SYNTHESIS OF ANTITUMORAL AGENTS FROM PODOPHYLLOTOXIN

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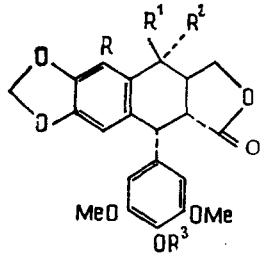
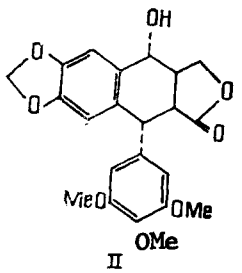
Syntheses based on podophyllotoxin have led to the inclusion among antitumoral agents valuable in practice of the preparations etoposide(I) and teniposide(II). These compounds are, respectively, the 4,6-ethylidene and 4,6-thenylidene derivatives of 4'-dimethylepipodophyllotoxin β-D-glucopyranoside. A review has been made of methods of synthesizing (I) and (II) and for their experimental investigation. Some conclusions are given from the results of clinical trials. Although (I) and (II) have been accepted for practical use, their clinical activity is limited. The principles of the experimental selection of antitumoral agents are considered particularly. Considerations are expressed concerning the possibilities of improving the results of the search for antitumoral agents of plant origin.

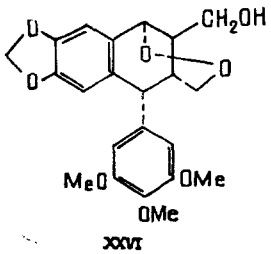
The chemotherapy of malignant tumors has been developed for more than 40 years. A definite success has been achieved in this area [1], particularly in the treatment of tumoral diseases of the hemopoietic and lymphatic tissues [2]. There have also been advances in the treatment of solid tumors. Antitumoral agents of plant origin have also acquired some recognition — the Catharanthus (Vinca) alkaloids vinblastine and vincristine, and also the autumn crocus alkaloid colchamine [3, p. 464]. In recent years, two preparations, etoposide and teniposide obtained by the chemical transformation of podophyllotoxin (I), the main agent of podophyllum, have been introduced into medical practice [1, p. 141].



	R	R^1	R^2	R^3
I	H	H	OH	Me
III	OMe	H	H	H
IV	OH	H	H	Me
V	H	H	OH	H
VI	H	H	H	Me
VII	H	OH	H	Me
IX	H	H	$OC_6H_{11}O_5$	Me
X	H	H	$OC_6H_7OAc_4$	Me
XI	H	H	$OC_6H_{11}O_5$	Me
XXV	H	H CN	CN H	or Me
For XII—XVI	H	H		Me



II



XXVI

XII	$R^4=C_6H_5$
XIII	$R^4=C_4H_3O$
XIV	$R^4=MeOC_6H_4$
XV	$R^4=C_4H_3$
XVI	$R^4=HOC_6H_4$

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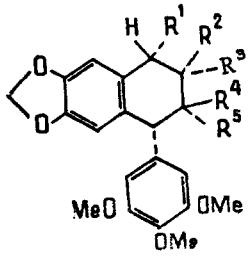


	R^1	R^2	R^3	R^4	R^5
VIII	H	CH_2OH	H	H	$COOC_6H_{11}O_5$
XVII	OH	CH_2OH	H	$CONHNH_2$	H
XVIII	OH	CH_2OH	H	H	$CONHNH_2$
XIX	H	H	CH_2OH	H	$CONHNH_2$
XXIV	OH	CH_2	H	H	$CONHNHC_2H_5$

Scheme 1

In the present review we consider investigations devoted to the synthesis of antitumoral compounds from podophyllotoxin and give some information on their properties and also the results of clinical trials. An attempt has been made to consider what has been achieved and, in connection with this, the prospects for finding antitumoral substances of plant origin. Such a consideration may be useful since the desirability of introducing new antitumoral agents into the practice is generally recognized [1, p. 264]. Plant substances could also, apparently, have some value in this respect.

Podophyllin is a water-insoluble resin obtained from the rhizomes of plants of the genus *Podophyllym* L., family Berberidaceae. Its use for medical purposes has long been known [4]. Podophyllin is still used at the present time [3, p. 468]. The inhibition of cell growth by podophyllin was discovered in the 1940's [5, 6]. Podophyllotoxin was first isolated during the last century [7]. The conversion of podophyllotoxin under the action of alkali into an isomer was established, and this isomer was acquired the name of picropodophyllin [7]. The isolation of other compounds of similar structure — substances accompanying podophyllin — and the structure of podophyllotoxin and the associated compounds, and also their biological activity, has been described in reviews and original papers [5, 6, 8-11]. Let us give some information. Accompanying substances related to podophyllotoxin have been isolated from podophyllin — α- and β-peltatins (III and IV), 4'-demethylpodophyllotoxin (V), and dioxypodophyllotoxin (VI) (scheme 1). In addition, glucosides obtained from the water-soluble fractions of the initial plant materials have been described. One of the main chemical properties is the above-mentioned conversion of podophyllotoxin (I) into picropodophyllin (II) [5, 7]. Epimerization at C-1 is of considerable value; the production of derivatives of epipodophyllotoxin (VII) will be reported below.

