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# Derivatives of Sorbic Acid–Thiol Adducts

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*ABSTRACT*

*Ethyl and methyl sorbate react with thiols in the corresponding alcohol as solvent and with sodium alkoxide as catalyst. Nucleophilic attack by alkylthiols leads to the formation of diadducts (2,3- and 4,5-addition of $H^+RS^-$) whilst reaction with mercaptoethanol, esters of 2-mercaptoacetic acid, and cysteine ethyl ester gives the 2,5-addition product. Detailed consideration is given to the location of the double bond in position 3. Attempted hydrolysis of the esters causes dissociation of monoadducts liberating sorbic acid. However, if sorbic acid is allowed to react with cysteine in a predominantly aqueous medium, and the product esterified, it is identical to that formed from the esters of sorbic acid and of cysteine.*

## INTRODUCTION

Sorbic acid reacts slowly with thiols (mercaptoethanol, cysteine) in aqueous solution at 80°C and pH 3·7–7·7 (Wedzicha & Brook, 1989) in a bimolecular reaction which is thought to involve nucleophilic attack by the thiolate anion at position 5 of the sorbic acid molecule. The reaction is completed by addition of $H^+$ to form either the substituted 2-hexenoic or 3-hexenoic acid. The inability of sorbic acid to form a 1:2 addition product with these thiols suggests that the product might be the latter but no systematic study of the structures of sorbic acid–thiol reaction products has been carried out.

The main reason for the lack of structural data is the fact that it is difficult to isolate the adducts in question in sufficient quantities; they tend to separate as viscous oils and there is a tendency for decomposition to take place yielding sorbic acid as one of the products, even under mild acid or



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alkaline conditions (Wedzicha & Brook, unpublished). In this paper we approach the problem by synthesis of methyl and ethyl esters of the adducts in question by nucleophilic addition of thiolate anion to the ester of sorbic acid. Such addition may be carried out in the corresponding alcohol catalysed by the appropriate sodium alkoxide. Thus, reaction products capable of being purified by distillation may be obtained, their structures established and compared with the corresponding esters of reaction products obtained in the reaction of sorbic acid in aqueous systems.

## MATERIALS AND METHODS

All thiols, cysteine ethyl ester hydrochloride, 2-hexenoic and 3-hexenoic acids, ethyl sorbate and mercaptoacetate were obtained from Aldrich, Gillingham, UK. Crotonic and sorbic acids and cysteine were obtained from BDH Chemicals Ltd, Poole, UK. Where not available commercially, ethyl and methyl esters were prepared by dissolving the acids in the appropriate alcohol and saturating the solution with dry HCl gas at 0°C. After 1 h the solvent was removed under reduced pressure (40°C) and the ester distilled *in vacuo*.

Reactions of the esters with thiols (ethanethiol, 2-methyl-2-propanethiol, butanethiol, mercaptoethanol, methyl mercaptoacetate and cysteine ethyl ester hydrochloride) were carried out by adding to a solution of the appropriate sodium alkoxide (prepared from 0·92 g Na, 40 mmol) in dry alcohol (ethanol or methanol, 50 ml), ester (40 mmol) and thiol (40–120 mmol). After stirring for a given time at room temperature the mixture was extracted with $CHCl_3$ (3 × 100 ml), the combined organic layers washed with water (50 ml) and dried ($MgSO_4$). The solvent was removed under reduced pressure (40°C) and the product isolated by bulb-to-bulb distillation (Büchi GKR-51 Kügelröhr distillation apparatus, Büchi, Switzerland) *in vacuo*.

To convert esters into free acids the product from the reaction of ethyl sorbate and cysteine ethyl ester (2 g) was boiled with NaOH (10% w/v, 50 ml, 15 min), cooled, acidified with conc. HCl and the precipitate filtered off. A reaction of sorbic acid (40 mmol) with cysteine (40 mmol) was also carried out in 10% ethanol (aqueous) (50 ml) at 80°C for 80–90 h. The solvent was removed under reduced pressure (40°C) and last traces of water by co-distillation with absolute ethanol. The product was dissolved in ethanol or methanol and saturated with dry HCl at 0°C. After 1 h, the solvent was removed under reduced pressure. The residue was dissolved in a small volume of $CHCl_3$, washed with $NaHCO_3$ (10% w/v), water and dried ($MgSO_4$). The product was distilled as described for esters above.

