COUMARINS OF THE ROOTS OF *PHLOJODICARPUS VILLOSUS* TURCZ.

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According to V. B. Kuvaev and K. F. Blinova [1], extracts of the roots of *Phlojodicarpus sibiricus* give reactions characteristic of coumarins, but the presence of the latter has not been confirmed preparatively.

From the roots of *Phlojodicarpus villosus* Turcz. collected by M. G. Pimenov in the Far East we have isolated a lactone A of composition $C_{21}H_{24}O_7$ which, from its chemical properties and UV spectrum, is a coumarin. Its IR spectrum has bands at 3105, 3065 (C—H bond of an aromatic nucleus), 1738 (C=O group of an α-pyrone nucleus), 1495 (aromatic nucleus), and 1238 cm^{-1} (C—O bond attached to an aromatic nucleus). A broad carbonyl band at 1738 cm^{-1} did not exclude the presence of one or more ester groupings.

Saponification of the lactone A with sulfuric acid gave a dihydroxycoumarin $C_{14}H_{14}O_5$ and substance B. The IR spectrum of the compound $C_{14}H_{14}O_5$ exhibited hydroxyl bands (3420-3360 cm^{-1}) in addition to the bands characteristic of coumarins, and this was supported by the determination of two mobile hydrogen atoms (Zerewitinoff method). This dihydroxylactone was identified as 2', 2'-dimethyl-3', 4'-dihydroxy-5', 6':8, 7-pyranocoumarin (a mixture of cis and trans khellalactones [7].

On the basis of the composition and constants, and also the production of a 2, 4-dinitrophenylhydrazone, substance B was identified as 3'-oxo-3', 4'-dihydroseselin [5].

Saponification of lactone A with caustic alkali gave a monohydroxycoumarin $C_{15}H_{16}O_5$, identified as 2', 2'-dimethyl-3'-hydroxy-4'-methoxy-5', 6':8, 7-pyranocoumarin (methylkhellactone), and also acetic and isovaleric acids.

Thus, a comparison of the physicochemical properties of lactone A and its saponification products with literature data has identified it as dihydrosamidin (2', 2'-dimethyl-3'-isovaleryl-4'-acetyl-5', 6': 8, 7-pyranocoumarin) which has been isolated from the fruit of *Ammi visnaga L.* [6]. It is known that samidin, dihydrosamidin, visnadin, and other similar acylcoumarins possess a pronounced spasmolytic action [6, 8].

Experimental

The paper chromatography (descending flow) was carried out in the following systems: A) cyclohexane—benzene—methanol (5:4:1), B) petroleum ether—(40-70°)—benzene—methanol (5:4:1). The paper was impregnated with a 10% solution of formamide in methanol, and the detection agent was diazotized sulfanilamide [3].

By means of a preliminary evaluation we established that *Phlojodicarpus villosus* contains 3.3% and *Phlojodicarpus sibiricus* 2.5% of coumarins consisting of a mixture of four substances. The quantitative determination of the combined coumarins in the plant raw material was carried out by a gravimetric method [2]. In the determination of coumarins containing ester groups, this method gives low results.

Isolation of dihydrosamidin. Four kilograms of the dried and comminuted roots was extracted three times with 24-*l* portions of ethanol. The resulting extract was evaporated in vacuum to 2 *l* and was diluted with 4 *l* of water. The precipitate which deposited was separated off and dissolved in 600 ml of ether, and the solution was washed with 0.5% caustic potash solution. Evaporation of the ethereal solution gave 151.6 g (3.77%) of a dark brown oily liquid. On standing in a refrigerator, 73 g of a crystalline substance with mp 109-111° (from methanol) deposited. This substance (0.4 g) was chromatographed on a column of alumina (Brockmann activity grade II) filled by the wet method. It was eluted with petroleum ether (40-70°). This gave a substance with mp 111-113°, $[\alpha]_D^{21}$ +64.5° (c 0.93; dioxane), R_f 0.92, readily soluble in ethyl and methyl alcohols, chloroform, and acetone, less readily in petroleum ether, and insoluble in water. UV spectrum: λ_{max} 219, 245, 257, 322 mμ (lg ε 3.9, 3.39, 3.35, 3.89). Found, %: C 64.68, 64.86; H 6.38, 6.31; C-CH_3 8.20, 8.62; mol. wt. 386 (Beckmann method). Calculated for $C_{21}H_{24}O_7$, %: C 64.95; H 6.23; C-CH_3 3.9.

