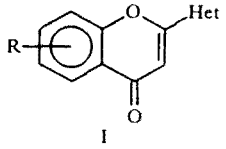
Chemistry of Heterocyclic Compounds, Vol. 32, No. 6, 1996

INVESTIGATION OF THE STRUCTURE OF MODIFIED FLAVONOIDS BY MEANS OF A LANTHANIDE SHIFT REAGENT AND RELATED ^{1}H NMR SPECTROSCOPIC METHODS (REVIEW)

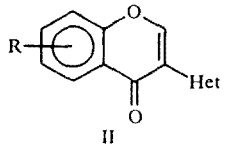
A. V. Turov and V. P. Khilya

An approach to determination of the structure of the heterocyclic analogs and derivatives of flavone, isoflavone, chalcone, and aurone by measurement of their ^{1}H NMR spectra in the presence of lanthanide shift reagents and in an aromatic solvent is described. The coordination of the lanthanide shift reagents with the substrate molecules is discussed, and a series of problems that can be solved most effectively by this method are identified. Examples of the solution of specific problems are given.

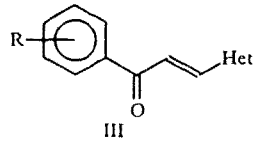
Flavonoids, which are constituents of widely encountered natural heterocyclic compounds, are present in many plants and perform a variety of physiological functions. This has led to investigations into the synthesis of flavonoids and the introduction of various functional groups into their molecules. The modified flavonoids obtained in this way have useful biological activity [1], and some of them have been used in medicine. Some of the most important types of modified flavonoids are the heterocyclic analogs in which the heterocycle occupies the position of the phenyl substituent. Other compounds include products having sugar-reducing, hepatoprotecting, anabolic, hypoglycemic, antiviral, and other types of biological activity [2-8]. Often the compounds have not one but several types of biological activity, thereby permitting the combined treatment of a number of diseases. The toxicity of the products here is extremely low. The nature of the biological activity of the flavonoids containing a heterocyclic substituent has given rise to vigorous researches into their synthesis and biological screening. In this connection it became important to develop effective methods for determining the structure and conformation of these compounds. The present article discusses one of the possible approaches to solution of the problem, involving the use of lanthanide shift reagents (LSR), the homonuclear Overhauser effect (NOE), and the shifts induced by an aromatic solvent (ASIS). We have used this method for a number of years in the study of various 2-heteroarylchromones (I), 3-heteroarylchromones (II), the heterocyclic analogs of chalcone (III) and aurones (IV), and also their hydrogenated analogs. Here we have attempted to summarize the obtained results.



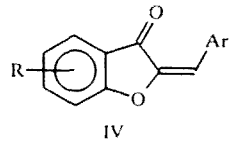
I



II



III



IV

R = Alk, OH, OAlk, OCOMe, OMe

Taras Shevchenko Kiev University, Kiev. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 6, pp. 723-737, June, 1996. Original article submitted January 15, 1996.

0009-3122/96/3206-0619$15.00

1. COMPLEXATION OF THE HETEROCYCLIC ANALOGS OF FLAVONOIDS WITH LANTHANIDE SHIFT REAGENTS

In recent years lanthanide shift reagents have been widely used to simplify the PMR spectra and the structural and conformational analysis of organic compounds. An essential condition for the success of this method is the presence in the molecule of the investigated compound of electron-donating groups capable of interacting with the LSR and their adequate solubility in such solvents as chloroform, benzene, and acetonitrile. As follows from the structural formulas, the molecules of the heterocyclic analogs of the flavonoids contain at least one center for coordination with the lanthanide shift reagent, i.e., the carbonyl group. This fact makes it possible to use the LSR in the study of a large number of compounds. In the presence of coordination with the LSR, strong paramagnetic shifts of the signals are observed in the ^1H NMR spectra (particularly for the protons close to the coordination center of the molecule), since the observed lanthanide-induced shifts (LIS) are mainly pseudocontact in nature. Their values for proton i can be calculated by means of the McConnel—Robertson equation [9], which has the following form for axially symmetric adducts:

$$\Delta H_i = (3 \cos 2\theta_i - 1)/r^3, \quad (1)$$

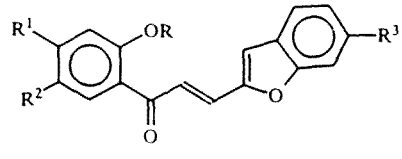
where r and θ are the polar coordinates of the proton in the molecule on the condition that the coordination center is at the origin. Many structural and conformational problems can be solved by calculations using this equation.

The position is complicated in cases where the flavonoid molecules contain phenolic hydroxyls. These groups on the one hand reduce the solubility of the compounds and, on the other, can lead to the destruction of such LSRs as $Eu(dpm)_3$ [21]. Although it was shown for the thiazole analogs of chalcone [10] that the use of $Yb(fod)_3$ as LSR gives a considerable LIS with retention of the multiplet structure, the alkylated or acylated derivatives of phenols are more suitable for experiments with the LSR. With such compounds it is usually possible to obtain fairly large lanthanide-induced shifts and to calculate the position of the lanthanide ion in the adduct [11, 12]. The calculations show that in the adducts with most flavonoids the lanthanide ion is at a distance of 2.3-2.7 Å from the oxygen atom of the carbonyl group and is deflected 10-30° away from substituents close to this oxygen atom. The presence of bulky substituents adjacent to the carbonyl group can greatly weaken its coordination with the LSR, leading to a decrease in the LIS [13].

The situation may become more complicated in cases where the molecule of the investigated compound contains other coordination centers in addition to the chromone carbonyl. Coordination may take place either at the most active center or at two (or several) centers to various degrees, depending on the ratio of the complexation constants of the LSR at each of the coordination centers. This can be judged from the LIS of the signals of the protons situated close to each of the coordination centers. If more than one coordination center is involved in complexation, calculation of the structure of the adduct by means of the McConnel—Robertson equation is not usually successful [10]. It should be noted that the situation where the molecule contains two coordination centers similar in complexing ability does not arise too often, and the adduct of the LSR with the most active coordination center is therefore formed in most cases. This can be used during the study of flavonoids containing simultaneously a phenolic hydroxyl and a substituent capable of complexation by the LSR method. As demonstrated for the 3-pyridine analog of 2′-hydroxychalcone [10, 14], the lanthanide-induced shifts are determined exclusively by the coordination of the pyridine ring at the nitrogen atom. Here the phenolic hydroxyl does not prevent complexation.

