FUROCOUMARINS OF CNIDIUM DUBIUM

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The three coumarins cnidin, cnidicin, and cnidilin have previously [1] been isolated from the fruit of Cnidium dubium (Schkuhr) Thell.

The present paper reports the results of a further study of the coumarin composition of the fruit of this plant. From the results of paper chromatography (figure, sample 1), the fruit of C. dubium contains not less than eight compounds of coumarinic nature, six of which have been isolated in the individual crystalline state (figures, samples 2–6, 11).

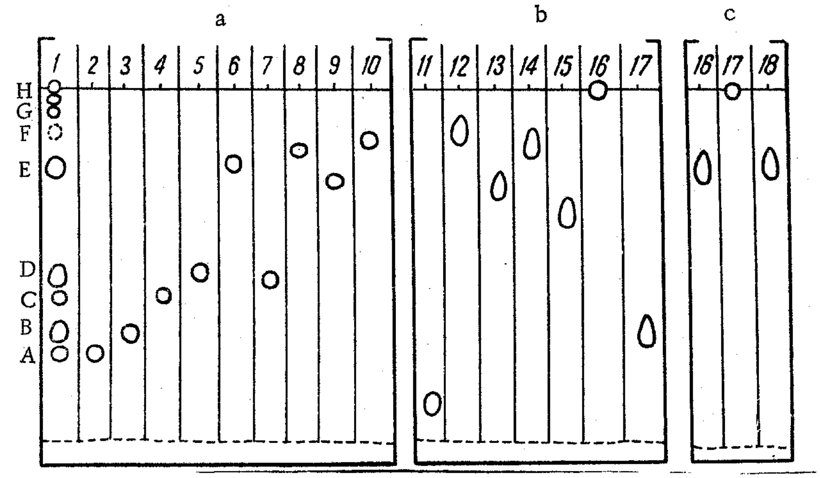

Fig. 1. Chromatogram of the furocoumarins of C. dubium: a) System: petroleum ether-formamide, 20°C, 1 hr 30 min. 1) Total coumarins of C. dubium; 2) cnidicin (I); 3) isoimperatorin; 4) cnidilin (II); 5) imperatorin (VIII); 6) oxypeucedanin; 7) phellopterin; 8) isopimpinellin (IV); 9) bergapten; 10) xanthotoxin. b) System: benzene-formamide, 20°C, 1 hr 30 min. 11) Substance H; 12) bergaptol; 13) xanthotoxol (VII); 14) 5-hydroxy-8-methoxypsoralen (V); 15) 5-methoxy-8-hydroxypsoralen; 16) 5, 8-dihydroxypsoralen (III); 17) the quinone (VI). c) System: 2% acetic acid, 20°C, 3 hr; 16) 5, 8-dihydroxypsoralen (III); 17) the quinone (VI); 18) the reduced quinone (III).

An investigation of the structure of these substances showed that substances B (cnidin) and D and E (figure, samples 1, 3, 5, and 6) were identical, respectively, with isoimperatorin, imperatorin, and oxypeucedanin obtained from certain species of the genera Cachrys [2] and Seseli [3]. Some of the properties of substance H were determined, while substance F, from its R_f value, the fluorescence of the spots in UV light before and after development, and the characteristic coloration with diazotized sulfanilic acid corresponds to prangenin (we obtained a sample of prangenin from G. A. Kuznetsova). Cnidicin (substance A) and cnidilin (substance C) are new compounds. (See Fig. 2.)

Cnidicin (I) readily decomposes under the action of acetic acid in the presence of concentrated sulfuric acid [4], forming substance (III) (figure, sample 16). The methylation [5] of (III) gave a substance (IV) which proved to be identical with isopimpinellin isolated from certain species of the genus Heracleum [6, 7]. The conversion of substance (III) into isopimpinellin shows that cnidicin (I) is a 5, 8-dihydroxy-substituted psoralen. To confirm this we carried out the directed synthesis of 5, 8-dihydroxypsoralen by the oxidation of xanthotoxol (VII) to the quinone (VI) and subsequent reduction of the latter with sodium borohydride. The resulting 5, 8-dihydroxypsoralen was identical with substance (III).

