

# Electron Impact Induced Elimination of HNO<sub>2</sub> from Trifluralin–Phenylenediamine Dimers — an *ortho*-Effect Resulting from a $\pi$ – $\pi$ Interaction Persisting into the Vapour Phase†

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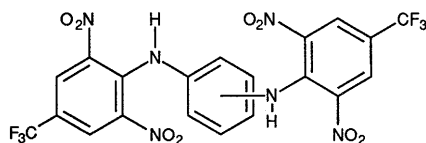
**In this paper we attempt to explain the two neutral HNO<sub>2</sub> losses observed from the *ortho* isomer of the title compounds in terms of a minimum energy conformation which we suggest persists into the vapour phase. This is supported to some extent by preliminary molecular modelling studies. The ‘splitpin’ shape of the *ortho* molecule brought about by  $\pi$ – $\pi$  stack interactions brings the nitrated rings together in a favourable orientation, allowing hydrogen transfer processes to occur. © 1997 by John Wiley & Sons, Ltd.**

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Trifluralin (2,6-dinitro-(*N,N*-dipropyl-4-trifluoromethyl)benzene) is a potent herbicide<sup>1</sup> that has recently been shown to selectively inhibit proliferation and differentiation of various protozoa such as *Leishmania mexicana*<sup>2</sup> and *Plasmodium falciparum*<sup>3</sup>, apparently because it binds to *Leishmania* microtubulin in preference to host tubulin. Our work has involved synthesizing several novel *N*<sup>1</sup>,*N*<sup>2</sup>-bis(2,6-dinitro-4-trifluoromethylphenyl)diamine type dimers<sup>1</sup> related to trifluralin with the aim of both probing putative drug-receptor interactions and screening them against *Leishmaniasis* or other tropical diseases such as malaria and trypanosomiasis in both animals and humans.



**Scheme 1.** *N*<sup>1</sup>,*N*<sup>2</sup>-bis(2,6-dinitro-4-trifluoromethylphenyl)diamine type dimers (1)

In the course of this work we made all three of the trifluralin adducts of the diphenylene diamines, the formulae of which are shown on Figs. 1, 2 and 3, by reacting 1-chloro-2,6-dinitro-4-trifluoromethylbenzene with the appropriate diamine. Whereas the most general features of the electron impact (EI) mass spectra of these diamine compounds are losses of 16 (O), 30 (NO), 46 (NO<sub>2</sub>) and 19 (F) mass units in various sequences, the *ortho*-phenylenediamine derivative alone showed intense ions resulting from two successive losses of 47 mass units, thought to be due to HON=O, followed by minor conventional NO, NO<sub>2</sub> and F losses, even though the possibility of similar HON=O elimination exists in all three isomers.

## EXPERIMENTAL

The mass spectra were determined using a VG Analytical (Floats Road, Altrincham, Manchester, UK) 70–250 MS, with a DEC PDP 11/24 data system, at 70eV and a source temperature of ~200 °C. Samples were admitted by a direct insertion probe heated to 250–300 °C. High resolution measurements were made at a resolving power of ~10 000, using a calibration with perfluorokerosene.

All materials used were of the highest purity commercially available from both Lancaster Synthesis (Eastgate, Lancashire, UK) and Aldrich Chemical Co. (Gillingham, Dorset, UK). Thin-layer chromatography (TLC), was carried out on pre-coated silica gel 60 plates and substances were detected using a UV light source (254 nm).

The diphenylenediamine adducts were prepared as follows: 4-chloro-3,5-dinitrotrifluoromethylbenzene (5.41g) was dissolved in dry toluene (40cm<sup>3</sup>) and added dropwise to a stirred solution of the diphenylenediamine (1.08g) and triethylamine (2.05 cm<sup>3</sup>) under nitrogen. The mixtures were heated for 4–5 h, until TLC showed that the reaction was complete, then cooled, filtered, washed with water, air-dried and recrystallized from methanol. The 1,2-adduct (58% yield), had a m.p. of 252–254 °C; the 1,3-adduct (70% yield) a m.p. of 264–266 °C, and the 1,4-adduct (45% yield) a m.p. of 316–318 °C. Fourier-transform infra-red spectroscopy was performed using a Mattson (Maddison, Wisconsin, USA) Galaxy series FT-IR 5000 instrument using a 1% compound/KBr mixture compressed into flats under vacuum. This analysis and <sup>1</sup>H, <sup>13</sup>C, Distortionless Enhancement by Polarization Transfer (DEPT), and deuterium oxide exchange NMR spectra (Bruker Spectrospin, Coventry, UK; 250 MHz FT Aspect 2000) were consistent with the expected structures.