2. CHELATION BETWEEN THE LSR AND THE HETEROCYCLIC ANALOGS OF FLAVONOIDS

A series of interesting features are observed during the complexation of LSRs with molecules containing two coordination centers in direct proximity to each other. This effect was discovered [15, 16] during a study of the analogs of chalcones having the following structure:



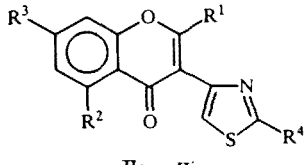
R – R^3 = H, Alk

The compounds with R = H hardly react with the LSR at all, and paramagnetic shifts are not observed in the ^1H spectra in the presence of $Eu(fod)_3$. On the other hand, with R = CH_2Ph effective complexation occurs, and the lanthanide-induced shifts for the signals of a series of protons can amount to 8 ppm. Thus, the introduction of a benzoyloxy group, which itself hardly interacts at all with the LSR, improved the coordination of the molecule with the LSR. The lanthanide-induced shifts indicate that the oxygen atoms of both the carbonyl and the ether groups take part in complexation, i.e., an adduct of the chelate type is formed (see also [18]).

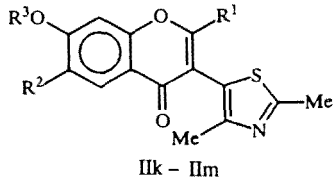
Chelation can not only improve the interaction between the molecule and the LSR but can also give rise to undesirable effects and, in particular, strong broadening of a number of signals. We studied in detail the interaction between the LSR and bidentate compounds as illustrated by substituted 3-thiazolylchromones (IIa-o).

The structure of the compounds was chosen in such a way that their molecules contained substituents differing in size and electronic nature in the chromone and thiazole rings. The point of fusion of the 3-chromone group with the thiazole ring was also varied. The reaction with such lanthanide shift reagents as $Eu(fod)_3$ and $Eu(hfbk)_3$ was also studied.

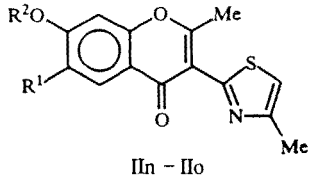
The specific lanthanide-induced shifts (the shifts extrapolated to an LSR—substrate molar ratio of 1:1) of the investigated 3-thiazolylchromones are given in Table 1. It is seen that the lanthanide-induced shifts depend very strongly on the substituents present in the molecule. Thus, in compounds (IIa-d), not containing substituents at position 2 of the thiazole ring, the largest lanthanide-induced shifts are observed for the signal of the 2-H proton of thiazole. This indicates that the LSR is coordinated with the nitrogen atom of the thiazole ring. The second potential coordination center of 3-thiazolylchromones, i.e., the oxygen atom of the carbonyl in the chromone ring, also makes some contribution to the coordination with the LSR, as seen from the appreciable lanthanide-induced shift of the signal for the 5-H proton of the chromone fragment, but the degree of coordination here is much smaller. This is also demonstrated by the absence of appreciable changes in the lanthanide-induced shifts with the introduction of the electron-withdrawing group CF_3 [compound (IIc)] and a hydroxyl group [compound (IId)], which sterically hinders coordination of the LSR at the chromone carbonyl, at position 2 of the chromone ring.



IIa – IIj



IIk – IIm



IIn – IIo

IIa $R^1 = R^2 = R^4 = H$, $R^3 = OCH_2COOC_2H_5$; b $R^1 = R^2 = R^4 = H$, $R^3 = OCOMe$; c $R^1 = CF_3$, $R^2 = R^4 = H$, $R^3 = OMe$; d $R^1 = CF_3$, $R^2 = OH$, $R^3 = Me$, $R^4 = H$; e $R^1 = R^2 = H$, $R^3 = OCOMe$, $R^4 = Me$; f $R^1 = R^4 = Me$, $R^2 = H$, $R^3 = OMe$; g $R^1 = H$, $R^2 = OCOMe$, $R^3 = R^4 = Me$; h $R^1 = H$, $R^2 = R^4 = Me$, $R^3 = OCOMe$; i $R^1 = R^2 = H$, $R^3 = OCOMe$, $R^4 = Ph$; j $R^1 = CF_3$, $R^2 = H$, $R^3 = OMe$, $R^4 = Ph$; k $R^1 = R^2 = H$, $R^3 = CH_2OCOC_2H_5$; l $R^1 = R^2 = H$, $R^3 = Me$; m $R^1 = R^2 = R^3 = Me$; n $R^1 = C_3H_7$, $R^2 = COMe$; o $R^1 = H$, $R^2 = Me$

The situation changes substantially in the case of the derivatives (IIe-g), containing a methyl group at position 2 of the thiazole ring. In the absence of further hindrances to complexation [compound (IIe)] larger lanthanide-induced shifts are observed for the signals both of the protons close to the chromone carbonyl (the 5-H protons of the chromone and the 5-H protons of the thiazole rings) and of the 2-CH_3 group of the thiazole ring. The introduction of substituents at position 5 of the chromone ring [compounds (IIg-h)] leads to a decrease in the lanthanide-induced shift of the 5-R signals of the chromone fragment and of the 5-H proton of the thiazole ring and to some increase in the LIS of the signal for the 2-CH_3 group in the thiazole ring. Thus, in the products (IIe-h), as also in the case of compounds (IIa-d), coordination with the LSR takes place at the two coordination centers present in the molecule. The effectiveness of coordination is determined by the presence of steric hindrances to complexation. Unexpected here are the large values of the lanthanide-induced shifts of the signal for the 2-CH_3 group in compounds (IIe-h) compared with the LIS of the signal for the 2-H group in the derivatives (IIa-d), although the

