APPLICATION OF ELECTRON SPIN RESONANCE TO CONFORMATIONAL ANALYSIS

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ABSTRACT

Conformational analysis by electron paramagnetic resonance study is naturally limited to free radicals. Very interesting information on these molecules is given by e.p.r., and with short-lived radicals e.p.r. is often the only method for conformational analysis. Then, one needs a complementary hypothesis of theoretical origin. For monoradicals, it can be theoretically and experimentally verified that hyperfine splittings are geometrically dependent. Moreover, one can observe dynamic processes corresponding to jumps between conformations, the critical frequency being about 10^7 Hz. Different examples are given for aromatic and aliphatic radicals.

For biradicals, other interactions are measurable; particularly the exchange interaction J and the dipolar interaction D. The variation of the exchange interaction J in terms of geometry is only known qualitatively. On the other hand, the simple variation of dipolar interaction D, which depends only on the distance between the two unpaired electrons, permits quantitative evaluations.

There are now many examples of the application of electron spin resonance (e.s.r.) to the study of conformational analysis. Geske[1] has summarized most of the work published up to 1966. In this paper, we shall try to show how original can be the contribution of e.s.r. to conformational analysis, compared to other physical methods.

One can correlate the various data obtained from e.s.r. (g-value, hyperfine splitting, line width) with structural parameters. However, a model is necessary and the conclusions obtained depend on the choice of the particular model. We shall study the following problems:
1. Determination of the geometry at the radical centre.
2. Conformational analysis of monoradicals.
3. Conformational analysis of biradicals.

1. GEOMETRY AT THE RADICAL CENTRE

Although the determination of a planar or distorted structure of a radical is not strictly conformational analysis[2], it is important as an illustration of the methods that can be used in e.s.r. for such a determination.

We should like to demonstrate the principles of the method using the CH_3 radical; in a simple description (*Figure 1*) the unpaired electron is

localized in a $2p$ carbon atomic orbital if this radical is planar, and in a hybrid orbital if the radical is pyramidal, the hybridization ratio s/p increasing as the carbon atom recedes from the hydrogen plane. Using e.s.r. it is possible to measure the hyperfine splittings or coupling constants of the unpaired
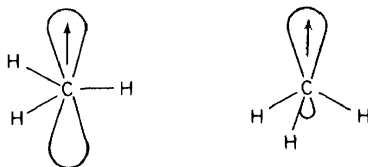

Figure 1

electron with non-zero spin nuclei. One must distinguish between the isotropic coupling constant a and the anisotropic coupling tensor **b**.

In the case of CH_3 it is possible in principle to measure the coupling constants a_c and $\mathbf{b}_c$ of the unpaired electron with ^{13}C. Qualitatively it is expected that the greater a_c, the greater the s percentage of the unpaired electron orbital, i.e. the more CH_3 is distorted. Conversely, the greater **b**, the greater the p percentage of the unpaired electron orbital, i.e. the more CH_3 is planar. Quantitatively, one must rely upon models that allow calculations.

Model 1. One calculates by approximate quantum mechanics methods[3] the expected coupling constant A_c (or $\mathbf{B}_c$) for the unpaired electron fully localized in a carbon $2s$ (or $2p$) orbital.

(*a*). Assuming that there is no delocalization of the unpaired electron over hydgogen, a_c/A_c is expected to give the s percentage of the unpaired electron orbital. This gives the hybridization ratio and hence the torsion angle.

(*b*). A more accurate method takes into account the possibilities of delocalization: the ratio of a_c/A_c to b_c/B_c gives the hybridization ratio, even if the unpaired electron is not fully localized on the carbon. It is then possible to deduce the torsion angle[3].

Model 2. There are now methods for calculating (with good approximation[4]) the spin densities at a given nucleus thus giving a measure of the isotropic coupling constants. For CH_3, this calculation can be made for different torsion angles[5] but, although it is known from i.r. analysis[6] that the methyl radical is planar, it is not possible to give an unambiguous answer by using only e.s.r. data.