Molecular modelling studies were performed on a Silicon Graphics Indy workstation and used the MM2\* forcefield of MacroModel Version 4.0<sup>4</sup> to predict global energy minimum conformations of the three isomers. Systematic conformational searching was performed at 15–30° intervals.

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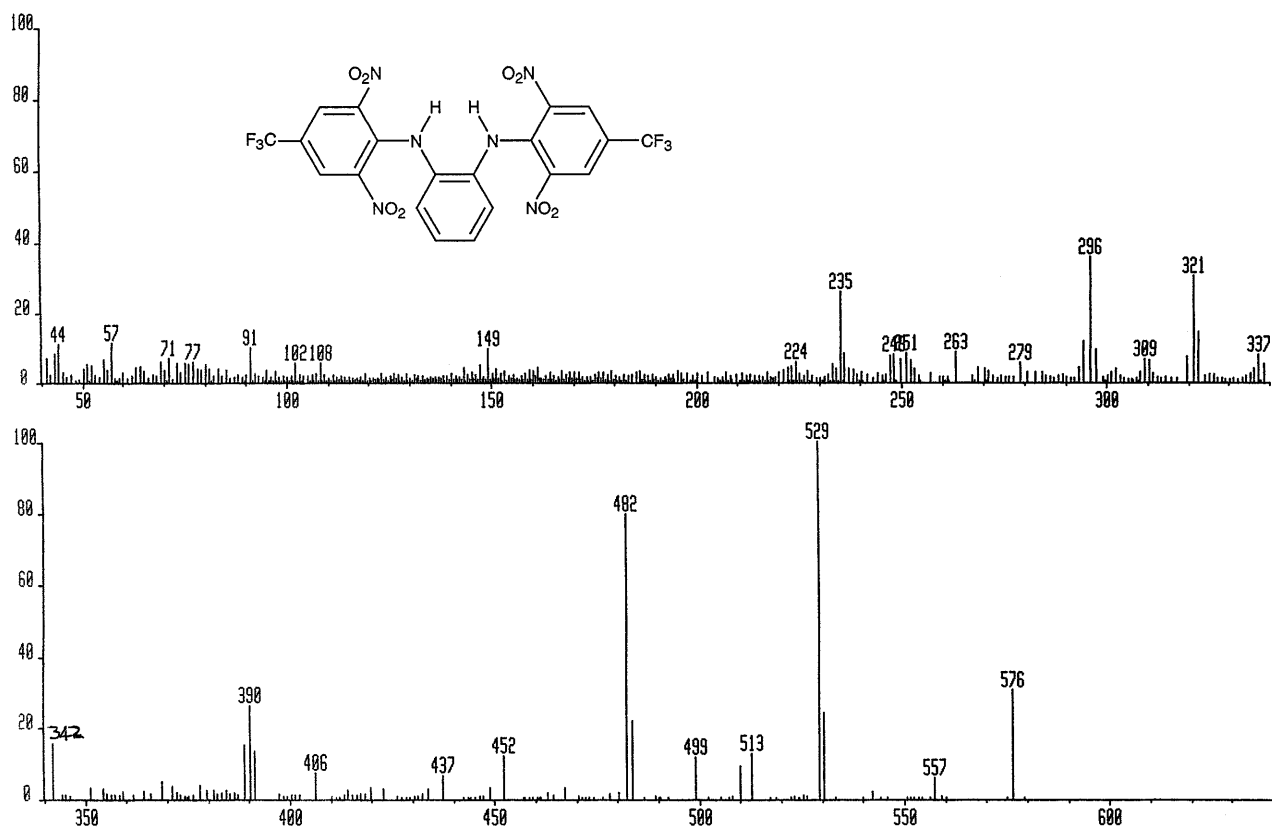


Figure 1.