Podophyllotoxin is sparingly soluble in water. This leads to some difficulties in the investigation of its biological action. Consequently, derivatives with ionic groups have been synthesized from (I), (III), and (IV) [5, 12]. Among cationic derivatives are pyridinioacetylpodophyllotoxin chloride (I, R^2 = $OCOCH_2NH_3C_5H_5^+Cl^-$), nicotinolypodophyllotoxin methiodide (I, R^2 = $OCOC_5H_4N\cdot CH_3I$), and the methosulfate methylate corresponding to this methiodide. Anionic derivatives are represented by, for example, the monoesters of succinic, glutaric, and phthalic acids (I, respectively, R^2 = $OCOCH_2CH_2COOH$, $OCOCH_2CH_2CH_2COOH$ and $OCOC_6H_4COOH$). All these substances are inferior to podophyllotoxin in the chemotherapeutic respect. Appropriate information is given in a review [5].

Another pathway for obtaining water-soluble derivatives of this series is a search for water-soluble active principles of plants of the genus *Podophyllum* L. For this purpose, the rhozomes of *Podophyllum emodi* Wall., growing in India, have been investigated for the presence of podophyllotoxin glycosides. It is assumed that not only a water-soluble but also a less toxic substance would be obtained. It was found from several aqueous fractions that no longer contained podophyllotoxin after the action of emulsion that it was possible to isolate this compound again. This result indicated its binding by a β-glycosidic bond, and this impelled Stoll et al. to isolate the glucoside [13]. Podophyllotoxin β-D-glucoside (IX) is capable of inhibiting mitosis and its toxicity is somewhat lower than that of the aglycon [4, 13]. The amount of glucoside in the raw material is 0.5-1.0%, its solubility in water is 2%, and the aqueous solutions are stable [13]. The solubility in water of podophyllotoxin is 0.01% [14]. A low toxicity of the glucoside on its peroral administration has been shown, which is due to its retention in the intestine, as had been established with the aid of the ^{14}C-labeled glucoside [4]. A label has been introduced into the methoxy group in position 4' [15]. The same paper gives information on the preparation of the labeled diazomethane. In addition to an in vivo evaluation of the antitumoral activity and toxicity of (IX), its favorable local action has been shown. One case of the heating of a carcinoma in a dog has been reported [4].

The isolation of α- and β-peltatin glucosides (III and IV) and also of 4'-dimethylpodophyllotoxin (V) has been reported. The amounts of these glucosides were, respectively, 0.05-0.1, 0.05-1.5, and 0.5% of the dry weight of the raw material (rhizomes) [16]. The glucosides mentioned are less active in the inhibition of mitosis than their aglycons [17]. It has been observed that the difference in the activities of the glucosides and aglycons of this series is not qualitative but quantitative [16]. In addition to the glucosides mentioned, the presence of another five similar glucosides has been shown chromatographically. Of these, three have been isolated, and for one of them the structure of the β-D-glucopyranosyl ester of deoxypodophyllic acid (VIII) has been shown [17] (Table 1). In further study of the glucosides of *Podophyllum emodi* Wall. and *Podophyllum peltatum* L., as a result of repeated chromatography, 4'-glucosyl-4'-dimethyldioxypodophyllotoxin, with a weak in vitro activity, was isolated [18].

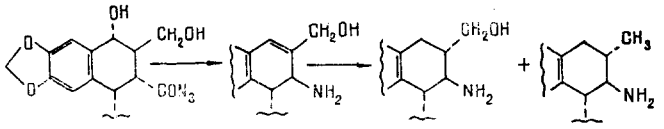
To protect the glucoside (IX) from the actions of enzymes, acyl groups have been introduced into the hydroxyls of the glucose residue. The acyl derivatives include the tetraacetyl- and tetrapropionyl-β-D-glycosides (X) and (XI), respectively), and also three substances with longer hydrocarbon chains in the acyl residues. The acyl derivatives were obtained by the ordinary action of acid anhydrides in pyridine [19]. The antimitotic activity of compounds (X) and (XI) was considerably less than that of the glucoside (IX). With an increase in the lengths of the acyl chains there was a further fall in activity [19]. It is obvious that the acyl derivatives, although they are resistant to the action of enzymes, did not give what was desired. Protection from enzymes was therefore brought about by another method: derivatives of the glucosides with two blocked hydroxyls were synthesized. This was achieved by condensation with carbonyl compounds. Depending on the initial aldehyde, benzylidene-, fufuryl-idene-, anisylidene-, thenylidene-, and salicylidene glucosides (XII-XVI), respectively) were obtained. The interaction of the glucoside with the aldehydes was performed in the presence of zinc chloride. The position of attachment of the aldehyde residue at C-4 and C-6 of the glucose residue was assumed by analogy with the known methyl benzylidene-α- and -β-glucosides. The initial glucoside can be regenerated by the hydrolysis of the benzylidene derivatives with acetic acid. Conversion into picropodophyllin β-D-benzylideneglucoside is achieved by the action of the alkalis [19].