Another example is the cyclohexyl radical (*Figure 2*). Following model Ia, Fessenden[7] has found that the isotropic ^{13}C hfs are consistent with a
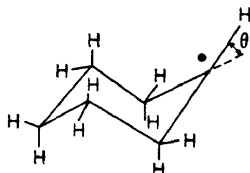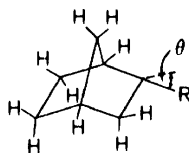

Figure 2

planar radical centre (H in the C_1, C_2, C_6 plane). Following model II, Hudson[8] has found that the experimental results are in agreement with a distorted radical centre.

Recently, Gloux, Guglielmi and Lemaire[9], have examined bicyclo(2,2,1)-heptyl radicals (*Figure 3*). Since the β hydrogens from α methylene have


A : R = H
A : R = OH

Figure 3

different coupling constants, they conclude that the radical centre is non-planar, the difference in coupling constants reflecting the different spatial position of the β hydrogens relative to the unpaired electron. Nitroxide radicals

$$R\text{---}N\text{---}R'$$
$$|$$
$$O$$
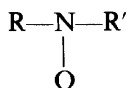
are expected to be non-planar[10, 11].

It has been shown by x-ray analysis that radicals I and II are distorted, the torsion angle being of the order of 20° in both radicals[12, 13]. However, e.s.r. measurements on radicals III, IV and V in a rigid matrix are consistent with a planar structure of the CNOC group[14].



I

CH₂I
II

III

IV

V

625
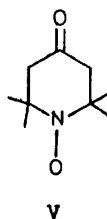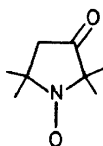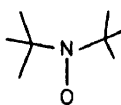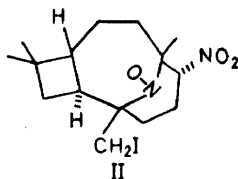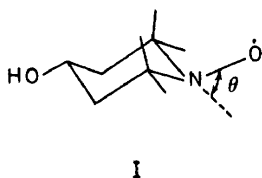
It is possible that this discrepancy stems from the fact that different molecules may have different geometries at the same centre. One must, however, remember that it is important to know the shape of the energy variation curve with torsion angle and this suggests that the energy may not be very sensitive to the torsion around the NO group.

## 2. CONFORMATION OF CYCLIC NITROXIDES

Piperidine nitroxides can be prepared and their conformation can be studied by e.s.r. Their spectra are sometimes temperature dependent and this dependence gives information about characteristic energy values.

Unstable piperidine nitroxides can be obtained by oxidizing the corresponding amines[15-17] or by photolysing the parent hydroxylamines[18]. The spectra obtained show coupling of the unpaired electron with the nitrogen nucleus and with the β hydrogens (from C_2 and C_6 methylene).
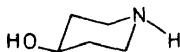
We have examined spectra obtained from piperidine VI, from 2-, 3-, or 4-methyl substituted piperidine (VII, VIII, IX), from 4-phenyl- (X), and 4-hydroxy-piperidine (XI) and from two 'rigid' amines, solasodine (XII)
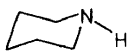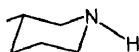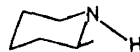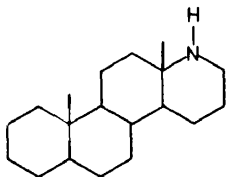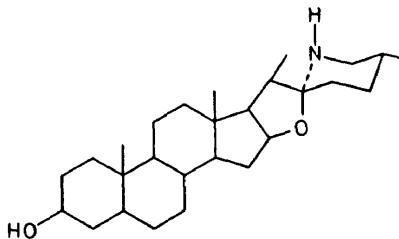
VI

X

XI

IX

VIII

VII

XIII

XII
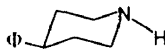
and 17a-aza-D-homo androstane (XIII). The results from rigid amines are in agreement with an angular dependence

$$a_H = A_0 + A_2 \cos^2 \theta$$

(θ being the angle of the HCN plane with the perpendicular to the CNC plane) (*Figure 4*).
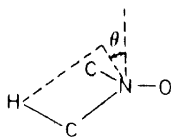

Figure 4

This relationship can be calculated by semi-empirical methods[19].

Using this relation, e.s.r. spectra of piperidine derivatives are interpreted by attributing a chair conformation to the piperidine ring. In this case, the measured hyperfine splittings are not exactly those expected for an ideal chair. However, it would be unsafe to attribute this non-correlation of the experimental results with calculated values to distortion of the molecule; the $\cos^2 \theta$ relationship is empirical and only gives a first approximation. The shape of the molecule is rather chain-like, since a twist conformation would give other hyperfine splittings and a boat conformation is less probable.