## RESULTS AND DISCUSSION

The EI mass spectra of the *ortho*-, *meta*- and *para*-phenylenediamine adducts of 2,6-dinitro-4-trifluoromethylbenzene are shown in Figs 1, 2 and 3 respectively. It is immediately apparent that the *meta* and *para*

derivatives give intense stable molecular ions with restricted losses of O, OH, F, NO and NO<sub>2</sub> neutral fragments, as expected for aromatic nitro compounds and a trifluoromethyl group.<sup>5</sup> There is some evidence for  $\alpha$ -cleavage at the imino groups with either hydrogen transfer or self-protonation to yield the M<sup>+</sup> ions of the

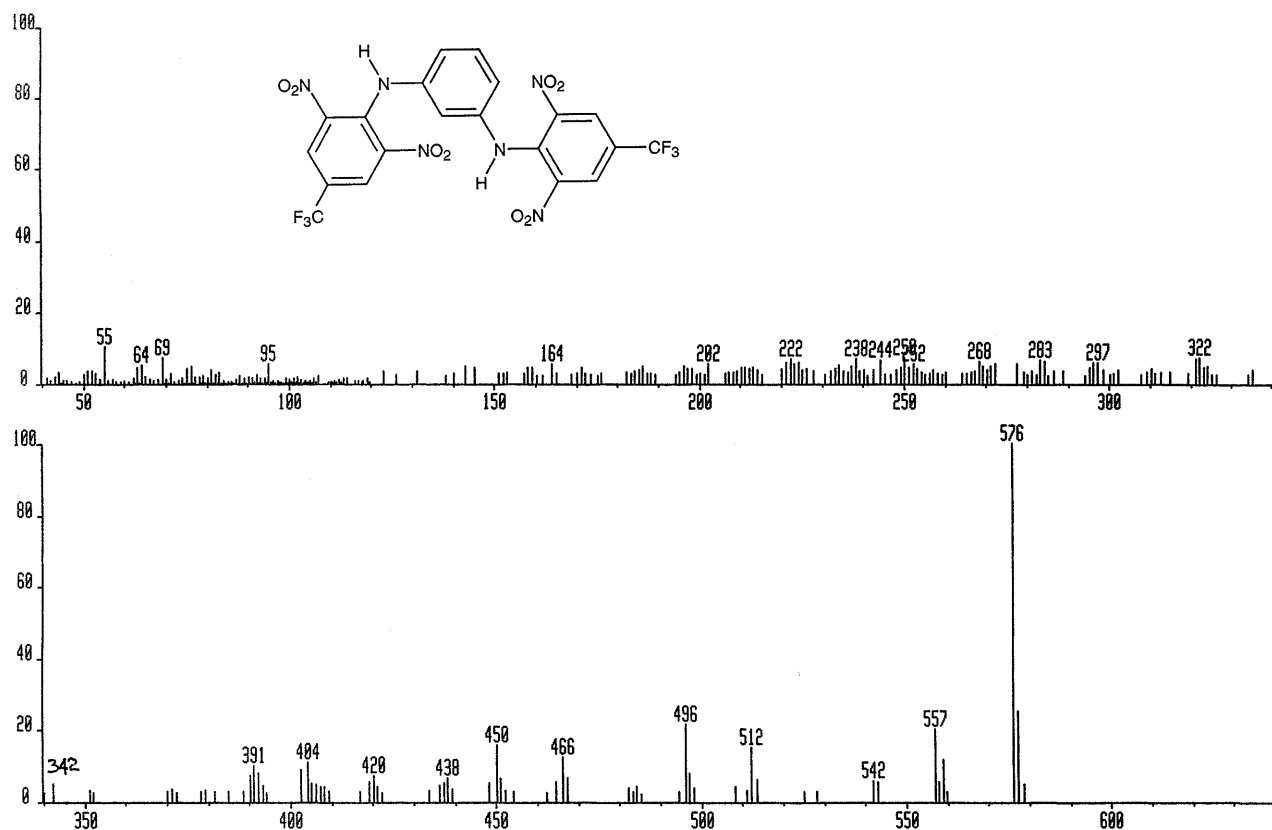


Figure 2.

