# Oxidative Degradation of the Piscicide Squoxin in Aerated River Water

James E. Oliver,* Claude N. Lamb,[1] and Richard H. Smith, Jr.[2]

The title piscicide squoxin [1 [1,1′-methylenebis(2-naphthol)]] rapidly decomposes in aerobic river water (but not in aerobic distilled water). Oxidation of 1 in river water, unlike its oxidation by most chemical reagents, evidently produces an unstable hydroperoxide that fragments to 1,2-naphthoquinone (5) and 1,2-naphthoquinone 1-methide (7). 1,2-Naphthoquinone (5) further decomposes to a number of products whereas 7 instantly hydrates to form the relatively stable 1-(hydroxymethyl)-2-naphthol (4). Further decomposition of 4 produces formaldehyde and probably additional 5.

1,1′-Methylenebis(2-naphthol) (squoxin, 1) was found by MacPhee and Ruelle (1969) to be a selective piscicide against the northern squawfish (*Ptychocheilus oregonensis*). Johnston (1972) described its effectiveness at concentrations in water as low as 6 ppb and also its lack of effect on desirable fish at concentrations up to 1 ppm. The ability to selectively control the northern squawfish is of interest because of the damage (both predatory and competitive) caused by this species to trout and salmon in the northwestern United States. Field tests with 1 were reported to confirm its usefulness (Keating et al., 1973; Terriere and Burnard, 1975).

The environmental acceptability of such a material depends, among other factors, on its stability and the nature of its transformation products. Keating et al. (1973) reported that 1 was readily oxidized to undetectable levels in fast-moving streams, and Terriere and Burnard (1975) found that decomposition of 1 in aerated tap water also occurred readily. The latter investigators also reported that as many as 12 oxidation products were detected by thin-layer chromatography (TLC). Decomposition products were not identified, although further studies in the same laboratory (Kiigemagi et al., 1975) suggested that 2-naphthol (2), 2-hydroxy-1-naphthoic acid, and bis(2-hydroxy-1-naphthyl) ketone were not important products.

Because the products of squoxin decomposition were not known, we began an investigation into the fate of 1 in aerobic water. The susceptibility of phenolic materials to photooxidation and to oxidation by peroxy radicals generated photochemically is well-known. Squoxin is almost certainly no exception, and because of its photolability, it is recommended that it be applied to streams after sundown (D. M. Baker, Jr., personal communication).

Decomposition of 1 in natural waters also occurs readily in the absence of sunlight, and in this paper we address only the nonphotochemical oxidation of 1 in water.

## MATERIALS AND METHODS

**Instrumentation.** High-performance liquid chromatography (HPLC) was performed on a Waters instrument equipped with a Model 440 absorbance detector (254 nm) and a 10 cm × 0.8 cm Radial-PAK A (C18) column with water and acetonitrile as primary and secondary solvents. A typical program consisted of an initial solvent composition of 80% $H_2O$ and 20% MeCN (3.5 mL/min); upon injection it was slowly (5 min) changed to 30% MeCN (elution of 4 and 5), more rapidly (1 min) increased to 60% MeCN (elution of 1), and finally (2 additional min) increased to 80% MeCN (elution of 6, if present). Mass spectra were obtained on a Finnigan 4000 mass spectrometer. Scintillation counting was performed on a Searle Mark II instrument using commercial general-purpose cocktails, and quench and background corrections were applied.

**Standards.** Unlabeled squoxin, 1, was prepared by standard methods (Ogata et al., 1969).

Commercial 1,2-naphthoquinone (5) was sublimed in vacuo prior to use.

1-(Hydroxymethyl)-2-naphthol (4) was prepared by adding excess sodium borohydride to an alcohol solution of 2-hydroxy-1-naphthaldehyde (Aldrich, recrystallized from acetic acid–water); after the yellow color was discharged (a few seconds), water was added, the solution was acidified with acetic acid, and 4 was isolated by extraction or with a C18 extraction cartridge. In one case, 4 was isolated as a white solid, but in a very short time the odor of formaldehyde was apparent and 1 was detected by HPLC. In most cases solutions of 4, eluted from the C18 extraction cartridges with ethanol, were used directly; these were reasonably stable for several days in the cold and dark. A mass spectrum was obtained from a freshly prepared sample: $m/e$ (rel intensity) 174 (24, $M^+$), 156 (69, $(M^+ - H_2O)$, 128 (100, $M^+ - H_2O - CO$).

Dehydrosquoxin (6) (spiro[naphthalene-1(2*H*),2′-(1′*H*)-naphtho[2,1-*b*]furan]-2-one) was prepared by oxidizing 1 with aqueous sodium hypochlorite (Shearing and Smiles, 1937).

**Ring $^{14}$C-Labeled Squoxin, 1a.** A solution of 2-[8-$^{14}$C]naphthol (5.15 μmol, 100 μCi, 19.4 μCi/μmol, Research Products International, Elk Grove Village, IL) in methanol was treated with 10 μL of 1 N NaOH; then the solvent was carefully evaporated with a stream of nitrogen. A solution (1 mL) of 5 mM formaldehyde in water was added, and the mixture was heated under nitrogen for 1.5 h (oil bath at 105 °C). After the mixture was cooled, the product was partitioned between ethyl acetate and water, and the ethyl acetate, after being dried and concentrated, was streaked on a 20 × 20 cm × 0.25 mm silica gel thin-layer chromatography plate. The plate was developed with benzene–hexane–methanol (9:1:1), and the band with $R_f$ 0.3–0.4 was scraped and extracted with ethyl acetate to provide 79.5 μCi (79.5%) of **1a** (specific activity 38.8 mCi/mmol).

