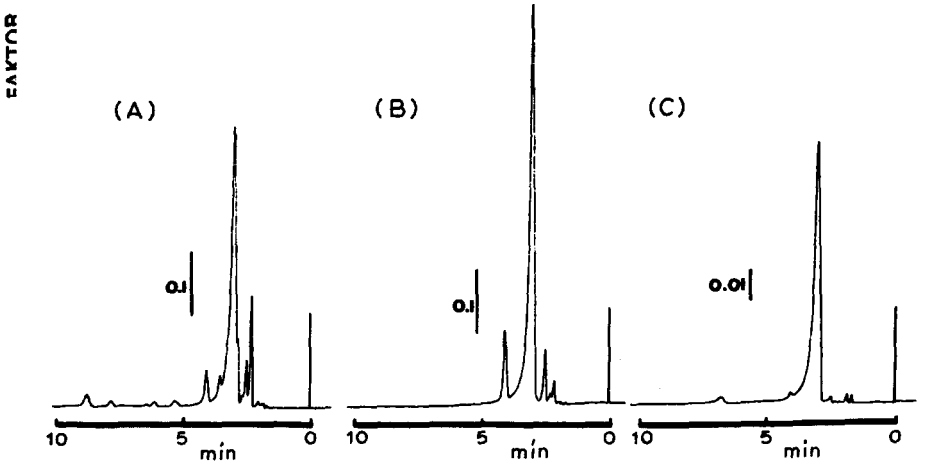
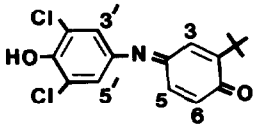


Fig. 1. HPLC separation of Gibbs reagent/phenol reaction products. Reactions were carried out as described in the text. Chromatographic conditions were: column, Alltech CN 250 × 4.5 mm; solvent, hexane–diethyl ether, 60:40; flow-rate, 2.0 ml/min. Detector: UV 254 nm. Scale bars indicate 0.1 absorbance unit (A, B), 0.01 absorbance unit (C). Injection volume was 5 μl. (A) Gibbs reagent + BHA; (B) Gibbs reagent + 2-*tert.*-butyl phenol; (C) Major product from A, purified by TLC (see text).

ditions. However, the product yield was about 50% higher with the latter compound, as measured by absorbance at λ_{max}.

The purple indophenolate anion was converted to the neutral indophenol by acidification, and extracted with ether. The solvent layer was decanted and dried, and the product was redissolved in chloroform for analysis. The indophenol solution was golden brown colored. TLC analysis of the 2-*tert.*-butylphenol Gibbs reaction product showed a single brown spot, R_F = 0.47. With BHA, the main product was identical, but additional minor products were seen (faint violet spots at R_F = 0.74 and 0.79).

HPLC analysis of the reaction of BHA with Gibbs reagent is shown in Fig. 1A. A single major product is found, along with some smaller peaks. Retention times of the starting materials were: BHA, 2.5 min; Gibbs reagent, 2.4 min. The major product had a retention time of 3.0 min. By comparison, the reagent of Gibbs reagent with 2-*tert.*-butylphenol (Fig. 1B) gave the same major product in higher yield, and with fewer additional peaks.



The identity of the major product obtained from BHA with the single product obtained from 2-*tert.*-butylphenol suggests the structure shown for the product. We used mass spectrometry and NMR to provide definitive evidence of the structure. The reaction of BHA with Gibbs reagent was repeated at 50-fold higher scale, and the product was purified by preparative TLC. The major product band was scraped off and eluted, and purity was checked by HPLC (Fig. 1C). An aliquot of the purified material was studied by optical spectroscopy: λ_{max} = 480 nm (chloroform solution) and, following addition of base, the purple indophenolate anion (λ_{max} = 588 nm) was recovered.

The electron-impact mass spectrum showed a parent ion peak at m/e = 323, intensity 3% of the base peak (135). There were ^{37}Cl isotope peaks at 325 and 327, and ^{13}C isotope peaks at 324 and 326, as expected for formula $C_{16}H_{15}Cl_2NO_2$. Other strong peaks included 308/310 (parent − CH_3), 150, and 107.

The aromatic region of the 400-MHz proton NMR spectrum of the purified product is shown in Fig. 2A. We used the two-dimensional homonuclear chemical shift-correlated (COSY) technique[10] as an aid to interpretation of the spectrum; a contour plot of the results is given in Fig. 2B. The peak assignments are tabulated in Table I.

The spectrum shows the presence of two isomers. These must reflect the possibility of E and Z configurations of the *tert.*-butyl group on the BHA-derived ring with respect to the Gibbs reagent-derived ring; the central C–N=C bond is nonlinear, and rotation about the N=C double bond is restricted. The intensities of the two isomers are quite different, allowing the assignment of peaks to each isomer without ambiguity. However, the absolute assignment of E and Z isomers cannot be made from this evidence.

