ELUCIDATION OF THE COMPOSITION OF PASTINACIN AND ISOLATION OF SPHONDIN

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The fruit of the cultivated parsnip contains a considerable amount of furocoumarin compounds. We have isolated some of them previously [1-4]. Two furocoumarin preparations of the fruit of the parsnip have found practical employment as medicinal substances: beroksan - a mixture of bergaptene and xanthotoxin possessing photosensitizing properties [5, 6], and pastinacin, which is a fairly active spasmolytic [7].

As a result of chemical investigation, the majority of the furocoumarin compounds of parsnip have been identified with furocoumarins known from other plants [2, 3] and only two - pastinacin and substance 7 - remain completely uncharacterized. It has been established only that pastinacin has the composition $C_{12}H_8O_4$, contains one methoxy group, forms furan-2, 3-dicarboxylic acid on oxidation with hydrogen peroxide, and differs from known monomethoxyfurocoumarins by its low melting point and its high spasmolytic activity.

Continuing investigations on the composition of pastinacin, we have compared pastinacin with authentic samples of monomethoxy derivatives of psoralen and angelicin with respect to the physicochemical constants and the chromatographic behavior of individual standard samples of the furocoumarins and mixtures of them. Simultaneously, several batches of commercially produced pastinacin marketed by the experimental factory of KhNIKhFI [Khar'kov Chemical and Pharmaceutical Research Institute] were analyzed (Table 1).

The data given in the Table indicate that the standard and commercial samples of pastinacin contain, in addition to the main component with R_X 1.58, a small amount of another substance or a mixture of substances with R_X 1.00.

Judging from the chromatographic behavior of known furocoumarins and synthetic mixtures of them, it may be assumed that the main component of pastinacin is bergaptene. The second component is possibly a mixture of sphondin and xanthotoxin, since their R_X values practically coincide.

In view of the fact that repeated crystallization of pastinacin does not lead to the separation of this natural mixture of furocoumarins, attempts were undertaken repeatedly to separate it chromatographically on acidic and neutral aluminas. The investigation showed that no separation of the furocoumarins contained in pastinacin takes place on neutral alumina, even though a large excess of adsorbent was used (1 : 1600).

Acidic alumina, prepared by a method described previously [8], permits the separation of pastinacin into three components to some extent (Table 2), two of these having similar chromatographic mobilities but being distinguished by fluorescence in ultraviolet after development of the chromatograms with a solution of an alkali. One of the two substances is similar to xanthotoxin, which has been previously isolated from the fruit of the parsnip, and the other is similar to sphondin. The latter furocoumarin has not previously been isolated from parsnip. However, the main component of pastinacin is a furocoumarin similar to bergaptene.

The furocoumarins isolated were identified with authentic samples of bergaptene, sphondin, and xanthotoxin. The preparative separation of these compounds showed that their ratio was 6.5 : 2.5 : 1.0.

Experimental

Preparation of the adsorbents. Neutral alumina. Alumina for chromatography was treated for 1 hour in a boiling water bath with 1% HCl solution, and was then washed with water to neutrality and, after drying, activated for 1 hour at 500°.

Acidic alumina. Alumina for chromatography was treated for 1 hour with a 1% HCl solution in boiling water bath, filtered, dried, and activated for 1 hour at 500°.

Separation of pastinacin in neutral and acidic aluminas. Three experiments were carried out on neutral alumina and three on acidic alumina.

A solution of 5.0 g of pastinacin in 200 ml of dichloroethane was diluted with 800 ml of petroleum ether and transferred to a column containing 8 kg of acidic (or neutral) alumina. Separation was carried out with a mixture of dichloroethane and petroleum ether in a ratio of 1 : 4 (by volume). The separation of 5.0 g of pastinacin consumed approximately 35 liters of the mixture of solvents. The separation was considered to be complete when the furocoumarin compounds were distributed over 2/3 of the column of the alumina.

TABLE 1

Physicochemical properties of monomethoxy derivatives of psoralen and angelicin.

Properties	Bergaptene (I)	Xantho-toxin (II)	Sphondin [9] (III)	Isoberg-aptene (IV)	Pastinacin		Mixture of (I), (II), (III), and (IV)
					Standard sample	Commer-cial product	
Mp, °C	189-191	145-147	189-193	218-222	132-140	124-138	116-132
Mol. wt.	216	216	216	216	216	216	216
Elementary composition, %							
C	66.71	67.10	66.70	66.98	66.68	66.62	66.88
H	3.70	3.92	3.73	3.85	3.75	3.78	3.82
Formula	$C_{12}H_8O_4$	$C_{12}H_8O_4$	$C_{12}H_8O_4$	$C_{12}H_8O_4$	$C_{12}H_8O_4$		$C_{12}H_8O_4$
OCH_3, %	13.2	12.9	12.7	12.6	12.7	12.8	12.9
Formation of furan-2, 3-dicarboxylic acid on oxidation with H_2O_2	+	+	+	+	+	+	+
Reaction with diazotized sulfanilic acid	+	+	+	+	+	+	+
R relative to xanthotoxin	1.58	1.00	0.99	2.03	1.00 Weak 1.58 (Main)	1.00 Weak 1.58 (Main)	1.00 1.58 2.03
Fluorescence of the spots in UV before development	Light yellow	Light yellow	Light blue	Light yellow	1-First spot light greenish 2-Second spot light yellow		1-First spot light greenish 2-Second spot light yellow 3-Third spot light yellow
After development with 10% KOH solution	Light blue	Orange-brown	Bright green-yellow	Light blue	1-Yellow-brown 2-Light blue		1-Yellow-brown 2-Light blue 3-Light blue
Spasmolytic activity*	1:250,000	1:250,000			1:1,000,000		—

*The biological activity was determined in the pharmacological laboratory by P. I. Bezruk.

