Action of Dry HCl on (IV) in Hexane. An excess of dry HCl was passed through a solution of 16.8 g (0.071 mole) of (IV) in 100 ml of hexane. The reaction proceeded with liberation of heat. The colorless crystals which separated were filtered off and washed with hexane to give 9.8 g of $(C_2H_5)_3N \cdot HCl$. The filtrate was evaporated, and the fuming viscous liquid residue was distilled *in vacuo* to give 7.6 g (62%) of (IV). The distillate crystallized in the receiver. The chloride was treated with methanol in the presence of $(C_2H_5)_3N$ to bind the HCl liberated. The resulting solid was filtered off, and the filtrate was distilled *in vacuo*. According to PMR, the resulting compound was (VII) [2].

Arginine-1-boradamantane (XXV). To a suspension of 3.1 g (0.018 mole) of arginine in 10 ml of CH_3OH was added a solution of 4.0 g (0.017 mole) of (IV) in 10 ml of CH_3OH, whereupon the arginine dissolved. Unreacted arginine was filtered off, and the filtrate evaporated to dryness to give 5.2 g (~100%) of (XXV). Following recrystallization from acetone–water mixture (1:1), (XXV) melted at >172°C (decomp.).

All the remaining compounds were prepared similarly. The solvents used can be hydrocarbons, ethers, methylene chloride, etc.

The properties of the compounds obtained are shown in Table 2.

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SYNTHESIS OF POTENTIAL DRUGS DERIVED FROM HYDROPHOSPHORYL COMPOUNDS. III. SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SCHIFF BASES OF RIFAMYCINS WITH A PHOSPHORUS–HYDROGEN BOND

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Semisynthetic antibiotics of the rifamycin series such as rifampicin [1], obtained by chemical modification with exclusively organic reagents (primary and secondary), carbonyl reagents, etc. [2], are widely used in medical practice. There is no information in the literature on the incorporation of phosphorus-containing groups into the rifamycin molecule. However, a variety of synthetic, biosynthetic, and semisynthetic organophosphorus compounds with biological activity are known, including antibiotics with various types of biological activity and containing different types of phosphorus bonds (P–C, P–O–C, P–N, P–S, etc.). Such compounds are octathione [3], cyclophosphan [3], mical [4], phosphaton [4], and phosphozal [5]. Increasing use had been made in recent times of drugs containing the phosphorus–carbon bond, such as phosphomycin [6], phosphonet [7], ethidronate [4], and phosphiin [4].

It was of interest to modify chemically one of the semisynthetic rifamycins [rifaldehyde (I)] with sodium 1-(4-aminophenyl)ethenephosphonite (II) in order to obtain a water-soluble azomethine with a phosphorus–carbon bond possessing antimicrobial activity. Reaction of the antibiotic (I) with the phosphonite (II) at 35-40°C gave the sodium salt of the azomethine phosphonite of rifamycin SV (III) in which the P–H bond was conserved.

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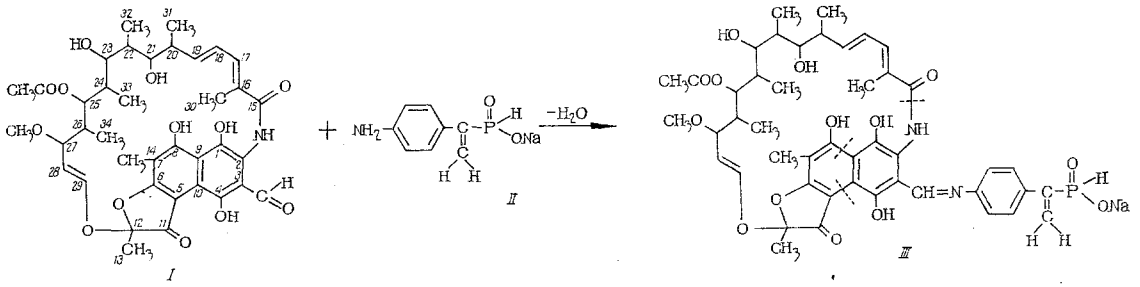
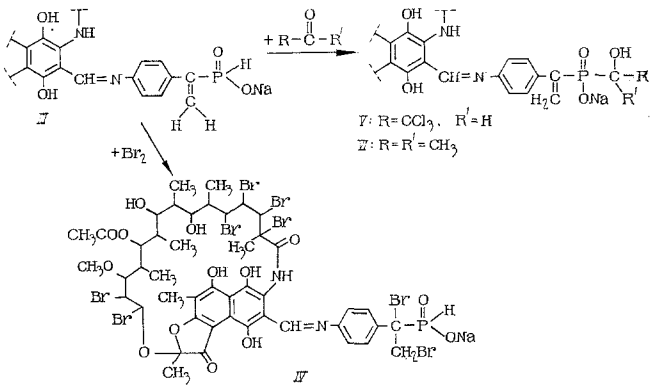