The acyl derivatives and cyclic acetals of podophyllotoxin β-D-glucoside are less soluble in water than the initial glucoside. Both types of compounds are resistant to enzymatic hydrolysis. The antimitotic activity of the acetals is, again, lower than that of podophyllotoxin [19].

The aim of another direction of syntheses was to obtain amines of this series [20]. In the opinion of the authors, such compounds would fall structurally between two groups of antimitotic compounds: the alkaloids of the autumn crocus genus — *Colchicum* L. — and the lignins of the *Podophyllum* L. genus [20]. With the aim of obtaining the corresponding amines via the hydrazides, the interactions of hydrazine with lignins was studied. Picropodophyllin gave a homogeneous hydrazide which was undoubtedly the hydrazide of picropodophyllic acid (XVII). A mixture was obtained from podophyllotoxin in which compound (XVII) predominated; the hydrazide of podophyllic acid (XVIII) was isolated from the mother solutions after recrystallization. When the basicity of the medium is lowered, isomerization in the direction of the picro series can be inhibited: in methanol, under the action of hydrazine and acetic acid (ratio by weight 1:1), compound (I) gave a good yield of the homogeneous product (XVIII) [20].

Acyl and alkyl derivatives of the hydrazide (XVIII) have been synthesized. The alkyl hydrazides were obtained by the reduction of the acyl hydrazides with sodium tetrahydroborate or by the alkylation of the hydrazides or by the action of alkylhydrazines on podophyllotoxin. Hydrazides of the podophyllic and picropodophyllic acid series have also been obtained from 4'-demethylpodophyllotixin, α- and β-peltatins, and epi-, deoxy-, and isodeoxypodophyllotoxins (for the last-mentioned compound, see formula (XIX)). Podophyllic acid azide (XIX) and picropodophyllic acid azide have been described. Curtius degradation is complicated by the presence of free hydroxyls. Dehydration takes place and the C—N bond arising is oriented as in picropodophyllin. As a result, apopodophyllamine (XXI) is obtained which, on hydrogenation, gives isodeoxypicopodophyllamine (XXII) and isobisdeoxypicrophdophyllamine (XXIII) [20] (Scheme 2).

Thus, starting from podophyllotoxin it was possible to synthesize amino compounds only with a spatial structure differing from that of the initial lignin. Amino compounds with



Scheme 2

the same orientation of the C—N bond and also of the C—O bond of the carbonyl group of podophyllotoxin are obtained in low yield from the lignins of this series not containing a hydroxyl at C-1 [20]. Nothing has been reported about the biological activity of the amino derivatives. As a result of this work [20] and also of an investigation of acetyl derivatives of podophyllotoxin glucoside [19]. compounds have been found that are favorably distinguished in a chemotherapeutic trial.

With approximately the same activities as those of the glucosides themselves, the products of the condensation of the podophyllotoxin glucosides with benzaldehyde cause fewer side effects [19]. Analogous derivatives of 4'-demethylphyllotoxin and of α-peltatin, showing advantages in a number of cases over the corresponding glucosides, have been synthesized [19]. Two products of the transformation of podophyllotoxin were selected for clinical trials: a cyclic acetal, podophyllotoxin benzylidene-β-D-glucoside (XII), and podophyllic acid ethylhydrazide (XXIV). The acetal derivative (XII was the main component of the drug SP-G. In addition, SP-G contained 4'-demethylpodophyllotoxin benzylideneglucoside, and also other accompanying substances originating in the plant raw material [21, 22]. For peroral use, SP-G was dissolved, with the use of Tween-80 as solubilizing agent. The second drug (XXIV), designated SP-I [21-25] has also acquired the names of Proresid [23, 26, 27], and mitopozide [27]. SP-I is comparatively readily soluble in water [21]. The action of both drugs has been studied in detail in vitro and in vivo. Like podophyllotoxin, they inhibit mitosis, being spindle poisons acting in vitro only in the dividing cell. Only in high concentrations do these drugs prevent the entry of cells into mitosis, exhibiting the properties of nonspecific cell poisons [21, 23]. Their interaction with tubulin has also been studied [22]. Like podophyllotoxin in binding with tubulin, they compete with colchicine. However, they are less active in this respect [28].

