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Rotational Barriers and the Number of Stereoisomers of Iodixanol, an X-Ray Contrast Agent

Rune Fossheim,[a] Trygve Gulbrandsen,[b] Hanno Priebe[a,†] and Arne Jørgen Aasen[c]

[a] Nycomed Imaging AS, Nycoveien 2, P.O. Box 4220 Torshov, N-0401 Oslo, Norway, [b] Hafslund Nycomed AS, P.O. Box 5010, Majorstua, N-0401 Oslo, Norway and [c] Department of Pharmacy, University of Oslo, P. O. Box 1068, Blindern, N-0316 Oslo, Norway

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The diastereomeric composition of iodixanol resulting from chiral centres and hindered rotation has been elucidated by two approaches. Rotational barriers for the nine bond types in iodixanol have been calculated by molecular mechanics and semiempirical quantum mechanical methods (MOPAC) as an aid in determining which bonds contribute to the diastereomeric composition by virtue of high rotational barriers. The results of the calculations suggest that rotation around the phenyl-*N* bond is sufficiently high (118–140 kJ mol^{-1}; 28–33 kcal mol^{-1}) to give rise to non-interconvertible rotamers at room temperature. In this case the diastereomeric composition of iodixanol will consist of six racemates and four *meso* forms, as opposed to three racemates and three *meso* forms if phenyl-*N* rotation is allowed.

X-ray contrast agents generally contain heavy atoms such as iodine as an X-ray absorbent and hydrophilic parts like carboxylic acid, carboxamide or hydroxy groups to achieve good water solubility. The type of hydrophilic group classifies a tri-iodinated aromatic compound as an ionic (1st generation) or non-ionic (2nd generation) contrast agent. Third generation X-ray contrast agents like iodixanol[1] are dimers of non-ionic tri-iodinated aromatic compounds in a pharmaceutical formulation[2] which are isotonic[3] and isoosmotic[4] with blood.

Isomerism in iodixanol has influence on the ^{1}H/^{13}C NMR spectra, HPLC retention time, dipole moment and the crystallization behaviour. Different isomers of iodixanol have identical constitutional formulas, but different three-dimensional shapes, thus making crystallization from solution difficult.

Iodixanol is a complex mixture of stereoisomers and rotational isomers due to several stereocentres and chiral axes arising from hindered rotation. Knowledge of the exact composition of iodixanol is important for several reasons, *e.g.*, compliance with the requirements of drug regulatory authorities that apply to documentation, and analytical aspects such as knowledge of the maximum number of separable entities on chiral or achiral chromatography. However, the assessment of the number of stereoisomers of iodixanol is an intricate[5] problem due to elements of symmetry that render the classical formulae of computation inapplicable. These formulae[6] are limited to molecules having *n* asymmetric atoms, [R-(CXY)$_n$-R′], and none, or one centre of symmetry only [R = R′].

Calculations of rotational barriers to determine rotamers of high stability at room temperature and the enumeration of the stereoisomers of iodixanol constitute the subject of the present paper.

Molecular structure and symmetry elements. Iodixanol accommodates five stereocentres carrying four different substituents and six chiral axes arising from hindered rotation caused by steric interactions between the bulky iodine atoms and the side chains attached to the aromatic moieties. However, at room temperature only the two chiral axes connecting the iodinated benzene rings with the tertiary nitrogen atoms are likely to contribute to the number of stable stereoisomers.

For an analysis of the symmetry elements[7] of iodixanol the molecule is stretched out into the form of highest symmetry. The phenyl-*N* nitrogen atom is treated as a planar sp^2 hybridized atom with its substituents in a plane orthogonal to the phenyl ring plane. The molecular structure and symmetry elements are shown in Fig. 1.

Whether the central carbon atom in the bridge is chiral or not is decided by the symmetry behaviour of all other parts of the molecule. Therefore the chirality of this carbon atom is treated last. The –CHOH– group in the bridge is temporarily replaced by the prochiral –CH$_2$– group only for the symmetry evaluation of all other parts of the iodixanol molecule. Only then has the molecule a

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[†] To whom correspondence should be addressed.

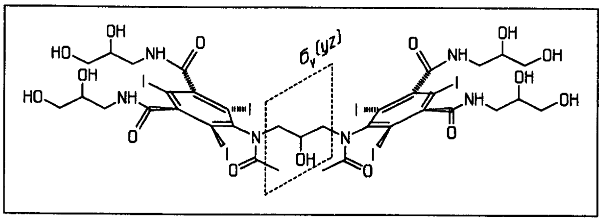

Fig. 1. Molecular structure of iodixanol and symmetry plane $\sigma_v(yz)$.

C_2 axis through the central carbon atom. Here it is important to emphasize that iodixanol itself does not have a C_2 axis. Symmetry operations are used to determine whether two isomers are identical, enantiomer or *meso* forms.[8] In order to discuss the properties and contributions of all stereogenic units it is useful to define parts of the iodixanol molecule as shown in Fig. 2.

Calculations of rotational barriers

Restricted rotation is the cause of axial chirality and some other forms of isomerism in iodixanol. Therefore a qualitative and quantitative description of rotational barriers is not only helpful but necessary for further analysis of isomerism types.

Methods. The rotational barriers for rotation around the nine different single bonds in iodixanol have been calculated using both the molecular mechanics method and semiempirical quantum mechanical methods (MOPAC 5.0).[9] The programs were used on a Silicon Graphics 4D/35 workstation, running under the IRIX 3.2.2 operating system. To reduce the size of MOPAC geometry calculations we have calculated the rotational barriers for the three smaller model molecules shown in Fig. 3. The model molecules contain all relevant partial structures of iodixanol.