The PMR spectrum of (III) contained all the signals characteristic of the starting materials, with the exception of the signal for the proton of the aldehyde group. There also appeared a multiplet for the aromatic ring protons and the methine proton (δ 6.85-7.48 ppm). The signal for the proton attached to the phosphorus atom appeared as a doublet with a spin–spin coupling constant of 568 Hz (δ 7.13 ppm). The signals for the terminal protons of the phosphonite (II) were conserved in the PMR spectrum of the rifamycin derivative (III) (a doublet of doublets), but they were shifted to lower field (δ H_aC 5.69; δ H_bC 5.76 ppm). In place of absorption bands for the formyl group in rifaldehyde and the primary amino group in (II), the IR spectrum contained bands attributed to the reactant groups P=O (1250) and P–H (2395-2300), together with new absorption for the azomethine group CH=N (1622 cm^{-1}).

A number of further reactions of the hydrophosphorylated Schiff base of rifamycin SV (III) were carried out, not only to provide further confirmation of its chemical structure, but also in the search for new phosphorus-containing potential drugs.



Reaction of (III) with Br_2 at 3-5°C in chloroform afforded, following elution from silica gel, the product of bromination at the olefinic bonds of both the phosphoryl moiety and of the whole rifamycin molecule (IV). The IR spectrum of (IV) contained no absorption for C=C bonds, and the PMR spectrum contained no signals for olefinic protons. This modification also afforded a number of by-products, the structures of which could not be conclusively established. This reaction presumably gives rise to oxidation products followed by recyclization of the macrolide ring of rifamycin in some of its positions.

The rifamycin SV azomethine phosphonite (III) readily adds aldehydes (chloral) and ketones (acetone) at the P–H bond with the formation of phosphates (V) and (IV). The IR spectra of (V) and (VI) contained no absorption characteristic of the phosphorus–hydrogen bond. There was, however, an increase in the intensity of the absorption in the OH region (3450-3200 cm^{-1}). In the PMR spectra, doublets appeared for the methine protons at the α-carbon atom (δ 4.48-4.61 ppm), and, in the case of (VI), a doublet for the methyl group (δ 1.09 ppm). In the PMR spectrum of (VI), the integral intensity of the signals for the methyl protons increased threefold (a doublet of protons for the two equivalent methyl groups of the phosphinate moiety, (δ 1.09 ppm)) and the adjacent doublet for the protons of one of the four methyl groups (CH_3CH; posns. 31, 33, and 34) of the ansa-ring of rifamycin SV (δ 1.00 ppm) [8] as compared with the spectrum of rifaldehyde itself at δ 1.00-1.09 ppm.

EXPERIMENTAL PHARMACOLOGY

The antimicrobial activity of the hydrophosphorylated rifamycin SV derivatives (III-VI)

TABLE 1. Antibacterial Activity of Rifamycin SV Azomethine Phosphonites

Compound	Minimum inhibitory concentration, μg/ml			
	E. coli, strain 60	Proteus, strain 247	blue-green pus bacillus, strain 0387	staphylococcus, strain 340
III	15	30	60	0,0125
IV	100	>100	>100	100
V	100	100	25	0,125
VI	50	100	25	0,06
Rifampicin	6	12,5	25	0,003

was determined by serial dilution in meat-peptone broth. The activity was examined against several strains of *Staphylococci, Proteus,* blue-green pus bacillus, and *E. coli* (see Table 1). THe results showed that the rifamycin SV azomethine phosphonite (III), containing a phosphorus—hydrogen bond, possesses high antibacterial activity in comparison with the rifamycin phosphinates (V) and (VI), but was less active than the commercial antibiotic rifampicin. Bromination of (III) resulted in a sharp drop in activity both against staphylococci and against gram-negative organisms, as shown by biological studies with (IV).

The acute toxicity of the best of the compounds (III) was slightly less than that of rifampicin. When administered intravenously to white mice, (III) had an LD_{50} of 1000 mg/kg and a maximum tolerated dose of 250 mg/kg, and by the oral route the LD_{50} was 2000 mg/kg. The animals tolerated well intravenous administration of this compound.

EXPERIMENTAL CHEMISTRY

The IR spectra of the compounds obtained were recorded on a UR-20 spectrophotometer (East Germany) in KBr disks, and [^{1}H]-NMR spectra on a Tesla BS-487 instrument (100 MHz) (Czech SSR), internal standard hexamethyldisiloxane. The purities of (III-VI) were established by TLC on Silufol UV-254 plates, solvent system chloroform—methanol (9:1).

Sodium [4-(1-Ethenephosphonite)phenylimino]-3-formylrifamycin SV (III). Sodium 1-(4'-aminophenyl)ethenephosphonite (0.01 mole) was dissolved in 20 ml of methanol, and added with stirring to a solution of 0.01 mole of rifaldehyde in 50 ml of THF. The solution was heated for 4 h at 40-50°C, and evaporated to dryness. The residue was dissolved in 100 ml of chloroform, and the solution was filtered free from accompanying impurities and added dropwise to 300 ml of hexane. The solid which separated was filtered off, washed with hexane, and dried to give (III) as a dark claret-colored solid, yield 41%, mp 156-167°C (decomp.). Found, %: C 61.21; H 5.85; P+Na 5.98. $C_{46}H_{54}N_2O_{14}PNa$. Calculated, %: C 60.53; H 5.92; P+Na 5.93. R_f 0.16.