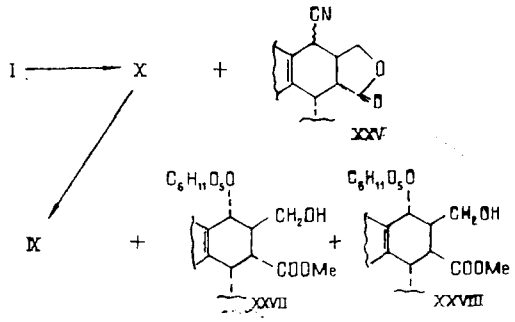
Information on the experimental antitumoral activity of both drugs is known [21]. So far as concerns their activity in the clinic, the initial judgment of doctors has not rejected the possibility of continuing trials [24, 29, 30]. There is information on the clinical doses of both drugs, for example in [24, 31]. In [31] it is concluded that they are unsuitable as therapeutic agents. In a review [32] the indeterminacy of the clinical trails of both drugs is reported. Another review [6] evaluated these substances as possessing a minimal clinical activity and as being excessively toxic. The result remains the same, although both drugs have been placed on the market as antitumoral agents since 1963 [10]. Consequently, there are grounds for stating that the hopes that arose in the course of the study of the podophyllotoxins are in ruins [6].

However, the work expended has proved useful subsequently for finding new compounds of the podophyllum lignin group and their chemotherapeutic study. In addition to the isolation of the natural podophyllotoxin glucoside, in the synthesis of SP-G the possibility of its production from the aglycon was studied. A glucose residue was introduced into the hydroxyl at C-1 [33] by a method proposed previously [34], consisting in the action of tetraacetylbromoglucose in the presence of caustic alkali in methanol. However, for this case, in order to exclude epimerization at C-3, alkali had to be eliminated, which was achieved by performing the reaction in acetonitrile in the presence of mercury cyanide [33]. Podophyllotoxin tetra-O-acetyl-β-D-glucoside (X) was obtained with a yield of 50%. By-products were: podopyllotoxin tetra-O-acetyl-α-D-glucoside and 1-cyanodeoxypodophyllotoxin (XXV), in which the cyano group possibly possessed the epi orientation [33].

The splitting out of the acetyl groups presented certain difficulties: alkaline agents are unsuitable: acids cause undesirable transformations at C-1; substitution or epimerization is possible, particularly at high temperatures. The hydroxyl in this portion is also insufficiently stable in hydrogenolysis. The transformations are due to the fact that the C-1 position is a benzyl position with respect to the benzene ring A (see structure (I)) [33]. The acetyl groups were split out by their trans-esterification in methanol in the presence of anhydrous zinc chloride [33]. The lactone group also took part in trans-esterification. When this reaction is performed on podophyllotoxin itself, an equilibrium is

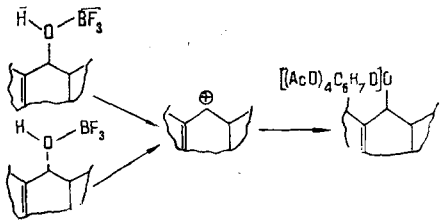
established of it with methyl podophyllate and an isomer of podophyllotoxin to which we have given the name neopodophyllotoxin (XXVI). The latter, thanks to the features of its spatial structure, does not undergo the picro rearrangement under the action of caustic soda but gives podophyllic acid [35].

It proved possible to perform the deacetylation of compound (X) under suitable conditions. On being boiled for 15 h, this tetraacetate gave compound (IX), identical with that isolated from *Podophyllum emodi* Wall., in a yield of 60%, by-products being the β-D-glucosides of methyl podophyllate (XXVII) and of methyl picropodophyllate (XXVIII), with yields of 15 and 10%, respectively [33]. The course of the synthesis of glucoside (IX) is shown in scheme 3 [33].



Scheme 3

Another method of introducing a carbohydrate residue was then investigated. It was found that the action of tetra-O-acetyl-β-D-glucopyranose in dichloroethane in epipodophyllotoxin (VII) or podophyllitoxin (I) in the presence of boron trifluoride etherate at −20°C leads to the formation of epipodophyllotoxin O-(2,3,4,6-tetra-O-acetyl-β-D-glucoside) [36]. The authors explained this result by the appearance of a stable carbonium ion (scheme 4) [36]. In this reaction, the anomeric structure of the initial carbohydrate component was retained, i.e., in this part, as well, the reaction was highly stereospecific [36]. Thus, in the case of the initial β-anomer, the reaction product contained not more than 10% of the α-anomeric glucoside and the yield of the β-anomer was 68%. This method of introducing a glucose residue, in which the oxygen atom of the glycosidic bond is introduced by the carbohydrate reagent was called "glycosidation" by the authors [36] in contract to the reaction described previously in which the carbohydrate residue is introduced via tetraacetobromoglucose [33], where the linking oxygen atom obviously belongs to the aglycon. The latter process was called "glycosylation" [36].



