

## STRUCTURE OF BRITANNIN.

### USE OF TWO-DIMENSIONAL $^1\text{H}$ NMR SPECTROSCOPY

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Two-dimensional  $^1\text{H}$  NMR spectroscopy (COSY, NOESY) in combination with the one-dimensional variant has been used to establish the conformation of the molecule of britannin - a sesquiterpene lactone of the ambrosane type. The comparative possibilities of one- and two-dimensional NMR spectroscopies in stereochemical investigations of sesquiterpene lactone with ambrosane skeletons are considered.

The isolation of britannin - a sesquiterpene lactone of the ambrosane type - and the establishment of its structure has been reported previously [1-3]. The spatial structure of this compound was not investigated in detail. Recently, in stereochemical investigations by the NMR method wide use has been made of COSY and NOESY two-dimensional spectroscopy [4], and we have attempted to extend these procedures to the given class of compounds. NOESY spectra, in which the nuclear Overhauser effect (NOE) appears in the form of signals, gives information directly on the distance between the atoms in the molecule, which makes NOESY spectroscopy an extremely effective method of studying the conformations of molecules. However, a traditional approach to the study of the stereochemistry of molecules, based on the use of stereochemical dependences of coupling constants, gives information which cannot always be obtained from NOESY spectra.

We have studied the spatial structure of britannin using both one-dimensional and two-dimensional  $^1\text{H}$  NMR spectroscopies. The britannin molecule consists of three condensed rings - a 7-membered and two 5-membered rings, of which one is a lactone ring - and five substituents (Fig. 1). Table 1 gives the values of the chemical shifts and coupling constants for a solution of britannin in  $\text{CDCl}_3$ , obtained from an analysis of monoresonance and COSY spectra (Fig. 2) at a frequency of 250 MHz. With the exception of the  $J_{8,9}$  constants, the value of which could not be determined because of pronounced overlapping of the signals of the H-8 and H-9 protons, the values of all the coupling constants greater than 1 Hz were determined. The assignment of the signals was made from an analysis of the cross-peaks in the COSY spectrum. In the NOESY spectrum (see Fig. 3) there was a large number of cross-peaks with different intensities which were due both to intra- and intermolecular interactions of protons. The dependence of the values of the latter on the concentration of britannin in solution enabled the cross-peaks corresponding only to the intramolecular interaction to be isolated. The most intense cross-peaks were given by the geminal protons of the methylene groups (positions 2, 7, and 13).

The cross-peaks of the methylene protons, the distance between which is 1.8 Å were taken as the initial ones for qualitative stereochemical analysis. The marked dependence of the NOE on the proton-proton distance ( $\text{NOE} \sim 1/\gamma^6$ ) [5, 6] permits the distance at which intense cross-peaks appear to be limited to two angstroms. The NOE for protons present at a distance of 2.0 Å is only half that for the protons of a methylene group. It follows directly from the NOESY spectra that in the britannin molecule the Me-15 group and the H-4 and H-6 protons are spatially close, i.e., they are present on the same side of the plane of the 7-membered ring. At the same time, the H-3 and the H-5 protons, are spatially remote from Me-15, as is shown by the absence of the corresponding cross-peaks in the spectrum. These facts demonstrate the trans-linkage of the lactone and cyclopentane rings and, correspondingly, the cis and trans positions of the hydroxy and acetyl groups with respect to Me-15. The information contained in the NOESY spectra proved to be inadequate to establish the type of linkage of the cyclopentane and cycloheptane rings and the configurations of the two other substituents.

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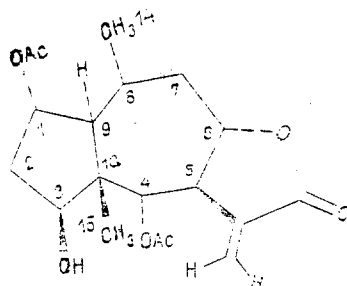


Fig. 1. Structure of britannin.