Dynamic processes

The variation of e.s.r. spectra as a function of temperature can be related to energy values. In the case of piperidine derivatives, the spectra we obtained permitted only qualitative conclusions, but Windle, Kuhnle and Beck[18] obtained better spectra by photolysing hydroxylamines and were able to obtain quantitative results.

The model used is a 'jump model'—the molecule 'jumps' from one conformation to another one, of identical energy in the case of piperidine, and of different energy in the case of substituted piperidines. The critical frequency of this process is of the order of 10^7 MHz.

Windle, Kuhnle and Beck used a model analogous to the one used in n.m.r.[20, 21] to relate the line width to the lifetime of different conformations, They found the following values for barriers to inversion between the two equal energy conformations:

For piperidinoxy   5.0 $\pm$ 0.1 kcal/mole
For morpholinoxy 5.7 $\pm$ 0.2 kcal/mole

For substituted piperidines there are two conformations of different energy: if there is 'fast exchange', the hyperfine splittings are the weighted average of the hyperfine splittings of the two conformers (axial and equatorial substituent). By assuming values for the fixed conformers, one obtains in these cases the difference in energy between the two conformers: for 4-methyl piperidine Windle, Kuhnle and Beck found an energy difference (ΔG at 80°C) of 1.6 kcal/mole between the two conformers.

The inversion barriers (calculated from line width) are 6.9 ± 0.2 and 5.3 ± 0.2 kcal/mole for one or the other conformation. Similarly, the energy difference between the two 4-phenyl piperidine conformers is estimated as 2.5 kcal/mole, the barriers to inversion being 8.8 ± 1.2 and 6.3 ± 0.2 kcal mole.

We have studied radicals from 2,3- and 4-methyl-piperidine[16]. Although our results are mainly qualitative, due to the low resolution of our spectra, these radicals show an interesting phenomenon: at room temperature the 4-methyl- and 3-methyl-piperidine show two strong hydrogen splittings and two smaller hydrogen splittings. On the other hand, in 2-methyl-piperidine two strong splittings and one smaller one are observed. But in this case the strong splittings are smaller (while the smaller splitting is larger) than in 4- and 3-methyl-piperidine. We attribute the larger splittings to axial hydrogen and the smaller splittings to equatorial hydrogen.

In 2-methyl piperidinoxy the axial splitting is smaller and the equatorial splitting larger than in 3- and 4-methyl piperidinoxy. We believe that this indicates that inversion is 'easier' in 2-methyl piperidinoxy than in the 3- and 4-methyl derivatives; inversion is already noticeable at 0°C. This 'easier' inversion is related to a smaller energy difference between conformers. We have estimated this energy difference to be in the order of 0.75 kcal/mole; much smaller than the 1.6 kcal/mole found in 4-methyl piperidinoxy.

This could be attributed to a '2-alkyl nitroxide' effect analogous to that which has been postulated for ketones[22] and which could originate in a repulsion between NO and methyl groups. Similarly, the conformation of conjugated nitroxides derived from N-hydroxy succinimide and H-hydroxy-glutarimide can be studied[23]. Different cases of ring inversion and of fixed conformation have been detected by e.s.r. In this case, the hypotheses involved are exactly the same as in the case of piperidine nitroxides. From line width variation, the barrier to inversion has been estimated to be 4.5 kcal/mole in N-oxy-3,3-dimethylglutarimide.

Conformational studies in stable free radicals

Usually nitroxide-free radicals can be isolated in a pure state when both α carbon atoms are tertiary[24]. Triacetone amine derivatives give stable free radicals, which can be isolated and studied at any concentration.

The e.s.r. spectra of these radicals show essentially three lines, attributed to spin one from nitrogen. Some satellite lines are also observed and are attributed to ^{13}C in natural abundance. In some cases weak couplings can be seen, as in *Figure 5*, and are attributed to γ and δ protons. These weak splittings are more easily studied by nuclear magnetic resonance (n.m.r.)[25]. Both ^{13}C satellites and γ and δ splittings can give information on the conformations of the molecules. These interpretations of ^{13}C splittings involve no new assumption and they are in good agreement with information obtained independently from other e.s.r. evidence[26, 27].