**Methylene[$^{14}$C]squoxin, 1b.** A procedure similar to that described for **1a** was used with unlabeled 2-naphthol and [$^{14}$C]formaldehyde (100 μCi, 35 μCi/μmol; Research Products International, Elk Grove Village, IL) to give 32.9 μCi (32.9%) of **1b** (specific activity 35 μCi/μmol). The

Pesticide Degradation Laboratory, AEQI, Agricultural Research Service, U.S. Department of Agriculture, BARC-W, Beltsville, Maryland 20705.

[1]Present address: Department of Chemistry, North Carolina A and T State University, Greensboro, NC 27411.

[2]Present address: Department of Chemistry, Western Maryland College, Westminster, MD 21157.

purities of **1a** and **1b** were confirmed by TLC–autoradiography.

**Degradation of 1a and 1b in Natural Waters.** Typical experiments were conducted by adding **1a** (1–2 μg, 0.05–0.1 μCi) in a few microliters of ethanol to 5 or 10 mL of water (0.1–0.4 ppm, (0.33–1.33) × $10^{-6}$ M) and then introducing a stream of oxygen through a sintered glass bubbler (usually ca. 20 mL/min; changes in $O_2$ flow rates did not seem to meaningfully alter rates of disappearance of **1**). Aliquots (usually 100 μL) of the solution were withdrawn at time zero and appropriate intervals, mixed with mixtures of unlabeled standards (usually **1**, **4**, and **5**; if appropriate, **6**, **2**, etc.), and injected onto the HPLC column. As the standards were eluted with a suitable solvent program, the appropriate fractions were collected in scintillation vials and counted for $^{14}C$. All experiments were performed at ambient temperature.

**Identification of 1,2-Naphthoquinone, 5, as a Product of 1.** In addition to coelution of radioactivity from **1a** with authentic **5**, a sample of **5** was isolated (preparative HPLC) during a larger scale (ca. 5 ppm) reaction of unlabeled **1**. The isolated **5** was identical by HPLC, gas chromatography, and mass spectrometry to an authentic sample of 1,2-naphthoquinone.

**Identification of 1-(Hydroxymethyl)-2-naphthol, 4, as a Product of 1 (in Addition to HPLC Coelution).** Because of the difficulties in handling **4** as a pure compound, we derivatized it as the cyclic boronic ester **15** as follows. A solution of **1b** (0.087 μCi) in Sheets River water was aerated as usual for 16 h. Two drops of acetic acid were added, and the mixture was passed through a C18 Sep-PAK followed by a few milliliters of $H_2O$; 13% of the radioactvity was found in the combined eluates. The Sep-PAK was eluted with freshly distilled dioxane, which was subsequently passed through a small column of anhydrous sodium sulfate and made up of 4.00 mL (63.5% of the expected radioactivity was found in this solution). Phenylboronic acid (**ca.** 5 mg) and acetic acid (1 drop) were added, and the solution was refluxed for 2 h under nitrogen in a small apparatus that allowed for occasional removal of distillate (dioxane plus $H_2O$). After being cooled, the solution was diluted with water and passed through another C18 Sep-PAK. The Sep-PAK was rinsed with aqueous $NaHCO_3$ (8% of the expected $^{14}C$ was found in the aqueous eluate) and then eluted with tetrahydrofuran (which contained 81% of the expected $^{14}C$). Unfortunately, we were unable to achieve HPLC separation of the expected boronic ester **15** from phenylboronic acid; therefore, the product was diluted with the unlabeled phenylboronic ester of 1-(hydroxymethyl)-2-naphthol (**15**, 159 mg; Nagata et al., 1979), and the mixture, after evaporation of tetrahydrofuran, was recrystallized 4 times from acetic acid. The specific activity (expressed as cpm/mg) showed no change between the third and fourth crystallizations (354 cpm/mg; 30.1 mg remaining), establishing the formation of radiolabeled **15** and, by inference, of radiolabeled **4**.

**Identification of Formaldehyde (3) as a Product of 1.** A solution of **1b** (0.19 μCi) in Sheets River water (50 mL) was aerated 48 h, and then ca. 10 mg of dimedone (5,5-dimethyl-1,3-cyclohexane-1,3-dione) was added. After 1.5 h at room temperature, the mixture was passed through a C18 Sep-PAK; after being rinsed with $H_2O$, the Sep-PAK was eluted with ethanol (25% of the radioactivity was in the aqueous eluates and 75% of the ethanol eluate). Aliquots of the ethanol eluate were coinjected onto a C18 HPLC column with a mixture of unlabeled **4** and the standard dimedone–formaldehyde adduct, and the appropriate fractions were collected and counted. Four and a half percent of the radioactivity coeluted with **4** and 67 with the dimedone–formaldehyde adduct. Similar experiments wherein formaldehyde was trapped as its 2,4-dinitrophenylhydrazone also confirmed its production from **1** (and by inference—see Results and Discussion—from **4**).

