THE STRUCTURE OF GROSSHEIMIN

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Grossheimin, which possesses antitumoral activity is isolated from the epigeal green part of Grossheimia macrocephala [1]. The structure that we originally proposed for grossheimin [2] we reconsidered on further study [3] and proposed a new one (Fig. 1).

The present paper gives additional information obtained by applying the INDOR method to the analysis of the NMR spectrum of grossheimin, confirming the reconsidered structure of the substance.

The NMR method is very widely used to study the structure and stereochemisty of sesquiterpene lactones [4]. Recently, the INDOR method [5], consisting in the observation of the peak intensity of certain lines in the spectrum when other lines are irradiated by a second radiofrequency field with $H_2 \approx \gamma T_2$ (where γ is the gyromagnetic ration and T_2 is the cross-relaxation time, which determines the width of the NMR signals) has been used to a greater and greater extent in the analysis of NMR spectra. In comparison with other two-frequency methods (total, selective, local, and double resonances) [6], the INDOR method allows a larger number of spectral parameters to be obtained and this, which is particularly important, it does regardless of whether the signals appear in a separate region of the spectrum or are masked by overlapping with other signals.

The acquisition of the largest possible number of parameters of the NMR spectrum is of great importance for determining the structure of natural compounds, which not infrequently tend to undergo rearrangements during the preparation of derivatives. However, in spite of the undoubtedly high informativeness of the INDOR method in the study of the NMR spectra of natural compounds, it is nevertheless not available to a wide circle of investigators. One of the reasons for this is the absence of a simple method of determining from the polarity of the INDOR signals the relative signs of the coupling constants, in spite of the fact that this must be given, for example, in the assignment of geminal, vicinal, and allyl protons.

Even for a system consisting of three nuclei (AMX system), the spectrum of which has twelve lines, in dependence on the ratios of the magnitude (six variants) and signs (eight variants) of the coupling constants, 2304 positive and negative INDOR signals can be obtained.

To select from the numerous possible INDOR spectra those which are realized at a given ratio of the magnitudes and signs of the coupling constants, it is convenient to use a matrix representation of the polarities of the INDOR signals.

The matrix of polarities given below for the INDOR signals for the lines of two nuclei of a three-spin system contains four rows and four columns, corresponding to the four lines of each nucleus. The sign of the matrix element a_{ij}(i, j = 1 − 4), +, −, or 0 corresponds to the polarity of the INDOR signals for line i of one nucleus and line j of the other nucleus. Thus, the sign of the matrix element a_{23} corresponds to the polarity of the INDOR signal for line 2 of nucleus T and line 3 of nucleus S.

$$
\begin{array}{c}
\quad S \\
\begin{array}{cccc} 1 & 2 & 3 & 4 \end{array} \\
\left|\begin{array}{cccc|c}
a_{11} & a_{12} & a_{13} & a_{14} & 1 \\
a_{21} & a_{22} & a_{23} & a_{24} & 2 \\
a_{31} & a_{32} & a_{33} & a_{34} & 3 \\
a_{41} & a_{42} & a_{43} & a_{44} & 4
\end{array}\right| \; T
\end{array}
$$

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TABLE 1. Polarity Matrices of the INDOR Signals for an AMX System

Number of the lines	1	2	3	4																
1	−	0	+	0	−	+	0	0	−	0	+	0	−	+	0	0	0	−	0	+
2	0	−	0	+	0	0	−	+	+	0	−	0	+	−	0	0	+	0	−	0
3	+	0	−	0	+	−	0	0	0	−	0	+	0	0	−	+	−	+	0	0
4	0	+	0	−	0	0	+	−	0	+	0	−	0	0	+	−	+	0	0	0
Matrix type	I				II				III				IV				I^a			

Number of the lines	1	2	3	4																
1	0	−	0	+	0	0	−	+	0	0	−	+	0	−	0	+	0	−	0	+
2	−	0	+	0	0	0	−	+	0	0	−	+	−	0	+	0	+	0	−	0
3	0	−	0	+	−	+	0	0	−	+	0	0	0	+	0	−	−	0	+	0
4	+	0	−	0	0	0	+	−	0	0	+	−	+	0	−	0	+	0	−	0
Matrix type	II				III				IV				II^a				III^a			

Number of the lines	1	2	3	4
1	0	0	−	+
2	0	0	+	−
3	−	+	0	0
4	+	−	0	0
Matrix type	IV^a			

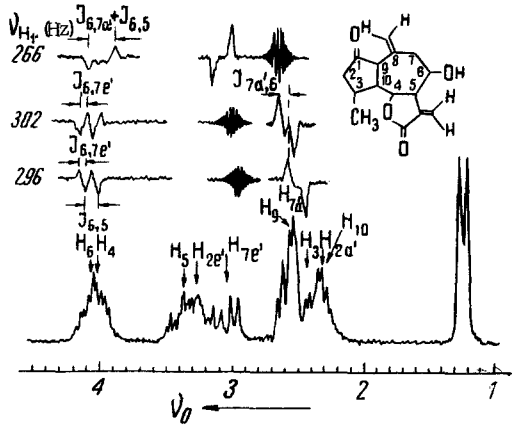

Fig. 1. Fragment of the NMR spectrum of grossheimin (100 MHz, pyridine) and the INDOR spectrum.

