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The *S*-oxidative degradation of a novel corticosteroid tipredane (INN). Part I: preliminary investigations into the hydrogen peroxide *S*-oxidation of tipredane

MELVIN R. EUERBY,* JOANNE HARE and STEVEN C. NICHOLS

Analytical Chemistry Department, Research and Development Laboratories, Fisons Pharmaceuticals PLC, Bakewell Road, Loughborough, Leicestershire LE11 0RH, UK

Abstract: The C-17 dithioketal moiety of the corticosteroid tipredane (INN, **I**) has been shown to undergo peroxide oxidation yielding an array of sulphoxide epimers. All epimers have been isolated and characterized by spectroscopic techniques. Oxidation of the C-17β methylthio- substituent appeared to occur exclusively giving a 4:1 ratio of the *S*:*R* configuration at the sulphoxide moiety. Both methylthiosulphoxide epimers (**V**) have been shown to be susceptible to thermolysis yielding the monoethylthio- derivative (**VI**) via elimination of methylsulphenic acid. As expected from stereochemical considerations at the C-17 position the *S* epimer has been found to be more susceptible than its corresponding *R* epimer towards thermolysis. The monomethylthiosulphoxide epimers (**III**) were produced in a 1:1 ratio, indicating that they were formed from the oxidation of the corresponding monomethylthio- derivative (**VII**). This probably arose from the elimination of ethylsulphenic acid from the α-ethylthiosulphoxide (**VIII**).

Keywords: *Corticosteroid; dithioketal;* S-*oxidation; peroxide; site and stereoselectivity; sulphoxide epimers.*

Introduction

Tipredane (INN,[11β,17α]-17[ethylthio]-9α-fluoro-11β-hydroxy-17-[methylthio]androstra-1,4-dien-3-one, **I**) is a novel corticosteroid [1], which possesses a C-17 asymmetric dithioketal grouping. The novelty of tipredane's chemistry resides in its reactivity at the dithioketal moiety. The dithioketal rapidly undergoes acid hydrolysis (pH <4.5) to give the parent C-17 keto derivative (**II**). However, it is the *S*-oxidation of this moiety which is of most interest. This paper therefore describes the *S*-oxidative degradation of tipredane induced by hydrogen peroxide and examines the site and stereoselectivity of the oxidation. Structures of the compounds mentioned in this paper are shown in Scheme 1.

Experimental

Chemicals

All chemicals and solvents used were of HPLC grade. Water was purified by means of a Milli-Q water system.

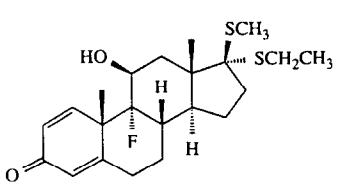
Spectroscopy

NMR spectra were recorded on a Bruker AM-360 spectrometer operating at 360.134 and 90.56 MHz for proton and carbon, respectively. FT-IR spectra were recorded on a Perkin–Elmer 17720X spectrometer. A Finnigan MAT TSQ700 mass spectrometer with a thermospray interface was used for the LC–MS investigations. Ammonium acetate was used in place of the phosphate-buffered mobile phase for this work.

Chromatography

HPLC analyses were performed using a Hewlett–Packard 1090M HPLC system equipped with a 1040 linear photodiode array UV detector. Data acquisition and integration was controlled by a Hewlett–Packard 79994A Chem Station. Chromatography was performed on a 5-μm Waters Nova Pak C18 (150 × 3.9 mm) column fitted with a cartridge guard column (5-μm Lichrospher 100 RP-18 end-capped, Merck). Detection was at 240 nm, based on the λ_{max} of tipredane. The eluent consisted of mobile phases A and B which were 0.025 M KH_2PO_4 and 0.025 M KH_2PO_4 in acetonitrile–water (65:35, v/v). The flow rate was 1.5 ml min^{-1} and the oven temperature was thermostatically held at 40°C. A linear gradient was run over 20 min from 10 to 95% mobile phase B; then the eluent composition was held for a further 10 min.

* Author to whom correspondence should be addressed.



