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# Solution Conformation of the Ionophore A23187 and Its Magnesium Salt

MARC J. O. ANTEUNIS

*Laboratory for NMR Spectroscopy, Department of Chemistry, State University of Ghent, Krijgslaan 271 (S4bis), B-9000 Ghent, Belgium*

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The analysis of proton nmr spectra at 300 MHz in $C_6D_6$ and in $CDCl_3$ of the divalent cation ionophore A23187 and its magnesium salt are reported and discussed in terms of configurational and conformational behaviour. The conformation of the free acid in solution was found to be almost identical to that described previously (M. O. Chaney, P. V. Demarco, N. D. Jones, and J. L.Occolowitz, *J. Amer. Chem. Soc.* **96**, 1932 (1974)) for the solid state, except that in apolar solvents no head-to-tail interaction is observed, in that free rotation around certain bonds (e.g., $C_{15}$–$C_{19}$, structures **1** and **2**) substantially prevents such quasi-cyclic forms. For the conformation of the magnesium salt, two monovalent units aggregate around the divalent cation, the two substructures displaying equivalent atoms in each of their corresponding positions. A compact sphere-like form having a $C_2$ axis and a cavity of about 3.5 Å is proposed, the cation being held in the middle by two carboxylate ions, carbonyl and *N*-benzoxazole units as the ligands. These conclusions result from consideration of the extracted parameters in the proton nmr spectrum and from model building. Thus, the most probable form of the Mg-salt in solution corresponds almost exactly to that of the Ca-salt in the solid state, as very recently revealed by the X-ray analysis of M. O. Chaney, N. D. Jones, and M. Debono (*J. Antibiot.*, in press).

## INTRODUCTION

In connection with our conformational studies of ionophores and their cation complexes in solution, including Lasalocid (*1a*) and the nactins (*1b*), the antibiotic A23187 (**1**) constitutes a very attractive member in this series because it is one of the most simple complexes of low molecular weight (MW = 523). Furthermore, it is able to clathrate divalent ions in a 2 : 1 ionophore : cation ratio (*2–4*). The compound, obtained (*5*) from cultures of *Streptomyces chartreusensis*,[1] displays as the free acid a relative simple 300-MHz $^1H$ nmr spectrum (Figs. 1 and 2), all patterns of which could be analyzed by simple first-order inspection, except for some couplings between protons H-16, H-17, and H-18 in $C_6D_6$ and between protons H-11, H-13, and H-12 in $CDCl_3$. The extracted values, obtained in $C_6D_6$ and in $CDCl_3$, are collected in Table 1. The spectrum of the magnesium salt is less clearly defined (see Fig. 3), and not all the spectral values could be extracted (Table 2).

[1] The present sample was a gift of Eli Lilly Company, Indianapolis, Ind. Lot No. 361-VO2-276-1.





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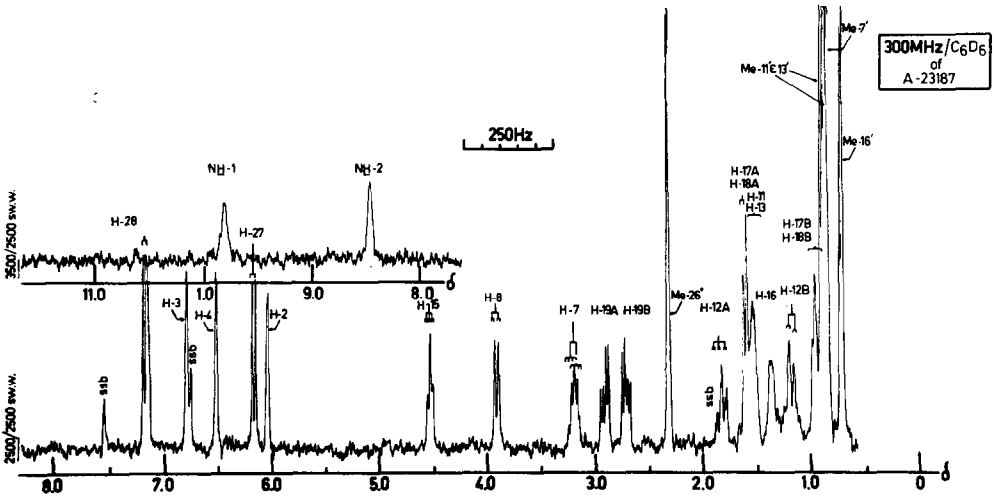



FIG. 1. Three-hundred-megahertz $^1H$ nmr spectrum of A23187, free acid, in $C_6D_6$. Proton absorptions are numbered according to structure **1**. The scale in ppm from TMS-internal is drawn with respect to the vertical mark line at the left, the first number indicating the offset from TMS in hertz at 300 MHz and the second number gives the sweep width. Spinning side bands are indicated by ssb.

