FLAVONOIDS OF Thermopsis alterniflora

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UDC 547.972.918

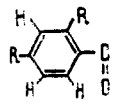
In the isolation of flavonoids from plants, use is made very frequently of a method based on the so-called "deresinification" of the raw material, i.e., its preliminary treatment with chloroform or gasoline. It has been considered that flavonoids are insoluble in these solvents, and therefore the "resin" fractions were not investigated. We have established that in plants of the family Leguminosae there is a considerable amount of lipophilic flavonoids that are readily soluble in benzene and chloroform, sparingly soluble in ethanol, and insoluble in water. The present work was devoted to a study of a flavonoid with mp 262°C [1] which we have isolated previously in the "deresinification" of the herbage of Thermopsis alterniflora Rgl. et Schmalh. [1, 2]. On paper chromatography and thin-layer chromatography in silica gel in various systems, it gave a single distinct spot, and on recrystallization from various solvents is retained a clear and unchanged melting point, which showed its individuality. At the same time, a comparison of its mass and NMR spectra showed their incompatibility with one another: The mass spectrum contained the peak of the molecular ion M^+ 300, while, judging from the number of protons, this flavonoid must contain not less than 32 carbon atoms and has a molecular weight of the order of 550-600. The absence of peaks with m/e greater than 300 excluded the possibility of a dimeric structure. On the basis of these results, compound (I) is an equimolar mixture or a molecular compound of two substances.

The NMR spectrum of (I) had a one-proton singlet at 8.2 ppm showing that one of the components belongs to the isoflavone group. It is known that on alkaline treatment under mild conditions isoflavones are cleaved with the formation of formic acid and the corresponding benzyl phenyl ketones. Since numerous attempts to separate (I) into its components were unsuccessful, we treated it with alkali, which led to the destruction of the isoflavone. Two substances were obtained: $C_{16}H_{12}O_6$ (II) with mp 325-327°C, UV spectrum λ_{max} 254, 271, and 353 nm (log ε 3.93, 3.90, and 4.03); and $C_{15}H_{14}O_5$ (III) with mp 138-139°C, UV spectrum 283 and 325 nm (log ε 4.25 and 4.07).

From its NMR and IR spectra, a mixed melting point with an authentic sample, and the formation of luteolin on demethylation, compound (II) was identified as chrysoeriol (4',5,7-trihydroxy-3'-methoxyflavone).

The second product of the destruction of (III) gave with vanillin–sulfuric acid and vanillin–hydrochloric acid a bright red coloration, and with ferric chloride a pale pink coloration. It possessed the properties of a ketone (2,4-dinitrophenylhydrazone with mp 236°C), and on alkaline fusion it formed β-resorcillic acid and resorcinol. When the UV spectrum was taken in the presence of sodium methoxide, it gave a bathochromic shift, but with boric acid and sodium acetate there was no such shift.

The NMR spectrum of (III) (in DMSO) showed the following signals: doublet at 7.75 ppm, J = 8.5 Hz; quartet at 6.3 ppm, $J_1 = 8.5$ Hz, $J_2 = 2.5$ Hz; and singlet at 6.22 ppm corresponding to two ortho- and one meta-interacting aromatic protons in the grouping



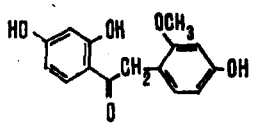
The considerable nonequivalence of the ortho protons is caused by the electron-accepting influence of the carbonyl group. In addition, the spectrum contained doublets at 7.8 ppm, J = 8.5 Hz, and 6.75 ppm, J = 8.5 Hz, and a singlet at 6.67 ppm also due to two ortho- and one meta-interacting protons of a second aromatic nucleus. Judging from the composition, the substituents in the aromatic nuclei are three hydroxy and methoxy groups.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 140-144, March-April, 1975. Original article submitted December 14, 1973.

The substance gave a bright brown coloration with Zimmerman's reagent ($CH_2-C=O$) grouping. The NMR spectrum taken in pyridine showed two singlets: at 4.09 ppm (2 H) and 3.50 ppm (3 H), which are due, respectively, to methylene protons at a benzyl carbon atom attached to a carbonyl group, and a methoxy group in an aromatic nucleus.

The mass spectrum of (III) showed the peak of the molecular ion M^+ 274 and fragments with m/e 258, 137, 121, and 109 formed as a result of the α- and β-cleavage of the ketone.

On the basis of the facts given, we propose the following structure for the ketone (III):



From the dried meal obtained after the extraction of the alkaloids with water, we isolated a flavonoid (IV) with the composition $C_{16}H_{12}O_5$, mp 275°C (from methanol), the nature of the UV spectrum of which (λ_{max} 251, 302 nm; log ε 4.43, 4.06) showed the presence of a 3',4',7- or a 2',4',7-trihydroxyisoflavone chomophore.

The IR spectrum of the substance showed absorption bands at (cm^{-1}) 1625 (isoflavone carbonyl), 1600, 1575, and 1520 (aromatic nucleus), and 3100-3200 (phenolic hydroxyl). On treatment with a 1% solution of caustic soda, it underwent cleavage with the formation of the ketone (III), and on alkaline fusion it yielded resorcinol and β-resorcylic acid.

The NMR spectrum of (IV) (in DMSO) showed a singlet at 8.41 ppm (H-2); a doublet at 8.26 ppm, J = 8.5 Hz; a doublet with secondary splitting at 7.1 ppm, J = 8.5 Hz; and a singlet at 7.18 ppm (H-5, H-6, and H-8 protons; 1 H each), doublets at 7.66 and 7.00 ppm, J = 8.5 Hz, and a singlet at 7.04 ppm (H-2, H-3, and H-5 protons).

