Pergamon

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 6, pp. 761–769, 1994

0731–7085/94 $7.00 + 0.00

0731–7085(93)E0031-H

The S-oxidative degradation of a novel corticosteroid tipredane (INN). Part II — Detailed investigations into the primary S-oxidation of tipredane using achiral oxidants

MELVIN R. EUERBY,* CHRISTOPHER M. JOHNSON, RICHARD J. LEWIS 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 site and stereoselective S-oxidations with a range of achiral oxidants. S-Oxidation was favoured on the methylthio substitutent (β-plane) in preference to the ethylthio substituent (α-plane) in a ratio of 1.8–3.5:1. S-Oxidation on both substituents appeared to be stereoselective yielding an approximate ratio of 4:1 and 1:3.5–6 for the *S*-:*R*-methyl and ethylsulphoxide diastereoisomers, (**II** and **VI**) respectively. The preferred sites for S-oxidation have been explained in terms of the preferred rotamers of the C-17 substituents and the steric factors imposed on the attack of the oxidant on the lone pair of electrons at each sulphur atom. Optimized HPLC conditions using a Hypersil ODS column and an acetonitrile–0.025 M NH_4OAc pH 7.2 gradient at 26°C were developed to prevent degradation of the ethylsulphoxide diastereoisomers (**VI**) occurring during chromatographic analysis, via elimination of ethylsulphenic acid to yield the corresponding C-17 vinylmethylthio derivative (**VII**).

Keywords: *Tipredane; corticosteroid; dithioketal; S-oxidation; sulphoxide diastereoisomers (epimers); site; stereoselectivity.*

Introduction

Tipredane (INN,[11β,17α]-17-[ethylthio]-9α-fluoro-11-hydroxy-17-[methylthio] androsta-1,4-diene-3-one, **I**) is a novel corticosteroid [1], which possesses a C-17 asymmetric dithioketal moiety. The novelty of the chemistry of tipredane resides in its reactivity at the dithioketal moiety. It has previously been shown that tipredane undergoes S-oxidation with hydrogen peroxide to yield the methylsulphoxide diastereoisomers (**II**, the steroidal diastereoisomers will be referred to as epimers) in a stereoselective manner (i.e. *S*:*R* ratio of 4:1) [2]. The tipredane methylsulphoxide epimers (**II**) undergo facile elimination of methylsulphenic acid to form the corresponding C-17 vinylethylthio derivative (**III**). The latter can then undergo a non-selective S-oxidation to yield the C-17 vinylethylsulphoxide epimers (**IV**) in a 1:1 ratio. In addition to the latter sulphoxides being formed a significant amount of the corresponding C-17 vinylmethylsulphoxide epimers (**V**) was produced. This suggested that they may be formed from an analogous reaction pathway originating from the corresponding tipredane ethylsulphoxide epimers (**VI**).

This paper establishes the existence of these extremely labile tipredane ethylsulphoxide epimers (**VI**) and describes appropriate HPLC conditions which must be employed to conserve the integrity of these compounds during chromatography.

In addition, the paper examines the site and stereoselectivity of the primary S-oxidation of tipredane using a variety of achiral oxidants and attempts to rationalise these findings in terms of the preferred rotamers of the C-17 alkylthio moieties and the steric impositions on attack of oxidants of differing steric bulk. The structures of the compounds mentioned in this paper are shown in Fig. 1.

Experimental

Chemicals

All chemicals and solvents used were of

* Author to whom correspondence should be addressed.

