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STUDY OF SOPHORICOSIDE DERIVATIVES WITH THE AID OF LANTHANOID SHIFT REAGENTS

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Acylated and methylated derivatives of sophoricoside and of genistein have been synthesized, and their interaction in solution with the lanthanoid shift reagent (LSR) Eu(FOD)$_3$ has been studied. For 5-O-alkyl derivatives of genistein an anomalous broadening of the ^1H NMR signals was observed in the presence of the LSR, which is explained by the formation with the LSR of a chelate having a low rate of dissociation. The temperature and concentration dependencies of the paramagnetic broadenings of the signals have been studied.

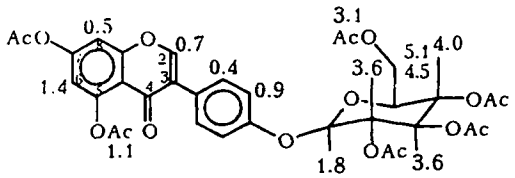
As a rule, the establishment of features of the structures of natural flavonoids causes serious difficulties connected with the diversity of the forms both of their carbohydrate residues and of the aglycons. Problems of this type are most frequently resolved by means of complex investigations, including purely chemical operations such as hydrolysis and the formation of functional derivatives of the isolated compounds, and physicochemical investigations of them, especially with the use of different variants of NMR spectroscopy.

One of the methods of structural investigation that is widely employed for other classes of compounds is the use of lanthanoid shift reagents (LSRs). In the PMR spectra of adducts of organic electron-donating molecules with LSRs large anisotropic paramagnetic shifts of signals having basically a pseudocontact nature are observed [1, 2]. From their magnitudes it is possible to make reliable deductions concerning the structures of the compounds under investigation [3].

Nevertheless, the use of LSRs for studying natural flavonoids and their analogs is beset by a number of difficulties due to an inadequate solubility of many natural flavonoids in suitable solvents (chloroform, benzene) and the absence from their molecules of effective centers of coordination with LSRs. This relates, in particular, to 5-R-substituted chromones, in which the coordination of LSRs with the carbonyl group of the chromone nucleus (one of the most effective coordination centers in a flavonoid molecule [4, 5]) proves to be sterically hindered. We have studied approaches to the investigation of the structures of such natural flavonoids with the aid of LSRs.

Sophoricoside (4′,5,7-trihydroxyisoflavone 4′-O-β-*D*-glucopyranoside, **1**), isolated from the Japanese pagoda tree *Sophora japonica* [6-8], forms a colorless microcrystalline powder practically insoluble in solvents suitable for working with LSRs. It is therefore impossible to study its interaction with LSRs directly. The hexaacetyl derivative (**2**) proves to be considerably more convenient.

On the interaction of sophoricoside acetate with Eu(FOD)$_3$ we found the following specific lanthanoid-induced shifts (LISs):



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Since the largest LISs were observed for the carbohydrate moiety of the molecule, it may be concluded that the most effective center of coordination with LSRs is present just here. The main contribution to coordination is most probably made

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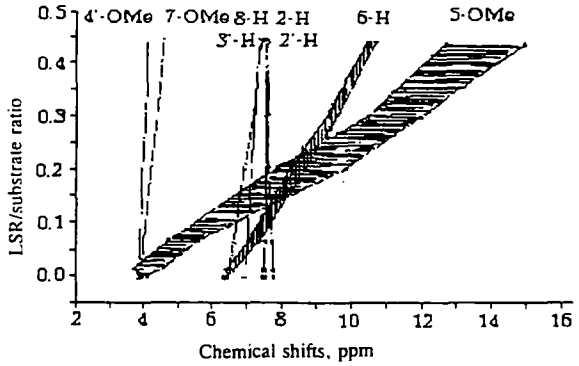


Fig. 1. Chemical shifts of the signals of the protons of tri-O-methylgenistein (**4**) on the addition of a LSR. Hatched areas correspond to the width of the signals at each LSR/substrate ratio.

by the carbonyl groups of the CH_2OAc fragments, for which the highest values of the LISs were found. As follows from the small values of the relevant LISs, the functional groups of the chromone fragment do not take a significant part in coordination with LSRs. In the PMR spectrum of hexacetylsophoricoside in the presence of a suitable amount of a LSR all the protons in the molecule give nonoverlapping signals from the SSCCs of which it is possible to judge the structure of the carbohydrate moiety of the molecule and, where necessary, to use double-resonance procedures — for example, in order to refine the configuration of the molecule.