What is specific to stable free radicals is the fact that they can be studied by n.m.r. at large concentrations. We have studied the following radicals: V, XIV, XV, XVI, XVII, XIV R = H, XV R = Ac, XV R = p-Br-$C_6H_4SO_2$. By analysing the number of different protons it is possible to obtain an idea of which molecules are in chair form and which are in non-chair form.
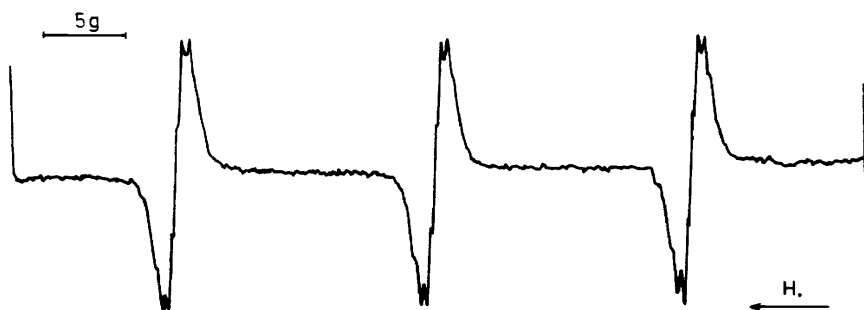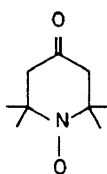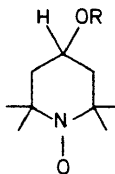
Figure 5
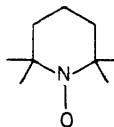
At room temperature the ketone V shows two different types of protons in a 12:4 ratio attributed by selective deuteration to 12 methyl-protons and 4 α-methylene protons. The saturated radical XVII shows three different couplings attributed to 4 equivalent methyl groups—4 equivalent protons from methylene in positions 3 and 5 (γ protons) and 2 equivalent protons attributed to methylene in 4 (δ protons). In the 4-substituted radicals XIV, XV and XVI, γ-methylene protons and methyl protons are different. This is in agreement with a chair form for these radicals, in which axial and equatorial positions can be distinguished. This chair form is in agreement with x-ray data[12, 28].

Only the non-substituted radical XVII shows equivalent methyl and methylene groups. Since in this radical the coupling constant for the methyl group (or methylene) is the *average* of what is found in XIV, XV and XVI, for axial and equatorial methyl groups (or methylene), we conclude that XVII is in 'fast' inversion between two chair conformations of equal energy.

The situation is different for the ketonic radical V: all methyl groups are equivalents, all methylene protons are equivalent but their coupling constants are different from those found in XVII. A non-chair conformation must be attributed to the ketonic radical V and we believe that this radical
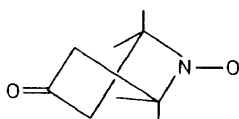
Figure 6

is in a twist conformation (*Figure 6*). This suggestion is in agreement with what is found by analysing ^{13}C splittings[26], assuming a 'cos^2 θ' variation for these splittings.

Conformation of aliphatic monoradicals

The observed hyperfine splittings of aliphatic radicals such as *t*-butyl ethylnitroxide indicate preferred conformations. Stone and Maki[29] have analysed the conformation of aliphatic nitroradical anions. We have another interpretation[30] of their findings: the favoured rotamer is one (*Figure 7*) in which the NO$_2$ group (assumed to be planar) eclipses a CH bond, the eclipsing of a C—C bond by NO being less favoured.
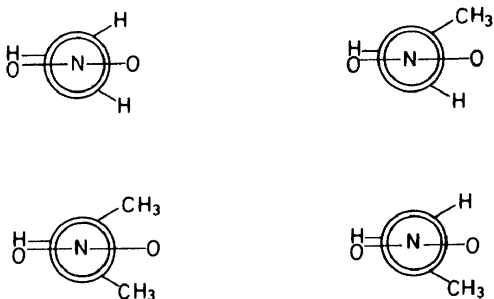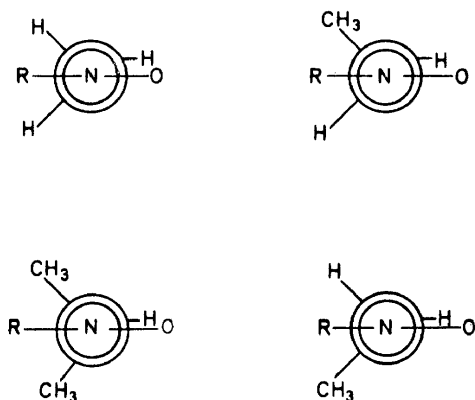