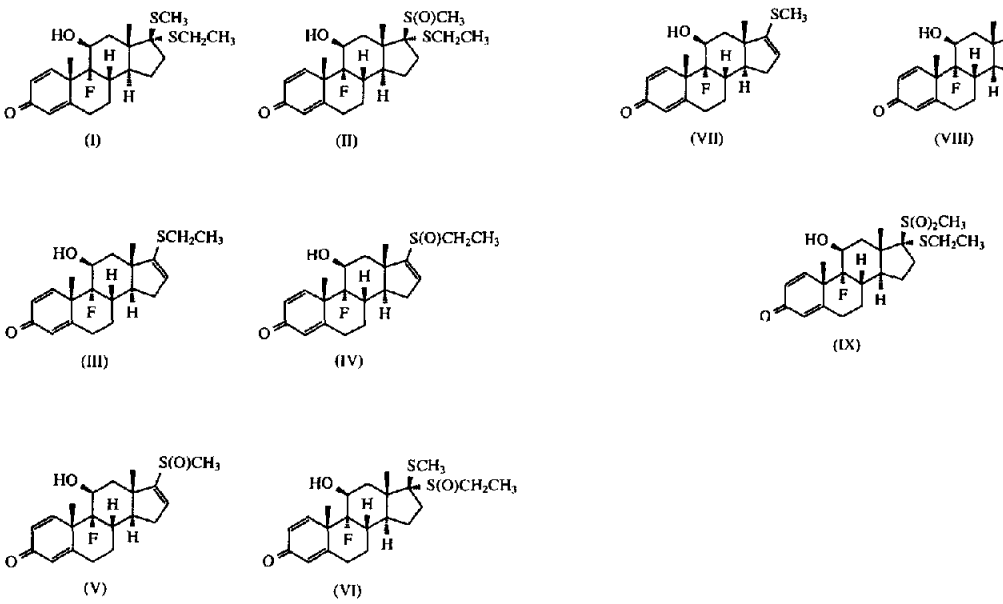


Figure 1
Structure of tipredane and related substances.

HPLC grade. Water was purified by an Egastat spectrum RO and ion exchange/carbon filter system supplied by Elga (High Wycombe, UK).

Spectroscopy

Thermospray (TP-MS) and fast atomic mass spectroscopic techniques (FAB-MS) were performed on a Finnigan MAT TSQ700, utilizing an ammonium acetate (0.4 M)–acetonitrile mobile phase, and a VG 70-250 SEQ mass spectrometer, respectively. For FAB-MS the sample was in a matrix of nitrobenzylalcohol and dimethylsulphoxide.

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. Detection was at 240 nm, based on the λ_{max} of tipredane.

HPLC conditions 1

Chromatography was performed on a Waters Nova-Pak C_{18} (5 μm, 150 × 3.9 mm) column fitted with a cartridge guard column (Lichrospher 100 RP-18 end-capped, 5 μm, Merck). 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; the final eluent composition was then held for a further 10 min.

HPLC conditions 2

Chromatography was performed on a Hypersil ODS 'Excel' column (5 μm, 100 × 4.6 mm, Hichrom, Reading, UK). The eluent consisted of mobile phases A and B which were 0.025 M NH_4OAc (pH 7.2) and 0.025 M NH_4OAc (pH 7.2) in acetonitrile–water (65:35, v/v). The flow rate was 1.5 ml min^{-1} and the oven temperature was thermostatically held at 26°C. A linear gradient was run over 10 min from 45 to 50% and then from 50 to 95% mobile phase B over 10 min; the final eluent composition was then held for a further 5 min.

Reaction mixtures were diluted with ethanol–ammonium acetate 0.1 M, pH 7.2 (1:1, v/v) to give a 0.5 mg ml^{-1} solution and 20 μl injections loaded onto the column.

Reaction of tipredane (**I**) with hydrogen peroxide or the hydrogen peroxide–urea adduct [3]

The oxidant (hydrogen peroxide (100 vol, 0.4 ml, 3577 μmoles) or hydrogen peroxide–urea adduct (335 mg, 3577 μmoles) was added to tipredane (20 mg, 49 μmoles) in ethanol

(8 ml) and sodium acetate (0.1 M, pH 7.2, 2 ml). The resultant solution was stored in the dark at room temperature and the reaction monitored by HPLC (conditions 1), for up to 33 days.

Reaction of tipredane (**I**) with potassium peroxymonosulphate (Oxone) or monomagnesium peroxyphthalic acid (MMPP)

Tipredane (2.5 μmoles) in ethanol (0.5 ml) stored at iced-conditions was added to a freshly prepared solution of ice-cold sodium acetate (0.5 M, pH 7.2, 0.5 ml) containing the oxidant (MMPP 0.6 μmoles or Oxone 0.9 μmoles). The reaction mixture was stored at 4°C and the reaction monitored by HPLC (conditions 2), over a period of 73 min.

Reaction of tipredane (**I**) with dimethyldioxirane

Tipredane (2.5 μmoles) in acetone (975 μl) stored at −18°C was added to a freshly prepared solution of dimethyldioxirane (1.2 μmoles) [4–6] in acetone (25 μl) stored at −18°C. The reaction mixture was stored at −18°C and the reaction monitored by HPLC (conditions 2), over a period of 68 min.

