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CONFIGURATION OF STEREOISOMERS OF NARINGENIN 5-GLUCOSIDE PRESENT IN FLAMIN

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The configurations of the C-2 chiral centers of the stereoisomers of naringenin 5-glucoside isolated from flamin by HPLC have been established by the method of circular dichroism. One of the stereoisomers is (−)-2S-naringenin 5-glucoside and the other has been characterized as a molecular compound containing the 5-glucosides of (+)-2R- and (−)-2S-naringenins in a ratio of ~2:1.

In an investigation of flamin by high-performance liquid chromatography (HPLC) it was established that chalcone-flavanone isomerization [1] took place in its water-containing solutions. In a model example with isosalipurposide it was shown that under suitable conditions the chalcone was converted into two stereoisomers of naringenin 5-glucoside in the course of a month.

The presence of two compounds corresponding to naringenin 5-β-D-glycoside was reported previously for yellow everlasting [2, 3] and in flamin [4]. It was assumed that these were either different hydrate forms [2] or substances having a stereochemical difference [3]. The compounds were separated by column chromatography and paper chromatography [3, 4]. It was established with the aid of chemical methods that one of them (helichrysin A) was a glucoside of (−)-naringenin and it was subsequently ascribed the 2S-configuration [5]. The other compound (helichrysin B) was characterized as a glucoside of racemic naringenin capable of being separated into diastereomers on further chromatographic separation [6]. In a later paper [7] doubt was cast on the existence of the 5-glucoside of racemic naringenin.

Since the pharmacological activity of flamin is due primarily to a chalcone-flavanone pair of compounds, we set ourselves the task of determining the configuration of the C-2 chirality centers of the stereoisomers of naringenin 5-glucoside by using a combination of one of the most informative chiroptic methods — circular dichroism (CD) — and HPLC. An important circumstance is the HPLC provides the possibility not only of effectively separating stereoisomers but also of performing clear qualitative and quantitative monitoring at any stage of the investigation.

1st I. M. Sechenov Moscow Medical Institute. M. V. Lomonosov Moscow State University. All-Union Scientific-Research Institute of Drug Chemistry and Technology, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 185-188, March-April, 1989. Original article submitted May 7, 1988.

0009-3130/89/2502-0157 $12.50

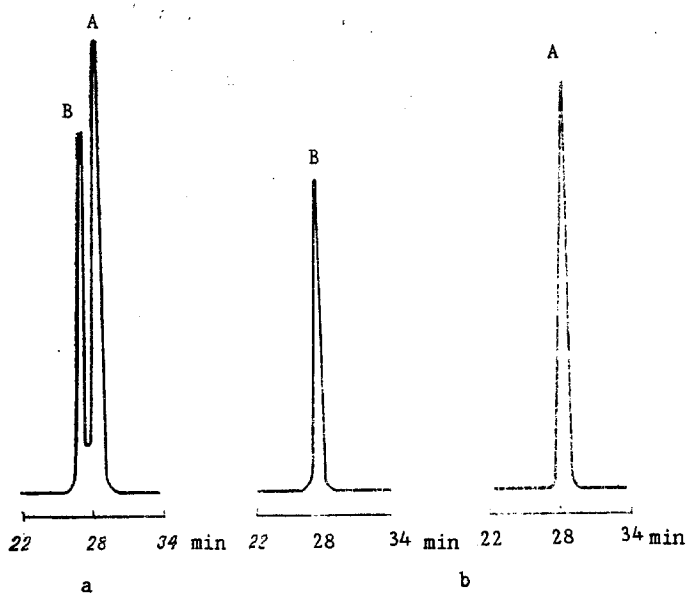


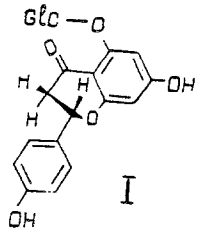
Fig. 1. Semipreparative separation of the stereoisomers of naringenin 5-glucoside (a) and a check on it (b).

