ment of unsaturation and substituent orientation but, with the exception of manoyl oxide acid (8,13-β-epoxy-14-labden-19-oic acid)[10], having the same carbocyclic skeleton.

From Table II it can be seen that growth inhibition occurs with all test compounds, and that except for the rather higher toxicity of isopimaric acid there are relatively slight differences from one to the other. This seems reasonable since examination of molecular models reveals essentially the same overall shape for these structures. Interestingly, this configuration differs appreciably from that of the sunflower acids which have the opposite stereochemical orientation at the A–B ring junction and also posses a more rigid system due to additional ring formation.

Table III. Larval growth of *Pectinophora gossypiella* on artificial diets containing isopimaric acid derivatives

Compound	Growth at dietary level[a] 0.05%	0.1%
Isopimaric acid	18	5
Dihydroisopimaric acid	23	10
Methyl isopimarate	71	68
Isopimarol	98	55

[a] Percent of control wt. after 14 days, 10 larvae per level.

Table IV. Larval growth of *Pectinophora gossypiella* on diets containing both levopimaric acid and cholesterol

Additive	Larval weight[a]
None	100
1% Cholesterol	125
0.2% Levopimaric acid	23
Same + 0.05% cholesterol	45
Same + 0.1% cholesterol	61
Same + 0.2% cholesterol	41
Same + 0.4% cholesterol	64
Same + 1.0% cholesterol	86

[a] Percent of control, 10 larvae per level.

Whether or not the carboxyl functionality of the resin acids is necessary for toxicity may be indicated by comparison of the respective ester or carbinol with the parent compound. Table III shows these results for the isopimaric series and includes data on the reduced side-chain form also. The latter compound, dihydroisopimaric acid, in which the vinyl group at C-13 is modified by hydrogenation, shows essentially unaltered activity which is consistent with the observations presented in Table II. Formation of the methyl ester on the other hand does reduce toxicity and loss of activity is also shown by the corresponding alcohol, isopimarol. Apparently the carboxyl group, while not absolutely essential for growth inhibition, does increase activity. Reduced effectiveness of the methyl ester might be partly counteracted by partial hydrolysis to the free acid during digestive processes. It is likely that the polar acid function facilitates transport and absorption of the compound.

Since the skeletal arrangement of the tricyclic resin acids resembles the ABC-ring system of common steroids, it seemed possible that these compounds interfere with steroid metabolism in the insect. Dietary phytosterols must be absorbed, transported and biochemically altered to produce essential hormones. Interference with any step by inhibitor action of the resin acid would certainly decrease vitality of the larva. We sought to examine this point by fortifying the test diet with increasing concentrations of cholesterol to determine whether growth inhibition could be reversed by mass action effect. The activity of levopimaric acid in the presence of added cholesterol is, in fact, reduced (Table IV). Normal growth was not completely restored, however, even at the 1% level. Larval growth in the absence of toxicant is accelerated by cholesterol resulting in average weights substantially greater than those of controls. The effect of the sterol is proportionally greater on those larvae consuming resin acid in the diet. Larvae fed the resin acid diet with 1% cholesterol showed a 270% weight increase while larvae fed the control diet augmented by 1% cholesterol showed only a 25% weight increase. It may therefore be concluded that there is antagonism between resin acid and steroid, and that the effect involves the hormonal system of the insect.

[10] Isolated from *Pinus resinosa* needles[11].

[11] D. F. ZINKEL and W. B. CLARKE, unpublished.

Formation of Monoanion Radicals in Reactions of Vitamin K_3 with Sodium Sulphite

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Summary. It is shown that the reaction between vitamin K_3 and sodium sulphite under physiological conditions leads to the formation of free radical intermediates.