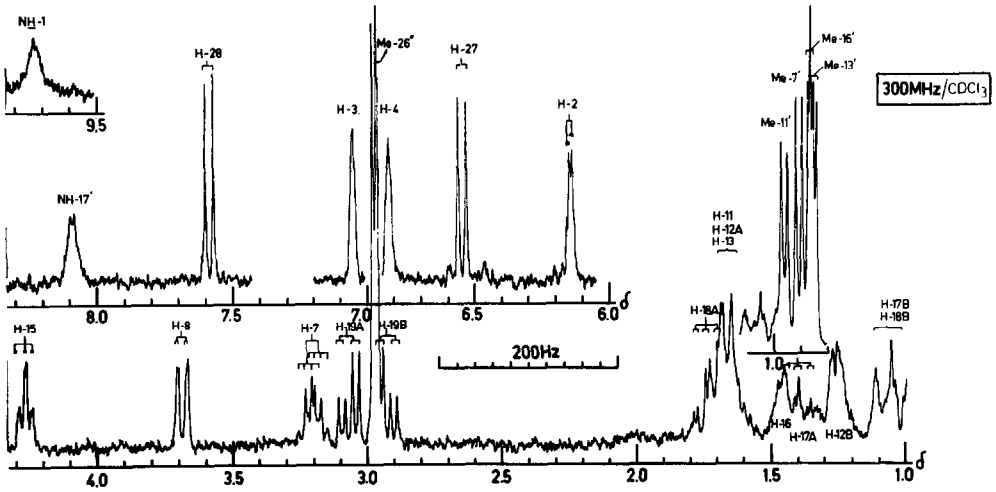



FIG. 2. Three-hundred-megahertz $^1H$ nmr spectrum of A23187, free acid, in $CDCl_3$. See Fig. 1 for further specifications.

## METHODS

All reported assignments and (relative signs of) coupling constants were confirmed by homo-INDOR and double irradiation experiments for the free acid and by double irradiation of the magnesium salt. Coupling constants were extracted from simple first-order analyses and were ascertained by simulations (SIMEQ-16/II), whereby it was necessary to consider only subspectral simplifications. Spectra were taken at 19°C and the concentration was about 0.1 m*M*.

TABLE 1

SHIFT VALUES IN ppm FROM TMS-INTERNAL OF A23187[a]

| | Proton | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Solvent | N*H*-1 | H-2 | H-3 | H-4 | H-7 | H-8 | H-11 | H-12A[c] | H-12B[d] | H-13 | H-15 | H-16 |
| $C_6D_6$ | 9.78 | 6.02 | 6.80 | 6.53 | 3.20 | 3.92 | 1.53 | 1.84 | 1.19 | 1.55 | 4.54 | 1.37 |
| $CDCl_3$ | 9.74 | 6.24 | 7.06 | 6.80 | 3.20 | 3.69 | 1.64 | 1.66 | 1.26 | 1.67 | 4.27 | 1.46 |
| $\Delta$(Asis)· × $10^2$ | 4 | −22 | −26 | −27 | 0 | 23 | −11 | 18 | −7 | −12 | 27 | −9 |

| | Proton | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H-17A[c] | H-17B[d] | H-18A[c] | H-18B[d] | H-19A | H-19B | N*H*-26′ | H-27 | H-28 | Me-7′ | Me-11′ | Me-13′ | Me-16′ | Me-26″ |
| $C_6D_6$ | 1.16 | 0.97 | 1.16 | ~0.95 | 2.92 | 2.72 | 8.43 | $6.14_5$ | 7.19 | 0.86 | 0.91 | 0.87 | 0.73 | 2.33 |
| $CDCl_3$ | 1.40 | $1.09_5$ | 1.73 | 1.02 | 3.07 | 2.94 | 8.09 | 6.65 | 7.56 | 0.91 | 0.97 | 0.86 | 0.87 | 2.97 |
| $\Delta$(Asis)· × $10^2$ | 24 | −12 | 57 | −7 | −15 | −22 | 34 | −50 | −37 | −5 | −6 | 1 | −14 | −64 |

Coupling constants (in Hz) of A23187

| | $J$ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $^2J$(12) | $\lvert^2J(17)\rvert$ | $\lvert^2J(18)\rvert$ | $^2J$(19) | $^3J$(1, 2) | $^4J$(1, 3) | $^4J$(1, 4) | $^3J$(2, 3) | $^4J$(2, 4) | $^3J$(3, 4) | $^3J$(7, 8) |
| $C_6D_6$ | >−13.3 | ~13.0 | ~13.5 | $-15.1_5$ | 2.40 | 1.40 | 1.10 | 3.70 | 2.40 | 2.4 | 10.3 |
| $CDCl_3$ | −13.5 | ~13.0 | ~13.5 | −15.3 | $2.4_5$ | 2.4 | 1.30 | 3.80 | $2.4_5$ | $2.5_5$ | 10.3 |

| | $J$ | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $^3J$(8, 13) | $\Sigma^3 J$(16)[e] | $^3J$(15, 16) | $^3J$(11, 12A) | $^3J$(11, 12B) | $^3J$(12A, 13) | $^3J$(12B, 13) | $^3J$(15, 19A) | $^3J$(15, 19B) | $^3J$(16, 17A) | $^3J$(16, 17B) |
| $C_6D_6$ | 2.30 | <10 | 2.2 | 12.4 | 4.2 | 4.5 | $2.6_5$ | $7.0_5$ | $7.1_5$ | — | — |
| $CDCl_3$ | 1.70 | ~8 | 2.1 | —[a] | —[a] | —[a] | —[a] | $7.2_5$ | $7.5_4$ | ≲4.0 | ~2.0 |