TABLE 1. Specific Lanthanide-Induced Shifts of the Protons in 3-Thiazolylchromones in Adducts with $Eu(fod)_3$

Compound	Protons of chromone ring					Protons of thiazole ring		
	2-R	5-R	6-R	7-R	8-H	2-R	4-R	5-H
IIa	H; 2,4	H; 2,8	H; 0,5	0,0*	0,2	H; 7,3	—	2,7
IIb	H; 2,6	H; 3,4	H; 0,6	CH_3; 0,2	0,5	H; 6,9	—	2,9
IIc	—	H; 3,1	H; 0,5	CH_3; 0,1	0,0	H; 6,5	—	4,3
IId	—	OH; 4,8	H; 0,4	CH_3; -0,1	0,1	H; 5,5	—	4,8
IIe	H; 2,9	H; 12,6	H; 2,1	CH_3; 0,6	2,2	CH_3; 9,4	—	8,9
IIf	CH_3; 1,3	H; 8,0	H; 1,7	CH_3; 0,5	0,8	CH_3; 13,3	—	4,5
IIg	H; -0,2	CH_3; 1,8	H; 2,4	CH_3; 0,7	0,9	CH_3; 12,8	—	0,0
IIh	H; -0,4	CH_3†	H; 2,3	CH_3; 0,5	0,9	CH_3; 10,6	—	0,0
IIi	H; 3,7	H; 11,1	H; 1,1	CH_3; 1,2	2,8	Ph; 0,6‡		10,5
IIj	—	H; 9,2	H; 0,6	CH; 0,6	2,1	Ph; 0,9‡		7,8
IIk	H; 2,8	H; 5,3	H; 0,4	CH_2; 1,5	1,8	CH_3; 0,2	CH_3; 3,0	—
IIl	H; 3,1	H; 6,0	H; 0,2	CH_3; 0,3	1,5	CH_3; 1,0	CH_3; 1,8	—
IIm	CH_3; 3,0	H; 3,7	CH_3; -0,4	CH_3; 0,6	1,0	CH_3; 1,5	CH_3; 1,1	—
IIn	CH_3; 1,1	H; 2,2	CH_2; 0,5	CH_3; 0,2	0,9	—	CH_3; 4,9	0,7
IIo	CH_3; 4,4	H; 8,6	H; 2,4	CH_3; 0,6	1,2	—	CH_3; 12,3	3.5

*The LIS of all the protons of the substituent.

†The signal is greatly broadened.

‡The signal of the *ortho*-protons of phenyl.

introduction of the methyl group at position 2 of the thiazole ring should impair the coordination with the LSR on account of the increase of the steric hindrances to complexation. This does not occur on account, probably, of the increase in the electron-donating nature of the thiazole ring in the products (IIe-h), due to the displacement of electron density from the methyl group.

The reaction of compound (IIh) with the LSR proved unique. With the addition of $Eu(fod)_3$ at a rate of less than 2 mole %, the signal for the protons of the 5-CH_3 group of chromone is broadened so much that it cannot be observed in the spectrum. At the same time the signal of the 2-CH_3 group of thiazole is broadened, although to a lesser degree. The signals of the other protons do not exhibit anomalous broadening. With increase in the amount of the LSR from 30 to 110 mole % the half-width of the signal for the 2-CH_3 group in the thiazole ring is reduced from 60 to 10 Hz. However, the signal of the 5-CH_3 group here does not appear in the spectrum. We consider that the observed effects are due to a decrease in the rate of exchange between the coordinated and uncoordinated molecules of the substrate and as a consequence to the appearance of exchange broadenings, which are particularly strong for the signals of protons with large lanthanide-induced shifts [10]. In turn, the retarded exchange may be due to stabilization of the adduct of the substrate molecule with the LSR as a result of chelation. The effect is not observed in the product (IIg) on account of the complete absence of interaction between the LSR and the chromone carbonyl as a result of the greater steric hindrances to complexation.

In the products (III-j), having a 2-phenyl substituent in the thiazole ring, the signals of the protons of this substituent are shifted very little by the LSR. This indicates the absence of coordination between the LSR and the heterocyclic nitrogen atom of the thiazole ring. Comparison of the lanthanide-induced shifts of these derivatives with the corresponding values in the methyl-containing analog (IIe) shows that the coordination of the LSR at the heterocyclic nitrogen atom has little effect on the signals for the protons of the chromone fragment (as a result, probably, of the specific mutual orientation of the thiazole and chromone fragments).

In the products (IIk-m), in which the chromone fragment is attached to position 5 of the thiazole ring, the lanthanide-induced shifts were smaller than in compounds (IIa-j). Here, the coordination at the heterocyclic atom of the thiazole ring is weak, as demonstrated by the small (less than 1.5 ppm) lanthanide-induced shift of the signal for the 2-CH_3 group, attached to this substituent. The formation of the adduct of the LSR with the chromone carbonyl is also less effective on account of the considerable steric hindrances due to the sulfur atom and the 4-CH_3 group of the thiazole ring.

In the position of the centers of coordination with the LSR, compound (IIo) is reminiscent of the chromone derivative (IIe). Accordingly, their coordination with the LSR takes place effectively both at the thiazole nitrogen atom and at the

chromone carbonyl. The largest lanthanide-induced shifts are observed for the protons of the 4-CH_3 group of thiazole and the 5-H proton of the chromone fragment. In the product (IIn), which contains a 6-propyl substituent that prevents coordination with the LSR at the chromone carbonyl, the lanthanide-induced shifts were much smaller.