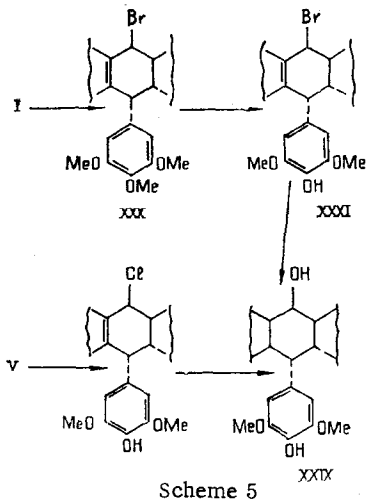
Scheme 4

On glycosidation, epimerization takes place at C-1 of podophyllotoxin. In contrast to glycosidation, on the glycosylation of epipodophyllotoxin the yield of the tetraacetyl derivative did not exceed 30%. Furthermore, as already mentioned, the 1-epi derivative of podophyllotoxin was formed. These facts are explained by the high reactivity of the secondary hydroxyl at C-1 and the stereochemistry of epipodophyllotoxin [36].

In glucosidation, the origin of the oxygen of the glucosidic bond is confirmed by the configuration at C-1 of the carbohydrate residue of the glucoside, corresponding to the pyranose anomer used for this reaction [36].

The synthesis of glycosides of 4'-dimethylepipodophyllotoxin was then investigated. The starting material could be the 4'-dimethylpodophyllotoxin β-D-glucoside isolated from the roots of *Podoplyllum emodi* Wall. [37] or of *Podophyllum peltatum* L. [16]. However, this was

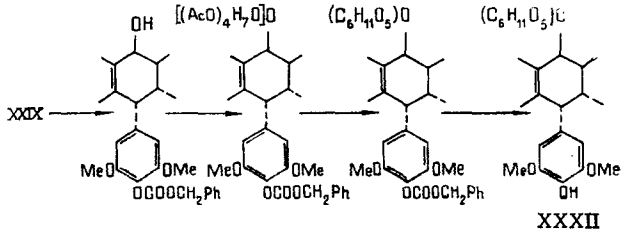
obtained from the raw material in negligible amounts. Although the amount of 4'-demethylpodophyllotoxin in the resin was 1.7%, it likewise cannot be considered as a starting material because of the poor availability of the raw material. However, epimerization to 4'-demethylepipodophyllotoxin (XXIX) was developed with it (scheme 5) [38].



Scheme 5

The conversion of the methoxyl at C-4' into a hydroxyl takes place in two stages. The 1-bromodeoxyderivative (XXX) is first formed under the action of hydrogen bromide in dichloroethane. Then the 1-bromodeoxy-4'-demethylepipodophyllotoxin (XXXI) is crystallized from the reaction mixture (scheme 5) [38]. Epimerization at C-1 of compound (XXXI) was shown unambiguously by its PMR spectrum. The bromide atom was replaced by a hydroxyl group under the action of barium carbonate (1 h at 40°C in an aqueous medium), giving the product (XXIX) (scheme 5) [38]. In addition, epimerization at C-1 by the action of hydrochloric acid in a mixture of acetone and water (boiling for 2 h) has been described (scheme 5). The PMR spectrum showed the epi configuration of the chlorine atom [38]; the PMR spectrum of the diacetyl derivative of (XXIX) confirmed the epi configuration of the substituting group at C-1 [38].

Compound (XXIX) was glycosylated with tetra-O-acetylglucose in the presence of boron trifluoride etherate. The hydroxyl at C-4' was first protected with a benzyloxycarbonyl group. After deacetylation of the tetraacetyl glucoside, 4'-benzyloxycarbonyldemethylepipodophyllotoxin β-D-glucopyranoside was obtained, and the protective group was split out by hydrogenation. This completed the synthesis of 4'-demethylepipodophylotoxin β-D-glucoside (XXII) (scheme 6) [39]. The galactoside was obtained similarly. In this case, immediately after glycosylation about 5% of the α-anomer was separated. The PMR spectrum of compound (XXXII) again confirmed the epi configuration at C-1 [39]. Epipodophyllotoxin (VII) and its glucoside, and also substance (XXIX) and its glucoside (XXXII), showed no appreciable advantage in relation to antimitotic action [25].



Scheme 6

Cyclic acetals were then synthesized, as in the preparation of the drug SP-G. The condensation of the pyranosides with carbonyl compounds was carried out in the presence of ordinary acids or of Lewis acids. If acetals or ketals were used, the reaction gave particularly favorable results for simple aliphatic carbonyl compounds. The formation of two isomers (epimers) at the carbonyl carbon atom is possible, but almost without exception the substance with the axial substituent in the aldehyde residue predominated [25]. Zinc chloride was used as a Lewis acid, the medium — the aldehyde — being taken in considerable excess.

The process was performed with shaking in an anhydrous medium in a current of nitrogen. The course of the reaction was monitored with the aid of thin-layer chromatography. In another method, the reactants were dissolved or suspended in nitromethane with p-toluenesulfonic acid or a cation-exchange resin as catalysts [25]. The number of derivatives of this type prepared was 48. Some of them showed appreciable antimitotic activity and antitumoral action [25]. Table 1 gives examples of the preparations with the most favorable experimental results [25].