| | $J$ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $^3J$(17A, 18A) | $^3J$(17A, 18B) | $^3J$(17B, 18A) | $^3J$(17B, 18B) | $^3J$(27, 28) | $^3J$(7, Me) | $^3J$(11, Me) | $^3J$(13, Me) | $^3J$(16, Me) | $^3J$(26′, Me) |
| $C_6D_6$ | —[b] | —[b] | —[b] | —[b] | $9.1_5$ | $6.8_5$ | $6.8_5$ | $6.8_5$ | $6.9_5$ | 4.5 |
| $CDCl_3$ | 13.5 | ≲4.0 | 4.5 | ~2.0 | $9.1_0$ | $6.9_0$ | $6.9_5$ | 6.1 | 7.0 | 4.8 |

[a] Degenerated: positions of H-11/12A and H-13/12A almost identical.
[b] Degenerated: positions of H-17A/18A and H-17B/18B almost identical.
[c] Axial proton, see text.
[d] Equatorial proton, see text.
[e] Excluding $J^{exo}$(16, 16′).
*Note:* See structure **1** for numbering. $\Delta$(Asis) = $\delta(C_6D_6) - \delta(CDCl_3)$.

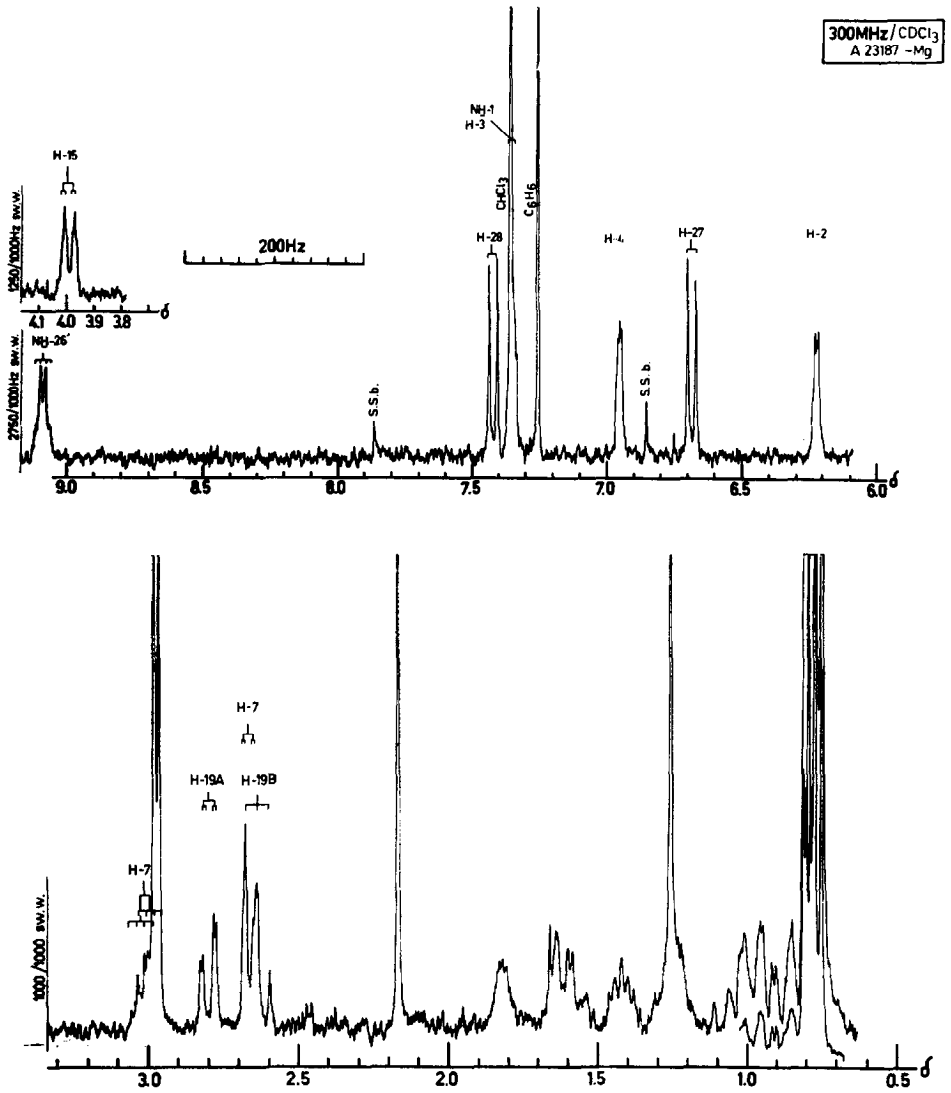



FIG. 3. Three-hundred-megahertz $^1H$ nmr spectrum of the magnesium salt of A23187 in $CDCl_3$. See Fig. 1 for further specifications.