Preparation and isolation of the ethylsulphoxide epimers (VI)

Tipredane (2.1 mg, 5.2 μmoles) in ethanol (0.5 ml) stored at iced-conditions was added to a freshly prepared solution of ice-cold ammonium acetate (0.5 M, pH 7.2, 0.5 ml) containing MMPP (83%, 1.7 mg, 2.8 μmoles). The reaction mixture was stored at 4°C for 15 min then 100 μl injections were chromatographed using HPLC conditions 2 and the fractions corresponding to the ethylsulphoxide epimers (**VI**) collected at 4°C.

The following isolation procedure was performed at 4°C, all solutions and equipment were equilibrated at 4°C prior to use. The collected fractions from four 100 μl injections were diluted 1 to 3 with ice-cold 0.5 M NH_4OAc (pH 7.2) and loaded onto a C_{18} Sep Pak cartridge (pre-conditioned with 0.5 M NH_4OAc (pH 7.2), ethanol and finally 20% v/v ethanol–0.5 M NH_4OAc, pH 7.2). The cartridge was washed with 20% v/v ethanol–0.5 M NH_4OAc, pH 7.2 and dried for 2 min by the passage of air through the cartridge, the ethylsulphoxide epimer (**VI**) was then eluted with methanol and the methanol removed under a stream of oxygen free nitrogen.

Results and Discussion

Isolation and characterization

Monitoring the reaction of tipredane (**I**) with hydrogen peroxide revealed the presence of two small peaks (R_t = 12.7 and 12.9 min using HPLC conditions 1), which could be attributed to the tipredane ethylsulphoxide epimers (**VI**, see Fig. 2(a) for a typical chromatogram). In addition, the presence of the C-17 vinylmethylthio derivative (**VII**) was established and as the reaction was further monitored, the unknown

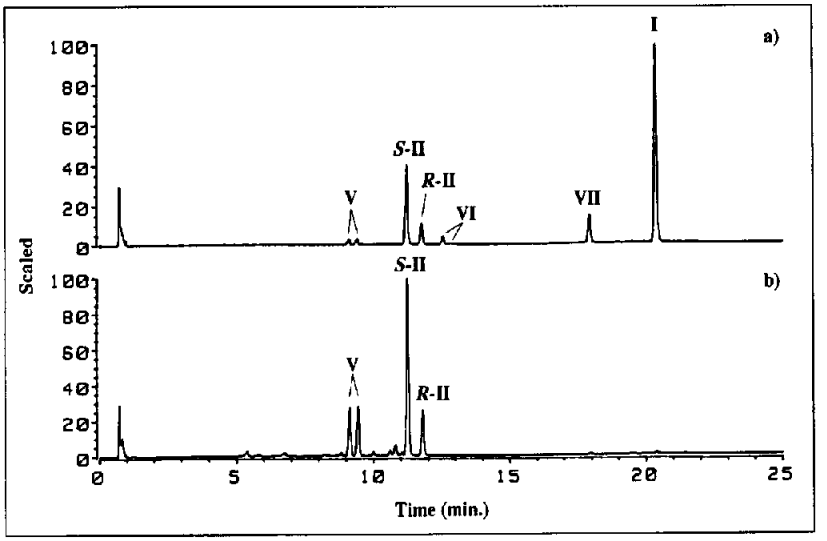
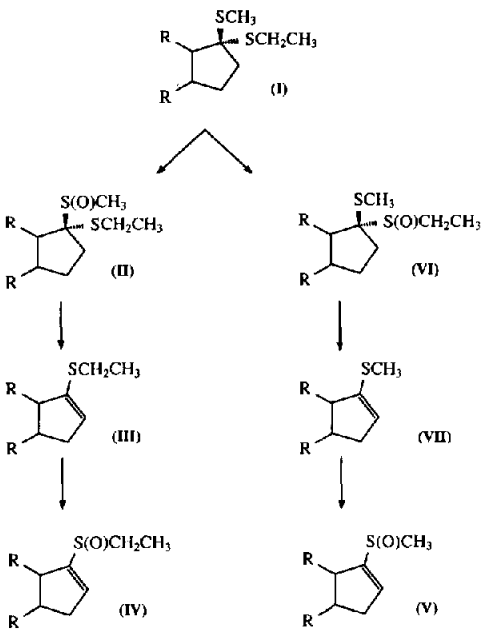


Figure 2
(a) Reaction of tipredane with hydrogen peroxide (7 days/room temperature), monitoring with HPLC conditions 1 (see Experimental section). (b) As (a) except 33 days/room temperature.