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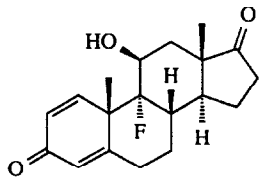

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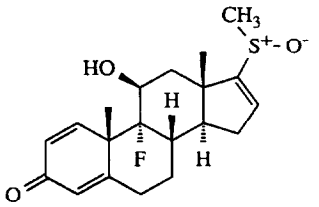

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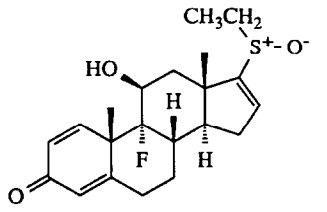

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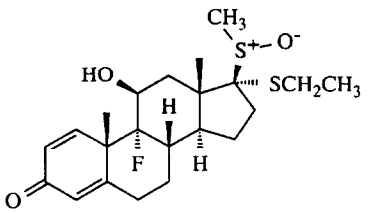

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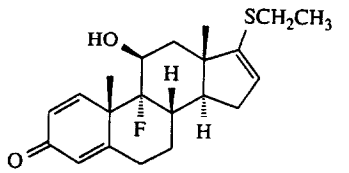

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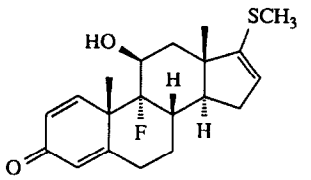

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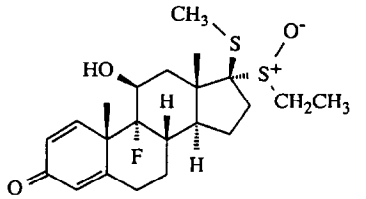

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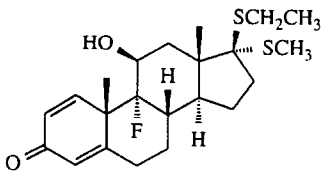

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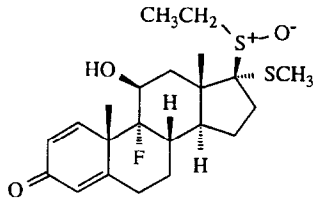

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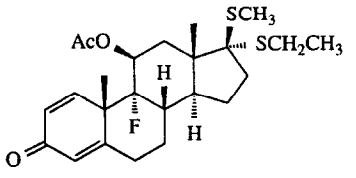

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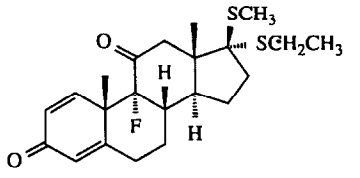


(XII)

Scheme 1
Structure of tipredane and related compounds.

Semi-preparative HPLC

This was performed using three Waters Model 510 pumps controlled by a Waters automated gradient controller, Hewlett–Packard HP 3390A integrator and Gilson 115 UV detector. The third pump was used to introduce the sample solution onto the column. Chromatography was performed on a 5-μm Rainin Dynamax-60A C-18 semi-preparative column (300 × 21.4 mm). Detection was at 280 nm to avoid saturating the detector.

The eluent consisted of mobile phases A and B which were 0.025 M ammonium acetate (pH 7.0) in acetonitrile–water (30:70, v/v for the sulphoxide epimers **IV** and **V** and 21:79, v/v for the sulphoxides epimers **III**).

The flow rate was 13 ml min^{-1}.

Preparation and separation of the sulphoxide epimers

Hydrogen peroxide (100 vol, 10 ml) was added to tipredane (0.5 g) in ethanol (200 ml) and sodium acetate (0.1 M, pH 6.5, 50 ml). The resultant solution was stored in the dark at room temperature for 30 days.

