THE ISOLATION OF DAUCOSTEROL FROM ACANTHOPANAX SESSILIFLORUM

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The isolation from Acanthopanax sessiliflorum (family araliaceae) of four glycosides called acanthosides A, B, C, and D has been reported previously [1]. The structure of two of them (B and D) has been completely established [2]. They proved to be, respectively, the mono- and di-β-D-glucopyranosides of a genin of the lingnan series, (−)-syringaresinol.

In spite of the fact that Acanthopanax belongs to the family araliaceae, we have not previously succeeded in finding in this plant triterpene glycosides similar to those obtained from other representatives of of the araliaceae: Aralia manshurica [3], Kalopanax septimlobus [4], and Panax ginseng [5]. However, in a more detailed investigation of the methanolic extract and also of the essential oil of Acanthopanax we have isolated and identified daucosterol, a glucoside of β-sitosterol found by Takahashi et al. [6] in the essential oil of ginseng. According to previous results, the extract of the roots of another representative of the family araliaceae, Eleutherococcus senticoccus, also contains daucosterol. Thus, the presence of the latter is possibly a general chemotaxonomic characteristic of this group of plants of the family mentioned.

We isolated daucosterol by two methods: from a methanolic extract of the roots of A. sessiliflorum by chromatographic separation, and from the essential oil by successive extraction with various solvents.

The hydrolysis of daucosterol with dilute sulfuric acid gave its genin, β-sitosterol, and glucose, which was identified chromatographically.

Fig. 1 gives our IR spectra of daucosterol, its acetate, and β-sitosterol. The IR spectrum of our sample of β-sitosterol and the IR spectrum of authentic β-sitosterol coincided completely as did their NMR spectra (Fig. 2). In the NMR spectrum, the peaks can be assigned to definite methyl groups by comparing the results obtained with the NMR spectra of other steroids with a long side chain [9]. One angular methyl group at C_{18} absorbs in a strong field (0.7 ppm), and another at C_{19} in a weaker field (1.2 ppm). The C_{21}, C_{26}, and C_{27} methyl groups, interacting with neighboring protons, give two signals at 0.79 and 0.89 ppm, and a triplet (0.96, 0.86, 0.77 ppm) is given by the protons of the C_{29} methyl group adjacent to a methylene group. The signal at 5.4 ppm belongs to a proton at a double bond. The width of the line (24 cps) with a center at 3.5 ppm corresponds to the signal of a proton at C_3. The other protons of the skeleton and of the hydroxyl group absorb in the 1-2.6 ppm region.

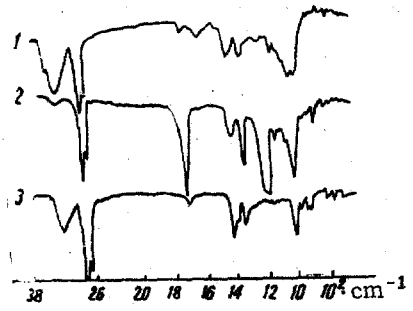

Fig. 1. IR spectra of daucosterol, daucosterol acetate, and β-sitosterol.

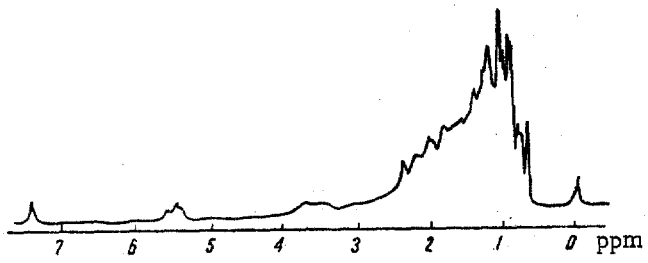

Fig. 2. NMR spectrum of β-sitosterol.

Experimental

Neutral alumina (Brockmann activity grade III) was used. The silica gel was 100-200 mesh for column separation, and greater than 270 mesh for thin-layer chromatography. The melting points of the substances were determined on a "Boetius" stage and are not corrected.

Isolation of daucosterol from the methanolic extract of the roots of Acanthopanax. The preparation of the

methanolic extract and the subsequent isolation of the glycoside fraction from it, together with the separation of the latter into acanthosides A, B, C, and D, have been described previously [1]. In such a separation, the daucosterol is eluted together with acanthoside B, and is crystallized directly from the fractions. The fractions containing the daucosterol were combined, and the daucosterol was separated off and recrystallized from a mixture of pyridine and methanol. The white crystals had mp 290°C, $[\alpha]_D^{20}$ −35° (c 6.19; in pyridine). Literature data: mp 295-297°C [7], 292-295°C [6]. The Liebermann-Burchardt reaction was positive.

Isolation of daucosterol from the essential oil of Acanthopanax. A solution of 100 g of the essential oil in 1.2 *l* of chloroform was extracted with 0.85 *l* of ethylene glycol. The ethylene glycol extract (1.5 *l*) was diluted with water to a volume of 10 *l* and the liquid, in the form of an emulsion, was extracted with chloroform (2 *l*). The chloroform extracts were evaporated. The resulting dark viscous mass was treated with isobutyl acetate and the precipitate which deposited was filtered off. Yield 200 mg (0.2% of the essential oil) of daucosterol.

Found, %: C 72.69, 72.49; H 10.27, 10.20; mol. wt. 525; 570 (isothermal distillation). Calculated for $C_{35}H_{60}O_6$, %: C 72.87; H 10.48; mol. wt. 576.83.

Daucosterol tetra-O-acetate. A mixture of pyridine and acetic anhydride (1:1) was boiled for 20 min. The reaction mixture was evaporated to dryness, and the residue was dissolved in boiling ethanol. On standing, white plate-like crystals with mp 156-158°C deposited; after recrystallization from a mixture of chloroform and methanol they had mp 162°C. Literature data: mp 167-169°C [7, 8].

Found, %: C 69.48, 69.20; H 8.56, 8.36. Calculated for $C_{43}H_{68}O_{10}$, %: C 69.42; H 9.08.

Hydrolysis of daucosterol. A mixture of 80 mg of daucosterol, 0.8 ml of concentrated H_2SO_4, and 20 ml of ethanol was boiled for 4 hr. The reaction mixture was evaporated to small bulk, diluted with 20 ml of water, and extracted with ether. After the elimination of the ether, the residue was crystallized from methanol. White needle-like crystals with mp 137-139°C were obtained. Literature data: mp 137-139°C [9]; 139-141°C [6, 7]. Chromatography in a thin fixed layer of silica gel in the benzene-ethyl acetate (8:2) and chloroform-ethyl acetate (1:1) systems with an authentic sample of β-sitosterol confirmed the identity of the substances.

Found, %: C 83.80, 84.01; H 12.05, 12.06. Calculated for $C_{29}H_{50}O$, %: C 84.07; H 12.07.

The IR spectra were taken by M. Yu. Nefedova in KBr on a UR-10 spectrophotometer, and the NMR spectra by A. K. Dzizenko on a JNM-C-60 spectrophotometer in $CDCl_3$. The analyses were performed by L. I. Glebko and Zh. I. Ul'kina.

Summary

Daucosterol, a glucoside of β-sitosterol, has been isolated from the roots of Acanthopanax sessiliflorum.

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