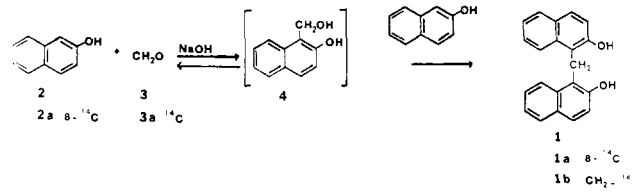


**Figure 1.** Synthesis of labeled squoxins **1a** and **1b**.

## RESULTS AND DISCUSSION

Squoxin (**1**) is prepared by simply condensing 2-naphthol (**2**) with formaldehyde (**3**) in the presence of base (Figure 1). Because each of the starting reagents was available labeled with $^{14}C$, we synthesized samples of **1** individually labeled at the 8-position of the aromatic ring (**1a**) or at the methylene position (**1b**). We then confirmed that dilute solutions of **1a** in either tap or distilled water were decomposed by bubbling oxygen or air through the medium (although the reactions were not as fast as we had anticipated) and that when assayed by TLC–autoradiography, a number of rather poorly resolved and poorly defined products seemed to have been found.

We then found that solutions of **1** in distilled water contained in rigorously cleaned glassware were stable to oxygen. Either aqueous nitric acid or solutions of ethylenediamine tetraacetic acid (EDTA) were capable of precleaning glassware so that no reaction occurred (in distilled water). Furthermore, addition of EDTA during the decomposition of **1** in river water inhibited further decomposition. Either EDTA or $HNO_3$, of course, would be expected to remove adsorbed metal ions from glass surfaces. A number of small-scale experiments were subsequently conducted that led to the following generalizations: (1) Oxygen is required for the decomposition of **1** in water. The piscicide is stable in natural or distilled water purged with nitrogen. (2) **1** is stable in aerated distilled water in the absence of metal ions. (3) Different metal ions, added individually, seem to be able to initiate *different* reactions (different rates and products). These reactions have not been studied in detail; $Cu^{2+}$ had little effect at low concentrations and gave unidentified products at high concentrations. Dehydrosquoxin (**6**) was a major product from **1** plus $Fe^{3+}$. (4) Monovalent alkali metal cations are at best marginally effective in catalyzing **1** decomposition. Ferrous ion (as $FeSO_4$) also had little effect (i.e., solutions containing **1** and $Fe^{2+}$ in distilled water were much more stable than solutions of **1** in river water). Slow decomposition of **1** occurred in aerated $10^{-3}$ M $CaCl_2$, but ca. 20 days were required to consume most of the added **1**. (5) Products from the above decomposition ($O_2$, $10^{-3}$ M $CaCl_2$) included spiro[naphthalene-1(2*H*),2′(1′*H*)-naphtho[2,1-*b*]furan]-2-one [**6**, called dehydrosquoxin (Kiigemagi et al., 1975)], 2-acetylcinnamic acid (both *E* and *Z* isomers), and 2-acetylcinnamaldehyde (presumably *E*). Subsequent results, however, have indicated that these products are of little or no environmental significance (i.e., none of these products were detected during any studies of **1** decomposition in natural waters). (6) Silica gel TLC is not a satisfactory analytical procedure for the oxidation products of **1** because some of them are susceptible to further decomposition during the chromatography.

Because of the diverse effects of additives as well as the difficulties in trying to individually assess all possible

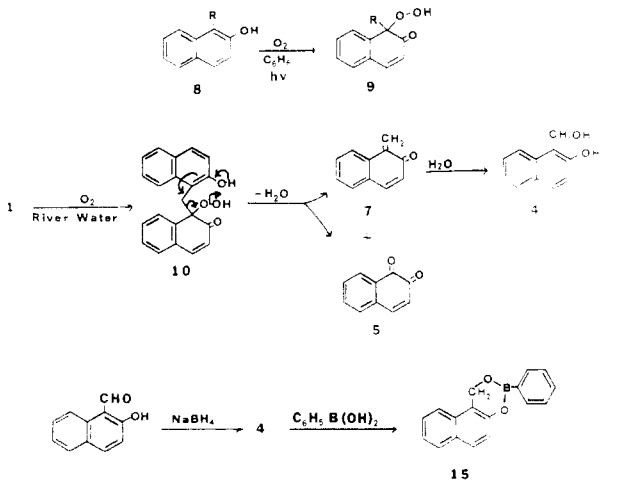

**Figure 2.** Oxidation pathway of squoxin and derivatization of 1-(hydroxymethyl)-2-naphthol.

components of natural waters, we were forced to alter our initial plans for a systematic study of 1 oxidation under a variety of conditions and elected to conduct further studies in natural water. We obtained a sample of water from the Sheets River in Oregon (a typical river in which 1 might find piscicidal use). Further discussions, unless otherwise specified, will describe studies conducted with that water.

