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Applications of X-ray diffractometric techniques in the analysis of drugs*

C. J. DE RANTER

Lab. Anal. Chemie en Medicinale Fysicochemie, Inst. Farm. Wet., K.U. Leuven, E. Van Evenstraat 4, B-3000 Leuven, Belgium

Abstract: The principles underlying the diffraction of X-rays by crystals and the strategy of determining crystal structure are discussed; special attention is given to results obtained by X-ray crystallography. Examples illustrate how detailed study of the topographical characteristics of drug molecules of a particular pharmacological class may furnish a set of structural parameters that are helpful in understanding a given pharmacological response.

Keywords: *Drug analysis; X-ray structure determination; X-ray analysis; crystal structure analysis.*

Introduction

What is a crystal? Depending upon the personal interest of the crystallographer the answer could be: "it is a well-shaped solid with symmetry-related faces"; or "it is a substance in which the atoms or molecules are packed closely together in such a way that the total potential energy is at a minimum".

These differences in definition clearly state the differences in objectives between those of a classical crystallographer (in previous times usually a mineralogist) whose main interests were the description and classification of crystal systems by the study of the external symmetry of crystals, and the so-called X-ray crystallographer, whose final aim is to understand the factors governing the internal periodic repetition in three dimensions of patterns of atoms or molecules.

In discussing X-ray diffraction by crystals it is convenient to consider first a crystal built up by a simple motif consisting of one atom. The periodic repetition of this motif in three directions generates an infinite collection of identical points; this is generally called a three-dimensional *lattice array*.

Within such a lattice a number of planes with different orientations may be drawn, with each plane containing some of the lattice points. The planes are labelled according to their intercepts on the a, b and c axes, measured from an arbitrarily chosen origin, e.g. 1a, 2b, 4c. By taking the reciprocals of these intercepts, 1/a, 1/2b, 1/4c, and by multiplying each quantity by the least common denominator, which is 4 in this case, three

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numbers (421) are obtained, which are known as the *Miller indices* of the plane. Generally, the Miller indices (*hkl*) of any plane give the orientation of that plane in the crystal with reference to its three internal axes. This notation for naming crystallographic planes was developed in the late 18th century and is based on the consideration that only those planes in a lattice are rational, and so have a real existence in the sense that the three integers have no common factor.

Parallel to any such rational plane there is a whole set of equidistant identical planes that can be generated from it by application of the unit lattice translation. How does such a stack of planes now react on an impinging wave front of X-rays?

The Diffraction Phenomenon

X-rays were discovered in Würzburg, Germany, on 8 November, 1895, by Wilhelm Röntgen while investigating the effects of cathode rays that were produced by electrical discharges through gases at low pressures. Although in 1912 it was not known whether X-rays had the proper wavelengths or not, Max von Laue, an instructor at Munich University, became so interested in the possibility of diffraction by a lattice array of atoms that he influenced two assistants, W. Friedrich and P. Knipping, to set up an experiment in which a pencil of X-rays fell on a crystal. Copper sulphate, found in the laboratory, was used as the crystal. A photographic plate was placed between the X-ray tube and the crystal on the assumption that the crystal would act like a reflection grating. The first exposure produced no effect. By placing the plate behind the crystal, as for a transmission grating, the second attempt was positive; surrounding the imprint of the direct or primary beam, rings of fuzzy spots appeared. Crude as the picture was, it contained an unmistakable proof of the correctness of von Laue's idea of the diffraction of X-rays by crystals. Considering the diffraction phenomenon as the resultant of diffraction from three non-coplanar rows of atoms, von Laue had difficulty in understanding which planes of atoms produced the spots on the film. By examining very carefully von Laue's theory of diffraction, Sir Lawrence Bragg came to the conclusion that the whole phenomenon is equivalent to the resultant reflection of X-rays by an infinite stack of parallel, equally spaced planes (Fig. 1a). This has led to the well known Bragg equation for diffraction: $2d_{hkl} \sin \theta = n\lambda$, where d_{hkl} is the interplanar distance for a set of (*hkl*) planes with identical atomic occupation, θ the scattering angle, λ the wavelength of the incident X-ray beam, and $n(=1,2,3,\ldots)$ the order of reflection. With this formulation Bragg was able to explain successfully all the spots on von Laue's diagrams.

