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LUCIDIN PRIMEVEROSIDE FROM RUBIA IBERICA. II

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In a study of the chemical composition of the roots of Rubia iberica C. Koch (Iberian madder), we isolated a substance (I) with the composition $C_{26}H_{28}O_{14}$, mp 210–212° C, having properties characteristic for anthraglycosides. The substance (I) is readily soluble in water, gives a red coloration with alkalis, and has a UV spectrum characteristic for hydroxyanthraquinones [1] (Fig. 1). In the IR spectrum of (I) (Fig. 2a) there is strong absorption in the region of O—H stretching vibrations (maxima at 3560 and 3410 cm^{-1}), which also shows the presence of one or more sugar residues in the molecule. In the region of the stretching vibrations of C=O and C=C bonds, there are bands at 1679 cm^{-1} (free quinoid C=O group) and 1638 cm^{-1} (quinoid carbonyl group bound by an intramolecular hydrogen bond with a hydroxyl in the α-position), and also bands at 1610, 1596, and 1585 cm^{-1}, due to the vibrations of aromatic rings [2,3].

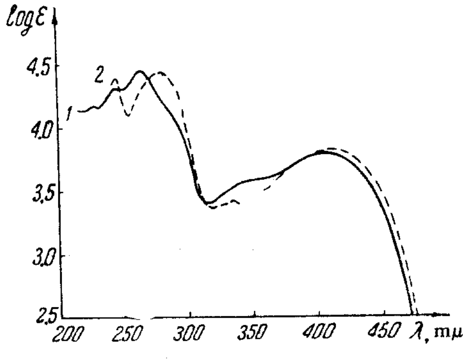


Fig. 1. UV spectra of lucidin primeveroside (1) and lucidin (2) in a mixture of dioxane and water (19 : 1).

The acid hydrolysis of (I) gave a genin (II) with the composition $C_{15}H_{10}O_5$ having mp above 330° C, and the hydrolysate was found to contain two sugars which were identified by their R_f values as glucose and xylose. The IR spectrum of (II) (Fig. 2b) had the band of a hydroxyl at 3435 cm^{-1} bound by an intermolecular hydrogen bond and a number of bands in the 3100–2600 cm^{-1} region probably due to a hydroxy group participating in the formation of an intramolecular hydrogen bond. The IR spectrum of (II), like that of (I), had bands at 1667 and 1625 cm^{-1} (free and bound quinoid carbonyls), and at 1597 cm^{-1} (vibrations of rings). The UV spectrum of (II) was similar to that of (I). In the literature a compound similar in all its properties to (II) is described under the name of lucidin [4]. A comparison of the IR spectrum of (II) with that of an authentic sample of lucidin kindly given to us by Prof. Thompson (Aberdeen University, Scotland) showed their identity.

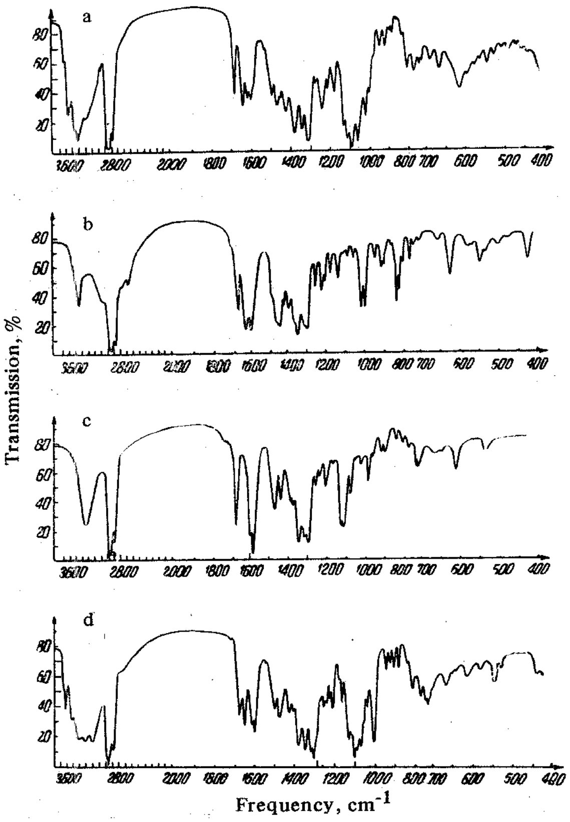
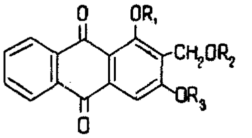


Fig. 2. IR spectra of lucidin primeveroside (I) (a), lucidin (II) (b), 3-hydroxy-1-methoxy-2-methoxymethylanthraquinone (III) (c), and the lucidin monoside (IV) (d).