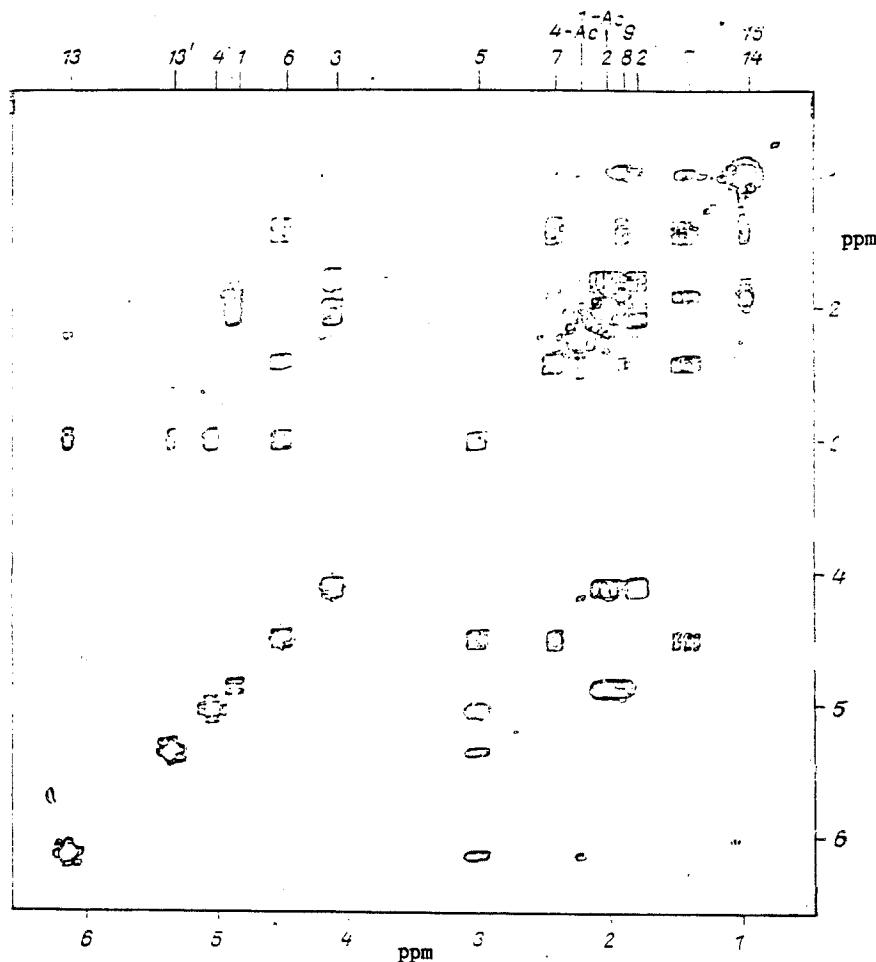


Fig. 2. COSY spectrum of britannin.

Additional information on the stereochemistry of the britannin molecule was provided by an analysis of the vicinal coupling constants. To determine the dihedral angles ( $\theta$ ) between the protons use was made of the following stereochemical relationship of the vicinal constant [7]:

$$^3J = 7 \cos 2\theta - \cos \theta + 5.$$

The application of this relationship to the coupling constants of the protons of the five-membered ring (see Table 1) showed that a C-2 proton of the methylene group is present in the cis-position with respect to the H-3 proton ( $J_{2,3} = 8.5$  Hz) and is trans-oriented with respect to the H-1 proton ( $J_{1,2} = 1.5$  Hz). Consequently, the acetyl and hydroxy groups have different orientations relative to the plane of the 5-membered ring.

