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Products from the Autoxidation of Δ^5-Avenasterol

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ABSTRACT

Comparison of the products from the oxidation of cholesterol and Δ^5-avenasterol indicates that similar oxidation products are formed by oxidation in the A and B rings of the sterols during the heating of solutions of these compounds in edible oils. However, the UV spectrum of the oxidation products indicates that at least one compound is formed from Δ^5-avenasterol which is not analogous to the oxidation products of cholesterol. The formation of 5,24(25),28-stigmastatrien-3β-ol is suggested as a possibility.

INTRODUCTION

Δ^5-Avenasterol occurs together with other sterols in many edible oils including olive oil and coconut oil (Itoh *et al.*, 1973). It is a particularly important unsaponifiable component because, unlike most other sterols, it acts as an antioxidant at elevated temperatures (Gordon & Magos, 1983). This antioxidant activity has been ascribed to the formation of an allylic free radical at C29 followed by isomerisation to a relatively stable tertiary allylic free radical at C24. This mechanism would require the formation of side chain oxidation products and therefore the products

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from the oxidation of Δ^5-avenasterol in edible oil have been studied. It is particularly important to identify the oxidation products formed by sterols, because some of the products are angiotoxic (Peng *et al.*, 1978) or carcinogenic (Bischoff, 1969).

MATERIALS AND METHODS

Materials

Cholesterol and trioleylglycerol, prepared from technical oleic acid, were purchased from BDH Chemicals Ltd. Δ^5-Avenasterol was isolated in the laboratory from green algae (*Ulva lactuca*) (Gibbons *et al.*, 1968) as reported in an earlier paper (Gordon & Magos, 1983).

Methods

Samples of the sterols (0·1 %) were heated in solution in trioleylglycerol at 180 ± 5 °C for a total of 72 h, comprising nine 8 h periods with cooling to room temperature at night. The product was saponified with alcoholic potassium hydroxide (1 M) and extracted either with diethyl ether (for TLC and GC analysis) or with light petroleum (40–60 °C, for UV analysis). TLC analysis was performed using 20 × 20 cm plates coated with Silica Gel H. Ethyl acetate–heptane (1:1) was used as the developing solvent, and spots were visualised by spraying the plates with sulphuric acid (50 %) and heating at 135 °C.

Gas chromatography was undertaken using a Perkin-Elmer Sigma 3B chromatograph equipped with an OV17 column (1·5 % on Chromosorb W, 80–100 mesh) at 250 °C. Electron impact mass spectra were recorded with a Kratos MS25 mass spectrometer equipped with a DS505 data system. UV spectra were recorded using a Perkin-Elmer spectrometer, model 124, with ethanol as the solvent.

RESULTS AND DISCUSSION

The unsaponifiable components of samples of trioleylglycerol heated at 180 °C for 72 h with either cholesterol (0·1 %) or Δ^5-avenasterol (0·1 %) were analysed by TLC (see Tables 1 and 2). The oxidation products of

TABLE 1
Oxidation Products of Cholesterol by TLC Analysis

R_m	$R_m(1)$	$R_m(2)$	*Colour*	*Identity*	*Expected* λ_{max} *(nm)*
132	144	110	Beige[a]	IV	277
119	129			VI	—
	115		Yellow/tan[b]	VII	234
	125			VIII	284
100	100	100	Magenta[a]	Cholesterol	—
79			Yellow/tan[b]	V	—
65	43	63	Tan[b]	III	236–238
40	33	49	Blue[c]	I	—
33	31	45	Blue[c]	II	—

$R_m(1)$, van Lier & Smith, 1968.
$R_m(2)$, Smith *et al.*, 1967.
[a] Colour appeared after spraying with sulphuric acid and heating for 3–5 min.
[b] Colour appeared after spraying with sulphuric acid and heating for 60 min.
[c] Colour appeared after spraying with sulphuric acid.

TABLE 2
Oxidation Products of Δ^5-Avenasterol by TLC Analysis

R_m	*Colour*	*Assignment*	*Expected* λ_{max} *(nm)*
132	Beige[a]	XII	277
119	Yellow/tan[b]	XIV, XV, XVI	—, 234, 284
100	Magenta[a]	Avenasterol	—
79	Yellow/tan[b]	XIII	—
65	Tan[b]	XI	236–238
40	Blue[c]	IX	—
33	Blue[c]	X	—

[a] Colour appeared after spraying with sulphuric acid and heating for 3–5 min.
[b] Colour appeared after spraying with sulphuric acid and heating for 60 min.
[c] Colour appeared after spraying with sulphuric acid.