The relative ease of hydrolysis of cnidicin (I) and also the difference between the elemental compositions of (I) ($C_{21}H_{22}O_5$) and its hydrolysis product, substance (III) ($C_{11}H_6O_5$), give grounds for suggesting that cnidicin contains isopentenyl residues $[-CH_2-CH=C(CH_3)_2]$ in positions 5 and 8. The formation of acetone after the oxidation [8] of cnidicin shows the presence of a double bond at the secondary carbon atom of an isopentenyl residue. On the basis of the chemical reactions carried out, the structure of cnidicin (I) may be represented as 5, 8-di-(γ, γ-dimethylallyloxy) psoralen.

Cnidilin (II) decomposes on acid hydrolysis to give substance (V) (figure, sample 14) which is converted after methylation into isopimpinellin (IV) (figure, sample 8). The difference between the elemental compositions of cnidilin (II) ($C_{17}H_{16}O_5$), its hydrolysis product (V) ($C_{12}H_8O_5$), and 5, 8-dihydroxypsoralen (III) ($C_{11}H_6O_5$) shows that the substance under investigation (II) is a methoxyisopentenyloxypsoralen. The double bond of the isopentenyl residue was demonstrated as for substance (I). 8-(γ, γ-Dimethylallyloxy)-5-methoxypsoralen (phellopterin) is known in the literature [9, 10]. However, the physicochemical properties and the results of paper chromatography of samples of phellopterin, cnidilin, and their hydrolysis products (figure, samples 4, 7, 14, 15) show that the substances compared are not identical. On the basis of what has been said above, the structure of cnidilin (II) may be represented as 5-(γ, γ-dimethylallyloxy)-8-methoxypsoralen.

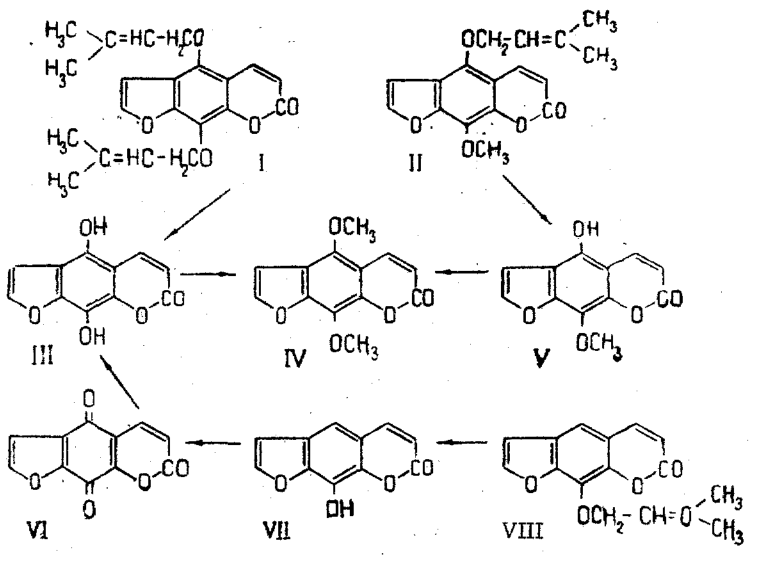

Fig. 2

Experimental

Acidic alumina was used to separate the mixture of coumarins [1, 5]. The melting points were determined in a Kofler apparatus. Goznak brand chromatographic paper was used for chromatography. Molecular weights were determined by Rast's method.

2. 5 kg of the comminuted fruit of C. dubium was extracted with 20 *l* of 96% ethonal. The extract was evaporated in vacuum to give a resinous residue which was then treated with 1 *l* of petroleum ether. The petroleum ether extract was evaporated to 100 ml and transferred to a column of alumina (60 × 6 cm). The column was first washed with petroleum ether. This gave 88. 6 g of a light yellow oil. Then it was eluted with petroleum ether-diethyl ether (2:1 and 1:1), with diethyl ether and, finally, with chloroform. A check on the separation of the coumarins in the column was carried out by means of filtered UV light, and the fractions collected (200 ml each) were analyzed by paper chromatography. Fractions having similar coumarin compositions were combined, the solvent was eliminated, and the residue was crystallized. Substances having only small amounts of impurities were purified by crystallization. Fractions containing mixtures of coumarins were reseparated on alumina columns. In this way six coumarins were isolated; they are designated on the chromatogram (figure, sample 1) by the symbols A, B, C, D, E, and H.