Water (750 ml) was added to bring the ethanolic content down to 20% (v/v). The solution was then passed through a Mega Bond Elut C18 (60 ml, Analytichem Ltd) column. Further quantities of 20% ethanol were passed down the column to remove the monomethylthiosulphoxide epimers (**III**, monitored by HPLC). Once these sulphoxides had been eluted, 40% ethanol (10 × 20 ml) fractions were used to elute the monoethylthiosulphoxide (**IV**) and methylthiosulphoxide (**V**) epimers. On cooling these fractions the methylthiosulphoxide *S* epimer crystallized. Further recrystallization from cold chloroform–hexane (1:4, v/v) afforded the pure *S* epimer of compound **V**. The monoalkylthiosulphoxide epimers (**III** and **IV**) and the *R* epimer of compound **V** were separated into their pure epimers by semi-preparative HPLC.

Polar monomethylthiosulphoxide epimer (III). Yield (19.5 mg, 4.4%); m.p. 278°C; ν_{max}(KBr)/cm^{-1} 3240(OH), 1660(1,4-dien-3-one), 1042(C—F), 1030(S—O); $\lambda_{max/nm}$ 240; $\delta_H(CDCl_3)$ 7.26(1H,d,J10,1-H), 6.42(1H,m, 16-H), 6.34(1H,dd,J10&2,2-H), 6.14(1H,s,H-4), 4.33(1H,m,H-11), 2.71(3H,s,20-MeS), 1.59(3H,s,19-Me), 1.41(3H,s,18-Me); MS *m/z* (%) 365(M^+ + 1,100), 345(7), 141(32), 125(11); HPLC R_t = 9.1 min.

Less polar monomethylthiosulphoxide epimer (III). Yield (8 mg, 1.8%); m.p. 275–281°C; ν_{max}(KBr)/cm^{-1} 3460(OH), 1660(1,4-dien-3-one), 1060(C—F), 1018(S—O); $\lambda_{max/nm}$ 240; $\delta_H(CDCl_3)$, 7.26(1H,d,J10,1-H), 6.34(1H,dd,J10&2,2-H), 6.21(1H,br,16-H), 6.13(1H,br,4-H), 4.33(1H,m,11-H), 3.20(1H, br,11-OH), 2.76(3H,s,20-MeS), 1.59(3H,s,19-Me), 1.46(3H,s,18-Me); MS *m/z* (%) 406(M^+ + 1,100), 155(6), 141(52); HPLC R_t = 9.4 min.

Polar monoethylthiosulphoxide epimer (IV). Yield (1.5 mg, 0.3%); m.p. 274°C; $\lambda_{max/nm}$ 240: $\delta_H(CDCl_3)$ 7.24(1H,d,J10,1-H), 6.41(1H, br,16-H), 6.35(1H,d,J10,2-H), 6.14(1H,s, 4-H), 4.33(1H,m,11-H), 2.92(1H,m,20-CHS), 1.59(3H,s,19-Me), 1.40(3H,s,18-Me), 1.30 (3H,t,J7,22-Me); MS *m/z* (%) 420(M^+ + MeCN,10), 379(M^+ + 1,100), 196(13), 155(13); HPLC R_t = 9.9 min.

Less polar monoethylthiosulphoxide epimer (IV). Yield (1 mg, 0.2%); m.p. 258°C; $\lambda_{max/nm}$ 240; $\delta_H(CDCl_3)$ 7.26(1H,d,J10,1-H), 6.35(1H,d,J10,2-H), 6.25(1H,br,16-H), 6.14(1H,br,4-H), 4.29(1H,m,11-H), 2.95 & 2.85(2 × 1H, 2 × m, 20-CH_2), 1.58(3H,s,19-Me), 1.44(3H,s,18-Me), 1.22(3H,t,J7.5,22-Me); MS *m/z* (%) 420(M^+ + MeCN,11), 379(M^+ + 1,100), 196(6), 155(18); HPLC R_t = 10.5 min.