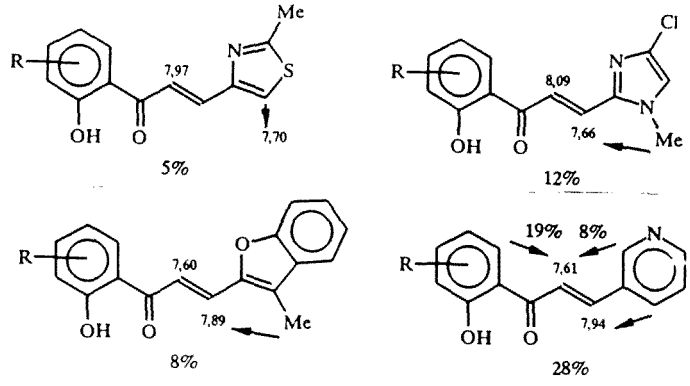
Thus, the presence and the nature of substituents close to the coordination centers of both heterocyclic fragments have a substantial effect on the coordination of the LSR with bidentate 3-thiazolylchromones. This conclusion is fairly general in nature for flavonoids, and it must be taken into account during work with other types of these compounds.

3. DETERMINATION OF THE STRUCTURE, SPECTRAL PARAMETERS, AND CONFORMATION OF CHALCONES AND THEIR EPOXIDES

During analysis of the PMR spectra of the heterocyclic analogs of chalcones, the question of the assignment of the signals of the olefinic protons often arises, since their chemical shifts can be used to reach conclusions about the electronic effects in the molecule. These signals take the form of two doublets with a spin—spin coupling constant of 14-15 Hz. It is usually impossible to say beforehand which of the olefinic protons is downfield. These signals are shifted almost identically with the addition of a lanthanide shift reagent. We found that if the chalcone contains the residue of electron-donating heterocycles the signals can be assigned most simply on the basis of ASIS. During measurement of the ^{1}H NMR spectra in benzene, small downfield shifts are observed for the α-proton (in relation to the carbonyl group) compared with its position in chloroform, while an upfield shift is always observed for the signal of the β-proton in benzene. The signals in the ^{1}H NMR spectra of the 1,3- and 1,4-benzodioxane and 1,5-benzodioxepane analogs of chalcone were assigned on this basis, and it was established that for these derivatives the signal of the α-proton is always upfield from the signal of the β-proton. The results were confirmed by synthesis and by measurement of the spectra of a of deuterated analogs [18].

The ASIS method proved ineffective during the investigation of chalcones with electron-withdrawing substituents, such as 3-pyridyl, 2-imidazolyl, and 2-benzofuryl, on account of the weak association of the benzene molecule with the carbonyl group of the chalcones. In this case the shifts of the signals of the substrate observed in benzene were due mainly to association of the benzene with the π-electron system of the heterocyclic substituent.

It was possible to assign the signals by means of the NOE [14, 25]. Thus, the following values were obtained for the increase in the intensity of the signals:

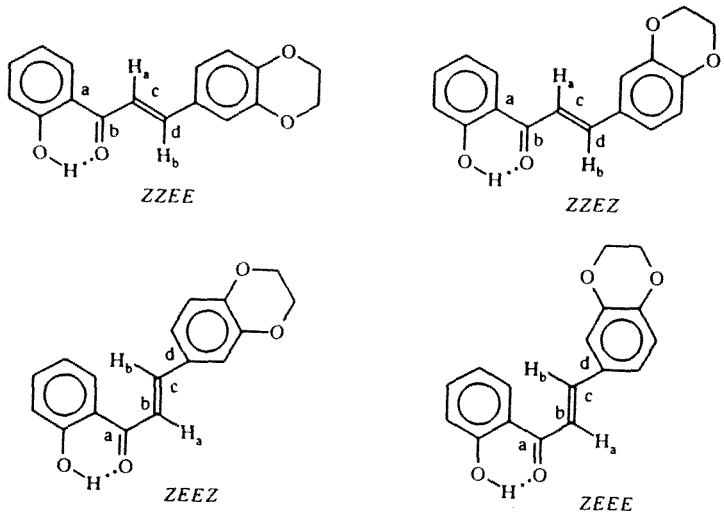


On the basis of this it was shown that the signal of the β-olefinic proton is in the upfield region in the derivatives of thiazole and imidazole and in the downfield region in chalcones with 3-pyridyl and 2-benzofuryl substituents.

An interesting aspect of the stereochemistry of the heterocyclic analogs of chalcones is the orientation of substituents at the olefinic double bond linking the heterocyclic fragments. We studied this aspect for chalcones containing the 1,3- and 1,4-benzodioxane and 1,5-benzodioxepane fragments, their 2-O-benzylated derivatives, and also their thiazole, imidazole, and pyridine analogs.

As follows from the obtained ^{1}H NMR spectra, on the basis of the chemical shifts it is impossible to reach any definite conclusions about the conformation of the conjugation chain present in the molecules of the chalcones. The only point not subject to doubt is the *trans* orientation of the olefinic protons, since the spin—spin coupling constants between these protons lie in the range of 14-16 Hz.

The lues of the ASIS for t.: derivatives of electron-donating heterocycles make it possible to reach definite conclusions concerning the conformation of the conjugation chain in the molecules of the chalcones. Here we used the model of Connoly and McGrindle [19] for the association of the carbonyl group with the benzene molecule. According to this model, if a plane perpendicular to the C=O bond is drawn through the carbonyl oxygen atom the protons located on the side of the carbonyl carbon atom in relation to this plane undergo negative ASIS (downfield shifts), while the protons on the other side of the plane undergo positive ASIS (upfield shifts). In the molecules of chalcones with a 2-OH substituent, rotation about one of the single chemical bonds of the conjugation chain (bond *a*) is greatly hindered on account of the formation of a strong intramolecular hydrogen bond. It is, therefore, possible to propose four planar conformations for such chalcones:

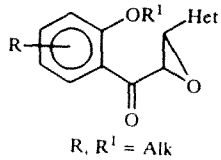


By molecular mechanics we found that the energy of the chalcone molecules changes during rotation about bonds *b* and *d*. It was found that the *ZZEE* and *ZZEZ* conformations were the most favorable, whereas in the other two conformations there was an energy maximum, due to steric hindrances arising between the olefinic proton and the close-lying 6-H proton of the benzene ring. Although the realization of a somewhat nonlinear conformation leads to a decrease in the steric hindrances, the energy of such a state is nevertheless considerably higher than for the *ZZEE* and *ZZEZ* conformations. The equality of the energies for the *ZZEE* and *ZZEZ* conformations indicates that the chalcone molecules exist in the form of equal amounts of the given conformers with a high rate (on the NMR time scale) of transition between them. Indirect evidence for this is provided by the absence of an appreciable increase in the intensity of the signals of the aromatic protons of the heterocyclic fragment of the chalcone molecules in NOE experiments with irradiation at the frequency of the H_b proton. Altogether the experiments that were carried out indicate that in chloroform and benzene the investigated 2-hydroxyl-containing chalcones represent a mixture of equal amounts of the *ZZEE* and *ZZEZ* conformers.