Cnidicin [A] (I). On the alumina column, the zone of this substance fluoresced brownish yellow in UV light. This zone was eluted from the column with petroleum ether-diethyl ether (2:1). The residue remaining after the evaporation of the eluate was crystallized from alcohol. This gave 359 mg of yellowish crystals melting at 77–78°C (figure, sample 2).

Found, %: C 70. 74, 71. 66; H 6. 45, 6. 32. Calculated for $C_{21}H_{22}O_5$, %: C 71. 16; H 6. 26.

Hydrolysis of cnidicin (I). 70 mg of the substance was dissolved in 1 ml of glacial acetic acid containing concentrated sulfuric acid (one drop of sulfuric acid was added to 10 ml of acetic acid), and the mixture was left for a day. The yellow crystals of substance (III) that deposited (31 mg) melted at 242–244°C (figure, sample 16). The solutions of the substance obtained darkened on standing.

Found, %: C 60. 48, 60. 71; H 2. 51, 2. 63. Calculated for $C_{11}H_6O_5$, %: C 60. 55; H 2. 27.

Methylation of 5, 8-dihydroxypsoralen (III) to isopimpinellin (IV). A solution of 80 mg of substance (III) in 1 ml of anhydrous acetone was treated with 1. 5 mg of anhydrous potassium carbonate and 0. 01 ml of freshly distilled dimethyl sulfate. The reaction mixture was boiled under reflux for 2 hr, after which, according to the results of paper chromatography, it contained a single substance (figure, sample 8). The precipitate of inorganic salts was filtered off and washed with acetone, the washings were filtered, and the residue obtained in evaporating the acetone was crystallized from ethanol in the cold. This gave 62 mg of yellowish needle-like crystals with mp 149–151°C.

Found, %: 63. 49; H 4. 03. $C_{13}H_{10}O_5$. Calculated, %: C 63. 49; H 4. 09.

A mixture of substance (IV) with an authentic sample of isopimpinellin gave no depression of the melting point.

Oxidation of xanthotoxol (VII) to the quinone (VI). With careful stirring, 2 ml of concentrated nitric acid was added dropwise to a solution of 250 mg of xanthotoxol (VII) obtained from imperatorin (VIII) [D], in 2. 5 ml of glacial acetic acid. After 30 min, the reaction mixture was poured into 10 ml of ice water. The orange crystals that deposited (189 mg) were filtered off and recrystallized from alcohol. Mp 260–262°C (figure, sample 17).

Found, %: C 61.18, 61. 07; H 1. 81, 1. 89. Calculated for $C_{21}H_{22}O_5$, %: C 61. 12; H 1. 86.

Reduction of the quinone (VI) to 5, 8-dihydroxypsoralen (III). A solution of 50 mg of substance (VI) in 5 ml of methanol was cooled to +3°C, 8 mg of sodium borohydride was added, and the mixture was left in the cold for 30 min. Then acetic acid was added to the reaction mixture to give it a weakly acid reaction, the methanol was distilled off, the residue was dissolved in 2 ml of ethyl acetate, and the solution was twice washed with water. The organic phase was evaporated and the residue was recrystallized from acetone and acidified with acetic acid. This gave 18 mg of crystals with mp 241–243°C. A mixture with an authentic sample of 5, 8-dihydroxypsoralen gave no depression of the melting point (figure, sample 18).

Oxidation of cnidicin (I). A solution of 300 mg of chromic anhydride in 2 ml of water was added to a solution of 100 mg of cnidicin in 6 ml of acetic acid, and the mixture was left for 3 days. After neutralization of the reaction mixture, 6 ml of distillate was distilled off, and to this was added 3 ml of freshly-distilled benzaldehyde and 3 ml of alkaline methanol. After a day, the crystals that had deposited were filtered off (mp 110°C). A mixture with an authentic sample of the substance obtained by condensing acetone with benzaldehyde gave no depression of the melting point.

Isoimperatorin (= cnidin, = substance B). The zone of substance B on the alumina column fluoresced bluish yellow in UV light, and it was eluted with petroleum ether-diethyl ether (2:1). After the eluate had been distilled, the sample was crystallized from alcohol to give 1. 86 g of colorless crystals with mp 108–109°C (figure, sample 3).

Found, %, C 70. 87, 70. 93; H 5. 18, 5. 29. Calculated for $C_{16}H_{14}O_4$, %: C 71. 09, H 5. 22.