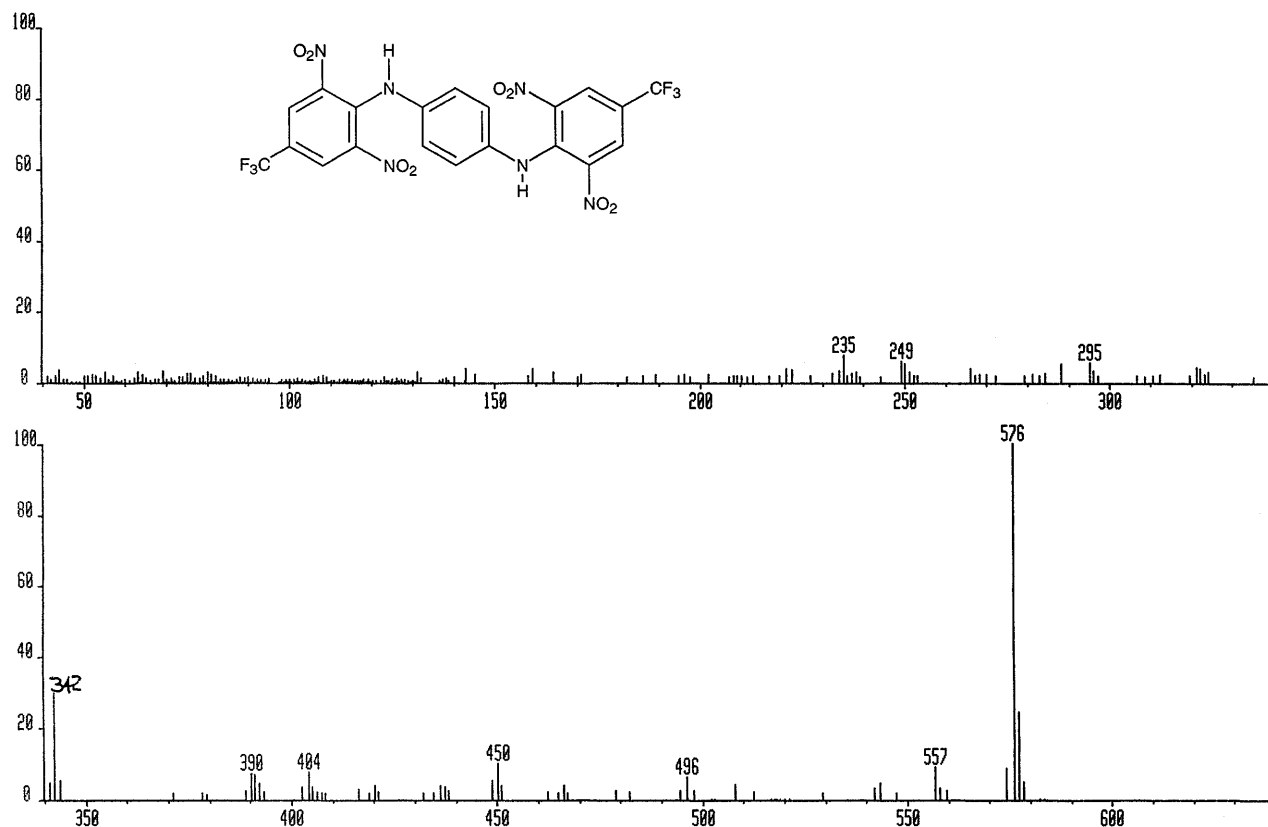
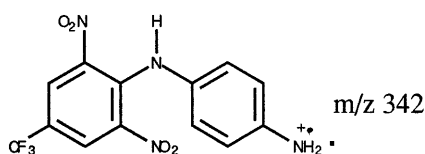


Figure 3.

mono-trifluorodinitrobenzene adduct of  $m/z$  342 in all three spectra; this ion could also be due to an impurity of the mono-adduct, though these are twice recrystallized samples.

