SPHAEROSIN AND SPHAEROSININ – NEW ISOFLAVANES FROM Sphaerophysa salsula

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In the roots of the salt globe-pea (Sphaerophysa salsula (Pall) D.C.) collected in the Sverdlovsk region of the Bukhara oblast in the flowering period, we have detected the presence of seven substances of phenolic nature. By chromatography we have isolated two of them, which we have called sphaerosin (I) and sphaerosinin (II). In addition to these we have isolated an unidentified coumarin with mp 61-62°C and β-sitosterol.

Sphaerosin is a colorless crystalline substance with the composition $C_{17}H_{18}O_5$, mp 151°C, M^+ 302, $[\alpha]^{20}_D$ +10.7° (c 0.7; acetone), readily soluble in caustic alkalis, ethanol, acetone, chloroform, benzene, and carbon tetrachloride, and insoluble in water. With ferric chloride, the substance gives a green coloration, but it does not react with carbonyl reagents or with m-dinitrobenzene ($CH_2{-}C{=}O$) group, nor does it give the cyanidin reaction, which shows that it is not a flavone, flavonol, or a chromone. With vanillin 2-hydrochloric acid, vanillin 2-sulfuric acid, and concentrated nitric acid it gives a red coloration, and it also gives the Goodhue [1] and Keller–Kiliani [2] reactions.

Its UV spectra has maxima at λ_{max} 282 and 289 nm (inflection) (log ε 3.81 and 3.70), showing the presence of an aromatic nucleus.

In the IR spectrum of (I) there are absorption bands at (cm^{-1}) 1620, 1590, 1500 (aromatic ring), 3300-3500 (hydroxy groups), 2840, 2870, 2885, and 2940, 2970, 2995 (methylene and methoxy groups), 810, 890 and 810, 840 cm^{-1} (1,2,4- and 1,2,3,4-substituted benzene rings, respectively). The presence of two hydroxy groups was confirmed by the preparation of a diacetate, $C_{21}H_{22}O_7$, M^+ 386 (Ib), and of a dimethyl ether, $C_{19}H_{22}O_5$, mp 73-74°C, M^+ 330 (Ia).

The mass spectrum of compound (I), with a molecular peak of maximum intensity (m/e 302) and a group of strong peaks in the region of moderate mass numbers, resembles the spectrum of the flavonols [3] or the flavans [4]. In the mass spectrum of (Ib) the successive elimination of two molecules of ketene leading to the formation of an ion with m/e 302, is observed. This corresponds to the presence in the molecule of (I) of two phenolic hydroxyls, as is confirmed by the signals of the PMR spectrum.

Thus, the aliphatic part of the molecule of (I) contains no OH groups, i.e., this compound can presumably be assigned to the flavans or isoflavans. According to Audier, these two groups of compounds decompose by similar pathways, the main one of which is connected with a retrodiene reaction of ring C (ions A and B, see Scheme on next page).

The splitting off of ring B with one of the carbon atoms of the dihydropyran ring led to the appearance of a pair of ions, G and H. The mass numbers of the fragments corresponding to the ions A, B, G, and H in the spectrum of (I) correspond to the presence in ring B of one hydroxy and two methoxy groups.

The deuteration of (I) with heavy water in the inlet system of the mass spectrometer led to the displacement of the ion A by two mass units. This means that in the formation of this ion the hydrogen of the hydroxyl migrates (in the case of the diacetate, (Ib), the transfer of hydrogen takes place after the ejection of ketene).

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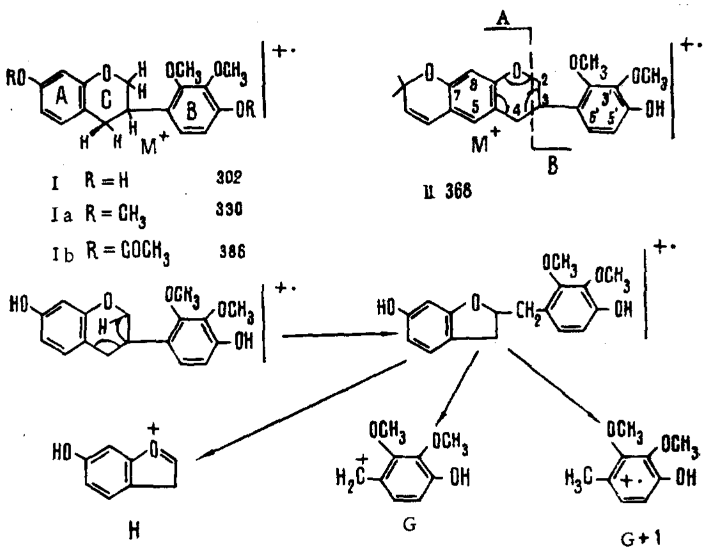

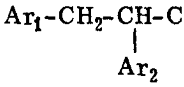

The presence in the NMR spectrum of (I) of the signals of the $Ar_1\text{-}CH_2\text{-}\underset{\displaystyle Ar_2}{\underset{|}{CH}}\text{-}CH_2\text{-}O\text{-}Ar$ grouping shows that the molecule of (I) is based on two benzene rings connected by a propyl chain. Consequently, (I) is an isoflavan. The alkaline fusion of (I) formed pyrogallol and β-resorcylic acid, which means that the oxygen substituents in one nucleus are present in the meta position to one another and in the second nucleus they are adjacent.