To each set of magnitudes and signs of the coupling constants for the three pairs of nuclei of an AMX system correspond sets of three matrices: the INDOR signals of the lines of the A and M nuclei – the matrix $A\hat{M}$: $A|^M|$, for the lines of the A and X nuclei the matrix $A\hat{X}$: $A|^X|$, and for the lines of the nuclei M and X the matrix $M\hat{X}$: $M|^X|$. In the matrices, the rows correspond to the lines of nucleus A and the columns to the lines of nucleus X.

The results of a consideration of the INDOR spectra for bound transitions for different ratios of the magnitudes and signs of the coupling constants using the diagram of the energy levels and the expressions for the energy levels [6] shows that all the sets of INDOR signals for the lines of three pairs of nuclei can be represented by combinations of three of the eight matrices. Tables 1 and 2 give the matrices of the polarities (I-IV^a) and the combinations of types of matrices for all the possible ratios of the magnitudes and signs of the coupling constants in an AMX three-spin system. It must be mentioned that matrices of types I^a-IV^a can be obtained from matrices of types I-IV by interchanging the rows and columns. In view of this, for the matrices of types I-IV the name basic matrix is used below, and for the matrices of types I^a-IV^a the name of derived matrix. It can be seen from Tables 1 and 2 that the sets of basic matrices correspond to three coupling constants of the same sign. Where the sign of one coupling constant is the opposite to the other two, sets of matrices consisting of one basic matrix and two derived matrices are obtained. Under these conditions, the basic matrix corresponds to the pair of nuclei the sign of the constant between which is the opposite to the others. The invariability of the types of matrices on passing from the case where all three coupling constants have the same sign to the case where the sign of one of them is the opposite to that of the other two clearly shows that for the lines of the nuclei between which the sign of the constant changed it is impossible to determine the latter.

In finding the signs of the constants using the polarity matrices, the relationship between the magnitudes of the constants is as follows:

$$|J_{MX}| > |J_{AX}| > |J_{AM}|.$$

For the lines 2 of nucleus A and 3 of nucleus M the INDOR signal is positive, and for the lines 2 A and 3 X there is no INDOR signal. Let us determine the relative signs of the constants J_{AM}, J_{MX}, and J_{AX}. We find from Table 2 that for the lines 2 A and 3 M the signal is positive for an $A\hat{M}$ matrix of type IV^a. There is no INDOR signal for lines 2A and 3X in the $M\hat{X}$ matrices of type I, the following set of matrices being realized: $A\hat{M}$ – IV^a; AX – II^a; and $A\hat{M}$ – I, corresponding to the sets of signs of the constants $J_{AM} > 0$, $J_{AX} > 0$, $J_{MX} < 0$ or $J_{AM} < 0$, $J_{AX} < 0$ and $J_{MX} > 0$.

We have used the matrix representation of the INDOR signals in a study of the structure of grossheimin.

Considerable difficulties in establishing the structure of grossheimin are caused by the determination of the positions of the substituents and of the keto group.

Additional information on the positions of the substituents in the grossheimin molecule were obtained by applying the INDOR method to an analysis of the NMR spectrum of grossheimin (see Fig. 1).

TABLE 2. Possible Combinations of Types of Matrices for an AMX System

Sign of constants J_{AM}, J_{AX}, J_{MX}	Type of matrices $\hat{AM}$, $\hat{AX}$, $\hat{MX}$																	
	1 $J^*_{MX}>J_{AX}>J_{AM}$			2 $J_{AX}>J_{AM}>J_{MX}$			3 $J_{AM}>J_{MX}>J_{AX}$			4 $J_{MA}>J_{AM}>J_{AX}$			5 $J_{AX}>J_{MX}>J_{AX}$			6 $J_{AM}>J_{AX}>J_{MX}$		
± ± ±	IV	II	I	III	I	IV	I	IV	III	II	IV	I	IV	I	II	I	III	IV
∓ ± ±	IV	II^a	I^a	III	I^a	IV^a	I	IV^a	III^a	II	IV^a	I^a	IV	I^a	II^a	I	III^a	IV^a
± ∓ ±	IV^a	II	I^a	III^a	I	IV^a	I^a	IV	III^a	II^a	IV	I^a	IV^a	I	II^a	I^a	III	IV^a
± ± ∓	IV^a	II^a	I	III^a	I^a	IV	I^a	IV^a	III	II^a	IV^a	I	IV^a	I^a	II	I^a	III^a	IV

*All the constants are comparable in absolute value.