Sodium [4-(1-Ethene-2,2,2-trichloro-1-hydroxyethanephosphinate)phenylimino]-3-formylrifamycin SV (V) and Sodium [4'-(1-Ethene-1-hydroxy-1-methylethanephosphinate)phenylimino]-3-formylrifamycin SV (VI). Sodium rifamycin SV azomethine phosphinite (III) (0.01 mole) and 0.01 mole of the carbonyl compound in 30 ml of methanol were heated for 8 h at 60°C. Addition of ether precipitated a solid which was filtered off and washed with hexane, to give (V), yield 52%, mp 188-197°C (decomp.). Found, %: P+Na 5.41; N 2.68. $C_{48}H_{55}Cl_3N_2O_{15}PNa$. Calculated, %: P+Na 5.10; N 2.64. Compound (VI) was obtained similarly, yield 39%, mp 163-181°C (decomp.). Found, %: P+Na 5.69; N 2.97. $C_{49}H_{60}N_2O_{15}PNa$. Calculated, %: P+Na 5.57; N 2.89.

Sodium {4'-[1-(1,2-Dibromomethanephosphonite)]phenylimino}-3-formyl-16, 17, 18, 19, 28, 29-hexabromorifamycin SV (IV). To 0.01 mole of sodium rifamycin azomethine phosphonite (III) in 40 ml of chloroform was added gradually dropwise at 3-5°C with stirring 0.02 mole of bromine. The solution was kept at 18°C for 4 h and at 35-40°C for 3 h, and when the reaction was complete the mixture was cooled and added dropwise to 120 ml of hexane. The solid which separated was filtered off, to give (IV) as light reddish-brown crystals by eluting the methanol solution of the filtered solid on a column of length 25 cm containing silica gel (μ 100-250). Yield 11%, mp 181-207°C (decomp.). Found, %: P+Na 3.71; Br 41.58. $C_{46}H_{54}Br_8N_2O_{14}PNa$. Calculated, %: P+Na 3.48; Br 41.24.

The use of sodium phosphonites for the modification of rifamycins therefore affords water-soluble antibiotics containing a phosphorus—hydrogen bond, which possess antimicrobial activity.

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PHARMACOLOGICAL STUDY OF OINTMENTS WITH TRIAMCINOLONE ACETONIDE

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To study the influence of the type of the ointment base, the concentration of active and auxiliary substances in it, and also the frequency of application of the ointment, the condition of the laboratory animals, and other factors on the pharmacological effect, we investigated the antiinflammatory activity (AIA) of medicinal forms in ointment form with triamcinolone acetonide (I). This was prepared on emulsion bases of the oil in water (OW) and water in oil (WO) type, and also on a Vaseline–lanolin base with a 0.025 and 0.1% content of of I.

EXPERIMENTAL

The experiments were carried out on male rats of two age groups: 60-90 days (body weight 80-140 g) and 180 days (body weight 260-320 g). The activity of the ointments was evaluated on models of dextran and aerosil induced edemas, caused by subplantation administration into the right hind paw of the rats of 0.08 ml of a 3% solution of dextran (polyglucin with mol. wt. 45,000-60,000 + water, 1:1) and a 2.5% suspension of hydrophilic aerosil in water [1].

In some of the experiments, the ointment was applied following the administration of antiinflammatory compounds, and in the others the rats were narcotized by sodium pentobarbital (35 mg/kg, intraperitoneally) before the application of the ointment. The ointments were applied in an amount of 100 mg on the skin at the site of the leg–paw joint immediately after narcosis occurred in rats (or after the introduction of a phlogogenic compound in non-narcotized animals). The ointment was applied again after 45 min (after the rats woke up) in the same amount. In separate experiments, we limited ourselves to a single application of the ointment, or the ointment was applied even for a third time 5-6 h after the first application. Nontreated rats, and also rats who received ointment bases, and also those who received the "Fluorocort" ointment (Hungary), served as the control. The results at the peak of the edema reaction were taken into account: 1 h and 24 after the introduction of dextran and aerosil, respectively. The rats were killed by chloroform, the paws were amputated at the site of the leg–paw joint, and their weight was determined. The antiinflammatory (AI) effect of the ointment was determined by comparing the increment in weight of the edematous paws in treated and control animals.

In a separate series of experiments, we studied the endurability of 0.1–0.025% ointments with I compared with "Fluorocort" ointment, by applying the ointment daily in an amount of 250 mg on the tail of the rats (area 20-25 cm^2) for two weeks or more.

RESULTS AND DISCUSSION

In the first series of experiments we studied the AIA of the ointment with I with re-

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