A series of experiments was performed wherein oxygen was bubbled through solutions of 1 in the river water. Labeled 1 and/or unlabeled 1 were used at concentrations ranging from a few ppm (where the UV detector on the HPLC was adequately sensitive to follow the initial stages of the reaction) to ca. 50 ppb (where collection and scintillation counting were necessary). At the higher concentrations, the disappearance of 1 and the appearance of what seemed to be a single, faster eluting product were easily detected. The reaction was fairly rapid, with a half-life of 1.5–3 h, depending somewhat on the concentration of 1. (At concentrations of 1 above a few ppm the apparent rate of reaction decreased; both the limited solubility of 1 and an overload of the unknown catalyst may have been factors, vide infra.) Collection of the new "peak" and analysis of the collected material by gas chromatography–mass spectrometry (and subsequent gas chromatographic and HPLC comparisons) demonstrated that the isolated product was 1,2-naphthoquinone (5). Interestingly, almost none of the "expected" dehydrosquoxin (6) (Figure 6) could be detected, either by UV detection or by liquid scintillation counting of collected fractions. This latter compound, the product of oxidation of 1 by several oxidants (vide infra), was found to be relatively stable to the reaction conditions and, therefore, was not a intermediate in the formation of 5 or other products. Barely detectable levels (<2%) of 2-naphthol (2) were observed (by scintillation counting; not confirmed chemically); 2 was also relatively stable to the conditions and, therefore, was not an important product or intermediate. The $^{14}C$ balance was acceptable through the first two half-lives of 1.

Mechanistic consideration to be discussed later suggested that 1-(hydroxymethyl)-2-naphthol (4) could be another (complementary to 5) oxidation product of 1. 1-(Hydroxymethyl)-2-naphthol (4) has appeared in the literature a number of times (Chauhan et al., 1973, and references cited therein; Casiraghi et al., 1978; Nagata et al., 1979), and Chauhan et al. (1973) discussed the limited stability of 4 (it tends to extrude formaldehyde and condense to 1) and suggested that most of the samples described in the literature as 4 were in fact mixtures of 4 and 1. Our initial preparations of 4 supported this observation; decomposition was especially troublesome when attempts were made to isolate pure 4. Crystalline 4 of high quality could be prepared by sodium borohydride reduction of the commercially available 2-hydroxy-1-naphthaldehyde (Figure 2), but within a few days the sample consisted largely of 1. However, solutions of 4 resulting from the $NaBH_4$ reduction of 11 in ethanol or 2-propanol were easily prepared, and when solutions of pure 4 were needed, it was convenient to add an aliquot of the $NaBH_4$ reduction product to dilute acetic acid, isolate 4 on a C18 extraction cartridge, and elute it with ethanol or acetonitrile for HPLC analysis. These solutions of 4 were relatively stable, remaining useful for several days if stored in the cold and in the absence of light. In contrast to its reported rapid destruction during chromatography on silica gel (Chauhan et al., 1973), 4 chromatographed without incident on reversed-phase HPLC columns (tailing could be a minor problem, but decomposition was not observed). It was not, however, stable to gas chromatography, which explains why it was not observed during the GC–MS identification of 5.

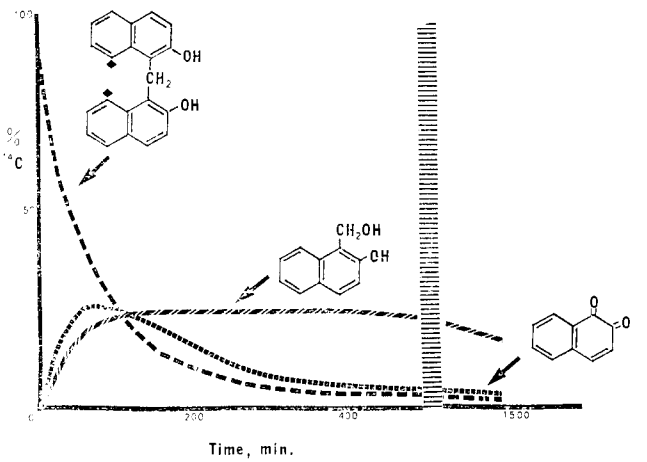


**Figure 3.** Decomposition of ring-labeled squoxin in river water.

With samples of 4 at hand, we found that the HPLC conditions we had been using did not adequately separate 4 from 5 (the problem was further complicated by the fact that the molar absorptivity of 5 at 254 nm is probably about 9 times that of 4). After developing a gradient elution program capable of resolving 1, 4, and 5 (as well as 2 and 6), we conducted some experiments wherein **1b** was oxidized in the usual way, and aliquots were taken at intervals and coinjected as described with mixtures of unlabeled standards onto the HPLC column. The appropriate fractions were collected in scintillation vials and counted. Plotting collected radioactivity vs. time provided data like those displayed in Figures 3 and 4.

These data clearly demonstrate the concomitant disappearance of 1 and appearance of 4 and 5. Interestingly, the naphthoquinone 5 rather rapidly disappeared, whereas 4, whose stability had been suspect, was in fact relatively stable to the reaction conditions and was only slowly degraded over a period of several days.

Because of its instability of GC–MS as well as to isolation, direct conformation of 4 as a product of 1 was not attempted. Its formation from 1 was confirmed, however, by trapping 4 as a phenylboronic ester (Nagata et al., 1979) as follows (Figure 2): A solution of **1b** in river water was aerated overnight, products were concentrated, and phenylboronic acid was added to convert 4 to 15, which was isolated, diluted with unlabeled 15, and recrystallized to constant specific activity.