The constants  $J_{1,2}$ ,  $J_{1,2'}$ ,  $J_{2,3}$  and  $J_{2',3}$  corresponded to dihedral angles of 100, 20, 155, and 35°. It follows from a consideration of Dreiding models that for the cyclopentane ring such values of the dihedral angles correspond to the half-chair conformation [8] with

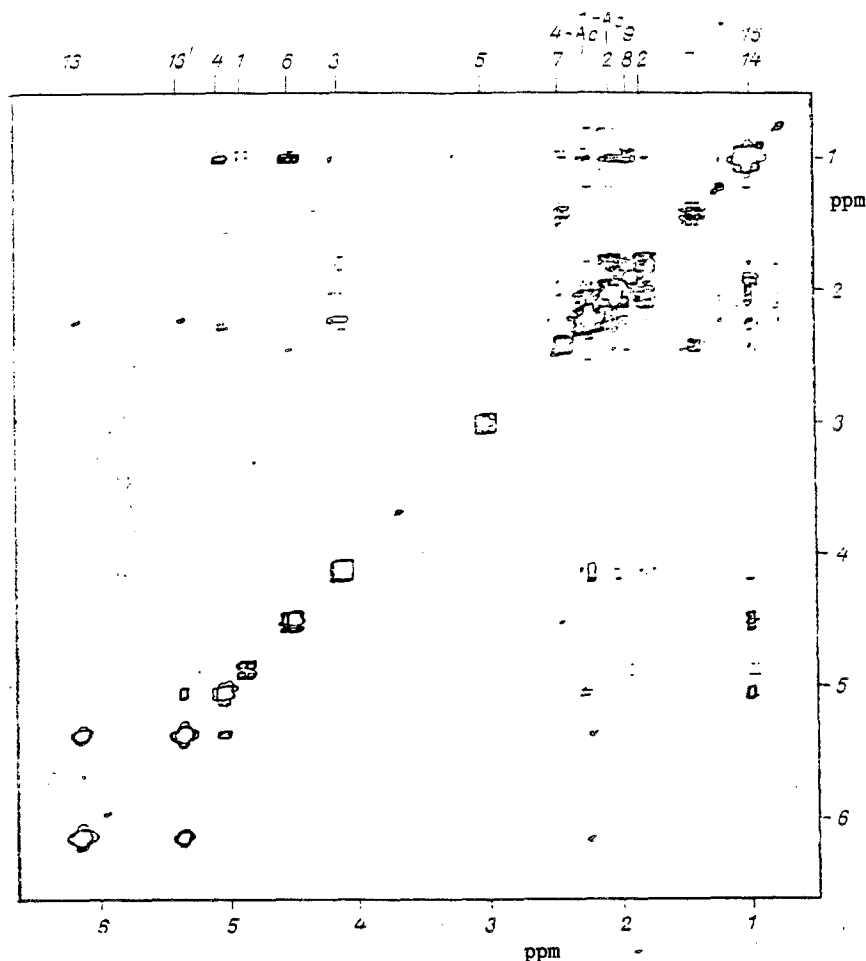


Fig. 3. NOESY spectrum of britannin.

TABLE 1. Chemical Shifts and Coupling Constants of the Protons of the Britannin Molecule ( $\text{CDCl}_3$ ,  $20^\circ\text{C}$ )

Proton No.	1	2	2'	3	4	5	6	7	7'	8	9	13	13'
$\delta$ , ppm	4.91	1.81	2.06	4.14	5.08	3.05	4.55	2.95	1.45	1.9	1.9	5.38	6.18
$^1J$ , Hz		15.0						12.3				0	
$^3J$ , Hz*	4.5	1.5	8.5	3.9	8.1	10.5	2.9		12.3	—			
	(9)	9.8	11.7 (OH)				11.8						
$^4J$ , Hz											3.1	3.3	
											(5)	(5)	

\*The upper figure relates to the number of the proton of the methylene group nearest in the table, and the figure in parentheses to the number of the vicinal proton: 4.5 is to (9)

be read  $J_{1,9} = 4.5$  Hz; 1.5 is to be read  $J_{1,2} = 1.5$  Hz,  $J_{1,2'} = 9.8$  Hz.

an axis of symmetry passing through the C-9 carbon atom and with the C-2 carbon atom separating from the plane of the ring in the direction of the Me-15 group. The value of the constant  $J_{1,9} = 4.5$  Hz for such a conformation corresponds to the interaction of protons present in the trans position with respect to one another. This shows the trans-linkage of the cyclopentane and cycloheptane rings.