Only a very few crystals are so simple, however. Most crystal structures are characterized by many atoms per unit cell and each of the atoms in the cell is repeated by the same translations. The entire crystal can therefore be thought of as "*m*" intermeshed lattice arrays, one for each of the *m* atoms in the cell. Each lattice array diffracts as described above. For a single lattice array which reflects X-rays under Bragg conditions, the scattering amplitudes are maximum for all reflections but this is not true for the net amplitudes resulting from the contributions by several lattice arrays (Fig. 1b). Indeed, the wavelets issuing from various lattice arrays arrive, in general, with phase differences in the direction of observation; thus the total amplitude received is a function of the angle of scattering and of the size and distribution of the atoms. This gives rise to the following important statement: the directions of the diffraction maxima depend on the geometry of the cell but the amplitudes depend on the locations of the atoms in the cell.

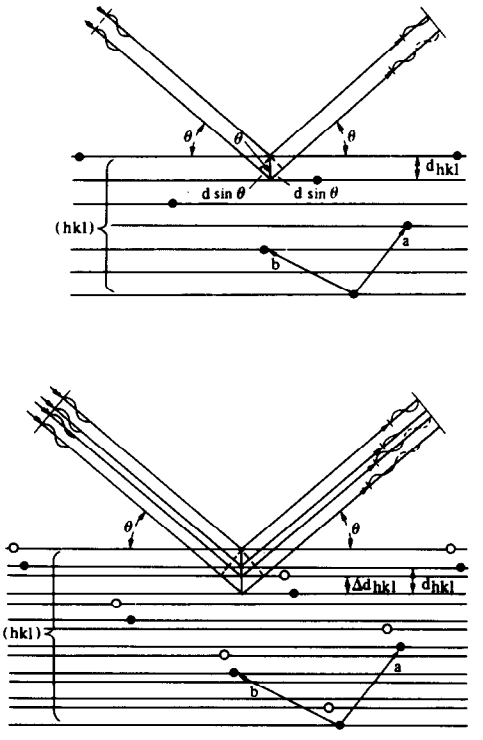


Figure 1
Resultant reflection of X-rays by (a) a single lattice array; (b) two different, intermeshed lattice arrays.

The amplitude, in general, is a complex quantity and is commonly known as the *structure factor,* F_{hkl}. Owing to the definition the structure factor has both magnitude $|F_{hkl}|$, and phase, Φ_{hkl}, and its formal expression is $F_{hkl} = |F_{hkl}|e^{i\Phi_{hkl}}$.

Experimentally, between 100 and 20,000 reflections may be observed, the higher number for the most complex crystals with large cells. If the structure factor itself (i.e. including the phase) could be used as weights, a Fourier synthesis, consisting of the summation of a series of sine and cosine functions with the F_{hkl} values as coefficients, would lead back to the electron and thus to the atom distribution in the crystal. However, this cannot be done. The quantity that can be measured is the intensity of the reflected X-ray beam, and that intensity is proportional to $|F_{hkl}|^2$. The fundamental problem is thus that the phases Φ_{hkl} corresponding to the observed magnitudes $|F_{hkl}|$, are lost.