The ethylidene (XXXIII) and thenylidene (XXXIV) derivatives were selected for clinical trials. The symbols VP-16 (VP16-213) and VM-26 are synonyms of the names etoposide and teniposide, respectively. For compound (XXXIII), in addition, the name vepiside is used [40]. Information on the antitumoral activities of both drugs in vivo on mouse plasmocytoma is given in [41]. The method of administering these substances has a substantial influence on their in vivo efficacy [32].

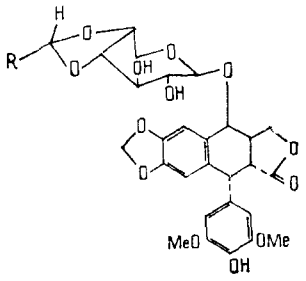
A feature of etopside and teniposide is the mechanism of their action. Depending on the antimitotic activity of podophyllotoxin derivatives, they prevent the interaction of colchicine with tubulin to a greater or smaller degree [28, 42]. While possessing the capacity for inhibiting cell division, neither of the drugs delays mitosis. They do not have the properties of spindle poisons, like podophyllotoxin or colchicine. No accumulation of cells at the metaphase stage is observed for them [43, 44]. Both drugs act on the cell cycle at stages preceding mytosis [44]. They differ from podophyllotoxin and other mitotic poisons by their capacity for cleaving DNA within cells [42, 45]; the corresponding experimental results [42] are given in Table 2. This table shows that the amount of the low-molecular-weight form of DNA rises under the action of the compounds, in the sequence podophyllotoxin—4'-dimethylpodophyllotoxin—4'-dimethylepipidiphyllotoxin—epoposide. Furthermore, a dependence of this activity sequence on the presence of a free hydroxyl in position 4' is obvious. It has also been established that etoposide inhibits the transport of nucleosides through the cell membrane somewhat more intensively than podophyllotoxin [42, 46]. The two effects are regarded as independent of one another [46, 47]. The difference in the mechanism of the action of podophyllotoxin and the majority of its derivatives, on the one hand, and etoposide together with tenoposide on the other hand, are obvious.

Table 2 gives information on the advantages of etoposide and teniposide over the podophyllotoxin derivatives with which they have been compared. With any changes in the structure of podophyllotoxin the activity in inhibiting cell division increases severalfold. The formation of benzylidene derivatives leads to a substantial increase in activity in all cases, and for dimethylpipodophyllotoxin β-D-glucopyranoside the order of magnitude of the activity becomes the same as for podophyllotoxin. Tenoposide is as active with respect to this index as podophyllotoxin, but etoposide is ∿10 times weaker.

in vivo experimenta on leukemia L-1210 also indicates a considerable superiority of the acetal derivatives of the glucosides. For example, for the benzylidene derivatives of

TABLE 1. Antimitotic and Antitumoral Activities of Acetals of the Glucoside (XXXII) [25]

R	Formula	DE-50*, mg/ml	ILS**, %
CH_3—(VP-16)	XXXIII	0,031***	167
$CH_2CH{=}CH_2$—		0,016	121
$(CH_3)_2CH$—		0,0055	121
		0,018	136
 (VM-26)	XXXIV	0,0048	121



*Concentration at which 50% of mouse mastocytoma P-815 cells were inhibited.
**Increase in the length of survival of mice with leukemia L-1210.
***According to other results, 0.046 mg/ml [44].

TABLE 2. Information on the Experimental Activity of Podophyllotoxin and Its Derivatives

Name of compound	Formula	DE-50 [49], mg/ml	ILS [49], %	k_i [27], M	DNA* [42], %
Podophyllotoxin	I	0,005	35	0,51	5
4'-Demethylpodophyllotoxin	V	0,007	10		33
Deoxypodophyllotoxin	VI			0,54	5
Podophyllotoxin β-D-glucopyranoside	IX	6,0	0	180	
Podophyllotoxin benzylidene-β-D-glucopyranoside (SP-G)	XII	3,8	5	39	
Epipodophyllotoxin	VII	0,034	11	1,7	3
Podophyllic acid ethylhydrazide (SP-I)	XXIV			4,5	
4'-Dimethylpipodophyllotoxin	XXIX	0,056	7	0,65	68
4'-Dimethylpodophyllotoxin glucoside		2,0	0		
Epipodophyllotoxin glucoside		20	7		
Demethylepipodophyllotoxin glucoside	XXXII	4,4	34		
Demethylpodphyllotoxin benzylidene-β-glucopyranoside		0,79	29		
Epipodophyllotoxin benzylidene-β-D-glucopyranoside		0,33	60		
Demethylepipodophyllotoxin benzylidene-β-D-glucopyranoside		0,0068	91		
4'-Demethyldeoxypodophyllotoxin					56
Etoposide**	XXXIII	0,046 [44]	200*** [44]		75
Teniposide	XXXIV	0,005 [43]	121–375 [43]		56

*The low-molecular-weight form of DNA is expressed in percentages of the radioactivity representing this fraction on centrifugation.
**On a single intraperitoneal injection, etoposide proved to be 3-4 times less toxic than teniposide [44].
***Perorally. For the DE-50 and the ILS, see the footnote to Table 1. k_i is the concentration at which inhibition of the binding of colchicine with tubulin is observed.

