activity while the larger the amount of nitrogen contained in a molecule of the substance the more strongly was its action expressed.

LITERATURE CITED

1. C. J. Pederson and H. K. Frendsdorff, Angew. Chem., 84, 16-26 (1972).
2. J. M. Timko and D. J. Cram, J. Am. Chem. Soc., 96, 7159-7160 (1974).
3. Synthetic Heterocyclic Compounds, Academy of Sciences of the Armenian SSR, 1958, Pt. 3, pp. 27-29.
4. O. Moldenhauer, G. Trautmann, W. Irion, et al., Ann. Chem., 580, 169-190 (1953).
5. A. A. Shulezhko, I. T. Rozhdestvenskaya, and A. I. Kipriyanov, Zh. Org. Khim., 6, 2118-2121 (1970).
6. Toshinori Iseki and Tukouru Sugiura, J. Biochem. (Tokyo), 30, 113-118 (1939).
7. S. A. Vartanyan, T. R. Akopyan, E. G. Paronikyan, et al., Arm. Khim. Zh., 32, 19-23 (1979).
8. S. A. Vartanyan, T. R. Akopyan, and E. G. Paronikyan, ibid., 31, 349-352 (1978).
9. G. M. Paronikyan, L. G. Akopyan, et al., Genetika, 13, 1621-1625 (1977).

SYNTHESIS AND ANTITUMOR ACTIVITY OF CERTAIN BERBERINE-ETHYLENAMIDES OF PHOSPHORIC ACID

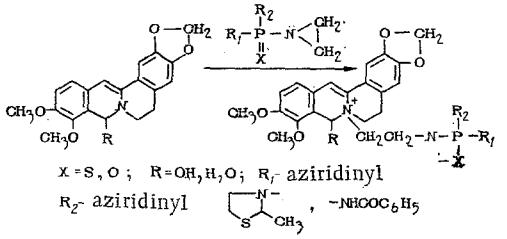
L. I. Petlichnaya, S. V. Ivasivka, and A. I. Potopal'skii

UDC 615.277.3:547.978.1].012.1

Substances have been discovered among berberine derivatives which possess antitumor properties [1-3]. The present work is devoted to the synthesis of new berberine derivatives, to a study of their toxicity, antitumor activity, and to the establishment of a relationship between the structure of the synthesized substances and their biological activity.

The compounds mentioned were obtained by the interaction of berberine, dihydroberberine, and hydroxyberberine with derivatives of phosphoric acid ethylenamides such as thiophosphamide, imiphos, and benzotef, in an organic solvent without a catalyst (compounds I-XI), the synthesis of some of which has been described previously [4, 5].

Under the conditions of the given reaction it is apparent that fission occurs of ethylenimine rings in thiophosphamide, imiphos, or benzotef by the withdrawal of electrons from the nitrogen atom to the oxygen or sulfur atom as a result of which a reactive ion is formed which interacts with reactive centers of the alkaloid possessing nucleophilic properties in accordance with the scheme:



Since thiophosphamide is a complex alkylating agent containing 3 aziridine groups in the molecule not all rings may be subject to fission at one time and depending on the reaction conditions there may be formed a mono-N'-[2-(N-berberinyl)ethylamide] of diaziridinylthiophosphoric acid (I) [4], compound (IX), di-N',N"-di-[2-(N-berberinyl)ethylamide of aziridinylthiophosphoric acid (II) [4], compound (X), and the trisubstituted derivatives N',N",N"'-tri-

L'vov Division of the A. V. Palladin Institute of Biochemistry, Academy of Sciences of the Ukrainian SSR.

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 15, No. 7, pp. 46-50, July, 1981. Original article submitted August 21, 1980.