## RESULTS AND DISCUSSION

### Analysis of reaction products

Reaction products which were isolated were subjected to microanalysis and identified by $^{1}H$ (60 MHz) and $^{13}C$ (90 and 400 MHz) NMR, mass spectrometry and infrared spectroscopy. Data for each compound are as follows:

*Ethyl sorbate-ethanethiol adduct* (Fig. 1, structure 10)
Reaction time, 20 h; yield, 63%, colourless oil (b.p. 130°C/1 mm Hg). IR (neat) 1738 $cm^{-1}$; mass spectrum *m/e* 264, 235 (-Et), 202 ($CH_3CH(SEt)CH_2CH{=}CHCOOEt$), 173, 157, 141, 127. Anal. calc'd for $C_{12}H_{24}S_2O_2$:C, 54·54; H, 9·09; S, 24·24. Found: C, 54·6; H, 9·25; S, 24·6.

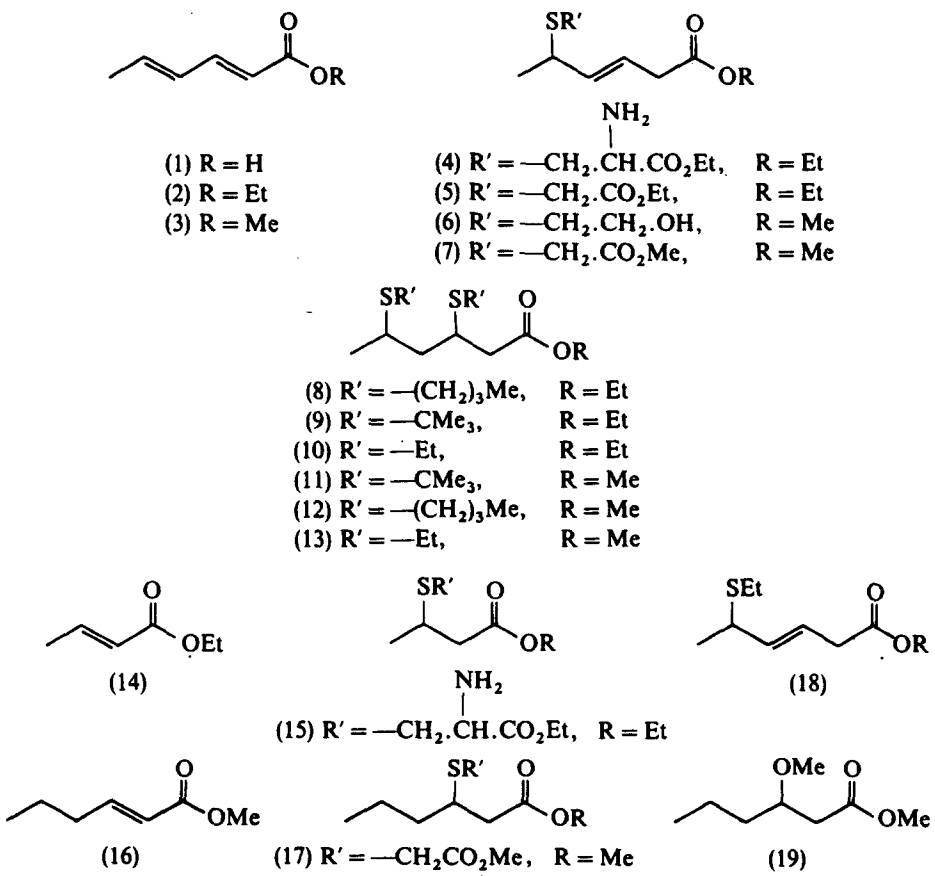


**Fig. 1.** Structures of reactants (1–3, 14, 16) and reaction products of reactions of unsaturated carboxylic acid esters with alkyl thiols, mercaptoethanol and esters of mercaptocarboxylic acids.