In order to determine the nature and position of the substituent, we used PMR spectroscopy. The spectrum of (I) shows the signals of five aromatic protons, including two pairs of ortho-interacting protons (doublets at 6.82 and 6.30 ppm, $J = 9$ Hz, 6.55 and 6.45 ppm, $J = 8.5$ Hz) and one present in the meta position (singlet at 6.25 ppm). It follows from a consideration of the PMR spectra of flavanones and flavanols [5] that the protons of an unsubstituted ring B give a signal in the 7.27 ppm region. The presence of donor substituents causes a diamagnetic shift of the protons located in the ortho position of about 0.5 and in the para position of about 0.3 ppm. Similar results were obtained by Clark-Lewis [6]. In the case of flavan derivatives, the presence in ring B of (I) of two methoxy groups and one hydroxy group in adjacent positions, i.e., at C'-2, C'-3, and C'-4 must cause the screening of the H'-5 and H'-6 protons, and the diamagnetic shift of their signals by approximately 0.8-0.9 and 0.3-0.4 ppm, respectively. On this basis, the doublets at 6.82 and 6.30 ppm can be assigned to H'-6 and H'-5. In the spectrum of the acetate (Ib), the signal corresponding to H'-5 is shifted downfield by 0.28 ppm, which is determined by the presence of an electron acceptor in the ortho position, i.e., at C'-4. This shows that the methoxy groups are located at C'-2 and C'-3, and the hydroxy group at C'-4.

The singlet at 6.28 ppm can be assigned only to H-8. The chemical shift of this signal is determined by the screening of the H-8 proton by the unshared pairs of electrons of the oxygen atoms present in the ortho position. On this basis, the doublet at 6.45 ppm must be assigned to the H-6 proton. In the spectrum of (Ib) the signals mentioned are shifted downfield by 0.33 and 0.35 ppm, respectively. Thus, the hydroxy group in ring A is located in position 7.

Furthermore, in the spectrum there are signals of five protons connected with the propyl link — a broadened two-proton doublet at 2.78 ppm, $J = 8$ Hz (H-4), a quartet at 4.20 ppm, $J_1 = 10$ Hz, $J_2 = 3.0$ Hz, and a triplet at 3.88 ppm, $J = 10$ Hz, which appears distinctly in the spectrum of the diacetate (H-2), and a multiplet in the 3.45-3.60 ppm region (H-3).

The spin—spin splitting constants given show the equivalence of the H-4 benzyl protons and the existence of vicinal coupling of them with H-3, and also the nonequivalence of the H-2a and H-2e protons, which is expressed both in their geminal and in their vicinal interaction. H-2a/H-3, $<\varphi \sim 180°$, $J = 10$ Hz, and H-2e/H-3, $<\varphi \sim 50°$, $J = 3.0$ Hz. The singlets at 3.82 and 3.77 ppm, of 3 H each, and at 5.68 and 5.86 ppm are due to the protons of the $Ar{-}OCH_3$ group and the phenolic hydroxyl (the latter disappears on deuterium exchange and in the spectrum of the diacetate).

The facts given above show that sphaerosin (I) is 4',7-dihydroxy-2',3'-dimethoxyisoflavan.

Sphaerosinin (II) is a colorless crystalline substance with the composition $C_{22}H_{24}O_5$, mp 97-98°C (from methanol), M^+ 368, $[\alpha]_D^{20}$ +37.5° (c 0.6; methanol), readily soluble in chloroform and ethanol, and insoluble in water. It gives all the reactions characteristic for (I). Its UV spectrum: λ_{max} 232, 282, 287 (shoulder), and 316 nm (log ε 4.22, 3.93, 3.77, and 3.42) differs from that of (I) by the presence of a long-wave maximum, which shows the presence in the molecule of an additional conjugated C = C bond.

In the IR spectrum of (II) there are absorption bands at 1610, 1580, and 1500 cm^{-1} (aromatic nucleus), and 1640 cm^{-1} (C = C bond) and 3400-3450 cm^{-1} (hydroxyl).

In the PMR spectrum of (II), basically the signals for (I) showing the presence of a substituted isoflavan ring are repeated: doublets at 6.75 and 6.29 ppm, J = 8 Hz, quartet at 4.25 ppm, J_1 = 10 Hz, J_2 = 3 Hz (H-2e), triplet at 3.90 ppm, J = 10 Hz (H-2a), doublet at 2.79 ppm, J = 8 Hz (H-4), and singlets at 3.83 and 3.79 ppm ($ArOCH_3$). In addition, there is a six-proton singlet at 1.37 ppm (gem-dimethyl group in a benzopyran ring), doublets at 6.60 and 5.48 ppm, J = 10.5 Hz (olefinic protons in a benzopyran ring). At the same time, the signal of one of the phenolic hydroxyls (5.86 ppm) is absent and in place of the doublets due to the H-5 and H-6 protons a two-proton singlet is observed at 6.60 ppm (H-5 and H-8). These facts show that a dimethylpyran grouping is condensed in position 6,7. The presence of ions with m/e 180 and 167, respectively, shows that ring B in (II) has the same structure as in (I). The disappearance of the ions with m/e 123 (A) and 135 (H) and the appearance of ions with m/e 189 and 201 shows that the alkenyl substituent is attached to ring A. The spectrum of the OD analog shows that in the molecule of (II) there is a hydroxyl only in ring B.