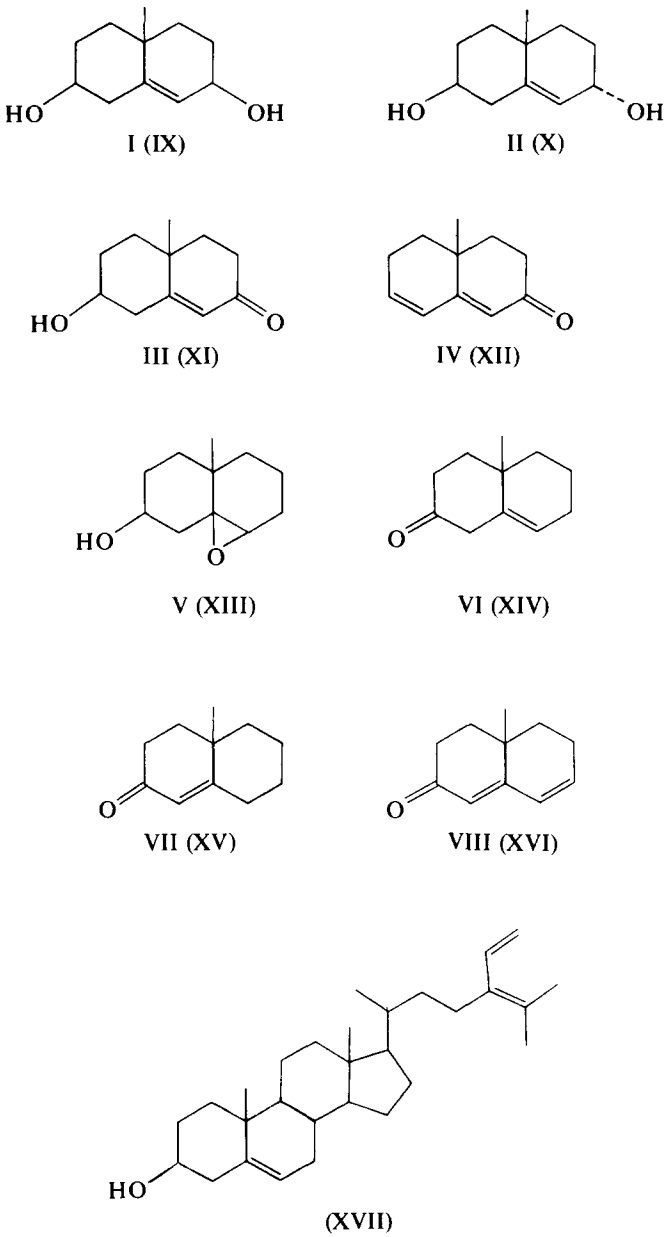

Fig. 1. Oxidation products from cholesterol and Δ^5-avenasterol. Only the A and B rings are shown for products I–XVI. The rest of the molecule is identical to the sterol used. Numbers in brackets refer to the products derived from Δ^5-avenasterol.

cholesterol were identified from the TLC data by comparison of the chromatographic mobility, R_m, with the values of van Lier and Smith (1968) and Smith *et al.* (1967). In addition, the colour developed and the conditions required for colour development were in agreement with the rules of Smith *et al.* (1967). Cholest-5-ene-3β,7β-diol (I), cholest-5-ene-3β,7α-diol (II), 3β-hydroxycholest-5-en-7-one (III) and cholesta-3,5-dien-7-one (IV) were identified unequivocally (Fig. 1). The spot at R_m 75 was probably cholesterol 5,6-epoxide (V), since this was the only cholesterol oxidation product with an R_m value between cholesterol and III found by other workers using a solvent system of similar polarity (Chicoye *et al.*, 1968). The spot at R_m 119 probably corresponds to one or more of the ketones 5-cholesten-3-one (VI), 4-cholesten-3-one (VII) or 4,6-cholestadien-3-one (VIII). The identification of III, IV and VIII is confirmed by the UV spectrum of the product mixture which shows two overlapping peaks with λ_{max} at 238 nm and 274 nm (relative intensities 89:37) together with a shoulder at approximately 280 nm. Compounds III, IV and VIII have UV spectra with λ_{max} at 236–238 nm (ε 12 500–15 500; Smith, 1981), 277 nm (ε 24 400; Scott, 1964) and 284 nm (ε 26 300; Scott, 1964), respectively. The observed spectrum indicates that III was the major UV absorbing compound present.

The R_m values and the colour development of spots after spraying with sulphuric acid were identical for the cholesterol oxidation products and the Δ^5-avenasterol oxidation products (see Tables 1 and 2). Therefore, the oxidation products of Δ^5-avenasterol were identified as compounds IX–XVI by analogy with those of cholesterol. However, the UV spectrum of the oxidised Δ^5-avenasterol sample was significantly different with two overlapping peaks at 232 nm and 274 nm (relative intensities 81:30), with a shoulder at approximately 280 nm. The shift of 6 nm towards lower wavelength in the spectrum compared with that of the cholesterol oxidation products indicates that at least one compound has been formed in the oxidation of Δ^5-avenasterol that is significantly different in structure from the oxidation products of cholesterol. Since cholesterol and Δ^5-avenasterol differ only in the side chain, it appears probable that oxidation of Δ^5-avenasterol has occurred in the side chain. This is particularly likely since Δ^5-avenasterol acts as an antioxidant in heated oils and is consumed more rapidly than other sterols that are ineffective as antioxidants (Boskou & Morton, 1975).

We have suggested that Δ^5-avenasterol acts as an antioxidant by forming the allylic free radical at C29 which then isomerises to the

tertiary allylic free radical at C24 (Gordon & Magos, 1983). This radical is relatively stable, but loss of a hydrogen atom can lead to the formation of the most stable diene which is 5,24(25),28-stigmastatrien-3β-ol (XVII). This is predicted to have λ_{max} at 232 nm, which corresponds to the precise wavelength observed in the UV spectrum of the Δ^5-avenasterol oxidation products. This compound is unlikely to be separated from Δ^5-avenasterol by either TLC or GLC under the conditions used in this study. However, positive identification of XVII requires the synthesis of this compound and comparison of its spectroscopic and chromatographic properties with those of the oxidation products of Δ^5-avenasterol.

GC–MS confirmed the presence of IV and XII in the oxidation products of cholesterol and avenasterol, respectively. IV showed peaks at *m/e* 382, 367, 270, 269, 229, 227, 187, 174 and XII showed peaks at *m/e* 408, 310, 269, 254, 242, 229, 187, 174. The M^+-98 peak in the mass spectrum of XII is characteristic of sterols with a $\Delta^{24(28)}$ bond (Bergman *et al.*, 1965; Benveniste *et al.*, 1966) and is due to the loss of C_7H_{14} by a McLafferty rearrangement. No other products could be identified from the GC–MS analysis.

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