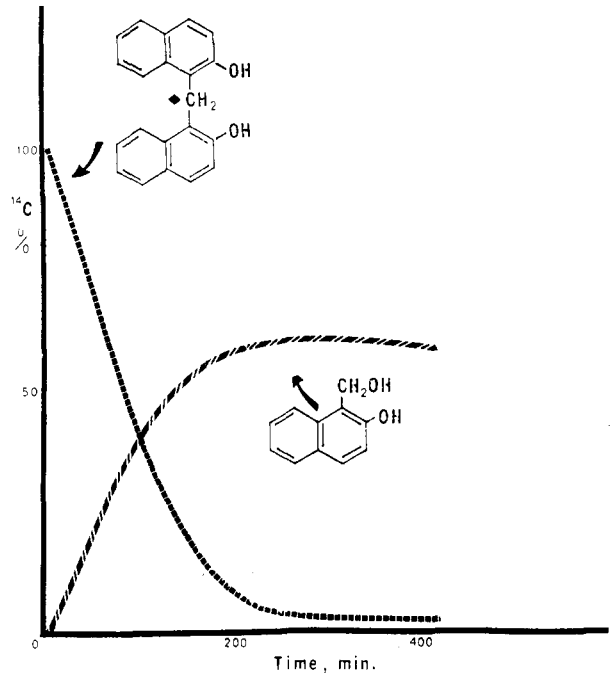


**Figure 4.** Decomposition of methylene-labeled squoxin in river water.

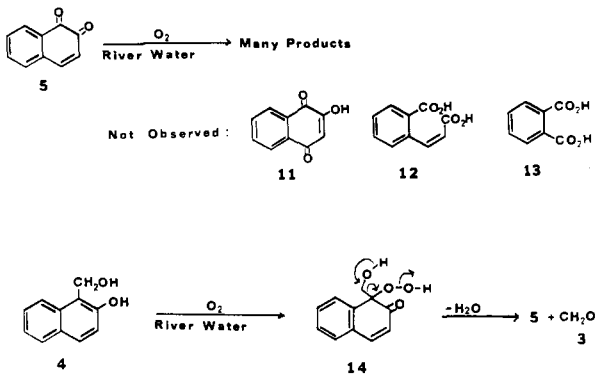


**Figure 5.** Further transformations of primary oxidation products.

Further decomposition of 4 in river water evidently led to loss of the hydroxymethyl group as formaldehyde, for addition of dimedone or 2,4-dinitrophenylhydrazine to decomposition products of **1b** allowed us to isolate the respective derivatives of [$^{14}$C]formaldehyde.

The fates of the primary products **4** and **5** have not been elucidated beyond the following observations: (A) 1,2-Naphthoquinone (**5**) fairly rapidly decomposes as illustrated in Figure 3. Several products seem to be formed, none representing a major portion of **4**. We looked for three likely oxidation products of **4**, 2-hydroxy-1,4-naphthoquinone (**11**), 2-carboxycinnamic acid (**12**), and phthalic acid (**13**) (Figure 5; Kawasaki, 1965) by the method of searching for radioactivity in the appropriate HPLC fractions; none of these compounds were detected, however. Similar experiments wherein HPLC fractions (various columns and solvent programs) were collected and counted at arbitrary intervals (e.g., 30 s) indicated that the radioactivity was "smeared" over the chromatogram with no fractions acquiring important amounts of the $^{14}$C. Of course, **5** is itself an oxidant and could decompose through combinations of reductive and hydrolytic processes (in fact, **5**, unlike 1, decomposed in river water in the absence of oxygen). Incidentally, solutions containing both **1** and **5** in organic solvents were relatively stable in the absence of light, suggesting that **5** did not by itself oxidize 1. (B) 1-(Hydroxymethyl)-2-naphthol (**4**), as mentioned, was in fact more stable to the reaction conditions than either 1 or **5**; it did further decompose, however, and from the reaction of **1b** (methylene-labeled 1) we were able to trap derivatives (dimedone or 2,4-dinitrophenylhydrazone) of [$^{14}$C]formaldehyde. Assays for $^{14}CO_2$ and $H^{14}CO_2H$ were negative.

## DISCUSSION

The reaction in question has at least two requirements—oxygen and some component of river water. The necessary ingredient of the water may be a metal ion; whatever it is, it seems to function as a catalyst as opposed to a consumable reactant. For example; it can be easily overloaded—the apparent rate of 1 disappearance decreased as the concentration of 1 increased (the limited solubility of 1 in water also became a factor at concentrations above a few ppm). On the other hand, the catalytic activity was not readily exhausted—river water in which 1 had previously decomposed was capable of supporting the decomposition of freshly added 1 at the normal rate.

Factors concluded to be *unimportant* were microbiological involvement (no induction period or other characteristics of microbiological reactions, no inhibition by Millipore filtration), particulate matter (no effect of Millipore filtration), dissolved organic matter (no observed effect of passing the river water through an XAD-2 column or through a C18 Sep-PAK, or light [almost all reactions were carefully guarded from light, but no difference in rate was observed when the solutions (in Pyrex vessels) were intentionally exposed to laboratory light].