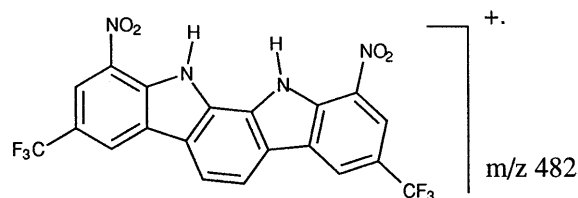


Scheme 2.

The spectrum of the *ortho*-bis-trifluorodiphenylenediamine adduct, by contrast is very distinctive (Fig. 1). About 25%  $M^{+\bullet}$ -ion is seen, but the base peak is  $m/z$  529  $[M-HNO_2]^{+\bullet}$  and the ion of  $m/z$  482, resulting from consecutive loss of the elements a  $HNO_2$  is of 80% relative abundance. Since we are aware of few, if any, literature reports of  $HNO_2$  losses from aromatic nitro compounds (loss of  $HO^\bullet$  is common<sup>5</sup>), we have confirmed these processes by high resolution mass measurements on  $m/z$  576, 529 and 482 (see Table 1).

Since the *ortho* compound shows the  $HNO_2$  losses and the *meta* and *para* isomers do not, it is tempting to ascribe them to an *ortho* effect and leave it at that. However, it is perfectly possible to devise novel pentacyclic structures for the  $(M-2 HNO_2)$  ions by elimination of both *ortho* hydrogens of the diamino

rings and a nitro group from each trifluralin ring in all three isomers. An example of such a structure is:



Scheme 3.

In the case of the *meta* isomer this leads to two structures depending on whether the 2,4- or 4,6-hydrogens are involved. There is no obvious reason why the *ortho*-phenylenediamine derived ion should be so intrinsically more stable than those of the *meta* and *para* isomers as to cause its domination of the mass spectrum to the extent observed.

Simple ball and stick models and molecular modelling of the un-ionized isomers showed that three possible factors might be involved. In the *ortho* isomer the preferred conformation was a hairpin shape brought about by  $\pi$ - $\pi$  stack interactions, bringing the nitrated rings together in a favourable orientation for single-electron transfer (SET) processes to occur. In the *meta* isomer steric overcrowding arising from the nitro groups of the two trifluralin rings could inhibit the transfer of the 2-hydrogen, though not the 4- and 6-hydrogens, while the *para* isomer's shape would not allow close approach of the nitro oxygens to the 2-hydrogen positions.

More sophisticated molecular modelling using MacroModel Version 4.0 sheds further light. Figure 4 shows the global minimum energy conformers with the key H—O distances in Ångstrom units. The sum of the van

Table 1.

Formula	Measured mass	Theoretical mass
$C_{20}H_{10}N_6O_8F_6$	576.0445	576.04643
$C_{20}H_8N_5O_6F_6$	529.0471	529.04570
$C_{20}H_6N_4O_4F_6$	482.0455	482.04497