*Ethyl sorbate-2-methyl-2-propanethiol adduct* (Fig. 1, structure 9)
Reaction time, 20 h; yield, 57%, colourless oil (b.p. 140°C/mm Hg). IR (neat) 1738 $cm^{-1}$, $^1H$ NMR: $\delta$ 1·24–1·44 (t, 3H), 1·44 (s, 21H), 1·68–2·0 (m, 2H), 2·64–2·72 (m, 2H), 3·0–3·52 (m, 2H), 4·0–4·36 (q, 2H); mass spectrum *m/e* 320, 263, 230, 207, 173, 161, 141. Anal. calc'd for $C_{16}H_{32}S_2O_2$: C, 60·00; H, 10·00; S, 20·00. Found: C, 60·2; H, 10·2; S, 19·7.

*Ethyl sorbate-butanethiol adduct* (Fig. 1, structure 8)
Reaction time, 20 h; yield, 49%; colourless oil (b.p. 170°C/1 mm Hg); IR (neat): 1738 $cm^{-1}$; mass spectrum *m/e*: 320, 263 ($M-(CH_2)_3CH_3$), 230($M-S(CH_2)_3CH_3$), 173, 157, 141, 127, 117, 99. Anal. calc'd for $C_{16}H_{32}S_2O_2$: C, 60·0; H, 10·0. Found: C, 60·25; H, 10·25.

*Ethyl sorbate-ethyl-2-mercaptoacetate adduct* (Fig. 1, structure 5)
Reaction time, 3 h; yield, 47%; colourless oil (b.p. 150°C/1 mm Hg); IR (neat): 1730 $cm^{-1}$ (b); $^1H$ NMR: $\delta$ 1·2–1·44 (m, 9H), 2·80 (dq, 4H), 3·4–3·8 (m, 1H), 4·20 (q, 4H), 5·48–5·78 (m 2H); mass spectrum *m/e*: 260, 214, 187, 172, 141 ($M-SCH_2COOCH_2CH_3$), 113, 99, 85. Anal. calc'd for $C_{12}H_{20}SO_4$: C, 55·33; H, 7·75. Found: C, 55·0; H, 7·75.

*Ethyl sorbate-cysteine ethyl ester hydrochloride adduct* (Fig. 1, structure 4)
Reaction time, 20 h; yield, 71%; pale yellow oil (b.p. 180°C/1 mm Hg); IR (neat): 1735 $cm^{-1}$; $^1H$ NMR: $\delta$ 1·2–1·4 (m, 9H), 1·80 (m, 2H), 2·68–3·0 (m, 2H), 3·4–3·70 (m, 2H), 4·0–4·40 (dq, 4H), 5·4–5·52 (m, 2H, olefinic); $^{13}C$ NMR: 173·74 (C=O), 171·34 (C=O), 136·17 (C–4), 122·65 (C–3), 61·10 and 60·64 ($OCH_2$), 54·44 ($CHNH_2$), 42·36 (CHS), 37·46 ($CH_2S$), 35·77 ($CH_2$–CH=CH), 20·39 (6–$CH_3$), 14·22 ($CH_3$); mass spectrum *m/e*: 290 ($M^+$). Anal. calc'd for $C_{13}H_{23}NO_4S$: C, 53·98; H, 7·96; N, 4·84; S, 11·07. Found: C, 52·15; H, 7·6; N, 4·85; S, 12·9.

*Methyl sorbate-ethanethiol adduct* (Fig. 1, structure 13)
Reaction time, 3 h; yield, 59%; colourless oil (b.p. 140°C/1 mm Hg); IR (neat) 1738 $cm^{-1}$; mass spectrum *m/e*: 250, 221, 188 ($M-SCH_2CH_3$), 159, 127 ($CH_3CHCH_2CH=CHCOOCH_3$).