The pattern observed during the decomposition of 1 in Sheets River water was not limited to that water. Waters from two streams in Maryland were also briefly examined for their ability to promote oxidation of 1, and in each, the same pattern (1 → 4 and **5**) was observed. In one case the reaction seemed somewhat more rapid than in the Sheets River water, and in the other case the rate was within experimental error of that in the Sheets water. Water compositions would vary with the time of year, rainfall, etc., and we can assign no significance to small rate differences; indeed, we emphasize the similarity of 1 behavior in the different waters.

The patterns of decomposition of 1 illustrated in Figures 3 and 4 are typical of those obtained from a number of experiments. The results in general were too imprecise for careful kinetic analyses (note the somewhat different shapes of the curves representing the disappearance of 1). The water employed was presumably saturated with oxygen throughout the experiments (varying the $O_2$-flow rate did not appear to change the rate of disappearance of 1), and it is tempting to assume that the decomposition of 1 should be a pseudo-first-order reaction. Plots of ln $[a/(a - x)]$ vs. time produced imperfect straight lines with no apparent pattern to the deviations from linearity, and we cannot judge whether they were due to inadequate methodology or to a more complex rate expression.

Carnduff and Leppard (1976) reported that 1-alkyl-2-naphthols **8**, wherein the 1-alkyl group was fairly bulky (e.g., isopropyl and cyclohexyl) were readily autoxidized (air plus daylight, organic solvents) to the hydroperoxides **9** (Figure 2). We found that solutions of 1 in organic solvents, when exposed to light and air, quickly developed a yellow color, and naphthoquinone **5**, formaldehyde (**3**), and 1-(hydroxymethyl)-2-naphthol (**4**) were all identified as products. Thus, the light-catalyzed reaction in organic solvents appeared to closely parallel the dark reaction in river water. Consideration of a hydroperoxide analogous to **9** that might be formed from 1 (1 → **10**) suggested that

10 was ideally constructed to undergo the fragmentation process illustrated in Figure 2, generating 5 and the quinone methide 7.

Quinone methide 7 has been generated by other means, and there is even one report of its having been oxidatively generated from 1 (Kasturi et al., 1979). It is a highly reactive compound that rapidly reacts with nucleophiles if they are available and with itself to form dimers in the absence of nucleophiles (Gardiner et al., 1959). Under our conditions, of course, it would be expected to immediately add water to produce 1-(hydroxymethyl)-2-naphthol (4). Indeed, approximately half of the radioactivity associated with the decomposition of 1a, and most of that was derived from 1b, coeluted with 4 during the early stages of the reaction in river water.

Thus, our data support 1 reacting with oxygen in river water to form 4 and 5 as the initial products, presumably via the hydroperoxide 10. Direct evidence for subsequent decomposition pathways was more difficult to obtain. As discussed, 5 rapidly decomposed, apparently to a number of products. The stability of 4 to the conditions was somewhat surprising, but its concentration did decrease with time. The formation of formaldehyde (3) from 4 (i.e., 1 → 4 → 3) would be consistent with a process very similar to that proposed for 1—i.e., hydroperoxide formation from 4 to give 14, which in turn can fragment to give the observed formaldehyde and another mole of 1,2-naphthoquinone (5) (Figure 5). Because 5 was less stable than 4, the latter process was difficult to establish. [Still another route by which both rings of 1 could be converted to 5 stems from the observation that 4, upon isolation, tends to self-condense to 1 (presumably 4 → 2 + 3 and 4 + 2 → 1—note the reversibility of the 2 + 3 reaction in Figure 1). If this reaction were to occur in river water, each mole of 1 could form 1 mol each of 5 and 4, and the latter could, in principle, self-condense to 0.5 mol of 1, which would then reinitiate the same cycle. Indeed, we observed that if unlabeled 1 was allowed to decompose in river water containing excess 2-[$^{14}$C]naphthol (2a), and the reaction was terminated before completion, recovered 1 had incorporated a small, but measurable, amount of $^{14}$C. It must be recalled, however, that the initial concentrations of 1 are in the 100-ppb range (ca. $3 \times 10^{-7}$ M), and concentrations of 4, and especially of 2, at any given time would be still lower. Thus, the likelihood of a second-order reaction occurring at a measurable rate seems negligible. This consideration, along with observed stability of 4 in the reaction medium, has prompted us to dismiss this scheme from consideration.]

A final observation, however, that would be consistent with the latter peroxide mechanism (4 → 14 → 5 + 3) involves the similarity between the dark oxidation of 1 → 10 → 4 + 5 and the photooxidation of 1 in organic solvents to give the same products. Like solutions of 1, solutions of 4 in acetonitrile or ethanol also became yellow upon exposure to air and light, and naphthoquinone 5 was found by HPLC to also be a product of 4. If 1 reacts via a common pathway in the two media, it seems reasonable that the structurally related 4 might also react by a similar pathway in the same two media. This suggests, then, that both naphthol rings of 1 may proceed through naphthoquinone 5 on their way to a number of subsequent products.