4'-dimethylepipodophyllotoxin glucoside the increase in the length of survival of experimental animals exceeds by almost three times this activity index of podophyllotoxin. For etoposide and tenoposide the superiorities are represented by factors of 5.7 and 10.7, respectively. The advantage of the 4-dimethylepi derivatives over the 4'-dimethylpodophyllotoxins proved to be unexpected [25].

Information on the inhibition of the binding of colchicine (see Table 2) shows that the mechanism of the antitumoral action of all the compounds active in this respect consists in binding with butulin, which is expressed in an inhibition of mitosis at the metaphase stage. There are such results, for example, for podophyllotoxin and the drugs SP-G and SP-I. It is characteristic that the introduction of a glucose residue into podophyllotoxin leads to a sharp decrease in the capacity of compounds for competing with colchicine, but the fall is considerably less for the acetal derivative (XII) or, in other words, the introduction of a benzylidene residue increases the activity of the glycoside. The ratio of the activities of podophyllotoxin, its glucoside, and the benzylidene compound with respect to other indices are similar (see Table 2).

Other workers [42] have deduced that etoposide and teniposide inhibit the cell cycle in the S or early G_2 phases. Such action is characteristic of many substances that inhibit the synthesis of DNA. Furthermore, etoposide causes disturbances in the chromosomes [42].

Although etoposide and teniposide have similar chemical structures and the same type of mechanism of action, their action spectra in vivo are different. Teniposide is more active on plasmocytoma [41] and on Walker's carcinoma [43]. On the other hand, etoposide is more active on sarcomas 37 and 180, and also on leukemia L-1210. In the case of the latter, in contrast to tenoposide, a stable increase in the survival time was observed on peroral administration, as well [43]. Reviews [6, 47, 48] have generalized the results of the experimental investigation of the mechanism of the action of etoposide, teniposide, and some other compounds of the podophyllotoxin series, as well. The two drugs possess advantages over other podophyllotoxin derivatives and have deservedly been sent for clinical trials, which began

in 1971 [50]. Information on medicinal forms is given in a number of papers [11, 32, 50-55]. Etoposide is sparingly soluble in water, but medicinal forms are obtained with the aid of solubilizing agents. This extremely instructive fact must be borne in mind before renouncing the experimental study of a new preparation because of its poor water solubility. For figures on clinical dosage, see [11, 32, 50-54, 56].

For a better understanding of the course and results of the clinical testing of etoposide and teniposide the chemist must know that the modern chemotherapy of malignant neoplasms, as a rule, uses several antitumoral drugs for each case [1, p. 50]. Monotherapy — with a single drug — is used mainly in the clinical trials of new medicinal substances. In the majority of cases, a new drug is given to patients the treatment of whom does not lead itself to the measures previously adopted. This circumstance is usually not taken adequately into account in the experimental search for new antitumoral drugs and this factor apparently hinders the evaluation of a drug in clinical investigation.

Favorable results of the use of etoposide in small-cell lung cancer have been reported [50, 51, 53, 54, 57]. The efficacy achieveable is 40% [50]. According to [53], an effect is observed in 50% of patients, with an average lengthening of their lives of eight months, the quality of these lives being practically normal. Three (6.4%) of the patients were alive and healthy after more than two years. A case of a cure (observed for more than six years) has been described [59]. The best results of the use of etoposide in this form of disease have been given by cooperative (in several medical institutes) clinical trials [60]. An effect was achieved in 12% of cases with a mean remission time of 35 weeks (nine weeks in the case of unsuccessful treatment). The more favorable results in cases described previously for monotherapy have been explained [60] either by a smaller number of patients observed at that time [53], or by the fact that those observed previously were not subjected to the treatment or it was less intensive.

There is information on the activity of both drugs on other types of human tumors [1, 11, 50, 57]. Estimates of difference in their clinical significance are regarded as unfounded [32]. In actual fact, for example, the opinion of the advantages of teniposide brain tumors according to [50] is based on 63 observations and those for etoposide is hardly convincingly based, all the more so since in the experiments the possibility of overcoming the blood–brain barrier was shown for etoposide [58].

Both drugs, but particularly etoposide, have also been tested in combination with others [40, 61-68]. In a number of cases advantages over monotherapy were reported [62, 64]. However, sometimes no such results were achieved orthe role of etoposide or of teniposide was a matter of doubt [40, 63, 65, 67].