*Methyl sorbate-2-methyl-2-propanethiol adduct* (Fig. 1, structure 11)
Reaction time, 24 h; yield, 47%; colourless oil (b.p. 140°C/1 mm Hg); IR (neat): 1740 $cm^{-1}$; $^1H$ NMR: $\delta$ 1·44 (s, 21H), 1·72–2·0 (m, 2H), 2·68–2·80 (m, 2H), 3·0–3·5 (m, 2H), 3·76 (s, 3H, $OCH_3$); mass spectrum *m/e*: 306, 249 ($M-C(CH_3)_3$), 216, 193, 127. Anal. calc'd for $C_{15}H_{30}S_2O_2$: C, 58·82; H, 9·80; S, 20·91. Found: C, 59·10; H, 9·90; S, 21·20.

*Methyl sorbate-butanethiol adduct* (Fig. 1, structure 12)
Reaction time, 3 h; yield, 50%; colourless oil (b.p. 150°C/1 mm Hg); IR (neat): 1740 $cm^{-1}$; mass spectrum *m/e*: 306, 249 ($M-(CH_2)_3CH_3$),

216 ($CH_3CH(SC(CH_3)_3)CH_2CH{=}CHCOOCH_3$), 127 ($CH_3CHCH_2$-$CHCHCOOCH_3$). Anal. calc'd for $C_{15}H_{30}S_2O_2$: C, 58·85; H, 9·80; S, 20·92. Found: C, 58·80; H, 9·75; S, 21·15.

*Methyl sorbate-mercaptoethanol adduct* (Fig. 1, structure 6)
Reaction time, 1·5 h; yield, 56%; colourless oil (b.p. 220°C, water pump pressure); IR (neat): 3420 $cm^{-1}$ (OH), 1738 $cm^{-1}$; $^1H$ NMR: $\delta$ 1·31 (d, 3H, J = 7 Hz), 2·48–3·28 (m, 7H), 3·60 (m, 1H), 3·68 (s, 3H), 5·40–5·64) (m, 2H olefinic); $^{13}C$ NMR: $\delta$ 171·6230 (C=O), 135·8928 (C–4), 121·4426 (C–3), 60·6836 ($CH_2OH$), 51·2593 ($OCH_3$), 40·9829 (CHS), 36·4819 ($SCH_2$), 32·4571 ($CH_2$), 19·8349 (6–$CH_3$); mass spectrum *m/e*: 204, 159 (M–$CH_2CH_2OH$), 127 (M–$SCH_2CH_2OH$). Anal. calc'd for $C_9H_{16}O_3S$: C, 52·94; H, 7·84; S, 15·68. Found: C, 52·64; H, 7·85; S, 15·50.

*Methyl sorbate-methyl 2-mercaptoacetate adduct* (Fig. 1, structure 7)
Reaction time, 2 h; yield, 54%; colourless oil (b.p. 130°C/1 mm Hg); IR (neat): 1735 $cm^{-1}$; $^1H$ NMR: $\delta$ 1·30 (d, 3H, J = 7 Hz), 3·28–3·44 (m, 4H), 3·60–3·84 (m, 1H), 3·88 (s, 3H, $OCH_3$), 3·92 (s, 3H, $OCH_3$), 5·44–5·88 (m, 2H, olefinic); $^{13}C$ NMR: $\delta$ 170·0020 (C=O), 170·2689 (C=O), 134·5412 (C–4), 122·8134 (C–3), 51·0756 ($OCH_3$), 51·5080 ($OCH_3$), 41·4522 (CHS), 36·4628 ($SCH_2$), 31·3997 ($CH_2$), 19·1316 (6–$CH_3$); mass spectrum *m/e*: 232, 159 (M–$CH_2CO_2CH_3$), 127 (M–$SCH_2CO_2CH_3$). Anal. calc'd for $C_{10}H_{16}SO_4$: C, 51·72; H, 6·89; S, 13·79. Found: C, 51·90; H, 7·05; S, 13·90.