Less polar methylthiosulphoxide epimer (V). Yield (37.5 mg, 7.2%); m.p. 275–281°C; λ_{max} (KBr)/cm^{-1} 3440(OH), 1680(1,4-dien-3-one), 1040(C—F), 1020(S—O); $\lambda_{max/nm}$ 240; $\delta_H(CDCl_3)$ 7.24(1H,d,J10,1-H), 6.33(1H,dd, J10&2,2-H), 6.12(1H,br,4-H), 4.35(1H,m,11-H), 3.30 & 2.75(2 × 1H, 2 × m, 21-CH_2), 2.66(3H,s,20-MeS), 1.57(6H,s,18 & 19-Me), 1.22(3H,t,J8,22-Me); MS *m/z*(FAB, %) 443(M^+ + NH_4,5), 427(M^+ + 1,3), 363(M^+-MeSOH,100), 343(16), 319(17), 238(6), 141(120); HPLC R_t = 11.7 min.

Polar methylthiosulphoxide epimer (V). Yield (182 mg, 35%); m.p. 274–284°C; λ_{max} (KBr)/cm^{-1} 3440(OH), 1665(1,4-dien-3-one), 1050(C—F), 1025(S—O); $\lambda_{max/nm}$ 240; MS *m/z*(FAB, %) 443(M^+ + NH_4,7), 427(M^+ + 1,18), 363(M^+-MeSOH,100), 343(5), 319(12), 155(7), 141(57); HPLC R_t = 11.2 min.

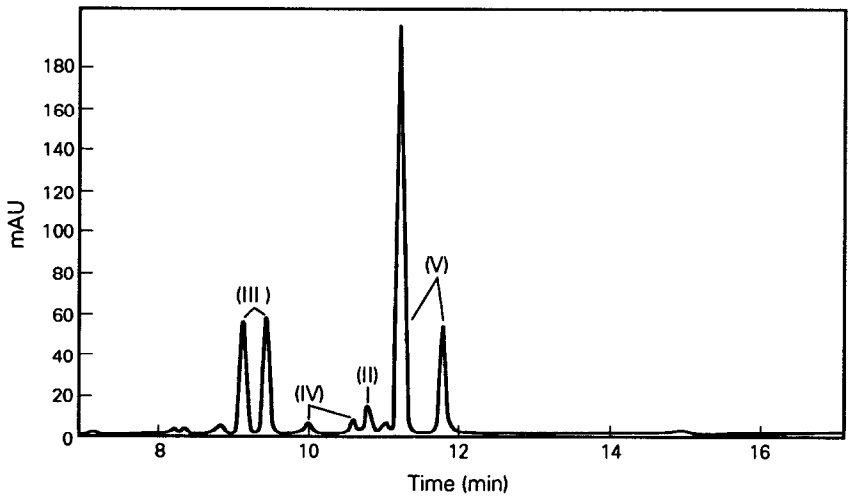


Figure 1
Section of a typical chromatogram of the tipredane sulphoxide epimers (**III–IV**).

Results and Discussion

Isolation and characterization

Tipredane has been shown to undergo oxidative degradation in various pharmaceutical formulations [2]. The same profile of oxidative products was seen on reacting tipredane with hydrogen peroxide at pH 6.5 at room temperature (Fig. 1). The oxidative products were initially identified by LC–MS as the monomethylthio- (**III**), monoethylthio- (**IV**) and methylthio- (**V**) sulphoxide epimers of tipredane. It has previously been postulated that these sulphoxides are metabolites of tipredane when incubated with liver homogenates, however their structures were not fully characterized [3]. Sulphoxides are chiral because of the stable pyramidal configuration about sulphur, bringing about the formulation of diastereoisomers (i.e. epimers). The separation of the epimers on a reversed-phase C18 column using a phosphate–acetonitrile linear gradient was facilitated by the close proximity of the two chiral centres. The molecular ion for the methylthiosulphoxide epimers (**V**) was very weak as a result of rapid fragmental loss of methylsulphenic acid in the mass spectrometer yielding a base ion for the corresponding C-17 monoethylthio derivative (**VI**) of tipredane.

Therefore, in order to confirm the postulated structures unequivocally and to determine the stereochemistry of the sulphoxides, sufficient tipredane was oxidized with hydrogen peroxide to allow isolation and characterization of the sulphoxides. The pH of the reaction medium was buffered to pH 6.5 in order to prevent acid hydrolysis of the resultant sulphoxides. The reaction was slow at room temperature taking approximately 30 days for all the tipredane to be oxidized.