Thus, substance (I) is a glycoside of lucidin, the sugar moiety of which consists of glucose and xylose. The position of attachment of the sugars was determined by the exhaustive methylation of (I) with dimethyl sulfate and subsequent hydrolysis of the methyl derivative. This gave a compound (III) with mol. wt. 298 (mass spectrometry) the IR spectrum of which (Fig. 2c) had bands at 3310 cm^{-1} (hydroxyl), 1678 cm^{-1} (nonbound quinoid carbonyl), and 1590 and 1575 cm^{-1} (vibrations of aromatic rings). The molecular weight and the IR spectrum of (III) show that this compound is lucidin in which two hydroxy groups have been methylated. Consequently, substance (I) is a bioside in which the position of attachment of the sugar moiety corresponds to the position of the hydroxy group in (III). Since (I) has an α-hydroxyl [on the basis of the IR spectra of (I) and (III)], the sugar moiety can be attached to the aglycone through the hydroxy group in position 3 or through the alcoholic hydroxyl of the hydroxymethyl group.

A study of the UV spectra of (III), 1-hydroxyanthraquinone, and 1,3-dihydroxyanthraquinone in ethanol and in a 0.05 N ethanolic solution of caustic soda permitted the nature of the hydroxy group in (III) to be determined. In the UV spectra of hydroxyanthraquinones, on passing from an ethanolic solution to a solution in ethanolic alkali there is a shift of the long-wave band into the red region due to the ionization of the alcoholic hydroxyls. For 1-hydroxyanthraquinone, this shift amounts to 93 mμ and for 1,3-dihydroxyanthraquinone to 78 mμ; in the case of (III), the shift in the long-wave band amounted to 90 mμ (Fig. 3), which could only be the result of the ionization of a phenolic hydroxyl. Consequently, the sugar residue in (I) is attached in position 3.

To determine the sequence of addition of the sugars to the aglycone molecule, we performed the stepwise hydrolysis of (I) to the monoside (IV) (IR spectrum, Fig. 2, d), and then to the aglycone. After the production of substance (IV), xylose was found chromatographically in the hydrolysate. On further hydrolysis of substance (IV), we obtained lucidin and glucose, which was identified chromatographically and from the melting point of the osazone. Enzymatic hydrolysis of the glycoside gave primeverose.



I $R_1 = R_2 = H$
R_3 = primeverose
II $R_1 = R_2 = R_3 = H$
III $R_1 = R_2 = CH_3$; $R_3 = H$
IV $R_1 = R_2 = H$
R_3 = glucose

The results presented permit the conclusion that the glycoside (I) is lucidin primeveroside.

Experimental

The IR spectra were obtained on a UR-10 spectrophotometer; the substances were studied in the form of mulls in paraffin oil. The UV spectra were taken on an SF-4A spectrophotometer. The mass spectrometric determination was performed on an MKh-1303 mass spectrometer.

Isolation of lucidin primeveroside. 2.5 kg of the comminuted roots of Iberian madder was extracted with two 10-liter portions of boiling water for 0.5 hr. The aqueous extracts, after filtration and cooling to room temperature, were treated successively with saturated solutions of lead acetate and basic lead acetate, and with 10% ammonia. The precipitate obtained on treating the filtrate with ammonia solution was suspended in 250 ml of saturated sodium sulfate solution and the suspension was acidified with dilute acetic acid. The precipitate of lead sulfate formed was filtered off, and the filtrate was transferred to a chromatographic column of polyamide. Elution was carried out first with water until the inorganic salts and acetic acid had been eliminated and then with 50% aqueous acetone. The aqueous acetonic eluate, colored intense yellow, was evaporated to dryness under vacuum at 60° C. The residue was dissolved in 200 ml of water, filtered, and then evaporated under vacuum to a volume of 50 ml.

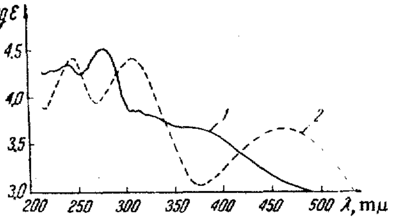


Fig. 3. UV spectra of 3-hydroxy-1 methoxy-2-methoxymethylanthraquinone (III) in ethanol (1) and in 0.05 N NaOH (2).