Crystal Structure Determination

The progress of crystallography from the 1920s consisted of developing procedures to find and record all the diffraction spots and to identify which ones were missing; it was also necessary to devise methods of estimating the relative values of the phases. Before 1935 it was general practice to solve problems of crystal structures by trial and error. In 1935 important progress in evading the "phase problem" was made by the work of Patterson [1], who defined a function $P(u,v,w)$ that uses the $|F_{hkl}|^2$ directly in combination with interatomic vectors. The method is especially helpful for structures that contain a heavy atom (e.g. an organic molecule with a Cl, Br or S atom). In a series

of papers from 1950 Karle and Hauptman [2] have developed a mathematical method of determining probable sign relations between structure factors. This method has now been recognized to be so powerful that in 1985 both authors have been awarded the Nobel Prize for Chemistry.

The limiting factor in X-ray diffractometric techniques is still the availability of good quality crystals, i.e. single crystals of suitable size (minimum 0.1 × 0.1 × 0.1 mm) and perfection. Data collection is executed by a fully-automated four-circle diffractometer comprising an X-ray source, a single crystal diffractometer, detector and counting chain, and a computer for control and storage of the data. High-precision crystal-structure analysis is capable in many cases of determining interatomic distances to a precision of 0.01 Å; with special care and use of low-temperature techniques, precisions of about 0.005–0.001 Å are attainable. Standard accuracies in bond angles are of the order of 0.5–0.1°. With these accuracies in measured C–C bond lengths and angles the crucial experimental evidence was provided for theories of aromaticity in various $4n$ and $(4n + 2)$ π-electron systems. In addition the crystallographic data supported the establishment of the topochemical concept and permitted valuable conclusions to be drawn concerning, for example, the ways in which the molecular architecture can influence the nature and direction of reactions.

The strength of X-ray diffractometric techniques in the analysis of drugs is the determination of the absolute configuration. The assignment of the configuration is based on the anomalous scattering from heavy atoms (frequently a Br or I-atom) present in a noncentrosymmetric space group (e.g. $P2_12_12_1$, $P2_1$,...). Slight differences in intensity will then be measured between a reflection (hkl) and its anti-reflection ($\bar{h}\bar{k}\bar{l}$). By comparing the observed differences, $\Delta F_o = |F_{hkl}|_o - |F_{\bar{h}\bar{k}\bar{l}}|_o$, with the corresponding calculated differences, the absolute configuration can be established.

Stereostructural Properties and Drug Action

The thalidomide tragedy (Contergan®, Softenon®) would probably never have occurred if, instead of using the racemate, the R-enantiomer had been brought on the market. For it was shown that after intraperitoneal administration only the S(−)-enantiomer exerts an embryotoxic and teratogenic effect [3, 4]. Very recently the synthesis and H_1-antihistaminic activity of four racemates of 1-[4-cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenyl-4-piperidine carboxylic acid, termed R 48756, R 49389, R 49429 and R 49549, were described [5]. In this particular series of compounds, R 48756 (cabastine) was found to be the most potent with oral ED_{50}-values of 0.002–0.003 mg/kg in both the histamine-induced lethality test (guinea-pig) and the compound 48/80 lethality assay (rats). The geometry of the four racemates could be derived from the NMR spectra of the benzyl esters. In all cases the cyano-group is axially oriented. In the specific case of cabastine the *cis*- (in the 1,4-substituted cyclohexane ring) and *trans*-configuration (in the 3,4-substituted piperidine ring) were confirmed by X-ray diffraction data (Fig. 2). The enantiomers (levocabastine and dextrocabastine) were synthesized and X-ray diffraction proved the absolute configuration of levocabastine to be 3S, 4R. The intravenous histamine-induced lethality test in guinea-pigs revealed that levocabastine (R 50547) was about 4 times (1 h) to 90 times (24 h) more potent than dextrocabastine (R 50554).