In the transition to the 2-O-benzylated analogs of the chalcones the ASIS values of the 6-H aromatic proton change sign and lie in the range of −0.2-0.4 ppm, while changing extremely little from compound to compound. This fact indicates a change in the orientation of the phenyl substituent where the 6-H proton is close in space to the carbonyl oxygen atom. It is clear that bond *a* has the *E* configuration in all the 2-O-benzylated chalcones.

In the analogs of chalcone with thiazole, imidazole, and pyridine fragments the conformation of the conjugation chain can be determined on the basis of the NOE values. Thus, the orientation of the heterocyclic substituent is determined unambiguously from the formulas of the compounds presented above, where the NOE values are indicated. As in the case of the benzodioxane derivatives, the orientation of the benzene ring here depends on the presence of the OH group at position 2 [14].

Another important type of intermediate products in the synthesis of flavonoids comprises the epoxides synthesized by the oxidation of chalcones [15, 16, 19]:

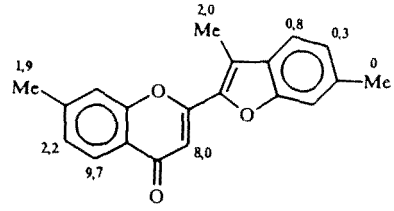


R, R^1 = Alk

These compounds are interesting in that they coordinate with the LSR at the epoxide oxygen atom. Here the lanthanide-induced shifts are extremely large. (For the protons of the oxirane ring they are greater than 40 ppm.) Such shifts are probably due to chelation effects involving complexation of the carbonyl group present in the molecules. It is possible to obtain first-order ^{1}H NMR spectra for most of the epoxides in the presence of the LSR.

4. STRUCTURE OF 2-HETEROARYLCHROMONES

During investigation of the ^{1}H NMR spectra of the 2-heteroaryl analogs of flavone the need arises to refine the assignment of a series of signals and also to determine the mutual orientation of the heterocyclic substituents [15, 20]. Both of these tasks can be solved by means of the LSR. All the 2-heteroarylchromones coordinate well with the LSR if their molecules do not contain phenolic hydroxyls. Thus, for example, the following lanthanide-induced shifts were obtained for the adduct of Eu(fod)$_3$ with the benzofuran derivative:



Since the only effective center of coordination with the LSR in the given compounds is the carbonyl oxygen atom of the chromone ring, it is possible to refine the assignment of the signals and conduct their conformational analysis on the basis of the lanthanide-induced shifts. For this purpose the lanthanide-induced shifts are calculated by means of Eq. (1) for the signals of the protons in a molecule with a different orientation of the heterocyclic substituent. The obtained values are compared with the experimental values, and the differences are expressed numerically in the form of the sum of the mean-square deviations or the associated correlation parameter. Figure 1 shows the curves for the dependence of the mean-square deviations on the angle of rotation ϕ between the heterocyclic fragments for the compound with the formula presented above. It is seen that the best correlation between calculated and experimental lanthanide-induced shifts is obtained for the conformation with $\phi = 0$.

During such calculations it is necessary to take account of the fact that the conformationally mobile group must not be situated near the coordination center of the molecule. Otherwise, the obtained orientation will only correspond to the substrate molecule combined into the adduct. In the case of sterically hindered 3-(1,3-benzodioxan-5-yl)chromones it was also shown [13] that such calculations cannot be used with such a reagent as Eu(hfbk)$_3$ [on account of the absence of axial symmetry in the obtained adducts and, accordingly, the unsuitability of Eq. (1) for the calculations].

5. STRUCTURAL FEATURES OF 3-HETEROARYLCHROMONES

Unlike the corresponding 2-derivatives, the 3-heteroarylchromones are more sterically hindered compounds, since the freedom of rotation of the 3-heteroaryl fragment is affected by the carbonyl group at position 4. Therefore, if the molecule contains a substituent R^1 at position 2, the chromone and heterocyclic substituent fragments cannot lie in one plane. Calculations by molecular mechanics show that, depending on the type of substituents close to the bond linking the heterocyclic fragments, the torsion angle between them will amount to 30-90°. 3-Heteroarylchromones react effectively with the LSR, although the LIS here is 2-3 times smaller than in the corresponding 2-heteroarylchromones. The temperature dependence of the lanthanide-induced shifts of the 3-heteroarylchromones proved abnormal. For the thiazole, benzofuran [22], and benzodioxane [13] derivatives, it was found that in contrast to the theoretical temperature dependence of the LIS [23] the series of lanthanide-induced shifts for the ^{1}H signals of the heterocyclic substituent decreases with decrease in temperature. This indicates that the temperature has an effect on the equilibrium conformation of the substituent.