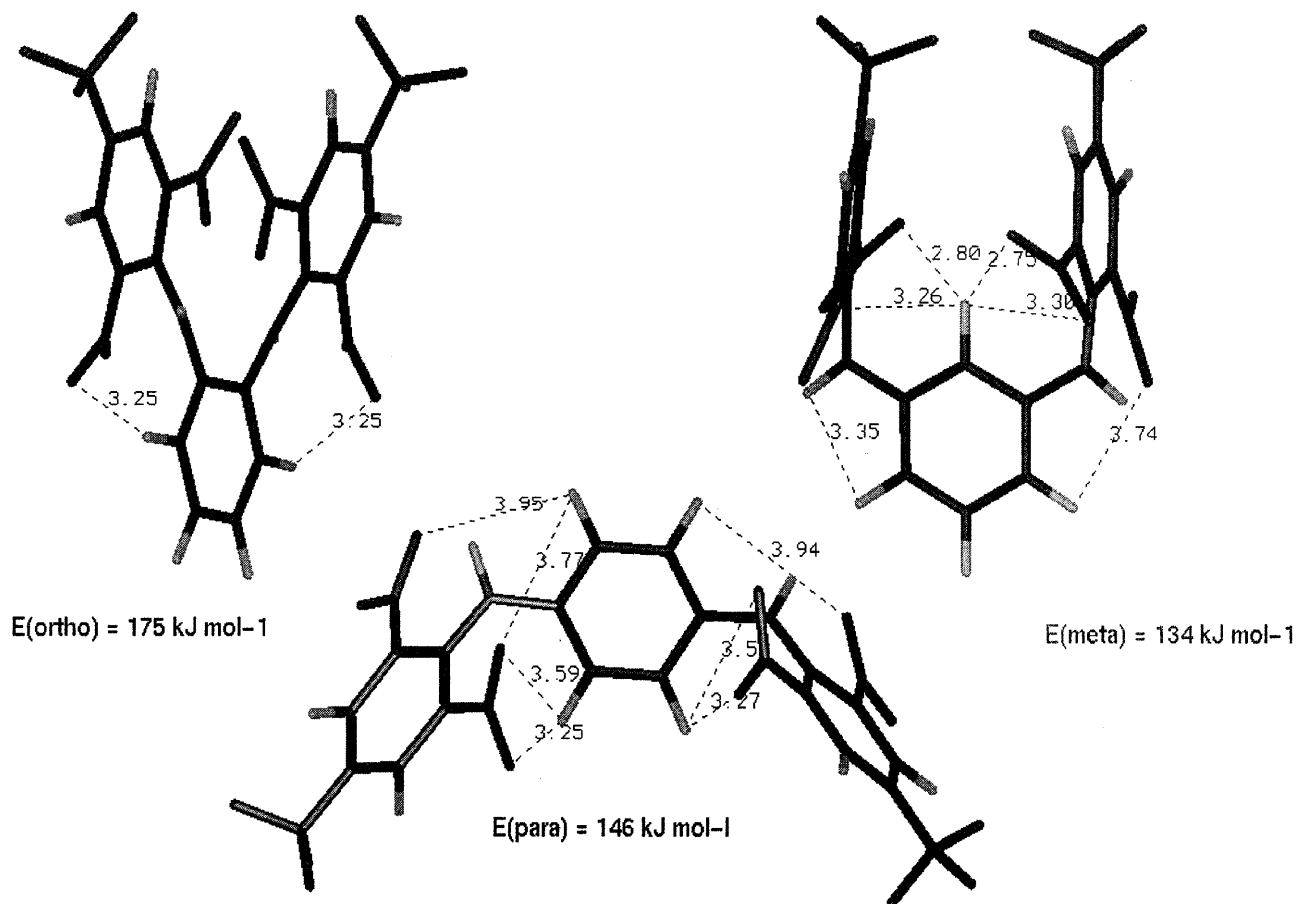


Figure 4. Global minimum energy conformers of the *o*-, *m*- and *p*-isomers

der Waals covalent radii for H and O is about 2.7 Å; thus, it is reasonable to assume that in those conformers where the *ortho* hydrogens and the nitro oxygens can approach one another to this extent hydrogen transfer could occur, but that transfer would be difficult or impossible where this distance was significantly larger.

The interesting points about these results are that the *meta* isomer has the lowest energy at 134 kJ mol<sup>-1</sup> and the closest H—O approach, to the 2-hydrogen, at

2.75–2.80 Å. In the *ortho* isomer, which has the highest energy by some margin, the closest H—O distance is 3.25 Å, similar to that for the *para* isomer which has an intermediate energy of 146 kJ mol<sup>-1</sup>. H—O distances to the 4- and 6-hydrogens in the *meta* isomer are also in the 3.3–3.8 Å range which would appear to rule out the involvement of facile SET processes. So, if these conformers were maintained in the gas phase, and after ionization, only the *meta* isomer appears to be able to