0091-150X/81/1507-0486$07.50

TABLE 1. Derivatives of Berberinylethylamides of Phosphoric Acid

Compound	Name	Yield, %	Mp, °C	Found, %			Empirical formula	Calculated, %			UV spectra λ_{max} (log ε), nm
				N	S	P		N	S	P	
IV	N',N"-Di-(N-berberinylethylamide) of 2-methylthiazolidin-3-yl-phosphoric acid	50,0	95—100	7,26	3,24 3,41	3,56 3,60	$C_{45}H_{53}N_8O_{11}PS$	7,45	3,41	3,30	228—233 (4,83), 290—300 (4,39)
V	N',N"-Di-(N-berberinylethylamide)-N"'-benzoylamide of phosphoric acid	60,6	66—70	7,46	—	3,00 2,92	$C_{51}H_{52}N_6O_{12}P$	7,31	—	3,23	228—30 (4, 8, 6), 261—4 (4,59) 341—(4,46) 409—413 (3,89)*
VI	N',N",N"'-Tri-(N-dihydroberberinylethylamide) of thiophosphoric acid	69,0	200—5 (decomp.)	6,65	2,54 2,47	2,35 2,40	$C_{66}H_{69}N_9O_{12}PS$	7,00	2,67	2,64	223—5 (5,09) 315 (4,59)*
VII	N',N",N"'-Tri-(N-hydroxylberberinylethylamide) of thiophosphoric acid	55,0	112—15 (decomp.)	7,08	2,30 2,47	2,31 2,40	$C_{66}H_{63}N_9O_{15}PS$	6,76	2,58	2,49	220—5 (4,98) 308—311 (4,48)
VIII	N',N",N"'-Tri-(N-berberinylethylamide) of thiophosphoric acid	68,5	190—2 (decomp.)	6,69	2,57 2,29	2,00 2,11	$C_{66}H_{68}N_9O_{12}PS$	6,88	2,62	2,54	228 (4,87), 331 (4,53)
IX	N'-[2-(N-Acetonylberberinyl)ethylamide] of diaziridinylthiophosphoric acid	51,0	152—4 (decomp.)	9,39	5,59 5,12	5,11 5,21	$C_{29}H_{35}N_4O_5PS$	9,62	5,50	5,32	230 (4,50), 265 (4,50) 349 (4,50) 420 (3,73)
X	N',N"-Di-[2-(N-acetonylberberinyl) ethylamide] of aziridinylthiophosphoric acid	59,5	142—6	6,88	3,35 3,41	2,98 3,07	$C_{52}H_{58}N_8O_{10}PS$	7,01	3,28	3,17	220 (4,57) 260 (4,33), 290 (4,21) 333—5 (4,12) 420 (3,51)
XI	N',N",N"'-Tri-[2-(N-acetonylberberinyl)ethylamide] of thiophosphoric acid	72,4	128—30	6,23	2,55 2,44	2,08 2,00	$C_{75}H_{81}N_9O_{15}PS$	6,14	2,34	2,26	228 (4,75), 264 (4,67) 346 (4,62) 418—19 (4,01)
XIV	Chloride of thiophosphoric acid N'-[2-(N-acetonylberberinyl)ethylamide-N",N"'-di-(2-chloroethyl)diamide	67,4	140—2 (decomp.)	8,38	4,51 4,60	4,00 4,07	$C_{29}H_{38}Cl_3N_4O_8PS$	8,10	4,64	4,48	2,25—7 (4,99), 267 (4,80) 352 (4,75) 430—2 (3,40)
XV	Dichloride of thiophosphoric acid N',N"-di-[2-(N-acetonylberberinyl)ethylamide]-N"'-(2-chloroethylamide)	60,2	120—5 (decomp.)	6,86	3,02 3,00	3,15 3,17	$C_{52}H_{61}Cl_3N_8O_{10}PS$	6,45	2,95	2,85	229 (5,05) 265—7 (4,97) 350 (4,87) 429—30 (4,32)

*Shoulder.

[2-(N-berberinyl)ethylamide of thiophosphoric acid (III) [4, 5], compounds (IV-VIII, XI). The remaining unbroken ethyleneimine rings in compounds (I, II, IX, X) were determined titrimetrically by the thiocyanate method [6]. The obtained results agreed satisfactorily with those calculated. Compounds (III-VIII, XI) could not be determined by the titrimetric method under these conditions.

In addition the obtained compounds were subjected to interaction with dry hydrogen chloride gas to confirm the structure [7]. In the case of compounds (I, II, IX, X), i.e., in the presence of unbroken ethylenimine rings in the molecule, the corresponding derivatives (XII, XIII) [4] and (XIV, XV) containing 2-chloroethylamine groups were obtained.

The UV absorption spectra of (IV, VI-VIII) had 2 maxima while compounds (I-III, IX-XI) were characterized by 4 absorption bands which also confirmed their structure. This is linked with the presence in their molecules of the corresponding polycyclic berberine grouping.

The properties of the obtained compounds are given in Table 1.

Study of the acute toxicity of the synthesized compounds showed the presence of an interconnection between their structure and biological activity. Thus the toxicity fell on going from (I) to (III-VIII) or from (IX) to (XI), i.e., it was reduced with an increase in the number of alkaloid molecules in the compound. The marked differences in toxicity were also characteristic for compounds obtained from various derivatives of berberine (III, VI, VII). Thus, the LD_{50} of berberinol was 22.0 mg/kg on intraperitoneal injection to mice, of dihydroberberine 190 mg/kg, and of ketoberberine 240 mg/kg. The chloro derivative products (XII-XV) were also less toxic in comparison with the initial substances (Table 2). The display of acute toxic action by the various berberine derivatives was of a single type and was expressed in a retardation of the locomotor activity of animals, isolated convulsions of the extremities, quickening of respiration, dampness of the coat, etc.