Although our data are consistent with hydroperoxide formation as the initial step in the oxidation, the mechanisitic details of this transformation remain obscure.

Particularly striking in this oxidation reaction was the absence of the spiro ketone 6, which is formed from 1 and

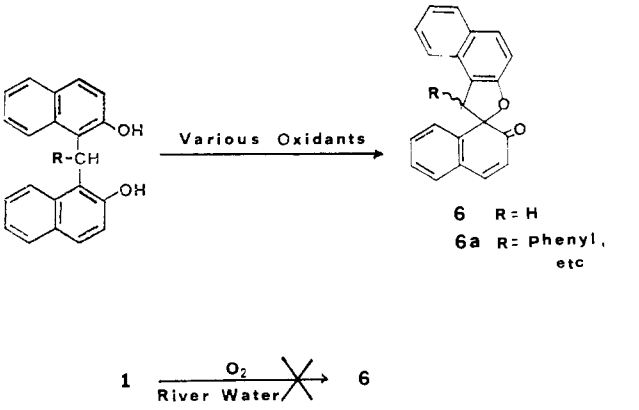


**Figure 6.** Spiro ketone formation from squoxin and derivatives.

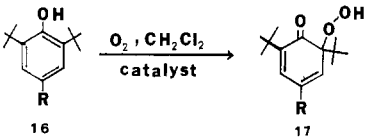


**Figure 7.** Catalytic oxygenation of hindered phenols.

a variety of oxidants—e.g., hypochlorite, halogens, ferricyanide, lead tetraacetate, and tetrachloro-*o*-benzoquinone (Shearing and Smiles, 1937; Abel, 1893; Pummerer and Cherbuliez, 1914; Toyama et al., 1977; Svestanj, 1951; Kasturi et al., 1979). Bennett et al. (1980) studied oxidations of derivatives of 1 substituted at the methylene position with aryl substituents; *no less than 24 oxidants plus a peroxidase enzyme were shown to convert these compounds to spirans 6a* (Figure 6). A wide variety of types of oxidants and reaction conditions were used, including some believed to generate aryloxy radicals and/or radical anions, and although yields varied, the work clearly demonstrated the generality and ease of spiran formation under oxidative conditions. We judge, therefore, that whatever mechanism is operative in the 1 → 4 + 5 reaction, it must be of a different nature than that produced by most common oxidants.

Environmental oxidations in water are common occurrences, but it is generally assumed that sunlight is initially required to generate free radicals that may react directly or indirectly with oxygen to ultimately lead to oxygen incorporation or otherwise oxidized products (Mill, 1980; Mill et al., 1980). In the absence of light, what provides the driving force for the reaction of 1 with oxygen?

Nishinaga et al. (1981, 1982) described their use of cobalt(II)–Schiff base complexes as catalysts in the selective oxygenation of 2,6-di-*tert*-butylphenols with molecular oxygen; the products were 2- or 4-hydroperoxycyclohexadienones (16 and 17, Figure 7). They liken their reactions to those of iron- and copper-containing enzymes and suggest that complexing oxygen with the already complexed metal may serve to activate the oxygen and thus facilitate its reaction with the phenol. At least two papers have described chelation of 1 with alkali metals (Evans and Smiles, 1937; Jensen, 1964), and although we are unaware of studies involving complexes of 1 with other metals, it is conceivable that the metal ion requirement for 1 oxidation might relate this process to the one described by Nishinaga (1981, 1982). The reaction conditions were admittedly quite different in the two cases, but the latter work (and studies cited therein) establish the possibility of hydroperoxide formation from phenols as a result of oxygen activation via organometallic complexes.

**Comments on and Implications of Analytical Methodology.** Kiigemagi et al. (1975) described two

analytical procedures for squoxin. The first consisted of methylation of 1 with diazomethane and gas chromatographic determination of the dimethyl ether of 1. In our limited experience with reactions of 1 with diazomethane, we noted a tendency for monomethylation and recommend that future investigators be aware of and check for that possibility. The second method was a colorimetric determination of 1 via an azo dye produced by coupling 1 with tetrazotized *o*-dianisidine. This would at first glance seem to be an unusual choice of reaction for 1 because 1-substituted 2-naphthols as a rule do not couple with diazonium ions. It was noted long ago, however [see, e.g., Venkataraman (1952)], that 1, as well as derivatives of 2-naphthol substituted in the 1-position with $-CO_2H$, –CHO, halogen, etc., do couple with diazonium ions at the 1-position with concomitant loss of the 1-substituent. [This phenomenon seems best explained by a fragmentation reaction resembling that of 10 (Figure 2)]. We verified that both 1 and 2-naphthol, 2, produced the same azo dye upon reaction with *m*-nitrobenzenediazonium chloride. More signficant, perhaps, was our further observation that 1-(hydroxymethyl)-2-naphthol (4) also produced the same azo dye. Thus, the colorimetric method would not distinguish between 1 and its relatively stable oxidation product 4.

Kiigemagi et al. (1975) mentioned an excellent correlation between fish mortality and 1 concentration in water (the latter determined by the colorimetric method). This observation would seem to recommend further experiments to answer such questions as whether the toxicity of 4 to fish might be similar to that of 1 or even whether 4, generated upon addition of 1 to natural waters, might be the principal biologically active agent.