The cis-position of the methyl and hydroxy groups, leading to the screening of the methyl group by the unshared pair of the oxygen atom was apparently the reason for the retardation of the intermolecular proton exchange of the hydroxy groups as result of which the signal of the hydroxy group appeared in the form of a doublet ( $J_{3,\text{OH}} = 3.9$  Hz) and not, as usual, in the form of a singlet as is the case for rapid proton exchange.

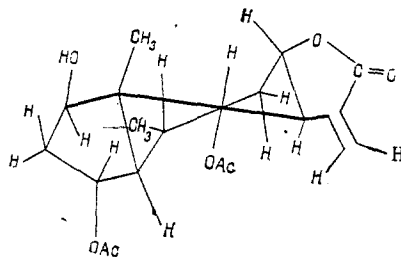


Fig. 4. Spatial structure of the britannin molecule.

With the mutual trans positions of the Me-15 group and the H-9 proton, of the two possible variants of the assignment of the cross-peak, to a 15-9 or a 15-8 interaction, the choice must be made in favor of the latter. Thus, in the seven-membered ring the H-4, H-6, and H-8 protons are spatially close to the Me-15 group. In view of the large values of the constants  $J_{6,7a}$  and  $J_{7a,8}$ , showing the closeness to  $180^\circ$  of the dihedral angles between the corresponding protons, it follows from the Dreiding model that the "chair" conformation is realized for the cycloheptane ring [9] with an axis of symmetry passing through the C4 atom and the equatorial arrangement of the Me-14 group. Figure 4 shows the conformation of britannin obtained from a consideration of the NOESY spectra and the coupling constants. The two methods gave agreeing results. However, the loss of information on the stereochemistry of britannin because of the overlapping of the signals in the NMR spectrum proved to be more substantial for the NOE than for the coupling constants. A large part of the information in the NOESY spectra is connected with the interaction of the Me-15 group with the other protons of the molecule, which may be characteristic for NOESY spectra of sesquiterpene lactones having an ambrosane skeleton. In the case of an unambiguous assignment of the cross-peaks, the interaction of this methyl group with the protons in position 8, the configuration of the substituent, and the mode of linkage of the rings in the britannin molecule can be established even without bringing in information on coupling constants.

#### EXPERIMENTAL

$^1\text{H}$  NMR spectra were obtained on a Bruker WM-250 spectrometer with an Aspect-2000 computer. Concentration of this compound in chloroform was 0.1 M. Chemical shifts were measured relative to residual chloroform, the chemical shift of which is 7.27 ppm. The COSY and NOESY two-dimensional spectra were obtained by means of standard programs in the regime of quadrature detection. In both experiments for each of the 256 values of  $t$ , the pulse sequence was repeated 16 times. The relaxation delay was 4 s. The value of  $F_m^*$  for the NOESY sequence was 1 s. To obtain the same digital resolution (4.8 Hz per point) the data matrix in the  $t_2$ -direction was zero-filled and multiplied by the sinefunction in the  $t_1$ -direction and by the  $\sin^2$  function in the  $t_2$  direction, respectively, before Fourier transformation. An additional improvement in resolution was obtained by symmetrization.

#### SUMMARY

1. The conformation of the molecule of a sesquiterpene lactone of the ambrosane type - britannin - has been established with the use of one- and two-dimensional  $^1\text{H}$  NMR spectroscopy (NOESY).
2. A comparative estimate has been made of the possibilities of one- and two-dimensional NOESY spectroscopy in the study of the stereochemistry of the britannin molecule.