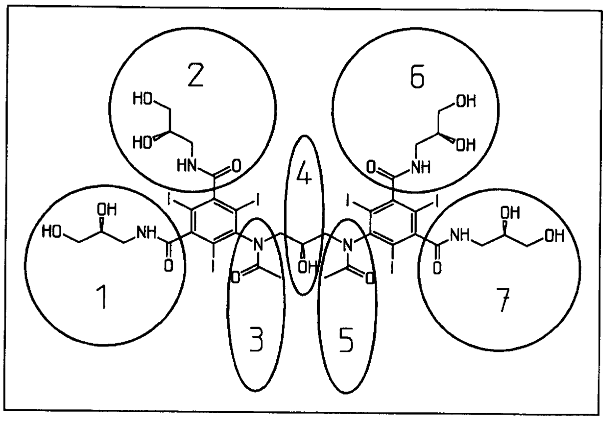

Fig. 2. Definition of groups in iodixanol: amide side chains = groups 1, 2, 6 and 7; *N*-acetyl functions = groups 3 and 5; central carbon atom = group 4.

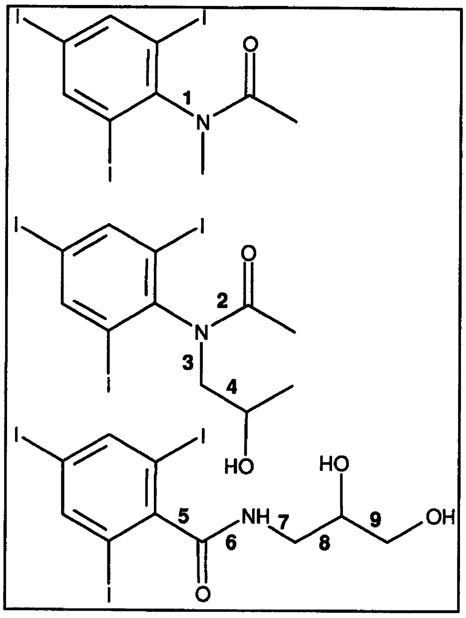

Fig. 3. Partial structures of iodixanol and bond number assignment used in rotational barrier calculations.

The rotational barrier of a single bond A–B is predominantly determined by the nature of the bond A–B and the groups attached to A and B. Therefore, the errors introduced by using these model molecules are judged to be small. Furthermore, by using two methods of calculation that are based on different theories, one can better assess the validity of the results.

Molecular mechanics.

The three molecules were built using the Builder option in INSIGHT 2.3.0.[10] The molecules were energy minimized with DISCOVER 2.9.5 using the CFF91 forcefield with supplied atomic charges. The VA09A minimization procedure was used until the gradient norm was less than 0.041 kJ Å^{-2} (0.001 kcal mol^{-2}). Rotational barriers were calculated using a dihedral driver to rotate the specific bonds in steps of 10^0. The structures were fully geometry optimized at each point, constraining only the specific torsion angle to the specific step value. All calculations were performed using a distance dependent dielectric function ($e = r_{ij}$).

MOPAC. The rotational barriers for bonds 1, 2, 5 and 6 were also determined by use of MOPAC 5.0, incorporating the SADDLE option by the following procedure. 1. The MM geometry optimized structures were re-optimized using MOPAC to obtain the starting geometry. 2. The respective bonds were rotated 180° and the structure's geometry optimized with MOPAC to obtain the final geometry. 3. The initial and final coordinates were used for SADDLE calculations. The keywords MMOK and XYZ were used, with default convergence criteria. 4. In the case of bond 1 the MM calculations strongly suggest that phenyl-*N* rotation is accompanied by amide rotation, i.e., an *exo*/*endo* transition. Therefore the amide bond was also rotated 180° under step 2. In the case of bond 5, two SADDLE calculations were performed, one with the amide bond rotated 180° in the final geometry (*cis*) and one keeping the amide bond *trans*.

The calculations 1–4 were performed using both the AM1 and the PM3 methods. The rotational barriers for the single bonds 3, 4, 7, 8 and 9 were calculated using the procedure described below. Only the AM1 method was used here. 1. The staggered forms of the molecules were geometry optimized. 2. A SADDLE calculation was then performed using all combinations of starting points. In the case of three starting points a, b and c this results in the SADDLE calculations ab, ac and bc. 3. The energy barrier was taken as the difference in energy between the highest energy transition-state structure and the lowest-energy staggered structure.

Results

The calculated energy barriers for the nine different bonds are given in Table 1, together with results from NMR and HPLC.

Although the three calculational methods are not always consistent with respect to the barrier heights, they do collectively give an ordering of the barriers that is consistent: $\text{Ph–N} > \text{PhN–COCH}_3 > \text{PhCO–NH} > \text{Ph–CO} \gg \text{CHOH–CH}_2\text{OH} \geq \text{PhNCH}_2\text{–CHOH} \geq \text{NHCH}_2\text{–CHOH} \geq \text{NH–CH}_2 \geq \text{PhN–CH}_2$. Furthermore, the calculated barriers and the experimental results support the conclusion that only Ph–*N* rotation will be disallowed at room temperature.

Discussion

The rotational barriers for the bonds 3,4 and 7–9 are all calculated to be below 44.7 kJ mol^{-1} (10.7 kcal mol^{-1}),

Table 1. Calculated energy barriers in kJ mol^{-1} (kcal mol^{-1}) for nine non-H bond types in iodixanol and related experimental data.