peaks and the C-17 vinylmethylthio derivative (**VII**) disappeared with the concomitant formation of the C-17 vinylmethylsulphoxides (**V**) in a ratio of 1:1 (see Fig. 2b). This strongly suggested that the S-oxidation with hydrogen peroxide, which was relatively slow at room temperature even with a large excess of oxidant, occurred at the ethylthio moiety (i.e. α-plane) as well as at the previously reported methylthio moiety (i.e. β-plane) [2]. The resultant ethylsulphoxide epimers (**VI**) then underwent a facile elimination of ethylsulphenic acid, even under the relatively mild reaction conditions, to yield the C-17 vinylmethylthio derivative (**VII**) which underwent further S-oxidation to yield the C-17 vinylmethylsulphoxide epimers (**V**) in a ratio of 1:1 (see Scheme 1).

Therefore, in an attempt to establish the existence of the tipredane ethylsulphoxide epimers (**VI**), the more powerful oxidants potassium peroxymonosulphate ($2KHSO_5.KHSO_4.K_2SO_4$) [7] and magnesium monoperoxyphthalic acid (MMPP) [8] were investigated allowing the reaction to be monitored at 4°C in the presence of an excess of tipredane (**I**). Monitoring both reactions by HPLC (conditions 1, i.e. 40°C column temperature and a mobile phase A of pH 4.5) indicated the production of relatively large amount of the suspected ethylsulphoxide epimers (**VI**).



Scheme 1
Proposed reaction pathway for the S-oxidation of tipredane with hydrogen peroxide.

However, HPLC results indicated that a significant amount of the C-17 vinylmethylthio derivative (**VII**) had been produced, this seemed unlikely to have been produced by elimination of ethylsulphenic acid in the reaction mixture due to the low reaction temperatures employed. This was later substantiated by the fact that chromatography of the pure *R*- and *S*-ethylsulphoxide diastereoisomers resulted in on-column elimination under these chromatography conditions (20 and 41%, respectively). In addition lowering the column temperature and using a neutral pH mobile phase minimized the degree of elimination from the ethylsulphoxide epimers (**VI**). Unfortunately, the ethylsulphoxide epimers (**VI**) appeared to co-elute using the modified HPLC conditions 1 (i.e. NH_4OAc pH 7 buffer and a column temperature of 26°C).

In order to achieve resolution of the ethylsulphoxide epimers (**VI**) and to prevent elimination of ethylsulphenic acid the following prerequisites were needed: a high efficiency column, low column temperatures and a neutral pH mobile phase. The mobile phase employed was as in HPLC conditions 1 with the exception that ammonium acetate pH 7 was used and the gradient started at a substantially higher percentage mobile phase B in order to elute the epimers quickly to prevent elimination of ethylsulphenic acid. A 10 cm Hypersil ODS 'Excel' column was employed since it had previously been observed that this type of column afforded the greatest separation of the analogous 6β-hydroxylated C16,17-vinylsulphoxide epimers of tipredane [9]. After extensive investigations, baseline resolution of the ethylsulphoxide (**VI**, R_t = 7.5 and 8.4 min) and methylsulphoxide (**II**, R_t = 4.4 and 5.6 min) epimers was obtained using HPLC conditions 2 (see Fig. 3).

The individual ethylsulphoxide epimers (**VI**) were isolated by heavily overloading the analytical HPLC conditions, both epimers were obtained in a purity in excess of 98.5% (see Fig. 3).

Subjecting the individual ethylsulphoxide epimers (**VI**) to TP-MS failed to yield a molecular ion corresponding to the proposed sulphoxide, instead the fragmental ion at *m/z* 349 ($M^+ + 1 - EtSOH$) was observed. In order to verify the molecular ion, FAB-MS was required. Working at 4°C the ethylsulphoxide