Much attention has been paid to sulphite and its organic addition compounds as they accumulate in foodstuffs[1–3]. Although there are many observations on biochemical alterations induced by sulphite, its role in biological systems is still subject to controversial interpretation[4]. Vitamin K_3 (menadione; 2-methyl-1,4-naphthoquinone) (**1**) has recently been shown to inhibit the microsomal lipid peroxidation and the iron-catalyzed lipid peroxidation of the liver cells[5,6]. It has also been shown that **1** reacts with sulphite to form a water-soluble bisulphite addition compound **2**, whose medicinal effectiveness presumably results from regeneration of the vitamin in basic intestinal fluid via elimination of sulphite[7]. So far no information is available describing these mechanisms. We have found that the attack of sulphite on **1** under physiological conditions (pH 7–8) produces free radicals which

were identified by in vitro ESR measurements. Some interesting results of the bisulphite elimination of the sulphonate **2** by OH^- are also presented here.

Materials and methods. Treatment of menadione (Merck, Darmstadt, BRD) with Na_2SO_3 was carried out as follows: A few crystals of the substance to be investigated were placed in the neck of an ESR cuvette and a small portion ($\sim$0.7 ml) of the chosen solution (Na_2SO_3 equivalent to 400 ppm SO_2) – previously freeze thawed to 10^{-5} torr – was allowed to trickle slowly onto them. A

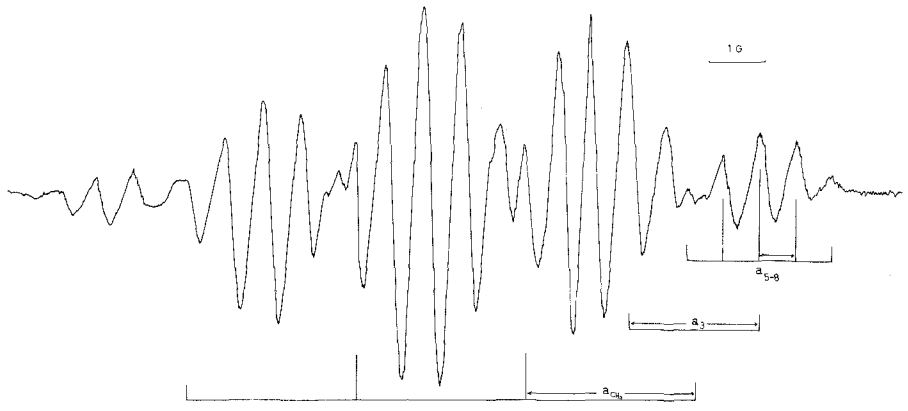


Fig. 1. ESR-spectrum obtained from reaction mixture $1/Na_2SO_3$(pH 7–8).

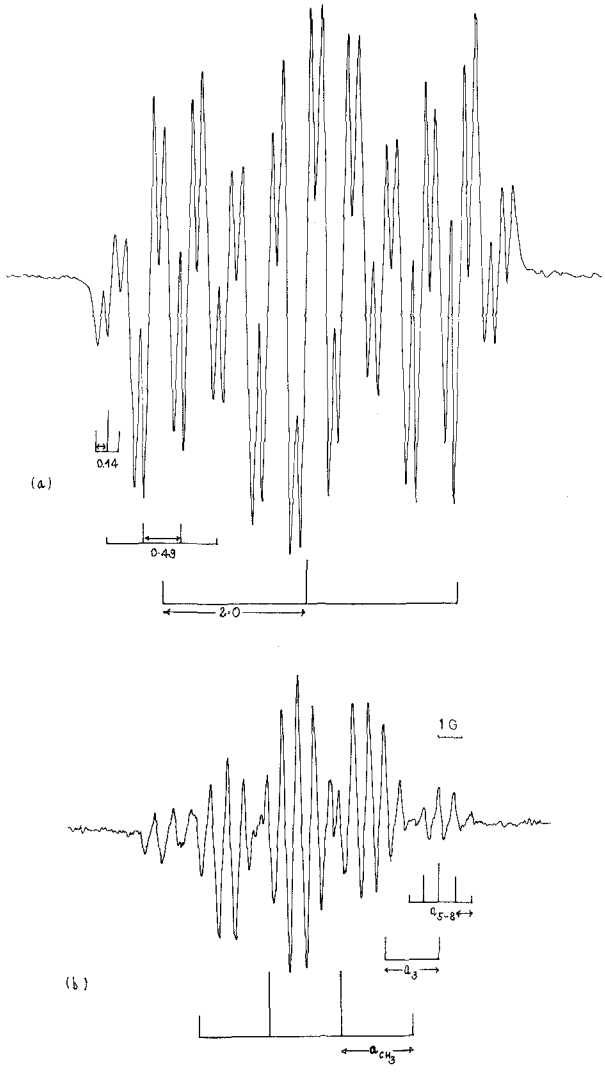
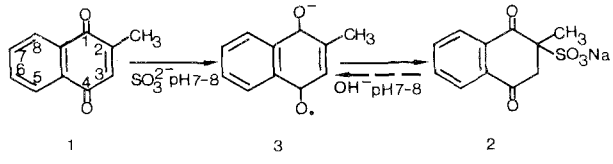


