We have found no information on the isolation on (I) in the literature.

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METHOD FOR THE QUANTITATIVE DETERMINATION OF THE ISOFLAVONES AND POLYHYDROXYSTILBENES OF Maackia amurensis

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Isoflavones (retusin, genistein, and formononetin) and polyhydroxystilbenes (resveratrol, piceatannol) from the heartwood of Maackia amurensis Rupr. et Maxim., family Fabaceae, [1] exhibit a specific physiological activity [2]. In view of this, we have developed a method for the quantitative determination of these groups of compounds which consists in the chromatographic separation of accompanying substances on a silica gel column and the spectrophotometry of the eluate at two wavelengths close to the isobestic points of the isoflavones and the polyhydroxystilbenes. We used stilbene as the comparison substance. Coefficients eliminating the mutual influence of the groups of compounds concerned on the results of their determination have been introduced into the formulas for calculation. A comparison of the results obtained by HPLC [3] and by the proposed method has shown that the latter permits the determination of the actual amounts of the desired components on the wood.

Procedure. Air dry sawdust (≤ 1 mm; 5 g) is steeped in 50 ml of ethanol at room temperature in a dark place. The extract is filtered, and 5 ml of the filtrate is evaporated to dryness. The residue is dissolved in ethanol (1 ml) and the solution is added to 1.5 g of silica gel L (Czechoslovakia), 60-100 mesh, impregnated with a solution of acetic acid (100 g of silica gel + 30 ml of a 3:7 solution of acetic acid in water). After the complete volatilization of the solvent, the silica gel with the sample is transferred to a column (1.5 × 7 cm) of the same silica gel (2.5 g) equilibrated with benzene. The isoflavones and the polyhydroxystilbenes are eluted with benzene—acetone (3:1) at the rate of 1-1.5 ml/min, 30 ml of the eluate is collected in a 50 ml measuring flask, the volume is made up to the mark with acetone (solution A), and 1 ml of solution A is transferred to a 25 ml measuring flask. The solvent is driven off with a stream of air, and the residue is dissolved in ethanol up to the mark (solution B). The optical densities of solution B at the wavelengths 272 and 320 nm — D_1 and D_2, respectively — are measured in a cell (1 = 10 mm) during the first 10 min after its preparation, against ethanol. The optical densities of a solution of stilbene at the wavelengths 272 and 320 nm — D_3 and D_4, respectively — are measured under similar conditions. The total amount of isoflavones (X) and the total amount of polyhydroxystilbenes (Y) in percentages on the absolutely dry wood are calculated from the formulas:

$$X = \frac{m \cdot (D_1 - 0{,}283 \cdot D_2)\,1250}{m_1 \cdot D_3 \cdot 0{,}936\,(100 - W)}, \quad Y = \frac{m \cdot (D_2 - 0{,}226 \cdot D_1)\,1250}{m_1 \cdot D_4 \cdot 0{,}936 \cdot (100 - W)},$$

where m is the weight of the stilbene, g; m_1 is the weight of the wood, g; 0.266, 0.283, and 0.936 are coefficients taking into account the mutual influence of the isoflavones and the polyhydroxystilbenes on the results of their determination; and W is the loss in weight on drying, %.

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The stilbene solution was prepared by dissolving 0.05 g of stilbene (TU [Technical Specifications] 6-09-4489-77) in ethanol in a 200 ml measuring flask and diluting 1 ml of the resulting solution to 50 ml with ethanol in a measuring flask.

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INDIRECT $^{13}C-^{1}H$ SPIN-SPIN COUPLING CONSTANTS IN 3-CARENE

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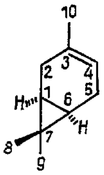
The potential value of the indirect constants $^{n}J_{CH}$ ($n \geq 2$) as powerful tools in the investigation of the compositions and spatial structures of organic compounds is generally known [1, 2], but the usual procedures for their measurement from proton-linked ^{13}C NMR spectra are ineffective in the case of terpenoid compounds in view of the complex multiplet structures of the signals. On the other hand, the determination of these constants is considerably more attractive from the point of view of the sensitivity of the NMR method than the $^{13}C-^{13}C$ SSCCs that we have obtained previously [3]. In the present paper, using (+)-3-carene as an example, the possibility is demonstrated of measuring the complete set of indirect $^{13}C-^{1}H$ SSCCs with the aid of two-dimensional J-resolved ^{13}C NMR with selective excitation of protons [4] (using the DANTE Procedure [5]). The values of the constants are given below [±0.1 Hz, apart from $^{1}J_{CH}$ (±1 Hz)], the other values of $^{n}J_{CH}$ being ≤0.3 Hz)



	H-1	H-2β	H-2α	H-4	H-5β	H-5α	H-6	Me-8	Me-9	Me-10
C-1	160	5,0	4,0	—	4,4	0,6	3,0	3,3	4,5	0,6
C-2	1,3	125	125	7,1	2,4	1,2	1,7	—	0,4	4,2
C-3	4,9	7,1	8,0	—	5,1	6,0	0,6	—	—	6,2
C-4	0,6	4,2	5,1	153	7,3	8,3	5,2	—	—	5,8
C-5	1,7	2,2	1,1	4,7	125	125	1,4	—	0,4	0,4
C-6	3,1	4,2	0,6	6,5	5,2	4,2	160	3,3	4,5	—
C-7	2,2	4,5	6,2	—	4,4	6,1	2,2	5,1	4,8	—
C-8	1,8	—	—	—	—	—	1,8	125	4,0	—
C-9	5,7	—	—	—	—	—	5,7	4,5	125	—
C-10	0,5	2,2	2,1	7,0	1,4	1,5	0,5	—	—	125

The signs of the $^{n}J_{CH}$ ($n = 2$ or ≥ 4) constants may be negative [1]. The qualitative analysis of the vicinal constants $^{3}J_{CH}$ showed that their values obeyed a Karplus-type dependence on dihedral angles [2], except for cases of the transmission of interaction through a path with a double bond (C=C-C-H). For a more detailed investigation of the dependence of the indirect constants $^{n}J_{CH}$ on structural and spatial factors a further expansion of the volume of experimental results on the constants is necessary.

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