The molecules of substituted 3-heteroarylchromones do not contain planes of symmetry and can exist in the form of two enantiomers (atropoisomers). We in fact discovered and studied the optical isomerism of such a type in the case of derivatives containing thiazole and benzofuran fragments [22]:

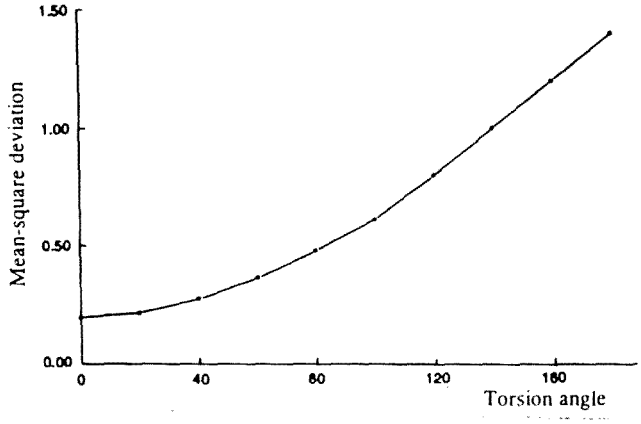
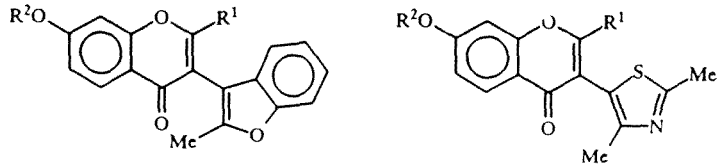


Fig. 1. Curve for the dependence of the sum of the mean-square deviations of the calculated from the experimental lanthanide-induced shifts for the adduct of $Eu(fod)_3$ with 2-(3,6-dimethyl-2-benzofuryl)-7-methylchromone. The angle $\phi = 0$ corresponds to the E conformation of the molecule.



R^1 = Me, CF_3; R^2 = Me, Bu

It was detected by the addition of the optically active LSR $Eu(HPBK)_3$ to their solutions. The action of this reagent leads to considerable changes in the positions of the signals, and the signals of the protons close to the carbonyl oxygen atom in the chromone ring are shifted particularly strongly. With LSR—substrate ratios of > 1:5 most of the signals in the ^1H NMR spectra are split, indicating the formation of diastereomeric LSR—substrate adducts. The reaction of the optically active LSR with isoflavones, in which there are no substituents in the heterocyclic fragments close to the point of fusion, does not lead to splitting of the signals. This indicates the absence of atropoisomerism in such compounds.

We established that the possibility of detecting atropoisomerism by means of an optically active lanthanide shift reagent depends largely on the temperature at which the spectrum is recorded. At temperatures above 333 K even with the maximum LSR—substrate ratio the signals in the spectrum are not split. Conversely, decrease in temperature leads to an increase in the difference between the chemical shifts of the protons in the diastereomers. Figure 2 shows the temperature dependence obtained for the benzofuran derivative given above. It is seen that at low temperatures the difference between the chemical shifts for the signals of the individual protons can exceed 2 ppm. From the figure it also follows that, as in the case of $Eu(fod)_3$, the shifts of the individual signals can both increase and decrease with change in temperature. It is possible to discover the reason for the anomalies in the temperature dependence on the basis of calculation of the geometric structure of the LSR—substrate adduct [22], but such calculations cannot be considered reliable on account of the possible effect of the LSR on the equilibrium conformation of the substrate molecule. Such an effect may be due to the steric proximity of the coordination center and the conformationally mobile group.

6. STRUCTURE OF 2- AND 3-HETEROARYLCHROMANONES

The hydrogenated analogs 2- and 3-heteroarylchromanones have a close genetic relationship with the 2- and 3-heteroarylchromones. Since the hydropyranone fragment has a nonplanar structure, the question of the orientation of the substituent at position 2 or 3 and the assignment of signals for the 3- or 2-methylene protons of the pyranone ring always arises during the synthesis of these compounds. For 2-heteroarylchromanones we found that the assignment can be made by the joint use of the LSR and ASIS [24]. The PMR spectra of the benzodioxane and benzodioxepane derivatives were measured.

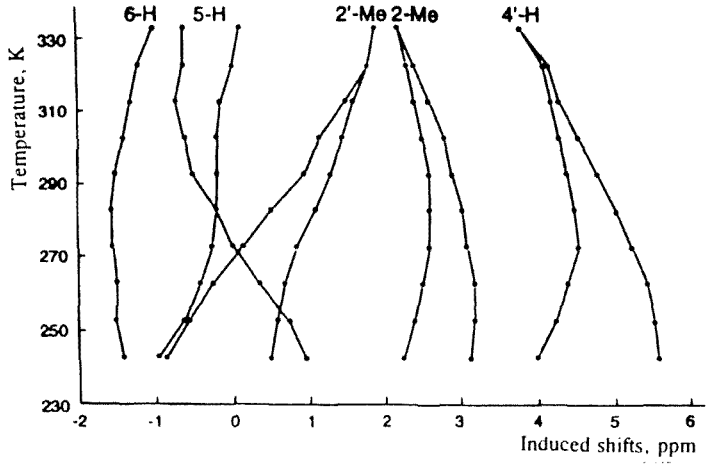
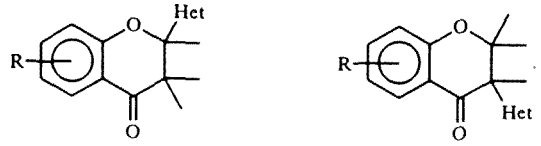


Fig. 2. Dependence of the lanthanide-induced shifts of the adduct of $Eu(hfbk)_3$ with 2-methyl-3-(2-methyl-3-benzofuryl)-7-butoxychromone on the temperature.



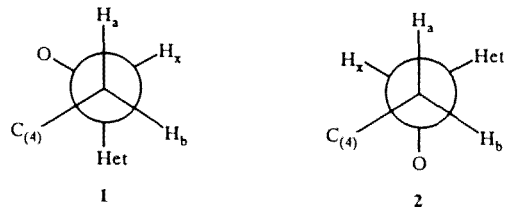
R = Alk, OAlk

The signals of the aromatic protons in the investigated compounds form multiplets in a fairly narrow region (6.5-7.2 ppm). Only the signal of the 5-H proton of the chromanone ring is shifted somewhat downfield and always appears in the region of 7.3-8.2 ppm.