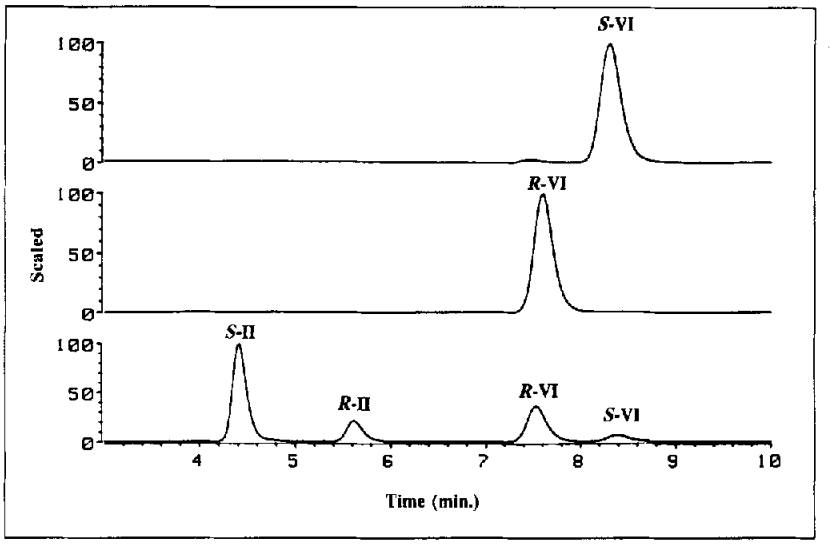


Figure 3
Resolution of the methyl and ethylsulphoxide epimers, isolation and purity checks of the *R*- and *S*-ethylsulphoxide epimers, using HPLC conditions 2 (see Experimental section).

epimers (**VI**, R_t = 7.5 min and R_t = 8.4 min) were desalted by loading the aqueous sample onto a C_{18} Sep Pak cartridge and washing with 20% v/v ethanol–0.5 M NH_4OAc, pH 7.2, the sulphoxides were then eluted with methanol and concentrated to yield a solid which was subjected to FAB-MS. Both ethylsulphoxide epimers (**VI**) produced a MH^+ ion at *m/z* 427. Addition of rubidium iodide further confirmed the molecular weight of 426 with the presence of the MRb^+ ion at *m/z* 511.

Stereochemistry

The configuration of the ethylsulphoxide epimers (**VI**) was proven by converting the individual epimers into their corresponding diasteroisomeric disulphoxides which are of known stereochemistry (paper in preparation, Part III). Therefore, the *R*- and *S*-configuration at the sulphoxide moiety could be assigned to the ethylsulphoxide epimers eluting at 7.5 and 8.4 min, respectively.

Stability of the ethylsulphoxide epimers (VI)

The individual ethylsulphoxide epimers (**VI**) in acetonitrile–ammonium acetate pH* 7.5 when exposed to temperatures up to 45°C for 18 h (see Fig. 4) and monitored using the optimized HPLC conditions 2, highlighted the extremely labile nature of both the ethylsulphoxides epimers (**VI**) towards elimination. Both epimers readily eliminated ethylsulphenic acid to yield the C-17 vinylmethylthio derivative (**VII**) which in turn partially hydrolysed to the C-17 keto derivative (**VIII**).

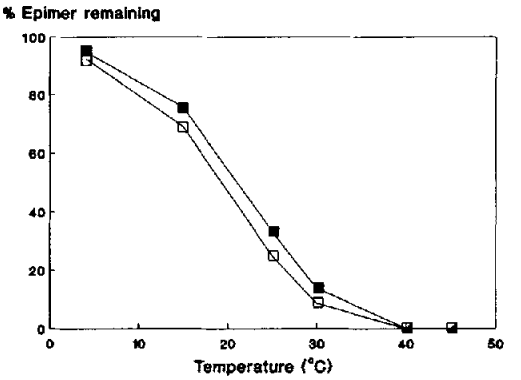


Figure 4
Thermal stability of *R* and *S*-ethylsulphoxide epimers in acetonitrile/ammonium acetate pH* 7.5 after 18 h. Key, -■-, *R*-epimer; -□-, *S*-epimer.

The *R*-epimer appeared to be slightly more stable than its corresponding *S*-epimer. Storage of the epimers at 4°C for 18 h produced 4.3 and 7.5% degradation in the *R*- and *S*-epimers, respectively (it is of interest to note that 0.8% epimerization of the *S*-epimer was also observed under these conditions). This instability in solution is in marked contrast to that of the corresponding methylsulphoxide epimers (**II**) [2] and presumably reflects the lack of steric impositions on forming the *syn*-periplanar transition state in the ethylsulphoxide epimers.

Site and stereoselectivity of the S-oxidation of tipredane (I)

This was investigated with the oxidants peroxymonosulphate, MMPP and dimethyldioxirane. A two-fold excess of tipredane was used to prevent over oxidation of the sulphoxides to the disulphoxides. Low reaction temperatures and the optimized HPLC conditions 2 were utilized to maintain the integrity of the sulphoxides formed.