The pair of doublets (6.4 and 6.5 ppm) is assigned to the 6 proton with 10 Hz

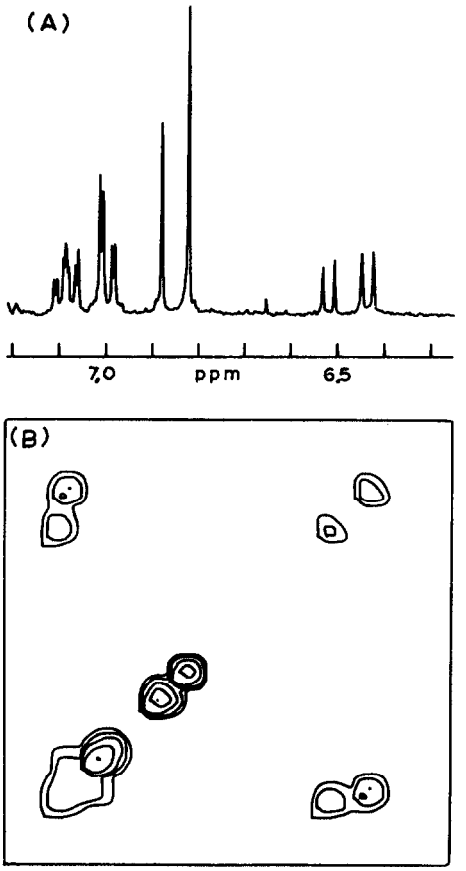


Fig. 2. (A) ^{1}H NMR spectrum (400 MHz) of product (16 scans). Solvent: carbon tetrachloride + 10% acetone-d_6. (B) Contour plot obtained from a 2D-COSY experiment. Only the aromatic region is shown; the scale is the same as in A. Digital resolution in each domain was 5 Hz per point.

TABLE I

400-MHz NMR SPECTRUM:ASSIGNMENTS

δ = ppm relative to TMS, mult. = multiplicity of peak.

Isomer 1		*Assignment*	*Isomer 2*	
δ	*mult.*		δ	*mult.*
1.323	s	*tert.*-Butyl	1.231	s
6.436	d, 10 Hz	6	6.519	d, 9.7 Hz
6.823	s	3', 5'	6.881	s
7.011	d, 2.6 Hz	3	6.985	d, 2.5 Hz
7.064	d, 2.3 Hz	5	7.109	d, 2.5 Hz
7.089	broad:	5 (isomers 1 and 2 overlap)		

splitting by the 5 proton. The chemical shift of the 6 proton is in agreement with that observed for unsubstituted 2,6-dichloroindophenol [δ = 6.44 and δ = 6.6 (ref. 11)]. The 5 proton is assigned using the COSY experiment results, in which the off-diagonal peaks indicate spin-spin coupling connectivities. The large coupling (c 10 Hz) is to the 6 proton, and the small coupling (c 2.4 Hz) is a long-range coupling to the 3 proton. The 3 proton was assigned on the basis of the 2.5 Hz coupling to the 5 proton, which is also detectable in the COSY plot. Finally, the 3′ and 5′ protons (equivalent because of free rotation about the C=N single bond) were assigned to the singlets at 6.82 and 6.88 ppm.

The ratio of the concentrations of the Z and E isomers was determined by integration of the strong singlets assigned to the *tert.*-butyl protons (see Table I). Although absolute assignments of the *E* and *Z* isomers was not possible, their ratio was 1.52:1. This corresponds to a free energy difference of 1.15 kJ/mole at room temperature.

We hope that a full understanding of the mechanisms and products of the reactions of Gibbs reagent will facilitate the interpretation of results obtained with this useful compound.

NOTE ADDED IN PROOF

We also studied the reaction of Gibbs reagent with 2,6-di-*tert.*-butylphenol. The major product was 2,6-di-*tert.*-butyl-2′-6′-dichloroindophenol, as anticipated. This compound is similar to the product obtained from BHA, but since it is symmetrically substituted, *E*/*Z* isomerism is not possible. Thus, the NMR spectrum of the di-*tert.*-butyl derivative shows only one component, but with distinct resonances for the 2 and 6 *tert.*-butyl groups (δ = 1.236 and 1.326) and 3 and 5 aromatic protons (δ = 6.919, d, J = 2.5 Hz, and δ = 6.868, d, J = 2.3 Hz). The 3′,5′ protons are a singlet, δ = 6.828. These results agree closely with the spectrum of the BHA product, and confirm the assignment of the 6 proton peak.

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