The facts given permit the conclusion that sphaerosinin (II) is 4'-hydroxy-2',3'-dimethoxy-2".2"-dimethylpyrano-5",6" : 6,7-isoflavan.

EXPERIMENTAL METHOD

The NMR spectra were taken on a JNM-4-H-100/100 MHz instrument (in deuterated chloroform and CCl_4); the chemical shifts are given in the δ scale from the signal of HMDS taken as 0; the UV spectra were taken on a Hitachi instrument, the IR spectra on a UR-20 instrument (KBr), and the mass spectra on an MKh-1303 spectrophotometer.

The substances were chromatographed by the descending method on type "S" ["medium"] paper impregnated with a 10% solution of formamide in methanol.

The mobile phase (A) was hexane–benzene–methanol (5 : 4 : 1), and the spots were revealed with diazotized sulfanilamide. The reaction products were chromatographed by the ascending method in system (B) – butan-1-ol – benzene–acetic acid–water (2 : 10 : 2 : 1).

Isolation of the Substance with mp 62°C. The air-dried comminuted raw material (4 kg) was extracted with methanol (3 × 15 liters). The extract was evaporated in vacuum to 1.5 liter, diluted with a twofold amount of water, and treated with chloroform (5 × 2 liters). The chloroform extracts were dried, and the solvent was distilled off. This gave 400 g of a colored oily substance which was dissolved in 150 ml of ethanol, and the solution was mixed with an equal volume of alumina, dired, and transferred to a column with the same adsorbent (1 : 20) (h 30, d 10 cm; activity grade II according to Brockmann). The substances were eluted with thiophene-free benzene, 500-ml fractions being collected. The separation of the substances was monitored by paper chromatography, the migration of the zones being observed in filtered UV light. The fractions 4-14 contained mainly a single substance. They were combined and crystallized from methanol. This gave a substance with mp 61-62°C, R_f 0.96 in system A.

Isolation of β-Sitosterol. On standing after the separation of the substance with mp 62°C, the filtrate deposited colorless crystals with mp 137-138°C giving with the Lieberman–Burchard reagent a pink coloration changing to blue-violet.

Isolation of Sphaerosinin. When the column was washed with ether, a mixture of substances was obtained which was reseparated on a column of KSK silica gel (h 90, d 14 cm) with elution by benzene (400-ml fractions). Fractions 9-20, containing substance (II) with R_f 0.94, were combined and evaporated to dryness, and the residue was crystallized from methanol. This gave colorless crystals of sphaerosinin with mp 97-98°C. Yield 15 g.

Isolation of Sphaerosin. Fractions 21-36 contained a substance with R_f 0.47 (in system A). When they were concentrated, colorless acicular crystals with mp 151-152°C (from benzene) were obtained. Yield 20 g.

Alkaline Decomposition of Sphaerosin. To a melt of 0.5 g of caustic potash with a few drops of water was added 0.05 g of the substance, and the mixture was heated for 3-4 min. Then the melt was cooled and was acidified with sulfuric acid, and the phenols were extracted with ether. The residue after the ether had been driven off was chromatographed on paper in system B in the presence of markers. When the spots were revealed, two were found with R_f 0.72 and 0.96, corresponding to β-resorcylic acid and pyrogallol.

Sphaerosin diacetate was obtained by heating the substance with a mixture of acetic anhydride and pyridine (1 : 1) for an hour. This gave an oily product with M^+ 386, R_f 0.95 in system B.

The Goodhue and Keller–Kiliani reactions were performed by known methods [1, 2]; a cherry-red coloration appeared.

Methylation of Sphaerosin. With stirring, over 30 min, 50 mg of sodium hydride was added to a solution of 50 mg of the substance in 4 ml of dimethyl sulfoxide, and this was followed by 5 ml of methyl iodide. The reaction mixture was stirred for 2 h and was poured into 40 ml of a saturated aqueous solution of sodium thiosulfate and extracted with chloroform (5 × 40 ml). The extracts were washed with water, the solvent was distilled off, and the residue was crystallized from 70% methanol. This gave colorless acicular crystals with mp 73-74°C.

SUMMARY

From the roots of Sphaerophysa salsula two new isoflavans have been obtained: sphaerosin, $C_{17}H_{18}O_5$, mp 151°C, $[\alpha]_D^{20}$ +10.7° (c 0.7; acetone) and sphaerosinin, $C_{22}H_{24}O_5$, mp 97-98°C, $[\alpha]_D^{20}$ +37.5° (c 0.6; methanol). On the basis of spectroscopic characteristics, the products of alkaline fusion, and derivatives, probable structures have been proposed for both substances.

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