ACKNOWLEDGMENT

We are grateful to Einor Wold of the National Marine Fisheries Service, U.S. Department of Commerce, for the shipment of water from the Sheets River in Oregon. We thank Dr. Drew M. Baker, Jr., for his assistance and interest, Prof. Leon Terriere for sharing experience and materials, and Dr. Ted Mill for helpful discussions. We appreciate the help of William Lusby and Marc Tischler in obtaining mass spectra and Paul Warfield and Gary Reitz for conducting experiments on the photodecomposition of 1.

**Registry No.** 1, 1096-84-0; **1a**, 87279-89-8; **1b**, 23355-82-0; **2**, 135-19-3; **2a**, 18698-20-9; **3**, 50-00-0; **3a**, 3046-49-9; **4**, 5386-25-4; **5**, 524-42-5; **6**, 65857-96-7; **7**, 5690-44-8; **10**, 87279-90-1; **11**, 83-72-7; **15**, 59648-25-8; $H_2O$, 7732-18-5; phenylboronic acid, 98-80-6; 2-acetylcinnamic acid, 4361-81-3; (*E*)-2-acetylcinnamaldehyde, 87279-91-2; 2-hydroxy-1-naphthaldehyde, 708-06-5.

LITERATURE CITED

Abel, J. *Chem Ber.* **1983**, *25*, 3477.
Bennett, D. J.; Dean, F. M.; Herbin, G. A.; Matkin, A.; Price, A. W.; Robinson, M. L. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1978.
Carnduff, J.; Leppard, D. G. *J. Chem. Soc.; Perkin Trans. 1* **1976**, 2570.
Casiraghi, G.; Casnati, G.; Cornia, M.; Pochini, A.; Sartori, G.; Ungaro, R. *J. Chem. Soc., Perkin Trans. 1* **1978**, 322.
Chauhan, M. S.; Dean, F. M.; Matkin, D.; Robinson, M. L. *J. Chem. Soc., Perkin Trans. 1* **1973**, 120.
Evans, W. J.; Smiles, S. *J. Chem. Soc.* **1937**, 727.
Gardiner, P. D.; Rafsanjani, H. S.; Rand, L. *J. Am. Chem. Soc.* **1959**, *81*, 3364.
Jensen, B. S. *Acta Chem. Scand.* **1964**, *18*, 739.
Johnston, J. M. *Prog. Fish-Cult.* **1972**, *34*, 122.
Kasturi, T. R.; Rajashekhar, B.; Shivaramakrishnan, R. *Indian J. Chem., Sect. B* **1979**, *18B*, 1.
Kawasaki, H. *Kogyo Kagaku Zasshi* **1965**, *68*, 675; *Chem. Abstr.* **1965**, *63*, 5569f.
Keating, J. F.; Kiigemagi, U.; Terriere, L. C.; Swan, R. L., Proceedings of the 52nd Annual Conference of the Western Association of State Game and Fish Commissioners, 1973, p 609; quoted in Terriere and Burnard (1975).
Kiigemagi, U.; Burnard, R. J.; Terriere, L. C. *J. Agric. Food Chem.* **1975**, *23*, 717.
MacPhee, C.; Ruelle, R. *Trans. Am. Fish. Soc.* **1969**, *98*, 676.
Mill, T. In "The Handbook of Enviromental Chemistry"; Hutzinger, O., Ed.; Springer-Verlag: Berlin, 1980; Vol. 2, Part A, p 77.
Mill, T.; Hendry, D. G.; Richardson, H. *Science (Washington, D.C.)* **1980**, *207*, 886.
Nagata, W.; Okada, K.; Aoki, T. *Synthesis* **1979**, 365.
Nishinaga, A.; Shimizu, T.; Toyoda, Y.; Matsuura, T.; Hirotsu, K. *J. Org. Chem.* **1982**, *47*, 2278.
Nishinaga, A.; Tomita, H.; Nashizawa, K.; Matsuura, T.; Ooi, S.; Hirotsu, K. *J. Chem. Soc., Dalton Trans.* **1981**, 1504.
Ogata, Y.; Kawasaki, A.; Goto, J. *Tetrahedron* **1969**, *25*, 2589.
Pummerer, R.; Cherbuliez, E. *Chem. Ber.* **1914**, *47*, 2957.
Shearing, E. A.; Smiles, S. *J. Chem. Soc.* **1937**, *1931*.
Svestanj, K. *Arh. Kem.* **1951**, *23*, 80; *Chem. Abstr.* **1952**, *46*, 11164*b*.
Terriere, L. C.; Burnard, P. J. *J. Agric. Food Chem.* **1975**, *23*, 714.
Toyama, M.; Motohashi, S.; Satomi, M.; Hugichi, K. *Nihon Daigaku Yakugaku Kenkyu Hokoku* **1977**, *17*, 5; *Chem Abstr.* **1978**, *89*, 108784k.
Venkataraman, K. "The Chemistry of Synthetic Dyes"; Academic Press: New York, 1952; Vol. 1, p 422.

Received for review April 7, 1983. Accepted August 2, 1983. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned. Part of this work was supported by the National Marine Fisheries Service, U.S. Department of Commerce.