## RESULTS

### *A23187, Free Acid*

The following spectral features are relevant to the conformation of A23187. (We follow an increasing numbering of the skeleton as indicated in structure **1**). A coupling of 10.3 Hz between H-7 and H-8 reveals their antiperiplanar relationship with a torsion angle of $\lesssim 180°$. The coupling $^3J(8, 13) = 2.30$ Hz discloses a small angle between both protons H-8 and H-13; and because $^3J(12A, 13)^2 \sim 4.5$ Hz and $^3J(12B, 13)^2 \sim 2.5$ Hz,

[2] We use the coding A and B for the designations of the partner at lowest and highest field position, respectively.

TABLE 2

$^1$H nmr PARAMETERS OF A23187, MAGNESIUM SALT

| Solvent | Proton | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N*H*-1 | H-2 | H-3 | H-4 | H-7 | H-8 | H-11 | H-12A | H-12B | H-13 | H-15 | H-16 |
| $C_6D_6$ | 7.71 | 6.19 | 7.16 | 6.83 | $2.94_0$ | 2.82 | ~1.3 | ~1.3 | ~1.1 | 1.02 | 4.30 | 2.20 |
| $CDCl_3$ | 7.36 | 6.22 | 7.35 | $6.95_5$ | 3.01 | 2.67 | 1.42 | 1.59[b] | ~0.85[b] | 1.82 | 3.99 | 1.22[b] |
| $\Delta$(Asis)·$10^2$ | 35 | −4 | −29 | −12.5 | −7 | 16 | ~−10 | ~−30 | ~25 | −80 | 71 | 98 |
| $\Delta(CDCl_3)\cdot 10^2$ | 238 | 2 | −29 | −15.5 | 19 | 102 | 22 | 7[b] | 37[b] | −15 | 28 | 24[b] |

| | Proton | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H-17A | H-17B | H-18A | H-18B | H-19A | H-19B | N*H*-26′ | H-27 | H-28 | Me-7′ | Me-11′ | Me-13′ | Me-16′ | Me-26″ |
| $C_6D_6$ | ≳1.0 | ≲1.0 | $1.51_4$ | ~1.0 | 3.12 | 2.64 | 9.89 | 6.46 | 7.20 | 0.87 | 0.81 | 0.57 | 0.70 | 2.62 |
| $CDCl_3$ | 1.01[b] | 0.94[b] | [c] | [c] | 2.80 | 2.64 | 9.09 | 6.68 | 7.42 | 0.80 | 0.76 | 0.79 | 0.76 | 2.97 |
| $\Delta$(Asis)·$10^2$ | ~0 | ~0 | — | — | 31 | 0 | 80 | $-22._5$ | −22 | 7 | 5 | −22 | −6 | $-35._5$ |
| $\Delta(CDCl_3)\cdot 10^2$ | 39[b] | 15[b] | — | — | 27 | 30 | −100 | −3 | 14 | 11 | 21 | 7 | 11 | 0.0 |

Coupling constants (in Hz) of A23187

$J$

| | $\lvert^2J(12)\rvert$ | $^2J(17)$ | $\lvert^2J(18)\rvert$ | $^2J(19)$ | $^3J(1,2)$ | $^4J(1,3)$ | $^4J(1,4)$ | $^3J(2,3)$ | $^4J(2,4)$ | $^3J(3,4)$ | $^3J(7,8)$ | $^3J(8,13)$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_6D_6$ | <11.5 | [c] | 13.5 | −12.8 | >1.6 | [a] | ≳1.1 | 3.8 | ≳1.6 | ≳1.6 | 10.0 | 1.8 |
| $CDCl_3$ | [c] | [c] | [c] | −13.0 | 1.5 | [a] | 1.1 | 3.8 | 1.6 | 1.6 | 9.8 | 2.1 |

$J$

| | $^3J(11,12A)$ | $^3J(11,12B)$ | $^3J(12A,13)$ | $^3J(12B,13)$ | $^3J(15,16)$ | $^3J(16,17A)$ | $^3J(16,17B)$ | $^3J(17A,18A)$ | $^3J(17B,18A)$ |
|---|---|---|---|---|---|---|---|---|---|
| $C_6D_6$ | [c] | [c] | [c] | [c] | ≲2.0 | Small[d] | Small[d] | ~13 (or 5) | 5 (or ~13) |
| $CDCl_3$ | large | [c] | [c] | [c] | 2.0 | [c] | [c] | [c] | [c] |

$J$

| | $^3J(15,19A)$ | $^3J(15,19B)$ | $^3J(27,28)$ | $^3J(7,Me\text{-}7')$ | $^3J(11,Me\text{-}11')$ | $^3J(13,Me\text{-}13')$ | $^3J(16,Me\text{-}16')$ | $^3J(NH,Me\text{-}26'')$ |
|---|---|---|---|---|---|---|---|---|
| $C_6D_6$ | 2.3 | 11.8 | 9.0 | 6.5 | 6.5 | 6.7[e] | 6.9[e] | 5.0 |
| $CDCl_3$ | 2.3 | 11.9 | 9.1 | 6.7 | 6.5 | 6.5 | 6.5 | 4.8 |

*Note:* Shift values (from TMS-internal) in ppm. See structure **1** for numbering. $\Delta$(Asis) = $\delta(C_6D_6) - \delta(CDCl_3)$. $\Delta(CDCl_3) = \delta(CDCl_3$, free acid) − $\delta(CDCl_3$, Mg-salt).