Bond		Molecular modeling				Related experimental data	
number and type		Average	MM	AM1	PM3	NMR	HPLC
1	Ph–N	118.0 (28.2)	140.1 (33.5)	109.3 (26.1)	104.7 (25.0)		
2	PhN–COCH$_3$	69.4 (16.6)	104.1 (24.9)	59.9 (14.3)	44.4 (10.6)		102.4; 105.8 (24.5;[d] 25.3[a])
3	PhN–CH$_2$	23.8 (5.7)	18.4 (4.4)	29.3 (7.0)			
4	PhNCH$_2$–CHOH	36.0 (8.6)	39.3 (9.4)	32.7 (7.8)			
5	Ph–CO	76.4 (18.2)	69.8 (16.4)	69.1 (16.5)	91.8 (21.9)[b]	73.3; 68–76 (17.5;[c] 16.3–18.2[d])	
6	PhCO–NH	84.2 (20.1)	69.4 (16.6)	92.0 (22.0)	91.3 (21.8)	87.8 (21.0)[c]	
7	NH–CH$_2$	27.4 (6.5)	24.7 (5.9)	30.2 (7.2)			
8	NHCH$_2$–CHOH	32.8 (7.8)	26.8 (6.4)	39.0 (9.3)			
9	CHOH–CH$_2$OH	39.7 (9.5)	44.7 (10.7)	34.8 (8.3)			

[a] Kinetic data by Hagen and Tønseth.[15] [b] Two different transition-state (TS) structures are arrived at when using the PM3 method. The lowest energy TS structure is arrived at from the *cis* final geometry. The *trans* TS-structure is 8.4 kJ mol^{-1} (2.0 kcal mol^{-1}) higher in energy. Both structures possess a pyramidal nitrogen. They differ mainly in the C–N–C–O torsion angle that is 34.4° in the *cis* TS-structure and −4.9° in the *trans* TS structure. [c] Determined for serinol derivatives.[17] [d] Kinetic data by Berg and Fagervoll.[16]

i.e., rotation around the bonds would be expected to occur readily at room temperature. The results from the MM and MOPAC methods are in only fair agreement, the average difference for the five bonds being 8.8 kJ mol^{-1} (2.1 kcal mol^{-1}). A closer examination of the relative barrier heights and comparisons to relevant experimental data for these bonds is outside the scope of this paper. For comparison, the rotational barrier around the central bond in butane is 25–29 kJ mol^{-1} (6–7 kcal mol^{-1}) and 260–264 kJ mol^{-1} (62–63 kcal mol^{-1}) in 2-butene.[11]

Bond 1 (Ph–N). Bond 1 is calculated to possess the highest barrier by all three methods. However the molecular mechanics method predicts a significantly higher barrier than MOPAC AM1 and PM3. Since it is known that the AM1 and PM3 methods usually underestimate barrier heights,[12] we believe the MM value 140.1 kJ mol^{-1} (33.5 kcal mol^{-1}) to be closest to the true value. This means that rotation around this bond should not occur readily even at elevated temperatures. All three methods agree that phenyl–*N* rotation is accompanied by amide bond rotation, i.e., an *exo–endo* transition. The concerted amide rotation avoids the severe I···Me contacts that would result from a planar transition-state (TS) structure. However, inspection of the calculated structures show that, in spite of amide bond rotation, there are large out-of-plane distortions of the iodine atoms. Since all other bonds can rotate more easily than the phenyl–*N* bond it is difficult to detect the phenyl–*N* bond rotation relative to an intramolecular reference position.

Bond 2 (PhN–CO). Bond 2 has a barrier[13,14] of 104.1 kJ mol^{-1} (24.9 kcal mol^{-1}) according to the MM method. The AM1 and PM3 barriers are not in keeping with this result since they are only half this magnitude. Using HPLC kinetic data for the *exo–endo* equilibrium in iodixanol[15] and iopentol [16] = 5-[*N*-(2-hydroxy-3-methoxypropyl)acetamido]-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodoisophthalamide and the Arrhenius equation we obtain activation energies for the *exo–endo* transition of 105.8 kJ mol^{-1} (25.3 kcal mol^{-1}) for iodixanol and 102.4 kJ mol^{-1} (24.5 kcal mol^{-1}) for iopentol. It thus appears that the molecular mechanics results are most reliable also for this bond. The barriers for bond 1 and 2 are in agreement with the finding that the barrier for bond 2 is superimposed on the barrier for bond 1. We know from experience that rotation around the PhN–COCH$_3$ bond in iodixanol occurs in aqueous solution even at room temperature.

Bond 5 (Ph–CO). The calculated barriers for bond 5 are fairly consistent, and also in keeping with experimental NMR results. All three methods agree that Ph–C rotation is not accompanied by amide bond rotation, i.e., there is no coupling between rotation around bonds 5 and 6. The fact that the AM1 method arrives at the same TS structure from both starting points as opposed to PM3, gives more credit to this method. It is also in better keeping with the MM result and the NMR result[17] 73.3 kJ mol^{-1} (17.5 kcal mol^{-1}) found for serinol (2-aminopropane-1,3-diol) derivatives such as iopamidol [= *N*,*N*′-bis-(2-hydroxy-1-hydroxymethylethyl)-5-(2-hydroxypropionamido)-2,4,6-triiodoisophthalamide].

Bond 6 (PhCO–NH). The AM1 and PM3 methods both give a barrier of 92.0 kJ mol^{-1} (22.0 kcal mol^{-1}), which is also in good keeping with NMR results for serinol derivatives containing the PhCO–NHR fragment 87.8 kJ mol^{-1} (21.0 kcal mol^{-1}).[13,17,18] The MM method slightly underestimates this barrier 69.4 kJ mol^{-1} (16.6 kcal mol^{-1}).

Types of stereoisomerism in iodixanol

In iodixanol three types of stereoisomer are possible: (A) diastereomers and enantiomers from, e.g., chiral carbon atoms in the side chains, the pseudoasymmetric carbon atom in the bridge and chiral axes; (B) torsion diastereomers (rotamers), e.g., *exo/endo* isomerism, *cis/trans* isomerism, *syn/anti* isomerism and chiral axes; (C) conformers.