Isolation of the individual sulphoxide epimers of compounds **III–V** could be achieved firstly by separating the monoalkylthio- sulphoxide epimers (**III** and **IV**) from the methylthiosulphoxide epimers (**V**) on a C18 Mega Bond Elut column followed by recrystallization (ethanol–water and chloroform–hexane) or semi-preparative HPLC using a C18 Dynamax column and acetonitrile–ammonium acetate buffer (pH 7.0).

The isolated sulphoxide epimers were then characterized by high-resolution NMR, FT-IR and MS. All compounds were isolated in a purity in excess of 98% (HPLC).

Spectroscopic characterization of the methylthiosulphoxide epimers (V)

Thermospray mass spectrometry of both epimers gave very weak responses at *m/z* 427 corresponding to the predicted sulphoxide molecular ion + 1. Therefore in order to verify the molecular ion the sulphoxides were subjected to fast atom bombardment mass spectrometry which produced a MH^+ at *m/z* 427 with the MNa^+ at *m/z* 449. Addition of rubidium iodide further confirmed the molecular weight of 426 with the presence of the MRb^+ ion at *m/z* 511. The more polar of the two sulphoxide epimers could be successfully recrystallized from ethanol–water and finally chloroform–hexane to yield crystals suitable for X-ray crystallographic studies to be per-

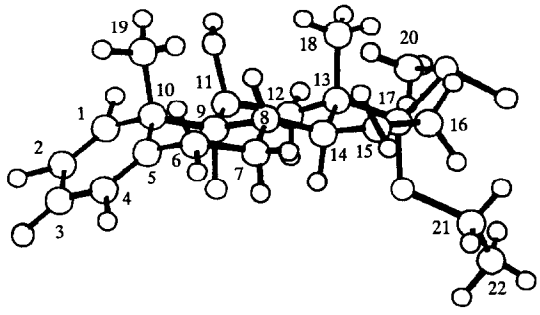


Figure 2
X-ray crystallographic structure of the methylthiosulphoxide *S*-epimer (**V**).

formed. Examination of the crystal structure established that the sulphoxide moiety possessed the *S* configuration (Fig. 2).

Detailed ^1H and ^{13}C NMR spectroscopic investigation of the S *epimer of compound V*

^1H NMR spectroscopy indicated that there was coupling between the *cis* olefinic protons H-1 and H-2 of 10 Hz and long-range coupling between H-2 and H-4 of 2.0 Hz. The signal for H-4 was broad owing to additional long-range coupling. The carbinol proton H-11 was observed as a complex signal owing to it being coupled to two methylene protons at C-12, the hydroxyl proton and also to the fluorine atom at C-9.

The *S*-ethyl group gave the expected triplet and two quartets owing to non-equivalence of the CH_2 caused by the close proximity of the sulphoxide moiety. The two methyl substituents C-18 and C-19 appeared as two singlets at δ1.26 and 1.56 ppm respectively. The C-20 methyl substituent in the *S*-sulphoxide (**V**) appeared as a single at δ2.68 ppm whereas in tipredane (**I**) it was observed at 2.12 ppm illustrating a paramagnetic shift on the C-20 methyl substituent on sulphoxide formation. The remaining proton signals lay between δ1.3 and 2.7 ppm and arose from the saturated ring protons. These required further two-dimensional NMR spectroscopic techniques to provide a full assignment. Twenty-two signals were observed in the broadband proton decoupled ^{13}C spectrum (see Table 1). In addition, ^{13}C spectra measured with DEPT pulse sequences provided a distinction between methyl, methylene, methine and non-protonated carbons. Five signals in the decoupled spectrum showed coupling to the fluorine atom at C-9. Assignments shown in Table 1 were based on ^{13}C chemical shifts, DEPT and C—F coupling constants. In addition, the ^{13}C–^1H correlated NMR spectra, which had been optimized for one-bond coupling, allowed the assignment of all the methyl carbons and the *S*-methylene carbon since the assignment of the corresponding proton signals had already been established. From the ^{13}C–^1H correlation spectra chemical shifts for the pairs of protons attached to each of the six ring methylene carbons could be established (Table 2). These methylene pairs were confirmed in the phase-sensitive ^1H–^1H correlated spectrum.