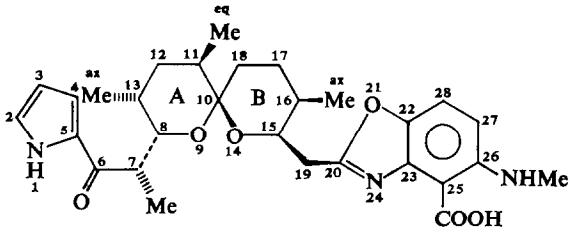
[a] H-3 hidden proton by solvent peak.

[b] Tentative values.

[c] Not known with certainty because of extensive overlap with other signals or because of degenerated character.

[d] $\Sigma^3$ (J(16) ~ 28 Hz.

[e] Broadened (axial Me).

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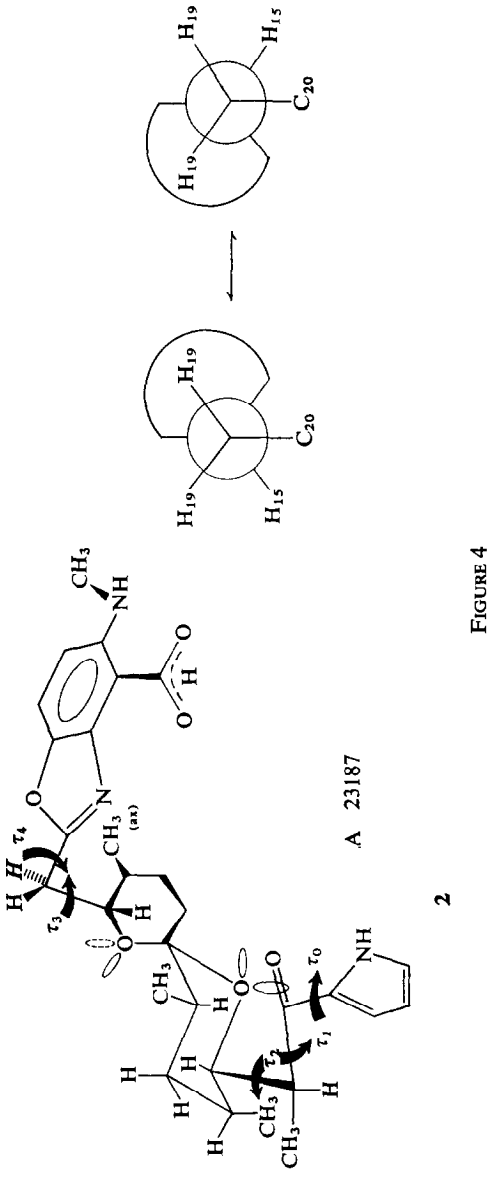
proton H-13 must be equatorial. From the values $^3J(11, 12A) = 12.4$ Hz and $^3J(11, 12B) = 4.2$ Hz, it is clear that H-11 is axial and, thus, Me-11′ is equatorial. It is concluded that Me-13′ stays axial, because in view of the small coupling the oxo-pyrrole moiety should otherwise be axial; and together with the spiro function at C-10 the A ring would become much too strained and therefore flip over into the inverse form. Hence Me-11′ would not be equatorial. Also the values of $^3J(12A, 13)$ and $^3J(12B, 13)$ are only conceivable in terms of the relative positioning of Me-13′ (and thus the $C_8$ fragment).[3] The relatively low values for $^3J(8, 13)$ (as low as 1.70 Hz in $CDCl_3$) and $^3J(12B, 13)$ may be partly explained by the fact that H-13 is a proton that stays in a planar M-mode pathway with respect to one of the ring $O_9$ lobes, a feature also observed in 1,3-dioxanes. (*7*). However, the relatively large value for $^3J(12A, 13)$ reveals, on the other hand, that ring A may be a somewhat flattened chair, especially around the torsions $C_8$–$C_{13}$–$C_{12}$. Following the same lines of reasoning and taking into consideration similar observations concerning $J(16, 17)$ and $J(17, 18)$, ring B also shows analogous behaviour. The value of $^3J(15, 16) \sim 2.2$ Hz clearly shows that both substituents, the benzoxazole moiety and Me-16′ are *cis* to each other. Because an axial benzoxazole substituent would cause an unacceptable strain (syn-axial strain with the spiro substitution at $C_{10}$), it is therefore clear that $C_{19}$ stays equatorial, hence Me-16′ must be an axial group. The couplings $^3J(16, 17A)$ and $^3J(16, 17B)$ are in accord with this conclusion, as certainly is the value of $\Sigma^3J(16)$. $^3J(15, 19A) = 7$–$7.2$ Hz and $^3J(15, 19B) = 7.2$–$7.5$ Hz can be interpreted as a result of an averaging between two torsion angles of clinal and antiperiplanar dispositions, respectively (Fig. 4). Therefore the molecule possesses freedom of rotation around $\tau_3$. Finally, $^2J(19) = 15.1$–$15.3$ Hz clearly demonstrates the relative spatial position of the $CH_2$–19 methylene grouping with respect to the plane of the benzoxazole moiety. Free rotation around $\tau_4$ would result in $^3J(19) \lesssim 14.3$ Hz (*8*). Therefore, one of the H-19 protons lies instead in the nodal plane of the aromatic $\pi$ system (*8*).