In the NMR spectrum of (IV) taken in pyridine there was a three-proton singlet at 3.50 ppm (methoxyl in an aromatic nucleus).

On the basis of these facts, it may be concluded that (IV) is a monomethyl ether of 2',4'7-trihydroxyisoflavone.

When the spectrum of (IV) was taken in the presence of sodium methoxide a bathochromic shift of the long-wave maximum by 43 nm took place, while with sodium acetate there was a shift of 6 nm, which unambiguously shows the presence of the methoxyl in position 2. In the mass spectrum of (IV) there were peaks with m/e 136 and 108, corresponding to fragments of ring A without and with the splitting out of the carbonyl group, and also peaks with m/e 147 and 133 from a methoxylated ring B.

Thus, the isoflavone isolated, which we have called teralin, is new and has the structure of 4',7-dihydroxy-2'-methoxyisoflavone. The IR and UV spectra and melting point of a synthetic mixture of pure chrysoeriol and teralin were identical with that of the flavonoid having mp 262°C.

EXPERIMENTAL METHOD

The UV spectra were taken on a Hatachi instrument (in ethanol), the IR spectra of a UR-20 spectrophotometer (tablets with KBr), the NMR spectra on a JNM-4H-100/100 MHz instrument (in deuterated DMSO and pyridine), the chemical shifts being given in the δ scale from the signal of HMDS taken as 0; and the mass spectra on an MKh-1303 instrument. The purity of the substances and the course of the reactions was monitored by paper chromatography and thin-layer chromatography on Silufol plates in the following systems: 1) butan-1-ol–acetic acid–water (4:1:5); 2) butan-1-ol–benzene–acetic acid–water (2:10:2:1); 3) benzene–dioxane–acetic acid (90:25:4); and 4) chloroform–acetone (97:3), and the spots were revealed with a 5% ethanolic solution of aluminum chloride and with diazotized sulfanilamide. The elementary analyses corresponded to the calculated figures.

Isolation of the Flavonoid with mp 262°C

The dried and comminuted herbage (1 kg) was treated with a mixture of methanol, ethanol, and acetone (1:2:1) at room temperature (3 × 4 liters). The extracts were evaporated in vacuum to 0.5 liter, the residue was treated with 0.9 liter of distilled water and 0.5 liter of benzene, the mixture was shaken, and the benzene layer was separated off. The extract was concentrated to 0.5 liter and was treated with a 5% aqueous solution of sodium carbonate. The alkaline solution was acidified with sulfuric acid, and the flavonoid

was extracted with ether. After elimination of the solvent, a greenish-yellow crystalline substance was obtained which was soluble only in pyridine, dimethylformamide, and dioxane, apart from being sparingly soluble in acetone, and had mp 262°C (from methanol); yield 3 g (0.3%), Rf 0.84 (system 1).

Alkaline Treatment of the Flavonoid

Isolation of the Ketone (III). A solution of 0.3 g of the substance with mp 262°C in 30 ml of 1% aqueous KOH was heated in the boiling-water bath for 30 min. Then it was acidified with hydrochloric acid and the degradation products were extracted with diethyl ether. The extracts were evaporated to dryness and the residue was chromatographed on a column of KSK silica gel (d 5 cm, h 15 cm). The column was eluted with benzene, 50-ml fractions being collected. When the solvent was eliminated from fractions 2-10, a substance was obtained with mp 138-139°C (from methanol), M^+ 274, colorless acicular needles readily soluble in acetone and ethanol, and insoluble in water. R_f 0.74 and 0.65 in systems 3 and 4, respectively (TLC).

Isolation of Chrysoeriol. When the column was eluted with a mixture of benzene and methanol (2 : 1), a dark yellow substance was obtained which, after recrystallization from methanol, had mp 325-327°C, M^+ 300, R_f 0.42 on TLC in system 3.

Demethylation was performed by a known method [4].

Alkaline Decomposition of the Ketone (III). A melt of 0.5 g of caustic potash with a few drops of water was prepared in a porcelain crucible, and then 0.05 g of the ketone was added and the mixture was heated for 2-3 min. After cooling, the alkaline melt was acidified with hydrochloric acid, and the phenols were extracted with diethyl ether. By PC in system 2 in the presence of markers, the ethereal extract was found to contain β-resorcylic acid (R_f 0.70) and resorcinol (R_f 0.57).

Zimmerman's Reaction [3]. A solution of 2 mg of the substance in 2 ml of methanol–dioxane (1 : 1) was treated with 0.5 ml of a 10% aqueous solution of caustic soda and with a saturated solution of m-dinitrobenzene in the same solvent. A bright red coloration appeared.

Isolation of Teralin. The meal dried to the air-dry state (factory waste after the preparation of cytisine) (8 kg) was steeped in 30 liters of methanol for 24 h three times. This gave 85 liters of extract, which was concentrated in vacuum to a volume of two liters. On standing, a resinous precipitate deposited, which was separated off and was treated with gasoline (6 × 2 liters). The purified dark yellow precipitate (180 g) was recrystallized from methanol. This gave a dark yellow crystalline substance with mp 275°C, M^+ 284, R_f 0.84 in system 1 (PC).

Alkaline Decomposition of Teralin. The reaction was performed by a known method; β-Resorcylic acid and resorcinol were identified by the PC method in system 2 (R_f 0.70 and 0.57, respectively).

SUMMARY

From the lipophilic fraction of the substances from the herbage of Thermopsis alterniflora we have isolated a molecular compound of two flavonoids, one of which has been identified as chrysoeriol, while the second, with the composition $C_{16}H_{12}O_5$, mp 274-275°C, is a new isoflavone which we have called teralin. On the basis of spectral characteristics and the products of alkaline fusion, we have proposed for it the structure of 4',7-dihydroxy-2'-methoxyisoflavone.

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