Comparison of the ^1H NMR spectra for the *S* and *R* epimers of compound **V** showed that the C-18 methyl protons were less deshielded (Δδ 0.3 ppm) in the *S* epimer than in the corresponding *R* epimer. The reason for this could be seen in the X-ray structure of the *S* epimer (Fig. 2) which showed that the oxygen of the sulphoxide was in closer proximity to the C-16 protons than to the C-18 methyl protons. Since a deshielding effect on the C-18 methyl protons in the *R* epimer was observed, it could be concluded that the sulphoxide oxygen was in close proximity to the C-18 methyl protons confirming the *R* configuration.

Spectroscopic characterization of the monoalkylthiosulphoxide epimers (III and IV)

Thermospray mass spectrometry gave good molecular ions as expected for the individual monoalkylthio sulphoxide epimers.

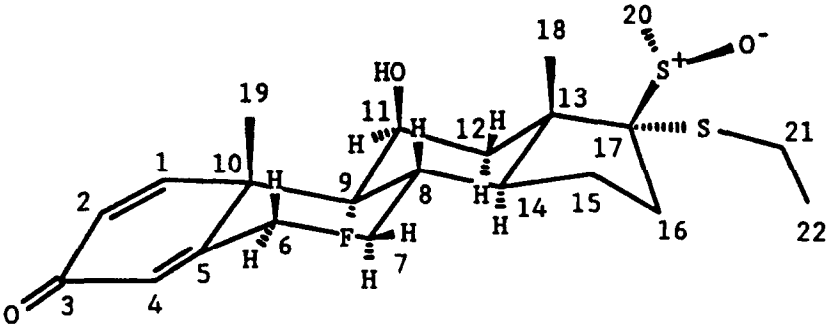
The C-16 protons of the more polar epimers of compounds **III** and **IV** in the ^1H NMR spectra experienced considerably more deshielding (Δδ 0.21–0.06 ppm) compared to the less polar epimers. In addition, the C-18 methyl protons of the less polar epimers also experienced more deshielding (Δδ 0.05 and 0.04 ppm) than their corresponding polar epimers.

Based on the X-ray structure and the ^1H NMR results of the *S* epimer of the methylthiosulphoxide (**V**), it is reasonable to conclude that sulphoxides causing a deshielding of the C-18 methyl substituent possess the *R* configuration whereas sulphoxides causing a deshielding of the C-16 proton would possess the *S* configuration.

Site selectivity of the S*-oxidation of tipredane using hydrogen peroxide*

It was surprising to note that oxidation appeared to have occurred exclusively on the C-17 methylthio- substituent (i.e. β-attack)

Table 1
^{13}C Chemical shifts and assignments of the methylthiosulphoxide *S* epimer (**V**)



Assignment	Chemical shift*	J C—F (Hz)
1	152.6	
2	129.5	
3	186.7	
4	124.9	
5	166.7	
6	31.0	
7	27.3	
8	33.9	19
9	100.1	176
10	48.2	24
11	71.3	37
12	37.2	
13	48.7	
14	45.3	
15	24.4	
16	30.0	
17	81.1	
18	18.0	
19	22.9	6
20	35.1	
21	25.3	
22	14.5	

*ppm from TMS ($CDCl_3$ set to 77.0 ppm).

when from steric considerations there would appear to be no preference for either α or β attack. A detailed LC–MS examination of the crude reaction mixture after 21 and 33 days failed to detect any of the corresponding ethylthiosulphoxide analogues of tipredane (**VIII**) arising from α-attack. The site of oxidation could be easily established from the LC–MS as the tipredane methylthiosulphoxide gave rise to the base ion corresponding to the loss of methylsulphenic acid. In an analogous manner, the ethylthiosulphoxides would be expected to give rise to a base ion corresponding to the monomethylthio- derivative of tipredane (this fragmentation pattern was observed with the ethylthiosulphoxides of the C-17 epimer of tipredane).