In the experiment with ASIS considerable changes occurred in the PMR spectra of the 2-heteroarylchromones in the transition from deuterochloroform to deuterobenzene. The signal of the 2-H proton in benzene is shifted 0.4-0.5 ppm upfield compared with its position in chloroform. The signal of one of the protons at the $C_{(3)}$ atom is shifted upfield by 0.35-0.4 ppm, while the signal of the other is shifted upfield by 0.12-0.2 ppm. Earlier it was shown for the case of ketosteroids that in the case of protons situated at the α position to the carbonyl group, larger chemical shifts are observed for H_a and smaller for H_e [25]. This makes it possible to assign the signals of the protons at the $C_{(3)}$ atom.

We used the Karplus relation to determine the orientation of the 2-H proton in 2-heteroarylchromanones. The alternative conformations of the chromanone ring are determined by the orientation of the substituents in relation to the C_2—C_3 bond.

Projection 1 corresponds to the axial orientation of the heterocyclic substituent, while projection 2 corresponds to the equatorial orientation:



1 2

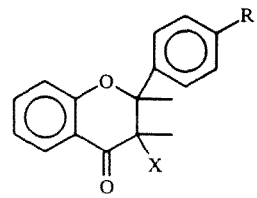
Here the H_a and H_b protons are linked to the $C_{(3)}$ atom, while the H_x atom is linked to the $C_{(2)}$ atom. As seen from Fig. 1, in projection 1 the H_x—C—C—H_b angle is equal to the H_x—C—C—H_a angle. Similar values must therefore be expected for the corresponding spin—spin coupling constants. In projection 2 the H_x—C—C—H_b torsion angle is close to 180°, while the H_a—C—C—H_x angle is close to 55°. According to the Karplus equation, the spin—spin coupling constants of the vicinal protons should amount to 11 and 2.5 Hz for this conformation. Thus, a conformation in which the heterocyclic substituent has the equatorial orientation is realized in the investigated 2-heteroarylchromanones. Similar conclusions about the orientation of the heterocyclic substituent were reached for substituted 2-imidazolylchromanones [26].

In 3-heteroarylchromanones the 3-H proton can also be found in the equatorial or axial position. A conclusion about the position of the proton can also be reached on the basis of ASIS as described above for the isomeric products. In the case under consideration the ASIS for the 3-H proton amounts to 0.12-0.17 ppm, indicating that it has the equatorial orientation and consequently that the heterocyclic substituent has the axial orientation. This conclusion is particularly important since it is not possible to use the vicinal spin—spin coupling constants to determine the structure of the compounds on account of the magnetic equivalence of the protons at $C_{(2)}$.

The reaction of 2- and 3-heteroarylchromanones with $Eu(fod)_3$ and $Eu(hfbk)_3$ was also studied. The largest lanthanide-induced shifts are observed for the signals of the protons close to the oxygen atom of the carbonyl group in the chromanone ring, indicating that the LSR is coordinated at this coordination center. The LSR was particularly useful for the analysis of the spectra of 3-heteroarylchromanones. In the presence of the LSR the methylene protons at $C_{(2)}$ become magnetically nonequivalent, and this makes it possible to determine the values of their vicinal spin—spin coupling constants with the 3-H proton. They amounted to 4.7 and 8 Hz. Thus, the $H_x—C—C—H_a$ and $H_x—C—C—H_b$ torsion angles do not differ too greatly, and this is only possible for the equatorial position of the 3-H proton. As we see, this conclusion agrees with the conclusion reached earlier on the basis of ASIS.

7. FEATURES OF THE STRUCTURE OF 3-SUBSTITUTED 2-ARYLCHROMANONES

During the synthesis of such compounds the question of the orientation of the substituents in the pyranone ring arises. The compounds can have the *cis* or *trans* configuration, and the axial or equatorial orientation of the substituents is possible in each of the isomers. The assignment of the products to the *cis* or *trans* series is made by their vicinal spin—spin coupling constants. It amounts to 9-11 Hz for the *trans* products and 2-3 Hz for the *cis* products. Since the compounds contain two asymmetric centers, it seemed of interest to study their chirality by means of an optically active LSR.



R = H, Cl, NO_2; X = OSO_2Me, NHCOMe, SCN, Br, Me

In the PMR spectra of the investigated products, measured in the presence of the LSR, strong lanthanide-induced shifts are observed for the signals of a series of the protons and particularly for those situated close to the carbonyl group. Such a situation is observed irrespective of whether the flavonone molecule contains other electron-donating groups capable of interacting with the LSR. It follows from this that the oxygen atom of the carbon group in the flavonoid ring is the most active center of coordination with the LSR in compounds of this type. The signals of the aromatic protons of the investigated flavonones (with the exception of the 5-H proton) are shifted weakly and in many cases form poorly resolved multiplets even with large LSR—substrate ratios.

For the *cis* compounds the lanthanide-induced shifts are on the whole larger than for the *trans* compounds, particularly if the substituent X contains an additional coordination center. Calculations of the structure of the adducts showed that the type of the substituents has little effect on their structure, with the exception of cases where there is an additional effective coordination center in the molecule.

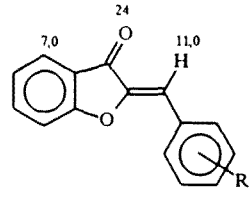
The molecules of all the investigated flavonoids contain two asymmetric carbon atoms (the $C_{(2)}$ and $C_{(3)}$ atoms) and can accordingly each have four optical isomers. With the addition of $Eu(hfbk)_3$ to their solutions, a series of the ^1H NMR signals are doubled (chiral splitting). The lanthanide-induced shifts here are of the same order of magnitude as in the case of the interaction of the flavonoids with $Eu(fod)_3$. The fact that splitting of the signals into four components was not observed in any of the investigated compounds indicates that $Eu(hfbk)_3$ makes it possible to distinguish between the optical antipodes only for the asymmetric carbon atom closest to the coordination center.

The chiral splittings proved extremely different: for some compounds they amounted to only a few Hz (the *trans* isomer with X = Br, R = H), and for the others they were 1.5 Hz (the *cis* isomer with X = SCN, R = Cl). To characterize the chiral splitting quantitatively it was proposed to use the ratio of the observed splitting of the signals to the average value of their lanthanide-induced shifts, expressed in the same units (the parameter χ).