Genistein (4′,5,7-trihydroxyisoflavone (**3**)) [8-11], like sophoricoside itself, is practically insoluble in deuterochloroform, and we therefore studied more soluble derivatives of it, namely: tri-O-methylgenistein (**4**), di-O-methylgenistein (**5**), genistein triacetate (**6**), and the newly synthesized tri-O-ethyl- and tri-O-benzylgenisteins (**7** and **8**).

For genistein triacetate we observed extremely weak LISs, which showed the practical absence of its coordination with the LSR. This is connected with the facts that, on the one hand, the carbonyl carbon atom of the chromone nucleus proved to be sterically hindered by the substituent in position 5, and, on the other hand, that the acetoxy groups in the aromatic nucleus likewise have practically no capacity for interacting with LSRs. It is therefore almost impossible to study flavonoid derivatives having such substituents with the aid of LSRs. In contrast, as was found, tri-O-methylgenistein interacts with LSRs extremely effectively, and its PMR spectra in the presence of $Eu(FOD)_3$ have a number of interesting features.

On the addition of even small amounts of the LSR (molar ratio LSR:tri-O-methylgenistein = 0.002) there was a pronounced broadening of the signal of the 5-OMe group, and on further addition of the LSR this signal broadened to such an extent that it was hardly visible in the spectrum. The signal of the 6-H proton also broadened appreciably. The form of the spectrum under the action of the LSR is shown schematically in Fig. 1.

So far as concerns the nature of the unusual signal broadenings observed in the spectrum, they may have either a paramagnetic or an exchange character. To answer this question, we studied the dependence of the change in the broadening of the signals on the temperature of the sample. The results are given in Fig. 2 in the form of three-dimensional diagrams. Here the widths of the signals of the 6-H proton and of the 5-OCH_3 group have been plotted along the vertical axis, and the temperature and the LSR/substrate ratio along the two horizontal axes. It can be seen that with a lowering of the temperature the width of the signals increased. With a rise in the molar fraction of LSR the width of the signals first increased (LSR/substrate ratio = 0.1-0.2) and then it decreased, and at a LSR/substrate ratio of 0.45 the width of the signals was small. It is interesting that with a rise in the temperature it was possible to achieve a practical disappearance of the broadening of the signals, but at the same time the LISs for the signals of the 6-H and 5-OAc protons remained constantly large. This shows the exchange nature of the observed broadenings, since, for europium complexes, paramagnetic signal broadenings change symbatically with the LISs and disappear only with the disappearance of the latter [12].

Exchange broadenings arise in those cases where the rate of exchange between substrate molecules bound into the adduct and uncoordinated molecules falls and becomes intermediate in the NMR time scale. In other words, the strength of the adduct of tri-O-methylgenistein with the LSR must be unusually high in comparison with adducts of the LSRs with other substrates in which the chromone carbonyl acts as the center of coordination (see, for example [5]). We assume that this effect is connected with the formation between the tri-O-methylgenistein and LSR molecules of an adduct of the chelate type