With respect to shift values of other protons, it is noteworthy that H-17A and H-18A, which are the axial counterparts of H-17B and H-18B, respectively, are located at *lowest* field. This is in each case the result of the presence of a syn-axial substituent. Exactly the same can be observed for H-12A with respect to H-12B.

We may perhaps also comment on the noticeably low field position of H-15 ($\delta$ 4.3 in $CDCl_3$ and $\delta$ 4.5 in $C_6D_6$). First, the model (**2**) predicts that this proton could be

[3] The usual criterion $^3J(Me_{ax}, H) > {}^3J(Me_{eq}, H)$ that can often be used for the assignment of the relative positioning (*6*) of side-chain methyl groups, cannot be used in the present case, because the methyl groups, being in the $\beta$-position to a ring oxygen atom, do not suffer from appreciable synaxial strain, which is the basis of this diagnostic discrimination (*6*).




2 A 23187

FIGURE 4

deshielded somewhat by the aromatic benzoxazole grouping. Also, a neighbouring axial substituent substantially deshields a proton (*9*), thus confirming that Me-16′ is axially positioned.

Using all the previous data, one may depict a rather detailed solution conformation. The conformation of A23187 in the solid state is known (*2*) (except that the absolute configuration has only been proposed in relation to the other polyetherin antibiotics). This spatial structure is shown as **2**. The present study, as discussed above, reveals that the solution conformation is very similar to that in the crystalline state. In solution, freedom of rotation around $\tau_3$, resulting in an almost equal population of the two rotamers depicted in Fig. 4, is allowed. This is at variance with the solid-state behaviour (*2*). We were unable to track the value for $\tau_0$, but it is clear that overlap between the carbonyl $\pi$ system and the pyrrole moiety should be maintained; and we accept that $\tau_0$ takes the value as depicted by **2**, because acyl-pyrroles are the most stable if the C=O dipole is pointing into the same direction as the N–H bond (*10*). Only $\tau_1$ remains unknown, and this might have several values in solution.

*A23187, Magnesium Salt*[4]

Although the $^1$H spectrum (Fig. 3) of the magnesium salt is much less well defined in the aliphatic region than is that of the free acid, we were able to extract most of the relevant data (Table 2) in order to deduce its spatial structure. Some protons have shifted quite appreciably (see Table 2, $\Delta(CDCl_3)$) and among these the *upfield* shift by 1.02 ppm for H-8 is noticeable. Also the ASIS-effect is quite different, and this may already point to changes in relative positions of one or both of the aromatic moieties. The largest difference is found for H-16, which now shows a greatly enhanced positive ASIS-effect and might indicate that the rotation of the benzoxazole moiety has been affected (*vide infra*) in the magnesium derivative. Although the locations in the spectrum could be tracked by homo INDOR or double irradiation for most of the protons, it was not always possible to obtain further detailed information about the coupling constants. It is, however, clear that the conformations of rings A and B have remained unaffected, on the basis of the facts that: (a) $^3J(8, 13) = 2.1$ Hz (almost unaffected); (b) the patterns of H-16 (observed in $CDCl_3$ and $C_6D_6$) and of H-13 (observed in $CDCl_3$) have band widths comparable to those in the free acid compound; (c) H-11 (observed in $CDCl_3$) is involved in a large vicinal coupling constant (thus is axial) and (d) H-18A in $C_6D_6$ could be observed to consist of a large triplet of doublets, identical to what is observed in the free acid and what is expected for an axial ring proton.

The most interesting feature, and difference with respect to the behaviour of A23187) itself, is found, however, in the values of $^3J(15, 19) = 11.9$ and 2.3 Hz, showing that free rotation around $\tau_3$ has disappeared and that H-19B stays almost antiperiplanar to H-15, while H-19A stays clinal. A second change is found in the torsion around $C_{19}$–$C_{20}$. In the free acid $^2J(19) \sim -15.2$ Hz, but in the magnesium derivative it is only 12.8

[4] The magnesium salt was obtained by passing a $CDCl_3$-solution of the free acid through a short magnesium carbonate column until the spectrum remained unchanged. Analysis by atomic absorption spectrometry indicated a 2:1 antibiotic:cation ratio. Ultradry solvents (molecular sieves) were used. The same sample was reused after evaporation. Spectral data obtained in methanol (not reported) indicated through the extracted *J*-values an unchanged conformational behaviour for the magnesium salt.