In addition, the reaction of tipredane with the slow reacting oxidant hydrogen peroxide was monitored. The reaction was performed at ambient temperature which would result in degradation of the ethylsulphoxide epimers (**VI**) and as a consequence, the ratio of the *R*-:*S*-epimers formed could not be determined. The ratio of α:β plane S-oxidation, in the case of the hydrogen peroxide reactions was determined as follows: The α-plane S-oxidation was calculated as the Σ ethylsulphoxide epimers + vinylmethylthio + vinylmethylsulphoxide epimers, the β-plane S-oxidation was calculated as the Σ methylsulphoxide epimers + vinylethylthio + vinylethylsulphoxide epimers.

The vinylalkylthio and corresponding sulphoxide derivatives can only be derived as shown in Scheme 1 as it has previously been shown that tipredane is stable under these conditions. From Table 1 it can be seen that for all the oxidants investigated there is a preference for β-plane S-oxidation. The degree of site selectivity is dependent on the size/bulk of the oxidant employed, i.e. the preference for β-plane S-oxidation is more pronounced with the bulky peroxymonosulphate oxidant.

These results suggest that the degree of S-oxidation observed on the α-plane is controlled by the size of the attacking oxidant and reflects the limited accessibility of the lone pair of electrons of the sulphur atom in the α-plane.

All the oxidants investigated, with the exception of dimethyldioxirane, yielded the *S*-epimer in preference to the *R*-methylsulphoxide in a ratio of approximately 4:1.

In contrast to the peroxymonosulphate and MMPP oxidations of tipredane, dimethyldioxirane [4–6] in addition to producing the sulphoxides (**II** and **VI**) yielded the highly labile methylsulphone of tipredane (**IX**), which eluted at 11.5 min using HPLC conditions 2 (see Fig. 5). The collected fraction corresponding to the methylsulphone of tipredane (**IX**) exhibited facile elimination of methylsulphinic acid when stored at 40°C for 30 min yielding the C-17 vinylethylthio derivative (**III**).

The production of the methylsulphone has additionally been observed in the Kagan S-oxidation [10] of tipredane (Euerby and Mariotte, personal communication). Isolation and purification of the sulphone was accomplished by normal phase open column chromatography. The structure was unambiguously confirmed from the FAB-MS which gave a MH^+ ion at *m/z* 443, addition of rubidium iodide further confirmed the molecular weight of 442 with the presence of the MRb^+ ion at *m/z* 527.

The production of the methylsulphone (**IX**) highlights the additional nucleophilic character of dimethyldioxirane compared to peroxymonosulphate and MMPP [5, 6]. The ratio for α:β plane attack for the dimethyldioxirane reaction was calculated as before with the exception that the Σ β-plane attack additionally contained the methylsulphone (**IX**) contribution. A similar α:β plane S-oxidation preference was observed for dimethyldioxirane as

Table 1
The effect of oxidant on the site and stereoselectivity of the S-oxidation of tipredane

Oxidants	Ratio* α:β S-oxidation	Ratio* *S*-:*R*-Epimers Methylsulphoxide (**II**)	Ratio* *R*-:*S*-Epimers Ethylsulphoxides (**VI**)
Hydrogen peroxide	1:2.1	4.0:1	N.D.
Urea–hydrogen peroxide adduct	1:1.8	3.9:1	N.D.
Monomagnesium peroxyphthalic acid (MMPP)	1:2.0	3.7:1	3.5:1
Peroxymonosulphate	1:3.5	3.9:1	6.0:1
Dimethyldioxirane	1:1.8	2.6:1	5.4:1

N.D., Not determined.
*Reaction distribution after 35 min at 4°C for the peroxymonosulphate and MMPP reactions, 37 min at −18°C for the dimethyldioxirane reaction and at ambient conditions for the hydrogen peroxide reactions, the ratios did not change as a function of time.