After the separation, the alumina column was cut mechanically into 20 equal parts, each of which was separately treated three times with 0.5-liter portions of chloroform. The chloroform extracts were evaporated to dryness. The dry residues were weighed and analyzed by paper chromatography in the petroleum ether-formamide system. The average results of the experiments on neutral and acidic aluminas are given in Table 2.

Xanthotoxin. Fractions 4-6 obtained from the column of acidic alumina were combined and were dissolved with heating in the minimum amount of ethanol. After cooling, crystals of xanthotoxin with mp 144-146° deposited. Yield 0.5 g from 15 g of pastinacin. The rechromatography of fractions 7-8 on acidic alumina gave an additional amount of 0.7 g of xanthotoxin. M 216.

Found %: C 67.11; H 3.92; OCH_3 12.9. Calculated % for $C_{12}H_8O_4$: C 66.71; H 3.70; OCH_3 12.60.

The furocoumarin isolated gave no depression of the melting point with an authentic sample of xanthotoxin.

Sphondin. Fractions 9-11 obtained from the acidic alumina column were combined and were dissolved with heating in the minimum amount of ethanol. On standing, crystals deposited from the solution in the form of needles with mp 189-193°. Yield 2.4 g from 15 g of pastinacin. The rechromatography of fractions 12-13 gave an additional 0.6 g of furocoumarin.

TABLE 2

Chromatographic separation of pastinacin on neutral and acidic aluminas

Fraction number	Neutral alumina					Acidic alumina				
		Paper-chromatographic analysis					Paper-chromatographic analysis			
	Weight of the residue, g	Spot with R_X 1.58 (I)	Spot with R_X 1.00 (II)	Fluorescence before (I) and after (II) development		Weight of the residue, g	Spot with R_X 1.58(I)	Spot with R_X 1.00(II)	Fluorescence before (I) and after (II) development	
				I	II				I	II
1	-					-				
2	-					-				
3	Traces					Traces				
4	"	+	+	Light blue	Yellow-brown	"	-	+	-	Orange-brown
5	"	+	+	"	"	"	-	+	-	"
6	0.27	+	+	"	"	0.18	-	+	-	"
7	0.34	+	+	"	"	0.24	-	+	-	Yellow-brown
8	0.60	+	+	"	"	0.32	-	+	-	"
9	0.53	+	+	"	"	0.37	-	+	-	Bright yellow-green
10	0.57	+	+	"	"	0.30	Traces	+	Light blue	"
11	0.56	+	+	"	"	0.27	+	+	"	"
12	0.59	+	+	"	"	0.33	+	+	"	"
13	0.48	+	+	"	"	0.65	+	Traces	"	-
14	0.36	+	+	"	"	0.62	+	-	"	-
15	0.27	+	+	"	"	0.66	+	-	"	-
16	0.24	+	+	"	"	0.45	+	-	"	-
17	Traces	+	+	"	"	0.16	+	-	"	-
18	"	+	+	"	"	Traces	+	-	"	-
19	-	-	-	-	-	"	+	-	"	-
20	-	-	-	-	-	-	-	-	-	-

The oxidation of 0.5 g of the furocoumarin with 3% H_2O_2 [2] gave 0.23 furan-2,3-dicarboxylic acid with mp 219-221°.

Found %: C 46.07; H 2.65. Calculated % for $C_6H_4O_5$: C 46.15; H 58.

The substance has R_X 0.99 and fluoresces in UV light, properties agreeing completely with those of an authentic sample of sphondin.

Found %: C 66.67; H 3.85; OCH_3 12.70. Calculated %: C 66.71; H 3.70; OCH_3 12.60.

Bergaptene. Fractions 13-18 obtained from the acidic alumina column were combined and were dissolved in the minimum amount of dichloroethane. When the solution was diluted with petroleum ether, needle-shaped crystals with mp 189-191° slowly deposited.

Found %: C 66.58; H 3.67; OCH_3 13.12. Calculated % for $C_{12}H_8O_4$: C 66.67; H 3.70; OCH_3 12.60.

In all its properties, the furocoumarin isolated is identical with bergaptene. 15 g of pastinacin gave 7.12 g of bergaptene.

Summary

A preparation of pastinacin has been separated by preparative chromatography on acidic alumina into three isomeric monomethylfurocoumarin components, bergaptene, sphondin, and xanthotoxin. Their ratio in the preparation was 6.5 : 2.5 : 1.0.

The angelicin derivative sphondin has been found in the fruit of the parsnip for the first time.

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