EVIDENCE FOR THIOETHER FORMATION BETWEEN 2-SOLANESYL-1,4-NAPHTHOQUINONE AND 2-MERCAPTOETHANOL*

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A variety of 2-β-alkenyl-1,4-naphthoquinones were synthesized by Fieser et al. (1940). The first evidence that such monosubstituted naphthoquinones are naturally occurring compounds was provided when a 2-β-alkenyl-1,4-naphthoquinone was isolated from the cytochrome-free microorganism Streptococcus faecalis (Baum and Dolin, 1963). This compound has now been identified as 2-solanesyl-1,4-naphthoquinone, a 1,4-naphthoquinone containing a 45 carbon polyisoprenoid side chain (Baum and Dolin, in preparation). Monosubstituted 1,4-naphthoquinones are not limited in distribution to the cytochrome-free bacteria, as shown by the recent isolation of such quinones from Hemophilus parainfluenzae (Lester et al., 1964) and Escherichia coli (Baum and Dolin, in preparation). The major isoprenolog present in H. parainfluenzae contains a 30 carbon polyisoprenoid chain. A new naphthoquinone considered to be a monosubstituted vitamin K_1 has been isolated from spinach chloroplasts (McKenna et al., 1964).

The fact that E. coli contains a 2-β-alkenyl-1,4-naphthoquinone in addition to the usual complement of vitamin $K_2C(40)$ and coenzyme Q (Baum and Dolin, in preparation) implies that the monosubstituted quinones have a special function that

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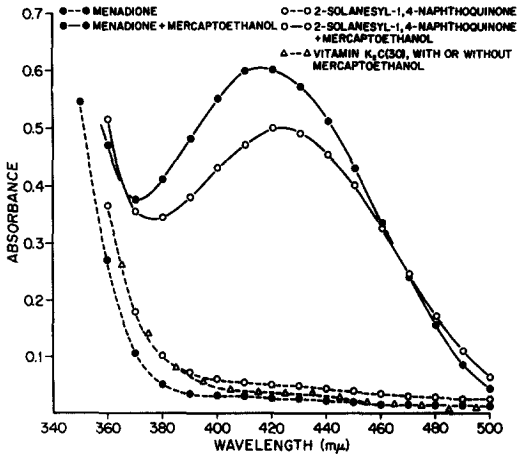
cannot be carried out by quinones in which all of the quinonoid hydrogen atoms have been substituted. The chemical reactivity of the unsubstituted position in monosubstituted 1,4-naphthoquinones is well known from work on model compounds. Fieser and Turner (1947) showed that a variety of mercaptans would add to 2-methyl-1,4-naphthoquinone (menadione) to form thioethers. Glutathione reacts with menadione by nucleophilic substitution to form a thiol addition compound that has an absorption band at 420 mμ (Nickerson et al., 1963). It was obviously of interest to see whether the lipophilic 2-β-alkenyl-1,4-naphthoquinones formed similar derivatives. Evidence for such a reaction is shown in Fig. 1.



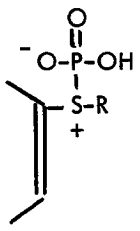
Synthesis of thiol addition compounds of monosubstituted 1,4-naphthoquinones.

The naphthoquinones were dissolved in ethanol to give a final concentration of 0.6 μmole/ml. 2-mercaptoethanol, where used, was added to a final concentration of 25 μmole/ml. The reactions were run in 10 X 75 mm test tubes. Corks covered with Parafilm were used to seal the tubes, and the tops of the tubes were further sealed with small squares of Parafilm. Incubation was carried out at room temperature in the dark. The reaction proceeds to its maximum extent in 48-72 hours. After 96 hours, the reaction mixtures were diluted 1:1 with ethanol and the spectra taken in micro cuvets (1 cm light path). 2-mercaptoethanol alone, at the concentration used above, has no absorption in the region shown.

Menadione and 2-solanesyl-1,4-naphthoquinone give similar yellow chromophores after reaction with 2-mercaptoethanol, except that the peak of the menadione derivative is at 417 mμ and that of the 2-solanesyl-1,4-naphthoquinone derivative is at 423 mμ. Vitamin $K_2C(30)$ does not react since both quinonoid positions are substituted

in this compound. The 2-mercaptoethanol derivative of 2-solanesyl-1,4-naphthoquinone was chromatographed on Whatman No. 1 paper, impregnated with Dow Corning silicone No. 550 (Lester and Ramasarma, 1959). The solvent system was n-propanol:water, 4:1. As expected, the thiol derivative is much more polar ($R_F = 0.67$) than the original naphthoquinone ($R_F = 0.27$). The thiol derivative can be detected by its yellow color, or by the formazan reaction of Lester and Ramasarma (1959). The latter reaction shows that the derivative is capable of being reduced and reoxidized. After chromatography on paper impregnated with Dow Corning silicone No. 200, as used by Lester et al. (1964) the yellow derivative can be eluted and shown to have the characteristic 423 mμ band.

In view of these results, it is interesting to note that biological activity has been demonstrated for thiol addition compounds of menadione. Stadtman (1959) has presented evidence that a phosphatase isolated from Clostridium sticklandii requires a menadione thioether as cofactor. At the time the phosphatase reaction was described, it was not known that monosubstituted naphthoquinones are naturally occurring compounds in bacteria. In the light of recent findings, this reaction takes on renewed interest. The function of 2-β-alkenyl-1,4-naphthoquinones is as yet unknown, however the finding that the lipophilic members of this class of quinones can form thiol addition compounds is suggestive. It is possible that the reaction described by Stadtman (1959) is a model for the function of thiol addition compounds of 2-β-alkenyl-1,4-naphthoquinones in a phosphate transfer process. The sulfur atom of the side chain might act as a phosphoryl acceptor through the formation of the sulfonium ion shown below.



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Oxidation of naphthoquinol monophosphate (Clark and Todd, 1960) is thought to yield monomeric metaphosphate as a transient intermediate. The oxidation of a naphthoquinol monophosphate containing a thioether side chain might take place with intramolecular transfer of metaphosphate, to form the sulfonium ion, I.

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