Comparison of the χ values for *trans*- and *cis*-flavonoids shows that the splitting is as a rule smaller in the *trans* compounds than in the *cis* compounds. However, no direct relation can be traced between χ and the type of substituents close to the coordination center of the flavonones. It is most likely that this parameter is affected simultaneously by several factors: The conformational mobility of the molecule, the stability constant of the adduct of the LSR with each of the antipodes, and the degree of chirality of the molecule [27].

8. STRUCTURAL FEATURES OF AURONES

The molecules of the aurones, which are isomers of the corresponding derivatives of 2- and 3-arylchromones, can exist in the form of two geometric isomers, differing in the orientation of the aromatic substituent.



It is possible to establish their structure reliably by means of an LSR [28]. During the action of $Eu(fod)_3$ particularly strong lanthanide-induced shifts are observed for the signal of the olefinic proton and for the 4-H proton of the heterocyclic ring. The presented formula shows the corresponding lanthanide-induced shifts for the compound in which R = H. It follows from this that the substituents have the *Z* configuration in relation to the exocyclic double bond of the aurone molecules. If the substituent R is at position 2′, the phenyl ring to which it is attached assumes a planar conformation, in which it is separated from the olefinic proton. (The lanthanide-induced shift of the signal for the 6′-H proton is larger than the lanthanide-induced shift of the substituent R.) In the absence of such a substituent a rapid transition between the planar conformations is observed for the phenyl ring. This follows from the equal values of the lanthanide-induced shifts for protons 2′ and 6′.

Thus, by using the lanthanide shift reagents it is possible to obtain valuable and comprehensive information on the detailed structure of flavonoid molecules in solutions. Together with the simplicity of the experiment this makes the LSR method extremely useful in structural investigations. In some cases the use of LSR is more expeditious than two-dimensional NMR spectroscopy.

The authors express their gratitude to the INTAS program and to the international Sorosov program ISSEP (grant No. SPU063069) for financial support.

REFERENCES

1. A. L. Kazakov, V. P. Khilya, V. V. Mezheritskii, and D. Litkei, Natural and Modified Flavonoids [in Russian], Izd. Rost. Un-ta, Rostov (1985).
2. N. V. Gorbulenko and V. P. Khilya, Ukr. Khim. Zh., **60**, 3 (1994).
3. A. Wander, British Patent No. 728,767; Chem. Abs., **50**, 7876 (1956).
4. J. Schmitz, R. Hirt, and F. Kinzle, Helv. Chim. Acta, **36**, 620 (1964).
5. W. Villiam and L. Bencze, Belgian Patent No. 633,436; Chem. Abs., **61**, 1840 (1964).
6. N. V. Gorbulenko, G. M. Golubushina, I. P. Kupchevskaya, and V. P. Khilya, Dokl. Akad. Nauk Ukr. SSR, Ser. B, No. 7, 623 (1978).
7. S. A. Vasil'ev and V. P. Khilya, Ukr. Khim. Zh., **58**, 1005 (1992).
8. P. Cozzi and A. Pillan, J. Heterocyclic Chem., **22**, 441 (1985).
9. N. M. McConnel and R. E. Robertson, J. Chem. Phys., **29**, 1361 (1958).

10. A. V. Turov and V. P. Khilya, Khim. Geterotsikl. Soedin., No. 4, 457 (1994).
11. M. Yu. Kornilov, V. V. Plaxhotnik, A. V. Turov, and V. P. Khilya, Ukr. Khim. Zh., **51**, 1026 (1992).
12. M. Yu. Kornilov, A. V. Turov, and V. I. Zamkovoi, Ukr. Khim. Zh., **41**, 769 (1975).
13. A. V. Turov, L. Tsao, and V. P. Khilya, Khim. Geterotsikl. Soedin., No. 12, 1631 (1993).
14. A. V. Turov, V. P. Khilya, and D. Litkey (Litkei), Khim. Geterotsikl. Soedin., No. 2, 244 (1994).
15. L. Grishko, A. Turov, V. Khilya, D. Litkey, and T. Patonay, Acta Chem. Hung., **112**, 401 (1983).
16. L. G. Grishko, A. V. Turov, M. G. Spasenov, and V. P. Khilya, Khim. Geterotsikl. Soedin., No. 9, 1202 (1981).
17. I. G. Marchenko, A. V. Turov, and V. P. Khilya, Dokl. Akad. Nauk Ukr. SSR, Ser. B, No. 1, 43 (1979).
18. A. V. Turov, A. Aitmambetov, and V. P. Khilya, Ukr. Khim. Zh., **59**, 62 (1993).
19. J. D. Connoly and R. M. McGrindle, Chem. Ind., No. 9, 3179 (1969).
20. L. G. Grishko, A. V. Turov, I. A. Potrusaeva, and V. P. Khilya, Ukr. Khim. Zh., **49**, 174 (1983).
21. V. P. Khilya, A. Aitmambetov, and A. V. Turov, Khim. Geterotsikl. Soedin., No. 2, 174 (1986).
22. A. V. Turov, I. V. Komarov, V. P. Khilya, and M. Yu. Kornilov, Teor. Éksp. Khim., No. 5, 629 (1989).
23. B. Bleany, J. Magn. Res., **8**, 91 (1972).
24. V. P. Khilya, D. Litkey, and E. N. Kovtun, Zh. Org. Khim., **27**, 690 (1991).
25. N. Bhacca and J. Williams, Application of NMR in Organic Chemistry [Russian translation], Mir, Moscow (1966).
26. V. P. Khilya, L. G. Grishko, G. M. Golubushina, N. N. Arkhipova, and A. V. Turov, Khim. Geterotsikl. Soedin., No. 8, 1063 (1994).
27. V. E. Kuz'min and I. B. Stel'makh, Zh. Strukt. Khim., No. 4, 50 (1987).
28. A. V. Turov, M. Yu. Kornilov, T. Patonai, and D. Litkey, Teor. Éksp. Khim., **21**, 108 (1985).