The convergence approach focuses on part A. Every amide side chain (groups 1, 2, 6 and 7) contains one asymmetric carbon atom. The number of isomers can be calculated from the formula 2^n, where n is the number of chiral elements but here the actual number is less because of *meso* forms. Torsion diastereomers (rotamers) are isomers resulting from restricted rotation. Here the expression rotamer is used for stereoisomers with a rotational barrier that is sufficiently high to prevent rapid equilibration at ordinary temperatures (bonds 1, 2, 5 and 6 in Fig. 4). Conformers are formed by rotation about all other single bonds excluding terminal single bonds and those that have been mentioned previously. However, there is no sharp borderline between rotamers and conformers.

Chiral axis through I-phenyl-N.[8] This exists only under *two* conditions: groups A and B are different (*condition 1*) and the phenyl–*N* bond rotation is highly restricted (*condition 2*). A high rotational barrier of the phenyl–*N* bond

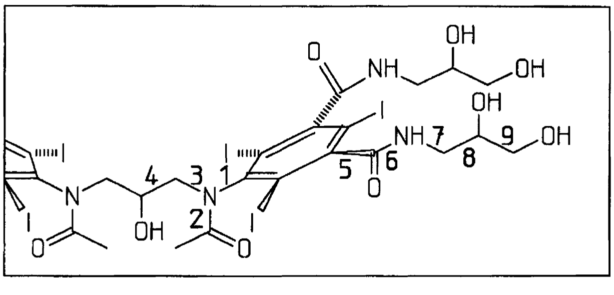

Fig. 4. Bond number assignment of single bond rotations in iodixanol.

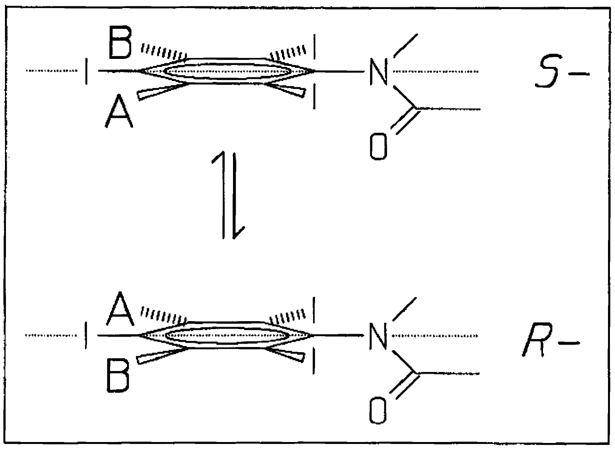

Fig. 5. Chiral axis through *I*-phenyl-*N*.

prevents the interchange of the side chain groups A and B (Fig. 5).

Any rotation around the phenyl-*N* bond will lead to racemization of the axial chirality. The priority of groups involved is A > B and $COCH_3 > CH_2$–C. The groups A and B in iodixanol correspond to the previously defined side chain groups 1 and 2 or 6 and 7 (Fig. 2). In iodixanol a difference in group A and B may result from different chiral carbon atoms in the side chains, *syn/anti* isomerism or *cis/trans* isomerism of two side chain carbonyl groups. Since axial chirality depends on two conditions, it is not treated as an independent type of isomerism by the symmetry species approach. But for every type of isomerism it is distinguished that *condition 1 is true* when group 1 + 2 or 6 + 7 are not equal; *condition 1 is false* when group 1 + 2 or 6 + 7 are equal (Fig. 2). The diastereomeric composition (optical isomers) of iodixanol is calculated separately for the two cases of condition 2: *condition 2 is true* when phenyl-*N* bond rotation is forbidden; condition 2 is false when phenyl-*N* bond rotation is allowed. Today there is not enough experimental evidence to answer the question, does the phenyl-*N* bond in iodixanol rotate or not? Therefore we distinguish and describe two borderline cases: the allowed and the forbidden phenyl-*N* bond rotation. The real diastereomeric composition of iodixanol must necessarily be between these two borderlines.

Chiral carbon atoms in the side chains. These have either *R* or *S* configuration.

syn/anti *Isomerism.* Restricted rotation about the amide PhCO–NH bond 6 (Fig. 4) may lead to two different forms as shown in Fig. 6. The primary amide functions have either *syn* or *anti* configuration.

The bond order of the CO–N bond is increased due to partial double bond character.[19,20] Higher carboxylic amides contain only about 3% *syn* isomer.[11]

cis/trans *Isomerism.* Restricted rotation about the Ph–CO bond 5 (Fig. 4) results in *cis/trans* isomerism of the carboxamido groups as shown in Fig. 7. The carbonyl oxygens of the carboxamido functions may be located on either the *upper* side of the phenyl ring as the *N*–acetyl carbonyl oxygen atom or on the *lower* side. Here the *U U* or *L L* locations of two carbonyl oxygens on the same phenyl ring both represent a *cis* configuration.

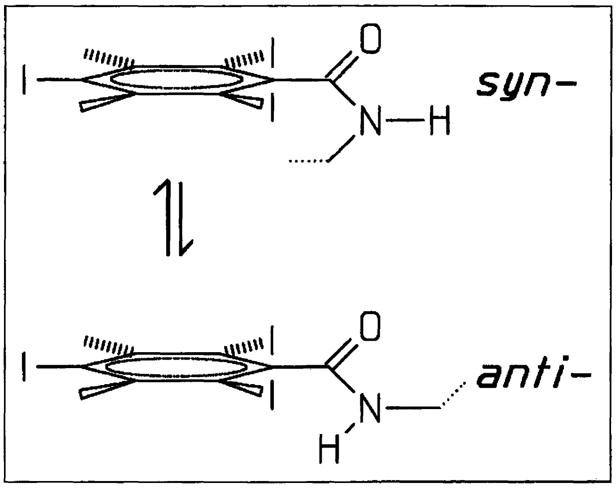

Fig. 6. *syn/anti* Isomerism.