Fig. 2. ESR-spectrum obtained from reaction mixture $2/OH^-$ (pH 7–8).

maximum ESR signal intensity was observed approximately concurrent with complete dissolution. For purposes of a quantitative evaluation of the role of free radicals in the reactions, weighed amounts of substance and solvent were employed, the procedure remaining as described above. For radical concentration measurements, the double integrated signal observed at maximum intensity was compared with that of a freshly calibrated standard solution of Fremy's salt recorded under identical spectrometer conditions. Each experiment was repeated 3 times at each concentration. The ESR spectrometer is described elsewhere[8]. The microwave fre-



quency was determined using a Hewlett-Packard 5246 L frequency counter and the magnetic field with an AEG nuclear magnetic resonance fieldmeter. Splitting constants were determined by comparison with an aqueous solution of Fremy's salt (a_N = 13.01 G).

Results and discussion. Assignment of the radical intermediate responsible for the spectrum in Figure 1 was not trivial, and hence will be elaborated in some detail. Superficially the spectrum could be analyzed on the basis of quintets arizing from 4H–4H proton interactions. However, departure from the expected binominal intensity relationships and the difficulty of assigning a realis-

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tic set of parameters for these interactions render this analysis suspect. We expect from the method of preparation and the overall chemistry (i.e. the known product being sulphited in the 2 position)[7] the anion **3**. For this, except for the protons of the methyl group, none are symmetrically equivalent and 6 different hyperfine splittings might be present ($a_{CH_3} \neq a_3 \neq a_5 \neq a_6 \neq a_7 \neq a_8$). Presupposing **1** anion structure and interpreting the spectrum on the basis of an accidental equivalence of protons 5, 6, 7, 8, provides the excellent fit shown in the stick diagram (Figure 1). The hyperfine splittings are in good agreement, though somewhat lower than those in the literature (Table I). There appears to be a solvent effect on the splitting constants as yet quantitatively unpredictable.

Table I. Proton hyperfine splittings in the ESR-spectra of vitamin K_3 intermediates

Method of preparation/solvent	HFs (gauss) at position numbers						Reference
	2	3	5	6	7	8	
red. with Na_2SO_3/H_2O	3.02	2.33	0.66	0.66	0.66	0.66	this work
K_3-sulphonate + OH^-/H_2O							
K mirror/EtOH	2.911	2.467	0.48	0.78	0.56	0.70	[9]
K mirror/EtOH/H_2O	3.01	2.38	0.64	0.64	0.64	0.64	[10]
electrochem. red. OH^-/H_2O	2.94	2.40	0.59	0.59	0.59	0.59	[11]

Table II. Radical anion yields in the system K_3–Na_2SO_3 ($Na_2SO_3 = 10^{-1}$ *M*)

Menadione conc. ($M/l \times 10^{-3}$)	Mean radical 3 on conc. ($M/l \times 10^{-3}$)	Conversion to anion (%)
2.70	2.38	88
1.40	1.30	93
0.70	0.65	92

We conclude, beyond all reasonable doubt, that it is the anion **3** with which we are concerned in these experiments. The role of the anion in the overall chemistry follows from the data summarized in Table II and shows that the reaction

$$K_3 + SO_3^{--} \rightarrow K_3^- + SO_3^- \qquad (1)$$

is essentially quantitative.