to 13 Hz. Therefore, the $CH_2$-19 function has rotated in a way that the nodal plane of the benzoxazole $\pi$ system almost bisects the H–C–H angle for which a value of about 13 Hz may be predicted (*8*). From the above conclusion and also from considerations of model constructions, two models may be proposed for the Mg-salt of A23187, taking symmetry elements into account in order to have identical shifts for similar protons in both units surrounding the cation (isochronous protons). Both models are characterized by a twofold axis. In the first, displayed as Fig. 5, the cavity is rather large (e.g., about 7 Å in diameter, ionic radius of $Mg^{2+} = 0.66$ Å) and is therefore believed to be less probable. The participating ligands in the octahedrally surrounded divalent cation are

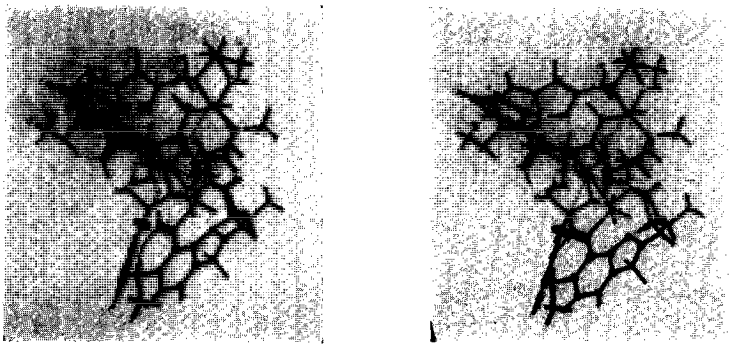


FIG. 5. Stereoscopic view of the less probable conformation (see text) of A23187: divalentcation complex consisting of two ionophore molecules surrounding a divalent cation (such as magnesium), thus forming a spherical structure of about 17 Å in diameter. The conformation of each of the individual moieties is nearly identical to the solution conformation of the free acid, except for the loss of rotational freedom around $\tau_3$ and $\tau_4$ (see structure **2**). Special features are (a) the coplanarity of $(C{=}O)_6$ with the pyrrole ring, whereby the C=O bond points in the same direction as the $N_1$–H bond; (b) the quasi-antiperiplanar disposition of H-7/H-8; (c) the chair conformations of both tetrahydropyran rings with Me-13′ and Me-16′ in axial positions; (d) one of the H-19 protons in antiperiplanar disposition with H-15 (not in the free acid compound); and finally (e) the nodal plane of the benzoxazole $\pi$ system bisecting the $CH_2$-19 grouping. In the free acid, the $CH_2$-19 grouping is oriented slightly differently, e.g., one of the C–H bonds of $CH_2$-19 is almost parallel with the nodal plane, as was also found in the crystalline state (*1*). The gray center atom shown in the stereoscopic view is the cation. The straight gray lines towards the center are the coordinative bonds and the black towards the center are the ionic bonds with the carboxylic function. The carbonyl function is represented by a double bend bond. The gray bend bonds represent hydrogen bonding. Short (black) bonds not ending on pins are free orbitals, e.g., of oxygen. For simplicity multiple bonds of the aromatic moieties are not explicitly displayed.

for each unit the carboxylate –25, O-14 and the keto function, respectively. The possibility remains that a weak hydrogen bond exists between $NH_1 \cdots N'_{26}$ (distance about 4 Å), thus holding the two units together. The model accounts for the relatively large upfield displacement of NH-1, because the amine proton is situated in the shielding cone of the (second) benzoxazole ring at a relatively close distance. This is at variance with the situation in the free acid. However, a contribution by interaction (*11*) of the various C–H bonds by the bound positive ion may intervene, especially when the latter is relatively close. The upfield shift of H-8 when passing from the free acid to the salt is nevertheless not well explained by this model.

A second model, shown stereoscopically as Fig. 6 is characterized by a much more compact overall structure (about 16 × 13 × 13 Å) with a cavity which is about 3.5 Å in diameter. Next to the carboxyl and carbonyl ligand, the third coordination site in each unit is the benzoxazole nitrogen. Hydrogen bonding between the pyrrole NH-1 of the first unit with $COO^-$-25 of the second one, and vice versa, is a typical feature, thus anchoring the two halves of the bimolecular structure together and forming a cavity

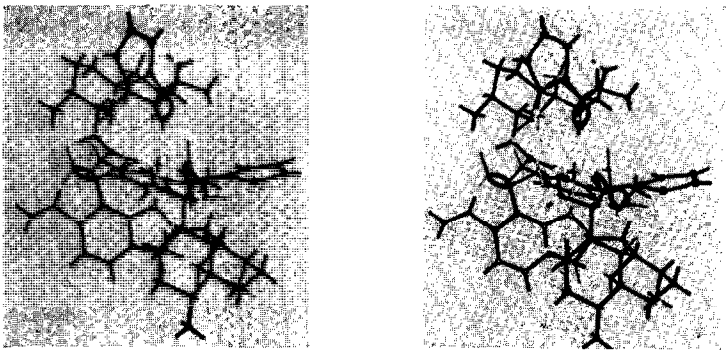


FIG. 6. Stereoscopic view of the more probable solution conformation of A23187, $Mg^+$-salt (2:1). Partial conformational peculiarities are the same as for the alternative form, displayed in Fig. 5, except that the benzoxazole nitrogen N-24 is the ligand instead of the O-14 of one of the spiro ring systems. Also, the carbonyl points directly toward the cation, i.e., the cation lays in the prolongation of the C=O axis, rather than at about 60°, as in the foregoing model. The cavity is much smaller (e.g., 3.5 Å), and the two halves are probably linked to each other by hydrogen bond bridging between NH-1 and $COO^-$-25′.

of more appropriate dimension than in the previous model. The upfield shift of H-8 in the salt is easily understood because it is situated above the benzoxazole system (in its own unit).