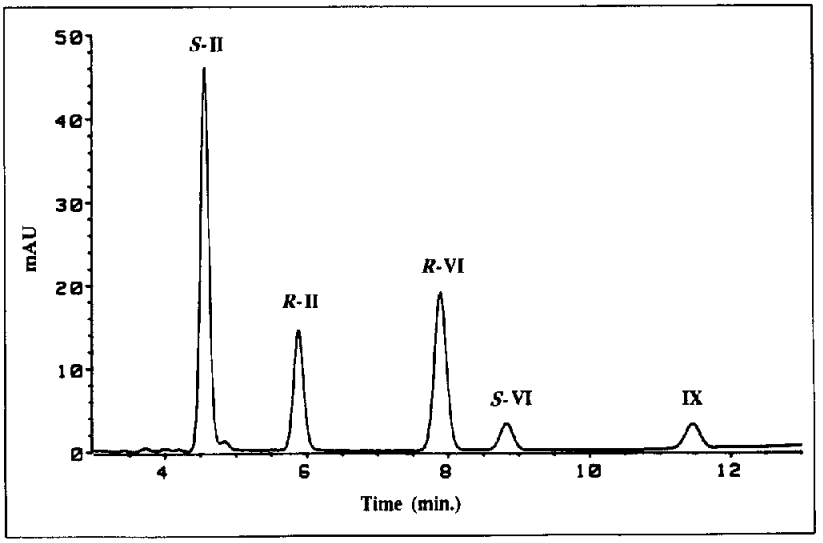


Figure 5
Chromatography of the dimethyldioxirane S-oxidation of tipredane, using HPLC conditions 2 (see Experimental section).

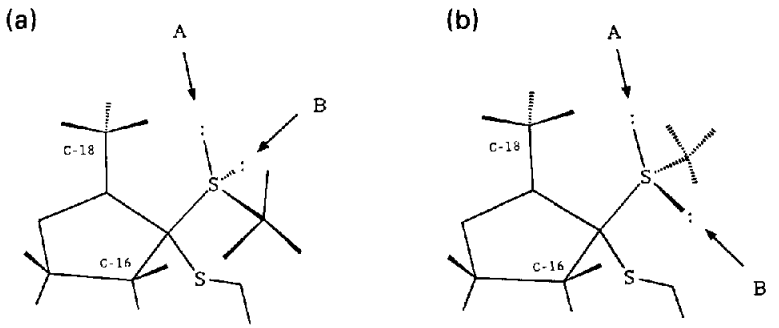


Figure 6
Three-dimensional representation of the preferred rotamers giving the stereoselectivity observed at the β-plane. (a) Rotamer 1 and (b) rotamer 2.

seen with the other non-sterically hindered oxidants (see Table 1). The *S*-:*R*-methylsulphoxide epimer production was significantly different from the ratio of approximately 4:1 observed with other oxidants. This may be attributed to the fact that more disulphoxide was formed using this powerful oxidant and that the *S*-methylsulphoxide epimer may be further oxidized to the sulphone more rapidly than its corresponding *R*-epimer, hence apparently yielding a lower preference for *S*-methylsulphoxide production.

The preference for *S*-methylsulphoxide production in favour of the corresponding *R*-epimer can be rationalized by considering the rotamers at the C-17 position. The preferred configuration around the C-17 position will be when steric interactions are minimum, i.e. when the methylthio and ethylthio groupings are furthest apart. If we consider rotamer 1 (see Fig. 6a), the oxidant would favour attack at position B as the oxidant would experience axial steric interactions with the C-18 methyl protons on approaching the sulphur lone pair of electrons from position A. Attack of the oxidant at the more accessible lone pair of electrons (position B) would yield the *R*-methylsulphoxide epimer. In rotamer 2 (see Fig. 6b), the same situation exists, i.e. the oxidant would prefer to approach the lone pair of electrons from position B which would yield the *S*-methylsulphoxide.

The *S*-methylsulphoxide is formed preferentially (see Table 1). This suggests that rotamer 2 would be more energetically favoured this is possibly due to the fact that it lacks the steric

interaction between the methylthio group and the C-16 β protons which is present in rotamer 1 (see Fig. 6(a) and (b)).

The ratio of *S*-:*R*-methylsulphoxide epimers produced appears to be independent of the type of oxidant employed, see Table 1 (the exception being dimethyldioxirane). This would not be the case for attack parallel to the C-18 methyl group (i.e. attack from position A in Fig. 6(a),(b)) which would be sensitive to the oxidant's size. This suggests attack parallel to the C-18 methyl group does not occur, and that the *S*:*R* ratio is due to the population distribution between conformations shown in Fig. 6(a)(b).

The same steric arguments can be used to explain the stereoselectivity observed of the S-oxidation at the ethylthio substituent (α attack). From Table 1 it can be seen that the *R*-ethylsulphoxide epimer is preferentially formed. This can be explained by considering Fig. 7(b) from which it can be seen that attack of the oxidant on the lone pair of electrons, from both positions A and B of rotamer 2, is restricted compared to attack at position B of rotamer 1 (see Fig. 7a). Attack at the latter position, of rotamer 1, would yield the *R*-ethylsulphoxide epimer whereas attack at the most accessible of the restricted lone pair of electrons of rotamer 2 (i.e. attack at position B, see Fig. 7(b)), which would be the most energetically favoured rotamer, would have yielded the corresponding *S*-ethylsulphoxide epimer.

