ETHYLENE FORMATION FROM METHIONAL

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SUMMARY: The biosynthetic precursor of ethylene is 3-methylthiopropanal (methional). It has been claimed that hydroxyl (HO·) radicals are involved in this biosynthetic sequence, and that the production of ethylene from methional can be used as a specific probe for the presence of the HO· radical. We have now shown, however, that a variety of organic radicals lead to the production of ethylene from methional. Clearly this reaction cannot be used to test for the presence of HO· radicals, and the mechanism for the conversion of methional to ethylene will have to be reexamined.

In free radical biology, it is important, but often difficult, to identify the radicals causing the changes that are observed. In enzyme systems, the radicals involved are often the superoxide radical and derived species such as hydrogen peroxide and the hydroxyl radical (1,2), although the mechanism of formation of the HO· radical is controversial (3). In systems in which lipid or drug peroxidation is observed, radicals involved in autoxidation (4) are implicated, including the alkoxy (RO·) and peroxy (ROO·) radicals (5–8). Thus, a general problem in free radical biology is identifying and distinguishing the various types of oxy radicals, including $O_2^{-}\cdot$, HO·, HOO·, RO·, and ROO· radicals.

The biosynthesis of ethylene involves methional as the immediate precursor of ethylene (9,10), and the mechanism of this conversion has been proposed to involve the HO· radical (11). Furthermore, the production of ethylene from this reaction, Eq 1, has been suggested as a specific test for the presence of the HO· radical (12–14). We have tested this hypothesis by studying the

$$CH_3SCH_2CH_2CHO + HO\cdot \longrightarrow CH_2=CH_2 + HCOOH + \tfrac{1}{2}CH_3SSCH_3 \qquad (1)$$
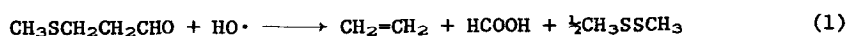
production of ethylene from methional in a variety of systems including those that do and do not involve HO· radicals. Our evidence makes it clear that

Eq 1 cannot be used as a test for the presence of HO· radicals. Furthermore,

the mechanism of the production of ethylene from methional must be reevaluated.

MATERIALS AND METHODS

Methional was obtained from Sigma and was redistilled. *tert*-Butyl perox-
ide and acetyl peroxide were purchased from Lucidol. Decane and *tert*-butyl
hydroperoxide were obtained from Aldrich. Azobisisobutyronitrile (AIBN) from
Eastman and benzoyl peroxide from MC/B were used without further purification.
Ethylene was obtained from Matheson. All the liquid reagents were redistilled
before used. Hydrogen peroxide (30%) was obtained from Fisher Scientific Co.

A solution containing the desired concentration of each of the reagents
was prepared. In a typical run, approximately 1.5 ml of the reaction mixture
in a 20 cm glass tube closed with a serum cap was incubated in an oil bath at
the desired temperature and for the desired time. At the end of each reaction,
the tube was allowed to cool to room temperature and a measured amount of gas
sample withdrawn with a gas syringe. The total volume of the gas phase and
the liquid phase in each ampoule was measured. The ethylene produced in the
reaction was identified with the use of a 6 ft porapak Q-S column and a flame
ionization detector in a Varian 1400 gas chromatograph. The amount of ethyl-
ene was calculated by comparison of the corresponding peak area of each injec-
tion with that of a known quantity of authentic ethylene. The carbon monoxide
generated in the reaction was verified by a Varian 920 gas chromatograph using
a 6 ft molecular sieve 5A column with a thermal conductivity detector. In the
photolysis of azo-*tert*-butane, the tube was irradiated by 15 uv lamps (8 watt
with maximum output at 350 nm) in a Rayonet apparatus. In the anaerobic runs
the reaction mixture was degassed by four freeze-pump-thaw cycles.