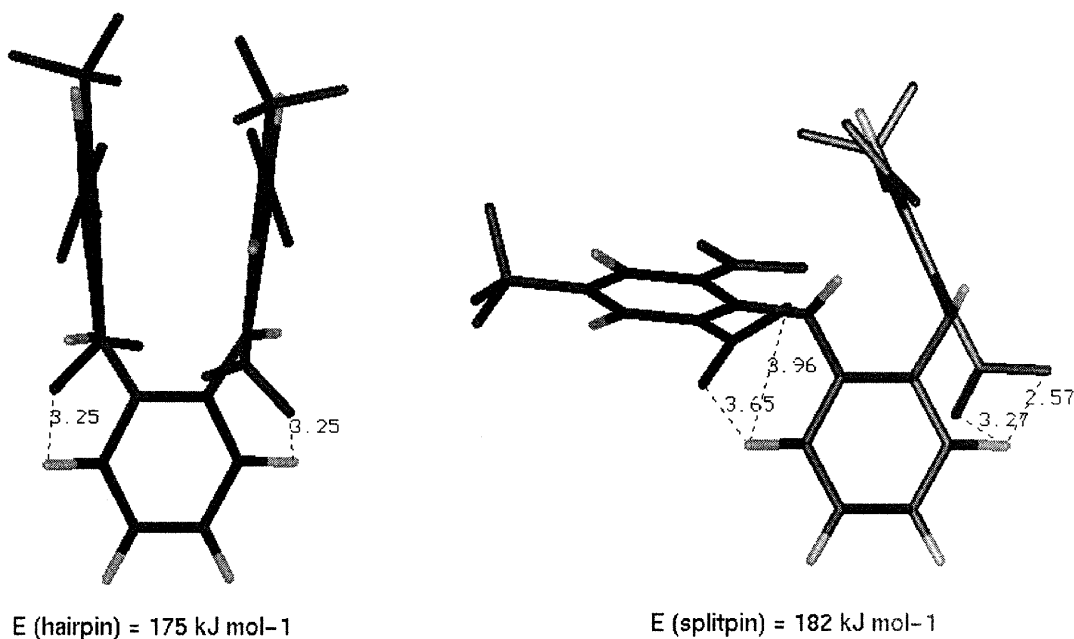


Figure 5. Closest H—O contacts in low energy conformers of the *ortho* isomer.

allow an *ortho* hydrogen to approach sufficiently close to a nitro oxygen to transfer and give rise to the HNO<sub>2</sub> fragment. If hydrogens as far away as 3.25 Å could transfer, all three isomers would lose HNO<sub>2</sub>.

Using the MacroModel Modelling package, it is possible to search for conformations, among the hundreds generated, that have a stipulated distance between selected atoms. When this approach was used it revealed that the next higher energy conformer of the *ortho* isomer, with an energy of 182 kJ mol<sup>-1</sup>, had a closest H—O contact to the 2-hydrogens of 2.57 Å, suitable for hydrogen transfer to occur. This conformer is no longer symmetrical, one of the legs of the hairpin being bent away from the other. Thus, it resembles the splitpin used to secure a wheel to an axle, and we call this the 'splitpin' conformation, as shown in Fig. 5.

We believe the explanation of the facile elimination of two HNO<sub>2</sub> fragments from the *ortho*-phenylenediamine adduct is that the splitpin conformation persists into the vapour phase, and the higher energy of this conformer, with its suitable van der Waals H—O distance of closest approach, promotes the SET processes leading to the formation of the highly stabilized pentacyclic azacycle molecular ion of *m/z* 482 shown in Scheme 3.

The *para* isomer is more stable and the minimum contact is too long for hydrogen transfer and, while for the *meta* isomer although the conformer is quite stable, the approach to the 2-hydrogen is too hindered by the other nitro group and the transition state may lack the necessary planarity. All other H—O distances are too long.

Modelling of the ground state conformations is clearly not conclusive and we intend to carry out further molecular orbital modelling studies on the molecular ions themselves. It would also be interesting to attempt to prepare the azacycles from the three adducts using radical-mediated cyclization, in the expectation that only the *ortho* compound will lead to the pentacyclic product which is related to the heterocyclic portion of several anticancer drugs including K-252a, staurosporine and rebeccamycin.<sup>6</sup>

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