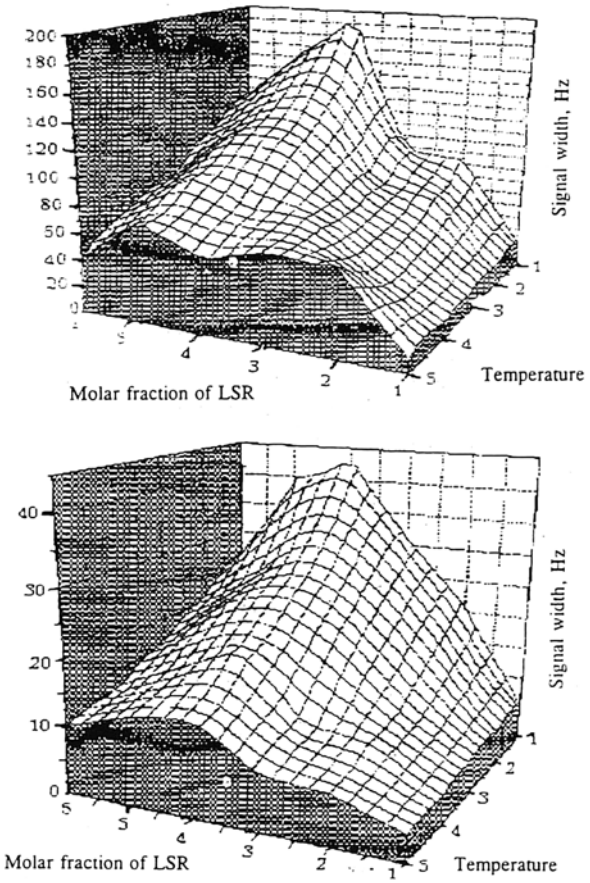
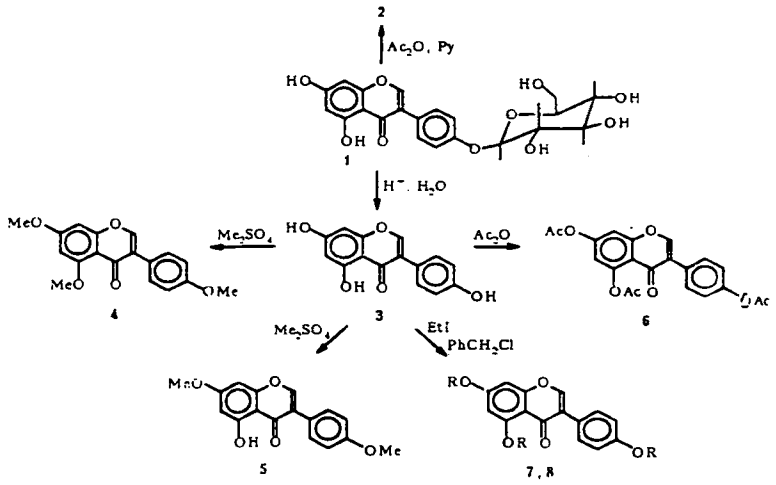


Fig. 2. Dependence of the width of the signals of tri-O-methylgenistein (**4**) on the temperature and the LSR/substrate ratio: *top*) for the signal of the 5-OCH_3 group; *bottom*) for the signal of the 6-H proton. Molar ratios of LSR to substrate: *1*) 0.002; *2*) 0.015; *3*) 0.07; *4*) 0.21; *5*) 0.31; *6*) 0.45; temperatures: *1*) 303K; *2*) 313K; *3*) 323K; *4*) 333K; *5*) 343K.

in which the europium ion is coordinated simultaneously with the chromone carbonyl and the 5-methoxy group, forming a stable six-membered ring.



We have attempted to find how coordination with the LSR is affected by an increase in the steric hindrance close to the chromone carbonyl. For this purpose we studied the interaction of the LSR with tri-O-ethyl- and tri-O-benzylgenisteins. It was found that the PMR spectra of such adducts also showed exchange broadening indicating chelate formation. The possibility of the formation of strong adduct between these isoflavones and LSRs is apparently determined by the fairly high

flexibility of the 5-O-ethyl and 5-O-benzyl fragments and the realization in the adducts of such an orientation as does not prevent interaction with LSRs.

Chelate formation was also observed between the LSR and 4′,7-di-O-methylgenistein, which contains a 5-OH group. However, in this case the LISs proved to be small, which shows weak coordination of the LSR with a chromone carbonyl in the *peri*- position to which there is a OH group.

Thus, the use of LSRs for elucidating details of the structure of methylated and acylated derivatives of sophoricoside and genistein has proved to be fairly effective. As a result of such investigations it is possible to determine the coordination features of such compounds, which is important for establishing the mechanisms of their biological activity.