Production of khellalactone. Four grams of dihydrosamidin was added to a solution of 4 ml of concentrated sulfuric acid in 72 ml of ethanol and the mixture was heated on a water bath under reflux for 3 hr. The reaction mixture was evaporated to 1/3 of its volume and was steam distilled. The residue from the distillation was evaporated to 40 ml, and the oily deposit which formed was separated. R_f of the oily deposit (system B) 0.73 with traces at 0.02, 0.12, and 0.9. Cooling the evaporated solution gave colorless crystals with mp 180-182° (from acetone—petroleum ether), R_f 0.02, 0.12. IR spectrum: 3420-3360, 3065, 1738, 1615, 1490, 1250 cm^{-1}. Found, %: C 63.39, 63.75; H 5.56, 5.68, H_{mobile} 0.9; mol. wt. 270, 257 (Rast). Calculated for $C_{14}H_{14}O_5$, %: C 64.11; H 5.38, H_{mobile} 0.77.

Production of 3'-oxo-3', 4'-dihydroseselin. Four grams of dihydrosamidin was added to a solution of 15 ml of concentrated sulfuric acid in 80 ml of ethanol. After heating on a water bath (3 hr), steam distillation, and concentration of the aqueous solution, the oily deposit was separated off and was crystallized from 96% ethanol in the presence of activated carbon. This gave colorless needles with mp 155-157°, R_f 0.73 (system B).

The 2, 4-dinitrophenylhydrazone of the ketone was obtained by a known method [7]. It was a dark yellow crystalline substance with mp 245° (decomp).

Preparation of methylkhellalactone. Four grams of dihydrosamidin was dissolved in 80 ml of a 10% solution of caustic potash in methanol and was heated on a water bath for 3.5 hr, after which the mixture was diluted with 160 ml of water and the methyl alcohol was distilled off in vacuum. The solution was acidified with sulfuric acid and was then exhaustively extracted with ether. The ethereal solution was treated with a saturated solution of sodium hydrogen carbonate (two 20-ml portions) and then with 30 ml of water (residue A). The ether was distilled off to give a crystalline residue with R_f 0.00, 0.05, 0.15, and 0.43 (system B). After repeated recrystallization from methanol, colorless crystals were formed (R_f 0.15), mp 159-160°, $[\alpha]_D^{22}$ +13.02° (c 0.96; ethanol). UV spectrum: 220, 247, 258, 327 mμ (log ε 4.09, 3.5, 3.45, 4.16). Found, %: C 65.21, 65.56; H 5.98, 6.00; $-OCH_3$ 10.71. 10.94, H_{mobile} 0.45, 0.52, mol. wt. 256 (Rast). Calculated for $C_{15}H_{16}O_5$, %: C 65.20; H 5.84; $-OCH_3$ 11.23, H_{mobile} 0.36.

Investigation of the residue A. The residue A was acidified with sulfuric acid and was steam-distilled. The distillate was extracted with ether and the ethereal solution was evaporated. This gave an oily liquid with a characteristic odor and an acid reaction. Part of the mixture of acids obtained was chromatographed in the form of the ammonium salts in the system consisting of butan-1-ol saturated with 1.5 N ammonia solution, using markers. The developing agent was a 0.2% solution of bromophenol blue in ethanol. Chromatography on paper showed two spots corresponding in R_f value to acetic acid (0.13) and isovaleric acid (0.53).

Acetic and isovaleric acids were isolated by the fractional distillation of the mixture of acids and were identified. The anilides of the acids under investigation (acetanilide and isovaleranilide) were obtained by a known method [4] as colorless scales with mp 112-114° and colorless crystals with mp 107-109°, respectively. Mixtures of these anilides with anilides of the authentic acids gave no depression of the melting points.

Type "M" Leningrad paper was used for the chromatography.

The UV spectra were taken on a SF-4 spectrophotometer (in 96% alcohol), and the IR spectra on a UR-10 spectrograph (mulls of the substances in liquid paraffin).

Summary

The roots of Phlojodicarpus villosus Turcz. have yielded 2% of an acylcoumarin identified as dihydrosamidin.

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