The extent of S-oxidation at the α-plane in rotamer 2, yielding the *S*-ethylsulphoxide, would be highly dependent on the steric nature of the attacking oxidant, i.e. the planer MMPP and the small peroxide (H_2O_2) can attack the lone pair of electrons more easily than the larger spherical oxidant (peroxymonosulphate), this is reflected in the larger *R*-:*S*-ethylsulphoxide ratio formed with peroxymonosulphate then MMPP (see Table 1).

Therefore in rotamer 2 there is a large preference for β-plane S-oxidation giving rise to the *S*-methylsulphoxide epimer than α-plane S-oxidation giving the *S*-ethylsulphoxide epimer, whereas in rotamer 1, there appears to be a more equal preference for both α and β plane attack giving the *R*-ethylsulphoxide and *R*-methylsulphoxide respectively (see Table 2).

This research into the site and stereoselectivity of the primary S-oxidation of tipredane

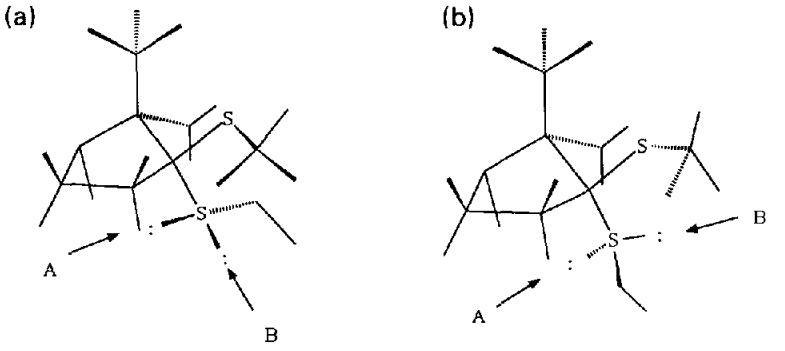


Figure 7
Three-dimensional representation of the preferred rotamers giving the stereoselectivity observed at the α-plane. (a) Rotamer 1 and (b) rotamer 2.

Table 2
The effect of oxidant on the percentage peak area of methyl and ethylsulphoxide epimers produced

	% Peak area*			
	Methylsulphoxides		Ethylsulphoxides	
Oxidants	*S*-Epimer	*R*-Epimer	*R*-Epimer	*S*-Epimer
Monomagnesium peroxyphthalic acid (MMPP)	25.3	6.9	12.8	3.4
Peroxymonosulphate	36.9	9.4	11.3	1.9
Dimethyldioxirane†	12.1	4.7	7.5	1.4

*Reaction distribution after 35 min at 4°C.
†Reaction distribution after 37 min at −18°C, in addition 1.4% methylsulphone (**IX**) produced.

will be extended to investigate a range of structurally diverse chiral oxidants, these findings will be reported at a later date.

Acknowledgements — We wish to thank the following for technical support and advice, Dr I.G. Beattie (MS), Dr C. Thomson, Mr D.J.P.N. Mariotte and Dr G.E. Taylor; Mr A. Morlin for the preparation of dimethyldioxirane in acetone.

References

[1] R.K. Varma, U.S. Patent No. 4361559 (1982) (to E.R. Squibb and Sons).
[2] M.R. Euerby, J. Hare and S.C. Nichols, *J. Pharm. Biomed. Anal.* **10**, 269–277 (1992).
[3] M.S. Cooper, H. Heaney, A.J. Newbold and W.R. Sanderson, *Synlett.* 533–535 (1990).
[4] R.W. Murray and R. Jeyaraman, *J. Org. Chem.* **50**, 2847–2853 (1985).
[5] W. Adam, R. Curci and J.O. Edwards, *Acc. Chem. Res.* **22**, 205–211 (1989).
[6] R.W. Murray, *Chem. Rev.* **89**, 1187–1202 (1989).
[7] B.M. Trost and D.P. Curran, *Tetrahedron Lett.* **22**, 1287–1290 (1981).
[8] P. Brougham, M.S. Cooper, D.A. Cummerson, H. Heaney and N. Thompson, *Synthesis* 1015–1017 (1987).
[9] M.R. Euerby, C.M. Johnson and S.C. Nichols, *Anal. Proc. (London)* **30**, 46–51 (1993).
[10] P. Pitchen and H.B. Kagan, *Tetrahedron Lett.* **25**, 1049–1052 (1984).

[*Received for review 16 September 1993; revised manuscript received 22 November 1993*]