Figure 7

Figure 8

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For the nitroethane radical anion, there are two favoured conformers in in which CH eclipses NO. Stone and Maki have found the barrier between these conformers to be 1.1 kcal/mole.

We have studied the conformation of aliphatic nitroxides[30]. Our results are rationalized by assuming (*a*), a planar NO group (i.e. O in the CNC plane), and (*b*), preferred conformations in which CH eclipses NO (*Figure 8*). These results are in agreement with the analysis of ^{13}C splittings[27, 31].

Polyradicals

As we have seen, the hypothesis and the models used in the conformational analysis of monoradicals are very similar to those used in nuclear magnetic resonance.

We would like to give an idea of some of the other methods that can be applied to the study of conformation analysis. Stable biradicals[32] are known, and in these compounds new parameters can be measured; these are the exchange interaction J and the dipolar interaction D. These can be visualized as interactions between the two unpaired electrons and they depend in a very sensitive way on the relative positions of these two electrons.

(a). *The dipolar interaction* has a very simple dependence on geometry: assuming that both unpaired electrons are localized, the dipolar interaction D (in oersteds) is related to the average distance r (in Ångströms) between unpaired electrons[33]:

$$\tfrac{2}{3} D = \frac{18\,542}{r^3}$$

This theoretical relation can be verified experimentally. In the rigid biradical derived from diaza-adamantane XVIII[34], one can measure a dipolar splitting of $2D = 510$ Oe. This implies an average distance of 4.8 Å between unpaired electrons. On a model one can measure 3.45 Å between two nitrogens and 5.9 Å between two oxygens, the average being 4.7 Å.
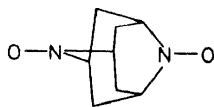
XVIII

We are currently applying this measurement of dipolar splittings to the structural analysis of new biradicals. One can prepare[35] biradical XIX starting from 1,4-cyclohexanedione XX. For this radical, assuming a chair form for the cyclohexane ring, there can be three conformers: ae (axial NO,

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equatorial NO), aa and ee. One can measure on models an average distance between unpaired electrons of 6.2 Å for ee, 4.6 Å for ae and 4.7 Å for aa. In a rigid solution, one measures $2D = 250$ Oe, corresponding to $r = 6$ Å, a result consistent with ee conformation. This method seems to be quite general:

XX   XIX

XIXee   XIXae   XIXaa
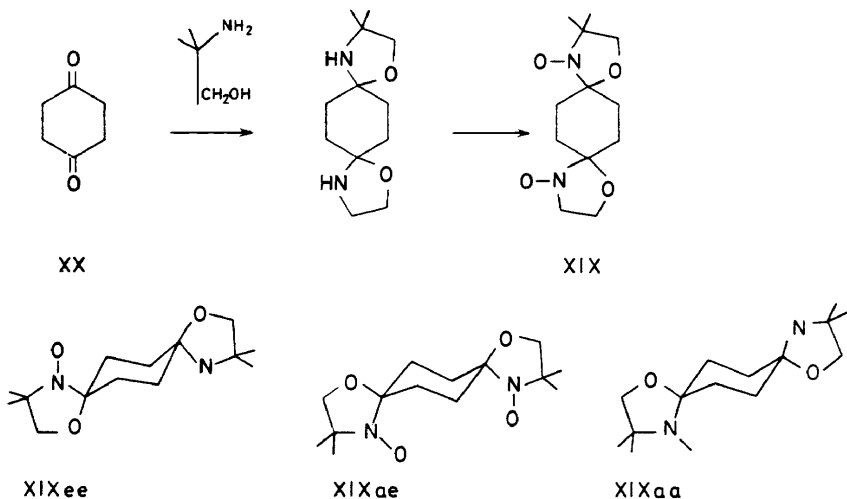
it is fairly easy to spin-label[36] a molecule with nitroxides. If it is possible to measure dipolar interaction, this gives the distance between the positions where the spin labels have been fixed.