Antimicrobial activity was investigated in liquid media on staphylococcus 209 and its UF-2 mutant by the generally used method. Data of the experiments made it possible to develop a series of preparations (I-X, XIII) which depressed growth of the UF-2 mutant, had no influence on the initial staphylococcus 209, and therefore may possess antitumor properties (Table 2).

The study of antitumor activity was carried out on random bred white mice and rats when using transplanted strains of S-180, LIO-1, RS-1, Guerin, etc.

The results of testing are given in Table 2 from which it is evident that the synthesized compounds displayed significant antitumor activity. The most marked action was recorded for (VIII) for which specific antitumor activity was studied on a wide spectrum of experimental tumors. It was established that (VIII) inhibited growth of the Harding-Passey melanoma by 80%, Guerin carcinoma by 95%, Shvets hemoblastosis by 100%, RS-1 by 83%, sarcoma 45 by 100%, and the Walker carcinosarcoma by 97%.

The influence of the synthesized substances was investigated on the process of blood formation at the same time as the antitumor action. It was noted that compounds (III-VIII, XI) did not cause appreciable changes in the characteristics of peripheral blood. Administration of (VIII) did not cause appreciable changes in the quantity of myelokaryocytes in the bone marrow, in myelogram, and in the histological structure of internal organs.

It is therefore possible to draw the conclusion that introduction of the alkaloid berberine in various forms to known antitumor preparations, particularly to phosphoric acid ethylenimides (thiophosphamide, benzotef, imiphos) led to the preparation of substances characterized by significant antitumor activity, lower toxicity, and also absence of a depressing action on blood formation.

EXPERIMENTAL (CHEMICAL)

TLC was conducted in system A in bound layers of KSK silica gel and gypsum with a mobile phase of benzene—methanol (8:2) (visualization in UV light with Dragendorf solution), system B on silufol with mobile phase n-butanol—acetic acid—water (4:1:5), or system C chloroform—acetic acid—methanol (75:5:20) (visualization in UV light or with iodine vapor). UV spectra were taken on a Spektromem-203 spectrophotometer (Hungary). Methanol solutions of the synthesized compounds were used.

TABLE 2. Antitumor and Antimicrobial Activity, and Acute Toxicity of the Synthesized Berberine Derivatives

Compound	Bactericidal concentration for UF-2	Animal species	LD_{50} on intraperitoneal injection, mg/kg*	Tumor strain	Therapeutic dose, mg/kg	Inhibition of tumor growth, %	P
I	1:1000	Mice	25,0 (19,5÷31,9)	RS-I hepatoma	15	90,5	<0,001
II	1:1000	"	30,0 (24,6÷36,6)	RS-I hepatoma	25	56,0	<0,02
				Guerin carcinoma	25	98,0	<0,001
III	1:5000	"	100,0 (62,5÷160)	Guerin carcinoma	25	95,4	<0,001
				RS-I hepatoma	25	69,0	<0,001
IV	1:1000	"	68,0 (42,5÷108,2)	Crocker sarcoma	25	61,4	<0,001
VIII	1:288000	"	148,0 (115,0÷175,0)	LIC-I lymphosarcoma	25	93,2	<0,001
		Rats	300,0 (238,0÷377,0)	Crocker sarcoma	25	66,2	<0,02
		Mice	320,0 (232,0÷441,0) †	Harding-Passey melanoma	25	80,5	<0,001
				Hertzen carcinoma	25	95,4	<0,001
				Shvets hemoblastosis	25	100,0	<0,001
				RS-I hepatoma	25	83	<0,01
				Sarcoma	25	100,0	<0,001
IX	1:1000	Mice	4,2 (2,86÷6,17)	Walker carcinosarcoma	25	97,1	<0,001
X	1:8000	"	12,9 (3,4÷52,8)	Shvets hemoblastosis	20	100,0	0,001
XI	1:10000	"	16,0 (12,5÷20,1)	" "	20		
XII			52,0 (38,2÷70,7)	" "	20		
XIII	1:10000	"	100,5 (63,0÷161,0)	" "	20		

*P = 0.05.

†Intramuscular injection. Limits of variation are given in parentneses.