In order to investigate this further the C-17 epimer of tipredane (**IX**) was subjected to similar oxidative conditions and the reaction mixture examined by LC–MS. Once again apparent exclusive β-oxidation occurred yielding the ethylthiosulphoxide epimers of tipredane (**X**).

It was postulated that the peroxide may hydrogen bond with the C-11β hydroxy substituent hence allowing oxidation to occur only on the C-17β substituent. In order to test this hypothesis, the C-11β O-acetyl (**XI**) and C-11 keto (**XII**) derivatives of tipredane were synthesized, both compounds lacking the hydrogen bonding capacity at the C-11 position. Hence oxidation of both compounds should result in non-exclusive β-oxidation.

However, the C-11β O-acetyl derivative, after oxidation and mild base hydrolysis of the O-acetyl moiety, gave an oxidative degradation profile the same as that for the oxidation of tipredane itself, illustrating that there is no anchimeric assistance from the C-11β hydroxy

Table 2
Proton chemical shifts and assignments of the methylthiosulphoxide *S* epimer (**V**)

Assignment	Chemical shift (ppm from TMS)
1	7.27
2	6.27
4	6.12
6A	2.39*
6B	2.65*
7A	1.60*
7B	1.86*
8	2.40*
11	4.38*
12A	1.50*
12B	2.25*
14	2.31*
15A	1.60*
15B	1.90*
16A	2.40*
16B	2.69*
18	1.26
19	1.56
20	2.68
21	2.72, 3.00*
22	1.20
11-OH	3.90

*Approximate value taken from the $^{1}H - {}^{13}C$ NMR correlation experiment.
No attempt has been made to distinguish between the protons of each methylene pair.

group hydrogen bonding with the peroxide. Examination of the C-11 keto oxidative reaction by LC–MS gave the same result, however a trace (approx. 2%) of the corresponding ethylthiosulphoxides was formed.

Further investigations are at present underway to examine the apparent site selectivity of the peroxide oxidation.

Stereoselectivity of the S*-oxidation of tipredane with hydrogen peroxide*

The epimeric methylthiosulphoxides were produced in a ratio of approximately 4:1 which did not change as a function of time. The ratio in the reaction mixture was the same after 9 months at room temperature. In addition, re-exposing the individual epimers to the reaction conditions and also to methanol (23 days room temperature) did not cause interconversion of the epimers indicating that the product distribution is under kinetic rather than thermodynamic control. From the X-ray structure the major epimer possesses the *S* configuration at the sulphoxide moiety. The same epimeric ratio was also seen with the C-11β O-acetyl tipredane oxidation.

In contrast, the monoalkylthio- sulphoxides were formed in an approximately 1:1 ratio indicating that they were formed from the corresponding monoalkylthio- derivatives in which peroxide attack from both planes was unrestricted (M.R. Euerby and C. Thomson, unpublished results). The monoalkylthio- derivatives could be formed as intermediates in the hydrolysis of tipredane or more probably from the degradation of the methylthio- (and ethylthio-) sulphoxide epimers; work is presently under way in an attempt to establish the existence of the α-ethylthiosulphoxide epimers (**VIII**).

Stability of tipredane sulphoxide epimers

The effect of pH. The monomethylthiosulphoxide epimers of tipredane (**III**) were shown to be more resistant to hydrolysis than their corresponding monomethylthio- derivatives (**VII**, R_t = 17.9 min). The monomethylthio- derivative degraded to the C-17 keto derivative (**II**, R_t = 10.8 min) at pH 2.5, stored at 45°C for 1 day whereas its corresponding sulphoxide was stable for 2 days at 60°C at the same pH.