(b). The variation of the *exchange interaction* J with geometry is more complicated. J decreases as the distance between unpaired electrons increases but at a given distance J may be zero if the unpaired electron orbitals are orthogonal. The e.s.r. spectra change very drastically when J is smaller, or is of the same order of magnitude, or is greater than the nitrogen hyperfine splitting a_N (*Figure 9*)[32].

If the biradical is non-rigid, the exchange interaction J will be different for different conformations. Biradical diesters such as XXI are examples of this case. Their e.s.r. spectra have been studied as a function of temperature[35].

Succinate ($n = 2$) is a typical example. At low temperatures it shows a three-line spectrum, characteristic of a 'weak exchange' spectrum. As the temperature increases, new lines become visible and above 100°C, the spectrum shows five lines, characteristic of a 'strong exchange' spectrum.

Qualitatively the experimental temperature dependence is in agreement with the expectation that stretched conformations of the diester are of lower energy than are those in which the two nitroxide groups are closed; one can assume that J is small in the stretched conformation and that it is large when the nitroxide groups are close together. This result could be interpreted by a detailed analysis using a jump model and matching the experimental e.s.r. curves with different values of J for different energy conformations.

Figure 9

XXI
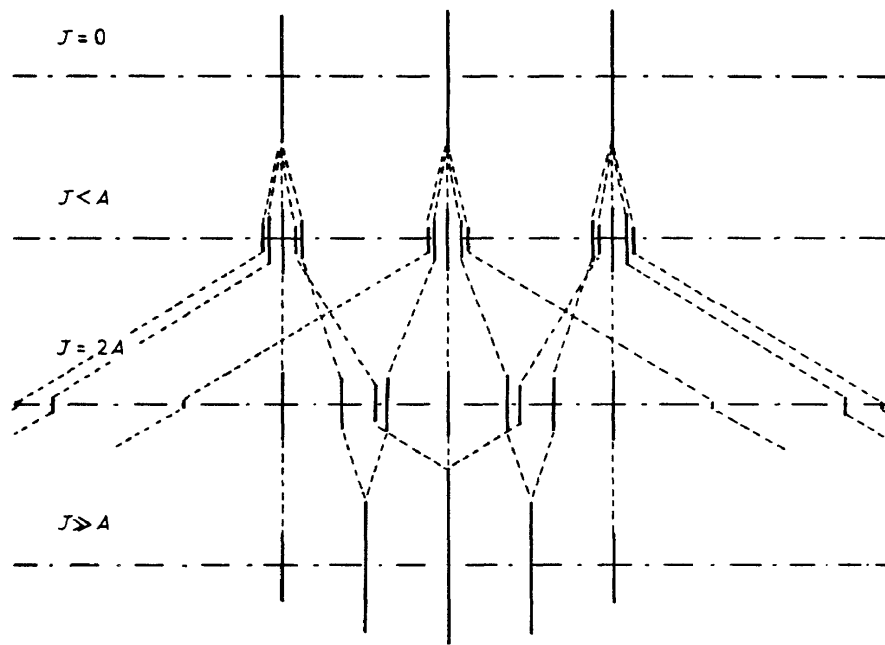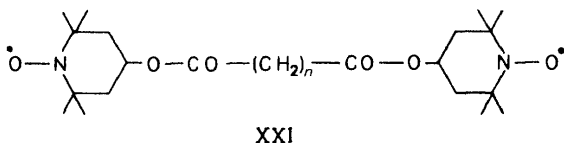
We have chosen[35] another, simplified, approach which gives an average energy between conformations with weak and strong exchange; this energy being a form of activation energy for the folding of the diester chain. The line width of the e.s.r. spectra can be related to the lifetime of one spin state for all conformations. One can obtain this average energy and an average value of J from an Arrhenius plot. With this model, average energies of the order of 4 to 5 kcal/mole are found, in qualitative agreement with what can be expected for folding a C—C chain.

ACKNOWLEDGEMENTS

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