Severely restricted rotation about the phenyl-*N* bond 1 combined with *cis/trans* isomerism results in several rotamers.

exo/endo *Isomerism.* Hindered rotation about the amide CO–N bond 2 (Fig. 4) results in two thermodynamically preferred orientations of the acetyl groups. As shown in Fig. 8 the carbonyl group may be directed away from the phenyl ring (*exo*) or inwards towards the ring (*endo*).

The pseudoasymmetric carbon atom. The central carbon atom in the bridge (group 4) is stereochemically similar to the central carbon atom in 2,3,4-pentanetriol (Fig. 9). The middle carbon atom is asymmetric when the 2- and 4-carbons have different configurational assignments. Such a

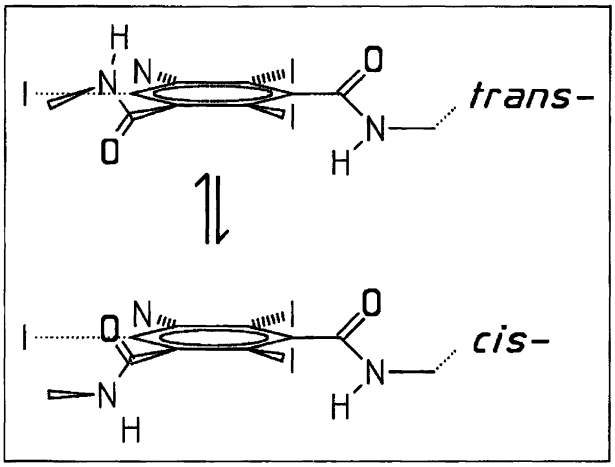

Fig. 7. *cis/trans* Isomerism.

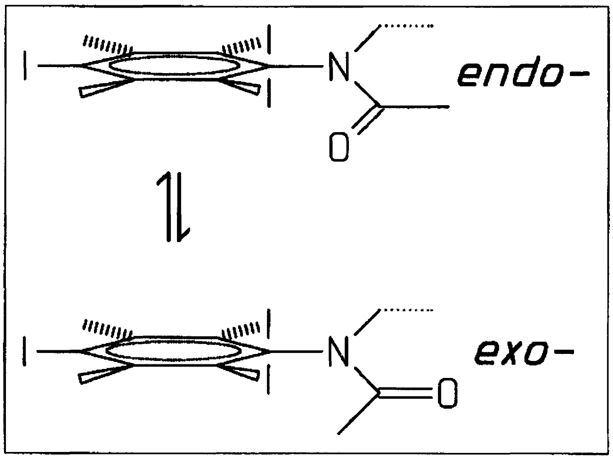

Fig. 8. exo/endo Isomerism.

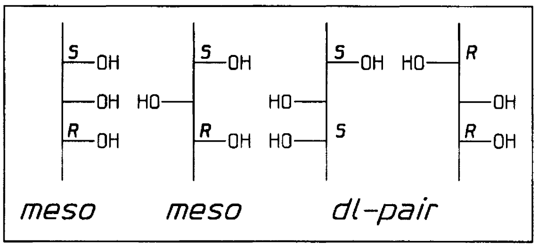

Fig. 9. 2,3,4-Pentanetriol, two *meso* forms and one (±)-pair

carbon is called a *pseudoasymmetric* carbon. The central carbon atom in the two *meso* forms is stereogenic and achirotopic, whereas the (±)-*pair* contain a center that is non-stereogenic and chirotopic.[21]

Molecular chirality is incompatible with any σ symmetry plane. In iodixanol the pseudoasymmetric carbon (group 4) is chiral when the adjacent groups are different. This is the case when the actual combination of all other types of isomerism form a structure with asymmetric behaviour under the $\sigma_v(yz)$ symmetry operation represented by the species A or B_1.

The adjacent groups are not equal when the actual combination of all other types of isomerism form a structure with symmetric behavior under the $\sigma_v(yz)$ symmetry operation represented by the species B_2. In these cases the central carbon is chiral giving two *meso* forms.

The adjacent groups are equal when the actual combination of all other types of isomerism form a structure with symmetric behaviour under the $C_2(z)$ operation represented by the species A_1 and A_2. In these cases the central carbon is achiral.

The number of stereoisomers of iodixanol

Two approaches have been employed to corroborate the number of stereoisomers: the symmetry species approach and the method of convergence. Characteristics of the symmetry species approach are as follows.[22] The behavior under symmetry operations correlates each isomer to a symmetry species. Symmetry operations are used to identify identical isomers and to simplify the mathematical combination of different types of isomerism. Potential stereoisomerism from restricted bond rotation and from chiral carbon atoms are discussed separately. Characteristics of the method of convergence:[23] step 1: tabulation of the configurational permutations for a compound having $n = 7$ stereocenters; step 2: assignment of achiral stereocenters in iodixanol; step 3: find equivalent stereocenters in iodixanol.