In the analytical regime for the HPLC of flamin that we selected [8] there is a partial separation of the two stereoisomers of naringenin 5-glucoside. For their accumulation we developed the conditions for their complete separation in a semipreparative variant (Fig. 1a). The individuality of the components was confirmed by various types of chromatographic analysis (Fig. 1b), and their constants are given below:

Compound	mp, °C	$[\alpha]_D^{20}$, deg (*c* 0,2; methanol)	λ_{max}, nm (ε)
A	159—160	—115 6	282 ($17{,}9\cdot10^3$)
B	221—222	— 86,9	282 ($15{,}7\cdot10^3$)
Aglycon A	251—252	— 28,6	287 ($17{,}8\cdot10^3$),
Aglycon B	251—252	+ 9,0	325 sh ($5{,}1\cdot10^3$)

To establish the configuration of the C-2 chirality center we measured in parallel the CD spectra both of the glycosides and of their aglycons under conditions of hydrolysis excluding racemization [9].

In the CD spectrum of compound A (Fig. 2) a positive Cotton effect was observed in the 330 nm region and a negative one in the 305 nm region. On the basis of known laws of the interrelationship of CD spectra and the configuration of the C-2 chirality center of the flavanones and their glycosides [10], we established the 2S-configuration for compound A. The CD spectrum of the aglycon of compound A was analogous to the CD spectrum of the (−)-naringenin isolated from naringin by enzymatic hydrolysis [10]. This confirmed that compound A that we had isolated from flamin was (−)-2S-naringenin 5-glucoside (I).



I

From a comparison with information in the literature it may be considered that it corresponds to helichrysin A [3].

In the CD spectrum of compound B, weak Cotton effects were observed in the 250-350 nm region, which is characteristic for glycosides of flavanones with racemic aglycons [10].

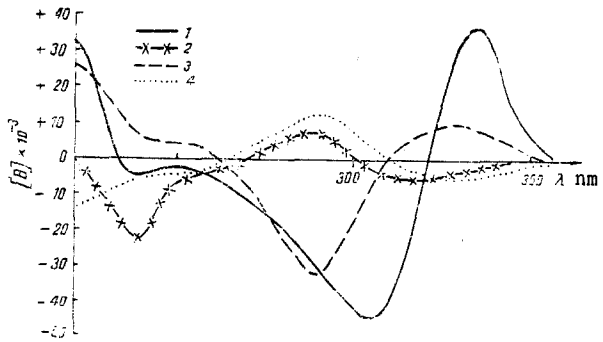


Fig. 2. CD spectra of the stereoisomers of naringenin 5-glucosides A (1) and B (2) and of the aglycons A (3) and B (4) corresponding to them.

However, the aglycon obtained from compound B was not racemic; it showed optical activity. In the CD spectrum of this aglycon, the same Cotton effects appeared as in the CD spectrum of (−)-2S-naringenin but with the opposite signs, which showed the R-configuration at C-2. From a comparison of the values of the molar ellipticities of (−)-2S-naringenin and the aglycon of compound B it was possible to conclude that the latter is not an optically pure enantiomer. Its optical purity calculated from the molar ellipticity of the aglycon of compound A ($[\theta]_{290} = -38.8 \cdot 10^3$) and that of the aglycon of compound B ($[\theta]_{290} = +12.5 \cdot 10^3$) was 32%, i.e., the 2R- and 2S-enantiomers were present in a ratio of ~2:1.

Thus, while compound A may be considered as identical with helichrysin A, compound B was not identical with the other stereoisomer – helichrysin B – described in the same paper [3].

The melting point of compound B remained constant on repeated recrystallization, and the compound was not separated chromatographically under various conditions, while helichrysin B is capable of chromatographic separation. On the basis of results obtained the hypothesis may be put forward that compound B consists of a molar compound including the 5-glucosides of (+)-2R- and (−)-2S-naringenins in a ratio of 2:1.