The methylthiosulphoxide epimers (**V**) were shown to be stable at pH values between 7 and 12 for 16 days at 25°C, a minor degree of degradation was noted for the *S* and *R* epimers at pH 4.5 (16 days/25°C) of 1.0 and 0.2%, respectively. It was of interest to note that the

Table 3
Effect of pH 2.5 on the individual R and S methylthiosulphoxide epimer (**V**)

	Degradants formed (%)					
	S Epimer			*R* Epimer		
Time period (days)	EtS (**VI**)	Keto (**II**)	Total	EtS (**VI**)	Keto (**II**)	Total
Initial	1.4	0.3	1.7	0.5	0.2	0.7
1	10.3	3.6	13.9	2.6	0.9	3.5
5	31.2	20.8	52.0	10.5	6.5	17.0
16	29.0	53.5	82.5	16.2	23.6	39.8

methylthiosulphoxide epimers showed differing susceptibilities to acid hydrolysis at pH 2.5 (see Table 3) with the *R* epimer being more resistant to hydrolysis. The acid hydrolysis initially resulted in formation of the C-17 monoethylthio- derivative (**VI**, R_t = 19.5 min) which then underwent hydrolysis to the C-17 keto derivative (**II**).

The effect of heat. The monomethylthiosulphoxide (**III**) has been shown to be stable for 24 h at temperatures up to 108°C in the crystalline state. Tipredane has been shown to be susceptible to thermolysis above temperatures of 100°C in inert solvents resulting in an approximate ratio of 85:15 of the monomethylthio- (**VII**): monoethylthio- (**VI**) derivatives indicating a preferential elimination of ethanethiol. The methylthiosulphoxide epimer (**V**) has been shown to be highly susceptible to thermolysis in the solid state yielding predominantly the C-17 monoethylthio- derivative (see Tables 4 and 5). The *S* epimer was greater than 90% degraded at 45°C/24 h whereas only 0.1% of the corresponding *R* epimer was degraded. Temperatures of above 82°C were required to bring about elimination of methylsulphenic acid from the *R* epimer. Surprisingly, the *S* epimer was more stable in ethanolic solution than in the crystalline form, only 0.25% was degraded at 58°C/24 h compared to over 90% in the solid state. The stability of the individual epimers towards thermal racemization confirms previous literature reports of the notable optical stability of sulphoxides, thermal decomposition often occurring at temperatures below that required for racemization [4].

The thermal instability of the *S* epimer could be attributed to the stereochemistry at the C-17 position. Both epimers could theoretically undergo a concerted *syn*-periplanar elimination of methylsulphenic acid where the dihedral angle between the C-16 C—H and the C-17β C—S bond approached zero. Examination of Dreiding models showed that a transition state of this type is less favourable for the conversion of the *R* epimer into the C-17 monoethylthio- derivative because of severe steric interaction between the methyl group of the methylsulphoxide moiety and the C-13β methyl substituent, whereas in the transition state from the *S* epimer these interactions were absent.

The pyrolytic elimination of alkyl and aryl sulphenic acid has previously been used to determine the absolute configuration at sulphur in a range of alkyl and aryl steroidal sulphoxides [5, 6]. In addition, the relative rate of fragmental loss of methylsulphenic acid, in

Table 4
The effect of temperature on the stability of the methylthiosulphoxide *S* epimer (**V**)

Temp. (°C)/24 h	Degradants formed (%)		Total impurities (%)
	Keto (**II**)	EtS (**VI**)	
Initial	0.0	0.5	0.5
25	0.5	2.1	2.6
45	7.2	90.1	98.9
58	6.1	91.5	98.9
82	2.7	95.9	99.6
106	2.1	96.6	99.9

Table 5
The effect of temperature on the stability of the methylthiosulphoxide *R* epimer (**V**)

Temp. (°C)/24 h	Degradants formed (%)		Total impurities (%)
	Keto (**II**)	EtS (**VI**)	
Initial	0.0	0.0	0.0
25	0.0	0.0	0.0
45	0.0	0.1	0.1
58	0.1	0.2	0.3
82	1.5	87.8	96.6
106	1.0	87.8	96.9

the mass spectrometer, from 20-methylsulphoxide pregn-5-ene derivatives, has been used to determine the absolute configuration at sulphur [7]. From these reports, the least stable of the methylthiosulphoxide epimers (**V**) would be expected to possess the *S* configuration at the sulphoxide moiety and this was indeed confirmed to be the case by X-ray crystallography.

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