Picenadol is a narcotic analgesic (LY 150720), the enantiomers of which were reported in 1982 to antagonize each other [6]. The D-isomer is a potent morphinomimetic whereas

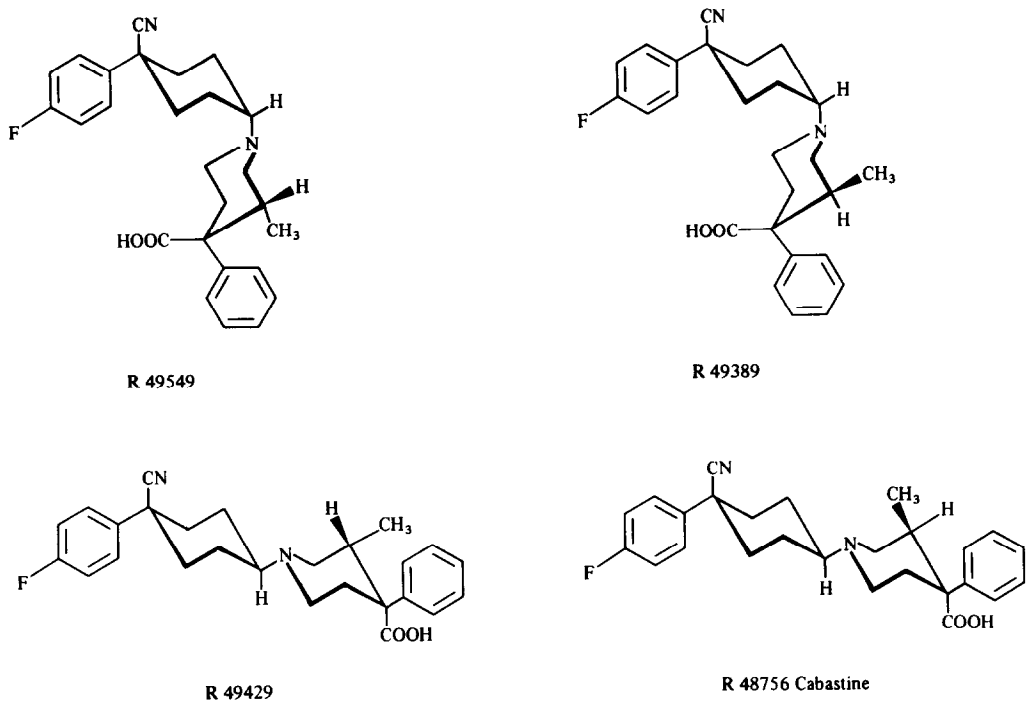


Figure 2
Four racemates of 1-[4-cyano-4-(4-fluorophenyl)cyclohexyl]-3-methyl-4-phenyl-4-piperidine carboxylic acid [5].

the L-isomer is a narcotic antagonist; the racemate is a partial agonist. In barbiturates the neuronal depressant activity generally predominates the S-(−) isomer whereas in the R-(+) isomer the excitatory effect is predominant.

In 1985 Baldwin and coworkers [7] studied the structure affinity relationships for yohimbine and its diastereoisomers at central α-adrenoceptors; they found that, in particular, α-yohimbine and corynanthine are of considerable interest as pharmacological tools for classifying α-adrenoceptors. Yohimbine itself preferentially antagonizes α_2-adrenoceptors; α-yohimbine (rauwolscine) shows even greater selectivity for α_2-adrenoceptors. In contrast the diastereomerically related molecule corynanthine discriminatively antagonizes α_1-adrenoceptors (Fig. 3).

In recent years there has been an increasing number of reports on the stereoselective biotransformation of enantiomers of drugs having asymmetric centres. For example for the anti-inflammatory drugs derived from 2-arylacetic acid (R-(−)-cycloprofen [8]) and 2-arylpropionic acid (R-(−)-ibuprofen [9], R-(−)-benoxaprofen [10], R-(−)-fenoprofen [11], R-(−)-ketoprofen and R-(−)-indoprofen [12]), preferential inversion of the R-(−)-isomer to the S-(+)-enantiomer has been reported in animals and man. Animal studies revealed stereoselective glucuronidation of oxazepam [13] and isomerization of R-(−)- to S-(+)-clidanac [14]. Although the S-(+)-enantiomer is consistently more active than the R-(−)-isomer, administration of the (R,S)-racemate in these cases will be definitely cheaper, and perhaps better. Recent developments in the use of crystallographic data originate from the now widely accepted fact that detailed study of the topographical