*Ethyl crotonate-cysteine ethyl ester hydrochloride adduct* (Fig. 1, structure 15)
Reaction time, 72 h; yield, 49%; pale yellow oil (b.p. 165°C/1 mm Hg); IR (neat): 1730 $cm^{-1}$; mass spectrum *m/e*: 263, 218, 190, 115. Anal. calc'd for $C_{11}H_{21}NSO_4$: C, 50·19; H, 7·98; N, 5·32. Found: C, 50·45; H, 8·20; N, 5·35.

*Methyl 2-hexenoate-sodium methoxide adduct* (Fig. 1, structure 19)
Reaction time, 2 h; yield, 88%; colourless oil (b.p. 75°C, water pump pressure); IR (neat): 1738 $cm^{-1}$; $^1H$ NMR: $\delta$ 0·96 (m, 3H), 1·48 (m, 4H), 2·48 (dd, J = 2 Hz), 3·36 (s, 3H), 3·72 (s, 3H), mass spectrum *m/e*: 160, 129 (M–$OCH_3$). Anal. calc'd for $C_8H_{16}O_3$: C, 60·0; H, 10·0. Found: C, 60·35; H, 10·35.

*Methyl-2-hexenoate-methyl-2-mercaptoacetate adduct* (Fig. 1, structure 17)
Reaction time, 3 h; yield, 64%; colourless oil (b.p. 110°C/1 mm Hg); IR (neat): 1740 $cm^{-1}$ (b); $^1H$ NMR: $\delta$ 1·04 (m, 3H), 1·68 (m, 5H), 2·76 (m, 2H), 3·40 (s, 2H), 3·80 (s, $OCH_3$), 3·84 (s, $OCH_3$); mass spectrum *m/e*: 234, 202, 161 (M–$CH_2COOCH_3$), 129 (M–$SCH_2COOCH_3$). Anal. calc'd for $C_{10}H_{18}SO_4$: C, 51·28; H, 7·69. Found: C, 51·6; H, 7·8.

The reaction times shown were those after which the product whose characteristics are given, was isolated. It was found that, at longer reaction times, no new reaction products were obtained. Shorter reaction times gave

lower conversions to product but in each case the product isolated was the same as that described above.

## Structures of reaction products

The structures of substrates for nucleophilic attack (1–3, 14, 16) and products of reaction identified in this work are shown in Fig. 1. The structures have been assigned on the basis of the analytical and spectroscopic data given above. It is seen that monoaddition products of sorbate esters are formed when the nucleophile is mercaptoethanol or esters of mercaptoacetic acid; no diadduct could be obtained even when the nucleophile was in 3-fold molar excess. On the other hand, alkyl thiols formed only diadducts and no monoadduct could be isolated when the molar ratio of thiol to sorbate was 1:1 or less.

The conclusion regarding stoichiometry is unequivocal because it may be reached on the basis of microanalysis data alone. The most important question concerns the location of the double bond of the monoadducts (4–7), shown in Fig. 1 as in position 3. $^{13}$C NMR data for the adducts of ethyl sorbate with cysteine ethyl ester and methyl sorbate with mercaptoethanol and methyl 2-mercaptoacetate shows the carbon atoms associated with this bond at $\delta$ 121·44–122·81 (C–3) and $\delta$ 134·54–136·17 (C–4). In spectra of 2-hexenoic and 3–hexenoic acids these carbon atoms are found (Barabas *et al.*, 1978) with the chemical shifts shown in Table 1.