A review [32] gives information not only on the clinical activity of both drugs but also on their toxicity. It has been reported that no clear difference is shown between them clinically, and it is also assumed to be undesirable to seek for new derivatives of this class of compounds: this would only complicate the clinical study of etoposide and teniposide [32]. But on the basis of the results of a study of the mechanism of the action of podophyllotoxin derivatives the consideration has been expressed that a further modification of the latter may give derivatives possessing new and unique properties [42]. Such a conclusion has been permitted, in particular, by the properties of 3'-dimethylepipodophyllotoxin, which causes inhibition of growth by two mechanisms — by being bound with tubulin, like podophyllotoxin, and by clearing DNA, like etoposide [42].

All that has been said above relative to the clinical testing of etoposide and teniposide illustrates the difficulties of evaluating its results and some obstacles arising at this stage of the development of a new antitumoral drug.

The efficacy of the antitumoral drugs in use and those being sent for clinical trials is limited. This situation must apparently be explained sometimes by the imperfection of the principles of the experimental chemotherapy of malignant neoplasms. Some results of the search for antitumoral plant compounds has been considered previously [69]. Certain prevailing views can be regarded as having been adopted without adequate foundation. Thus, the principle of seeking only substances interfering with dividing cells may be doubted. Then the unproved view holds sway that the higher the antitumoral activity in an experiment the more does a preparation deserve to have a clinical trial. Also adopted a priori is the requirement of the chemical individuality of a plant substance selected for investigation.

The search for valuable substances with the aid of experiments on transplantable tumors is regarded as basic in the untested assumption of the correspondence of laboratory experi-

ments to clinical results [70]. A rise in the demands on the activity indices of substances selected on the basis of transplantable tumors in vivo appears dangerous because of possible loss of substances valuable for clinical purposes [71, 72]. In [73], doubt is expressed about the universal principle of using the maximum tolerated dose, and it is also shown that in the selection of plant antitumoral substances the criteria for evaluating activity must be different from those used for synthetic compounds.

The evaluation of combinations of transplantable tumors used for selecting new drugs is carried out by determining the capacity of the test for showing the antitumoral activity of drugs already used in clinical practice [74, 75]. Apparently, such an evaluation of tests predetermines the similarity of newly found substances to those already in use, i.e., it predetermines their limited efficacy in clinical medicine.

The principles and methods of the preclinical search and study of antitumoral substances is also important for substances of plant origin. In the USA, substantial innovations are repeatedly introduced into the selection system. Thus, since 1975, in addition to other changes in the system of selection of transplantable human tumors in thymusless nude (having no immunity) mice have been included among the tests [76]. In 1982, the system of tests underwent further changes [77, 78]. Other principles of action on the malignant cell [77] and also of selection [79] are being investigated and discussed. It is proposed to use an in vitro test on clone-forming cells of human tumors [71, 80]. It has been established that in this method sensitivity to the action of drugs is confirmed clinically in 70% of cases [80]. Thus, it is possible by the system of screening used in the USA to detect an activity of substances having no antitumoral action [81]. The method is being intensively discussed and investigated [72, 80-82], and this has also found its reflection in the domestic literature [83]. The method of clone-forming cells of human tumors can also be used for detecting calmodulin antagonists [80]. Calmodulin is a protein which promotes malignant growth by binding the calcium ion; for its properties and structure, see [85].

The methods of screening used have apparently far from exhausted the possibilities of detecting effective substances in plants. New approaches in selection should disclose new possibilities. Some prospects are also being opened up by the plant raw material itself. It is not excluded that in a number of cases it will be useful to investigate not plant materials dried by the usual methods but fresh or lyophilized materials. The choice of individual sections of plants or plant products could probably give new results. In this respect, apparently, it could be of interest, for example, to investigate the flower pollen, the collection of which is, in a number of cases, not a technically insoluble problem.

Plant extracts or more or less narrow fractions of them could prove to be useful in the oncological clinic. Certain considerations could be expressed in favor of this. In the majority of cases, the active substance of any plant is accompanied by other compounds likewise possessing antitumoral action. The question arises: is it necessary to separate the natural mixture of such substances? A combined chemotherapy is most frequently used. It is quite possible that in the plant some physiological function is performed not by the main active principle but by its combination with accompanying compounds possessing the same activity or with plant substances having a different type of action. The preparation of such extracts of fractions and their standardization and toxicological study is a faster process than the search for, and study of, an individual active agent. Consequently, assistance to patients can be provided considerably earlier than an individual substance would be ready for passing on for clinical use. At the same time, it is possible that the efficacy of an individual compound may prove not to correspond to the antitumoral action of the initial plant, for which see [73]. However, the considerations expressed do not exclude the desirability and usefulness of the isolation and complete study of the main active principle.

The considerations given above for improving the search for plant drugs are not, of course, undisputed and far from exhaust the possible approaches in the search for new antitumoral drugs of plant origin.

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