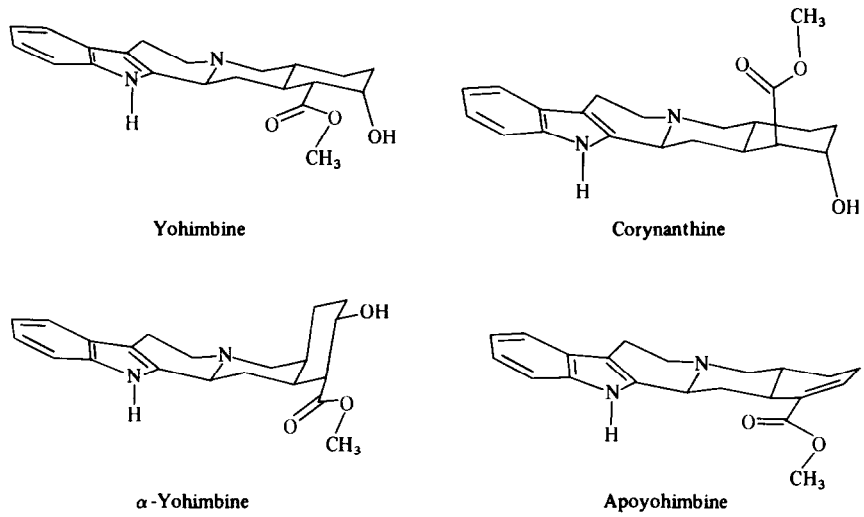


Figure 3
Chemical structures of yohimbine stereoisomers and analogues [7].

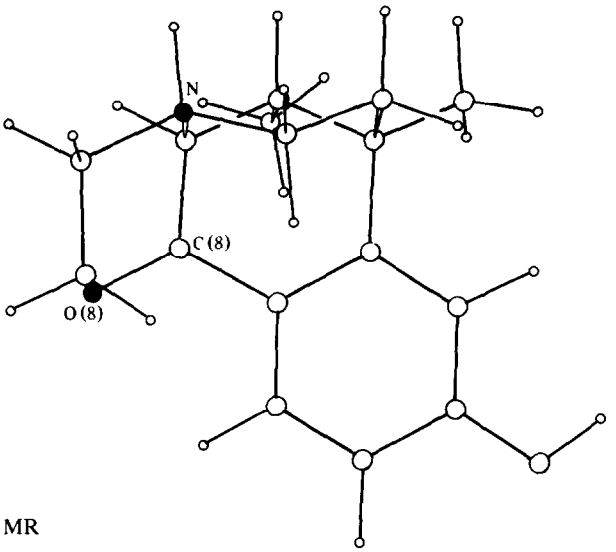
characteristics of drug molecules of a particular pharmacological class putatively interacting with the same receptor, may furnish a set of structural requirements (the so-called "pharmacophore") necessary to elicit a given pharmacological response. During the last decade ample evidence has been accumulated for the existence of multiple opioid receptors (μ, δ, κ, ϵ). The first compounds characterized as κ-agonists belong to the 6,7-benzomorphan class, i.e. ketazocine, bremazocine and MR 2034.

A striking feature is, that, compared with most other benzomorphans, all those showing kappa activity possess in addition to the phenolic hydroxy-group in the 2′ position an extra oxygen atom in either the crucial substituent at N(2) or in position 8 [15] (Fig. 4a).

In order to investigate the influence of that extra oxygen, net charges, bond polarities and proton affinities were calculated by using (PCILO) on the basis of the crystal structures [15].