After the acid hydrolysis of substance B (cf. the hydrolysis of cnidicin), bergaptol of composition $C_{11}H_6O_4$ with mp 278–281°C was obtained (figure, sample 12). Methylation of the latter with dimethyl sulfate led to the formation of bergapten (figure, sample 9). A mixture of substance B with an authentic sample of isoimperatorin gave no depression of the melting point.

Cnidilin [C] (II). A mixture of substances C and D was eluted by petroleum ether-diethyl ether (1:1) and was reseparated on a column of alumina (30 × 3 cm). The zone of substance C fluoresced brownish yellow in UV light. The eluate deposited 713 mg of yellowish acicular crystals with mp 117–118°C (from alcohol) (figure, sample 4).

Found, %: C 67. 84, 67. 89; H 5. 35, 5. 41. Calculated for $C_{17}H_{16}O_5$, %: C 67. 99; H 5. 37.

Hydrolysis of cnidilin (II). 100 mg of the substance was hydrolyzed similarly to cnidicin (I). This gave 52 mg of yellowish crystals of substance (V). At 190°C, crystals sublimed which melted at 282–283°C (figure, sample 14).

Found, %: C 61. 99, 62. 12; H 3. 55, 3. 50. Calculated for $C_{12}H_8O_5$, %: C 62. 67; H 3. 47.

The 8-hydroxy-5-methoxypsoralen (figure, sample 13) with mp 222–223°C obtained after the hydrolysis of phellopterin (figure, sample 7) (we obtained the sample of phellopterin from Yu. A. Dranitsina) differed from the product of the hydrolysis of cnidilin.

Methylation of 5-hydroxy-8-methoxypsoralen (V) to isopimpinellin (IV). The methylation was carried out as described for 5, 8-dihydroxypsoralen (III). The crystals obtained melted at 149–151°C. From its R_f value (figure, sample 8) and a mixed melting point, the methylation product, $C_{13}H_{10}O_5$ (IV), was identical with isopimpinellin.

Oxidation of cnidilin (II). This process was carried out as for substance (I). The acetone formed was distilled off and condensed with benzaldehyde. The crystals melted at 110°C.

Imperatorin [D] (VIII). When the mixture of the two substances (C and D) was reseparated, substance C was eluted first and then diethyl ether eluted substance D, which fluoresced bluish yellow on the column of alumina in UV light. After crystallization from alcohol, 4. 12 g of crystals with mp 102°C was obtained (figure, sample 5).

Found, %: C 71.15, 70.95; H 5.12, 5.31. Calculated for $C_{16}H_{14}O_4$, %: C 71.09; H 5.22.

The acid hydroloysis of substance D gave xanthotoxol $C_{11}H_6O_4$ with mp 249–251°C (figure, sample 13), and methylation of the latter gave xanthotoxin (figure, sample 10). A mixture of substance D and imperatorin gave no depression of the melting point.

Oxypeucedanin (E). The substance was eluted from the column with diethyl ether after substances C and D. Crystallization from alcohol and then from acetone gave 0.91 g of crystals with mp 142–143°C (figure, sample 6).

Found, %: C 67.25, 67.18; H 5.01, 4.88. Calculated for $C_{16}H_{14}O_5$, %: C 67.12; H 4.93.

The acid hydrolysis of substance E yielded bergaptol of composition $C_{11}H_6O_4$ with mp 278–280°C (figure, sample 12), the methylation of which gave bergapten (figure, sample 9).

Substance H. After the elution of the column with chloroform, 0.2 g of a substance fluorescing bluish yellow in UV light was obtained. The substance was crystallized from alcohol and then from acetone, mp 145–147°C (figure, sample 11). With diazotized sulfanilic acid, it gave a red coloration.

Summary

1. Paper chromatography has shown that the fruit of C. dubium contains not less than eight substances of coumarinic nature; six of these (A, B, C, D, E, and H) have been isolated in the crystalline state.

2. Substances B, D, and E are identical with isoimperatorin, imperatorin, and oxypeucedanin, respectively. Some properties of substance H have been determined. Prangenin [F] has been detected in the fruit of C. dubium by paper chromatography.

3. The structure of two new compounds has been established: substance A (cnidicin) (I) is 5, 8-di-(γ, γ-dimethylallyloxy) psoralen, and substance C (cnidilin) (II) is 5-(γ, γ-dimethylallyloxy)-8-methoxypsoralen.

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