EXPERIMENTAL

PMR spectra were obtained on a WP100-SY spectrometer (Bruker) with TMS as internal standard. A commercial LSR was used without additional purification. For processing the experimental results we used the SURFER and GRAFER program packages.

The methods of synthesizing the compounds studied are shown in the scheme. Sophoricoside (**1**), isolated from natural raw material, was converted into the hexaacetate (**2**) by the action of acetic anhydride in pyridine. Acid hydrolysis gave genistein (**3**). Derivatives (**4**)-(**8**) were obtained by the action of alkylating and acylating agents [8-11].

4′,5,7-Tri-O-ethylgenistein (7). To a solution of 1.5 g (5.5 mmole) of genistein in 10 ml of absolute dimethylformamide were added 4.6 g (33 mmole) of freshly calcined potash and 2.5 ml (19.8 mmole) of diethyl sulfate. The mixture was stirred at 70°C for 6 h. The solution was poured hot into 5% acetic acid. The resulting precipitate was filtered off, washed with water, and crystallized from 70% ethanol. Colorless needles with mp 113-114°C. Yield 1.45 g (75%). ^{1}H NMR (m $CDCl_3$): 7.72 (s, 2-H); 7.44 (d, ^3J = 8 Hz, 2H, 2′, 6′-H); 6.91 (d, 2H, 3′, 5′-H); 6.40 (d ^4J = 2.5 Hz, 1H, 6-H); 6.34 (d, 1H, 8-H); 4.09 (m, 6H, CH_2); 1.46 (m, 9H, CH_3).

4′,5,7-Tri-O-benzylgenistein (8). To a solution of 1.5 g (5.5 mmole) of genistein in 30 ml of absolute acetone were added 4.6 g (33 mmole) of freshly calcined potash, 1.5 g (9 mmole) of KI, and 2.3 mg (16.5 mmole) of benzyl chloride. The mixture was boiled for 30 h and was then poured into 100 ml of 5% acetic acid. The resulting precipitate was filtered off, washed with water, and crystallized from ethanol. Yield 1.5 g (51%). Colorless needles with mp 151–152°C. ^{1}H NMR (in $CDCl_3$): 7.73 (s, 1H, 2-H); 6.9-7.5 (m, 15H, Ph); 6.51 (s, 2H, 6.8-H); 5.21 (s, 2H, CH_2); 5.10 (s, 4H, CH_2). The results of elementary analyses corresponded to the calculated figures.

REFERENCES

1. K. A. Kime and R. E. Sievers, Aldrichimica Acta, **10**, 54 (1977).
2. M. Yu. Kornilov and A. V. Turov, Khim. Geterotsikl. Soedin., No. 10, 1299 (1979).
3. M. Yu. Kornilov, V. V. Plakhotnik, A. V. Turov, and V. P. Khilya, Ukr. Khim. Zh., **58**, No. 11, 1026 (1992).
4. L. G. Grishko, A. V. Turov, I. A. Potrusaeva, and V. P. Khilya, Ukr. Khim. Zh., **49**, No. 2, 174 (1983).
5. A. V. Turov, I. V. Komarov, V. P. Khilya, and M. Yu. Kornilov, Teor. Éksp. Khim., No. 5, 629 (1989).
6. V. A. Bandyukova, Rast. Res., **4**, No. 1, 97 (1968).
7. R. Paris, G. Faugeras, and J. F. Dombremez, Planta Med., **29**, No. 1, 32 (1976).
8. R. Bognar and V. Szabo, Acta Chim. Acad. Sci. Hung., **4**, 383 (1954).
9. J. B. Harborne, Phytochemistry, **8**, 1449 (1969).
10. M. Tofck, Fitoterapia, **47**, No. 5, 195 (1976).
11. A. L. Kazakov, V. L. Shinkarenko, and É. G. Oganesyan, Khim. Prir. Soedin., 804 (1972).
12. R. E. Lenkinski and J. Reuben, J. Magn. Reson., **21**, 47 (1976).