EXPERIMENTAL

The investigations were carried out on a Spectra Physics 8700 liquid chromatograph with a SP 8400 UV detector (analytical wavelength 282 nm). An analytical column (4.6 × 250 mm) filled with the reversed-phase sorbent Nucleosil C_{18} with a particle size of 10 μm was used, and also a Partisil 10 ODS M (9.4 × 500 mm) semipreparative column and the eluting system water–acetonitrile–tetrahydrofuran–acetic acid in a ratio of 71.5:20:3.5:5 (by volume). The areas of the peaks on the chromatograms and the retention times were measured with the aid of a SP 4100 integrator.

The substances were extracted from solutions in the eluting system with the aid of a Sep-Pak C_{18} cartridge, followed by elution with methanol and freeze-drying; melting points were determined on an instrument of the Boëtius type. Samples were weighed on a Sartorius 1201 MP2 balance, and solutions were prepared with the aid of Gilson pipettes.

The UV spectra of the compounds under investigations were recorded on a Cary 219 spectrophotometer. The CD spectra of the solutions of the substances in methanol (concentrations from 100 to 400 μg/ml) were taken on a J-20 spectropolarimeter in a cell 1 mm thick. Optical rotations were determined on a EPO-1 polarimeter in 0.25- and 1-dm cells.

SUMMARY

The configurations of the stereoisomers of naringenin 5-glucoside isolated from flamin by the HPLC method have been investigated. One of the stereoisomers is (−)-2S-naringenin 5-glucoside and the other has been characterized as a molecular compound consisting of (+)-2R- and (−)-2S-naringenin 5-glucosides in a ratio of 2:1.

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CHEMICAL MODIFICATION OF FURANOEREMOPHILAN-14β,6α-OLIDE

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On performing the modification of furanoeremophilan-14β,6α-olide at the most reactive fragments of the molecule, the lactone and furan rings, it was established that in an alkaline medium furanoeremophilan-14β,6α-olide isomerizes into the 14α,6α-olide; the oxidation of furanoeremophilan-14β, 6α-olide to a dilactone in the presence of Rose Bengal takes place with high yield under mild conditions – atmospheric oxygen, without heating and irradiation; on the interaction of furanoeremophilan-14β,6α-olide with selenium dioxide in glacial acetic acid a selenium-containing dimer is formed.

Golden ray roots contain a considerable number of furanoeremophilanes [1-12]. In greatleaf golden ray Ligularia macrophylla D. C. Prodr. six substances have detected [8, 11], one of which, furanoeremophilan-14β,6α-olide (I) is present in predominating amount. We have carried out a modification of this compound at the most reactive fragments of the molecule – the lactone and furan rings.

Under the action of potassium carbonate, the lactone ring of (I) opened, as a result of which a water-soluble salt of the corresponding 6-hydroxyfuranoeremophilane-4-carboxylic acid (II) was formed. On subsequent relactonization, instead of the expected initial (I), we obtained a substance (III) of the same composition but differing with respect to temperature and optical activity: (I) – mp 136-137°C $[\alpha]_D^{20}$ –45°C (c 0.45; dioxane); (III) mp 124-125°C $[\alpha]_D^{20}$ –121° (c 0.33; dioxane).

A difference in the structures of (I) and (III) followed from a comparison of their PMR spectra, which showed a change in the multiplicity of the signal of the C_4–H proton. In the spectrum of (I) it was represented by a doublet with J = 8 Hz due to e/e- and e/a-interactions in a three-spin system formed by the methylene protons at C_3 and the methine proton at C_4, which corresponds to the equatorial position of the latter and to an axial C_4-carboxy group. In the spectrum of (III), this signal was represented by a quartet with J_1 = 16 and J_2 = 6 Hz, which showed the axial position of C_4–H and an equatorial C_4-carboxy group. Thus, the opening of the lactone ring of (I) was accompanied by isomerization of the furanoeremophilan-14β,6α-olide into the 14α,6α-isomer, as a result of which the salts obtained had a configuration corresponding to (III).

We obtained readily water soluble crystalline potassium and sodium salts with mp 218-219°C and 188-189°C. Their IR spectra showed an intense absorption band at 1570 cm^{-1} which is characteristic for the carboxy group in salts.

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