The symmetry species approach. The symmetry elements of the iodixanol molecular structure were analyzed. Symmetry operations are used to *recognize whether two isomers are identical, enantiomer or meso forms.* Some molecular properties change their sign under a certain symmetry operation, *e.g.*, R configuration becomes S under a σ operation. In the point groups C_1, C_2, C_s or C_{2v} properties can only show symmetrical (+1) or asymmetrical (−1) behaviour (also called character). A combination of characters +1 and −1 relative to the elements of the point group is called symmetry species *e.g.*, A_1 or B_1. The symmetry species is an irreducible representation of the symmetrical behaviour of any stereoisomer.[7] Low symmetry combined with higher symmetry forms low symmetry products. All possible types of stereoisomerism of iodixanol form partial structures that are members of the point groups C_1, C_2, C_s or C_{2v}. The C_2 axis is identical with the (z)-coordinate axis and goes through the central carbon atom. The hydrogen and hydroxy group attached to the pseudoasymmetric carbon atom are not symmetrical under C_2 or $\sigma_v(xz)$ symmetry operations. The diastereomeric composition of iodixanol can be described as a combination of all types of isomerism mentioned above. The combination starts with the chiral carbon atoms in the side chains as the outer shell, then the inner shells and finally the pseudoasymmetric carbon atom. Each type of isomerism is treated separately. Its *contribution to the total number of stereoisomers* (Σ) is evaluated according to the total geometry of the molecule. Identical forms are ruled out at an early stage to simplify the mathematical combination of different types of isomerism. The following rules were found.

Chiral carbon atoms in the side chains. For forbidden phenyl-*N* bond rotation: $\Sigma_{R/S} = 2A_1 + 2A_2 + B_1 + B_2 + 4A = 10$ isomers. For allowed phenyl-*N* bond rotation: $\Sigma_{R/S} = A_1 + 2A_2 + 0B_1 + B_2 + 2A = 6$ isomers. Since *meso* forms of different species A_1 and B_1 equilibrate by phenyl-*N* bond rotation their contribution is represented by the species with highest symmetry $A_1 + 0B_1$. Since the chiral carbon atoms in the side chains are the first type of isomerism evaluated as the outer shell, there are no combination partners and no combination rules yet.

syn/anti *Isomerism.* Rules for forbidden phenyl-*N* bond rotation: in combination with A_1, A_2, $\Sigma_{syn/anti} = 2A_1$

$+ 2A_2 + B_1 + B_2 + 4A = 10$ isomers. In combination with B_1, B_2 or A, $\Sigma_{syn/anti} = 2A_1 + 2A_2 + 2B_1 + 2B_2 + 8A = 16$ isomers. Rules for allowed phenyl–N bond rotation: in combination with A_1, A_2, $\Sigma_{syn/anti} = 2A_1 + 0A_2 + B_1 + 1B_2 + 4A = 8$ isomers. In combination with B_1, B_2 or A then $\Sigma_{syn/anti} = 2A_1 + 0A_2 + 2B_1 + 1B_2 + 4A = 9$ isomers. Since forms of different species A_2 and B_2 equilibrate by phenyl–N bond rotation their contribution is represented by the species of highest symmetry $0A_2 + 1B_2$.

cis/trans *Isomerism.* Rules for forbidden phenyl–N bond rotation: in combination with A_1, A_2, $\Sigma_{cis/trans} = 2A_1 + 2A_2 + B_1 + B_2 + 4A = 10$ isomers. In combination with B_1, B_2 or A then $\Sigma_{cis/trans} = 2A_1 + 2A_2 + 2B_1 + 2B_2 + 8A = 16$ isomers. Rules for allowed phenyl–N bond rotation: in combination with A_1, A_2 then $\Sigma_{cis/trans} = 1A_1 + 2A_2 + 0B_1 + B_2 + 2A = 6$ isomers. In combination with B_1, B_2 or A, $\Sigma_{cis/trans} = 1A_1 + 2A_2 + 0B_1 + 2B_2 + 4A = 9$ isomers. Since forms of different species A_1 and B_1 equilibrate by phenyl–N bond rotation their contribution is represented by the species of highest symmetry $1A_1 + 0B_1$.

exo/endo *Isomerism.* Rules for forbidden phenyl–N bond rotation: in combination with A_1, A_2, $\Sigma_{exo/endo} = 2A_1 + B_1 = 3$ isomers. In combination with B_1, B_2 or A, $\Sigma_{exo/endo} = 2A_1 + 2B_1 = 4$ isomers. Rules for allowed phenyl–N bond rotation: in combination with A_1, A_2, $\Sigma_{exo/endo} = 1A_1 + 0B_1 = 1$ stereoisomer. In combination with B_1, B_2 or A, $\Sigma_{exo/endo} = 1A_1 + 0B_1 = 1$ stereoisomer. Since forms of different species A_1 and B_1 equilibrate by phenyl–N bond rotation their contribution is represented by the species of highest symmetry $1A_1 + 0B_1$.

The pseudoasymmetric carbon atom. In combination with A or B_1 the carbon atom is chiral and $\Sigma_{PC} = 2A = 2$ isomers. In combination with B_2 the carbon atom is chiral and $\Sigma_{PC} = 2B_2 = 2$ isomers. In combination with A_1 or A_2 the carbon atom is achiral and $\Sigma_{PC} = B_2 = 1$ stereoisomer.

Theoretical considerations. The theoretical considerations are based on the following hypothesis. All rotational diastereomers are stable and do not interchange and the phenyl–N bond rotation is forbidden. If you combine the total contribution Σ of each type of isomerism straightforward by and with no differentiation, then the maximum number of combinations N(max) is calculated from eqn. (1).

$$N(\text{max}) = \Sigma_{R/S}(\text{max}) \cdot \Sigma_{syn/anti}(\text{max}) \cdot \Sigma_{cis/trans}(\text{max}) \cdot \Sigma_{exo/endo}(\text{max}) \cdot \Sigma_{PC}(\text{max}) \qquad (1)$$

$$N(\text{max}) = 16 \cdot 16 \cdot 16 \cdot 4 \cdot 2 = 32768.$$

However the real number of stereoisomers is lower because combinations with A_1 or A_2 form two identical products. This is due to the symmetrical behaviour of A_1 or A_2 under the $C_2(z)$ operation. Σ is then less than Σ(max). Since all other types of isomerism determine in combination whether the pseudoasymmetric carbon atom is chiral or not, the pseudoasymmetric carbon is treated at last.