RESULTS AND DISCUSSION

Table I shows typical results. Most notable are the following points:

(i) Initiators that yield RO· radicals convert methional to ethylene in

excellent yield whether aerated (run 1) or deaired (run 2) solutions are

used. *(ii)* Under anaerobic conditions, alkyl radicals lead to the formation

of ethylene only at higher concentrations of initiator. (Compare runs 4 and

6 as well as 8 and 10.) *(iii)* In the presence of oxygen, alkyl radicals give

ethylene whether the concentration of the initiators is high or low. (See

runs 3 and 5 as well as 7 and 9.) These results indicate alkyl radicals

convert methional to ethylene with poor efficiency, but in aerated solutions,

as would be found in cells, R· is rapidly converted to ROO· and RO· radicals,

and these oxygenated radicals produce ethylene with higher efficiency.

(iv) Initiators that produce ArCO-O· or RCO-O· radicals produce ethylene,

but in poor yield. (See runs 11 and 12.)

Table I. Ethylene Formation from Methional

Run	Initiator	Molarity	Solvent	Reaction Conditions	% Yield[a]
1	*tert*-Butyl Peroxide Methional	0.11 0.11	Decane	130°C for 6.4 hrs	42 ± 3
2	*tert*-Butyl Peroxide[b] Methional (Deaired)	0.11 0.11	Decane	130°C for 6.4 hrs	40 ± 5
3	Azobisisobutyronitrile Methional	0.12 0.11	Benzene	70°C for 4.8 hrs	4.7 ± 0.3
4	Azobisisobutyronitrile Methional (Deaired)[b]	0.11 0.13	Benzene	70°C for 4.8 hrs	0.00
5	Azobisisobutyronitrile Methional	0.52 0.10	Benzene	70°C for 4.8 hrs	6.7 ± 0.3
6	Azobisisobutyronitrile Methional (Deaired)[b]	0.52 0.10	Benzene	70°C for 4.8 hrs	0.33 ± 0.03
7	Azo-*tert*-Butane Methional	0.10 0.10	Benzene	Photolyzed for 2 hrs at room temp.	5.9 ± 0.3
8	Azo-*tert*-Butane Methional (Deaired)[b]	0.12 0.12	Benzene	Photolyzed for 2 hrs at room temp.	0.00
9	Azo-*tert*-Butane Methional	0.50 0.10	Benzene	Photolyzed for 2 hrs at room temp.	7.2 ± 0.1
10	Azo-*tert*-Butane Methional (Deaired)[b]	0.50 0.10	Benzene	Photolyzed for 2 hrs at room temp.	2.0 ± 0.3
11	Acetyl Peroxide Methional	0.10 0.11	Benzene	85°C for 1.1 hrs	0.34 ± 0.03
12	Benzoyl Peroxide Methional	0.11 0.11	Benzene	85°C for 1.4 hrs	0.25 ± 0.04

[a]The percentage yield of ethylene was calculated based on the amount of methional present. The data show average deviation of duplicate runs.

[b]The reaction was run under anaerobic conditions.

We have also performed some work in aqueous solution, as shown in Table

II. The production of $HO\cdot$ radicals from H_2O_2 and of $RO\cdot$ radicals from organic

hydroperoxides in the presence of iron (II) is well documented (4,15). Table

II shows that $HO\cdot$ radicals produce ethylene in yields slightly greater than

do $RO\cdot$ radicals. The $HO\cdot$ radical abstracts hydrogen atoms with rate constants
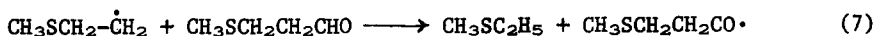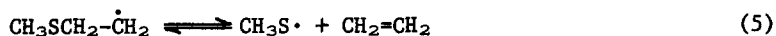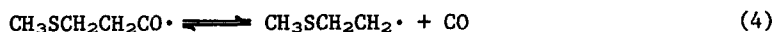
Table II. Ethylene Formation by Ferrous Ions

