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NMR Silent, Naturally-Occurring Quinones: A Case of Radicals

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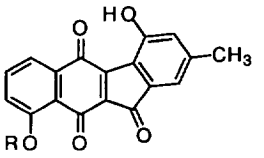
Abstract: Kinobscurinone, a benzo[*b*]fluorenone quinone previously reported to be "NMR silent," affording neither ^{1}H- nor ^{13}C NMR spectra under a variety of conditions, has now been shown to exist in part as a free radical under ambient conditions. The *g* value for kinobscurinone was found to be 2.0052; no hyperfine splitting was observed. ESR signals were similarly observed for three other substituted benzo[*b*]fluorene quinones. © 1997 Elsevier Science Ltd. All rights reserved.

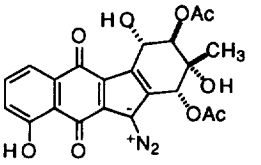
We recently reported the synthesis of kinobscurinone, **1**, and of stealthin C, **2**, and the intermediacy of each in the biosynthesis of kinamycin D, **3**, by *Streptomyces murayamaensis*.[1,2] Surprisingly, both **1** and **2** proved to be "NMR silent." They afforded neither a ^{1}H NMR nor a ^{13}C NMR spectrum, regardless of the conditions used.

In the case of **1**, normal spectra were obtained from the tetra-acetate **4** of the reduced form, **5**.[1] A similar behavior has been reported for the aminobenzo[*b*]fluorenes stealthin A (**6**) and stealthin B (**7**) which also afforded NMR spectra only after derivatization, in this case using *N*- and *O*-methylation (e.g., **8**).[3] Similar treatment of **2** also yielded an *N,O*-dimethylated derivative that afforded normal NMR spectra.[2] In contrast to these findings, all the kinamycins (e.g., **3**)[4,5] and the benzo[*b*]fluorene **9**[5] yielded NMR spectra without derivatization.

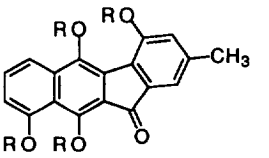
Kinobscurinone is a sparingly-soluble, intensely purple-red compound that yields a single peak by HPLC and exhibits a UV/vis spectrum with characteristic maxima at 488 and 580 nm. Seto *et al.* proposed that NMR spectra for stealthins A and B were unobservable due to severe line-broadening resulting from rapid interconversion of the many possible tautomeric forms of these compounds.[3] Changing the NMR solvent for kinobscurinone from DMSO-d_6 to either TFA-*d* or to pyridine-d_5, either of which might have disfavored rapid tautomerization, still did not provide NMR spectra. To investigate whether the lack of NMR signals was due to paramagnetic metal ions causing rapid relaxation of the nuclei excited in the NMR experiment, solutions of kinobscurinone were treated with Chelex® resin or washed with aqueous EDTA. New NMR samples were then prepared with acid-washed glassware, but still no spectra were observed.