#### LITERATURE CITED

1. K. S. Rybalko, V. I. Scheichenko, G. A. Maslova, E. Ya. Kiseleva, and I. A. Gubanov, *Khim. Prir. Soedin.*, 251 (1968).
2. P. V. Chugunov, V. I. Sheichenko, A. I. Ban'kovskii, and K. S. Rybalko, *Khim. Prir. Soedin.*, 276 (1971).
3. K. S. Rybalko, *Natural Sesquiterpene Lactones* [in Russian], Meditsina, Moscow (1978), p. 25,
4. R. R. Ernst, G. Bodenhausen, and A. Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Clarendon Press, Oxford (1987).
5. R. A. Bell and J. K. Saunders, *Can. J. Chem.*, **46**, 3421 (1968).
6. J. H. Noggle and R. E. Schirmer, *The Nuclear Overhauser Effect*, Academic Press, New York (1971), p. 51.

\*Possibly  $T_m$  - Translator.

7. A. A. Bothner-By, *Adv. Magn. Reson. Spectrosc.*, 1, 195 (1965).
8. H. Booth, *Prog. Nucl. Magn. Reson. Spectrosc.*, 5, 212 (1968).
9. E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, *Conformational Analysis*, Interscience, New York (1965) [Russian translation, Mir, Moscow (1969), p. 254].

# AMOUNT OF STEROLS AND TRITERPENOIDS IN LEAVES AND PETIOLES OF LEAF-SHEDDING AND STANDARD LINES OF COTTON PLANT

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The amount of free and bound sterols and triterpenoids in the leaf blades and petioles of the variety Tashkent-1 and the deciduous lines L-275 and L-470 in the budding-flowering, maturing, and leaf-fall phases have been compared. In the leaf blades, the maximum accumulation of free sterols in the deciduous lines is observed in an earlier phase than in the variety Tashkent-1, and this is most characteristic for sterols saturated in the C-17 side chain. The dynamics of the change in the content of unsaturated sterols is individual for each line. The nature of the change in the amount of free sterols in the petioles is typical for each line and does not depend on the nature of the sterol for the variety Tashkent-1 this index falls sharply in the leaf-fall phase while for L-275 the process is retarded between the second and third phases, and for L-470 in the same period the amount of these substances rises sharply. In the leaf blades, as a rule, the level of sterol esters changes in parallel with the levels of free sterols, and in the petioles in the antiparallel direction between the first and second phases. In all the samples free and bound amyirin was detected. The dynamics of the change in the amounts of these substances in the three lines were different.

One of the alternatives in the solution of the problem of pre-harvesting defoliation of the cotton plant is the creation of selection lines with earlier times of leaf-fall. Since mutant genes affect the biosynthesis of secondary metabolites [1], there is a definite point in studying the qualitative and quantitative composition of these compounds in lines possessing a known genetic characteristic and comparing it with the corresponding characteristic of lines (varieties) of cotton plant that have not been subjected to mutations. One of the results of the comparison may be the creation of methods for acting on industrial varieties with nontraditional ecologically pure substances the purpose of which consists in changing the balance of leaf-fall regulators in the required direction.

Searches for a natural "aging factor" (AF) performed on other plants [2] have not given clear-cut results. Attempts to ascribe this property to abscisic acid [3] have not yet undergone development. Only the role of ethylene and of compounds producing it in the process of leaf-fall is not a matter of doubt [2, 4].

Nevertheless, some authors tend to consider that the AF is an integrated influence of the change in the levels of a whole set of compounds [2] plus the stress action of the environment, including day length [3]. We set ourselves the task of performing experiments permitting the combination of both directions of the searches - attempts to reveal a concrete carrier of the AF and a correlation of the levels of individual compounds of nonhormonal nature accessible for analysis in standard and selection lines.

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