The *maximum theoretical* number of combinations C is calculated from eqn. (2)

$$C = \Sigma_{PC} \cdot \{\Sigma_{exo/endo} \cdot [\Sigma_{cis/trans} \cdot (\Sigma_{syn/anti} \cdot \Sigma_{R/S})]\} \qquad (2)$$

by successive combination of the upper matrices giving eqn. (3) as a result.

$$C = 128\,B_2 + 16000\,A = 16128 \text{ isomers.} \qquad (3)$$

Symmetry species such as A or A_2 with no σ symmetry plane form enantiomers whereas B_1, B_2 or A_1 form *meso* forms. Therefore C represents 8000 pairs of enantiomers and 128 *meso* forms. The maximum theoretical diastereomeric composition of iodixanol is summarized in Table 2. Nevertheless, the premises for this result are unrealistic since rotational diastereomers are not stable and can interchange. It is useful to calculate the number of combinations separately for permanent (optical) and temporary (rotational) diastereomers.

Permanent diastereomers / optical isomers. Permanent diastereomers are formed from the chiral carbon atoms in the side chains and the pseudoasymmetric carbon atom in the bridge. They are not able to equilibrate in solution. Axial chirality is only preserved when the phenyl–N bond rotation is forbidden. However, there are no exact borderlines between the bond rotation attributes: 'forbidden; never' or 'a scarce event' or 'possible at high temperature' or 'allowed'. The way to distinguish between allowed and forbidden phenyl–N bond rotation describes two borderlines. The real diastereomeric composition of iodixanol must necessarily be between these two borderlines.

Based on forbidden phenyl–N bond rotation the number of *maximum permanent* combinations M is calculated from $M = \Sigma_{PC} \cdot \Sigma_{R/S} = 12A + 4B_2$. M represents six pairs of enantiomers and four *meso* forms (Table 2).

Based on allowed phenyl-N bond rotation the number of permanent combinations P is calculated from $P = \Sigma_{PC} \cdot \Sigma_{R/S} = 6A + 3B_2$. P represents three pairs of enantiomers and three *meso* forms (Table 2).

Temporary diastereomers/rotational isomers. Temporary diastereomers (rotamers) are formed from restricted rotation (Fig. 4) about the amide CO–NH bond 4 (*syn/anti*), the phenyl–CO bond 3 (*cis/trans*), the amide CH_3CO–N bond 2 (*exo/endo*) and the phenyl–N bond 1. Rotational isomers can equilibrate.

Based on forbidden phenyl–N bond rotation the number of *maximum temporary* combinations T is calculated from $T = \Sigma_{exo/endo} \cdot (\Sigma_{cis/trans} \cdot \Sigma_{syn/anti})$. $T = 8A_1 + 24A_2 + 24B_1 + 12B_2 + 436A = 504$ isomers. T represents 230 pairs of enantiomers and 44 *meso* forms (Table 2).

Based on allowed phenyl–N bond rotation the number of temporary combinations L is calculated from $L =$

Table 2. The diastereomeric compositions of iodixanol. Abbreviations: *R*/*S*=configuration of chiral carbon atoms.

	Result conditions				
Diastereomeric composition name	phenyl–*N* bond rotation	evaluated types of isomerism	Number of isomers and isomer type	Point group	Symmetry species
maximum theoretical	forbidden	all	8000 pairs of enantiomers	C_1	A
			128 *meso* forms	C_s	B_2
maximum permanent	forbidden	only *R*/*S*	6 pairs of enantiomers	C_1	A
			4 *meso* forms	C_s	B_2
permanent	allowed	only *R*/*S*	3 pairs of enantiomers	C_1	A
			3 *meso* forms	C_s	B_2
maximum temporary	forbidden	all except *R*/*S*	8 *meso* forms	C_{2v}	A_1
			12 pairs of enantiomers	C_2	A_2
			24 *meso* forms	C_s	B_1
			12 *meso* forms	C_s	B_2
			218 pairs of enantiomers	A	C_1
			total:		
			230 pairs of enantiomers		
			44 *meso* forms		
temporary	allowed	all except *R*/*S*	2 *meso* forms	C_{2v}	A_1
			4 pairs of enantiomers	C_2	A_2
			1 *meso* form	C_s	B_1
			4 *meso* forms	C_s	B_2
			40 pairs of enantiomers	C_1	A
			total:		
			22 pairs of enantiomers		
			7 *meso forms*		

Table 3. Chiral carbon atom configuration and isomer types of the maximum permanent and permanent diastereomeric composition (Table 2); abbreviations: E=pair of enantiomers, M=meso form, *R*/*S*/a=configuration, a=achiral, —=identical form.