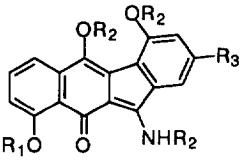
1, R=H
14, R=CH_3



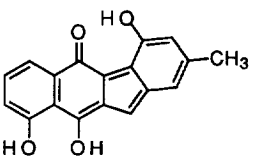
3



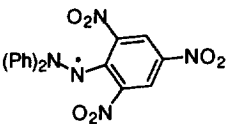
4, R=Ac
5, R=H
11, R=CH_3



2, R_1=R_2=H, R_3=CH_3
6, R_1=R_2=H, R_3=CH_2OH
7, R_1=R_2=H, R_3=CHO
8, R_1=H, R_2=CH_3, R_3=CH_2OH



9

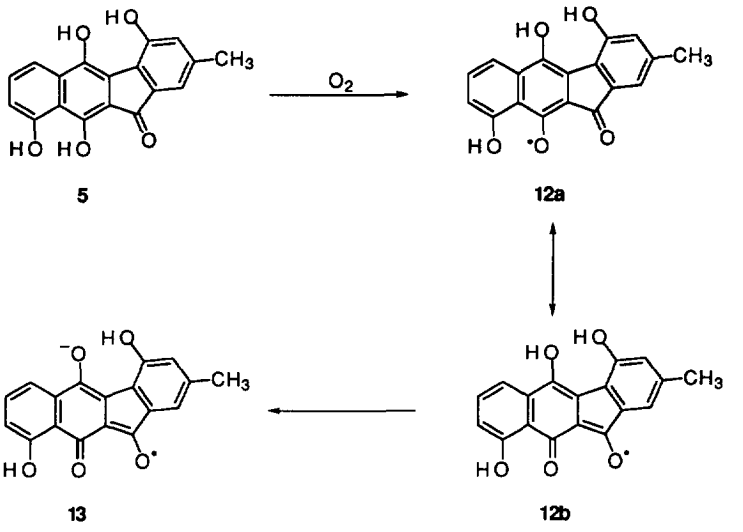


10

To test the possibility that kinobscurinone contains an unpaired electron, a sample in THF was examined by ESR and a single line was, indeed, observed. No hyperfine splitting of the ESR signal was observed, indicating that most of the spin density must be on atoms without protons. The derivative full-width was ~3.5 gauss. This was also true when a solid sample of kinobscurinone was examined. In order to quantitate the signal, solutions of kinobscurinone and of DPPH, **10**, were prepared in THF and measured by ESR.[6] Using the value for DPPH as a calibration (g = 2.0036), the g value for kinobscurinone was found to be 2.0052. The spin concentrations of kinobscurinone samples (1.1 x 10^{-5} M, 3.5 x 10^{-5} M) relative to solutions of the DPPH standard (8.7 x 10^{-8} M, 2.0 x 10^{-7} M) were calculated by double integration of the signals. Approximately 6% (± 0.8%) of the kinobscurinone molecules contained an unpaired electron. This should have been sufficient to broaden the NMR signals by relaxation to the point where they could not be observed.[7]

Efforts were made to exclude oxygen during deprotection of **11** in the preparation of kinobscurinone,[1] but an NMR silent product was still obtained. An ESR signal was also observed from a solid sample of kinobscurinone in an N_2-purged tube. As shown in Scheme 1, either the delocalized radical **12a,b**, or the radical anion **13** may represent the species present. These may be formed in parallel with oxidatioin of **5** to **1**. These are consistent with other substituted semiquinone radicals.[9,10] ESR data have been collated for a large number of monocyclic and bicyclic semiquinone radicals and, to a lesser extent for those of tricyclic semiquinones.[11] Whether in the neutral or anion form, the g values are generally in the range of 2.004-2.005. Thus, it is not possible to say whether the kinobscurinone radical is better represented by the neutral or anionic species.

Scheme 1



Since an ESR signal was observed for kinobscurinone, we similarly examined samples of kinafluorenone, **14**,[8] stealthin C,[2] and the nitroso analog of stealthin C.[2] Although these have not yet been examined quantitatively, they all exhibited ESR signals, and the g values were found to be 2.0068, 2.0067, 2.0029, respectively.

The observations reported here readily explain the previously-reported lack of NMR spectra for stealthins A and B, which are potent free radical scavengers.[3] Fredericamycin A,[12] a polycyclic quinone, was previously shown to exist in the presence of oxygen as a semiquinone radical (g = 2.004).[10] In this case, major portions of the ^{13}C- and ^{1}HNMR spectra were not observed, but complete spectra were observed after the addition of a trace of TFA. However, this had no effect for kinobscurinone. It is clear that a variety of polycyclic aromatic quinones can exist at a radical oxidation state under ambient conditions and appear to be "NMR silent." It is likely that other natural products will be found that behave similarly.

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