Run	Reagent	mMole^a	Reaction Conditions	% Yield
1	$CH_3SCH_2CH_2CHO$ $FeSO_4$ H_2O_2	0.22 0.26 2.00	1 hr at 40°C	17.7 $\pm$ 0.4
2	$CH_3SCH_2CH_2CHO$ $FeSO_4$ H_2O_2	0.21 0.25 0.40	1 hr at 40°C	16.7 $\pm$ 0.3
3	$CH_3SCH_2CH_2CHO$ $FeSO_4$ $(CH_3)_3COOH$	0.21 0.25 2.00	1 hr at 40°C	2.04 $\pm$ 0.14
4	$CH_3SCH_2CH_2CHO$ $FeSO_4$ $(CH_3)_3COOH$	0.22 0.26 0.40	1 hr at 40°C	1.51 $\pm$ 0.02

^{a}In a mixture of H_2O–acetic acid (2:1).

that are much greater than those for the comparable reactions of RO· radicals, and it is probable that the RO· radicals undergo more side reactions in our system. However, the differences between HO· and RO· radicals shown by the data in Tables I and II appear to be merely quantitative, rather than reflecting qualitatively different behavior.

These results require that the mechanism proposed (11) for the conversion of methional to ethylene must be expanded, and we tentatively suggest the mechanism shown in Eqs 2-7. The P· radical in Eq 2 is the primary radical responsible for initiation. In the absence of significant side reactions by P· or other paths for the decomposition of the $CH_3SCH_2CH_2CO$· radical, one ethylene is produced for each P· radical introduced. However, if Eq 7 competes with Eq 5, a chain occurs and each P· can yield more than one $CH_2{=}CH_2$ molecule.

$$Initiation\ process \longrightarrow P· \qquad (2)$$

$$CH_3SCH_2CH_2CHO + P· \longrightarrow CH_3SCH_2CH_2CO· + PH \qquad (3)$$

$$CH_3SCH_2CH_2CO\cdot \rightleftharpoons CH_3SCH_2CH_2\cdot + CO \tag{4}$$

$$CH_3SCH_2\dot{C}H_2 \rightleftharpoons CH_3S\cdot + CH_2{=}CH_2 \tag{5}$$

$$2CH_3S\cdot \longrightarrow CH_3SSCH_3 \tag{6}$$

$$CH_3SCH_2\dot{C}H_2 + CH_3SCH_2CH_2CHO \longrightarrow CH_3SC_2H_5 + CH_3SCH_2CH_2CO\cdot \tag{7}$$

This mechanism is consistent with the known behavior of aldehydes and β-thiyl-substituted compounds in radical systems. Equation 3 shows abstraction of the aldehydic hydrogen, a reaction known to be fast (16). The resultant acyl radicals then rapidly decarbonylate, Eq 4, yielding alkyl radicals and carbon monoxide (17). For methional, these steps give the β-methylmercaptoethyl radical which can eliminate $CH_3S\cdot$ to give ethylene, Eq 5 (18).

Our mechanism predicts that CO is the 1-carbon fragment formed, and we have verified that CO is produced in nearly quantitative yield in runs like 2 and 10, Table I. In the presence of air, CO_2 is formed and less CO is found. It is possible that CO is further oxidized by the reaction system to compounds of higher oxidation state.

At present, we propose that the varying yields of ethylene shown in Tables I and II simply reflect the differing ability of the $P\cdot$ radicals to abstract the aldehydic hydrogen, Eq 3, in competition with other reactions available. The ethylene yields roughly parallel the reactivity of radicals in hydrogen abstraction reactions: $HO\cdot > RO\cdot > ROO\cdot > RCO{-}O\cdot > R\cdot$

Clearly, more work will be necessary to elucidate the mechanism of ethylene production from methional. We intend to obtain complete product and material balance data, as well as detailed kinetics. However, the work reported here makes it clear that ethylene production cannot be used as a test for the presence of $HO\cdot$ radicals, and, by implication, suggests the biosynthetic route to ethylene also must be reexamined.

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