Configuration in group No.							Point group and symmetry species		Isomer type; forbidden Ph-*N* bond rotation	Isomer type; allowed Ph-*N* bond rotation
1	2	3	4	5	6	7				
R	*R*		a		*R*	*R*	C_1	A	E1	E1
S	*S*		a		*S*	*S*	C_1	A	E1	E1
R	*R*		*R*		*S*	*R*	C_1	A	E2	E2
S	*R*		*R*		*S*	*S*	C_1	A	E2	E2
R	*R*		*S*		*S*	*R*	C_1	A	E3	E3
S	*R*		*S*		*S*	*S*	C_1	A	E3	E3
S	*R*		*R*		*R*	*S*	C_1	A	E4	—
R	*S*		*S*		*S*	*R*	C_1	A	E4	—
R	*R*		*R*		*R*	*S*	C_1	A	E5	—
R	*S*		*R*		*S*	*S*	C_1	A	E5	—
R	*R*		*S*		*R*	*S*	C_1	A	E6	—
R	*S*		*S*		*S*	*S*	C_1	A	E6	—
R	*R*		*R*		*S*	*S*	C_s	B_2	M1	M1
R	*R*		*S*		*S*	*S*	C_s	B_2	M2	M2
S	*R*		a		*S*	*R*	C_s	B_2	M3	M3
R	*S*		a		*R*	*S*	C_s	B_2	M4	—

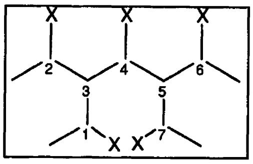

Fig. 10. Simplified model of iodixanol; 'X' = OH; sites 3 and 5 denote stereoaxes.

$\Sigma_{exo/endo} \cdot (\Sigma_{cis/trans} \cdot \Sigma_{syn/anti})$. $L = 2\,A_I + 4\,A_2 + B_I + 4\,B_2 + 40\,A = 51$ isomers. L represents 22 pairs of enantiomers and seven *meso* forms (Table 2).

The convergence approach. A simplified model of iodixanol with five stereocenters carrying four different substituents, and two stereoaxes arising from restricted rotation around the phenyl–*N* bonds, is depicted in Fig. 10.

The convergence approach consists of two steps: (i) tabulation of all possible stereoisomers ($2^7 = 128$), and (ii) elimination of all isomers that are identical because of achirality or inherent symmetry.

The central stereocentre of iodixanol, site No. 4 in Fig. 10, is achiral when corresponding chiral centers (1/7, 2/6, and 3/5) of its two large substituents possess identical configurational notations, *e.g.*, 1-(*R*)/7-(*R*), 2-(*S*)/6-(*S*), etc. The chirality of the stereoaxes vanishes when the side-chains of the aromatic moieties are allocated the same configurational assignments, *e.g.* axis No. 3 is achiral when sites 1 and 2 both attain the *R*-configuration.

Pairs of stereocenters in iodixanol are interchangeable, *e. g.* centers 1 and 2. Equivalent stereocenters are readily disclosed on rotation around the 3–4 or the 4–5 bond, or a 180 degrees rotation as outlined in Fig. 11. Consequently, several isomers receive identical configurational assignments. All but one are deleted from the initial list of 128 possible isomers.

In conclusion, in the event of two permanent stereoaxes, iodixanol consists of sixteen stereoisomers comprising six racemates and four *meso* forms. If both stereoaxes are ignored — implying free rotation around the phenyl–*N* bonds — the number is reduced to nine. These stereoisomers are three pairs of enantiomers and three *meso* forms.

Experimental

In the synthesis of iodixanol[24] only racemic forms of epichlorohydrin and 3-amino-1,2-propanediol were used. No optical activity was found in iodixanol drug substance. The population of *exo* and *endo* rotamers of iodixanol in D_2O was measured by means of ^1H NMR spectroscopy (300 MHz) and found to be 77.8% *exo* and 22.2% *endo* after reaching a state of equilibrium between isomers. The equilibrium was reached after a period of about 30 h at 25 °C. The establishment of an isomeric *exo/endo* equilibrium in both DMSO-d_6, DMF-d_7 and CD_3OD was accompanied by an increase of the *exo* isomer and a corresponding decrease of the *endo* isomer. Neither pH nor concentration affected the *exo/endo* population ratio of iodixanol in D_2O. Distinct *exo* and *endo* isomers were preserved in the temperature range 25–130 °C.

exo/exo- and [*exo/endo* and *endo/endo*]-isomers were separated and isolated by preparative HPLC on RP-18. The fractions were immediately frozen during collection and freeze dried. However, the high isomeric purity achieved through separation was not maintained during lyophilization.

The isomerization of isolated *exo/exo* iodixanol to an equilibrium of *exo/exo*, *exo/endo* and *endo/endo* isomers was measured by analytical HPLC at 10, 25 and 40°C. The results clearly show that the isomerization rate is temperature dependent with the *exo/exo* isomer as the most stable and the *endo/endo* isomer as the least stable. The reaction rate constants, the equilibrium constants as well as the time to reach equilibrium have been determined. The time to reach equilibrium was found to be 250 h at 10°C, 27 h at 25°C, 4 h at 40°C.

The X-ray crystal structure[25] of iodixanol is reported separately.

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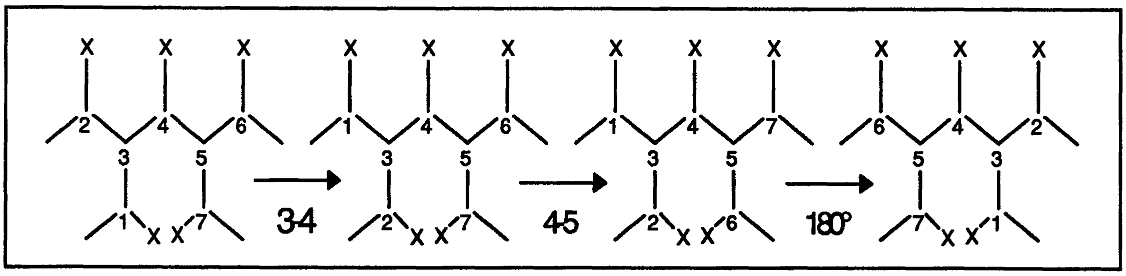

Fig. 11. Rotations indicating equivalent stereocenters in iodixanol.

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