Differences in rotation angles exist for the two models, but they are around bonds that do not display interproton interactions and therefore cannot be tracked by $^1H$ nmr spectroscopy. Recently (*3*, *4*) the crystal structure of the calcium complex of A23187 was elucidated, and the second model proposed in the present paper is identical to this solid-state Ca-salt structure proposed by Chaney *et al.* (*3*), where calcium coordination is octahedral and the solvent molecule is hydrogen-bonded to the carboxylic function. In the hydrated crystal structure proposed by Smith and Duax (*4*) the coordination number of calcium is seven (the seventh ligand being $H_2O$, and the ligand bond forming the pseudo-twofold axis) and can be obtained from the present model by twisting (*3* and Fig. 6) the plane formed by the nitrogen atoms of benzoxazole and the calcium ion so as to *bisect* the carbonyl oxygen–calcium–carboxyl oxygen angles of each subunit. In the model of Ref. (*3*), this plane is identical to that formed by the carbonyl (of the first unit)–calcium–carbonyl (of the second unit) oxygen atoms. From the data extracted from the solution conformation, it is not possible to decide between these slightly different modifications. The model proposed by Smith and Duax (*4*), however, does not account well for the burial of the ion away from its surroundings.

The alternative way to decide would be to answer the question whether it would matter much for complexation ability if the ion remains solvated or not, which would be reflected, e.g. in $K_c$ values for different ion species. Thus, in nonactin it has been clearly demonstrated (*12*) that specific complexation between $K^+$ and $Na^+$ is *not* the result of a geometric factor, but lies in differences in hydration energies. $K_c$ values for the two ions and nonactin do not differ greatly in dry acetone, but affinity is largely in favour of $K^+$ in wet acetone.

It must be emphasized, however, that the gross change in conformation, when passing from the free acid antibiotic to the salt and as found in the crystalline state (*2–4*), is entirely in agreement with those in solution. Thus, major changes occur (*4*) around $\tau_1$ and $\tau_3$ (structure **2**), reflecting the relative orientations of the pyrrole and benzoxazole moieties in space, both approaching each other and forming half the required cavity for ion trapping.

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## REFERENCES

1. a. M. J. Anteunis, *Bioorg. Chem.* **5**, 327 (1976); b. M. J. Anteunis and A. De Bruyn, in progress.
2. M. O. Chaney, P. V. Demarco, N. D. Jones, and J. L. Occolowitz, *J. Amer. Chem. Soc.* **96**, 1932 (1974).
3. M. O. Chaney, N. D. Jones, and M. Debono, *J. Antibiot.*, in press, 1976. We are much indebted to Dr. Noel D. Jones (Physical Chemistry Research, Lilly Research Laboratories, Indianapolis, Ind. (46206) for his communication of the solid-state data prior to publication. He also pointed out that the magnesium and calcium crystal structures are isomorphous.
4. G. D. Smith and W. L. Duax, *J. Amer. Chem. Soc.* **98**, 1578 (1976).
5. R. L. Hamill, M. Gorman, R. M. Gale, C. E. Higgins, and M. H. Hoehn, Abstracts, 12th International Conference on Antimicrobial Agents and Chemotherapy, Atlantic City, N.J., p. 65. September 26–29, 1972.
6. M. Anteunis, *Bull. Soc. Chim. Belg.* **80**, 3 (1971).
7. M. Anteunis, D. Tavernier, and F. Borremans, *Bull. Soc. Chim. Belg.* **75**, 396 (1966).
8. M. Barfield and D. Grant, *J. Amer. Chem. Soc.* **85**, 1899 (1963).
9. D. Danneels and M. Anteunis, *Org. Magn. Reson.* **6**, 617 (1974); cf. D. Danneels and M. Anteunis, *Tetrahedron Lett.* 687 (1975).
10. Chr. Garbay-Jaureguiberry, Ph.D. thesis, Paris VI, September 1975, No. CNRS A.O. 11.744, and personal communication; cf. R. A. Jones and A. G. Moritz, *Spectrochim. Acta* **21**, 295 (1965); M. K. A. Khan and K. J. Morgan, *J. Chem. Soc.* 2599 (1964).
11. A. D. Buckingham, *Canad. J. Chem.* **38**, 300 (1960).
12. J. H. Prestegard and S. I. Chan; *J. Amer. Chem. Soc.* **92**, 4440 (1970).