These results, taken with the observation for near quantitative sulphitation of **1** by Na_2SO_3, strongly suggest that **3** is an intermediate en route to the sulphonate **2**. Similarly the rapid radical build-up and its exceptional stability suggests that the rate-determining step is the subsequent sulphitation of the radical anion by SO_3^{--}.

Figure 2 shows the ESR-spectra observed on treating the sulphonate with NaOH solution (pH 7–8). Unfortunately we have been unable to interpret the spectrum 2a, except to point out that it is of a completely different nature to that shown in Figure 1 (spectral width of only 5–6 G) and undisturbed by the latter. It can be analyzed on the basis of 2H (aromatic ring protons) – 3H (CH_3) – 2H (aromatic ring protons) interactions leading to overlapping patterns of 2.0, 0.49, 0.14 G hyperfine splittings. We can tentatively ascribe this to a semiquinone derived directly from the sulphonate without fission of the SO_3Na group, where the accidental equivalences present in the **1** anion are removed by this group. It can, however, be said with certainty that a longer reaction-time leads to formation of the **1** anion as observed in Figure 1, possibly in parallel or consecutive reactions but more likely caused by the slow removal of the SO_3Na group by OH^-.

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Structure and Stereochemistry of Coccuvine (*Cocculus laurifolius* DC)

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Summary. Coccuvine $C_{17}H_{19}NO_2$, m.p. 137–38°, a new alkaloid from *Cocculus laurifolius* DC has been assigned the structure and stereochemistry as (I) on the basis of spectroscopic studies and chemical correlation.

Confirmation of hypotensive activity[1] in *Cocculus laurifolius* DC (Menispermaceae) prompted its reinvestigation, which resulted in the isolation of new dibenz(d,f)-azonine bases[2] and the abnormal *Erythrina* alkaloids[3]. Formerly, from this plant, 1-benzyltetrahydroisoquinoline[4–6], aporphine[7,8], *bis*benzylisoquinoline[9,10] and *Erythrina* alkaloids[11–13] had also been isolated. Continued search for the active principle(s) from the alkaloidal fraction from the leaves of the plant has now yielded a new base named Coccuvine. The present communication reports essential data which have led to the assignment of structure **1** with stereochemistry as shown for the coccuvine.

The phenolic fraction of the alkaloidal mixture from the leaves of *C. laurifolius* DC was carefully chromatographed on neutral Al_2O_3 column. Elution with chloroform/methanol (98:2) yielded coccuvine m.p. 137–38°. The molecular formula $C_{17}H_{19}NO_2$ (M^+ 269) for the base emerged from its elemental analysis[14] and was confirmed by mass spectrometry. Its IR-spectrum had absorption band at 3450 cm^{-1} for a hydroxyl function and the UV-spectrum (λ_{max} 228 and 282 nm) was very similar to that of aromatic *Erythrina* alkaloids[15], having a 1,6-diene system. The NMR-spectrum of the base was almost identical with erysotrine[15] and erythraline[15], the only apparent difference being in the number of signals for methoxyl and methylenedioxy groups. In coccuvine (**1**) there was no signal for an aryl methoxyl function, a 3 proton signal for –CH–O–*Me* resonated at τ 6.74. Of the 3 aromatic protons, 2 *meta* oriented protons were centred at τ 3.24 (J = 2.0 Hz) and an *ortho* oriented proton clear of others at τ 2.92 (1H, d, J = 9.5 Hz); 3 olefinic protons, with one at low field τ 3.45 (1H, d, d, J = 11.0 Hz and J_2 = 2 Hz), the other at τ 4.02 (1H, d, d, J_1 = 10 Hz and