Proton NMR of 5-mercapto-3-hexenoic acid and its methyl ester shows the olefinic protons at $\delta$ 5·60(m) whilst the corresponding protons of authentic 5-(ethylthio)-3-hexenoic acid are reported at $\delta$ 5·57 (Blenderman & Joullie, 1983). On the other hand, Camps *et al.* (1982) report the olefinic protons of 5-methylsulphonyl)-2-hexenoic acid at $\delta$ 6·2–6·8. In the present investigation the monoadducts show olefinic protons at $\delta$ 5·4–5·9. The observed chemical shifts of $^{13}$C and $^{1}$H associated with the double bond are, therefore, consistent with the monoadducts being derivatives of 3-hexenoic acid.

The fragmentation of methyl esters of $\alpha$-,$\beta$- and $\beta$,$\gamma$-unsaturated carboxylic acids during mass spectrometry (Lauwers *et al.*, 1973) shows features which

**TABLE 1**
$^{13}$C NMR Chemical Shifts of Carbon Atoms in 2-Hexenoic and 3-Hexenoic Acids

| | $\delta$ (*ppm*) | | |
|---|---|---|---|
| | *C-2* | *C-3* | *C-4* |
| 2-hexenoic acid | 122·5 | 151·8 | |
| 3-hexenoic cid | | 120·3 | 137·2 |

depend on the location of the double bond. Cleavage of the molecule, α to the carbonyl group, is seen. Loss of a methoxy group is likely to give rise to a resonance stabilised ion in the case of the α,β-unsaturated compound and hence, a more intense M–31 peak (e.g. intensity = 47 and 12% for methyl 2-hexenoate and methyl 3-hexenoate, respectively). This species loses CO (to give M–59) and it is regarded that the ratios of intensities at M–31 to M–59 (e.g. 3 and 0·5 for the two esters, respectively) are a guide to the initial location of the double bond. Also, the α,β-unsaturated esters give rise to intense peaks at *m/e* 113 and 87 (57 and 100% for methyl 2-hexenoate, respectively, compared with *c.* 10% for both ions in the case of methyl 3-hexenoate).

In the case of the monoadducts investigated here the mass spectrum is dominated by ions formed as a result of the cleavage of S-linked side chains as reported in the data above. However, ions at M–31, M–59 and *m/e* 113 and 87 are observed for methyl esters of sorbic acid-thiol monoadducts with the intensities (% of base peak at *m/e* 85) shown in Table 2.

The low intensities of these ions and the ratio of intensities at M–31 to M–59 (ratio = 1) is not inconsistent with derivatives of 3-hexenoic acid but may also be related to preferred fragmentation of the chain attached to position 5.

Ethyl crotonate (14) and methyl 2-hexenoate (16) react readily with thiols to form the expected reaction products on the same timescale as when ethyl or methyl 2,4-hexadienoate are used. Thus, the tendency to formation of monoadducts 4–7 further supports location of the double bond in position 3. It could, however, be argued that addition of nucleophile at position 5 causes steric hindrance for further addition at position 3 to a product with a double bond at position 2. However, reaction of product 7 with ethanethiol in the presence of sodium methoxide gave product 18 ($^1$H NMR: δ 1·25 (m, 6H), 2·41 (q, 2H), 3·15 (d, 2H), 3·68 (m, 1H), 5·57 (m, 2H) and identical to the $^1$H NMR spectrum of an authentic sample reported by Blenderman & Joullie (1983)) indicating that displacement of the original nucleophile had taken place and that the $CH_3CH_3S^-$ group had not added to the free double bond.

**TABLE 2**

Abundance (% of base peak at *m/e* 85) of Certain Ions in the Mass Spectrum of Nucleophile–Methyl Sorbate Adducts

| | *Nucleophile* | |
|---|---|---|
| | *Mercaptoethanol* | *Methyl-2-mercaptoacetate* |
| M-31 | 0·4 | 4·7 |
| M-59 | 0·4 | 4·7 |
| *m/e* 113 | 5 | 5·4 |
| *m/e* 87 | 5 | 2·5 |

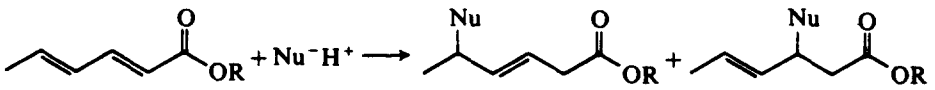

Fig. 2. Alternative monoaddition products when nucleophile Nu reacts with sorbate ester.