To the cooled evaporated solution was gradually added 30 ml of acetone. A crystalline precipitate in the form of yellow needles deposited. After two recrystallizations from aqueous acetone (1 : 4) the substance was dried in a vacuum pistol at 100° C for 6 hr. Yield 2.3 g, mp 210–212° C.

Found, %: C 53.62, 53.79; H 5.21, 5.35; mol. wt. 582 (spectrophotometry). Calculated for $C_{26}H_{28}O_{14} \cdot H_2O$, %: C 53.60; H 5.15; mol. wt. 572.3.

Enzymatic hydrolysis of lucidin primeveroside. One hundred milligrams of the glycoside was ground with 200 mg of an enzyme preparation from the roots and 5 ml of water. The mixture was treated with 2 ml of ethyl ether and left to stand at 35° C for 24 hr. Then 25 ml of water was added, and the mixture was filtered through a layer of alumina. The aqueous solution was evaporated to dryness under vacuum and the residue was recrystallized from ethanol. This gave 30 mg of a white crystalline substance the IR spectrum of which was identical with that of authentic primeverose, mp 193° C.

A mixture with authentic primeverose showed no depression of the melting point.

Hydrolysis of lucidin primeveroside. The monoside. A mixture of 196 mg of lucidin primveroside and 15% sulfuric acid was boiled for 7 min. The solution was cooled and the precipitate that deposited was filtered off. After recrystallization from dioxane, 137 mg of a substance with mp 216–217° C was obtained.

Found, %: C 58.27, 58.38; H 4.68, 4.80; mol. wt. 434 (spectrophotometry). Calculated for $C_{21}H_{20}O_{10}$, %: C 58.33; H 4.62; mol. wt. 432.

The added hydrolysate was passed through Dowex 1 × 4 and the aqueous solution was evaporated to a volume of 0.5 mm. Xylose was detected in the hydrolysate by paper chromatography in the systems 1-butanol–acetic acid–water (4 : 1 : 5) and 1-butanol–benzene–dioxane–water (5 : 1 : 3 : 3).

The molecular weights of the lucidin primeveroside (I) and the monoside (IV) were determined spectrophotometrically from the value of the molar absorption coefficient of lucidin at 411 mμ. The optical density of the solution of lucidin primeveroside was determined at 411 mμ and that of the monoside (IV) at 408 mμ. In all cases the measurements were carried out in a mixture of dioxane and water (19 : 1).

Lucidin. One hundred milligrams of the monoside was boiled with 30 ml of 15% acid for 6 hr. The solution was cooled and the precipitate was filtered off and recrystallized from dioxane. The resulting yellow needle-like crystals were sparingly soluble in acetone and dioxane. The substance, dried at 100° C for 6 hr, had mp > 330° C (decomp.).

Found, %: C 66.79, 66.50; H 3.95, 4.01; H_{labile} 1.18. Calculated for $C_{15}H_{10}O_5$, %: C 66.66; H 3.70; H_{labile} 1.11.

The acid hydrolysate was passed through Dowex 1 × 4 and evaporated to a volume of 0.5 ml. Glucose was detected in the 1-butanol—acetic acid—water (4 : 1 : 5) system. The melting point of the osazone of the sugar obtained was 221° C.

Methylation and hydrolysis of lucidin primeveroside. A suspension of 1 g of the glycoside in 80 ml of acetone was treated with 10 g of anhydrous potassium carbonate and 5 ml of dimethyl sulfate and the mixture was boiled for 12 hr. The precipitate was filtered off, and the acetonic solution was evaporated to small bulk and treated with 30 ml of 15% sulfuric acid. The solution was boiled for 3 hr and extracted with ether; the ether was evaporated off and the residue was dissolved in 5 ml of toluene and transferred to a chromatographic column of polyamide. Elution was performed with toluene. The eluate was evaporated to a volume of 1 ml and left for crystallization. This furnished 10 mg of pure needle-like crystals (the very small yield of methylated product is due to the poor solubility of the glycoside in acetone and the difficulty of methylating the hydroxyl in the α-position).

Found: mol. wt. 298 (mass spectrometry). Calculated for $C_{17}H_{14}O_5$, mol. wt. 298.

Conclusions

A new anthraglycoside with the composition $C_{26}H_{28}O_{14}$ and mp 210–212° C, lucidin primeveroside, has been isolated from the roots of Rubia iberica C. Koch.

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