The net atomic charges and bond polarities in the nitrogen region are very similar to those previously calculated for morphine-like opiate narcotics [16]. The proton affinities of MR 2034, bremazocine and MR 2549 are the same and exceed by 6.6 kcal that of morphine; ketazocine has the same proton affinity as that of cyclazocine (4.4 kcal above that of morphine).

A molecular modelling study on the basis of calculated energy maps was undertaken for the two molecules with the conformationally most restricted orientations of the "kappa oxygen" lone pairs (i.e. ketazocine and MR 2034). This study revealed that, with the benzomorphan skeleton fitted and with varying torsion angles of the N-side chain, the oxygen atoms in both molecules can form a hydrogen bond to a same group in the receptor (in this study an OH-group). Only the area with torsion angles C(1)–N(2)–C(1″)–C(2″) and N(2)–C(1″)–C(2″)–O(3″) at 240 and 95°, respectively revealed such a fit. An energetically possible fit of the almost inactive 2″ R diastereoisomer of MR 2034 on this model was not found. This was also the case for the 2″ S methylated derivative of MR 2034, which possesses analgesic properties but is devoid of kappa activity [17]. In



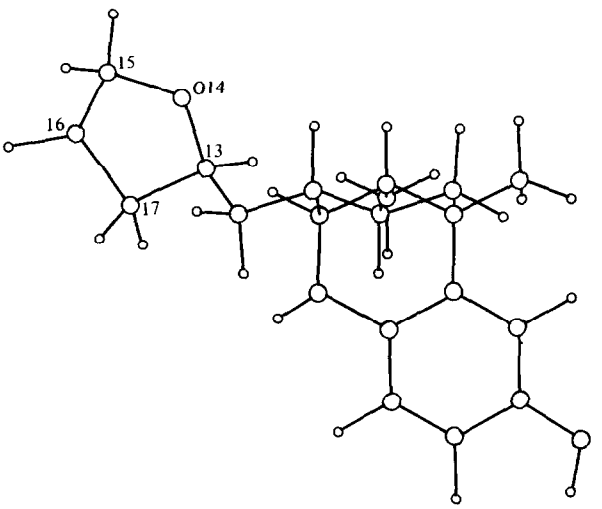


Figure 4
Three-dimensional plots of (a) MR 2034, (b) MR 3159.

Table 1
PCILO-calculations on some benzomorphans compared with data for morphine-like opiate narcotics [16]

Sum of charges in the cationic nitrogen region			
Ketazocine	0.610	Morphine	0.670
Bremazocine	0.607	Nalorphine	0.642
MR 2034	0.614	Mr 2547	0.624
MR 3159	0.616		
Proton affinities (kcal mol^{-1})			
Ketazocine	240.2	Morphine	236.3
Bremazocine	243.1	Fentanyl	236.0
MR 2034	242.9	Naloxone	238.6
MR 2549	242.6	Cyclazocine	240.6
MR 3159	240.2		

both cases steric hindrance of the side-chain with the protonated nitrogen is the main reason. In an attempt to obtain new kappa agonists with the oxygen in C(8), as in ketazocine, MR 3159 was synthesized (Fig. 4b), but found to lack kappa agonist properties. Subsequently a crystal structure analysis was undertaken and PCILO calculations were carried out. The most striking differences, and the possible explanation for the lack of kappa activity, lie in the structural area: steric hindrance of C(13) for interaction with the receptor; the distance from N to the centre of the aromatic ring in MR 3159 is 4.325(7) Å whereas in the other compounds it is in the range of 4.37–4.54 Å; the orientation of the lone pair on N is slightly different from that in the other compounds.

Conclusions

The objection that all this information is gleaned from a static representation of the crystal, can be refuted by the established fact that the conformation found in the solid state is always one of the minimal energy states of the molecule, in the gaseous state as well as in solution (NMR-data). Furthermore, in all determinations of crystal structure by modern methods, the calculations include a "temperature factor" for each atom (the Debye–Waller factor) which allows for the effect of thermal motion on the accuracy of the deduced atomic positions and bond lengths.

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