It seems that the only *simple* way of preparing the monoadduct when alkyl thiols are the nucleophiles is to first use a reagent known to form a monoadduct and then to displace it with the alkyl thiol.

The monoaddition reaction of a nucleophile, $Nu^-$ and $H^+$, can, in fact, lead to 2,3- or 2,5-addition as shown in Fig. 2. The 2,3-addition product also has a double bond which is not conjugated and, therefore, incapable of reacting further in a nucleophilic reaction. We dismiss the possibility of 2,3-addition because the $\delta$ value of the methyl protons (C–6) (1·2–1·44) is generally too low for $CH_3$—C=C (for which $\delta \approx 1{\cdot}6$) and, in the case of the methyl esters investigated (products 6,7) the C–6 doublet which could be clearly resolved at $\delta$ 1·30–1·31, is similar to $\delta$ 1·25 obtained by Blenderman & Joullie (1983) for methyl 5-(ethylthio)-3-hexenoate. Indeed, the fact that this product is formed when compound 7 is allowed to react with ethanethiol is ample evidence that the 2,3-addition of thiol to the sorbate ester had not taken place.

## Mechanism of addition

A mechanism for the atack on sorbate esters by thiolate anion is given in Fig. 3. The anionic intermediate combines with hydrogen ion to form either B or C. Obviously, B is required if a diadduct is to be formed. One question is whether, in the presence of a strong base, C may be converted to B. Our

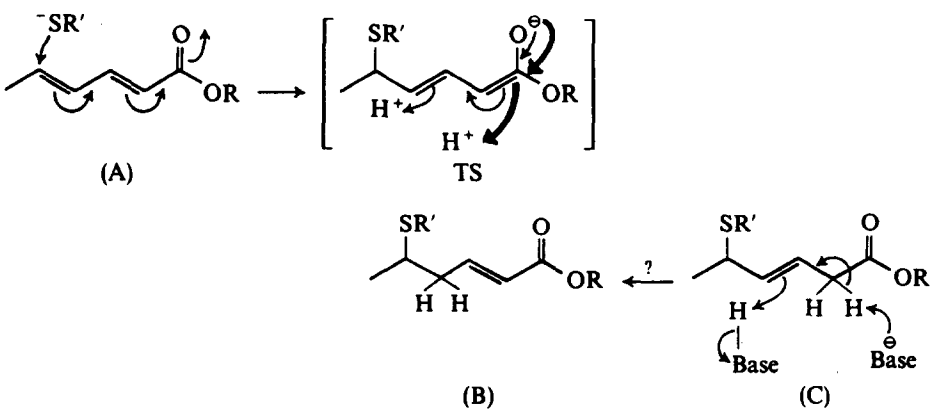

Fig. 3. Electron shifts when thiolate anion $R'S^-$ attacks sorbate ester, A, to form the anionic transition state, TS, and illustration of pick-up of $H^+$ in positions 2 or 4 to form products C and B, respectively. A speculative scheme for the acid/base-catalysed isomerisation of 5-substituted 3-hexenoate, C, to the corresponding 2-hexenoate, B, is shown.

results show that methyl 3-hexenoate is completely unreactive towards thiols under the same conditions as used for synthesis and, unless there is some participation from the substituent in position 5, the isomerisation of the 3-ene to the 2-ene seems an unlikely possibility. When methyl 2-hexenoate is treated with sodium methoxide in the absence of any other nucleophile, product 19 is obtained.