N'-[2-(N-Acetonylberberinyl)ethylamide of Diaziridinylthiophosphoric Acid (IX). Thiophosphamide (0.04 mole) in dioxane (100 ml) was placed in a flask fitted with a reflux condenser, dropping funnel, and mechanical stirrer. A solution of acetonylberberine (0.01 mole) dissolved in dioxane (300 ml) was slowly added dropwise over 2 h. Afterwards the reaction mixture was heated on a water bath for 1 h, purified with active carbon, and the solvent distilled off in vacuum. The residue was transferred to a dish and rubbed many times (10) with small portions of ether (40 ml). The obtained product was dissolved in acetone and purified on a chromatographic column packed with KSK silica gel. Compound (IX) was obtained in 51% yield. R_f 0.22 (A), 0.30 (B).

N',N"-Di[2-(N-acetonylberberinyl)ethylamine] of Aziridinylthiophosphoric Acid (X). Acetonylberberine (0.02 mole) was boiled with thiophosphamide (0.015 mole) in acetone (200 ml) for 3 h. The product (X) was isolated as described above. R_f 0.18 (A), 0.28 (B).

N',N",N"'-Tri-(N-dihydroberberinylethylamide) of Thiophosphoric Acid (VI). A mixture of dihydroberberine (3.37g,0.01 mole) and thiophosphamide (0.66g,0.003 mole) was boiled in methanol (100 ml) for 15 h. The reaction mixture was purified with activated carbon, the solvent distilled off, and the residue rubbed many times with ether. The product in form of a brown powder was purified by precipitation with ether from chloroform solution. R_f 0.11 (B), 0.77 (C). Compounds (IV-VIII, XI) were obtained similarly.

Chloride of N'-[2-(N-Acetonylberberinyl)ethylamide N",N"'-Di-(2-chloroethyl)diamide of Thiophosphoric Acid (XIV). Compound (IX) (1 g) was dissolved in dry chloroform (30 ml), dry benzene (30 ml) was added, and the mixture cooled to a temperature —7 to -10°C. Dry hydrogen chloride gas was passed into the well cooled mixture for 1 h. Precipitation of a resiny solid was observed. The mixture was left overnight in the refrigerator, the solvent was then poured off, the gum dissolved in methanol, purified with active carbon, the methanol partially evaporated, and the residue rubbed with ether. Compound (XIV) was obtained having R_f 0.20 (B), 0.60 (C). Compound (XV) was obtained in a similar manner.

EXPERIMENTAL (BIOLOGICAL)

The toxicity of the compounds being tested was studied on intraperitoneal injection by the graphical method of Litchfield and Wilcoxon [8].

The usual nutrient medium was used for culturing staphylococci and a special medium from [9] for the mutant when studying antimicrobial activity. The initial concentration of the preparations being studied was 1 mg/ml, further dilution in the case of a positive result was carried out until bactericidal action disappeared.

When studying antitumor activity preparations were injected dissolved in the solvent mixture dimexide—polyethyleneglycol-400 (1:3), on the 4-6th day after implantation of the tumor, at the maximum tolerated dose (1/6 to 1/8 LD_{50}) subcutaneously with an interval of 24 h. The course consisted of 10 injections.

To investigate the influence of preparations on the blood forming system the usual analysis of peripheral blood was carried out, the quantity of myelokaryocytes in 1 μliter bone marrow from the sternum was determined, and a myelogram was calculated.

We thank I. Slavik and L. Slavikova of the Institute of Medicinal Chemistry of Brno University for kindly giving to us some alkaloids of high purity.

LITERATURE CITED

1. N. M. Turkevich, M. S. Oleiovskaya, Yu. M. Pashkevich, et al., Inventor's Certificate No. 368254 (USSR); Otkrytiya, No. 9, 92 (1973).
2. Japanese Patent No. 52-31360.
3. Japanese Patent No. 52-398.
4. L. I. Petlichna and N. M. Turkevich, Farm. Zh., No. 6, 40-43 (1975).
5. L. I. Pelichnaya and N. M. Turkevich, in: Synthesis and Mechanism of Action of Physiologically Active Substances [in Russian], Odessa (1976), pp. 82-83.
6. M. T. Bushkova, T. V. Koval'chuk, and Ts. I. Shakh, Farm. Zh., No. 1, 24-30 (1972).
7. L. D. Protsenko and N. Ya. Skul'skaya, Zh. Obshch. Khim., 35, 715-717 (1965).
8. M. L. Belen'kii, Elements of Quantitative Assessment of the Pharmacological Effect [in Russian], Leningrad (1963).
9. G. F. Gauze, Antibiotiki, No. 1, 62 (1960).