Information on whether there is a proton (and, consequently, a CH_3 group) at C_8 is included in the signals of the protons at C_7. However, the signals of these protons in the spectrum of grossheimin are overlapped by the signals of other protons (see Fig. 1), which does not permit the structure of the signals of protons at C_7 to be determined directly from the spectrum of grossheimin. The lines relating to these protons can be isolated by the INDOR method. An investigation of the peak intensities of each of the four lines of the quartet at 3.05 ppm (proton M) on irradiation of the other protons with a second radiofrequency field has shown that heteropolar signals (INDOR signals) arise in the 3.9-4.2 ppm (proton A) and 2.4-2.7 ppm (proton X) regions of the spectrum (see Fig. 1). Spin-spin coupling takes place between the three protons giving signals in these regions. A consideration of the features of the structure of the INDOR spectra [6] (see also above) shows that the distance between the first heteropolar signals corresponds to the coupling constant of the protons the peak value of the line of which is recorded and the proton the line of which is irradiated with the H_2 field. The distance between the first heteropolar signals of the nucleus X = 12.5 Hz, and therefore J_{AM} = 6.3 Hz and J_{MX} = 12.5 Hz.

The INDOR spectra of the nucleus X obtained for lines 3 and 4 of nucleus M are shifted by the constant J_{AM} = 10 Hz.

The H_4 and H_6 protons give signals in the 3.9-4.2 ppm region in the spectrum of grossheimin. The signals of the lactone proton form either a quartet or a triplet, since there are two protons in the vicinal position to it. At the same time, it can be seen from a consideration of the INDOR signals in the 3.9-4.2 ppm region for the lines of the M and X protons that the signal of the A proton consists of 8 lines, i.e., this signal belongs to the H_6 proton. The presence of 8 lines in the spectrum of the H_6 proton shows that there are three protons in vicinal positions to it (H_5, H_7, and $H_{7'}$). Under double resonance conditions, fusion of the lines in the spectrum of the exocyclic methylene group takes place at ν_{H_2} 335 Hz, i.e., δ_{H_2} = 3.35 ppm. Consequently, the signals in the 2.9-3.2 ppm and 2.4-2.7 ppm regions belong to the protons of the methylene group. These signals are quartets, which is possible only if there are no other protons besides the H_5 proton in the vicinal position. A confirmation of this assignment is the negative sign of the coupling constant between the M and X protons, which is realized for geminal protons. The sign of the J_{MX} constant is determined by an analysis of the polarities of the INDOR signals.

On the basis of the structure of the signals of the AM and X nuclei the relationship $|J_{MX}| > |J_{AX}| > |J_{AM}|$ is realized for the coupling constants. The value of J_{AM} = 6.3 Hz, which is comparatively large for long-range constants and comparatively small for geminal constants in any systems, shows that the J_{AM} constant is vicinal, i.e., it is positive. The X proton is also vicinal to the A proton. As follows from Tables 1 and 2, it is possible to determine the sign of the constant J_{MX} relative to the signs of the constants J_{AX} and J_{AM} for the lines of the A and M or the A and X nuclei. For the lines of the M and X nuclei the INDOR spectra are identical, both in the case of J_{AX}, J_{AM}, $J_{MX} > 0$ and in the case J_{AM}, $J_{AX} > 0$, $J_{MX} < 0$. For line 1X in the spectrum of the nucleus A, above the last line of the signal of A, which corresponds to line 4A of an AMX system, a positive INDOR signal appears (see Fig. 1). In this spectrum, the absence of two signals (positive and negative) among the signals present is the result of the superposition of positive and negative signals $|J_{AX}| \simeq |J_{AH_5}|$.

The presence of a positive signal above line 4A (AMX system) according to the information of Tables 1 and 2 shows that the set of polarity matrices $\hat{MX} - I$, $\hat{AX} - II^a$, $\hat{AM} - IV^a$ is realized for the signs of the constants. Consequently, the sign of J_{MX} is opposite to the sign of J_{AX}, i.e., J_{MX} is negative and is, therefore, a geminal constant.

Thus, of the two possible positions for the methyl and vinyl methylene groups the following are in fact realized: the methyl group is present at C_3 and the methylene group at C_8.

SUMMARY

1. It has been shown by means of the INDOR method that in the grossheimin molecule the methyl group is located at C_3 and the exocyclic methylene group at C_8.

2. The matrix presentation of the INDOR signals for bound transitions has been considered for the three-spin AMX system. The INDOR signals for all the possible relationships of the magnitudes and signs of the coupling constants in the AMX system have been given in compact form.

LITERATURE CITED

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