An alternative approach to the mechanism is to say that, whilst the 3-hexenoate derivative is undoubtedly the thermodynamically favoured reaction product, the 2-hexenoate derivative may be formed as an initial kinetically favoured product. Thus, if we have a very reactive nucleophile, e.g. alkylthiol, the formation of diadduct is possible; in the event of a less reactive nucleophile an irreversible isomerisation to the more stable product predominates. Whilst such an explanation provides a route to the expected products, it is still unsatisfactory in one respect; the reaction of the intermediate in question with alkylthiol will be competitive with reaction of the thiol with unchanged sorbate ester. Thus, some monoadduct should remain at the end of the reaction and it should be the main product when the molar ratio of reactants is 1:1.

The main difference between nucleophiles which cause monoaddition and those which cause diadducts to be formed is the presence of oxygen atoms in the former. It is hard to envisage that the electronegativity of the oxygen might be responsible for the creation of a field which causes repulsion of negative charge within the anionic transition state (TS in Fig. 3) away from position 4 to facilitate pick-up of a hydrogen ion at position 2. However, the fact that the oxygen atom in question is at the end of a 3 or 4 membered chain in the case of the products described here, could allow the oxygen to approach position 4 sufficiently closely to exert such an effect and prevent the formation of a diadduct. We are currently investigating reaction products from longer chain mercapto-acids to attempt to verify this possibility and to identify the chain length required before the oxygen-containing nucleophile behaves as an alkylthiol.

Attempts to hydrolyse the esters to obtain the reaction products expected if sorbic acid and cysteine or mercaptoethanol had undergone reaction, led to the formation of sorbic acid in high yield. However, if the reaction between sorbic acid and cysteine were carried out in an aqueous medium and the resulting product esterified with ethanol, the resulting compound was identical to the reaction product obtained using ethyl sorbate and cysteine ethyl ester (compound 4). Thus, the reactions considered here represent the formation of derivatives of products formed in aqueous systems without the need for a strongly basic catalyst, though the rates of these reactions are much slower. However, instability of products towards hydrolysis is marked. This observation explains why in previous work (Wedzicha &

Brook, unpublished) steam distillation of mercaptoethanol-sorbic acid reaction mixtures to remove any unchanged reactants led to poor yields of products. It is possible that the hydrolysis reaction is driven by the thermodynamic stability of the conjugated-diene product. The diadducts formed from ethyl and methyl sorbate and alkylthiols could not be decomposed to sorbid acid.

## CONCLUSION

It is established that products of monoaddition of thiols to sorbic acid have a double bond in position 3 and that alkyl thiols lead to the formation of diadducts. A simple synthetic pathway for the formation of pure samples of reaction products could involve the use of ethyl or methyl sorbate and thiols or esters of thiols where appropriate (e.g. cysteine ester). However, a satisfactory hydrolysis procedure is now required to obtain the free acids in the case of monoadducts.

If reactions between thiols and sorbic acid take place in foods, the nucleophile may be a cysteine residue on a protein or an alkylthiol present as a food flavour component. Glutathione, perhaps the simplest naturally occurring cysteine-containing peptide, is known to react with sorbic acid in a reaction which is kinetically similar to that of cysteine (Wedzicha & Zeb, 1990). Also, sorbic acid is known to inhibit a small number of sulphydryl-containing enzymes, e.g. fumarase, aspartase, succinic dehydrogenase, alcohol dehydrogenase (York & Vaughn, 1964; Whitaker, 1959; Martoadiprawito & Whitaker, 1963). It has been suggested (York & Vaughn, 1964) that the cause of enzyme inhibition is reaction of sorbic acid with thiol groups of the protein. Reaction of food proteins with sorbic acid, according to the mechanisms discussed here, would give rise to modified cysteine residues. Having identified the specific cysteine-sorbic acid reaction product, we are now considering the extent to which sorbic acid molecules become attached to typical food proteins and the effects of proteolytic enzyme action on the stability of the adducts in question.

The results obtained here suggest that cysteine and alkylthiols may give rise to significantly different types of reaction product. More extensive studies of mono- and diadduct formation in aqueous systems are now required. A synthetic route to specific reaction products by hydrolysis of ester should also provide pure samples for toxicological evaluation.

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